Estimation and prediction for the BSE epidemic in Great Britain using a stochastic model

Christine Jacob, Laurence Maillard-Teyssier, Jean-Baptiste Denis, Caroline C. Bidot

BOOK OF ABSTRACTS

Prion 2008

8th-10th october

SPAIN

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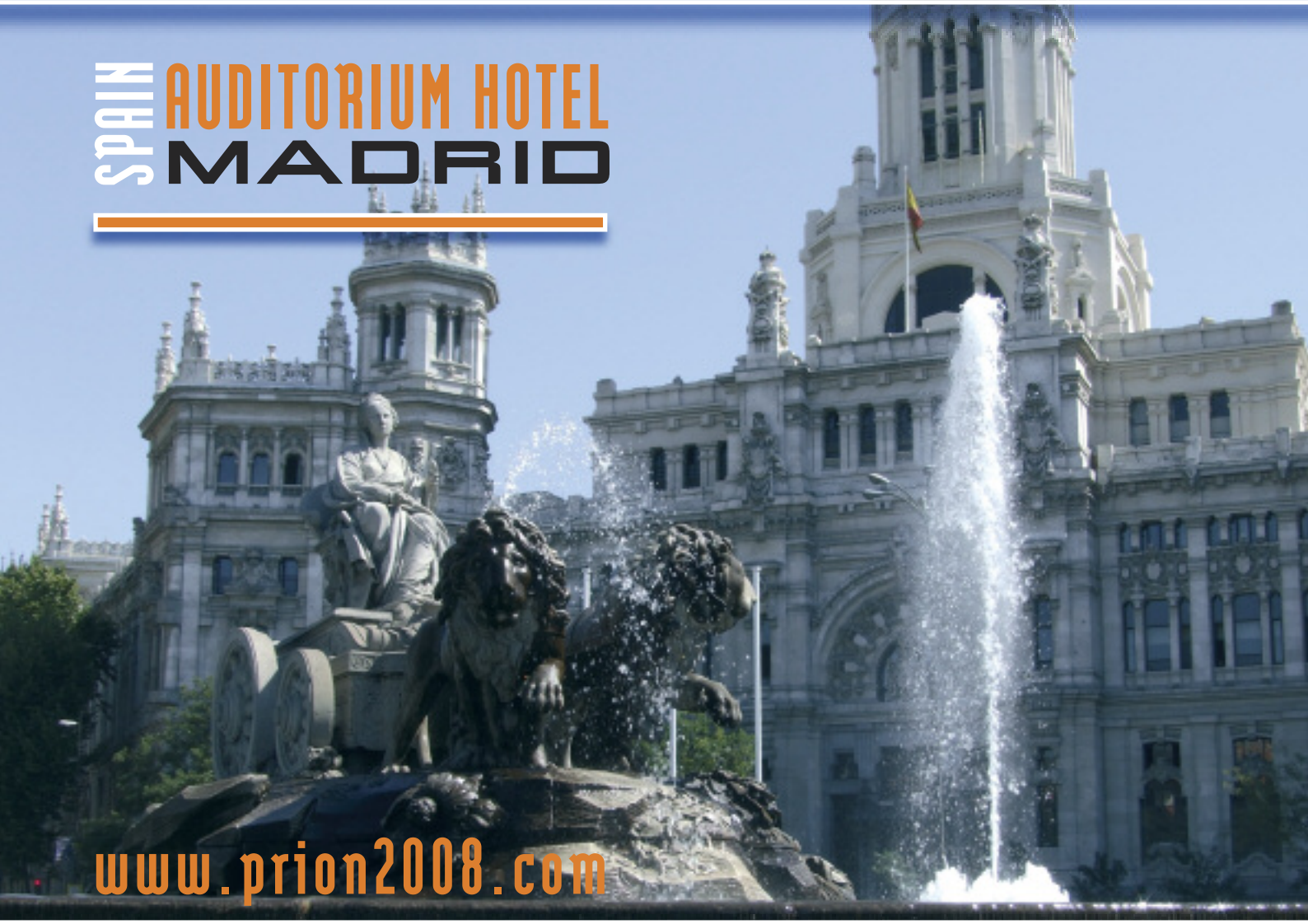

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SIXTH FRAMEWORK PROGRAMME

NeuroPrion

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INVITED SPEAKERS
abstract

S.01 The secret life of prion strains

Authors
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Content

Prions occur in the form of different strains, classically distinguished by the clinical and histopathological effects they elicit, for example, incubation time and location of the lesions and of PrP_{Sc} deposition: different strains target distinct brain areas, suggesting that cells differ in their susceptibility to prion strains. Interestingly, many strains can be propagated in inbred mouse lines or in cell lines that express only a single PrP sequence, leading to the well-substantiated hypothesis that strain specificity is encoded by different conformations of PrP_{Sc}, and that as host PrP_C adds onto the invading PrP_{Sc} it adopts their strain-specific conformation. It has been shown that some prion strains are very stable and can be transmitted across species without affecting their strain-ness, while others "mutate" to novel forms.

We have developed a cell-based assay, the Cell Panel Assay (CPA), that allows discrimination of prion strains by virtue of their cell tropism, i.e., their capacity to chronically infect certain cell lines but not others. With it, we can readily distinguish 22L, RML, Me7 and 301C on a panel of 4 cell lines (Mahal et al., PNAS 104:20908 (2007)). Using the CPA we have found that the transfer of 22L propagated in brain to the neuroblastoma-derived cell lines N2a or R33 changes the cell tropism of these strains, however, when the cell-derived prions are again propagated in mouse brain, they regain their original properties. We consider two possible explanations. (1) Host cells can influence the tropism of prions by causing a change in their structure (for example, the glycosylation pattern of PrP_{Sc}) or by adding to them a host-derived component (for example, membrane fragments, or a small RNA). (2) Prion strains are relatively mutable and exist in the form of biased quasi-populations (Domingo et al., Cell, 13:735 (1978); Collinge & Clarke, Science, 318:930 (2007)) from which different sub-populations are selected in brain cells and cell lines, respectively. Thus, a "brain-adapted" quasi-population would shift to a "cell-adapted" quasi-population on being transferred to cells, and vice-versa. We are currently examining whether the change of cell tropism is rapid, i.e. occurring immediately after the strain is replicated in the new host, as would be the case if tropism is imparted by the host cell, or gradual, as would be the case if selection occurred over several generations, as expected from the "quasi-population" hypothesis.

S.02 Yeast prion structure explains how proteins can be genes

Authors
Reed B. Wickner, Frank Shewmaker, and Rob Tycko
Laboratory of Biochemistry and Genetics, NIDDK and Laboratory of Chemical Physics; NIDDK, NIH, Bethesda, MD USA.

Content

The yeast prions [URE3], [PSI+] and [PIN+] are self-propagating amyloid forms of the Ure2, Sup35 and Rnq1 proteins, respectively. For each protein, a restricted region(the prion domain)is primarily responsible for the prion properties. We showed that deletion of the prion domain of Ure2p results in partial impairment of the nitrogen regulation function of the protein due to failure to interact with certain other proteins and destabilization of the protein. Deletion of the Sup35p prion domain produces several phenotypes indicating a functional role. Although [URE3] and [PSI+] arise de novo and are infectious, they are not found in wild strains, indicating that their net effect on their host is detrimental.

Surprisingly, shuffling the prion domain of either Ure2p or Sup35p does not prevent either from being a prion (or forming amyloid). This result proves that it is the amino acid composition, and not the sequence of the prion domain that determines prion-forming ability. It also suggests a parallel in-register beta sheet structure for the prion.

Amyloid structure cannot be studied by X-ray diffraction, because it cannot be crystallized, nor by solution NMR because it is not soluble. However, solid-state NMR has been used effectively to obtain detailed structures of amyloids formed of peptides as large as the 42 residue Abeta Alzheimer's disease amyloid. Using specifically 13C-labeled molcules, we showed that amyloid of the prion domains of Ure2p, Sup35p and Rnq1p are each in parallel in-register beta sheet structure. This means that, for example, valine35 of one molecule is aligned with valine35 of the next and the preceding molecules in the filament, with beta-strands perpendicular to the long axis of the filament. The line of valine35's is along the filament axis.

The parallel in-register beta sheet structure of these amyloids can explain how prion variant (strain) information can be inherited, that is, how a protein can be a gene.

S.03 Knock-in mice for the fatal familial insomnia mutation of PrP develop spontaneous, infectious prion disease

Authors
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Content

A critical tenet of the prion hypothesis is that misfolding of PrP is sufficient to create an infectious agent. Mutations in PrP can thereby cause disease by increasing the likelihood of spontaneous misfolding and the de novo creation of infectious prions. In order to study the events that lead to inherited, rather than sporadically acquired, prion disease we genetically engineered mice to express PrP from the endogenous locus carrying a mutation linked to FFI (fatal familial insomnia), an inherited human prion disease specifically characterized by thalamic neuronal loss and reactive gliosis. Our FFI mice develop a similar neuropathology to human FFI patients, as well as dysautonomia, sleep abnormalities, cerebellar atrophy and enlarged ventricles. These symptoms are very distinct from those described for other infectious and transgenic mouse prion models but are highly reminiscent of human FFI, establishing that our mouse model recapitulates the unique pathology associated with this mutation. Furthermore, the disease phenotype was readily transmitted to mice expressing wild-type PrP. The mutant PrP in our FFI mice carried an amino acid substitution that introduced a strong transmission barrier against established mouse prion isolates, eliminating the possibility that the mice were infected by contamination with exogenous prions to which the FFI mutation had simply rendered them more susceptible. These data demonstrate that a single amino acid change in PrP is sufficient not only to cause a distinct neurodegenerative disease but also the spontaneous appearance of prion infectivity.

S.04 Towards imaging prion replication and spread

Authors

Gerald S. Baron

Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, NIAID, NIH; Hamilton, MT 59840, USA.

Content

Background: Prion infections involve the spread of PrP_{res} within and between cells. Cellular mechanisms mediating the initiation, propagation, and intercellular spread of PrP_{res} are poorly understood. Among protein misfolding diseases, only prion diseases are capable of transmission between hosts under natural conditions.

Objectives: Our objective is to determine the mechanisms by which prions replicate and spread between host cells using imaging-based approaches. We are also investigating factors that contribute to the unique transmissibility of prion diseases, focusing on the role of GPI-anchored membrane association. Highlights of other RML work can be presented.

Methods: We have developed IDEAL-labeling, a novel technique that allows rapid and specific fluorescent labeling of cell-surface proteins containing the tetracysteine (TC) tag. We have created several TC-tagged PrP_C constructs. We are also expressing the amyloid domains from various amyloidogenic proteins, such as Sup35NM, as GPI-anchored fluorescent protein fusions in neuroblastoma cells.

Results: We show that IDEAL-labeled TC-tagged PrP_C molecules are converted to fluorescent PrP_{res} in live cells. Chase studies with anti-prion compounds post IDEAL-labeling have provided insight into their mechanisms of action. We also show inducible formation of self-propagating Sup35NM aggregates in cells expressing GPI-anchored Sup35NM-GFP. Aggregate formation can be initiated by treatment with pre-formed recombinant Sup35NM fibrils or co-culture with cells producing aggregated Sup35NM-mCherry-GPI. These events have been visualized by live cell imaging.

Discussion: With the advent of IDEAL-labeling and TC-tagged PrP_C, we have created the tools necessary for the first ever experiments aimed at imaging the propagation and intercellular spread of newly formed PrP_{res} in live cells. We have visualized related processes in a new model system utilizing a GPI-anchored version of another amyloidogenic protein. Our data advance our understanding of mechanisms by which GPI-anchored protein aggregates form and spread between cells and support the concept that GPI-anchoring modulates the transmissibility of amyloidogenic proteins. This provides a potential explanation why other protein misfolding diseases are not efficiently transmissible.

S.05 Reflections on a half-century in the field of Transmissible Spongiform Encephalopathy (how to endure without being brilliant)

Authors

Paul Brown, M.D.

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Content

The subject of transmissible spongiform encephalopathy may properly be said to have begun with the experimental transmission of scrapie by Cuillé and Chelle in 1936, although Creutzfeldt and Jakob had described the disease that bears their names in 1920-21. Thirty more years passed before the human disease was also shown to be transmissible, in 1966, and the following half century has seen the field move from classical biology to molecular biology and genetics, and from 'slow virus' to host-encoded 'prion' protein.

Because nothing is more important to the research scientist than the process of seeing a problem and devising ways of solving it, and because we live and die by our publications, as much care should be given to these vehicles of our work and reputations as to the research itself. Four aspects have been chosen for comment: authorship, abbreviations, data presentation, and references.

In addition to the science of research' there are several 'para-scientific' activities that may be categorized as 'the politics of research', which include administrative duties, committees (e. g., scientific meetings, grant organizations), journal/book editing, peer reviewing, and public relations Many young scientists are either unaware or dismissive of the importance of these 'scientific distractions', but their potential for influencing the direction of a field of research becomes increasingly evident as careers unfold. They are subject to uses and abuses, and some guidance and examples are given by way of illustration, particular attention being paid to the process of manuscript review which, because of its anonymity, is the most vulnerable to abuse.

As public and government interest in prions wanes in parallel with the disappearance of iatrogenic and variant Creutzfeldt-Jakob disease, the flow of money to sustain research is in evident jeopardy. With an uncertain future, it nevertheless seems possible that one of two things may breathe new life into the field: either an unforeseen new outbreak of human disease will occur (as has happened in the past), or a cross-fertilization between prions and the larger family of protein misfolding diseases, especially Alzheimer's disease, will bear fruit. For obvious reasons, we should hope for the botanical alternative.

ORAL COMMUNICATION

OC1.01 Mutant prion protein expression causes motor and memory deficits and abnormal sleep patterns in a new transgenic mouse model

Authors

Dossena, S.[1]; Imeri, L.[2]; Mangieri, M.[3]; Garofoli, A.[1]; Ferrari, L.[2]; Senatore, A.[1]; Bianchi, S.[2]; Balducci, C.[4]; Fioriti, L.[1]; Pincherle, A., Marcon[3]; Villani, F.[3]; Carli, M.[4]; Forloni,G.[4]; Tagliavini, F.[3]; Chiesa, R.[1]

[1]Dulbecco Telethon Institute - Mario Negri Institute, Milan; [2]Institute of Human Physiology, University of Milan; [3]Carlo Besta Neurological Institute, Milan; [4]Mario Negri Institute, Milan.

Content

A familial form of Creutzfeldt-Jakob disease (CJD) is linked to the D178N/V129 prion protein (PrP) mutation. We generated transgenic Tg(CJD) mice expressing the mouse homologue of this mutant PrP. These mice synthesize a misfolded form of the mutant protein in their brains, which is aggregated and protease resistant. They develop clinical and pathological features reminiscent of CJD, including motor dysfunction, memory impairment, cerebral PrP deposition and gliosis. Tg(CJD) mice also display electroencephalographic abnormalities and severe alterations of sleep-wake patterns strikingly similar to those seen in a patient carrying the D178N/V129 mutation and in sporadic CJD. This is the first transgenic animal model of a genetic prion disease recapitulating cognitive, motor and neurophysiological abnormalities of the human disorder. Tg(CJD) mice have the potential for giving greater insight into the spectrum of neuronal dysfunction in prion diseases.

OC1.02 Identification of neuron-specific gene regulatory circuits involved in prion-induced neurodegeneration

Authors

Booth, S.; Medina, S.; Saba, R.; Parchaliuk, D.; Frost, K.; Robertson, C.

NML.

Content

Identification of neuron-specific gene regulatory circuits involved in prion-induced neurodegeneration Sarah Medina1, Reuben Saba1,2, Debra Parchaliuk1, Kathy Frost1, Catherine Robertson1 and Stephanie Booth1,2 1Prion Diseases Program, Public Health Agency of Canada, 2Department of Medical Microbiology, University of Manitoba Pathways triggered by prions that lead to the damage, and ultimate death of neurons are as yet unidentified.

A number of studies have identified global gene expression changes in prion-infected brain; these have revealed multiple genes and signaling pathways that may be involved in pathogenesis. It is extremely important to determine which of these genes and pathways have a 'cause', rather than 'effect', role in the disease. The development of micro-dissection techniques to isolate and study the genetic content of individual cells allows us to specifically target degenerating neurons. Mice were infected with the RML strain of scrapie, or mock-infected with normal brain homogenate. Brains were removed from mice at time-points throughout disease incubation and placed in OCT in preparation for cryostat sectioning. We used Laser Capture Microdissection and Agilent whole genome microarray technology to determine temporal changes in gene expression specifically in degenerating neurons of the hippocampus and cerebellum during the course of disease. The recent identification of small non-coding RNAs, the microRNAs, has revealed an important new layer of gene regulation. We have previously determined that deregulation of specific microRNAs occurs in the brains of scrapie-infected mice. We therefore simultaneously identified microRNA expression changes in the RNA samples collected from microdissected degenerating neurons. Bioinformatics analysis based on 3'untranslated sequence homology to miRNAs was used to identify the potential gene targets of deregulated miRNAs. A combination of these data and gene expression data was used to predict regulatory circuits involved in prion-induced neurodegeneration.

It is now apparent that the vast majorities of genes do not operate alone within the cell, but interact in intricate networks of proteins and non-coding RNAs. Genome-scale experiments targeting temporal changes in individual infected cells will lead to the identification of these networks, thus providing the information necessary for the development of targeted therapies.

OC1.03 Cyclin dependent kinase 5 phosphorylation induces prion protein conformational change

Authors

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Content

Background: In prion diseases, prion protein (PrP) converts into proteinase K resistant, aggregate and fibril forms.

While several treatments such as acidic pH and mild denaturant conditions have been shown to convert prion protein in vitro, the cause of the conformational change of normal cellular PrP into its disease-associated form in vivo is unknown.

Objectives: Here, we examine if phosphorylation could be responsible for PrP conversion because PrP contains several kinase motifs and has recently been found in the cytosol, where kinases reside.

Methods: *In vitro* kinase assays were performed with purified recombinant and bovine brain extracted cyclin-dependent kinase 5 (Cdk5). Conversion of prion protein was examined by proteinase K digestion, Congo Red staining and immunoelectron microscopy with 3F4, 6H4 and anti-phosphoPrP antibodies. Phosphorylated proteins from PrP and Cdk5 co-transfected N2a cells and human adult brain protein extracts were purified with phospho-columns. The presence of PrP in the eluted phospho-proteins was verified by western blotting with 3F4, 6H4 and anti-phosphoPrP antibodies.

Results and Discussion: We show by in vitro kinase assays that Cdk5 phosphorylates recombinant PrP_{23-231} at the S43P44 motif. Cdk5-phosphorylated PrP becomes resistant to proteinase K (PK) digestion, forms Congo Red positive fibrils, and anti-PrP and anti-phosphoPrP$_{S43}$ (anti-pPrP$_{S43}$) immunopositive aggregates. PrP and pPrP$_{S43}$ are detected in PrP and Cdk5 co-transfected N2a cells and in adult human brain protein extracts. Roscovitine inhibition of Cdk5 phosphorylation or PrP S43A transfection in N2a cells eliminates the anti-pPrP$_{S43}$ immunopositive protein. We conclude that Cdk5-dependent phosphorylation induces a conformational change of PrP. These results raise the possibility that *in vivo* phosphorylation could participate in the conversion of PrP.

OC2.01 Monitoring prion protein stability by NMR

Authors
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Content

Background: : Prion diseases, or transmissible spongiform encephalopathies (TSEs), are a group of fatal neurological diseases that affect both of humans and animals. There are two major forms of prion protein: the native non-infectious form (PrP_C), and the misfolded infectious form (PrP_{Sc}). The structure of PrP_C is mainly alfa-helical with the exception of a small anti-parallel β-sheet, whereas PrP_{Sc} corresponds to an assembly of β-sheet forming amyloid fibrils. Since the first NMR structure of PrP_C, about 30 structures of the globular portion of PrP have been characterized for diverse organisms with different TSE strain susceptibilities. The few, minor structural differences between the PrP_C protein suggests that the key to understanding prion formation lies in the conversion between PrP_C and PrP_{Sc}.

Objectives: But how is the normal prion protein converted into an unfolding fibril state? We plan to acquire local structural information (i. e. region specific information) during the conversion of the protein to from its normal to an infectious state.

Methods: To identify the possible regions responsible for initiating amyloid fibril formation, we are using nuclear magnetic resonance (NMR) spectroscopic methods to characterize the stability of PrP_C and the intermediate state(s) between PrP_C and PrP_{Sc}. We are using specific ^{15}N and ^{13}C residue labeling in order to monitor chemical changes in different regions of the bovine prion protein residues 121 to 230 ($bPrP_{121-230}$) using urea as a denaturant.

Results and Discussion: We have monitored the chemical shift changes of $bPrP_{(120-231)}$ during unfolding using one- and two-dimensional (1D and 2D) ^1H NMR spectroscopy. Our results suggest that the stability of the small β-sheet of PrP_C is perturbed early in the denaturation process compared to the overall structure of PrP. It is possible that destabilization of the β-sheet is a required step in order for PrP_C to structurally transform into the misfolded form PrP_{Sc}. This could have significant implication in our understanding of the different polymorphisms and mutations that occur naturally in the *PRNP* gene, where a destabilized β-sheet would promote conformational changes that lead to the misfolded form of PrP.

OC2.02 Limited proteolysis of PK-sensitive PrP_{Sc} further supports a structure featuring beta sheet stretches interspersed with loops/turns for PrP_{Sc}

Authors
Sajnani, G.[1]; Pastrana, M. A.[1]; Ramos, A.[1]; Dynin, I.[2]; Vazquez, E.[1]; Onisko, B.[2]; Requena, J.[1]
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Content

Background: Partial resistance of PrP_{Sc} to PK revealed early on that it contains two domains, a highly sensitive one spanning up to residue ~90, probably structured as a random coil, and a very compact one spanning from that position to the C terminus. Using more resolutive techniques, we identified regions exhibiting intermediate susceptibility to PK within the compact core, suggesting that the central part of PrP_{Sc} (residues~90-178) conforms to an alternation of resistant beta stretches and more labile loops/turns. In the present study, we have aimed at extending our analysis to a PK-sensitive fraction of PrP_{Sc}, which can be completely and readily cleaved by PK, allowing a more extensive application of limited proteolysis protocols.

Objectives: While we have established that PK-sensitive PrP_{Sc} has converting activity *in vitro*, we first sought to confirm that it is infectious, to ensure that structural studies using it are fully relevant. After this, we probed its structure by limited proteolysis.

Methods: We isolated a PK-sensitive fraction of PrP_{Sc} by intermediate force centrifugation as described (Pastrana et al. Biochemistry, 45:15710-7, 2006). For infectivity studies, we inoculated 50/200 ug of it or of PK-resistant PrP_{Sc} to Syrian hamsters intracranially or intraperitoneally, respectively. For limited proteolysis studies, we treated PK-sen PrP_{Sc} with different concentrations of PK, and analyzed reaction products by WB and by mass spectrometry techniques.

Results and Discussion: *All animals innoculated with PK-sen PrP_{Sc} succumbed to scrapie with similar/lower incubation times than to those inoculated with PK-resistant PrP_{Sc}. Analysis of proteolytic fragments at different PK concentrations and times confirmed the same regions of relative susceptibility and resistance previously seen in total PrP_{Sc}, albeit with different, faster kinetics. These results lend further support to the notion that the central structure of PrP_{Sc}consists of short beta sheet stretches interspersed with loops/turns.*

OC2.03 Different strains have different "most infectious" particles

Authors
Beringue, V.; Tixador, P.; Herzog, L.; Chapuis, J.; Le Dur, A.; Reine, F.; Laude, H.
INRA.

Content

Background: *Much remains to be learned about the physical relationship between infectivity and aggregated PrP, and to which extent it varies according to the prion strain. Transmission of typical and atypical (Nor98) sheep scrapie and BSE infectious sources to transgenic mice expressing the VRQ allele of ovine PrP (tg338 line) has allowed us to establish a panel of biologically cloned strains that have been shown to produce stable and clearly distinct phenotypes based on the electrophoretic profile of PrP_{Sc}, its regional distribution in the brain, and the incubation time.*

Objectives: We have sought to further compare these strains according to sedimentation properties of the PrP_{Sc} aggregates and to examine the relationship with infectivity.

Methods: We have analysed the sedimentation properties of abnormal PrP and infectivity of each strain by velocity gradient centrifugation, by using detergent-solubilised, infected brain homogenate, instead of PrP_{res}-enriched material as generally done in such kind of studies. The detergent treatment conditions were optimised so as to separate PrP_C from the bulk of PrP_{res}. The gradient fractions were analysed for PrP_C, PrP_{Sc} and PrP_{res} content by ELISA and western blotting, and for infectivity by bioassay in tg338 mice.

Results and Discussion: We found that most PrP_{res} molecules sedimented in the middle region of the gradient. In this region however, the position and shape of the peaks noticeably differed among the strains, likely reflecting PrP_{Sc} aggregates of different sizes. Examining the distribution of infectivity revealed even more striking differences. Whereas the distributions of PrP_{res} and infectivity were largely overlapping in the case of *BSE* and *Nor98* strains, they were mostly decoupled for *127S* and *LA19K* strains. Thus most of *127S* infectivity was retained in the buoyant fractions of the gradient while a significant part of *LA19K* infectivity settled on the bottom. Ongoing studies aimed to examine the physicochemical nature of *127S* infectious particles and data about whether their disconnection from PrP_{res} constitutes a general characteristic of other fast strains will be presented.

Conclusion: These results show that the relationship between infectivity and pathological PrP multimers is not univocal and that the size and/or the physical nature of the most infectious particles may substantially differ among prion strains.

OC2.04 In vivo transmission of Alzheimer's disease by a prion like mechanism

Authors
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Content

Background: Misfolding and aggregation of proteins is the main feature of a group of diseases, termed Protein Misfolding Disorders (PMDs). PMDs include Transmissible Spongiform Encephalopaties (TSEs), Alzheimer's and Parkinson's diseases, among many others. Compelling evidences show that the only component of the infectious agent in TSEs is the misfolded form of the prion protein. Interestingly, the molecular mechanisms responsible for prion replication are very similar to the process of amyloid formation in all PMDs, suggesting that all these diseases have the inherent capability of being transmissible. In the case of high prevalent diseases such as Alzheimer's (AD), the study of a possible infectious origin is extremely significant.

Objectives: Demonstrate that AD pathogenesis can be transmitted in vivo by a similar phenomenon of prion propagation as occurring in TSEs. Our hypothesis is that administration of amyloid-β (Aβ) misfolded oligomeric seeds might accelerate amyloid plaque deposition *in vivo*.

Methods: Transgenic mice expressing wild-type human amyloid precursor protein gene (hAPPwt) produce soluble Ab with the human sequence, but do not develop any detectable Aβ deposition during their life span (even after >800 days old). To assess the putative transmission of AD, these animals were inoculated intra-cerebrally with brain extracts from AD patients and healthy individuals. The animals were sacrificed at different times after inoculation in order to analyze the deposition of Aβ in a time dependent manner as well as other histopathological features of AD.

Results: Histological analysis of brain tissues show that hAPPwt animals develop typical amyloid plaques only when inoculated with AD brain extracts. No deposition was detected in any of the control groups challenged with healthy extracts. Interestingly, Aβ deposition follows a time dependent kinetics by analysis of percentage of incidence and plaque burden. Other features of AD as brain inflammation, accumulation of Aβ-40, Aβ-42 and alteration in synaptophysin levels were also observed.

Conclusions: Our results show that AD pathogenesis can be induced by exogenous addition of "infected" tissues from AD patients in a similar way as occurring for TSEs. Currently we are studying whether AD pathogenesis can be induced by more relevant routes of administration of AD samples. Our findings urge to explore the possible infectious origin of AD and other PMDs.

OC2.05 Infectious and non-infectious and non-infectious aggregaters of the Prion Protein share several conformational epitotes

Authors

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Content

The prion hypothesis proposes that the central molecular event in all prion diseases is the conformational conversion of PrP_C into PrP_{Sc}, an aggregated form rich in β-sheets. There is evidence that PrP_{Sc} propagates its altered conformation via a templating mechanism in which PrP_{Sc} binds specifically to PrP_C. A great deal of effort has been devoted to developing antibodies that specifically recognize PrP_{Sc} but not PrP_C, as such antibodies would have enormous diagnostic and experimental value. A mouse monoclonal IgM antibody (designated 15B3) (Korth, C. et al. [1997] Nature 390, 74-77) and three PrP motif-grafted monoclonal antibodies (referred to as IgG 19- 33, 89-112, and 136-158) (Solforosi, L., et al. [2007] J. Biol. Chem. 282, 7465-7471) have been previously reported to react specifically with infectious PrP_{Sc} but not PrP_C. We report here further characterization of the reactivity of 15B3 and the three motif-grafted antibodies. We demonstrate that all four of these antibodies have a much wider reactivity than previously appreciated: they immunoprecipitate not only PrP_{Sc}, but also aggregates of PrP carrying several disease-associated mutations, as well as wild-type PrP molecules that have been induced to aggregate in vitro by chemical manipulation. In addition, we show that the motif-grafted antibodies are capable of visualizing PrP aggregates in situ in several different immunostaining formats, including sections of brain tissue, cultured cells, and unfixed, purified proteins immobilized on a glass coverslip. The results presented here suggest that 15B3 and the motif-grafted antibodies are specific for conformational epitopes that are common to both infectious and non-infectious aggregates of PrP. Our study extends the utility of these antibodies for diagnostic and experimental purposes, and it provides new insight into the structural changes that accompany PrP oligomerization and prion propagation.

OC3.01 Transmission of atypical BSE to Microcebus murinus, a non-human primate: Development of clinical symptoms and tissue distribution of PrP_{res}

Authors

Baron, T.[1]; Biacabe, A.[1]; Rouland, S.[2]; Verdier, J.[2]; Mestre-Francés, N.[2]

[1]AFSSA; [2]INSERM U710-EPHE-UM2.

Content

Background: Atypical BSE cases have been observed in Europe, Japan and North America. They differ in their PrP_{res} profiles from those found in classical BSE. These atypical cases fall into 2 types, depending on the molecular mass of the unglycosylated PrP_{res} band observed by Western blot: the L-type (lower molecular mass than the typical BSE cases) and H-type (higher molecular mass than the typical BSE cases).

Objectives and Methods: In order to see if the atypical BSE cases were transmissible to primates, eight animals (were intracerebrally inoculated with 50 µl of a 10% brain homogenates of two atypical French BSE case, a H-type (2 males and 2 females) and a L-type (2 males and 2 females).

Results: Only one of the four lemurs challenged with H-type BSE died without clinical signs after 19 months post inoculation (mpi), whereas all the 4 animals inoculated with L-type BSE died at 19 mpi (2 males) and 22 mpi (2 females). Three months before their sacrifice, they developed blindness, tremor, abnormal posture, incoordinated movements, balance loss. Symptoms got worse according to the disease progression, until severe ataxia. The brain tissue were biochemically and immunocytochemically investigated for PrP_{res}. For the H-type, spongiform changes without PrP_{res} accumulation were observed in the brainstem. However Western blot analysis did not allow to detect PrP_{res} into the brain. For the L-type, severe spongiosis was evidenced into the thalamus, the striatum, the mesencephalon, and the brainstem, whereas into the cortex the spongiosis was evidenced, but the vacuolisation was weaker. Strong deposits of PrP_{res} were detected by western blot, PET-blot and immunocytochemistry in the CNS: dense accumulation was observed into the thalamus, the striatum, and the hippocampus whereas in the cerebral cortex, PrP_{res} was prominently accumulated in plaques. Western blot analysis also readily confirmed the presence of protease-resistant prion protein.

Conclusions: L-type infected lemurs showed survival times considerably shorter than for classical BSE strain, indicating that the disease is caused by a very virulent distinct prion strain in a model of non human primate.

OC3.02 Oral transmission of BSE in resistant ARR/ARR sheep: involvement of lymphoid tissues

Authors

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Content

Background: Sheep susceptibility to TSE is linked to the *PRNP* gene polymorphism. Results from case-control studies and experimental infections have supported the implementation of genetic selection for the Scrapie resistance allele $A_{136}R_{154}R_{171}$. The possibility of BSE transmission in small ruminants raised the question of their genetic susceptibility to this agent.

Objectives: To study the physiopathology of BSE infection in sheep after an oral inoculation at birth with an ovine isolate in order to mimic natural transmission and to determine the infectious status of tissues at 4 and 10 months and at clinical stage in susceptible ARQ/ARQ and resistant ARR/ARR & ARR/ARQ sheep.

Methods: New Zealand TSE free lambs (n=27) were *PRNP* sequenced and orally inoculated at birth with a BSE infected sheep brain pool; 5 ewes were kept as sentinels. PrP_{res} tissue content was investigated by ELISA, Western blot and IHC; key samples were bio-assayed in reference and transgenic mice.

Results: ARQ/ARQ sheep had PrP_{res} deposits at 4 months of age in lymphoid and enteric nervous tissues and at 10 months in most tissues. They developed the disease at 19 months. Tissues of resistant animals sacrificed at 4 and 10 months were negative (at the exception of one spleen). ARR/ARR sheep developed the disease at 48 months (4/4) and ARR/ARQ at 52 months (1/3). PrP_{res} was detected in their lymphoid, nervous and muscle tissues, which transmitted the BSE strain in mice. Two sentinel ewes contracted the disease with an incubation period of 23 months.

Discussion: BSE was orally transmitted to susceptible and resistant sheep. The few exceptions were heterozygous or carried the mutation M112T. Without compromising the recognised efficiency of the genetic selection to eradicate classical Scrapie, these data point out the carrier status of ARR/ARR sheep, and the need for a TSE strain survey and for an evaluation of new resistance alleles.

Supported by EU contract QLRT-01309; sheep and mice were maintained at level 3 of bio-confinement by INRA-PFIE Tours.

OC3.03 Prions hi-jack tunneling nanotubes for intercellular spread

Authors

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Content

Background: In the infectious variant of Creutzfeldt-Jacobs disease (vCJD), prions (PrP_{Sc}) enter the body by oral exposure with contaminated foodstuffs. Prions may then spread from the intestinal entry site to the central nervous system (CNS) by intercellular transfer from the lymphoid system to the peripheral nervous system (PNS). Several mechanisms have been proposed for these intercellular transfer events, including hitch-hiking on membrane-coated viruses, transfer via exosomes or by GPI-painting and different cell types such as dendritic cells, follicular dendritic cells or macrophages have been proposed to be involved. However, the mechanism of cell-to-cell spread remains elusive. Furthermore, tunneling nanotubes (TNTs) have recently been identified as a novel means of cell-cell communication both in vitro and in vivo.

Objectives: In this work we wanted to understand whether TNTs could be involved in the transfer of PrP_C and PrP_{Sc} between different cells and therefore be a mean for spreading the infection from the periphery to the CNS. Furthermore we wanted to analyze whether dendridic cells (DCs) where involved in this event.

Methods: To this aims we followed in living cells the intercellular transfer of GFP-PrP and of fluorescently labelled PrP_{Sc} (from purified infected brain homogenate). To this aim we set different type of cell co-culture and used state-of-the-art real time imaging approaches.

Results: Here we show that TNTs transfer cellular PrP (PrP_C) and PrP_{Sc} between cells of the same and different origin both on their suface and via intracellular vesicles. Significantly, we observed fluorescently-labelled PrP_{Sc} transferring via TNTs from dendritic cells (DCs) to primary neurons. We also characterized the type of movement and the molecules involved, and we are currently analyzing the transmission of the infection between DCs and primary neurons.

Discussion: Since DCs can interact with peripheral neurons in lymphoid organs, TNT mediated intercellular transfer would allow neurons to retrogradely transport prions to the CNS. We propose that TNTs are involved in the spreading of infectious prions from the peripheral site of entry to the PNS by neuroimmune interactions and within the CNS.

OC3.04 New hospital prion decontamination procedures using copper-hydrogen peroxide formulations with optimal chemical compatibility and reduced safety risks

Authors

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Content

Background: With the appearance of variant Creutzfeldt-Jakob disease (vCJD) and the spreading of infectious prions in peripheral organs, development of efficient decontamination methods for both conventional and TSE agents compatible with medical equipments, has become a major issue. While studying the role of metal ions in prion biology, we observed that PrP^{c}, as well as PrP^{Sc}, undergoes a specific cleavage on exposure to copper (Cu) and hydrogen peroxide (H_2O_2). This seemed related to the high affinity of the protein for Cu that triggers, in presence of reactive oxygen species, an ion-mediated cleavage. This reaction produced also a dramatic reduction effect on the infectivity present in scrapie infected brain homogenates.

Objectives: We aimed at further validating the prion decontamination effect of Cu formulated i) with H_2O_2 in combination or not with detergent, and ii) with peracetic acid (PAA) (which results of an equilibrium between acetic acid and H_2O_2).

Methods: An *in vitro* evaluation of the different solutions was performed on PrP^{Sc} present in brain homogenates, or adsorbed on stainless steel wires using western blot and different prion strains (RML, 22L, 263K, vCJD, sCJD, BSE). This *in vitro* analysis was completed using the model of golden Syrian hamsters infected with the 263K scrapie strain to specify the effect on TSE infectivity.

Results and Discussion: *In vitro* data on both homogenates and contaminated steel wires confirmed the effect of the formulations on the different strains with a reduction in PrP^{Sc} western blot signal higher than 3 $\log_{10}$. Animal steel wire assay demonstrated the prion decontamination efficacy of Cu and H_2O_2 alone or in combination with detergent (reduction factor RF>5 $\log_{10}$). PAA formulated with Cu exhibited a RF>4 $\log_{10}$ compatible for TSE risk management in washer disinfectors. These new decontamination processes were also validated for conventional infectious risks.

Conclusion: These new approaches in prion decontamination represent a major breakthrough in medicalpractice by proposing efficient procedures at ambient temperature that can be routinely used in healthcare facilities for preventing conventional and prion infectious risks. Moreover, formulations present better chemical compatibility than alkaline reagents for non thermostable medical devices and reduced safety risks in handling chemicals.
The Cu-H_2O_2 prion decontamination effect is the basis of an international patent from the CNRS.

OC3.05 Detection of CWD Prions in Urine and Saliva of Deer by Transgenic Mouse Bioassay

Authors

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Content

Background: Chronic Wasting Disease (CWD) is a naturally occurring prion disease with highly efficient transmissibility among and between cervid species. Accumulating evidence points to a role for bodily fluids, i.e. saliva and urine, in the transmission of CWD and other prion diseases. Here we report new bioassay data on this subject.

Objectives: This study addresses two objectives: (1) to examine anew whether CWD prions may be shed in urine; (2) to examine with additional methodologies the presence of CWD infectivity in saliva[1].

Methods: Because any potential infectious prion protein in bodily fluids is below the maximal sensitivity of traditional assays (e.g. western blot [WB], ELISA], urine and saliva from CWD+ deer was concentrated by lyophilization prior to intracranial inoculation into cervid-PrP expressing line 1536 transgenic mice [Tg(CerPrP)]2. Mice were monitored for clinical disease and brains examined for PrP^{res} by WB, immunohistochemistry (IHC), and PMCA[3].

Results: At this writing, 7 of 9 saliva-inoculated mice and 2 of 9 urine-inoculated mice have developed neurologic signs consistent with a TSE; in all PrP^{res} has been demonstrated by WB and IHC. Mean survival times have been 378±4d for urine and 323±97d for saliva inoculated mice. PMCA studies are in progress and will be reported.

Discussion: These results demonstrate for the first time the presence of infectious prions in urine and confirm the shedding of prions in saliva of CWD+ deer. Our findings expand the proven potential routes for CWD prion shedding, transfer, and environmental dissemination and help to explain the facile transmission of this TSE.

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(2) Browning SR, Mason GL, Seward T et al. (2004) J Virol, 78(23):13345-50
(3) Kurt TD, Perrott MR, Wilusz CJ et al. (2007) J Virol, 81(17):9605-8

OC3.06 Neuroinvasion in sheep TSEs: the role of the haematogenous route

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Content

Background: It is generally believed that, after oral exposure to transmissible spongiform encephalopathy (TSE) agents, neuroinvasion occurs via the enteric (ENS) and autonomic (ANS) nervous systems. As a result, the dorsal nucleus of the vagus nerve (DMNV) is the initial point of disease-associated PrP (PrP_d) accumulation in the brain.

Hypothesis and Objectives: If direct ENS invasion following oral infection results in the initial PrP_d accumulation occurring at specific sites, such neuroanatomical points should be different when infection occurs by other routes. Within this study we also wanted to determine whether or not the circumventricular organs (CVOs) were involved in the early deposition of PrP_d in the brain. Methods: An immunohistochemical study has been conducted on the brain of 67 preclinically infected sheep exposed to natural scrapie or to experimental TSE infection by various routes. Tissues collected at post-mortem representative of the lymphoreticular system (LRS), enteric nervous system (ENS), peripheral nervous system (PNS) and central nervous system (CNS) were examined and scored for presence of PrP_d.

Results: Initial PrP_d accumulation consistently occurred in the DMNV followed by the hypothalamus, regardless of the breed of sheep, the PrP genotype, the TSE source and, notably, the route of infection; these factors did not appear to affect the topographical progression of PrP_d deposition in the brain either.
Moreover, PrP_d aggregates appeared consistently in the CVOs at very early stages, in most instances before than in the neighboring areas of the neuroparenchyma.

Discussion: Current evidence from blood transfusion studies indicates that prions are present in the blood stream, and our study provides evidence for the early involvemenent of the CVOs in animal TSEs. As the brain-blood barrier is absent in these organs, they can provide a portal for entry of prions and play a role in the spread of PrP_d throughout the brain. Overall, we suggest that the haematogenous route can represent a parallel or alternative pathway of neuroinvasion to ascending infection via de ENS/ANS.

OC3.07 The Structure of Rabbit PrP_C: Clues into TSE fibril formation and the species barrier

Authors
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Content

The transmissble spongiform encephalopathies (TSEs) are characterized by a toxic gain of function of a normal host protein, PrP. This toxic gain of function occurs when the normal, monomeric, predominantly alpha-helical cellular form of PrP (PrP_C) undergoes a conformational change to a polymeric, predominantly beta-sheet infectious form (PrP_{Sc}) commonly referred to as a prion.
The TSE species barrier describes the long incubation periods or absence of infectivity when prions are transmitted between certain species. Interspecies transmission experiments have shown that rabbits appear to be less susceptible to prion infection and single amino acid differences present in the rabbit PrP_C sequence can confer resistance to PrP_{Sc} conversion. One difference of particular interest, N174S. is located in a loop between the second beta strand and helix-2 (the X-loop).
We have solved the structure of the globular domain (121-231) of wild-type and S174N mutant rabbit PrP_C, both to 2.0 angstrom resolution using X-ray crystallography. The overall folds are similar to other monomeric PrP_C structures solved by NMR and crystallography. The wild-type S174 forms 4 additional hydrogen bonds within the X-loop compared to the S174N mutant. Urea induced unfolding at low pH showed that the S174N mutant is more prone to formation of an oligomeric species than wild-type, which work in one of our labs has shown to correlate with increased scrapie susceptibility. This indicates that conformational restriction in the X-loop could be a possible source of the species barrier. Additionally, the asymmetric unit of both crystals contain a dimer. These dimers associate along one axis of the crystal lattice forming a cross-beta spine, reminiscent of an amyloid fibril. Analysis of the crystallographic intermolecular contacts and the change in conformation of the X-loop in rabbit PrP_C, suggest a novel mechanism of PrP_{Sc} fibril formation and the nature of the species barrier that satisfies a great deal of previous structural and biochemical data.

OC3.08 Prions in milk from ewes incubating natural scrapie

Authors
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Content

Since infectivity was never reported in milk, dairy-food originating from ruminant flocks affected with Transmissible Spongiform Encephalopathies enter into the food chain. Disease associated prion protein (PrPSc) accumulation was recently reported in mammary gland from three scrapie-affected ewes. Deposits were associated to mammary ectopic lymphoid follicles that develop in response to retroviral infection (Maedi). However, no PrPSc was identified in milk or milk ducts from these animals.
Here we report abnormal Prion protein and consistent levels of infectivity, in colostrums and milk from sheep incubating natural scrapie. In PrPSc positive mammary glands, abnormal PrP accumulation mainly occurred in ectopic lymphoid follicles.
However, in half of the examined cases, PrPSc positive cells or free granules were observed in milk ducts and the lumen of acini. Identification of PrPSc in milk duct was limited to ewes affected with Maedi chronic lymphp-proliferative mastitis. Double labelling indicated that these PrPSc positive cells were also positive with CD68.
Prion infectivity was detected not only in ewes with chronic mastitis but also in ewes displaying healthy mammary gland. Highest titres were observed in the cellular fraction, but cream and casein-whey were also found infectious. Considering the shortest mean incubation period in our samples, infectivity levels were comparable to that of 0.02 g of posterior brainstem from a terminally affected sheep scrapie (106.6DI50 i.c/g of brain tissue in Tg338 mice), corresponding to an approximate infectious titre of 104DI50 i.c in Tg338 mice/liter.

OC3.09 TSE neuroinvasion is dramatically impaired in aged mice

Authors
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Content

Background: Most clinical cases of variant Creutzfeldt-Jakob disease (vCJD) have occurred in young adults, although the reasons behind this apparent age-related susceptibility are uncertain. Following peripheral exposure, many transmissible spongiform encephalopathy (TSE) agents accumulate first in lymphoid tissues before spreading to the brain where they cause neurodegeneration. TSE agent accumulation upon follicular dendritic cells (FDCs) in lymphoid follicles appears critical for efficient neuroinvasion. Host age has a significant influence on immune function. As FDC status in aged mice (600 days old) is reduced, we hypothesized that this decline in FDC function in aged individuals might impair TSE pathogenesis.

Objectives: To determine the influence of a senescent immune system on TSE pathogenesis.

Methods: We infected aged mice with the ME7 strain of scrapie using 3 different routes; the intraperitoneal, oral or intracerebral. At specific points following infection tissues were collected for infecivity bioassay and immunohistochemistry. Aged and young mice were maintained until the clinical endpoint of disease to determine the effect of age on pathogenesis

Results and Discussion: We show that coincident with the effects of host age on FDC status, the early TSE agent accumulation in the spleens of aged mice was significantly impaired. Furthermore, following peripheral exposure, none of the aged mice developed clinical TSE disease during their lifespans, although histopathological signs of TSE disease were detected in the brains of most mice. Our data imply that the reduced status of FDCs in aged mice significantly impairs TSE agent accumulation in lymphoid tissues and subsequent neuroinvasion.
Inefficient neuroinvasion in aged individuals may lead to considerable levels of sub-clinical TSE disease in the population.

OC3.10 Reversible changes of PrP^{Sc} after transmission in animals with mismatched prion proteins

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Content

Background: The size and glycoform pattern of PrP^{Sc} of a prion strain appear to be fairly stable upon transmission to defined host species, and they are thought to be reliable markers for prion strain identification. However, it is known that the PrP^{Sc} glycoform ratio of a prion strain may change after transmission in hosts with different PrP sequence. It remains to be determined whether the original PrP^{Sc} features are restored when the altered PrP^{Sc} is passaged back to the original host species.

Objectives: To demonstrate that the glycoform ratio and gel mobility of protease-resistant PrP^{Sc} fragments associated with human sCJDMM1 are stable upon transmission to hosts expressing homologous PrP, even after PrP^{Sc} alteration due to passage in hosts expressing mismatched PrP.

Methods: A sCJDMM1 isolate was subjected to primary and secondary transmission in three transgenic mouse lines expressing wild type human PrP-129M, elk PrP, or mutated human PrP-129M, respectively. The glycoform patterns and sizes of protease-resistant PrP^{Sc} from these transgenic mice after primary and secondary passages were compared with each other and with those of elk CWD and the original sCJDMM1 inoculum.

Results: Our data shows that the PrP^{Sc} glycoform ratio and sizes of the PK-resistant PrP^{Sc} fragments associated with sCJDMM1 were reproduced after passage in Tg mice expressing wild type human PrP-129M. In contrast, these features changed dramatically after primary passage in Tg mice expressing either elk PrP or mutated human PrP, and these changes were maintained after secondary passage in the same Tg lines. However, further passage of the changed PrP^{Sc} in Tg mice expressing wild type human PrP-129M restored the original features of sCJDMM1 PrP^{Sc}.

Discussion: Our results demonstrate that glycoform ratio and size of PK-resistant PrP^{Sc} fragments associated with sCJDMM1 are stable markers of the sCJDMM1 strain when propagated in hosts expressing homologous PrP^{C}; in contrast, these PrP^{Sc} features are susceptible to change when the host PrP^{C} sequence is different. These findings may have significant implications concerning the origins of prion strains and cross-species prion transmissions. Supported by NINDS NS052319 and NIA AG14359.

OC3.11 Strain targeting without strain-specific axonal transport or neuronal tropism

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Content

Background: Prion strains are operationally distinguished by the regions of the central nervous system (CNS) where PrP^{Sc}, the prion disease specific isoform of the prion protein, accumulates. While it is known that prions are transynaptically transported along defined neuroanatomical pathways in the CNS, the mechanism responsible for prion strain targeting within the CNS is not known.

Objectives: To investigate if prion strain targeting is due to strain-specific differences in transport along neuroanatomical pathways and/or differences in the neuronal susceptibility to infection.

Methods: Hamsters were inoculated in the sciatic nerve with either the hyper (HY) or drowsy (DY) strains of hamster-adapted transmissible mink encephalopathy (TME). Following inoculation with these two strains of TME, animals were sacrificed at selected time points postinfection. To determine the distribution of PrP^{Sc} within the CNS, immunohistochemistry was performed using a monoclonal antibody specific for the prion protein.

Results: In both strains of TME, sciatic nerve inoculation resulted in initial detection of PrP^{Sc} in the dorsal root ganglia and ventral motor neurons of the lumbar spinal cord, ipsilateral to the side of inoculation. The subsequent temporal and spatial deposition of PrP^{Sc} for both strains was consistent with slow axonal transport in the retrograde direction via the corticospinal, rubrospinal, vestibulospinal, and reticulospinal descending motor tracts. Prior to clinical stage of disease, PrP^{Sc} was not detected in any CNS structure associated with ascending sensory pathways. At the clinical stage of disease differences in the deposition patterns between the two strains appeared, as DY PrP^{Sc} was detected in every structure of the CNS, whereas HY PrP^{Sc} was absent from the hippocampus and several white matter tracts. However, following i.c. inoculation with the HY TME agent, PrP^{Sc} was detected in the hippocampus and white matter tracts.

Conclusion: These data demonstrate that strain-specific transport along neuroanatomical pathways is not involved in prion strain targeting in the CNS, and that initial PrP^{Sc} transport is predominately, if not entirely, retrograde. This also suggests that all populations of neurons are susceptible to prion infection and thus, differential neuronal susceptibility to infection is not solely responsible for prion strain targeting.

OC3.12 vCJD in primate: new insights for blood risk assessment

Authors

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Content

Background: Infection of cynomolgus macaques with BSE agent is a unique model for human vCJD, and brought the first evidence of transmissibility of BSE to primate, the infected animals presenting clinical pictures, distribution of infectivity and lesions representative of those observed in vCJD-infected patients.

Objectives: Since incubation of BSE in macaque exceeds several years, we initiated long-time experiments many years ago with experimental assessment of secondary transmissions via transfusion which results become available only now.

Methods: Cynomolgus macaques were infected by intracerebral and oral routes with various doses of BSE infectivity, or inoculated by intravenous or intracerebral routes with blood products from various sources. Clinical monitoring was performed, presence of PrP_{res} regularly assessed in inguinal lymph nodes, and distribution of PrP_{res} in several organs was determined in deceased animals.

Results: Animals with long incubation periods following exposure to a low infectious dose, showed a physiopathology, clinical picture and peripheral replication that differed from what was seen in earlier transmission experiments. In the present study, we also have used incubation period time to estimate levels of infectivity among different blood fractions on one hand, and along the incubation period on the other hand.

Discussion: These new observations in infected animals with long incubation periods have interesting medical and public health implications, at a time when any BSE-infected people would also have long incubation periods.

OC3.13 The Sup35p NM domain propagates as a prion in mammalian cells

Authors

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Content

Background: Prions can be defined as self-propagating, infectious aggregates of misfolded proteins that have been identified in mammals and fungi. In Saccharomyces cerevisiae an aggregated amyloid-like form of the translation termination factor Sup35p acts as a non-chromosomal element of inheritance that can exist as different variants. While molecular chaperones necessary for mammalian prion formation have not been identified, the yeast disaggregase Hsp104 is crucially involved in maintenance of the prion phenotype.

Objectives: To understand general mechanisms of prion formation, we have studied the aggregation propensities of the Sup35p yeast prion domain NM in mammalian cells.

Methods: A HA epitope-tagged NM was stably expressed in N2a mouse neuroblastoma cells via a lentiviral vector system. Aggregation was examined using sedimentation assays and immunofluorescence analysis.

Results: Ectopically expressed HA-tagged NM remained soluble in the cytosol of N2a cells. Surprisingly, addition of in vitro generated NM fibrils to the cell culture medium resulted in endogenous NM-HA aggregation. Furthermore, NM-HA aggregates were heritable and infectious, indicating that mammalian cells promote yeast prion propagation. Interestingly, phenotypically distinct aggregates faithfully propagated in individual cell clones, indicating that cellular factors might determine phenotypical variant selection.

Discussion: As the Sup35p prion domain aggregates appeared to propagate as prion variants in the absence of any Hsp104 orthologs, other cellular mechanisms must enable prion propagation in the mammalian cytosol. In summary, we have established a potent model system to study general aspects of prion formation in mammalian cells.

OC3.14 The influence of PrP glycosylation for the spreading of the TSE infectious agent and in determining the strain characteristics

Authors

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Content

Background: Numerous transmissible spongiform encephalopathy (TSE) strains exist, but to date the underlying nature of these strains remains elusive. However, it has been proposed that the variation in PrP molecules arising from differential glycosylation may contribute to the TSE strains and their characteristics.

Methods and Objectives: We have generated three lines of gene targeted transgenic mice with mutations at the first (G1), second (G2) or both (G3) glycosylation sites of PrP thus preventing the addition of glycans at these sites. To establish the contribution of different glycosylated forms of PrP in the infectious process, we have performed several experiments inoculating different TSE-strains into these mice.

Results: We have established that despite differences in cellular location, mono and un-glycosylated PrP can support both clinical and pathological disease. We have also shown that different TSE agents have dramatically different requirements for glycosylation of host PrP. Interestingly, glycosylation of host PrP has a profound effect in the spreading of infectivity from periphery to the CNS. We have also elucidated the role of PrP-glycosylation in determining TSE strain characteristics by passaging infected brains from PrP-glycosylation deficient mice into wild type animals. We have demonstrated that TSE strain properties can be modified when passaged through hosts with different PrP-glycosylated isoforms. Moreover, our data suggest that while strains such as ME7 and 301C are not affected by lack of sugars in their ability to infect the host, 79A targeting and incubation time are profoundly modified when sugars are partially or completely absent.

Discussion: With these models we are elucidating the effect of PrP-glycosylation in the TSE infectious process. Our results show that glycosylation of host PrP is more important than glycosylation PrP in the inoculum to regulate susceptibility to infection, transport of infectivity and finally strain characteristics.

OC3.15 The glycosylation of prion protein is not required for the prion strain formation

Authors

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Content

Background: The PrP conversion plays the key role in prion diseases. PrP^{c} carries two N-linked glycan chains at amino acid residues 180 and 196 (mouse). Previous data indicated that the conversion process may not require a glycosylation of PrP. However, it is conceivable that these glycans function as intermolecular binding sites during the de-novo infection of cells on susceptible organisms and/or play a role for the interaction of both PrP isoforms. Such receptor-like properties could contribute to the formation of specific prion strains.

Objectives: Determination of the role of the PrP glycosylation for the formation of prion strains.

Material and Methods: By introducing mutations at the glycosylation recognition sites we generated three different transgenic mouse lines which express non- or underglycosylated mouse PrP^{c} (on PrP-KO background). These mice were challenged with mouse adapted BSE, Me7 or Chandler scrapie. Brain material of the infected mice was eventually inoculated into wildtype RIII, C57Bl and VM mice as well as into the corresponding transgenic mouse line expressing non- or underglycosylated PrP^{c}. All mice were analysed for the presence of PrP^{sc} depositions by immunoblot and/or IHC. The wildtype mice were further used for straintyping studies (lesion profiles, IHC, PrP^{sc} glycotyping and PK cleavage site determination, long-term PK resistance).

Results and Discussion: All transgenic mice propagated BSE, Me7 and Chandler prions, irrespective of whether they expressed nonor underglycosylated mouse PrP^{c}. Incubation times in the nonglycosylated PrP^{c} expressing mice were extremely long (à observation of subclinical infections), which was most likely due to the only faint PrP^{c} expression on the cell surface. All infections were sustained in subpassages in the homologous non or under-glycosylated PrP^{c} expressing Tg mouse lines. Non- and under-glycosylated PrP^{sc} was infectious for wildtype mice. Despite having been passaged in glycosylation deficient transgenic mice, PrP^{sc} from all three strains retained/regained their distinct biochemical properties (glycosylation patterns / PK cleavage site) upon reisolation in wildtype mice. Based on these results we postulate that the PrP^{sc} glycosylation patterns do not encode intrinsic strain characteristics but are rather epiphenomena of prion strains.

OC4.01 Comparative analysis of the trend of the BSE epidemic across Europe, in relationship with control measures

Authors
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Content

Background: A comprehensive surveillance system of BSE has been place in the EU15 since 2001, based on the systematic testing of fallen stock and slaughtered cattle. It has provided the opportunity to analyze the trend of the BSE epidemic in these countries. Such a study of the trend may help understanding the effect of risk factors or risk management measures. Moreover, taking into account the length of the incubation period of BSE, a deeper insight in the epidemiology of the disease may be obtained when considering the temporal variations of the rates along with the age distribution of the populations of cases and susceptible cattle.

Objectives: The goal of the study was to compare the trend of the BSE epidemic in the EU15, based on the surveillance data and adequate and standardized methods, and to interpret the results with regard to the control measures implemented in the nineties.

Methods: Three methods were used in parallel: Age Period Cohort models, modelling of the reproduction ratio of BSE (R^0), and plot of the average age of cases by year of detection.

Results: A comparative analysis of the results obtained in the Ireland, Germany, the Netherlands, France and Italy is presented. All studied countries show a favourable situation with regards to the decrease of the epidemic, and a constant increase of the average age of the cases from year to year. Furthermore, the results show that a significant decrease of the risk was observed for cohorts born between 1994 and 1997, with the peak birth cohort varying between countries, and the reproduction ratio became significantly below one (meaning a fade out of the epidemic) between 1997 and 2001.

Discussion: These results will be discussed per se and with regards to the different control measures that were taken at different points in time in the studied countries.

Acknowledgements: The work was carried out under the auspices of MoE Neuroprion.

OC4.02 Increasing the age limit in active BSE surveillance to 48 months: How many cases will we miss?

Authors
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Content

Since 2001, EU member states are required to do active surveillance for BSE, which includes testing of all risk animals over 24 months of age and all animals at slaughter over 30 months of age. In the last 3 years, only one case was found under the age of 48 months.

This leads to the question whether it is worthwhile to continue testing this very large group of young animals.

We know that they carry an extremely small probability of giving a positive test result. Therefore their testing contributes minimally to the monitoring of the BSE epidemic. Recently EFSA has been asked to assess this issue. (See abstract by Barrizone/Arnold/EFSA, Prion 2008).

Modelling has previously shown that the age distribution of cases in a test year shifts to higher ages when an epidemic is declining. Further modelling of BSE cases based on active surveillance data predict extremely low numbers of young BSE cases, because all the EU countries which started their active surveillance in 2001 show a decline of the BSE epidemic (See abstract by Ducrot et al, Prion 2008).

We present a prognosis for these EU countries jointly and a few countries separately, using three extrapolation scenarios. These scenarios range from worst case (no decline of cases in future cohorts) via a pessimistic scenario (continued decrease as seen in cohorts up to 2002) to a most likely scenario (effect from extra control measures in 2001 quantified and incorporated in future cohorts). We show that the probability of finding BSE test positive animals in cattle under 48 months of age is presently much less than 1 per million and this soon declines below 1 per 10 million under the pessimistic and most likely scenarios.

OC4.03 Scrapie control through selective breeding in the Netherlands: when will it be achieved?

Authors
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Content

Scrapie control through selective breeding in the Netherlands: when will it be achieved?

Background: Exclusive use of ARR/ARR rams for breeding is the basis of a breeding program towards scrapie control that has been in place in The Netherlands since almost 8 years. Initially participation to this program was voluntary, but it has become compulsory (for most sheep flocks) in 2004.

Objectives: 1. To provide an epidemiologically underpinned assessment to which extent scrapie control has been achieved as a result of the program.
2. To assess how many more years of selective breeding would be required to achieve scrapie control in The Netherlands.

Methods: In this study we collect extensive data on (in particular) the genotype and "contact" structure of the national flock, and use mathematical modeling of scrapie transmission based on these and other data.
Animals were genotyped on 168 randomly selected farms with sheep, and a questionnaire provided information on breed(s), size, management and recruitment characteristics of these and control farms.

Results: On the basis of the new and the existing data and a mathematical modeling analysis we find that:

- Important differences exist between farms in implementation and management of the breeding program which are related to differences in genotype profile.
- Currently the overall resistance level to scrapie in The Netherlands is still (far) below the level required for scrapie control.
- One important underlying cause of this is the fact that between flocks there is a significant variation in genotype frequency distribution and thus in sensitivity to within-flock scrapie transmission.

Discussion: We discuss:

- How differences in farm-level implementation of the breeding program affect its effectiveness.
- How many years of selective breeding would be required to achieve scrapie control.

OC4.04 A case control study on atypical scrapie in French sheep

Authors
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Content
The existence of atypical scrapie was revealed recently. Whereas transmission and epidemiological pattern of classical scrapie has been documented for a long time, knowledge on this new disease is still very limited, especially regarding the aetiology and the possible risk factors associated with farming practices.
A case control study was conducted in 2007 among French sheep farms to investigate the risk factors of atypical scrapie at the flock level.
Cases were selected among farms from which atypical scrapie cases were detected in 2006 and begining of 2007 and control flocks were selected among farms involved in the surveillance system during the same period. Risk factor hypotheses were focussed on the birth cohort of the index case or a matching cohort for controls. Data collected included general information on management of the flock, contacts with animals from other flocks, lambing and feeding practices and exposure to toxics. Associations between study variables and disease status were checked by univariate models before a multivariate model was fitted. The matching design was accounted for by using generalised linear mixed models in the two steps of the analysis.
In total, 95 cases and 225 controls were included. The number of animals tested from the flocks since the beginning of the active surveillance was associated with the risk of disease and was therefore included as a covariate. Dairy flocks were at a higher risk than meat flocks, whereas organic producers were at a lesser risk. The feeding of corn silage and of minerals and vitamins supplements reduced the risk. The decrease of the risk by a supplementation in minerals and vitamins was higher in dairy flocks.
This study did not evidence any risk factor associated with sheep to sheep transmission or any potential source of oral infection. In the absence of a clear infectious pattern, one can hypothesize that atypical scrapie has an endogenous origin and that the metabolic condition of the animals could modify the chance to develop the disease. Our findings suggest that changes in the balance of minerals due to feeding practices and/or milk production could be involved in the development of the disease.

OC4.05 Diagnostic profile of young patients referred to the CJD Italian surveillance unit

Authors
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Content
Background: Patients younger than 50 years and with a clinical suspicion of Creutzfeldt-Jakob disease (CJD) deserve special attention from neurologists because they might represent variant CJD (vCJD) cases.

Objectives: The aim is the correct classification of patients aged <50 years to exclude or to confirm the diagnosis of vCJD.

Methods: Three hundred and three patients aged <50years were referred to the CJD Italian surveillance unit between 1993 and 2007. In only about 30% the diagnosis of CJD was finally confirmed, but the number of CJD patients has substantially increased in with time. In most suspected cases the diagnosis of CJD was dropped mainly because of clinical improvement, clinical diagnosis of Alzheimer""s disease, or encephalitis. At notification, vCJD was suspected in 18% of these cases, but only a few patients were classified as possible (13) or probable (1) vCJD during clinical course. Finally, in all possible v CJD cases an alternative diagnosis was made.

Discussion: The increased recognition of CJD in <50-year-age group was mainly through neuropathology evaluation since most of them were not clinically classified as probable CJD. This finding confirms the importance of performing autopsy in all suspected CJD to avoid a misinterpretation of surveillance data.

OC4.06 Human transmissible Spongiform Encefalopaties in Spain, 1993-2008

Authors
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Content
Objectives: To describe the epidemiology of Human Transmissible Spongiform encefalopaties (HTSE) in Spain.

Methods: Data obtained from the Spanish HTSE Registry for cases with disease onset during 1993-2008 were analyzed. Specific data on bovine spongiform encephalopaty (BSE) identified in Spain and bovine meet exports from the United Kingdom were collected.

Results: For the 1993-2008 period, the Registry collected data with defined clinical onset from 839 individuals suffering probable or definite HTSE: Creutzfeldt-Jakob Disease (CJD) – 752 sporadic, 40 genetic, 6 iatrogenic– and 38 Fatal Familial Insomnia cases. In the same period, 141 non-case were notified. The incidence of sporadic probable or definite CDJ increased, particularly until 1998 (1.47 per million), with an improvement in proportions of genetically or post mortem studied cases which reached 68% and 55%, respectively, in 2007.Compared to Catalonia, regional incidences for 1998-2008 ranked from 0.60 per million (RR 0.35; 95%CI 0.14-0.85) in Balearic Islands to 2.32 per million (RR 1.34; 95%CI 0.96-1.87) in Basque country. The most frequent mutations were D178N and E200K (n=42 and 30). Genetic HTSE clustered in the Basque Country with 4 CJD and 16 IFF cases. Iatrogenic cases were due to dural implant. Three confirmed vCJD cases, who had no clear history of risk factors, had onset in 2004, 2006 and 2007 at ages of 26 y. (Madrid), 49 and 40 y. (Northwest). The first case was blood donor.

Conclusions: The Spanish HTSE pattern corresponds to: 1) sCJD incidences and trends similar to those of large EU country members with a North to South gradient; 2) a high incidence cluster of sCJD and genetic HTSE in the Basque Country; 3) iatrogenic CJD related to dura implant; 4) a low vCJD incidence fitting low bovine UK-imports and local BSE.

OC4.07 Combining Quantitative Models for BSE and vCJD: eveloping an Accessible Tool for Prion Disease Risk Analysis

Authors
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Content
Background: Research over the last two decades has advanced our understanding of biological and epidemiological aspects of prion diseases. It is imperative to utilize such knowledge in developing robust quantitative models that can accurately project future trends of the disease and assess human risk of vCJD due to past exposure from contaminated meat products as well as secondary transmission potential through blood, tissue, organs and surgical instruments.

Objectives: To develop an integrated quantitative model that quantifies prion disease risk in animals and humans.

Methods: The model uses experimental and epidemiological data inputs as independent Poisson variates to obtain maximum likelihood estimates of risk.

Results: Phase 1 of the modeling extends a method that estimates the retrospective and current time-dependent risk of BSE infection, and projects the future trend of BSE in the cattle herd. Phase 2 relates the past pattern of human exposure from infected bovine tissue to the future risk of primary vCJD infection. Phase 3 assesses the risk of secondary vCJD infection due to blood transfusion, transplantation of infected tissues and exposure to contaminated surgical instruments.

Discussion: We devised an integrated model for quantitative risk assessment of prion diseases. The model incorporates, as inputs, important parameters influencing the spread of prion infectivity in the cattle herd and the risk of transmission to humans from primary and secondary infection. The resulting model is adaptable to new situations and urgencies. It can be used to model real-time changes that influence the risk of BSE and vCJD. Examples of such changing risk situations include meat and bone meal infectivity, feed ban efficacy, removal of infectious tissues from human food chains, age heterogeneity effects and genotype-dependent susceptibility to vCJD infection. Overall, the integrated model provides decision-makers with a more intuitive and direct quantitative risk tool.

OC4.08 TSE Roadmap – Results of a comparative study of risk perception and risk communication of stakeholders within European countries

Authors
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Content
The TSE roadmap, published by the European Commission 15th July 2005, suggests relaxations of BSE measures in the short, medium and long-term. According to TSE roadmap "any relaxations of BSE measures following the scientific assessment should be initiated by an open discussion with all stakeholders and supported by a strong communication strategy". This social scientific project addresses the issue by directly involving stakeholders of five European Member States (Belgium, France, Germany, Italy and United Kingdom) as well as the European level and investigates their perceptions of the TSE roadmap and its implications for precautionary consumer protection.
Knowledge about the risk perception was gathered by interviewing stakeholders representing the public (such as consumers and farmers) and juxtaposed by the risk perception of food industry and the respective government.
Results of the comparative study will show how different risk management and risk communication strategy of the Member States clearly influence how the risks of TSEs are perceived by the various stakeholders. The results of the project support a better understanding of the meaning of adequate risk communication. Recommendations on good practices of risk communication, taking into account public risk perceptions, will be given.

OC5.01 Association of cellular prion protein and sphingolipid-mediated signalling pathways

Authors
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Content
Background: The physiological function of the cellular prion protein (PrP^c) remains unclear. PrP^c associates with lipid rafts, containing a variety of signaling molecules, e.g. sphingolipids (SL). SL can act as signalling molecules influencing cell fate: while ceramide, generated by sphingomyelinases (SMases), mediates apoptotic responses, sphingosine-1-phosphate (S1P), is related to cell proliferation/survival. S1P, synthesized by sphingosine kinase (SphK) and degraded by S1P-lyase and/or -phosphatase, has a unique role as dual signaling molecule: acting either as intracellular second messenger or extracellular mediator via G-protein coupled cell surface receptors ($S1P_{1-5}$), which contribute to multiple effector systems including phospholipases C and D, Akt, Erk, and others.

Objectives: In this study, we investigated possible connections between PrP^c and sphingolipid associated signaling pathways.
Methods Using QRT-PCR, Western Blot, thin layer chromatography and enzyme activity assays, we elucidated critical parameters in the SL-rheostat and downstream signaling molecules in PrP^c-k.o. and -wildtype (wt) cells as well as mice.

Results: Enzyme activity assays determined higher activity of neutral and acid SMases in PrP^c-k.o. groups, while ceramide and sphingomyelin levels were unchanged. Despite lower basal SphK expression levels in PrP^c-k.o. mice, the levels of its metabolite S1P were increased in these mice. Furthermore, S1P3-receptor expression was higher in PrP^c-wt groups. In addition, we detected enhanced activity of phospholipase D1 (PLD1), an enzyme that seems to be a suitable connector between the S1P3-receptor and continuative signaling. Finally, evidence for an impact on downstream signaling cascades, especially activation of the PI3K/Akt pathway, was found.

Conclusion: In summary, our data suggest that PrP^c is involved in sphingolipid-associated signaling, modulating pathways that exert anti-apoptotic functions, hence indicating that PrP^c plays a role in neuroprotection.

OC5.02 Dose independent modulations of glutamate receptors expression and neuronal excitability by PrP^c *in vivo*.

Authors
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Content
Creutzfeldt-Jakob disease (CJD) is a neurodegenerative disease characterized by spongiform brain degeneration in humans. Although some patients have controversial symptoms, Diagnosis of this disease is based basically on characteristic triad of myoclonus, dementia, and periodic EEG activity. The agent causative of the spongiform encephalopathy in humans (CJD), scrapie in sheep and bovine spongiform
encephalopathy (BSE) and other animals is an abnormal conformational isoform (PrP^{sc}) of the normal cellular prion protein PrP^c. First characterization of physiological function of PrP^c in null mice (*PRNP-/-*) seemed to be minor. However, detailed studies using mutant mice point to a relevant synaptic function of PrP^c in excitability under pharmacological treatments with glutamate agonist (Rangel et al., 2007). However, a putative relationship between PrP^c expression levels and excitability is lacking. With this in aim, we decide to study in detail the susceptibility to kainate in several PrP^c mice strain with different doses of PrP^c, Our results suggest that PrP^c can modulate the expression of glutamate receptors and determinate the extent of of neuronal excitability but do not fully correlate in a dose dependent manner.
Supported by Contract grant sponsor: BFU2006-1356. Contract grant numbers: SGR2005-0382, SGR2005-00830.

OC5.03 Signalling and endocytosis: coupled mechanisms in cellular prion physiological functions

Authors

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Content

Background: Several biological functions of PrP^c have been uncovered, and the idea that PrP^c loss-of-function may have a role in the pathogenesis of prion diseases is plausible. PrP^c binds to Stress Inducible Protein 1 (STI1), which is secreted by astrocytes, and mediates neuronal survival and differentiation through the activation of PKA and ERK1/2 respectively.

Objectives: Define the role of PrP^c endocytic traffic on cellular signaling and physiological functions.

Material and Methods: PrP^c and STI1 were labeled with different fluorophores and their traffic was followed using specific cell compartment markers. PrP^c-STI1 signaling (PKA and ERK1/2) was measured in PrP-null cells transfected with DNA expressing a wild-type or an internalization defective PrP^c.

Results: STI1-induced signaling did not occur in cells devoid of endogenous PrP^c, however heterologous expression of PrP^c reconstituted both PKA and ERK1/2 activation. STI1, but not a STI1 mutant unable to bind PrP^c, induced PrP^c endocytosis. Transient ERK1/2 activity induced by PrP^c interaction with either recombinant STI1 or endogenous STI1 secreted by astrocytes depended on the endocytosis of PrP^c, while activation of PKA was not affected when PrP^c trafficking was impaired. Accordingly, a PrP^c mutant lacking endocytic activity was unable to promote ERK1/2 activation induced by STI1, whereas it reconstituted PKA activity in the same condition. The activation of ERK1/2 was transient and appeared to depend on the interaction of PrP^c and STI1 at the cell surface or shortly after internalization. Inhibition of dynamin activity by expression of a dominant-negative mutant caused the accumulation and colocalization of these proteins at the plasma membrane, suggesting that they use a dynamin-dependent internalization pathway.

Discussion: These results show that STI1, a protein ligand of PrP^c, triggers PrP^c endocytosis and this event is a physiological step necessary to modulate ERK1/2 signaling.

OC5.04 Effect of disease-associated mutations in the prion protein on the beta-secretase cleavage of the amyloid precursor protein

Authors

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Content

The cellular form of the prion protein (PrP^c), the causative agent of the transmissible spongiform encephalopathies such as Creutzfeldt-Jakob disease (CJD) and Gerstmann-Scheinker-Straussler (GSS) disease in humans, has been shown to inhibit the initial step of the proteolytic processing of the Alzheimer's amyloid precursor protein (APP). PrP^c inhibits the β-site APP cleaving enzyme (BACE1) resulting in the reduction of the amyloid β (Aβ) peptides, which are involved in the pathogenesis of Alzheimer's disease (AD).

In SH-SY5Y cells, PrP^c expression reduced secretion into the conditioned medium of Aβ40 by 92% and Aβ42 to undetectable levels, and reduced sAPPβ shedding by 98%. Studies using PrP null mice showed a significant increase in Aβ40 and Aβ42 in the brain, providing direct evidence that PrP^c regulates the production of Aβ in vivo. Two mutants of PrP, PG14 and A116V, which are associated with CJD and GSS, respectively, did not inhibit the β-cleavage of APP when expressed in SH-SY5Y cells. Here we have examined the effect of other disease-associated mutations in PrP^c on the BACE1 cleavage of APP.

SH-SY5Y cells expressing APP and various mutant PrP constructs associated with human prion diseases were established. Cell lysates and conditioned medium were subjected to western blot using antibodies specific to PrP, APP and sAPPβ, and BACE1 activity was assayed using a quenched fluorescent peptide substrate.

Two mutants of murine PrP^c (W144stop and Q159stop equivalent to Y145stop and Q160stop in human PrP^c that are associated with familial prion diseases) were rapidly metabolised by the proteasome and failed to inhibit the BACE1 cleavage of APP. The effect of further mutants of PrP^c, including P101L and D177N, on the BACE1 cleavage of APP is currently being examined. In addition, the levels of Aβ peptides in the brains from transgenic mice expressing some of these mutants will be reported.

The mechanism by which PrP^c inhibits Ab peptide production and the lack of this regulatory control by mutants of PrP^c associated with prion diseases suggests that an increase of Aβ peptides could contribute to disease pathogenesis. These observations have implications for both prion disease research and AD.

OC6.01 Transcriptional Regulation of Prion Protein Gene Expression by SP1 and MTF1

Authors
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Content

Background: The normal functions of the cellular prion protein (PrP_C) are still unknown, but numerous studies support a role in copper metabolism. PrP_C can bind copper in the N-terminal octapeptide repeat region and reversibly stimulate endocytosis of PrP_C from the cell surface, suggesting PrP_C acts as a receptor for cellular copper uptake or efflux. In vitro, copper can convert PrP_C into a detergent-insoluble and PK-resistant infective isoform (PrP_{Sc}), suggesting that copper may have a role in the conversion process and the infectivity of PrP_{Sc}.
In addition, copper has been shown to induce prion gene expression in neurons. Based upon bioinformatics analysis of the prion gene promoter we identified two potential transcription factors, SP1 and MTF1, which may be involved in copper-dependent regulation of the prion gene.

Objectives: To investigate the role of transcription factors SP1 and MTF1 in the copper-dependent regulation of the prion gene.

Methods: A novel fibroblast cell culture system, that has high and low copper levels due to varied expression of the Menkes copper efflux protein, and human neuroblastoma cell line SH-SY5Y were utilised to determine the effects of copper, SP1 and MTF1 on the regulation of prion gene expression. SP1 and MTF1 were expressed by transient transfection, PrP_C protein levels were detected by western blotting, while gene expression was determined by qRT-PCR.

Results: We observed that in cells with "extremely low copper" levels that PrP_C was undetectable compared to normal and "high copper" level cell lines. The addition of copper, SP1 and MTF1 failed to induce any change in PrP_C and prion gene expression in the "extremely low copper" cell line. In contrast, "high copper" levels with transient expression of transcription factors SP1 or MTF1 induced a significant 2-3 fold increase in *PRNP* gene expression resulting in increased PrP_C protein levels.

Discussion: These data support a novel mechanism for the involvement of SP1 and MTF1 in the copper-dependent regulation of prion gene expression. Expression of PrP_C is vital for propagation and infectivity of PrP_{Sc}, thus SP1 and MTF1 represent new targets in the development of key therapeutics in the prevention of prion disease.

OC6.02 Cathepsin D C224T polymorphism in sporadic and genetic Creutzfeldt-Jakob disease

Authors
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Content

Background: The endosomal-lysosomal system is involved in the processing of the cellular and disease-associated prion protein. Our recent neuropathological study indicated that accumulation of cathepsin D immunoreactive lysosomes is a marker of neuronal vulnerability in sporadic Creutzfeldt-Jakob disease (sCJD). Cathepsin D is also suggested to participate in the clearance of protein aggregates including Amyloid-beta. C224T polymorphism in the *Cathepsin D* gene was recently shown to be a risk factor in variant CJD.

Objectives: We examined the C224T polymorphism in the Cathepsin D gene to evaluate whether it is a risk or prognostic factor in sCJD and genetic CJD.

Methods: Blood samples taken from 541 sCJD, 102 genetic CJD and 726 control samples were tested for *the Cathepsin D* C224T and *PRNP* M129V polymorphisms together with Apolipoprotein E *(ApoE)* alleles. Genotype data and duration of illness were compared using multiple logistic regression.

Results: The distribution of alleles of the cathepsin D gene was approximately the same in all groups examined. We observed a trend for shorter duration of illness (25-35% reduction) in sCJD patients with *PRNP* M129M genotype and M129M type 1 sCJD patients showing the CT genotype at codon 224 of the *Cathepsin D* gene. A 35% reduction of survival was significant for individuals showing the constellation of *PRNP* codon 129 MM and Cathepsin D codon 224CT and carrying the ApoE epsilon 4 allele.

Discussion: In contrast to variant CJD, the examined *Cathepsin D* polymorphism is not associated with higher risk of developing sCJD. However, in M129M sCJD cases C224T heterozygosity carries a worse prognosis in particular in individuals with the *ApoE* epsilon 4 allele. Combined evaluation of polymorphisms in *PRNP*, *ApoE* and Cathepsin D genes might be useful for prediction of survival that is important for the assessment of potential therapeutic agents.

OC6.03 Investigation of protein-protein interactions from scrapie infected mice brain tissue using NAPPA

Authors

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Content

Investigation of protein-protein interactions from scrapie infected mice brain tissue using NAPPA A. Yansanjav1, U. Basu[1], S. Booth[2], J. Labear[3], S. S. Moore1 and L.L. Guan1

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Background: Transmissible Spongiform Encephalopathies (TSE) are fatal neurodegenerative diseases which occur in humans and various animal species. It is known that abnormal prions cause plaques to form on the neurons preventing them from functioning properly. However, it is not yet clear whether other proteins are affected by the alteration of prion protein in whole protein network of central nervous system. Objective: In this study, we aimed to identify protein-protein interactions that may have involved in TSE disease progression using NAPPA (nucleic acid programmable protein array) technology.

Methods: A mouse model infected with a scrapie strain was used. The same age mice C57/BL6 were injected with the scrapie RML strain. The infected mice showed clinical signs of disease, 150 days post-inoculation.

The total RNA, extracted from infected and control mice respectively, was used for full-length cDNA library construction to identify the differentially expressed genes by comparison of the transcripts from both libraries.The proteins encoded by these genes were expressed using NAPPA.

Results and Discussion: Twenty full-length transcripts were identified from infected mice after screening of 384 clones. Sequence analysis showed the presence of genes that are involved in protein folding (cyclophilin) or Alzheimer's disease (metallothionein). Furthermore, genes, previously reported (citation) as their normal functions altered in brains of scrapie-infected mice, such as cystatin, heat shock protein, ATP-ase Na+/K+ transporting, mitochondrial ribosomal protein and glycoprotein, have been found in this study. Some of the genes e.g. trha protein, SH3 domain binding glutamic acid-rich protein, ribosomal protein L4, L27, L28 and L39, cytocrome C oxidase, basigin isoform 2 and endocrine-specific proteins have not been reported. These full-length transcripts are cloned into a pANT7-cGST vector and NAPPA technology was used to express proteins for prediction of probable protein-protein interactions. The understanding of the protein-protein interactions will assist us to elucidate the molecular mechanisms of TSE disease.

OC7.01 BASE in sheep displays strain features distinct from BSE and scrapie

Authors

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Content

Background: In recent years two atypical forms of BSE have been recognised in cattle, namely BASE and H-type. Recent studies showed that BASE isolates acquire strain features similar to those of BSE upon interspecies transmission in wt mice and in tg mice expressing ovine PrP_{VRQ}.

Objectives: To investigate the transmissibility and the disease phenotype of BASE in sheep, in comparison to BSE and scrapie.

Methods: Sheep were challenged by the i.c. route with BASE, BSE or scrapie. Western blot was carried out to study the molecular features of brain PrP_{Sc}. PrP_{res} fragments were characterised with several mAbs and under varying PK conditions. The accumulation of PrP_{Sc} in brain, peripheral nervous system (PNS) and lymphoid tissues (LRS) was studied by IHC.

Results: Sheep with the ARQ/ARQ genotype showed terminal disease after 29±3 months post inoculation (m.p.i.) for BASE (n=8), 16±0 m.p.i. for BSE (n=2) and 15±1 m.p.i. for scrapie (n=5). At 50 m.p.i., 4 over 8 ARQ/ARR sheep (45±3 m.p.i.) have succumbed to BASE, and none to BSE (n=5) or scrapie (n=13). PrP_{res} fragments of BASE displayed molecular sizes similar to BSE, but they were significantly less glycosylated than in BSE and similar to scrapie, allowing a clear cut discrimination from BSE. PrP_{Sc} was more susceptible to PK in BASE than in BSE. Furthermore, under stringent PK, BASE samples showed a glycosylated, C-terminal PrP fragment of ~12 kDa, which was absent in BSE and scrapie.

PrP_{Sc} deposition in BASE was mainly intraneuronal and intraglial. Extracellular, astrocyte-associated, subependymal, submeningeal and perivascular PrP_{Sc} deposits lacked in BASE, but were present in BSE and scrapie. Finally, PNS and LRS of sheep with BASE were devoid of PrP_{Sc} deposits, contrary to what observed in BSE and scrapie.

Discussion: Our findings show that BASE behaves as a distinct strain in sheep, which is different from BSE and classical scrapie based on PrP genotype targeting, disease phenotype and tissue tropism. The molecular features of BASE could be exploited for a rapid discrimination from BSE and classical scrapie, but are reminiscent of those observed in CH1641-like isolates recently recognised in Europe during large scale molecular strain typing. Studies aimed at comparing the biological and molecular characteristics of BASE and CH1641-like isolates are underway.

OC7.02 When Atypical Scrapie cross species barriers.

Authors

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Content

Atypical scrapie is a TSE occurring in small ruminants and harbouring peculiar clinical, epidemiological and biochemical properties. Currently this form of disease is identified in a large number of countries. In this study we report the transmission of an atypical scrapie isolate through different species barriers as modelled by transgenic mice (Tg) expressing different species PRP sequence.

The donor isolate was collected in 1995 in a French commercial sheep flock. Inoculation into AHQ/AHQ sheep induced a disease which had all neuro-pathological and biochemical characteristics of atypical scrapie.

Transmitted into Transgenic mice expressing either ovine or PrP_C, the isolate retained all the described characteristics of atypical scrapie.

Surprisingly the TSE agent characteristics were dramatically different when passaged into Tg bovine mice. The recovered TSE agent had biological and biochemical characteristics similar to those of atypical BSE L in the same mouse model. Moreover, whereas no other TSE agent than BSE were shown to transmit into Tg porcine mice, atypical scrapie was able to develop into this model, albeit with low attack rate on first passage.

Furthermore, after adaptation in the porcine mouse model this prion showed similar biological and biochemical characteristics than BSE adapted to this porcine mouse model.

Altogether these data indicate.

(i) the unsuspected potential abilities of atypical scrapie to cross species barriers
(ii) the possible capacity of this agent to acquire new characteristics when crossing species barrier

These findings raise some interrogation on the concept of TSE strain and on the origin of the diversity of the TSE agents and could have consequences on field TSE control measures.

OC7.03 Mutational changes affecting thermostability of TSE agent-strains?

Authors

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Content

Strains of TSE agents can be differentiated by a series of phenotypic properties, (similarly to other infectious organisms), in this case by relative incubation periods in panels of inbred mouse strains (some differing in PrP genotype), by the relative amount and distribution of pathological lesions, and by differences in the degree to which PrP is glycosylated and its migration on SDS-PAGE gels. We have also shown that TSE strains differ in their resistance to inactivation with heat, a property that is intrinsic to the structure of these agents.

Experimental primary passage of a TSE isolate into inbred mice usually results in prolonged incubation periods. On serial passage incubation periods often shorten dramatically, although this is not always the case. In most cases incubation periods and other phenotypic properties for subsequent passages are relatively stable but in some cases they change. From 19 titrations of serially passaged 87A, 12 cases of breakdown to ME7 were detected. The titres of ME7 were found to fit a random distribution, indicative of mutation, with a mutation rate of 4.6 x 10-4. We have observed the appearance of the 79A strain from 139A or vice versa from passages at high dilution, after boiling or after passage in another PrP genotype. Two passage lines of ME7 established after heat selection at high dilution differ in thermostability properties.

Cattle BSE was found to be highly thermostable, mouse derived BSE strains were less thermostable and showed even greater thermolability on subpassage in alternate PrP genotypes. Differences in thermostability and in thermal inactivation rates indicate a diversity of thermostability properties within strains, suggesting two or more populations of sub-strains differing in their thermostability.

These examples illustrate changes in TSE agent properties which have occurred independently of the host (including host PrP genotype). They are most readily explained by mutation of TSE agent genomes, with no direct influence from the host. Selection of mutants depends on the experimental conditions which may include host PrP genotype, inoculum treatment and dilution. The virus quasi-species hypothesis provides a working model for these changes to TSE agent strains.

OC7.04 North American Cervids Harbor Two Distinct CWD Strains

Authors

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Content

Despite the increasing geographic distribution and host range of CWD, little is known about the prion strain(s) responsible for distinct outbreaks of the disease. To address this we inoculated CWD-susceptible Tg(CerPrP)1536+/- mice with 29 individual prion samples from various geographic locations in North America. Upon serial passage, intrastudy incubation periods consistently diverged and clustered into two main groups with means around 210 and 290 days, with corresponding differences in neuropathology. Prion strain designations were utilized to distinguish between the two groups: Type I CWD mice succumbed to disease in the 200 day range and displayed a symmetrical pattern of vacuolation and PrP_{Sc} deposition, whereas Type II CWD mice succumbed to disease near 300 days and displayed a strikingly different pattern characterized by large focal accumulations of florid plaques distributed asymmetrically. Type II CWD bears a striking resemblance to unstable parental scrapie strains such as 87A which give rise to stable, short incubation period strains such as ME7 under certain passage conditions. In agreement, the only groups of CWD-inoculated mice with unwavering incubation periods were those with Type I CWD. Additionally, following endpoint titration of a CWD sample, Type I CWD could be recovered only at the lowest dilution tested (10^{-1}), whereas Type II CWD was detected in mice inoculated with all dilutions resulting in disease. Although strain properties are believed to be encoded in the tertiary structure of the infectious prion protein, we found no biochemical differences between Type I and Type II CWD. Our data confirm the co-existence of two distinct prion strains in CWD-infected cervids and suggest that Type II CWD is the parent strain of Type I CWD.

OC8.01 In vitro amplification of PrP_{Sc} derived from brain and blood of sheep infected with scrapie

Authors
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Content

Background: TSE infectivty circulates in blood during the protracted, pre-clinical phase of scrapie infection, providing a bio-marker for development of an ante-mortem preclinical diagnostic test. Highly efficient in vitro conversion of host PrP_C to PrP_{Sc} has been demonstrated in models of scrapie and in natural prion diseases by protein misfolding cyclic amplification (PMCA).

Objectives: To apply PMCA to brain and blood from scrapie infected sheep.

Results: We have demonstrated amplification, by serial PMCA, of PrP_{Sc} from the individual brains of scrapie infected sheep, the efficiency of which is greatly enhanced by the addition of a synthetic polyanion (polyadenylic acid). PrP_{Sc} has also been amplified from blood leucocyte preparations from VRQ/VRQ sheep with terminal scrapie.

Discussion: This study demonstrates that highly efficient amplification of PrP_{Sc} (>100,000 fold) can be achieved for ovine scrapie from both blood and brain from naturally infected sheep. Coupling this technique to conventional rapid tests offers a realistic chance of a long sought after ante-mortem method of screening TSE susceptible sheep for evidence of infection.

OC8.02 Generation of a constellation of new prions in vitro leading to the emergence of novel prion diseases

Authors
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Content

Background: Prions are unconventional infectious agents responsible for prion diseases, which are composed exclusively by a misfolded form of the prion protein (PrP_{Sc}) that replicates in the absence of nucleic acids. We have recently described that PrP_{Sc} can be propagated indefinitely *in vitro* to generate infectious material using the protein misfolding cyclic amplification (PMCA) technology.

Objectives: The main goal of this study was to attempt the generation of novel prion strains *in vitro* and study the characteristics of the disease produced by inoculation of these newly generated infectious foldings of PrP_{Sc}.

Methods: New prions strains were generated in vitro by PMCA using three alternative approaches: In vitro crossing the species barrier; Mixing PrP_C and PrP_{Sc} containing different polymorphisms or mutations; Spontaneous "de novo" generation of infectious prions. Infectivity and disease features of the novel prions were studied by inoculation into wild type animals and assessment of incubation period, clinical signs, histopathological brain damage and biochemical characteristics of PrP_{Sc}.

Results: Our data showed that using PMCA we can faithfully replicate diverse prion strains and generate a variety of new prions by inter-species conversion, de novo formation of PrP_{Sc} or using polymorphic variants of PrP. In vitro generated PrP_{Sc} were infectious when inoculated to wild type animals producing a large variety of new diseases with unique and previously not described clinical, neuropathological and biochemical features.

Discussion: Our results represent a strong evidence to support the prion hypothesis and suggest that the universe of possible prions is not restricted to those currently known, but that likely many new forms of infectious protein foldings may be produced, resulting in completely novel diseases. This is worrisome, because it raises the possibility that new and perhaps more aggressive infectious prion foldings may originate in diverse species, leading to the emergence of new and unpredictable forms of transmissible diseases.

OC8.03 Persistent propagation of mouse-adapted vCJD and Fukuoka-1 agents in murine stromal cell cultures of bone marrow and spleen origin

Authors
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Content

Background: Attempts to infect cell cultures with human transmissible spongiform encephalopathy (TSE) agents have been unsuccessful, apart from the Fukuoka-1 (Fu), mouse adapted human isolate propagated in murine cells of neuronal origin, N2a and GT1, and rabbit epithelial cells, RK13 overexpressing mouse PrP_C. In particular, no cell culture capable of persistently propagating the variant CJD (vCJD) agent has yet been developed.

Objectives: To establish a cell culture persistently propagating the vCJD agent.

Methods: SJL/Ola mice that develop spontaneous B-cell lymphoma with aging were used for generating cell cultures from bone marrow (BM) and spleen (SP). Under conditions requiring presence of IL3 we selected adherent cells that became spontaneously immortalized after approximately 50 days. Cells were exposed for 72 hours to 1% inoculum from brain tissues of mice infected with either mouse-adapted vCJD (mo-vCJD) or Fu, or healthy controls, and cells were then propagated through multiple passages. In some cases, single-cell cloning was performed. The presence of PrP_C and PrP_{TSE} was confirmed by western blotting (WB) using 6D11 antibody. Cell cultures were characterized for the presence of various markers by FACS analysis. Lysates of BM stromal cells (BMSC) persistently propagating PrP_{TSE} were tested for infectivity by intra-cerebral inoculation of FVBn mice.

Results: Morphological features and data from FACS analysis indicated that cultures of BM and SP origin fulfill the criteria of stromal cell cultures, with some characteristics of mesenchymal stem cells. The major population of cells expressed a significant level of PrP_C which was maintained through multiple passages. In three experiments we were able to infect SP cell culture with Fu and in one experiment with mo-vCJD.
Infected cultures continued to propagate PrP_{TSE} through over 60 (Fu) and 45 (mo-vCJD) passages. In two experiments we infected BMSC culture with Fu. PrP glycosylation profiles were different between Fu and mo-vCJD. The Fu-infected BMSC culture was highly infectious in bioassay.

Discussion: This is the first report on successful propagation of vCJD in cell culture, a model that could be particularly relevant to studies of disease pathogenesis, validation of agent reduction methods, and comparatively rapid ex-vivo therapeutic assays.

OC8.04 The same primary structure of the prion protein yields two distinct self-propagating states

Authors
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Content
Background: The question of whether distinct self-propagating structures could be formed within the same amino cid sequence in the absence of external cofactors or templates has important implications for a number of issues ncluding the origin of prion strains.

Objectives: to test whether chemically identical prion protein can give rise to conformationally distinct,self-propagating states in the absence of cellular cofactors, post-translational modification, or PrP_{Sc}-specified templates.

Methods: *in vitro* fibrillation, atomic force microscopy, electron microscopy, FTIR, immunoconformational assay for probing conformation within individual fibrils, hydrogen-deuterium exchange ultraviolet Raman spectroscopy.

Results and **Discussion:** In the current study, we showed that two self-propagating amyloid structures could be generated from the same pool of highly purified full-length recombinant PrP under identical solvent conditions but different shaking modes. Individual prion conformations were inherited by daughter fibrils in seeding experiments conducted under alternative shaking modes, illustrating the high fidelity of fibrillation reactions. Raman spectroscopy, FTIR, immunoconformational assay and AFM revealed fundamental differences between two amyloid strains with respect to the cross-b core structures and surface-exposed epitopes. Our study showed that the ability to acquire conformationally different self-propagating states within the same amino acid sequence is an intrinsic ability of protein fibrillation and strongly supports the hypothesis that conformational variation in self-propagating protein states underlies prion strain diversity.

OC8.05 In vitro studies of the transmission barrier

Authors
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Content
Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative disorders affecting both humans and animals. TSEs can be of genetic, sporadic or infectious origin. The infectious agent associated with TSEs, termed prion, appears to consist of a single protein, an abnormal conformer (PrP_{Sc}) of a natural host protein (PrP_C), which propagates by converting host PrP_C into a replica of itself. One of the characteristics of prions is their ability to infect some species and not others. This phenomenon is known as transmission barrier. In general, the transmission barrier is expressed by an incomplete attack rate and long incubation times which becomes shorter after serial inoculation passages. Compelling evidence indicates that the transmission barriers are closely related to differences in PrP amino acid sequences between the donor and recipients of infection.

Unfortunately, the molecular basis of the transmission barrier phenomenon is currently unknown and we cannot predict the degree of a species barrier simply by comparing the prion proteins from two species. We have conducted a series of experiments using the Protein Misfolding Cyclic Amplification (PMCA) technique that mimics in vitro some of the fundamental steps involved in prion replication in vivo, albeit with accelerated kinetics. We have used this method to efficiently replicate a variety of prion strains from, among others, mice, hamsters, bank voles, deer, cattle, sheep, and humans. The *in vitro* generated prions possess key prion features, i.e., they are infectious in vivo and maintain their strain specificity. We are using the PMCA to study which amino acids in the PrP_C sequence contribute to the strength of the transmission barrier. These studies are proving very useful in evaluating the potential risks to humans and animals, of not only established prion strains, but also new (atypical) strains. In addition, we have also generated prions that are infectious to species hitherto considered to be resistant to prion disease. The correlation between in vivo data and our in vitro results suggest that PMCA is a valuable tool for studying the strength of the transmission barriers between diverse species and different prion strains.

OC8.06 Scrapie production occurs in the the perinuclear recycling compartment

Authors
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Content
Background: Trafficking of PrP_C plays an essential role in its conversion to PrP_{Sc}. In particular it has been shown that the surface expression of PrP_C is important for scrapie production and it has been suggested that the conversion event requires that the protein enter the endocytic pathway. However, the precise subcellular compartment where prion conversion occurs has not been identified yet. Several lines of evidence suggest that the late endosomal/lysosomal compartment is a likely location for scrapie production because in some cell lines PrP_{Sc} has been found to accumulate in late endosomes. Conversely, other studies implicate the Golgi as a candidate for scrapie synthesis and accumulation. Despite these observations, none of these organelles has been demonstrated to be directly involved in the conversion of PrP_C to PrP_{Sc}.

Objectives: The main objective of this work was to identify the subcellular compartment/s in which scrapie production occurs.

Methods: Using a combination of pharmacological and genetic approaches we selectively impaired trafficking between different endocytic compartments and analyzed their impact on PrP_{Sc} levels.

Results: We found that impaired exit from EE and accumulation of PrP_C in this compartment caused a decrease in PrP_{Sc} levels suggesting that PrP_C had too exit this compartment to be converted. We also found that impaired biogenesis of late endosomes/lysosomes and reduction in these organelle number did not affect PrP_{Sc} production indicating that LE are not needed for the conversion process. On the contrary, impaired sorting to the perinuclear recycling compartment (ERC) caused a decrease in PrP_{Sc} levels while delayed exit from this compartment increased PrP_{Sc} levels.

Discussion: Our results clearly indicate that the perinuclear recycling compartment is intracellular site for PrP_C to PrP_{Sc} conversion. The mechanism of this process however, remains still unknown. Because multiple proteins coming from the cell surface as well as from the secretory pathway traverse the perinuclear recycling compartment, one hypothesis is that PrP could meet and interact there with PrP_{Sc} and with specific factors needed for its conversion.
On the other hand the recycling compartment could represent the propitious environment for conversion.

OC8.07 De novo formation of purified infectious prions from minimal components

Authors
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Content
The conformational change of a host protein, PrP_C, into a disease-associated isoform, PrP_{Sc}, appears to play a critical role in the pathogenesis of prion diseases. To investigate the mechanism of prion formation biochemically, we conducted a series of experiments using the Protein Misfolding Cyclic Amplification (PMCA) technique with a preparation of native PrP_C molecules purified from hamster brain, which was >95% pure relative to other proteins and which contained equimolar quantities of 20-carbon fatty acids [Deleault, et al., Proc Natl Acad Sci U S A. (2007)104:9741]. Initially, we used infectious prions to seed serial PrP_{Sc} propagation reactions containing this purified substrate preparation. These experiments showed that successful PMCA propagation of PrP_{Sc} molecules in a purified system requires accessory polyanion molecules. We also subjected mixtures of purified PrP_C plus synthetic poly(A) RNA molecules to serial PMCA propagation experiments in the absence of infectious prion seed. Remarkably, in two independent unseeded serial propagation experiments conducted in rigorously prion-free environments, we observed the spontaneous generation of autocatalytic PrP_{Sc} molecules. Intra-cerebral inoculation of samples containing either prion-seeded or spontaneously generated PrP_{Sc} molecules into normal hamsters caused scrapie, which in both cases was transmissible upon second passage. Additional analyses revealed size-selective incorporation of fluorescent and radioactive polyanions into nuclease-resistant complexes with PrP molecules during the process of prion formation in vitro [Geoghegan, et al., J Biol Chem. (2007)282:36341]. Collectively, these studies suggest that infectious mammalian prions may contain multiple components, namely PrP, lipid, and polyanion molecules.

OC9.01 BSE / Scrapie intracerebrally co-infected sheep: Scrapie hides BSE in the brain, not in lymphoid tissues

Authors

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Content

Background: Background. Differentiation between Scrapie and BSE infection in small ruminant flocks is currently based on the use of discriminating biochemical tests that have been validated on experimentally BSE-infected sheep. The wide variety of TSE agents isolated from small ruminants makes BSE/Scrapie co-infections possible. In such context the discriminating capacity of available tests to identify BSE in co-infected animals appears crucial.

Objectives: Identification of BSE and Scrapie strains in tissues from co-infected sheep.

Methods: Three groups of 2-3 susceptible ARQ/ARQ sheep (n=8) from a TSE free flock (from Defra), were intra-cerebrally inoculated with ovine BSE and a Scrapie strain (vol/vol of ARQ/ARQ sheep brain homogenates). Scrapie strains were 3 field isolates differing from each other and from BSE by their incubation period, PK sensitivity, WB profiles and mouse bioassay results. Sheep were necropsied at clinical stage of the disease and sampled tissues were tested by ELISA, WB and IHC using discriminating anti-PrP antibodies and/or PK digestion conditions.

Results: Clinical onset occurred in all co-infected sheep in 10-12 months, i.e. earlier than Scrapie (13-15 months) and BSE (16 months) controls. In the 8 sheep, PrP_{Sc} was detected in the brain by IHC, ELISA and WB, this late assay showing a typical Scrapie PrP_{res} signature (21 kD). From this nervous tissue, discriminating Scrapie/BSE WB and ELISA were unable to identify PrPBSE. Typical BSE PrP_{res} profile could be observed from lymphoid tissues, indicating that the BSE agent has a high ability to disseminate in peripheral organs of co-infected sheep.
Ongoing mouse bioassays on various *PRNP-transgenic* mouse lines, revealing either Scrapie or BSE, should confirm our immunochemical results.

Discussion: Despite the small size of this sheep experimentation, our results clearly indicate the limits of the currently applied BSE/Scrapie discriminating diagnostic tests.
Supported by EU QLRT-01309; Defra is acknowledged for supplying TSE free sheep; INRA-PFIE for their maintenance at level 3 bio-confinement.

OC9.02 Sensitive Biomarker Detection of Protein Folding Disorders; Proximity Ligation-based detection of protein Oligomers

Authors

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Content

Background: The sensitive and specific detection of oligomeric conformation of proteins can provide opportunities for early detection of neurodegenerative disorders such as prion and Alzheimer's diseases. Our aim is to develop very sensitive and specific molecular tools for quantitative measurement of oligomeric and aggregated conformations of proteins at very low concentration, using proximity ligation assay.

Methods: In proximity ligation assay (PLA) affinity binders are coupled to ssDNA arms to form proximity probes. Once target molecules are recognized by two or more of these probes, the DNA arms are brought in proximity and can be hybridized to a connector oligonucleotide, allowing the ends to be joined by enzymatic DNA ligation. Only the ligation products are then amplified by PCR.

Results: Here, we present the application of PLA for sensitive identification of oligomers and aggregated proteins. For specific detection of oligomeric states of proteins we use a single monoclonal antibody conjugated to two different ssDNA arms, respectively. In this format of PLA only oligomers are detected while monomers with only one available epitope will be excluded. This is illustrated here with specific detection of PrP_{Sc} and Aβ oligomers spiked in different biological matrixes as well as detection of endogenous oligomeric conformations of these proteins.

Conclusions: The combination of efficient PCR amplification and the use of two or more binding reagents provide very high sensitivity and specificity of detection, surpassing the conventional protein detection methods. Furthermore, the proximity ligation technique can be carried out as in the homogenous assay –requiring very small amount of materials to be tested –, or in a heterogeneous format in which the target molecules to be detected are immobilized on a surface using affinity probes, while other materials are washed away. Proximity ligation can, therefore, provide a powerful molecular tool for detection and study of the biology of protein folding disorders.

OC9.03 The Identification of Biomarkers in the Urine of BSE Infected Cattle

Authors

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Content

Background: The bovine spongiform encephalopathy (BSE) epidemic and the emergence of a new human variant of Cruetzfeldt-Jakob Disease (vCJD) have led to profound changes in the production and trade of agricultural goods. The rapid tests currently approved for BSE monitoring in slaughtered cattle are all based on the detection of the disease related isoform of the prion protein, PrP_d, in brain tissue and consequently are only suitable for post-mortem diagnoses.

Objectives: In instances such as assessing the health of breeding stock for export purposes where post-mortem testing is not an option, there is a demand for an ante-mortem test based on a matrix or body fluid that would permit easy access and repeated sampling. Urine and urine based analyses would meet these requirements.
Results: Two dimensional differential gel electrophoresis (2D-DIGE) and mass spectrometry analyses were used to identify proteins exhibiting differential abundance in the urine of BSE infected cattle and age matched controls over the course of the disease. Multivariate analyses of protein abundance data identified a single protein able to discriminate, with 100% accuracy, control from infected samples. In addition, a subset of proteins were able to predict with 85% +/- 13.2 accuracy the time post infection that the samples were collected.

Conclusions: These results suggest that in priinciple it is possible to identify biomarkers in urine useful in the diagnosis, prognosis and monitoring of disease progression of transmissible spongiform encephalopathy diseases (TSEs).

OC9.04 Protein misfolding cyclic amplification combined with the conformation dependent immunoassay as a potential confirmatory blood test for variant Creutzfeldt-Jakob Disease

Authors
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Content

Background: To date there have been four cases of variant Creutzfeldt-Jakob disease (vCJD) infection associated with blood transfusion, which highlights the need for screening test for donor blood to protect public health. Several commercial tests may soon become available but imperfect specificity combined with the large numbers of cases to be screened would inevitably result in a significant number of patients being suspected of incubating vCJD. Therefore, a confirmatory assay is necessary to confirm the results from any rapid screening test.

Objectives: Our objective has been to develop a confirmatory blood test for vCJD by using protein misfolding cyclic amplification (PMCA) of PrP_{Sc} in combination with the conformation dependent immunoassay (CDI) for the detection of PrP_{Sc}.

Methods: We have ascertained that human platelets can be used as substrate source of PrP_C for PMCA. Once amplified, the PrP_{Sc} can be detected by CDI on the basis of an enhancement of immunoreactivity with the antibody 3F4 upon denaturation with a chaotrope to reveal an epitope that is hidden within the native structure of PrP_{Sc}.

Results: PMCA of vCJD PrP_{Sc} is most efficient when the source of the platelet substrate used are from individuals who are methionine homozygous (MM) at codon 129 of the *PRNP* prion protein gene which is compatible with all known clinical vCJD patients. Using this set-up a single round of PMCA (48 cycles of incubation and sonication) amplifies vCJD brain PrP_{Sc} by 100-fold as detected by CDI and additional 10-fold amplifications of PrP_{Sc} are attained by successive new rounds of PMCA after dilution of the reaction product in fresh platelet substrate.

Discussion: We propose that the above methodology opens the way to a confirmatory assay that is sufficiently sensitive to detect the very low levels of PrP_{Sc} thought to be present in the blood of patients incubating vCJD. Using CDI has the advantage that it allows the detection of protease-sensitive forms of PrP_{Sc} that may exist in blood. Platelets from apheresis donors are a readily available from the blood transfusion services, which obviates that problems associated with other PMCA substrate sources such as brain. The *PRNP* codon 129 compatibility of seed and substrate needs to be considered as BSE infection may have occurred in people who are valine homozygous or heterozygous at this codon. The length of time required (24h per round of PMCA and 24h for CDI) is not prohibitive as a confirmatory assay would only be performed on the limited number of samples flagged as positive by a primary high-throughput test.

OC9.05 High resolution quantification and differentiation of PrP_{Sc} forms in mixed BSE and scrapie samples by N-TAAP demonstrates potential for detection of co-infection.

Authors
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Content

BSE and classical scrapie can be distinguished by Western blotting (WB) due to the characteristic differential migration of the unglycosylated band of proteinase K (PK) digested PrP_{Sc} (PrP_{res}), attributed to a difference in PK cleavage sites [1]. N-Terminal amino acid profiling (N-TAAP) of PrP_{res} by mass spectrometry (MS) provides a higher resolution means of TSE differentiation [2,3], enabling precise identification and quantification of cleavage sites. N-TAAP is applied here to train differentiation and its capability compared with WB for detection of the co-presence of scrapie and BSE PrP_{Sc} using a simple 'mixed-infection' model.

Homogenates were prepared from brain from sheep infected naturally with scrapie or experimentally with BSE [2,3]. The scrapie [PrP_{Sc}] was adjusted by addition of genotype matched negative control brain homogenate to give [PrP_{Sc}] equivalent to the BSE sample. Homogenates were then mixed (0, 10, 25, 50, 75, 90 and 100 % BSE) and PrP_{res} prepared and characterised by WB [1] and N-TAAP analysis [3].

WB using 6H4 antibody showed the expected lower molecular mass of PrP_{res} in the 100% ovine BSE sample.

With increasing proportions of scrapie PrP_{res} in the mix, the molecular mass increased gradually, though at no point could two distinct unglycosylated bands be seen. Blotting with P4 antibody demonstrated that a 100% ovine BSE sample was only weakly detected, and that from >10% scrapie homogenate content, a dominant scrapie profile was obtained.

N-TAAP analysis enabled the proportions of each PrP_{res} type in the mixed samples to be determined and the profiles indicated a linear quantitative relationship for BSE ($r2 = 0.992$) or scrapie characteristics ($r2 = 0.986$).

The MS based approach thus offers much potential for discriminating strains and for complex differential diagnosis.

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(2) Howells, L.C. et al, Biomarkers (2008), in press.
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OC9.06 Specificity and capacity of the P-Capt® filter for prion protein and prion infectivity

Authors

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Content

Specificity and capacity of the P-Capt® filter for prion protein and prion infectivity
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Background: Prion infectivity is present in blood and is transmissible by blood transfusion. We have developed a device, the P-Capt® filter (manufactured by MacoPharma, Inc.), to reduce endogenous infectivity from human leucoreduced red blood cells (RBC). The P-Capt® filter is composed of eight membrane layers, each containing an embedded resin that adsorbs the prion protein and infectivity from brain-spiked RBC and from endogenously infected blood.

Objectives: The resin was developed in column format using the hamster 263K scrapie model. The studies presented here were conducted to assess the P-Capt® filter implementation for binding specificity for PrP from various animal models and prion strains. We have also investigated the prion binding pattern across the membrane layers of the filter.

Methods: Specificity was assayed by measuring PrP binding from brain homogenates of human vCJD and sCJD, uninfected macaque, mouse-adapted BSE, hamster-adapted BSE and scrapie, and natural sheep scrapie.
Each brain homogenate was spiked into a unit of leucoreduced RBC and applied to a P-Capt® filter. The filter was cut open and the resin from each membrane layer was analyzed by Western blot of the resin-bound proteins.
In the case of hamster scrapie, 1.5x107 infectious doses50 (ID50) were loaded on the filter.

Results: The Western blot analysis of the resin-bound proteins in the filters indicated that each layer of the filters behaved as an independent chromatographic component. The first few layers were saturated with PrP and the successive layers removed the remaining PrP signal to below the limit of detection of the Western blot.
The same binding behavior was observed for all prion strains tested.
All Western blot-detectable PrP was captured by the P-Capt® filter challenged with hamster scrapie brain homogenate, consistent with published removal values obtained for the resin in column format and corresponding to a capacity of approximately 1.5x107 ID50 per filter.

Discussion: The P-Capt® filter demonstrated broad specificity for PrP of various prion strains including human vCJD and sCJD confirming its suitability for the reduction of risk from blood transfusion. The capacity of the P-Capt® filter for prion infectivity is several orders of magnitude greater than is estimated to be present in a unit of human RBC.

OC10.01 An Update on the First U.S. Treatment Trial for Sporadic Jakob-Creutzfeldt Disease (sCJD)

Authors

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Content

Background: Quinacrine has been shown to eliminate prions in vitro. In 2005, we began the first U.S. treatment trial for sCJD.

Objectives: To provide our experience in designing and implementing this trial and report mid-study results.

Methods: This NIH-sponsored trial, "A randomized, double-blind, controlled study of the efficacy of quinacrine in the treatment of sporadic CJD," is advertised through the NIH (www.clinicaltrials.gov) and our own websites, and through letters sent to all U.S. and Canadian neurologists. Study staff includes two neurologists, an internist, three research coordinators, a research study nurse, a pharmacist and an administrative assistant. Design is randomized, double-blinded, placebo-controlled (50:50) with optional open label at 2 months. Primary outcome is survival from randomization. Other outcomes include change in neurological exam, neurocognitive testing, brain MRI (atrophy, diffusion changes, etc.), EEG, and magnetoencephalography (MEG). Referred subjects with suspected sCJD are evaluated at the University of California San Francisco (UCSF) on an inpatient research ward to confirm the diagnosis, randomized to the study drug, monitored for 1-2 days, discharged to home and then return to UCSF at months 2, 6, and 12. All subjects who return to UCSF at 2 months are offered open-label quinacrine. Biweekly or monthly safety monitoring (blood work & phone caregiver assessment) is also conducted locally.

Results: Since February 2005, we have been referred 351 suspected CJD cases. After record review and/or inpatient evaluation, diagnoses were: 44% (N=153) sCJD, 32% (N=113) potential CJD (insufficient records), 4% (N=15) genetic prion disease and 19% (N=68) non-prion diagnoses (many of which were treatable). 59 subjects have been consented and 47 randomized to the study drug. For those not randomized, 1 consented subject chose not to be randomized, 6 had CJD but were ineligible, 4 were not CJD, and 1 did not meet our sCJD diagnostic criteria and refused brain biopsy. Three randomized subjects were later found to have *PRNP* mutations. Study mid-point survival and other data will also be presented.

Discussion: Design and implementation of a treatment trial for a rare and rapidly fatal disease can be problematic, but methodologies have been developed for this and future sCJD trials.

OC10.02 Single localized treatment with RNAi against prion protein rescues early neuronal dysfunction and prolongs survival in mice with prion disease.

Authors

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[1]MRC PRION Unit.

Content

Background: Prion diseases are fatal neurodegenerative conditions for which there is no effective treatment. Prion propagation involves the conversion of cellular prion protein, PrP_C to its conformational isomer, PrP_{Sc}, which accumulates in disease. Knockout of PrP_C expression in transgenic mice during prion infection reverses early spongiform pathology and early cognitive and neurophyioslogical deficits and results in long term survival (Mallucci et al; Science, 2003 and Mallucci et al; Neuron 2007).

Objectives: The therapeutic effects of PrP knockout in the above model were achieved by germline manipulation and transgene expression and did not constitute a treatment. We now aimed to achieve a therapeutic PrP knockdown using RNA interference.

Methods: Mice were infected with RML prions at 1 week of age. 8 weeks later, when early prion infection and neuropathology is established, and early behavioural abnormalities appear, they were injected with lentivirus expressing a short hairpin (sh)RNA targeting PrP into each hippocampus. A second group of mice was injected with and 'empty' entivirus with no shRNA; and a third group received no virus at all. Animals were tested for behavioural changes and examined neuropatholgically at the time of death.

Results: A single administration of lentivirus expressing a short hairpin (sh)RNA targeting PrP into each hippocampus of mice with established prion disease significantly prolonged survival time. Treated animals lived 19% and 24% longer than mice given an 'empty' lentivirus, or not treated, respectively.
Lentivirally-mediated RNAi of PrP also prevented the onset of behavioural deficits associated with early prion disease, reduced spongiform degeneration and protected against neuronal loss. In contrast, mice receiving empty virus or no treatment developed early cognitive impairment and showed severe spongiosis and neuronal loss.

Discussion: The focal use of RNAi therapeutically in prion disease further supports strategies depleting PrP_C, which we previously established to be a valid target for prion based treatments. This approach can now be used to define the temporal, quantitative and regional requirements for PrP knockdown for effective treatment of prion disease, and to explore mechanisms involved in pre-degenerative neuronal dysfunction and its rescue.

OC10.03 Therapeutic benefit from gene therapy in the curative treatment of prion diseases

Authors

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Content

Several compounds possessing anti-prion activity are relatively effective in prolonging the incubation period of prion diseases and retarding the occurence of clinical symptoms in mice and hamsters experimentally infected with scrapie. However, the maximum of effectiveness of these molecules only occurs when the treatment is administered at or near the time of prion inoculation, before it can reach the central nervous system.
To overcome these limits, it is necessary to develop radically different strategies. Our objective is to evaluate, in vivo, the therapeutic potential of lentiviral vectors containing prion "resistant" polymorphisms. These "resistant" polymorphisms naturally exist in humans and sheeps and prevent the development of prion diseases. Since in humans treatments are started belatedly, once the clinical symptoms appeared, we focused on the development of a therapeutic protocol targeting the late stage of prion diseases.
For the first time, we developed a system of cannula implantation allowing chronic injections directly into the brain of prion-infected mice, combined with the use of lentiviral vectors carrying the prion-resistant PrPQ167R gene. After only two injections of PrPQ167R virions into the brain of prion-infected mice at 80 and 95 days post-infection, we observed a prolongation of 30 days of the incubation time, accompanied by substantial improvement in behaviour.
This delay was correlated with: (i) a strong reduction of spongiosis in the treated side of the brain; and (ii) a remarkable decrease in astrocytic gliosis in the whole brain. These results show that chronic injections of dominant negative lentiviral vectors into the brain are a promising approach for a curative treatment of prion diseases.
These data suggest that the gene therapy approach could be a promising treatment for humans suffering CJD.

OC10.04 Antibodies directed against the prion protein receptor LRP/LR provide alternative tools in prion diseases

Authors

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Content

The 37kDa/67 kDa laminin receptor (LRP/LR) acts as the cell surface receptor for the cellular prion protein (PrP_C) (1) and the infectious prion protein (PrP_{Sc}) (2). Antibodies directed against LRP/LR hamper the binding of PrP_{Sc} to the cell (3) and therefore prevent the propagation and spread of the infectious prion agent. We proved that the polyclonal anti-LRP/LR antibody W3, is able to abolish PrP_{Sc} propagation in scrapie infected neuroblastoma cells (3), demonstrating that the disruption of the LRP-PrP interaction is a relevant strategy in therapy against TSEs (for review (4, 5,6)). We injected W3 intraperitoneally into scrapie infected mice and observed in the spleen 90 days post infection a significantly reduced PrP_{Sc} content by approximately 66%, demonstrating a strong reduction of the peripheral PrP_{Sc} propagation. In addition, the survival of the scrapie infected mice was 1.8 fold prolonged compared to the control group suggesting that disruption of the LRP/LR-PrP interaction by antibodies is a promising therapeutic strategy (7).

We developed single chain antibodies directed against LRP/LR, able to pass the blood brain barrier (BBB) employing a phage display technique. Two scFvs termed N3 and S18 have been selected and characterized (8). A therapeutic effect of the scFvs on scrapie infected mice was investigated by passive immunotransfer resulting in a reduction of the peripheral PrP_{Sc} propagation in scrapie infected mice by approx. 40%, without a significant prolongation of the incubation time and survival. An improved scFv version, termed iS18, displaying a ten fold higher KD towards LRP, has been generated. Although we observed a curing effect of this iS18 on scrapie infected cells, the scFv did not influence the survival of scrapie infected mice when administered intraperitoneally. To improve the curing effect of anti-LRP antibodies for therapy of prion disease, stability and half life of the antibodies was enhanced by the design of a synthetic full length immunoglobulin, which is based on the variable chains of the scFv iS18 (9). This IgG1-iS18 can be produced in high yields in mammalian cells and is currently delivered by passive immunotransfer into scrapie infected mice to investigate a prolongating effect on incubation time and/or survival.

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OC10.05 Italian trial with doxycycline in Creutzfeldt-Jakob disease: background, study protocol and update on study population

Authors

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Content

Background: Previous studies from our group led to recognize that tetracyclines are able to (i) revert the protease-resistance of the disease-associated prion protein isoforms extracted from brain tissue of patients with all forms of CJD and cattle with BSE, (ii) reduce the infectivity titer in prion-contaminated material, and (iii) prolong survival of prion-infected animal models. On this ground, a series of CJD patients received compassionate treatment with doxycycline at a daily oral dose of 100 mg from the time of diagnosis to death. The retrospective analysis revealed that the subjects treated with doxycycline (n=21) survived significantly longer than untreated CJD patients (n=21) equivalent for sex, age at disease onset and codon 129 *PRNP* polymorphism (treated: 13.9 ± 3.8 months, untreated: 6.1 ± 0.5 months, Log Rank test: $p<0.01$), which are major predictors of survival in CJD.

Objectives: To determine the effectiveness of doxycycline in CJD through a controlled clinical trial.

Methods: We have designed a phase II, multicenter, randomized, double-blind study of doxycycline versus placebo, that has been approved by the Italian Agency of Drug (AIFA) and the ethical committees of the five participating clinical centers. The study population will comprise 60 patients fulfilling the criteria of probable or definite CJD. Randomization will be balanced on gender, age, time from disease onset and *PRNP* codon 129 genotype. The treatment (daily oral dose of 100 mg doxycycline or placebo) will continue until death.

Results: The study started on April 2007 and, at the time of writing, 22 CJD patients entered into the trial. We will illustrate the study design, efficacy evaluation criteria and the electronic CRF developed for data collection, and will report the number and characteristics of patients recruited into study.

Discussion: A positive outcome of this trial would activate similar studies in other neurodegenerative disorders due to protein misfolding such as Alzheimer's disease, since the effects of doxycycline seem to be dependent upon a direct interaction with abnormal protein conformers having an extensive beta-sheet conformation rather than a specific amino acid sequence. (Supported by AIFA, project FARM573ME8, and NoE NeuroPrion).

OC10.06 High Throughput Screening to Identify Therapeutics for Human Prion Disease

Authors

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Content

Background: Prion Diseases are a class of fatal neurodegenerative disease with no known cure that are initiated by the misfolding of a normal host-encoded protein, the prion protein (PrP). As PrP populates no folding intermediate, a small molecule which binds to and stabilizes the structured domain of the cellular form of PrP (PrP^C), and thereby reduces its unfolding, should be able to prevent misfolding and therefore to stop disease progression.

Objectives: The primary requirement for therapeutic stabilisation is the binding of any potential therapeutic compounds to PrP^C. We have developed a series of biophysical assays to directly detect interactions between recombinant prion protein and small molecule compounds, and we have validated these assays using a selection of putative therapeutic compounds identified from published literature.

Methods: We are currently undertaking a major project to identify novel compounds that stabilise PrP^C and prevent its conversion to PrP^{Sc} using High Throughput Screening (HTS). Three in vitro assays that monitor aggregation of recombinant human PrP were designed and tested. A fluorescence polarisation (FP) assay was superior to traditional light scattering assays in terms of sensitivity, reproducibility, throughput and cost.

Results: We have screened approximately one million compounds from the GlaxoSmithKline library for their ability to prevent PrP aggregation using the FP assay. Potential hit molecules were confirmed it triplicate and their XC50 quantified with a dose response. Hit compounds, and their analogues, have been tested for PrP^C binding using biophysical techniques and screened for anti-prion activity in a cell-based assay. We have now identified a number of novel pharmacophores which bind to PrP^C and have nanomolar efficacy in cells.

Discussion: The novel compounds discovered are the first to be identified by such a large-scale experimental screen and will now be optimized as lead molecules.

IPFA.01 Do sporadic or genetic CJD pose a risk through blood or blood products?

Authors
Will, R. G.
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Content
The demonstration of transmission of variant CJD through blood transfusion has raised the question as to whether other human prion diseases might be transmissible by a similar mechanism or through exposure to plasma derived products. In considering these issues there are a number of critical questions:
1. Are prion diseases transmissible through blood infectivity?
2. Does blood in sporadic or genetic CJD contain infectivity?
3. Will infectivity be cleared by production processes?
4. What is the potential individual exposure to infectivity?
5. What is the population risk?
6. Does epidemiological evidence preclude the possibility of transmission by these mechanisms?
7. What is the balance of risk and benefit of blood transfusion and plasma derived products?
The scientific evidence relevant to these questions will be considered and a tentative conclusion presented.

IPFA.02 Update on blood screening tests (yet again), with some 'strategic' comments

Authors
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Content
Screening tests for the detection of misfolded 'prion' protein (PrP_{TSE}) have still not reached the goal of human trials, but a number of laboratories continue to work through the stages stipulated by the UK regulatory committee with varying degrees of success. Detection strategies fall into the following four categories:
1) PrP_{TSE}-specific antibodies.
2) denaturation of the protein to reveal epitopes not accessible in the native state.
3) PrP_{TSE}-specific ligands to concentrate the protein to detectable levels.
4) PMCA to increase the concentration of protein to detectable levels.
The unexpected discovery that a proportion of normal brains subjected to many cycles of PMCA can yield both PrP_{TSE} and infectivity has created the need to establish a threshold to distinguish normal from infected specimens. It is also increasingly evident that any single test will not suffice to identify a true positive, and that two tests using different strategies will be mandatory for human use.

IPFA.03 Blood based assays for infection with the agent of vCJD

Authors
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National Institute for Biological Standards and Control (NIBSC) UK.

Content
A number of developers continue to work on promising blood based assays for infection with the agent of vCJD. The process for evaluating them elaborated by the committee that is overseeing the CJD Resource Centre at NIBSC is more developed, not trivial and will be reviewed. It includes assessment of analytical sensitivity using panels of plasma spiked with infected brain or spleen, an assessment of performance on panels from scrapie infected and uninfected sheep to estimate the degree to which normal and clinical samples overlap in the assay, an assessment of normal human plasma to assess specificity and the spreas of signal recorded on negative samples, followed by the results obtained on the few specimens available from vCJD cases. Assessment of clinical sensitivity may involve sequential bleeds from primates, sheep or exposed human subjects and is extremely difficult.

IPFA.04 Spiking for blood product validation

Authors
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Content
Background: Concern about the safety of blood, blood components, and plasma-derived products with respect to prions has increased since the report of four blood-related infections of variant Creutzfeldt-Jakob disease in the United Kingdom.
The development of protocols to remove or inactivate prions from blood components or plasma-derived products have used brain fractions of transmissible spongiform encephalopathy (TSE)-infected rodents as spiking materials.
Such spiking materials contain misfolded protein (PrP_{TSE})aggregates that are unlikely to be present in blood, and may thus be inappropriate for validation studies. We have prepared a novel spiking material from ultracentrifuged 263K scrapie-infected brain homgenates that is free of PrP_{TSE} aggregates.

Objectives: The objective of this study is to compare the performance of nanofilters, heating, and PK treatment using standard 10% brain homogenate (containing infectivity and PrP_{TSE}) and the ultracentrifuged water-soluble preparation that does not contain highly aggregated PrP_{TSE} molecules.

Methods: Water-soluble 263K scrapie infectivity was prepared according to a published protocol (Berardi VA et al., Transfusion 2006). Briefly, 263K infected hamster brains were homogenized in 9 volumes of sterile phosphate-buffered saline, sonicated by 10 short pulses and centrifuged at 825 g for 15 minutes. Low-speed supernatant was then ultracentrifuged at 220Ù000 g for 30 minutes. This high-speed supernatant contains about 105 LD50 of infectivity without any PrP_{TSE} aggregates.

Results: Removal of PrP_{TSE} aggregates renders nanofiltration less effective in removing infectivity. However, "soluble" infectivity is more easily inactivated by heating and PK digestion.

Discussion: "Soluble" infectivity preparationsi are likely to be the most relevant material for spiking of human blood in validation experiments aimed at demonstrating procedures to remove or inactivate TSE infectious agents.

OC11.01 X-ray fiber diffraction reveals major structural differences between brain-derived prions and recombinant prion protein amyloid

Authors

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Content

Background: X-ray fiber diffraction can be used to study the structure of amyloid fibrils and other helical polymers.

Periodic features in the amyloid fibrils produce signatures in the diffraction patterns, e. g., the cross-β reflection at ~4.7 Å that characterizes amyloid. So far, the literature contains no useful fiber diffraction data obtained from infectious prions.

Objectives: To analyze and compare the structures of brain-derived prions with those of recombinant prion protein (PrP) amyloid.

Methods: We prepared partially oriented, dried fibers of brain-derived PrP 27-30 and PrP_{Sc} as well as of amyloid formed from recombinant PrP(89–230). Fiber diffraction patterns were collected at the Stanford Synchrotron Radiation Laboratory. The data were analyzed and used to interrogate different molecular models for the structure of PrP_{Sc}.

Results and Discussion: Fiber diffraction patterns of recombinant PrP(89–230) amyloid displayed characteristic meridional reflections at ~4.7 Å and equatorial reflections at ~10 Å. This pattern is very similar to those of many other amyloids. Diffraction patterns from brain-derived PrP 27-30 and PrPSc also displayed strong meridional reflections at ~4.7 Å, but lacked the typical equatorial reflections at ~10 Å; instead, a series of equatorial reflections that characterize the diameters of amyloid fibers and of individual protofilaments was produced. Negative-stain electron microscopy was used to confirm these measurements independently.

A detailed analysis of the data indicated that the diffraction pattern of the recombinant PrP amyloid fits best with a silk-like beta structure. The brain-derived prions do not fit this structure, and are more consistent with a beta-helix. Other models were excluded by extensive modeling and simulation of diffraction.

We acknowledge generous support from the Sherman Fairchild Foundation and the National Institute onAging (Grants AG02132 and AG10770).

OC11.02 Exposure to small ruminants TSE agents: a threat for animal and human health?

Authors

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Content

A decade ago, a new variant form of CJD was identified. The emergence of this TSE form in human was the consequence of the zoonotic transmission of BSE through dietary exposure to contaminated animal products. Since then, control of the BSE exposure risk has become a priority and a sanitary policy, aimed at its eradication in food producing animals, was implemented.

A beneficial effect of the BSE eradication policy was an enhanced control of other TSE agents circulating in farm animals (i.e mainly Scrapie in small ruminants). While the ability of BSE to cross species barriers is well documented, it is uncertain that Scrapie might represent a similar risk.

Available epidemiological studies indicated an absence of correlation between Scrapie exposure and TSE occurrence in human.

However,

(i) The active surveillance program carried out since 2001 in the EU and other countries,

(ii) The quantification of prion infectivity distribution in tissues from scrapie incubating individuals, evidenced how biased could be our perception of the level of exposure to small ruminants TSE agents.

While for several centuries scrapie was considered to be a unique disease, it is now recognized to be caused by a complex mosaic of prions, each harbouring unique biological properties. Transmission of TSE small ruminants isolates across various species-barrier models has been achieved. However, the capacity of such TSE agents to cross these species-barriers under natural exposure conditions remains uncertain.

The cattle BSE epizootic is now under control and fading not only in EU herds but also in memory. Measures that were implemented a decade ago to prevent recycling of TSE agents in animal feed and to limit human exposure risk, are currently under re-evaluation.

The capacity of policy makers to balance their decisions according to both the uncertainties in TSE field and economic constraints, remains the key-parameter for the control of the human and animal TSE exposure risk.

OC11.03 PMCA and bank voles: an update

Authors

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Content

Protein Misfolding Cyclic Amplification (PMCA) is an innovative in vitro technique that, by alternating cycles of incubation and sonication, speeds up the conversion of PrP^{C} from healthy brain homogenates triggered by undetectable amounts of PrP^{Sc}. Conceptually, PMCA is reminiscent of Polymerase Chain Reaction but the molecular events leading PrP^{C} conversion and amplification in the reaction tubes are much less understood. This makes the technique rather empirical. Nevertheless, PMCA is at present one of most promising technology in prion science.

We recently proposed the bank vole *(Myodes glareolus)* as an additional laboratory model for prion diseases. Considering the high susceptibility of voles to a range of human and animal prion diseases, we aimed at improving the potentialities of PMCA by using the brain of bank voles as a substrate for PrP^{Sc} amplification.

We proved that vole's normal brain homogenate is an extremely efficient substrate for PMCA, transposing in vitro the characteristics of plasticity and efficiency in propagating TSEs that we observed *in vivo*.

However, we also observed the production of pK-resistant PrP from healthy vole brain homogenates (unseeded negative controls) after multiple rounds PMCA. These products proved to be highly infectious following inoculation into voles and to encode for different TSE strains. Either the hypothesis that this newly formed strains represented de novo generated prions or the result of "in vitro" replication of minute amount of contaminating prions have been deeply investigated. Unfortunately, we haven't been able to confirm previous suspects of de novo generation. In contrast, we had evidence that contamination is a critical point of PMCA and that when it is set up for ultrasensitive detection, the risk to amplify and reveal minimal contaminants is extreme. We carried out several experiments dissecting the process with the aim to identify and remove the source(s) of contamination. Appropriate technical improvements allowing ultrasensitive and reliable detection of prions by PMCA will be presented and discussed.

OC11.04 Genome-wide association study identifies genetic risk factors for variant Creutzfeldt-Jakob disease

Authors

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Content

Human and animal prion diseases are under strong genetic control. In a tiered genome-wide association study of variant Creutzfeldt-Jakob disease (vCJD) risk we analysed a thousand samples from multiple categories of human prion disease, and over four thousand control samples from the UK and Papua New Guinea, including three thousand UK control individuals genotyped by the Wellcome Trust Case Control Consortium (WTCCC). Unsurprisingly, the *PRNP* locus was strongly associated with risk across multiple markers and all categories of prion disease (best single SNP association in vCJD P = 1 x 10-17; best haplotypic association in vCJD P = 1 x 10-24). Although the major contribution to disease risk was conferred by *PRNP* polymorphic codon 129, an additional nearby SNP conferred increased risk of vCJD. Aside from *PRNP*, one technically validated SNP association achieved nominal genome-wide significance, upstream of *RARB* (P = 1.9 x 10-7). A similar association was found in a small sample of iatrogenic CJD (P = 0.030), but not sporadic CJD or kuru. In cell culture, retinoic acid has been shown to regulate prion protein expression. Upstream of *STMN2* we found evidence of an association in acquired prion disease, including vCJD (P = 5.6 x 10-5), kuru incubation time (P = 0.017) and resistance to kuru (P = 2.5 x 10-4). The risk genotype was not associated with risk of sporadic CJD, but conferred an earlier age of onset. Further, we found *STMN2* expression was reduced thirty-fold by infection in a mouse prion disease cell model. These findings justify functional analysis of the implicated biological pathways in prion disease.

OC11.05 Prion Protein-deficient Cattle are Resistant to Prion Disease

Authors

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Content

Background: Transmissible spongiform encephalopathies (TSEs) or prion diseases are caused by the propagation of a misfolded form (PrP_d) of the normal cellular prion protein, PrP^{C}. Disruption of PrP^{C} expression in the mouse results in resistance to PrP-propagation and disease. However, the impact of the ablation of PrP^{C} function in a natural host species of prion diseases is unknown. Recently, we have reported the generation and characterization of PrP^{C}-deficient cattle (PrP-/-) produced by a sequential gene targeting system.

Objectives: In this study we wanted to determine whether PrP-/-cattle are susceptible or resistant to prion diseases.

Methods: Five *PRNP*+/+ wild-type and five *PRNP*-/- cattle were inoculated intracerebrally with a 10% brain homogenate derived from a bovine infected with a cattle-adapted transmissible mink encephalopathy (TME) isolate. Six other cattle (three each *PRNP*+/+ and *PRNP*-/-) were inoculated with normal brain material.

Results: *PRNP*-/- cattle inoculated intracerebrally with a cattle-adapted TME isolate (i) did not replicate abnormal PrP_d in their central nervous system (CNS) for at least 23 months post inoculation (MPI) and (ii) are clinically normal at least 28 MPI. In contrast, all five *PRNP*+/+ cattle inoculated with TME were euthanized with clinical signs within 18 MPI and contained abnormal PrP_d in their CNS tissues. Interestingly, *PRNP*-/- cattle inoculated with TME (2 animals sacrificed at 32 month of age and 23 months post inoculation) showed multifocal areas with Purkinje cell degeneration, whereas none of the *PRNP*+/+ cattle inoculated with TME had significant Purkinje cell degeneration. This was further analyzed using Fluoro-jade staining (which identifies dying brain cells) employing confocal microscopy and Northern Blot analysis determining the mRNA expression of Doppel (dpl), a PrP-like protein. Results of these studies will be discussed.

Discussion: Our results for the first time determine that PrP^{C} is a critical component in the pathogenesis of TSE disease of a natural host. The observed degeneration of Purkinje cells in the two 32 month old but not the 14 month old *PRNP*-/- animals suggests a role of PrP^{C} in the long-term survival of Purkinje neurons.

POSTER

P1.01 Expression and localization of cellular prion protein in the human central nervous system. A comparative study

Authors

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Content

Background: The cellular prion proteín (PrP_c) is a cell membrane glycoprotein abundant in the central nervous system (CNS). The scrapie prion protein (PrP_{Sc}) is responsible for transmissible spongiform encephalopathies (TSE) - neurodegenerative diseases that affect humans and other mammals-requiring the presence of PrP_c for its establishment and evolution. It has been described the coincidence of PrP_c in humans' brains from Alzheimer disease (AD).

Objectives: The main objective was to compare previous findings in healthy rats, cats and cows with humans (healthy and AD cases).

Methods: In this work we studied the expression and localization of PrP_c in healthy humans and in some cases of AD. Western blot and immunohistochemistry were performed.

Results: We have observed a decrease in a rostrocaudal shift of the amount of PrP_c in the CNS of human and of species mentioned above, both in terms of the western blot and the immunohistochemistry. The proportion of PrP_c in AD cases was similar to healthy cases; but some differences were encountered comparing western blot and immunohistochemistry.

Discussion: The fact that the presence of PrP_c is greater in some areas than in others could mean that PrP_{Sc} could be transmitted and could be replicated more intensely on and from such areas.
Our data are congruent with a possible retrograde transport of prions, that there was a diminution of a subpopulation of GABAergic cells in TSE, and help to explain some of the pathogenic mechanisms more controversials in prion's diseases, such as the loss of selective neuronal cells surrounded by a special form of extracellular matrix [1, 2]. The presences of PrP_c in AD cases contribute to a pathophysiological explanation of such clinical entity.

Acknowledgements: Supported by PIUNA (University of Navarra) and BMH4-CT96-856(EU). FJMoleres was a recipient of a FPU grant 2003-5033 (Spanish government).

[1]: Moleres, J. and Velayos, J. L., Brain Research, 1056 (2005) 10-21
[2]: Moleres, J. and Velayos, J. L., Brain Research, 1174 (2007) 143-151

P1.02 Role of ERK1/2 and p38 in the anti-prion activity of minocycline

Authors

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Content

Background: In the past years, we developed an experimental model to assess PrP_{Sc} neurotoxicity using a recombinant polypeptide encompassing the amino acids 90-231 of the human PrP sequence (hPrP90-231). This peptide corresponds to the protease-resistant core of PrP_{Sc} that accumulates into the brain of prion disease patients. We set up an experimental protocol to convert hPrP90-231 from a PrP_c-like to a PrP_{Sc}-like conformation. In virtue of these structural changes, hPrP90-231 powerfully affected the survival of SH-SY5Y cells, inducing a caspase 3 and p38 dependent apoptosis, while in the native a-helix-rich conformation, hPrP90-231 did not induce significant cell toxicity.

Objectives: The aim of this study was to identify drugs able to block hPrP90-231 neurotoxic effects. In particular, we focused on minocycline, a second-generation tetracycline, with a known neuroprotective activity, on several pro-apoptotic conditions.

Results and Discussion: We determined the efficacy of minocycline in inhibiting SH-SY5Y cell death induced by hPrP90-231 and the molecular mechanisms involved. We report that hPrP90-231 induces a caspase 3-dependent apoptosis via the blockade of ERK1/2 activation induced by neurotrophic factors, and the subsequent activation of p38 MAP kinase. Thus, we propose that hPrP90-231 induced apoptosis is dependent on the inhibition of ERK1/2 responsiveness to neurotrophic factors, removing a tonic inhibition of p38 activity that, in turn, results in caspase 3 activation. Minocycline prevented hPrP90-231-induced toxicity interfering with this mechanism: the pretreatment with the tetracycline restores ERK1/2 activity, reverts p38 activation and blocks caspase-3 activity. Conversely, the effects of minocycline, were neither mediated by the prevention of hPrP90-231 structural changes nor by cell internalization.

Acknowledgements: This work was supported by grants from Italian Ministry of University and Research (MiUR-PRIN2006) and Compagnia di San Paolo to TF.

P1.03 Prion protein fragment 90-231 induces microglia activation: Role OF ERK1/2 AND p38 map kinases in nitric oxide production and CCL5 secretion

Authors
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Content

Background: Gliosis is a common feature in prion diseases, causing the release of chemoattractant and proinflammatory factors as well as reactive free radicals, involved in neuronal degeneration.

Aim: This study was aimed to evaluate the direct contribution of the extracellular accumulation of PrP_{Sc} in the gliosis, analyzing the biological activity of the PrP fragment 90–231 (PrP90-231) on glial cell activation and the role of MAP kinases in this phenomenon.

Results and Discussion: Treatment with PrP90-231 induces a dose-dependent cerebellar granule neurons (CGC) apoptosis (as evidenced by reduction of cell viability in the MTT assay and nuclear condensation at the bis-benzimide staining) and microglial cells activation. Microglial exposure to PrP90-231 elicits growth arrest as well as a number of molecular events such as ERK1/2 and p38 MAP kinases activation, nitric oxide (NO) production and chemokines secretion. We achieved a semi-quantitative analysis of a large number of cytokines released by microglial cells in the medium, both in basal conditions and upon exposure to PrP90-231. We observed that the release of proinflammatory cytokines and chemokines CCL5 G-CSF, GM-CSF and IL-12 was selectively increased by PrP90-231; therefore we investigated the role of ERK1/2 and p38 in NO and CCL5. We show that NO release and the secretion of the chemokine CL5 were under the opposing control of the MAP kinases ERK1/2 and p38. Indeed, ERK1/2 blockade reduced iNOS expression, but enhanced CCL5 release in PrP90-231-treated microglial cells. Conversely, p38 block reduced PrP90-231-dependent CCL5 release and augmented iNOS expression. In conclusion, we show that prion fragment PrP90-231 induces neuronal death either exerting a direct proapoptotic activity on neuronal cells or sustaining microglial release of neurotoxic molecules.

Acknowledgements: This work was supported by grants from Italian Ministry of University and Research (MiUR-PRIN2006) and Compagnia di San Paolo to TF.

P1.04 Correlation between neuronal loss and accumulation of prion protein (PrP) in the brain of patients with sporadic Creutzfeldt-Jakob disease

Authors
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Content

Background: Neuronal death is a central component of the neuropathological pattern in human prion diseases but the mechanisms involved in neurodegeneration are still poorly understood. The toxicity of disease-related prion protein (PrP_{Sc}) has been proposed to play a central role in the degeneration of neurons in transmissible spongiform encephalopathies. However, whether neuronal loss correlates with PrP deposition remains an open question since in some experimental models PrP cannot be detected although pathology does occur while in other models PrP does accumulate with no clinical signs and no pathology.

Objectives: We therefore investigated the loss of cerebellar granule neurons and PrP accumulation in the cerebellum of patients with sporadic Creutzfeldt-Jakob disease because both a severe neuronal depletion and PrP accumulation can be observed in some cases in this brain region.

Methods: To classify patients according to their genotype and biochemical characteristics of prion protein, the polymorphism at codon 129 of the *PRNP* and the type of proteinase K resistant PrP present in the cerebellum were determined (Western blotting). Neuronal loss was estimated with a computer-assisted image analysis system. PrP deposition was quantified as well as glial reaction and spongiosis.

Results and Discussion: Precise quantifications of neurons and scores of abnormal PrP accumulation showed significant statistical correlations between neuronal loss and the type of abnormal PrP deposition. As measured by the correlation coefficients, the relationships between the two variables depended on the type of PrP deposits and cerebellar subregions where PrP did accumulate. The results of this investigation support the hypothesis of putative pathogenic roles for disease-related PrP through neurotoxic mechanisms ending in neurodegeneration within the human brain.

P1.05 Early synaptic changes in the hippocampus underlying the onset of neurodegeneration in prion disease: an electron microscopy study

Authors
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Content

Recent evidence supports the hypothesis that synaptic alterations are one the earliest events proceeding neuronal degeneration in chronic neurodegenerative disease (Wishart et al., 2006; Yoshiyama et al., 2007). Intrahippocampal injection of ME7 prion agent leads to the accumulation of protease resistant prion protein (PrP_{Sc}), activation of microglia, astrocytes and neuronal death. Interestingly, the first behavioural changes (12 weeks) are associated with a decrease in synaptophysin staining in the hippocampus, a major presynaptic component (Cunningham et al., 2003). We have used transmission electron microscopy to study synaptic degeneration at 10 and 12 weeks postinjection with either ME7 or normal brain homogenate. An unbiased stereological analysis of the stratum radiatum in the CA1 region of the hippocampus has revealed 22% loss of asymmetric (excitatory) synapses in ME7- injected animals at 12 weeks. Furthermore, presynaptic terminals in these animals are smaller by 35% and postsynaptic density by 20% when compared to control animals. The majority of asymmetric synaptic contacts in the CA1 region come from the CA3 projecting excitatory axonal terminals synapsing onto pyramidal neurons. This study indicates involvement of presynaptic terminal at the onset of the synaptic loss and reduction of CA1 excitatory input in the further development of the disease.

P1.06 Proteomic analysis of neuroblastoma cells expressing a mutant prion protein reveals alterations in gdi alpha and Rab11

Authors
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Content

Inherited prion diseases are associated with mutations in the prion protein (PrP) gene on chromosome 20. These mutations are thought to favour the conformational conversion of PrP into a misfolded, pathogenic isoform. It was previously shown that in transfected cells mutant PrP molecules misfold soon after synthesis in the endoplasmic reticulum (ER) and are delayed in their trafficking to the cell membrane. The D178N mutation in coupling with methionine at polymorphic site 129 is linked to fatal familial insomnia. Here we show by immunogold electron microscopy, that the mouse homologue of this mutation (D177N) accumulates abnormally in the ER and Golgi of transfected neuroblastoma N2a cells. To investigate the impact of intracellular PrP accumulation on cellular homeostasis, we carried out a proteomic analysis of N2a cells expressing D177N PrP. To resolve accurately both acidic and basic proteins, we used two wide-range immobilized pH gradient strips (pH 4-7 and 6-11) coupled to SDS-polyacrylamide linear gradient gels. Quantitative differential analysis of protein maps revealed alterations of 45 proteins in cells expressing D177N PrP compared to wild-type controls. Among these, we found proteins involved in energy metabolism, protein folding, and cellular trafficking. In particular, we found a 3-fold increase of Rab GDP dissociation inhibitor (GDI) alpha, which modulates the activity of Rab proteins by regulating their association with cellular membranes. Interestingly, we found that Rab11, a small GTPase involved in vesicular trafficking between the Golgi and the plasma membrane, was increased in the cytosolic fraction of D177N N2a cells. This result is consistent with evidence that GDI alpha overexpression alters vesicular trafficking between the Golgi and the plasma membrane by sequestering Rab11 in the cytosol. Our data suggest that misfolding and intracellular accumulation of mutant PrP may induce abnormal cellular responses leading to altered vesicular trafficking.

P1.07 Investigating the role of vesicle-associated membrane protein-associated proteins in the ME7 model of prion disease

Authors
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Content

Background: The vesicle-associated membrane protein-associated proteins (VAPs) VAP-A and VAP-B are ubiquitously expressed, type II integral membrane proteins that localize to the endoplasmic reticulum (ER) and pre-Golgi intermediates, and have been proposed to regulate transport between the ER and the Golgi. Genetic mutation in VAP-B has been linked to motor-neuron degeneration in affected amyotrophic lateral sclerosis (ALS) patients Both VAP-A and VAP-B were reduced in human ALS patiets and superoxide dismutase 1 (SOD-1)-ALS transgenic mice in which over expressed protein accumulated within neurons to bring about neurodegeneration. These two lines of evidence suggest that VAP family proteins are involved in the pathogenesis of sporadic and SOD-1-linked ALS. As in ALS, several other neurodegenerative diseases such as prion diseases are also characterised by an increased accumulation of pathologically conformed protein.

Objectives: We are interested in understanding underlying mechanisms of neurodegeneration in prion disease associated with the extracellular deposition of misfolded proteins.

Methods: To investigate whether VAP was impacted on by prion pathogenesis, we have used the hippocampal pathology following injection of brain homogenates harbouring a murine form of scrapie (ME7) to initiate a staged in vivo model of prion disease in which accumulation of misfolded prion protein during disease progression correlates with a synaptic dysfunction.

Results and Discussion: By biochemically quantifying the levels of VAP-A and VAP-B in hippocampi of the mouse model of scrapie (ME7) showing an increasing burden of misfolded prion (early-, mid- and late-stage disease), we failed to detect significant changes in the levels of VAP-A or VAP-B. This contrasts observations in mouse models of ALS, and suggest that VAP-A or VAP-B and the compartments they are associated with, are differentially impacted during degeneration due to intracellular and extracellular insults.

P1.08 Gene expression and protein distribution of four Heat Shock Proteins in CNS of sheep infected with natural scrapie

Authors
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Content

Background: Background: Heat shock proteins (HSP) play cytoprotective roles such as apoptosis regulation and inflammatory response control. These proteins can be secreted to the extracellular medium acting as inflammatory mediators. Their chaperone activity allows the correct folding of proteins and avoids the aggregation of aberrant isoforms. Several studies have proposed the implication of HSPs in prion diseases, however it is unknown if these proteins play a cytoprotective role or they are involved in TSE pathogenesis facilitating the conversion of PrP^{c} into PrP^{sc}.

Objective: The aim of this study was the analysis of gene expression and protein distribution of HSP27, HSP72, HSP73 and HSP90 in the central nervous system of sheep naturally infected with scrapie.

Methods: Expression of chaperones genes was analyzed in four regions (medulla oblongata, diencephalon, prefrontal cortex and cerebellum) of control and scrapie infected sheep.
Quantification of mRNA was performed by real-time quantitative PCR and normalized against a factor based on the geometric mean of the expression of three housekeeping genes. Five micrometer sections obtained from paraffin embedded brain sections of scrapie and control central nervous system were prepared and processed for Hsp immunostaining. Staining was measured in morphologically identifiable neurons and neuropile using subjective scoring from 0 to 5.

Results and Discussion: Different gene expression profiles were observed in the analysed areas. Whereas changes in transcript levels were not observed in cerebellum and medulla oblongata, a significant decrease of HSP27 and HSP90 expression was detected in prefrontal cortex of scrapie infected sheep. On the contrary, HSP73 was over-expressed in diencephalon of positive animals. Immunohistochemistry did not reveal quantitative changes in Hsp90 protein; however a different distribution was observed in cerebellum. An intense Hsp90 immunostaining was observed in Purkinje cells of scrapie infected sheep; in contrast controls displayed little or null staining in these cells. The differences observed in gene expression and protein distribution suggest that the analysed heat shock proteins could play a role in the natural form of the disease.

P1.09 A co-ordinated and selective small heat shock protein response in the ME7 model of prion disease

Authors
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Content
Intrahippocampal injection of brain homogenate containing the ME7 murine modified scrapie leads to a progressive and chronic neurodegeneration that is associated with an increasing burden of misfolded PrP_{Sc}. Part of the degeneration involves a relatively early activation of microglia and astrocytes and a selective loss of stratum radiatum synapses in the CA1 sub-field of the hippocampus. These cellular and sub-cellular changes apparent at 13 weeks after prion inoculation increase and eventually overlap with a late stage (18-21 weeks) cell loss that is restricted to the CA1 and dominated by pyramidal cell death. This selective cell loss occurs despite significant deposition of misfolded prion across all subfields of the hippocampus and supports the use of this in vivo model to investigate the emerging concept of targeted neurodegeneration. This selective susceptability may be intrinsic to the degenerating cells, driven by a differential toxicity from the localized cellular environment or both. Comparative analysis of the mRNA extracted from microdissected control (NBH) or late stage (18 weeks post ME7 inoculation) hippocampus on Affymetrix gene chips revealed differential regulation of several transcripts encompassing a number of chaperone pathways. In particular, members of the small heat shock protein (sHSPs) of the alpha-crystallin family showed a greater than two-fold increase in their expression at this stage of disease. This gene family is of particular relevance as they impart regulation of protein (mis-) folding per se and/or key cellular responses that contribute to susceptibility or protection from neurodegeneration.

We extended this initial observation by systematically profiling the gene and protein expression of all ten members of the sHSP family across the time-course of the ME7 model. This revealed that a cohort of 3 sHSPs (HSP27, HSP22 and alphaB-crystallin) that are found in control hippocampus show induced expression at 13 weeks which is further increased at later time points. These increases are selective as neither HSP20 that is otherwise expressed in control brains, nor the other 6 sHSPs that are not seen in controls are induced or change expression during ME7 induced degeneration. The time-course of the increased sHSP gene and protein expression coincides well with astrocytosis and microglial activation. This suggests that the co-ordinated HSP response reflects a potential role in modulation of the neuroinflammtory response underpinned by these cell types. However, by microdissecting the hippocampus we evidence a selective induction of the prion disease induced sHSP expression within the CA1 subfield suggesting a potential role in cellular processes that directly underlie the synaptic loss that precedes CA1 cell loss. We are currently defining the precise cellular location of these increased sHSP levels to ascertain if these modulators of protein misfolding are mediating their effects in neurons or the non-neuronal cells. Detailing the nature of this sHSP response will contribute to a better understanding of prion disease and the processes that mediate or modulate protein misfolding induced neurodegeneration.

P1.10 Hyperphosphorylated MAP-tau immunoreactivity around amyloid plaques in prion diseases

Authors
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Content
Amyloid plaques are the hallmark of some transmissible and non-transmissible brain amyloidoses, and in human transmissible spongiform encephalopathies they occur in sporadic CJD (sCJD), kuru (both of which may contain kuru plaques), Gerstmann-Sträussler-Scheinker disease (GSS) (multicentric plaques) and variant CJD (vCJD) (florid plaques). Amyloid plaques were studied by immunohistochemistry and laser confocal microscopy in cerebral cortex and cerebellum in 3 cases of variant CJD, 3 cases of GSS, 3 cases of sCJD, 1 case of kuru and 5 cases of Alzheimer's disease. Additionally, all types of amyloid plaques were studied by electron microscopy. In vCJD there was significant hyperphosphorylated MAP-tau immunoreactivity around the plaques and in the neuropil. In sCJD, GSS and kuru minimal immunoreactivty for MAP-tau was also observed at the periphery of the plaques. However, paired helical filaments were observed by electron microscopy only in Alzheimer's disease. Our results suggest that yperphosphorylated tau is present in all prion diseases with amyloid laques, although the distribution and amount of the protein is variable. This phenomenon may result from the close proximity of amyloid, but also various factors, such as duration of the disease, may influence tau hyperphosphorylation.

P1.11 Histochemical approach to central nervous system pathogenesis of ovine field cases of classical scrapie

Authors
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Content
Histochemical approach to central nervous system pathogenesis of ovine field cases of classical scrapie.

Background: Neuroimflammation elicited by PrP_{res} deposits in the central nervous system has been proven to involve cellular and oxidative stress phenomena in cattle BSE and in different TSE mouse models. Also, deregulation of water homeostasis has been suggested as a possible explanation for the spongiform changes observed in TSEs.

Objectives: To characterise the pathogenetic events occurring in the brains of classical scrapie field cases.

Methods: An immunohistochemical and histochemical study has been carried out on the brains of seven field cases of scrapie, in different clinical stages, and in two control sheep. A semiquantitative evaluation of PrP_{res} deposits and spongiform change throughout the encephalon has been performed. Furthermore, distinct pathogenetic mechanisms taking place in the brain have been investigated. The glial response associated to the presence of PrP_{res} has been characterized using antibodies against astrocytic glial fibrillary acidic protein, vimentin and lectin from Griffonia simplicifolia to identify microglia. The presence of oxidative stress phenomena (antibody against metallothioneins I and II), cellular stress proteins (heat shock protein 25) and the membrane associated water channel Aquaporin 1 has also been assessed throughout the encephalon.

Results and Discussion: The present study corroborates that in scrapie field cases PrP_{res} deposition elicits an important astroglial and microglial reaction evidenced by an increase in number and change of morphology of these glial cells; in the case of astrocytes, involving an up regulation of vimentin, a component of the glial cytoskeleton typical of immature astrocytes also involved in this cell type motility. This neuroinflammatory reaction entails cellular and oxidative stress phenomena as shown by the increase in immunostaining of both methallotionein and heat shock protein 25 even though sheep, as compared to cattle or mice, show considerably higher basal levels of those proteins. On the other hand the immunolabelling of the water channel aquaporin1 was not altered by the presence of PrP_{res} in the brain.

P1.12 Toxicity of PrP106-126 prion peptide on retinal cells of a prosimian primate

Authors
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[1]INSERM U710; [2]INSERM U583.

Content
An experimental primate model is needed to design and rapidly test prion treatment conditions for humans. Our recent studies of TSE transmission to a prosimian primate, the gray mouse lemur *(Microcebus murinus)*, showed that these animals are especially sensitive to the new variant of Creutzfeldt-Jakob disease (nvCJD). Here, we hypothesize that the retina of gray mouse lemur will be a useful model to study *in vitro* and *in vivo* the effects of prion protein in primate nervous system, and consequently, lead toward effective therapeutic measures. We choose the retina of this animal because it affords several important advantages: a) gray mouse lemur is a useful compromise between practicality [it is a small animal (~12 cm, 60-100 g)] and relatedness to humans [it is a prosimian primate], circumventing the drawbacks of monkey use; b) retina is a part of the central nervous system and is located adjacent to a closed chamber with a low content of proteolytic enzymes, thus facilitating the delivery and lifespan of prions and anti-prion compounds after intravitreal injection; c) pathological effects, and therefore effective therapeutics can be evaluated electrophysiologically and immunohistologically.
We have investigated whether monolayer cultures of *Microcebus* retinal cells (mixed neuronal-glial cultures) could provide an adequate in vitro preparation to address the toxicity of $PrP_{106-126}$, and whether this peptide-mediated toxicity could be observed in vivo in the retina of this non-human primate model after intraocular injection.
Double-labelling experiments with TUNEL and cell-specific antibodies showed that $PrP_{106-126}$, but not scrambled, triggered rod photoreceptor cell loss by apoptosis in vitro. Immunocytochemical staining revealed that the morphology of astrocytes and microglial cells were also adversely affected. In addition, we directly administrated $PrP_{106-126}$ into *Microcebus* eyes. Immunohistological studies performed on slides from peptide treated retinas showed an increase in the number of apoptotic nuclei on photoreceptor cells. This work provides a baseline for the evaluation of nvCJD cytotoxicity and for future therapeutic studies in a primate model.

P1.13 Alteration of glutamate exocytosis and calcium dynamics in a transgenic mouse model of a familial prion disease

Authors

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Content

Tg(PG14) mice, expressing a nine-octapetide PrP insertion associated with an inherited prion disease, accumulate in their brains a misfolded form of the mutant protein that is aggregated and protease-resistant. As this form accumulates, the mice develop a neurological disorder characterized clinically by ataxia, and neuropathologically by synaptic-type PrP deposition, associated with loss of synapses and granule neurons in the cerebellum. To explore the hypothesis that mutant PrP induces synaptic dysfunction as a primary event in the pathogenesis, we carried out functional studies in isolated nerve endings (synaptosomes) from mice at different stages of their neurological illness.
Analysis showed a marked alteration of glutamatergic neurotransmission. Depolarization-evoked release of glutamate was already significantly reduced in cerebellar synaptosomes from presymptomatic mice, and was completely impaired at the time mice had advanced clinical disease. Defective depolarization-induced release was also observed in primary cultures of cerebellar granule neurons from newborn Tg(PG14) mice. The calcium ionophore ionomycin efficiently induced glutamate release, indicating no impairment of the exocytotic machinery. To explore the possibility that the reduction of glutamate release was due to defective calcium influx upon depolarization, we measured intracellular calcium levels in cerebellar synaptosomes and in cultured neurons, and performed whole cell patch clamp recordings to measure calcium currents. Analysis indicated that depolarization-induced calcium transients were significantly reduced in synaptosomes and cells from Tg(PG14) cerebella, possibly due to defective function of voltage-gated calcium channels. Studies are in progress to characterize the channel subtype specifically affected and define the molecular mechanism at the basis of this defect.

P1.14 Autophagy in Purkinje cells overexpressing Doppel in Ngsk *PRNP*-deficient mice

Authors

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Content

In prion protein (PrP)-deficient mice of the Ngsk strain (*NP0/0*), ectopic expression of PrP-like protein Doppel (Dpl) in central neurons induces significant Purkinje cell (PC) death as early as six months after birth resulting in late-onset ataxia. We have recently shown that PC death in Ngsk mice can be partly prevented by either knocking-out the pro-apoptotic factor BAX or overexpressing the anti-apoptotic factor BCL-2. This suggests that apoptotic mechanisms could be involved in Dpl-induced death of these PCs.
To investigate mechanisms that could contribute to *NP0/0* PC loss, we performed Western blotting, immunohistofluorescence and immunoelectron microscopy to examine the expression by *NP0/0* PCs of the autophagic markers LC3B and LC3-binding adaptor protein p62 and of the scrapie responsive gene 1 (SCRG1) potentially associated with autophagy.
Upregulation of the autophagic specific proteins LC3B-II and p62 was detected as soon as 4 months in the cerebellum of the NP0/0 mutant mouse by Western blotting whereas immunofluorescence revealed increased expression of Scrg1 and LC3B-II and p62 in 4-8 month-old *NP0/0* PCs. At the ultrastructural level, Scrg1-immunogold labelled Golgi-derived autophagic profiles in the mutant degenerating PCs. Autophagic profiles were not found in wild-type PCs. Furthermore, the most robust autophagy was observed in axons and presynaptic terminals of PCs in the deep cerebellar nuclei of the *NP0/0* mice suggesting that it is initiated in the axons.
Altogether, our previous and present data indicate that autophagic and apoptotic processes are activated by Dpl-induced stress in *NP0/0* PCs. Whether autophagy is activated as a neuroprotective reaction or as a neurodegenerative sequence contributing to neuronal death remains to be elucidated.

P1.15 Molecular etiologies underlying GSS syndrome revealed by a Drosophila model

Authors

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Content

Background: The neurodegenerative mechanisms of Gerstmann-Sträussler-Scheinker (GSS) syndrome remain unclear, even though mutant prion protein (PrP) transgenic animals have been generated for studying the biology and etiology of this disease. Drosophila has been utilized as a powerful genetic model to study unknown molecular etiologies underlying various neurodegenerative diseases in humans.

Objectives: This study was focused on evaluating unknown molecular etiologies underling GSS syndrome using Drosophila model systems.

Methods: Transgenic flies expressing wild type PrP or GSS mutant PrP were used to investigate cellular and molecular etiologies of GSS disease.

Results: Several molecular and cellular key features of GSS syndrome were recapitulated in flies expressing GSS mutant mouse PrPs. We found that GSS mutant mouse PrP expressing flies showed predisposed behavioral defects that were not shown in wild type mouse PrP expressing flies. In addition, we found that GSS mutant PrP expressing flies showed loss of motor control and deposit of insoluble PrP.

Discussion: These PrP transgenic Drosophila models may be useful tools to investigate unknown molecular etiologies underlying neurodegenerative mechanisms of prion disease.

P1.16 The role of cytosolic phospholipase A2 in neurodegenerative disease

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Content

Background: It has previously been demonstrated that the activation of phospholipase A_2 (PLA_2) and the subsequent metabolism of arachidonic acid to prostaglandins (PGs) could play a role in neuronal death in neurodegenerative disease. Moreover, pharmacological manipulation of the phospholipase A_2 (PLA_2) cascade invokes a sustained reduction in PrP^{Sc} formation in prion disease and a diminution in overall PrP^{C} in neuroblastoma cell lines. A similar effect has been shown for amyloid-β peptides used to model Alzheimer's disease (AD) in cell lines. However, the exact mechanism by which $cPLA_2$ causes this change is unknown.

Methods: NB41A3 and SH-SY5Y neuroblastoma cells and primary cortical neurons were treated with synthetic prion and amyloid-β peptides. Effects on $cPLA_2$, arachidonic acid and PGE_2 were determined by western blotting, [$_3$H] thymidine incorporation assays and ELISA respectively. In addition, changes in phosphorylated $cPLA_2$ localisation after stimulation of cells with either peptide were further investigated by confocal microscopy.

Results: Neither prion or amyloid-β peptides altered total $cPLA_2$ levels or induced its phosphorylation in neuroblastoma cells. In addition, no increase in the levels of arachidonic acid and PGE_2 was observed. In contrast, prion peptides increased ERK1/2 and p38MAPK phosphorylation in primary cortical neurons and induced relocation of phosphorylated $cPLA_2$ to stress fibre like structures within 30 minutes.

Discussion: The exact mechanism of neuronal death in prion disease and AD is complex and could involve multiple cell signalling pathways and re-arrangement of neuronal cytoskeleton. Further work is needed to define if $cPLA_2$ is the prominent PLA_2 isoform in neurodegenerative disease and how it relates to intracellular structural changes occurring in neurodegeneration. These studies also indicate that care should be taken when selecting sources of neuronal cells for use in studies of prion disease neuropathogenesis.

P1.17 Microglial activation in sporadic Creutzfeldt-Jakob disease: regional variability and the effect of disease subtype

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Content

Background: PrP_{Sc} deposition in the central nervous system is accompanied by early microglial cell activation and spongiform changes, whereas nerve cell loss occurs later. Despite the absence of a classic inflammatory response, microglial cell activation is involved in both brain damage and spread of the disease. A previous study (Puoti et al, 2005) reported that in sporadic Creutzfeldt-Jakob disease (sCJD) the extent of microglia activation is dependent on the biochemical type of PrP_{Sc}.

Objectives: To investigate the relationship between PrP_{Sc} deposition and microglial cell activation in human TSEs, focusing on the comparison among the different sCJD subtypes.

Methods: We studied 28 sCJD cases, including 6 cases from each of the most common subtypes (MM1, VV2, MV2) and a total of 10 cases with the rarest phenotypes (MM2T, MM2C, VV1). We evaluated semiquantitatively by western blotting the relative amount of PrP_{Sc} and of the [beta]-chain of human HLA-DR, -DQ, -DP in 8 brain regions using the monoclonal antibodies 3F4 and CR3/43.

Results and Discussion: Microglial activation showed striking differences according to the brain region examined, which significantly matched the lesion profile and the regional distribution of PrP_{Sc}. Nevertheless, the overall extent of microglia activation between the three most significant sCJD groups (total microglia activation "load"= MM1=528 ± 179 AU, VV2=401±216 AU, MV2K=385±247 AU) was not significantly different. Our preliminary results, based on western blot data, could not confirm the finding that differences in the overall extent of microglia activation observed among patients with the most common sCJD subtypes are related to the biochemical type of PrP deposits. The collected data indicate, however, that the study of microglial cell activation in different brain region may represent an alternative valuable tool to evaluate the lesion profile in prion disease and discriminate among prion strains or disease subtypes.

P1.18 A peptide-based experimental strategy to identify PrP-interacting molecules

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Content

The identification of proteins that may play a role in transducing the signals that emanate from PrP is essential to dissect the molecular pathways involved in prion-induced neurodegeneration. A synthetic peptide homologous to region 106-126 of human PrP is toxic to cells expressing PrP, but not to PrP knockout neurons. We have hypothesized that PrP106-126 may exert toxicity by disrupting the interaction of PrP with a hypothetical cell membrane receptor, in such a way that a neurotoxic signal is produced (Fioriti et al., Mol. Cel. Neurosci. 28:165-176, 2005). Consistent with this idea, transgenic mice expressing a mouse PrP molecule deleted for the 105-125 region (homologous to human sequence 106-126) develop a severe neurodegenerative illness that is reversed by co-expression of wild-type PrP (Li et al., EMBO J. 26: 548-558, 2007). In this study, we describe experimental approaches that exploit the 106-126 domain of PrP as a molecular probe to identify cell membrane proteins whose aberrant interaction with PrP may play a role in triggering neurodegeneration.

P1.19 Hippocampal region correlation of phenotypic diversity in human prion diseases

Authors
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Content

Background: Molecular classification of human prion diseases (HPD) accounts for a large proportion of phenotypic diversity among different entities and subtypes (e.g. sCJD). However, the finding of an increasing number of cases harbouring multiple PrP_{res} types, together with the recent description of molecular strain-like features characteristic of every case, suggest that a detailed phenotypic analysis, both global and regional, may contribute to elucidate the single or multiple strain-like nature of human CNS involvement by prion diseases.

Objectives: To describe the lesional pattern of the hippocampal region in a series of HPD cases, and to analyse it in correlation with neuropathological and molecular phenotype.

Methods: A total 75 cases with postmortem diagnosis of HPD performed in a single center were included: 58 sporadic CJD (40 MM1/MV1, 9 VV2, 5 MV2, 3 MM2, 1 VV1), 8 familial CJD (E200K), 4 FFI, 3 variant CJD and 2 iatrogenic CJD (dura mater). All cases had a complete molecular study, with PrP_{res} typing in cerebellum and frontal cortex, and a neuropathological profile of 40 brain regions and all sectors of the hippocampal region. The series includes 6 sCJD cases (10,3%) with combined 1+2A PrP_{res} types (3MV1+2, 3MM1+2).

Results: Three patterns of hippocampal involvement were identified. Pattern 1, (87% of sCJD MM1/MV1 cases), showed severe involvement of entorhinal cortex and presubiculum, and variable involvement of subiculum, stratum lacunosum-moleculare and dentate gyrus (DG). Pattern 2, (100% VV2 and 60% of MV2 cases) presented additionally severe subicular, hippocampal and DG involvement. Pattern 3, represented by a minority of cases in most MM groups (all etiologies) showed minimal involvement of entorhinal and hippocampal structures. Pattern 2, irrespective of molecular or etiological classification, was associated to type 2A-like global phenotypic features. Pattern 1 was associated to type 1-like features.

Discussion: The hippocampal region develops a few well-defined lesional patterns in HPD reflecting selective involvement of extrinsic (perforant pathway) and/or intrinsic circuits, with a consistent correlation with global phenotypic features, irrespective of molecular classification. Our results support a single-strain-like model of prion propagation in each HPD case.

P1.20 Genome-wide Association Study Identifies Genetic Risk Factors for Variant Creutzfeldt-Jakob Disease

Authors

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Content

Background: The widespread exposure of the UK and other European populations to Bovine Spongiform Encephalopathy (BSE) and its dietary or blood-borne transmission as variant Creutzfeldt-Jakob disease (vCJD) causes ongoing public health concern. There is strong evidence for genetic control of incubation time in mammalian prion diseases.

Methods: In a tiered genome-wide association study of vCJD risk we analysed over a thousand samples from multiple categories of human prion disease, and over four thousand relevant control samples, including three thousand UK control individuals genotyped by the Wellcome Trust Case Control Consortium.

Results: The *PRNP* locus was strongly associated with risk across multiple markers and all categories of prion disease (best single SNP association in vCJD P = 1 x 10-17; best haplotypic association in vCJD P = 1 x 10-24). Although the major contribution to disease risk was conferred by *PRNP* polymorphic codon 129, additional nearby factors were identified in vCJD. Aside from *PRNP*, one SNP association was validated and achieved nominal genome-wide significance (P < 5 x 10-7); a similar association was found for the risk allele in a small sample of iatrogenic CJD (P = 0.02), but not sporadic CJD or kuru. We tested 50 additional top-ranked SNPs in all prion disease categories. One from this list showed strong evidence of replication in acquired prion disease (combined P < 5 x 10-7), conferring risk of vCJD, modifying kuru incubation time and the low risk allele conferred resistance to kuru in elderly women survivors of mortuary feasts. Bibliometric and/or gene expression data support the involvement of the nearest genes to these SNPs in mammalian prion disease.

Conclusions: The first genome-wide association study of human prion disease thus provides evidence for novel genetic risk in acquired prion disease and targets functional genetic approaches to understanding prion pathobiology.

P1.21 Modelling prion neurotoxicity in organotyopic hippocampal slices

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Content

Background: In prion disease, PrP^{c} is converted to its conformational isomer, PrP^{Sc}. The accumulation of PrP^{Sc} is associated with synaptic dysfunction, neuronal vacuolation, and ultimately neuronal death. Adult onset knock down of PrP^{c} in prion infected mice reversed early spongiform pathology and neuronal loss and prevented progression of the disease,resulting in long term survival. However, the mechanisms by which prion conversion triggers neurotoxicity, and the nature of early molecular and synaptic events leading to hippocampal dysfunction, are still unknown, as are mechanisms and timing for their rescue. Organotypic slice cultures reproduce most anatomical and functional properties of the corresponding circuits in vivo. Recently, organotypic cerebellar slices have been used as an assay for prion replication.
Such systems allow easy access in vitro to circuits and cells involved in pathology in whole animals.

Objectives: We plan to use organotypic hippocampal slice cultures (OHCs) as an ex vivo system to characterise the early molecular, cellular and synaptic events involved in prion neurotoxicity and its rescue, aiming to define the point up to which these can be rescued before neurodegeneration is inevitable.

Methods: OHCs were prepared from five day old mice and infected with RML prion at day 0 in vitro. Slices were collected at different time points and analyzed for PrP^{Sc} production and deposition. Early changes in protein expression, neurotransmitter realease, synaptic vesicle trafficking, morphology and neurophysiological function are in progress. The effect of PrP knockdown on these functions will be assessed using lentivirally mediated knock down of PrP by RNAi.

Results: We have established OHCs that remain viable in culture long term, with preservation of hippocampal architecture, cell population pattern and neuronal structure. Slices have been infected with RML prions for the above studies, and in parallel, we have infected slices with lentiviruses for shRNA expression and knockdown of PrP. Data will be presented.

Discussion: OHCs represent a powerful tool to study mechanisms and treatment strategies for neurodegenerative disease, as they preserve in vivo characteristics, but with the advantages of an in vitro system. We aim to use them to analyze early molecular, cellular and synaptic changes involved in prion neurotoxicity and its rescue.

P2.01 Amyloid-like toxicity triggered by bacterial inclusion bodies.

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Content

Background: The aggregation of proteins as amyloid fibrils or plaques is characteristically related to some degenerative diseases, such as Alzheimer's, Parkinson's and prion diseases, affecting the central nervous system or some peripheral tissues (1). The nature of the pathogenic species and the mechanism by which the aggregation process results in cell damage are, however, the subject of intense debate (2). Some clues to the molecular basis of amyloid disease and the biological significance of protein aggregation have been provided by recent observations that protein aggregates, not related to neurodegenerative diseases, can also impair cell viability and, interestingly, that early aggregates of amyloid structures are more toxic than mature amyloid fibrils. The toxicity of these early aggregates appears to result from an intrinsic ability to impair fundamental cellular processes by interacting with cellular membranes, causing oxidative stress and increases in free Ca2+ that eventually lead to apoptotic or necrotic cell death (3). Inclusion bodies (IBs) are protein aggregates commonly occurring in recombinant bacteria upon targeted gene overexpression.
Results shown previously demonstrate that in vivo aggregation into IBs is in fact a very selective reaction, mechanistically similar to amyloid formation (4). However, the cytotoxicity associated to inclusion bodies and its possible impairment to cellular processes have been poorly studied so far (5).

Objectives: In this study we analyzed the cellular toxicity associated with differently aged-bacterial inclusion bodies on mammalian cells in order to compare their effect with other proteinaceous aggregates presenting amyloid-like properties. Also, we wanted to further explore if cytotoxicity of protein aggregates could be governed by general rules of aggregation and structure.

Methods: Bacterial IBs formed in Escherichia coli cells by the misfolding-prone polypeptide VP1LAC, an N-terminal β-galactosidase fusion to the food-and-mouth disease virus capsid VP1 peptide (6), were produced during either 1 or 5 hours. We also used as a control thermal aggregates of purified β-galactosidase obtained in vitro by a temperature shift to 96ºC and further incubated at room temperature. Both IBs and thermal aggregates were added to NHI-3T3 cell cultures at different concentrations (from 1μM to 8.5μM) and the cytotoxicity was evaluated by MTT reduction assay. Confocal microscopy was used to study the integrity of the cell membrane, the cell viability, and to localize IB in the cell.

Results: The experiments revealed that IBs, mainly those formed during 5h, significantly impaired the viability of cultured cells. Moreover, we demonstrated by confocal microscopy that bacterial IBs bound and entered into the cells. Interestingly, the thermal aggregates of β-galactosidase did not significantly alter the cell viability. Furthermore, thermal aggregates also entered into the cell although presenting a temporal difference in crossing plasma membrane when compared to IBs.

Discussion: In the present study, we have proved that bacterial IBs formed by an aggregation-prone β-galactosidase are clearly toxic for mammalian cells. Interestingly, the same protein deposited in the more structured thermal aggregates, which have been shown to present an amlyloid-like fibrilar pattern, does not impair cell viability. Overall, these results support the hypothesis that different kinds of aggregates with different structural patterns are deleterious in a different extent. Besides, the data demonstrate that the simply interaction between the protein aggregate and the cell membrane does not imply necessarily deleterious effects to the cell.

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P2.02 PrP N-terminal domain triggers PrP^{Sc}-like aggregation of Dpl

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Content

Background: Doppel (Dpl) is a protein that shares significant biochemical and structural homology with PrP^{c}. In contrast to its homologue PrP^{c}, Dpl is unable to participate in prion disease progression or to achieve an abnormal PrP^{Sc} -like state. However, the ectopic expression of Dpl in the brain of some PrP devoid mice leads to severe ataxia and Purkinje cell loss.

Objectives: To test the hypothetical ability of Dpl to be converted into a PrP^{Sc}-like isoform, we have grafted the N-terminal domain of PrP^{c} to the C-terminal domain of Dpl, and obtained several chimeric constructs. In the present work, we have looked for the structural and biochemical features of these recombinant proteins (Chi) as compared to PrP.

Methods: Purified recombinant proteins were aggregated in presence of NaCl, and structural changes were analysed by size exclusion chromatography, circular dichroism and electron microscopy. Furthermore, kinetics of amyloid formation was followed by thioflavin T fluorescence. Resulting aggregated species were subsequently subjected to pepsin proteolysis and N-terminal sequencing of proteolysed fragments.

Results and Discussion: These chimeric proteins display PrP-like biochemical and structural features; when incubated in presence of NaCl, the alpha-helical monomers form soluble beta-sheet-rich oligomers which acquire partial resistance to pepsin proteolysis *in vitro*, as do PrP oligomers. Moreover, the presence of aggregates akin to protofibrils is observed in soluble oligomeric species by electron microscopy. Our findings underline the possibility of obtaining a "Dpl^{Sc}" isoform and point out the role of the N-terminal segment of PrP in general aggregation process. It would be of interest to verify if such chimeric proteins could behave as PrP and support infectivity in an in vivo experimental situation.

P2.03 The monoclonal antibody P1:1: a PrP^{Sc}-selective reagent recognising full length, native abnormal prion protein from idiopathic, acquired and genetic human prion diseases

Authors

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Content

Background: Detection of disease-associated prion protein (PrP^{Sc}) typically relies on antibodies that recognise both PrP^{Sc} and PrP^{c}, but in assay formats that allow a distinction to be made between the two forms. In practice, this has commonly meant exploiting the partial protease-resistance of PrP^{Sc} by the detection of the protease-resistant core fragent of the abnormal prion protein (PrP^{res}) on a Western blot following digestion of tissue homogenates with proteinase K. We have produced a monoclonal antibody (P1:1) to an aggregated peptide (PrP106-126) from the human prion protein sequence and determined that it selectively recognises both the aggregated form of the peptide and disease associated PrP^{Sc}.

Objective: To test the selectivity of P1:1 for the different isoforms of PrP^{Sc} found in different human prion diseases.

Methods: We prepared brain homogenates from well characterised cases of variant Creutzfeldt-Jakob disease (vCJD),all of the sporadic Creutzfeldt-Jakob disease (sCJD) subtypes, Gerstmann-Straussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI) and from patients with other neurological conditions. P1:1 was used to immunoprecipitate PrP from these homogenates, with and without prior proteinase K digestion, and immunoprecipitated PrP was then detected by conventional Western blotting.

Results: P1:1 efficiently immunoprecipitated PrP from all tested cases of sCJD, vCJD and GSS, but failed to immunoprecipitate PrP from patients with other (non-prion disease) conditions or FFI. Immunoprecipiated PrP was shown to be proteinase K resistant, however, significantly more PrP was immunoprecipitated from brain homogenates that had not been digested with proteinase K compared to those that had.

Discussion: P1:1 selectively recognises full-length PrP^{Sc} found in a range of human prion diseases (vCJD, all of the sCJD subtypes and GSS) each associated with different physico-chemical forms of PrP^{res} (type 1, type 2A, type 2B, 8kDa fragments). The cognate conformational epitope recognised by P1:1 is therefore a common feature of native PrP^{Sc} in different prion diseases and is of considerable interest. P1:1 provides an attractive avenue for the development of diagnostics and therapeutics.

P2.04 The PrP-related Shadoo protein exhibits amyloid formation at neutral pH and in vivo metabolic lability

Authors

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Content

Background: In prion infections cellular prion protein (PrP^{c}) is refolded into an infectivity-associated form (PrP^{Sc}). The related Shadoo protein (Sho) has sequence homology to PrP^{c} and is also expressed in the adult central nervous system as a GPI-anchored glycoprotein. Wt Sho consists of an Arg-rich basic region encompassing up to six tetra-repeats, a hydrophobic central domain of tandem Ala/Gly AAG amino acid repeats (with similarity to the containing AGAAAGA palindrome of PrP^{c}), and a less conserved C-terminal domain containing one N-linked carbohydrate.

Objectives: We investigated the properties of wt Sho since alanine-rich proteins are prone to form amyloids and since the domain architecture of wt Sho also resembles to amyloidogenic "stop codon" mutant forms of PrP. Moreover we examined other aspects of biochemical behaviour for GPI-anchored proteins including rafts association, internal proteolytic cleavage and shedding.

Methods: Recombinant MoSho was expressed in *E.coli* and purified by standard procedures. Some experiments were performed by using murine N2a neuroblastoma stable clones expressing FLAG-MoSho or FLAG-MoPrP. Results were collected by using MALDI-MS, UV circular dichroism (CD) spectroscopy, SEM and Western Blotting.

Results and Discussion: In mammalian cells the GPI-linked Sho is associated with cholesterol-rich rafts, with a fraction of the full-length molecules and an "N1" fragment released into the extracellular space. Sho exhibits a shorter half-life, and *in vitro* is more protease-sensitive than PrP^{c}. An endoproteolytic C-terminal species was also apparent *in vivo*. CD spectra of MoSho(25-122) and its truncated version MoSho(25-102) detected a high population of random-coiled structure, and the ability to assemble *in vitro* into fibrils was verified by immunoelectron microscopy and by a fluorometric thioflavine T assay. The latent ability for wt Sho to form amyloid fibrils *in vitro* may be offset *in vivo* by rapid turnover.

P2.05 Intracellular delivery of T182A mutant PrP to lysosomes by autophagy

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Content

Background: Autophagy is a cellular response to starvation, wherein intracellular debris is surrounded by a membrane and transported to lysosomes for degradation and conversion to raw nutrients. In recent years, several neurodegenerative-related proteinopathies have been shown to employ autophagy in the removal of aggregated proteins; however, its role in prion disease has not been established.

Objectives: To study the potential role of autophagy in the intracellular degradation of a PrP mutant linked to familial prion disease and known to exhibit aberrant trafficking.

Methods: N2a and HeLa cells were transiently transfected with wild type (WT), and the T182A mutated (Mut) PrP, treated within 4-5 h with inhibitors and inducers of autophagy, and analyzed by either live cell imaging or fixed immunofluorescence microscopy, or fractionated to study their subcellular localization.

Results: Confocal immunofluorescence microscopy showed Mut-PrP did not label the plasma membrane (PM), but it did accumulate within ER (PDI). It also incompletely colocalized with Golgi (Giantin), yet accumulated in lysosomes (LAMP-1). GFP-tagged Mut-PrP significantly colocalized with LysoTracker Red in live cells and lysosomal inhibitors (Leupeptin, Bafilomycin) increased steady state levels of Mut and WT PrP. Cellular fractionation studies revealed differences in the lysosomal populations occupied by Mut and WT PrP. This persistent co-localization of Mut-PrP with lysosomes in the absence of PM localization suggests bulk intracellular transport, such as autophagy. In support of this, live cell imaging revealed Mut-PrP::GFP strongly colocalized with monodensyl cadaverin (MDC), a marker for autophagic vesicles. In the presence of 3-MA, a known inhibitor of autophagy, the level of Mut-PrP increased in a dose-related manner, whereas activation of autophagy with rapamycin reduced it. In addition, treatment with 3MA shifted Mut, but not WT, PrP from thelysosomal fractions.

Discussion: These results suggest a role for autophagy in the intracellular transport of misfolded PrP to lysosomes. Based on recent evidence that ER stress can trigger autophagy, we hypothesize that ER accumulation of misfolded/aggregated Mut-PrP stimulates autophagy, leading to its packaging and transport to lysosomes for elimination.

P2.06 Spontaneous and seeded fibrillization of the two variants of human recombinant prion protein

Authors
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Content

Background: The conversion of the cellular isoform of the prion protein PrP^{c} to the pathogenic isoform PrP^{Sc} is the fundamental event in prion diseases. Creutzfeldt Jakob disease (CJD) as human prion disease can occur spontaneously, genetically caused or by infection. The polymorphism M129 is associated with susceptibility of infection by BSE contaminated food leading to new variant CJD, whereas V129 is not susceptible.

Objectives: The aim of this study is to characterize the aggregation of recombinant human prion protein (PrP) and compare the mechanistic details with those of hamster and bovine PrP. The aggregation of both variants of the 129 polymorphism will be compared.

Methods: We used our *in vitro* conversions system established with hamster recPrP (1), which is based on well balanced concentrations of SDS and NaCl. Structure a mechanism of fibrillization was analyzed by Thioflavin-T (ThT) fluorescence, Circular Dicroism Spectroscopy (CD) and solubility by differential ultracentrifugation.

Results and Discussion: Optimal conditions for fibrillization were established for both 129 variants. The structure of the soluble intermediates was compared by CD. Kinetics of fibrillization were analyzed for both variants separately and for the mixture. Beta-sheet rich aggregates of PrP and natural prions from different systems were applied as seeds and the specificities of seeding were analyzed.

(1) Stöhr J., Weinmann N., Wille H., Kaimann K, Nagel-Steger L., Birkmann, E., Panza G., Prusiner SB., Eigen M. & Riesner D. (2008) Mechanisms of prion protein assembly into amyloid, Proc Natl Acad Sci U S A.,105(7): 2409-2414

P2.07 Peculiar properties of recombinant bovine PrP

Authors
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Content

Background: Conversion of recombinant PrP (rPrP) to amyloid fibrils is one of the possible ways for study of prion proteins physicochemical and biological properties and development of new effective antigens for PrP_d –specific antibodies generation. Up to date, majority of investigations, including our research, have shown that partially denaturing conditions are required for the formation of amyloid fibrils *in vitro*.

Objectives: The goal of the present study was to realize the fibrillization of bovine truncated rPrP at different (high) concentrations of urea, as a denaturing agent. The other goal of the study was the development of new types of PrP_d-like antigens, for overcoming of the immune response tolerance at hybridoma production.

Methods: Several samples of bovine (102-240 a.a.) rPrP proteins, produced in similar conditions of *Escherihia coli* expression system, were purified, using metal-affinity chromatography. Solutions of bovine rPrP at concentration 400μgDml were incubated in 0.1 M sodium acetate buffer, pH 4.5, containing 2-8 M urea in conical plastic tubes, at 4ºC during 3 months. The kinetics of fibrils formation was monitored by electron and atomic force microscopy. Resistance to PK-digestion of developed fibrils was controlled by PAGE and WB. Solutions of bovine (102-240 a.a.) rPrP proteins, containing 2-8 M urea, were used as antigens for immunization of standard Balb/c mice and appropriate Mabs generation.

Results: Puzzle data was obtained for three rPrP protein samples after 3 month of incubation in 8 M urea. Two types of SAF-like fibrils were revealed in each sample, presented as straight and ribbon-like twisted structures. The length of fibrils reached up to 400nm. Straight fibrils had diameter ~7,5 nm. Ribbon-like twisted fibrils had periodicity~ 170nm and width ~25nm. Narrow twisting place of ribbon fibrils had diameter 7 nm. Partial resistance to standard PK-digestion of fibrils has been demonstrated. Panel of generated anti-rPrP Mabs showed specificity against human and animal pathogenic prion isoforms, including BSE, scrapie, sporadic CJD, nvCJD.

Discussion: In the present study we have demonstrated certain peculiar propensity of bovine (102-240 a.a.) rPrP to fibrillize at high denaturing conditions. Up to date the aggregation of prion proteins in irregular structures in 8M urea was demonstrated only for fungus HET-s(218-289) prion protein. At present, we have no evident explanation for the observed phenomenon of fibril formation in 8,0 urea in vitro and high aggregates heterogeneity in samples, prepared and incubated in similar conditions. This data may be a result of partial degradation of denaturing agent at acidic pH (urea to ammonium), or by peculiarities, inherent to BSE causative infectious agents.

P2.08 Ultrastructures and strain comparison of under-glycosylated prion fibrils

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Content

Background: Mammalian prions, composed mainly of misfolded polymers of prion protein (PrP), have poorly understood structures and diverse fibril morphologies. PrP protofilaments, which variably combine to form fibrils, could be strain-defining elements.

Objectives: The heavy surface glycosylation of wild-type PrP may visually obscure protofilament features. Therefore, we used severly under-glycosylated PrP protofilaments, isolated from scrapie-infected transgenic mice expressing only anchorless PrP, as a basis for strain comparison. These mice develop large, fibrillar, PrP_{res} amyloid plaques *in vivo* and their brains retain strain characteristics and infectivity.

Methods: Standard PrP_{res} preparations are not designed to preserve amyloid plaque integrity. To circumvent this issue, we used low speed centrifugation over an iodixanol cusion to reduce disruption and shearing of fibrils. Transmission electron and atomic force micrscopy were then used to examine the fibrils.

Results: We measured prion protein protofilaments which variably intertwined to form scrapie fibrils. Protofilaments tended to associate laterally to form fibrils, although occasional isolated protofilaments were observed. Strain comparisons revealed basic structural differences; ME7 and 22L fibrils contained thinner protofilaments than RML fibrils, 22L fibrils were less likely to contain right-handed twisting protofilaments, and 22L fibril periodicities were 106 nm per half turn, compared with 64 nm and 66 nm for RML and ME7 fibrils, respectively. Overlapping fibril morphologies were also found within the ultrastructural variants of these strains.

Discussion: Our measurements provided new insights into the existing models of protofilament formation.

(1) The predominant strain-dependent difference among anchorless fibrils was their range of periodicities which may reflect a fundamental difference in protofilament structure.
(2) Wild-type and anchorless RML protofilaments were thinner than their 22L counterparts, suggesting that different widths may also reflect strain-dependent core variations.
(3) Obtaining detailed measurements of the protein core of scrapie amyloid fibrils should aid the development of new models of prion structure and strain determination.

P2.09 Folding studies of ovine prion proteins

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Content

Prion diseases are a group of fatal neurodegenerative diseases. Although the mechanisms of neurotoxicity still remain elusive, the misfolding of a naturally occurring prion protein (PrP) from its normal cellular form (PrP_C) to a virulent scrapie form (PrP_{Sc}) seems to be responsible for prion pathogenesis. In sheep, polymorphisms of PrP at positions 136 (A/V), 141 (L/F), 154 (H/R), and 171 (Q/R) are found to be associated with different scrapie susceptibilities. It has been reported that the $V_{136}L_{141}R_{154}Q_{171}$ and $A_{136}L_{141}R_{154}Q_{171}$ genotypes confer the highest susceptibilities to classical scrapie, but atypical scrapie cases are linked to the $A_{136}F_{141}R_{154}Q_{171}$ and $A_{136}L_{141}H_{154}Q_{171}$ genotypes. In the current study, we aim to elucidate the mechanisms underlying this genetic modulation. We hypothesize that different folding kinetics explain the different susceptibilities associated with ovine PrP (OvPrP) polymorphisms. Kinetics measurements have been carried out on $A_{136}F_{141}R_{154}Q_{171}$ and $A_{136}L_{141}R_{154}Q_{171}$ variants, and our preliminary data indicate that both variants exhibit two exponential folding phases. (One is on the ~40 μs time scale; another is on the millisecond time scale.) In conclusion, the susceptibilities of $A_{136}F_{141}R_{154}Q_{171}$ and $A_{136}L_{11}R_{154}Q_{171}$ variants to atypical scrapie show no correlation with their folding kinetics. More experiments will be carried out soon on other three variants ($A_{136}L_{141}R_{154}R_{171}$, $A_{136}L_{141}H_{154}Q_{171}$, and $V_{136}L_{141}R_{154}Q_{171}$) to correlate the folding kinetics with classical scrapie.

P2.10 An estimate of PrP_C beta sheet dissociation Gibbs free energy: implications for prion conversion

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Content

Background: The problem of how PrP_{Sc} induces PrP_C to misfold at a molecular level remains unsolved. The short PrP_C beta sheet is relatively unstable compared to the rest of the PrP_C globular domain tertiary structure. Recent evidence suggests that dissociation of the PrP_{Sc} beta sheet may be an early event in prion conversion. If so, the Gibbs free energy of beta sheet dissociation in PrP_C is one determinant of the likelihood of conversion.

Objectives: The Gibbs free energy of PrP_C beta sheet dissociation was estimated by calculating separately the effects of increased configurational freedom, increased solvation, and hydrogen bond and salt bridge breakage arising from dissociation.

Methods: The configurational entropy change was calculated with a Monte Carlo simulation of accessible elf-avoiding walks using a coarse-grained PrP model. The solvation and hydrogen bonding energy changes were found by determining the PrP surface area newly exposed to solvent on unfolding, using human prion protein NMR structures. Salt bridge strength estimation assumed a simple Coulombic energy relationship.
The unfolding energy was calculated in a stepwise fashion that involved sequential unfolding of one amino acid at a time from the N-terminus of the folded domain to find free energy as a function of extent of unfolding.

Results: The free energy change involved in the first strand of the beta sheet separating from the second is 2.7 – 5 kcal/mol, so the prion has a 0.02 - 1% chance of being unfolded to this point at 37C. Continued unfolding beyond the beta sheet yields a local minimum in free energy at the C-terminal end of the first alpha helix, suggesting that unfolding of the prion protein to this point may represent a metastable state that exposes many of the previously buried residues.

Discussion: This analysis helps to elucidate the early events in prion conversion. Future work will seek to confirm it experimentally by microcalorimetry and nanopore force spectroscopy.

P2.11 The Effect of PrP Polymorphisms on In Vitro Protein Misfolding

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Content

The prion hypothesis states that the infectious agent of transmissible spongiform encephalopathies is predominantly composed of misfolded prion protein (PrP). The identification of a neurotoxic species, responsible for the characteristic neuropathology, remains elusive, however, the presence of low molecular weight oligomeric species is believed to be important in neuronal cell death. *In vitro* protein misfolding assays are useful tools to study oligomer formation of recombinant PrP, which may, in turn, reveal the biological role of misfolded isoforms of the prion protein *in vivo*. We have been analysing the oligomerisation of various forms of mammalian PrP. In the presence of heat and acidic pH, recombinant PrP refolds sequentially into two oligomeric species with defined molecular weights. Size exclusion chromatography is used to separate these species and, using UV-absorbance at 280 nm, the rate of oligomerisation can be calculated. We will report results demonstrating the effect that certain disease-linked polymorphisms have on rates of oligomerisation and will compare and contrast these effects with the effect that the polymorphisms have on other misfolding assays and on in vivo disease.

P2.12 In vitro fibrillation of recombinant human PrP90-231 elucidates the role of position 129 in prion conversion into amyloid

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Content
Background: The role of the polymorphism M129V in the human PrP gene is well documented. Most cases of sporadic CJD afflict homozygous individuals. Differences in codon 129 genotype give rise to differences in phenotype regarding plaque and clinical symptoms. Despite this, little is known about the molecular background to this phenomenon.

Aims: In this study we wanted to elucidate the molecular mechanism of PrP *in vitro*. By investigating amyloid fibrillation kinetics and seeding propensity of different 129-mutants we aimed to discover differences on the molecular level. The variants used in this initial study were 129A, 129V, 129L, 129M, 129W, 129P, 129E and 129K. Three mutants were chosen to vary hydrophobicity, and in addition the tryptophan mutant was chosen due to its bulkiness and the proline was chosen for its constraint of the polypeptide backbone. 129E and 129K may give information regarding the effect of charge in this position.

Methods: After recombinant expression and purification the HuPrP90-231 mutants were subjected to agitation under physiological conditions regarding pH, temperature and buffer salt. Seeds of 129M mature fibrils or fibrils produced from the mutant itself were added. The fibrillation kinetics was followed by Thioflavin T(ThT) fluorescence. Initial aggregation was followed by measuring the turbidity.

Results: All mutants formed amyloid like fibrils within a few hours. Hydrophobicity did not appear to be rate determining for HuPrP90-231 fibril conversion. A tryptophan in position 129 interfered with fibrillation regarding both rate and ThT binding capacity. Uncharged variants were seeded equally well with both wild 129M seeds and seeds produced from the mutant itself. Fibrils with low ThT binding proved to be equivalent or better seeds than thioflavinophilic variants. 129E and 129K were more readily seeded by wild type seed than by self seed. Furthermore, stoichiometric blends of 129E and 129K resulted in enhancement of their fibrillation kinetics.

P2.13 Assessing the role of Hsp70 in prion propagation

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Content
Sup35p is a *S. cerevisiae* protein involved in termination of translation. In a state referred to as [*PSI+*], a significant portion of the Sup35 protein in the cell coalesces into nonfunctional, self-propagating, amyloid-like polymers.
Thus, yeast strains that are [*PSI+*] show increased levels of nonsense suppression. We can monitor the presence of the prion in our yeast strains by a simple nutritional assay based upon read through of the ade2-1 allele. Once present, [*PSI+*] propagates by recruitment of the soluble form of Sup35p into the aggregate in a manner analogous to that of mammalian prions. A search for genetic factors affecting propagation and maintenance of [*PSI+*] has identified an essential role for molecular chaperones, namely Hsp70 and Hsp104. Unlike Hsp104, the Hsp70 chaperone family and its associated cochaperones are highly conserved from yeast to mammals. In addition, the ability to separate Hsp70 essential and prion- related functions suggests that the Hsp70 chaperone machinery may be a good target for possible therapeutics for prion and other amyloid diseases.
We have undertaken a number of genetic screens and a domain targeted approach in order to characterize the role of cytosolic Hsp70 in yeast prion propagation. A random genetic screen of the Hsp70-Ssa family has highlighted the importance of the ATPase domain and ATPase regulation in prion propagation, and has implicated the role of nucleotide exchange factors as a particularly important feature in efficient prion propagation. By specifically targeting the Hsp70 peptide-binding domain (PBD) for mutagenesis we show the importance of a localized region in allowing efficient prion propagation. When mapped onto the Hsp70 PBD crystal structure the location of this region suggests it has the ability to influence Hsp70 substrate specificity.
To assess whether Hsp70 also plays an integral role in the propagation of mammalian prions we have constructed a number of murine Hsp70 mutants equivalent to dominant yeast Hsp70 mutants known to impair yeast prion propagation. When transiently over-expressed in ScN2a cells, a sub-set of wild type Hsp70 isoforms and Hsp70 mutants appear to cause a reduction in PrP_{Sc} levels.
These results highlight the use of yeast prions as models to study the molecular events of prion propagation and also suggest a role for Hsp70 in mammalian prion propagation.

P2.14 Characterization of different scrapie types using the highly sensitive PET blot method

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Content
Background: Scrapie has been reported in sheep and goats for more than 150 years and is often regarded as the prototype of prion diseases. Scrapie isolates can be characterized by transmission to certain inbred mouse lines where they produce distinct incubation periods and lesion profiles. Different sources of scrapie have produced a large number of so-called "strains" in the same mouse lines (Bruce et al., 2002) indicating that the scrapie agent seems to be rather heterogeneous. Ten years ago, a new type of scrapie, called Nor98, was reported for the first time in Norway. This type did not match any formerly established criteria of sheep scrapie, e.g. it had a distinct clinical, biochemical and epidemiological properties (Benestad et al., 2008). Nor98, also called atypical scrapie, is now reported in many different countries (EFSA opinion 2005, Epstein et al., 2005). Several studies indicate that distinct properties of the infectious agent might be enciphered in the conformation of the respective disease-associated form of the prion protein (Telling et al., 1996; Peretz et al., 2001, Safar et al., 1998).

Objectives: Characterization of different scrapie types.

Methods: PET blot (paraffin-embedded-tissue blot) method which provides a sensitive and specific topographic detection of PrP_{Sc} (Schulz-Schaeffer et al., 2000).

Results: We will present the resulting deposition patterns and the forms of PrP_{Sc} deposits that clearly distinguish between classical scrapie and Nor98 using this highly sensitive method.

Discussion: Our results support the hypothesis that different forms of PrP_{Sc} deposits are caused by different conformations of PrP_{Sc}.

P2.15 Spontaneous and prion-seeded amyloid formation of recombinant bovine and hamster prion protein

Authors
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Content
Background: The conversion of the cellular isoform of the prion protein PrP_C to the pathogenic isoform PrP_{Sc} is the key event in prion diseases, which can occur spontaneously, genetically caused or by transmission of PrP_{Sc}. The mechanism of prion protein conversion is not understood in detail. In earlier studies we have presented an *in vitro* conversion system which simulates the structural transition in rec PrP by varying low concentrations of SDS.

Objectives: : Within our *in vitro* conversion system we want to analyze the mechanism of prion protein assembly into amyloid. We focus on the characterization of the pre-amyloid state and the different kinetics of de novo and seeded fibril formation as model system for the spontaneous and infectious disease mechanism in respect to different species.

Methods: The amyloidic character was verified by electron microscopy, congo red staining and Thioflavin T fluorescence. Fibril formation was obtained by Thioflavin T as specific amyloid marker. The secondary structure of soluble PrP was analyzed by circular dichroismus and the aggregation state was characterized by analytical ultracentrifugation.

Results: In this study we present a detailed characterization of the pre-amyloid state of recombinant hamster PrP and bovine rec PrP(25-241). We compared the pre-amyloid states of bovine and hamster PrP and could obtain differences in the secondary structure. In contrast to the a/random dominated pre-amyloid state of SHaPrP(90-231) the bovine pre-amyloid state has higher b-sheet content. Furthermore we generated amyloid fibrils de novo as well as by seeding with PTA-precipitated prions from scrapie-hamster as well as BSE-cattle.

Discussion: We applied the conversion assay established in our group for SHa rec PrP(90-231) and hamster scrapie-prions (Stohr et al PNAS) also to bovine PrP and BSE-prions. This seeded amyloid formation assay avoids any PK-treatment, and does not require cellular compounds. In seeded and spontaneous assay the pre-amyloid state is different between SHaPrP and bovPrP.

P2.16 Interaction of the 37kDa/67kDa laminin receptor with the amyloid β precursor protein (APP)

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Content
Alzheimer´s and prion diseases, respectively, belong to the group of fatal neurological disorders. Both have in common to form amyloid plaques on neuronal tissues. In the case of Alzheimer´s disease (AD) the cleavage of the amyloid β precursor protein (APP) is thought to be the critical step causing AD. In the non-amyloidogenic pathway APP is cleaved by the alfa- and gamma-secretase and the emerging soluble peptides are secreted into the extracellular matrix. However, in case the proteolytic processing of APP is performed by β- and gamma-secretase, the emerging β-peptide (Aβ) which is secreted into the extracellular matrix, accumulates in amyloid plaques on neurons, accompanied by neurotoxic effects and neurodegeneration.

In prion diseases, misfolding of cellular prion protein (PrP^{c}) to a proteinase K resistant and infectious isoform (PrP^{Sc}) is the causative pathogenic event of TSEs (transmissible spongiform encephalopathies).
It has been shown that overexpression of cellular PrP has a regulatory effect on the β-secretase cleavage of APP 1 inhibiting β-secretase and reducing Aβ formation in neuronal cells, respectively. Vice versa, downregulation of PrP^{c} by RNAi (RNA interference) leads to an increased secretion of Aβ into the cell culture medium (1). Additionally, PrP^{c} is suggested to inhibit β-secretase (BACE1) activity involving interaction with glycosaminoglycanes (GAGs) 1.
Previously, we demonstrated that LRP/LR acts as receptor for the prion protein 2,3,4. Since APP and LRP share the same subcellular localization at the cell surface, we investigated whether both proteins might interact with each other. Employing an in vitro pulldown assay, we observed a specific binding between LRP::FLAG and APP. Furthermore, APP and LRP::FLAG partially co-localize at the cell surface of co-transfected human embryonic kidney cells, also implicating a direct or indirect interaction.
To obtain further insights into a possible regulatory effect of the 37kDa/67kDa LRP/LR on the proteolytic processing of APP we currently investigate whether LRP/LR has an influence on β-secretase cleavage.

(1) Parkin et al., PNAS 2007, 104, 11062-11067
(2) Gauczynski et al., EMBO J. 2001, 20, 5863-5875
(3) Morel et al., Am. J. Pathol. 2005, 167, 1033-1042
(4) Gauczynski et al., J. Infect. Dis. 2006, 194, 702-709

P2.17 High titres of TSE infectivity associated with extremely low levels of PrP^{Sc} in vitro

Authors
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Content
Background: The prion hypothesis predicts that the aetiological agent of the Transmissible Spongiform Encephalopathy (TSE) diseases is an abnormally folded isoform of a host glycoprotein, PrP. This abnormal isoform (PrP) is partially proteinase K (PK) resistant, deposited in infected tissue, and co-purifies with infectivity.However the true relationship between PrP and TSE infectivity is still unclear. Current diagnostic methods are based on the detection of PrP in postmortem brain tissue, and its identification is taken as indicative of the presence of TSE infectivity. If PrP is not the TSE infectious agent, its reliability as a diagnostic marker may be questionable.

Objectives: This work aims to study the relationship between misfolded forms of PrP and the TSE infectious agent, and determine whether the presence of misfolded PrP is always a relaible marker for TSE infectivity.

Results: P101L transgenic mice inoculated with classical human P102L GSS or hamster 263K scrapie developed clinical signs of disease and spongiform degeneration in the brain, yet most mice contained low or undetectable levels of PK-res PrP in the brain. The disease was transmissible to 101LL transgenic and wild type mice, and levels of infectivity in these tissues were shown to equivalent to or higher than mouse scrapie laboratory strains, despite the apparent lack of PrP. No evidence of PK-sensitive forms of PrP was shown by either limited PK digestion assays, immunoprecipitation with PrP-specific monoclonal antibodies, or Conformation Dependant Immunoassay (CDI). The infectious agent in these tissues was shown to be PK-resistant, and localised within the synaptosomal fraction following crude cellular fractionation studies. Bioassay following sucrose gradient fractionation of this preparation is ongoing. Brain tissue from several infected mice was however identified as TSE infected using the IDEXX HerdChek assay (IDEXX Laboratories), indicating the presence of aggregated forms of PrP in these samples.

Discussion: Further analyses of these models are essential to identify the specific conformer of PrP associated with TSE infectivity and its abundance in diseased tissue, or alternatively the presence of other molecules associate with TSE infectivity in the absence of PrP.

P2.18 Aggregation and amyloid conversion kinetics of recombinant human prion protein under native conditions in vitro

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Content
Aims: In this work we investigated if it was possible to induce amyloid fibril formation of recHuPrP90-231 starting from native folded protein under physiological salt, pH and temperature. We also wanted to investigate if we could prevent the amyloid formation by introducing molecular chaperones.

Results: Intense shaking of the folded protein under these native conditions induced irreversible conversion into aggregates composed of amyloid-like fibrils within hours. Formed aggregates were positive using ThT fluorescence, showed Congo red birefringence and bound luminescent conjugated polymers. Fibrillar morphology was verified by TEM, where conversion of amorphous aggregates into amyloid-like structures was observed. Under these conditions the kinetic profile of aggregation showed that within minutes the protein assembled into large amorphous non-thioflavinophilic aggregates which after >2h converted into amyloid-like fibrils. The amyloid fibrillation kinetics followed a traditional sigmoidal trajectory with a lag phase, a growth phase and an equilibrium phase. The lag phase of the ThT assay showed unusual variability indicating a stochastic onset of fibril conversion. That the lag phase was due to nucleation was evident because seeding with preformed fibrils shortened the lag-phase.
When different chaperones were present during the fibrillation process, the formation of fibrils was delayed or even totally prevented. The protein formed aggregates that was non-thioflavinophilic, and remained in this state instead of converting into the fibrillar forms that binds ThT.

Conclusion: Amyloid formation of HuPrP90-231 can be achieved starting from the native protein under gentle conditions without addition of denaturant or altered pH. The process is catalyzed by addition of preformed amyloid seeds. It is plausible that amyloid seeding reflect the mechanism of transmissibility of prion diseases. The fibrillation process can be prevented to various degrees in the presence of different chaperones.

P2.19 Pressure-induced aggregation pathways of prion protein

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Content
As limited structural information is available on prion protein (PrP) misfolding and aggregation, a causative link between the specific (supra)molecular structure of PrP and transmissible spongiform encephalopathies remains to be elucidated.
Another question arises concerning the role that some proposed cofactors such as copper ions (Cu_{2+}) and heparan sulfate (HS) can play in the structural conversion of PrP.
In this study, high pressure was utilized as an approach to perturb protein structure to characterize different morphological and structural PrP aggregates, as well as to study the effect of Cu_{2+} and HS on PrP conformational changes and assembly.
Full-length recombinant PrP undergoes beta-sheet aggregation on high-pressure-induced detabilization. By varying the physicochemical conditions, the assembly process evolves through two distinct pathways. One pathway, at alkaline pH, leads to oligomers of beta-sheet secondary structure, which take the form of large spherical particles of 20 nm in diameter. They are preceded by amorphous aggregates made up of partially unfolded protein, mostly conserving its native a-helical secondary structure. The other pathway, at neutral pH, leads to amyloid beta-sheet fibrils. This amyloidogenic pathway is preceded by the formation of a reversible reaction intermediate.

Cu_{2+} and HS, by their ability to bind to PrP, influence its assembly process. Cu_{2+} and HS may be considered as cofactors inducing either amorphous protein aggregation or amyloid fibril formation. High-pressure favors fibril formation in the presence of Cu_{2+}, and amorphous aggregation in the presence of HS. In the presence of both cofactors, amorphous aggregates are formed, regardless of the pressure. Both cofactors appear to compete for the same binding site, since HS-supported fibril formation is quenched by copper. Inversely, copper-mediated fibril formation under high-pressure is inhibited by HS.

P2.20 Lectin binding profiles of pathological PrP in scrapie-infected hamsters

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Content
Background: There is a wide agreement on the involvement of PrP^C glycosylation in the pathogenesis of prion disease, however little is known about the precise role of the N-linked glycans in the neurodegenerative process.

Objective: In this study we investigated lectin binding properties toward glycoproteins of brains of scrapie infected hamsters, PrP, and PrP.

Methods: We used seven biotinylated lectins and 2 capturing monoclonal antibodies raised against different epitopes of PrP in an enzyme-linked immunosorbent assay (Lectin-ELISA).

Results: We found that glycosylation of total proteins was generally reduced in brains of 263K-infected compared to non-infected hamsters. When PrP was captured by the monoclonal antibody 6H4 it showed an increasing binding to Peanut Agglutinin, which recognizes galactose, and Wheat Germ Agglutinin, which recognizes N-acetylglucosamine residues, as compared with PrP^C. When PrP was captured by the monoclonal antibody 3F4 it showed a reducing binding to Soybean Agglutinin, which recognizes N-acetylgalactosamine residues, and Ulex Europaeus Agglutinin, which recognizes fucose, in comparison with PrP.

Discussion: The observed changes in lectin binding pattern of PrP, which occurred by changing the capturing antibody, may reflect different affinities of 6H4 and 3F4 antibodies for different glycoforms of PrP, or it might be due to different exposure of lectin binding sites on PrP upon binding to capture antibody. Treating with denaturing agents such as guanidine hydrochloride and with proteinase-K may help to discriminate between these two possibilities.

P2.21 In vivo generation of neurotoxic prion protein isoforms: role for Hsp70 in prion protein conversion

Authors
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Content
Background: In humans, prion disorders such as CJD present typically with sporadic origin, where wild type PrP spontaneously aggregates in the brain. Unfortunately, major gaps exist in the knowledge of how wild type PrP undergoes conformational changes and how ultimately kills neurons. Recent in vitro studies suggest that the chaperone Hsp70 may be a key factor mediating PrP misfolding, however, its activity has not been manipulated in animal models of prion disease. Aims: To what extent is Hsp70 implicated in PrP-mediated neurotoxicity? Can Hsp70 modify the subcellular localization, aggregation or physicochemical properties of PrP? Is there a direct interaction between Hsp70 and PrP during disease progression?

Methods: We created a fly model of sporadic prion disease by expressing wild type PrP from Hamster in the Drosophila brain. The role of human Hsp70 in PrP-expressing flies was determined using a variety of genetic, biochemical and histopathological approaches (targeted expression, insolubility, immunoprecipitation, conformational antibodies, immunohistochemistry, axonal labeling, TEM, and locomotion imaging).

Results and Discussion: Initially, flies express wild type PrP with properties of the benign PrP^C. Then, PrP progressively misfolds, acquires biochemical and structural properties of PrP^{Sc}, and induces locomotor dysfunction and severe spongiform degeneration of brain neurons. However, flies do not generate the signature protease-resistant core of PrP^{Sc}, suggesting that this isoform is not necessary for neurodegeneration. When human Hsp70 was expressed in our PrP flies, we found that Hsp70 co-localizes and physically interacts with PrP in an ATP-dependent manner. Strikingly, Hsp70 also prevents the accumulation of PrP^{Sc}-like conformers and suppresses spongiform neurodegeneration. These results provide new insight into the mechanisms of spontaneous accumulation of neurotoxic PrP and uncover the potential therapeutic role of Hsp70 in prion diseases.

P2.22 Characterization of the molecular diversity of mutant prion protein aggregates.

Authors
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Content
Approximately 15% of human prion diseases display autosomal dominant inheritance and are linked to point or insertional mutations in the gene encoding the prion protein (PrP). Different PrP mutations are associated with distinct clinical and neuropathological phenotypes: Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker (GSS) syndrome or fatal familial insomnia (FFI). The molecular basis of such heterogeneity is not clear. It is postulated that different pathogenic mutations induce PrP to fold into distinct abnormal structures that are selectively toxic to specific subpopulations of neurons in the brain. We have been interested in characterizing the structural diversity of mutant PrPs, with the objective of identifying features that determine their distinct pathogenic properties. We generated transgenic mice expressing the mouse PrP homologues of a nine-octapeptide insertion associated with a mixed CJD-GSS phenotype, and of the D178N/V129 and D178N/M129 mutations linked respectively to CJD and FFI. We have found that these mutant proteins acquire biochemical properties typical of pathogenic PrP, including insolubility in non denaturing detergents and protease resistance. Here we describe a procedure for purifying detergent-insoluble aggregates of PrP from the brains of transgenic mice by performing sequential centrifugations followed by immunoprecipitation with the 15B3 monoclonal antibody. By applying a panel of biochemical and biophysical tests, we are now starting to reveal the molecular properties of different mutant PrP aggregates. In contrast to previous work focused on the characterization of mutant PrPs expressed in bacteria, our approach offers the opportunity of defining the structural features of PrP aggregates underlying inherited prion diseases using highly purified molecules from a mammalian source.

P2.23 Unravelling Prion Misfolding Pathway using NMR spectroscopy

Authors
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Content
Prion diseases are fatal neurodegenerative disorders of humans and animals. These diseases can be genetic, infectious or sporadic disorders but all of these involve misfolding and aggregation of ubiquitous Prion protein (PrP_C). The sequences of PrP_C and PrP_{Sc} (from amyloidal plaques) are identical and a purely structural change is thought to cause aggregation and fibrillogenesis. Though NMR and X-ray structures for PrP_C are determined still high resolution structure for PrP is unavailable. The pathways of conformational change of prion protein are poorly understood.It is believed that misfolding and aggregation of PrP_C to form PrP_{Sc} have a path through partially or completely unfolded prion protein. In our lab, we have established the non-native conditions and were able to get fibril from recombinant human prion protein and synthetic peptides. The fibrils were characterized by electron microscopy and congo red absorption. NMR spectroscopy is used as primary tool to investigate the aggregation behavior of recombinant human prion protein. We have applied numerous NMR spectroscopic methods like deuterium exchange and relaxations, to understand pathway of misfolding. Our results indicate crucial role of YYR/X motifs as well as toxic palindrome sequence AGAAAAGA. We are still in process of analyzing these data. Our results agree with previous studies which indicate crucial but structurally unchanged behavior of helix1.

P2.24 Prion protein-derived cell-penetrating peptides with anti-prion action

Authors
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Content
We have previously shown that peptides derived from the prion protein N-terminus have potent anti-prion effects (Löfgren, K., et al., FASEB J., in press 2008). These peptides are composed of a hydrophobic sequence followed by a basic segment, and have cell-penetrating ability like regular CPPs, short peptides capable of penetrating cellular membranes. Treatments of RML-infected mouse neuronal hypothalamic cells (GT1-1) with the prion protein-derived CPPs mouse $mPrP_{1-28}$ or bovine $bPrP_{1-30}$, significantly reduces PrP_{Sc} levels whereas PrP_C levels in healthy GT1-1 cells are not affected by such treatments. Our present study concerns further characterization of the anti-prion properties of the prion protein-derived CPPs, using modified peptides and additional prion strains.

P2.25 Fragment Molecular Orbital Calculations Reveal Local Structural Instability in the Human Prion Protein Carrying an E200K Variant

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Content
The point mutation at E200K of the human prion protein (PrP) is known to cause familial Creutzfeldt-Jakob disease (fCJD). NMR analysis revealed that an E200K mutation affects the electrostatic surface potential. However, the tertiary structures of the wild-type PrP and the variant carrying the E200K mutation closely resemble each other, except for minor differences. The fragment molecular orbital (FMO) method is an ab initio quantum chemical approach used to analyze biomolecules. It enables the identification of minor structural differences with high accuracy, through quantitative evaluation of the molecular interactions between amino acid residues or secondary structural elements in a protein. Here, we used FMO calculations to investigate the structural stability of the wild-type PrP and the E200K-variant form. The wild-type and E200K-variant PrPs did not differ distinctly with regard to the total interfragment interaction energies determined for residue pairs. This suggested that the global stability of the E200K-variant PrP is similar to that of the wild-type PrP. However, we found that the E200K mutation induced complex alternations in the intermolecular interactions of the PrP. The calculations revealed that this mutation markedly weakened the interactions between 5 secondary structural element pairs, namely, a2 (172–194) and a3 (200–224), L1 (132–143) and a3, L2 (154–160) and a3, L1 and L2, and L2 and L4 (193–199). This indicated that the substitution of Gly200 with Lys significantly destabilizes the local structures of PrP. On the basis of our calculations, we propose the possibility that alterations in the stability of local structures in the PrP carrying the E200K mutant may facilitate the incidental denaturation and the subsequent conversion of the protein.

P2.26 Insight into the mechanism of thermal prion inactivation

Authors
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Content
The unusually high resistance of prions against thermal inactivation is still unresolved on a molecular level. Thus, insight into the mechanism of thermal prion inactivation is most worthwhile. Since it is known that the decontamination of prions is influenced profoundly by the molecular environment, we realised a biophysical analysis of heat inactivation of prions under varying solution conditions. In our contribution, a qualitative and quantitative comparison of degradation of PrP 27_30 backbone integrity and inactivation of prion infectivity, both determined with the most stable form of the TSE agent, i.e. prion rods, will be given under a systematic variation of decontamination conditions. A detailed analysis of heat-mediated prion destruction in presence or absence of water, fat, fatty acids, and glycerol yielded degradation and inactivation differences of up to five orders of magnitude. Combined with quantitative phase distribution experiments as well as an analysis of heat-induced structural changes of PrP 27-30 as determined by circular dichroism (CD) spectroscopy, electron microscopy (EM), Congo Red birefringence, thioflavin T (ThT) assay, solubility assay, and PK resistance, a model for the at least two-step mechanism of prion inactivation could be derived. Additionally, an explanation for the phenomenon of more resistant prion subpopulations will be given.

P2.27 Immunological mimicry of PrP_C-PrP_{Sc} interactions: PrP unfolds to misfold.

Authors
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Content
Background: Prion diseases are associated with the conversion of cellular prion protein (PrP_C) to an abnormal protease-resistant conformational isoform PrP_{Sc} by template directed conversion. The interaction between PrP_C and PrP_{Sc} is mediated by specific sites which have been mapped to six putative binding and conversion domains (BCD) through peptide and antibody competition studies. We have found that monoclonal antibodies
(mAbs) directed against Tyr-Tyr-Arg (YYR) specifically recognize PrPSc and other misfolded PrP species (Paramithiotis et al, 2003).

Objective: To mimic and monitor the molecular dynamics of PrP conversion in a tractable non-infectious system.

Methods: A series of defined mAbs whose epitopes overlap or are immediately contiguous to the BCD were covalently coupled to magnetic beads and incubated with mouse brain homogenates. Flow cytometry for fluorochrome-labeled IgG mAb 4C2 and competition immunoprecipitations were used to monitor the binding-induced exposure of bi-tyrosine epitopes.

Results: We report that select bead-bound BCD mAbs induce exposure of YYR epitopes on mouse brain PrP. By competition immunoprecipitation, we show that BCD mAb-induced YYR exposure is predominantly due to alfa-helix 1 unstructuring, in contrast to the pattern of bi-tyrosine motif exposure in native PrP_{Sc}. BCD mAb-induced misfolded PrP is neither aggregated (as determined by the lack of recruitment of additional PrP molecules to the bead surface) nor is it resistant to protease K digestion, suggesting that YYR exposure in alfa-helix 1 is an early event in PrP conversion.

Discussion: These results indicate that PrP_{Sc} -mediated conversion of PrP_C is accompanied by increased YYR accessibility, indicating a loss of structure in cognate bi-tyrosine domains. PrP conversion in disease may comprise three mechanistic steps: 1) PrP_C binding to PrP_{Sc} via PrP BCD; 2) partial PrP unfolding triggered by binding, enabling sampling of conformation space by part of the previously structured domain; and 3) conformational selection of nascent PrP_{Sc} molecules by intermolecular backbone interactions with the template. Molecular dissection of PrP_C -PrP_{Sc} interactions can be mimicked by substrate-bound BCD mAbs and monitored by misfolding-specific probes.

P2.28 M129T mutation reduces the susceptibility of prion proteins to conversion induced by oxidative stress

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Content
Background: The enlightenment of the mechanisms of PrP conversion will pave the way to understand the molecular principles of prion disease, enabling the design of therapeutic strategies. Considering that oxidative stress has been proposed to be a pivotal event in the pathology of TSEs, we investigate the structural changes during PrP aggregation using a novel *in vitro* conversion assay based on oxidative damage of PrP molecules. We already reported that the rate of aggregation differs between species depending on the amount of Met/His residues, which are highly susceptible to oxidation.

Objective: To evaluate the molecular mechanisms of oxidative PrP conversion, we are on the way to systematically mutate the Met/His residues of human and murine PrPs. The subsequent analysis of the aggregation characteristics of the mutants will reveal the impact of each specific residue to the oxidative-induced conversion of PrP.

Methods: Initially, M129T mutation was induced into cDNA of human and murine PrP. The expressed proteins were subjected to oxidative damage by metal-induced oxidation (Redecke et al. 2007) followed by quantification of the amounts of aggregated PrP. Structural changes during aggregation have been analyzed by CD spectroscopy and dynamic light scattering (DLS) measurements.

Results: CD spectra revealed that the overall structure of the prion proteins was not affected by the mutations. However, the aggregation rates of the M129T mutants of human and murine PrP were significantly reduced compared to that of the wild-type proteins. As reported for the wt-PrPs, stable oligomers were detected on the pathway of aggregation, characterized by similar hydrodynamic radii.

Discussion: We clearly show that Met129 has a significant impact on the oxidative aggregation of PrP. This result strengthens the proposed link between protein oxidation and prion diseases. Structural analysis of the C-terminal domains by X-ray crystallography is on the way to understand the changes in the molecular interactions.

P2.29 UV radiation induces specific conversion and aggregation of prion proteins

Authors
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Content
Background: Substantial evidence supports a causative link between oxidative stress and the pathogenesis of prion disease. Oxidative modification of PrP molecules in vitro provoked conformational changes, which are characteristic features associated with TSEs. Ultraviolet (UV) light has a great potential to cause oxidative damage in biological samples by direct photo-oxidation of proteins and via the formation and subsequent reactions of singlet oxygen and free oxygen radicals. The formation of aggregates consisting of unfolded protein is a common consequence of photo-oxidation.

Objective: We analyzed for the first time the effect of UVB radiation on prion proteins to demonstrate if UV light has the ability to convert the proteins into a PrP_{Sc}-like isoform.

Methods: UV radiation at a wavelength of 302 nm was generated using an argon-ion laser adjusted to an intensity of 8.6 mW within the samples. Recombinant human and mouse PrP (residues 90 to 230 and 121 to 230) were irradiated for up to 420 min. Aliquots have been removed for detailed analysis including quantification of aggregated PrP, secondary structure analysis (CD), determination of hydrodynamic radii (DLS), and detection of oxidative modifications by MALDI-TOF MS.

Results: Depending of the pH of the applied buffer and the PrP sequence, two different aggregation pathways have been observed: (i) a rapid disordered protein denaturation process, and (ii) a PrP specific conversion and aggregation process. Free radicals and oxidative damage of residues was detected within all samples, indicating radical mediated mechanisms.

Discussion: We revealed that UV light has indeed the ability to convert PrP. The susceptibility depends directly on the primary structure. These results strengthen the proposed link between protein oxidation and prion diseases. Moreover, we established a photo-trigger suitable for time-resolved investigation of the dynamic PrP conversion process, which is required for the design of therapies.

P2.30 Extraction and purification of cellular prion protein from bovine brain

Authors
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Content
The post-translational conversion of the normal cellular prion protein (PrP_C) into the pathology-associated form (PrP_{Sc}) changes its biological, physical and chemical properties. Both proteins have the same amino acidic sequence and sugar content, but they differ in the structure of the carbohydrate chains. In addition PrP_{Sc} has a higher content of β-sheets than alfa-helices in the secondary structure, it is partially resistant to proteinase K and insoluble in non-ionic detergents. These differential biochemical properties have been widely profited for the extraction, purification and detection of PrP_{Sc}. In contrast, the isolation of PrP_C is more challenging.
In this work, we set out to develop an extraction and purification procedure for PrP_C from bovine brain obtained from the BTAC (Animal Tissue Bank of Catalunya) in order to get a standard for future studies of its properties and structure, and its conversion to PrP_{Sc}. The extraction started with the homogeneization of the bovine brain in a Tris-HCl buffer. The homogenate was centrifuged in two-steps at different centrifugal forces to separate the membrane fraction. PrP_C was solubilized with a non-ionic surfactant. The extracts were purified by size exclusion chromatography (SEC) followed by immobilized metal-ion affinity chromatography (IMAC). When it was necessary, the extracts and the fractions resulting from the purification were concentrated with centrifugal filter devices. The purification protocol was monitored by western blot.
The results were promising, and the presence of PrP_C was confirmed in the final purified sample, but several optimizations are needed before it could be used for obtention of PrP_C at large scale.

P2.31 DNA aptamer and antibody based biosensor for detection prions

Authors
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Content
We developed EQCM biosensors based on DNA aptamers and antibodies immobilised on carbon nanotubes for rapid detection of cellular prions (PrP_C). The biosensor allowed to detect PrP_C with detection limit between 20-50 pM.

Background and Objectives: Recently it has been reported possible transmission of Creutzfeld-Jacob disease (CJD) by transfusion [1]. It is therefore urgent need to develop rapid prion test in the blood. So far mostly post mortem test of brain tissues were available based on protein kinase (PK) resistance of PrP_{Sc} and on immunochemical reagents. These procedures are rather time consuming. The alternative rout of detection can be based on biosensors composed of DNA aptamers or antibodies. The objective of this work is in development of biosensors based on DNA aptamers and monoclonal antibodies for rapid detection of PrP_C. Another objective is comparative study of the binding properties of both types of biosensors.

Methods: The biosensor was based on multiwalled carbon nanotubes (MWNTs) formed by electropolymerisation at the surface of AT-cut quartz crystal onto which the DNA aptamers or monoclonal antibodies against recPrP were immobilised. The biotinylated DNA aptamer against recPrP was created based on the work by Takemura et al. [2]. We also used monoclonal antibody BAR233 (SPIbio, Montigny, France) that selectively bind to 141-152 amino acid residues of recPrP. The interaction of recPrP (Human PrP (23-230), Alicon, Switzerland) with the sensor surface was performed by means of electrochemical quartz crystal microbalance (EQCM).

Results and Discussion: The binding of PrP to a sensor surface resulted in decrease of the oscillation frequency of the crystal that served as a sensor response. The plot of frequency changes vs PrP concentration allowed us to determine dissociation constants for both aptamer [KD=(1.72±0.37) nM] and antibody [KD=(1.64±0.53) nM] based biosensors. The dissociation constants for both aptamer and antibodies were comparable and rather low, which suggest similar and high affinity of PrP to both types of biosensors. The limit of detection (LOD) was rather low: 50 and 20 pM, for aptamer and antibody biosensors, respectively. The non-specific interactions of the sensor with considerably higher concentrations of human serum albumin (HSA) as well as with blood serum from health individuals suggests high selectivity of developed biosensors.

Acknowledgements: This work was financially supported by European Commission under 6 FP program NoE NeuroPrion (Contract No. FOOD-CT-2004-506579), by Agency for Promotion Research and Development under the contract No. APVV-0362-07, by Slovak Grant Agency (Project No. 1/4016/07) and by Russian Foundation for Basic Research (grant 06-03-32217). We thank to Dr. J. Grassi for helpful discussion.

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P2.32 Detection of prion proteins using antibody based biosensor and amplification by gold nanoparticles

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Content
We report development and comparative analysis of the properties of affinity biosensor based on immobilisation on dendrimers of various antibodies against recombinant human prion protein (recPrP). Amplification of detection using gold nanoparticles allowed us to increase sensitivity of detection.

Background and Objectives: Ante mortem detection of prion proteins is one of the most important problem in current diagnostics of prion diseases. The urgent needs in the development of the fast test of the prions is connected also with necessity to control donor blood in transfusion stations that may be potentially infected by pathological (scrapie) forms of prions (PrP_{Sc}) from individuals with Creuzfeld-Jacob disease (CJD). The possibility to develop rapid detection of prions appeared thanks to recently developed series of monoclonal antibodies. The objective of this work is development of biosensor for detection cellular prions (PrP_C) based on various monoclonal antibodies and to develop assay for amplified detection of prions using gold nanoparticles.

Methods: The following antibodies were used: PRI 308 and BAR223 that selective bind to 106-126 and 141-152 amino acid residues of recPrP, respectively (SPIbio, Montigny, France). We used also AG4 and AH6, antibodies that selective bind to N or C terminal of recPrP, respectively (TSE Resource Center, Newbury Breks, UK). The antibodies were immobilized on a surface of AT-cut quartz crystal covered by thin gold layer that was modified by poly(amidoamine) (PAMAM) dendrimers of fourth generation (G4) and by protein A. The interaction of recPrP (Human PrP (23-230), Alicon, Switzerland) with the sensor surface was performed by means of quartz crystal microbalance (QCM).

Results and Discussion: The binding of PrP to a sensor surface resulted in decrease of the oscillation frequency of the crystal that served as a sensor response. The detection limit depended on the antibody used and was 6.4 nM for PRI 308 and BAR223, 21 nM (AG4) and 43 nM (AH6). Considering the fact that PRI308 and AG4 bind to different part of recPrP we used gold nanoparticles for detection amplification. For this purpose the PRI308 was immobilized on a surface of quartz crystal and AG4 was conjugated with gold nanoparticles of the average diameter 10 nm (Sigma-Aldrich). In this assay first the recPrP was added in flow format to a sensor surface. After the changes of frequency were stabilized the Au-AG4 conjugate was added. This method allowed to substantially amplified PrP detection (detection limit 1.6 nM).

Acknowledgements: This work was financially supported by European Commission under 6 FP program NoE NeuroPrion (Contract No. FOOD-CT-2004-506579), by Agency for Promotion Research and Development under the contract No. APVV-0362-07 and by Slovak Grant Agency (Projects No. 2/7134/7 to M.S. and 1/4016/07 to T.H). We thank to Dr. J. Grassi for helpful discussion.

P2.33 Detection and characterization of proteinase K-Sensitive Disease-Related prion protein with thermolysin

Authors

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Content

Disease-related prion protein, PrP_{Sc}, is classically distinguished from its normal cellular precursor, PrP_C by its detergent insolubility and partial resistance to proteolysis. Although molecular diagnosis of prion disease has historically relied upon detection of protease resistant fragments of PrP_{Sc} using proteinase K (PK), it is now apparent that a substantial fraction of disease-related PrP is destroyed by this protease. Recently, thermolysin has been identified as a complementary tool to PK, permitting isolation of PrP_{Sc} in its full-length form. Here we show that thermolysin can degrade PrP_C while preserving both PK-sensitive and PK-resistant isoforms of disease-related PrP in both rodent and human prion strains. For mouse RML prions, the majority of PK-sensitive disease-related PrP isoforms do not appear to contribute significantly to infectivity. In variant Creutzfeldt-Jakob disease (vCJD), the human counterpart of bovine spongiform encephalopathy (BSE), up to 90 % of total PrP present in brain resists degradation with thermolysin whereas only ~15 % of this material resists digestion by PK. Detection of PK-sensitive isoforms of disease-related PrP using thermolysin should be useful for improving diagnostic sensitivity in human prion diseases.

P2.34 NMR studies of prion protein-detergent micelle interactions

Authors

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Content

Transmissible spongiform encephalopathies such as the Gerstmann-Sträussler-Scheinker syndrome and the Creutzfeldt-Jakob disease in humans are assumed to be caused by a self-propagating process involving the conversion of the cellular prion protein, PrP_C, into an aggregated isoform, PrP_{Sc}. Besides the secretory form, $SecPrP_C$, that is located on the cell surface, two alternative topological variants of the cellular prion protein, $CtmPrP_C$ and $NtmPrP_C$, have been reported [1]. These variants span the lipid bilayer with the highly conserved hydrophobic polypeptide segment 111-134. Thereby, the pathogenic variants of the human PrP containing the amino acid replacements P102L, P105L or A117V were found to have significantly increased populations of the $CtmPrP_C$ form. We report here on NMR studies of the interactions with DPC micelles of the polypeptide segment 90-231 of the recombinant mouse prion protein carrying the aforementioned amino acid replacements, mPrP[P102L](90-231), mPrP[P105L](90-231) and mPrP[A117V](90-231). We further investigated two designed variants, mPrP[A113V,A115V,A118V](90-231) and mPrP[K110I,H111I](90-231). Whereas wild-type mPrP(90-231) and mPrP(90-231)[M129V] show no appreciable DPC-dependent 15N chemical shift changes at pH = 7.0, the variant proteins all show significant chemical shift changes for the polypeptide segment 111-134 and some immediately adjoining residues upon addition of DPC. These data indicate that amino acid substitutions near to or in the hydrophobic region might cause interactions of variable strength with cellular membranes, which in term might induce conformational changes that facilitate the conversion of PrP_C into PrP_{Sc}.

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P3.01 Experimental transmission of transmissible spongiform encephalopathies (scrapie, chronic wasting disease, transmissible mink encephalopathy) to cattle and their differentiation from bovine spongiform encephalopathy

Authors
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Content

Background: Experimental cross-species transmission of TSE agents provides valuable information for identification of potential host ranges of known TSEs. This report provides a synopsis of TSE (scrapie, CWD, TME) transmission studies that have been conducted in cattle and compares these findings to those seen in animals with BSE.

Objective: The primary objectives of this study were to determine if the TSE agents (scrapie, CWD, TME) could be transmitted to cattle and to provide information about clinical course, lesions and suitability of currently used TSE diagnostic procedures for detection of these agents in cattle.

Methods: Generally 6-month-old bull calves were obtained and assigned to inoculated and control groups. Inoculated calves were housed in a Biosafety Level 2 isolation barn at the National Animal Disease Center (NADC), Ames, Iowa. Calves were inoculated intracerebrally with 1 ml of a 10% TSE brain inoculum.

Results: Results of various TSE cattle experiments with intracerebral inoculation of scrapie, CWD and TME will be documented.

Discussion: It was concluded that:
1. All three TSEs agents (scrapie, CWD and TME) are capable of propagating in cattle tissues when administered intracerebrally.
2. All three TSEs can be distinguished from each other and from BSE when inoculated intracerebrally by histopathology, immunohistochemistry and Western blot techniques.

P3.02 Enhanced PrP^C (CD230) fluorescence intensities on blood lymphocytes in BSE-infected non-human primates (simian vCJD)

Authors
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Content

Background: [1] Paul-Ehrlich-Institut (PEI), Federal Agency for Sera and Vaccines, Paul-Ehrlich-Str. 51 – 59, 63225 Langen, Germany, [2] Commissariat à l'"Énergie Atomique (CEA), Fontenay-aux-Roses, France, [3] Department of Virology and Immunology, German Primate Centre (DPZ), Göttingen, Germany, [4] Astrid Fagraeus Laboratorium, Swedish Institute for Infectious Disease Control (SIIDC), Solna/Stockholm, Sweden Background: The cellular prion protein (PrP^C) plays a central role in prion diseases and BSE-infection of cynomolgus monkey (*Macaca fascicularis*) represents a relevant animal model to study the pathogenesis of variant Creutzfeldt-Jakob disease (vCJD). The significance of the blood circulation in spreading prions is controversially discussed.

Objective: To study the course of PrP^C expression on blood cells in a simian vCJD model.

Methods: First, we evaluated different protocols and monoclonal antibodies for immunophenotypic analysis of simian PrP^C on blood lymphocytes (PBL) by flow cytometry. We then applied a routine whole blood lysis protocol (8% CV) using mAbs 12F10 and 3F4 to investigate cell associated PrP^C expression profiles (> 3 years) by flow cytometry in intracerebrally BSE-infected macaques (n = 6) and non-infected age-/sex-matched controls (n = 8).

Results: In non-infected macaques, PBL fluorescence intensities were one log. step lower compared to those in healthy human donors. Mean fluorescence intensity ratios (MFIRs) of simian blood lymphocytes were significantly lower in density-gradient purified cell preparations compared to cells analyzed in whole blood samples. B-cells showed the highest fluorescence intensities of all subsets. In BSE-infected macaques, PBL MFIRs were significantly higher compared to age- and sex-matched controls ($p_{F\text{-}test} < 0.0001$) years before the onset of clinical signs.

Discussion: PrP^C fluorescence intensity of simian PBLs is lower than in humans, but much higher compared to rodent TSE models indicating that macaques can be used to study the role of blood in the vCJD pathogenesis. However, high individual and seasonal variations, ageing effects, and technical factors had a tremendous effect on CD230 profiles, thereby limiting its use as a biomarker in field studies. Finally, the fact that blood-based changes were detectable and PrP^C fluorescence was lost during density-gradient purifications shed light on both the role of blood in the early spread of the agent and the impact of sample preparation to detect infectivity in different blood compartments.

P3.03 In vitro model of prions neuroinvasion mediated by dendritic cells

Authors
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Content
Background: Dendritic cells could, due to their location and their migratory capacity, contribute to the uptake of prions and the spread of the agent from the periphery to the central nervous system. Objectives: In order to analyze mechanisms by which these cells may transmit prions to the peripheral nervous system, we studied in vitro the effect of exposing such peripheral neurons to scrapie infected dendritic cells.

Methods: We have developed an in vitro model of coculture which mimics the neuroimmune interfaces.

Results: In this system, dendritic cells, which establish numerous contacts with neurites, are able to spread the PrP_{Sc} to neurons. DCs which are sufficient to induce neuroinvasion in vivo, are also capable to transfer the infectious agent *in vitro*.

Discussion: Our model suggests that only infected-dendritic cell and nude nerve fibres are required to induce prion neuroinvasion.

P3.04 Proteomics analysis of prion infected cells confirmed the involvement of chaperone molecules in prion propagation, and bringing to light the role of Hsp70 proteins in the cellular response to the agent

Authors
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Content
Background: Prion infected cells are cellular models that allow the molecular investigation of the pathologic conversion of the cellular prion proteins, PrP_C into its rogue scrapie isoform, PrP_{Sc}. One salient observation in these models is the apparent lack of toxicity for the cells despite an important accumulation of PrP_{Sc} and prion infectivity. In some experimental condition however, infected cells appeared more susceptible to insults such as oxidative stress.

Objective and Methods: In the present work, we relied initially on a bidimensional gel electrophoresis proteomics analysis of prion infected cells in a rigorous experimental protocol to identify proteins affected by prion propagation. We then confirmed the results using biochemical and cell biological approaches to progress towards the understanding of the molecular mechanisms of prion propagation.

Results and Discussion: Proteins already involved in prion biology like Grp58, Hsp60, Hsp70 or LRP were among differential proteins identified by mass spectrometry. We decided to focus our analysis on Hsp70 proteins and we confirmed that they were affected by prion replication not only in cell culture but also in animals models. To understand the role of these proteins in prion propagation, we are now investigating their molecular interactions with normal and pathologic prion, and their role in prion mediated toxicity. This work was supported by a grant from the EU Commission: NEUROPRION, FOOD-CT-2004-506579.

P3.05 Transmission of scrapie and BSE prions from sheep to mice expressing elk prion protein

Authors
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Content
Background: Chronic wasting disease (CWD) is a fatal and extremely transmissible prion disease of cervids. Unlike scrapie, a prion disease of sheep that has been known for over two centuries and occurs worldwide, CWD was recognized only 40 years ago and its distribution is limited largely to North America. It is uncertain whether CWD is a disease of spontaneous origin or transmitted to cervids at some point by cross-species infection.

Objective and Methods: To investigate the possibility of cross-species prion transmission to cervids, we inoculated scrapie, bovine spongiform encephalopathy (BSE), and sheep-passaged BSE prions into transgenic mice expressing elk prion protein, denoted Tg(ElkPrP) mice.

Results: We report that Tg(ElkPrP) mice were susceptible to 1 of 2 scrapie isolates and 1 mouse-passaged scrapie strain: SSBP/1 and RML caused disease with median incubation times of 270 d and 398 d, respectively. While BSE prions isolated from cattle did not transmit to Tg(ElkPrP) mice, sheep-passaged BSE prions caused disease in these mice in 300 d. Neuropathologic changes in these diseased Tg(ElkPrP) mice were distinct from those of mice inoculated with CWD prions.

Discussion: Our data suggest that cross-species transmission of sheep prions to cervids may be possible and might offer a possible explanation for the origin of CWD.

P3.06 Immunohistochemical and biochemical characteristics of BSE and CWD in experimentally infected European red deer (Cervus elaphus elaphus)

Authors
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Content
Background: The major cause of the UK bovine spongiform encephalopathy (BSE) epidemic was the feeding of contaminated meat and bone meal (MBM) in the protein rations fed to cattle. The use of MBM in animal feed was not restricted to cattle rations and it is known that MBM was included in the concentrates fed to farmed deer. BSE has been shown to be naturally or experimentally transmissible to a wide range of different ungulate species and, in North America, mule deer, white-tailed deer, and Rocky Mountain elk are known to be susceptible to an endemic transmissible spongiform encephalopathy (TSE) termed chronic wasting disease (CWD). However, to date, no natural TSE infections of European deer have been reported but should BSE infection have been transmitted into the UK red deer population, the CWD precedent would suggest that there is a great danger for both spread and maintenance of the disease in both free living and captive UK deer populations. This study compares the immunohistochemical (IHC) and Western immunoblotting (WB) characteristics of red deer infected with BSE to red deer infected with CWD.

Results: This study shows that BSE in deer more closely resembles infection in cattle than infection in sheep and goats. The pathology of BSE in cervids resembles that of CWD in most major respects but BSE can be clearly differentiated from CWD by existing immunohistochemical and biochemical methods that are currently in routine use.

Conclusions: European red deer are susceptible to both BSE and CWD infections however the IHC and WB phenotypes of BSE infected red deer are unlike those seen in CWD infected red deer and more closely resembles infection in cattle.

P3.07 Modification of human prion strains upon serial passage

Authors
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Content

Background: Creutzfeldt-Jakob disease (CJD) can present clinically in myriad ways, which are associated with prion strain types. These strains types are believed to arise from differences in the structure of PrP_{Sc} and can be differentiated biochemically and neuropathologically. The M/V polymorphism at human PrP residue 129 is a major determinant of strain type. The most common strains of sporadic (s) CJD are MM1 and VV2, while variant (v) CJD has only been observed as the MM2 subtype.

Objective: To evaluate the molecular mechanisms of oxidative PrP conversion, we are on the way to systematically mutate the Met/His residues of human and murine PrPs. The subsequent analysis of the aggregation characteristics of the mutants will reveal the impact of each specific residue to the oxidative-induced conversion of PrP.

Methods: We produced new transgenic (Tg) mouse lines, expressing chimeric human/mouse (Hu/Mo) PrP, and inoculated them with a range of human prion strains. Mice were monitored for onset of neurological disease. Brain tissue was analyzed for strain type by Western blot and immunohistochemistry (IHC), and select samples were then serially passaged.

Results: One new Tg line, expressing Hu/Mo PrP with M129, was susceptible to human sCJD(MM1) prions in 80 d and to vCJD(MM2) prions in ~200 d. PrP_{Sc} in the brains of vCJD-inoculated mice could be divided into two subgroups based on Western blot and IHC analyses, which correlated well with "slow" and "fast" incubation periods. The fast strain had similar characteristics to PrP_{Sc} in the brains of sCJD-inoculated mice. Serial passage of the "slow" vCJD substrain occasionally resulted in the appearance of a "fast" strain. Tg lines expressing Hu/Mo PrP with V129 were not susceptible to sCJD(VV2) prions; when transmission did occur, the strain characteristics appeared to be altered. Surprisingly, these Tg lines were more susceptible to sCJD(MM1) prions.

Discussion: Our data suggest that serial transmission of vCJD could present like sCJD. Moreover, slowly replicating prions strains may be modified on serial passage, presumably by changes to the PrP_{Sc} structure, allowing the more rapidly replicating strains to predominate. Furthermore, the codon 129 polymorphism plays a role in strain susceptibility, but other residues are important for the propagation of the sCJD(VV2) strain.

P3.08 Resistance of prion strains to inactivation

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Content

Background: Prion inactivation studies have generally been performed on rodent-adapted strains, which provide experimentally tractable models. Rodent-adapted prion strains are often assumed to maintain the characteristics of the original prions from which they were derived, but this is based on neuropathological evidence rather than biochemical data.

Objective: To assess resistance to inactivation, we studied four prions strains: two naturally occurring (BSE and sCJD) and two rodent-adapted (301V and Sc237). The 301V strain was produced by passage of BSE in mice; the Sc237 strain was originally derived from sheep scrapie, subsequently passaged in hamsters.

Methods: Prion strains were exposed to sodium dodecyl sulfate (SDS) at various pH and temperatures. Reduction in PrP_{Sc} was measured by Western blot, and reduction in infectivity was measured by bioassay in transgenic (Tg) mice susceptible to each strain. To quantify inactivation by bioassay, we derived statistical models based on the inoculation of serially diluted samples. By stratifying comparisons, we were able to determine values for the differences between strains with confidence intervals.

Results: Western immunoblotting showed that that it was easier to reduce the PrP_{Sc} level of BSE prions than 301V prions, for low levels of inactivation. At higher levels of inactivation, bioassays demonstrated that BSE prions are up to 1,000-fold more resistant to inactivation than 301V prions. Our findings argue that despite being derived from BSE prions, mouse 301V prions are not always a reliable model for cattle BSE prions. Extending these comparisons to human sCJD and hamster Sc237 prions, we found that BSE prions were 10x and 1,000,000x more resistant to inactivation, respectively.

Discussion: Our studies contend that prion inactivation procedures must be validated by bioassay against the prion strain for which they are intended to be used.

P3.09 Species and tissue-specific variations in scrapie pathology of lymphoid tissues

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Content

Background: TSEs or prion diseases often result in FDC and TBM associated accumulation of PrP_d in the Lymphoid tissues. In previous studies of sheep Mesenteric lymph node (MLN) and mouse spleen, we have shown changes that are common to both sheep MLN and mouse spleen while some changes are either species or tissue specific.

Objective: To determine whether these changes are species-specific or due to different processing pathways present within different tissues, we have studied the morphological response to scrapie infection, and the subsequent sub-cellular location of PrP_d and immunoglobulin in scrapie-infected sheep and murine MLNs.

Methods: Spleens and MLNs were removed from Me7 infected mice killed at the terminal stage of disease and processed into araldite resin. Tissues were sectioned and immunolabelled using 1A8 anti PrP serum and an anti IgG antibody, and studied by light and electrion microscopy. Results were compared with previous data obtained from sheep scrapie.

Results: Within scrapie-infected MLN follicles, PrP_d was present at the plasmalemma of the majority of FDCs, ranging from relatively immature with no loss of intermediate dense line, to larger FDC complexes, again with an intermediate dense line, to large hypertrophic complexes similar to those identified within the spleens of scrapie-infected mice. No vesicular structures were associated with FDC dendrites. In contrast, scrapie-infected sheep MLNs showed frequent B cell emperipolesis by PrP_d-expressing FDC dendrites though B cells did not accumulate PrP_d. IgG co-localised with the intermediate dense line between dendrites and was identified within the extracellular space surrounding hypertrophic FDC dendrites, confirming abnormally-accumulated electron dense deposit in both sheep and mice contains IgG.

Discussion: The differences highlighted suggest that both species (or strain) and tissue influence the cellular response to infection.

P3.10 Susceptibility to prion infection is controlled by the highly conserved glycine rich region of PrP

Authors
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Content
Background: While the prion protein (PrP) is a key molecule required for prion infection, the mechanism of propagating infection has yet to be determined at the molecular level. PrP contains a glycine rich region (GRR) spanning residues 118-130 of mousePrP that is perfectly conserved across a range of mammalian species and lies within a hydrophobic domain that has been linked to prion toxicity.

Objective: To determine the influence of the GRR of PrP on prion infection by introducing mutations in this region and assaying in cell and animal models.

Methods: Wildtype and GRR mutants of moPrP were transfected into rabbit kidney epithelial cells (RK13) which have previously been shown to propagate prion infection. PrP localisation was determined using confocal microscopy and the trafficking of wildtype and mutant PrP was assayed, including monitoring the secretion of PrP in association with exosomes. Cells were infected with a mouse-adapted human prion strain (M1000), and the propagation of de novo infectivity was determined by cell immunoblotting and infection of cells with lysates from the previously infected lines. Infectability studies in cell cultures were also verified by bioassay in Tga20 indicator mice.

Results: The susceptibility of RK13 cells expressing mutations in the GRR of moPrP to infection with the M1000 prion strain correlated with the level of disruption to the GRR. Conservative substitution of glycine to alanine residues in this region diminished infection whilst the infection was completely abolished when more significant mutations (leucine or proline) were introduced. Verification of this diminished propagation by cellular assay and animal bioassay demonstrated that prion infectivity was no longer present. Despite this, no differences in localisation or trafficking of MoPrP and mutant MoPrP could be observed.

Discussion: The GRR is highly conserved across all species, and demonstrates a powerful effect on prion infection. Similar motifs in other proteins have been shown to have roles in protein-protein interaction. The role of protein-protein interaction with respect to the PrP GRR will be discussed.

P3.11 The potential role of phagocytic cells in resistance to scrapie in sheep

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Content
Resistance and susceptibility to scrapie in sheep are associated with specific alleles of the gene encoding PrP^{c}. However, the mechanisms by which amino acid substitutions in the PrP protein result in relative resistance to infection are unknown. They may directly alter the stability and conformation of the protein, or produce indirect functional changes through interactions with other molecules. Phagocytic cells, such as macrophages and myeloid dendritic cells, are likely to be among the first cells encountered by the scrapie agent at the site of infection. There is evidence that they may be involved in the uptake and transport of the scrapie agent to local lymphoid tissues, but also that they can contribute to its destruction. In resistant sheep, it is possible that more efficient degradation of the agent by phagocytes could prevent infection from becoming established in lymphoid tissues and progressing to clinical disease. The aim of this project is to test this hypothesis by comparing the efficiency with which phagocytes from scrapie susceptible and resistant sheep ingest and degrade PrP^{Sc} in vitro. Macrophages obtained by bronchoalveolar lavage or in vitro differentiation of blood monocytes are being tested initially. Macrophages from susceptible and resistant sheep are cultured in the presence of scrapie-infected brain homogenate, and the breakdown of PrP^{Sc} is measured by cell immunoblots and/or Western blotting. The study will be further extended to examine PrP^{Sc} degradation by e. g. myeloid dendritic cells, neutrophils.

P3.12 Uptake of amyloid protein in the murine and bovine intestines before and after weaning: a model for the enteric invasion of infectious prion protein

Authors
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Content
Background and Objective: Transmissible spongiform encephalopathies (TSEs) are a group of fatal neurodegenerative diseases. The transmission is conducted by oral exposure to abnormal prion protein (PrP^{Sc}); however, the process of uptake and dynamics of PrP^{Sc} in the intestines, especially the intestinal epithelial invasion of PrP^{Sc}, are poorly understood. Epidemiological studies reported that cows might be exposed to the agent at the first 6 months of life in most cases of BSE. In the present study, such age-dependent uptake mechanism was analyzed using amyloid-β (Aβ) protein and PrP^{Sc}.

Materials and Methods: A fusion protein (Aβ-EGFP) was produced by massive incubation of the transformed E. coli with the fusion gene. Aβ-EGFP or PrP^{Sc} from scrapie-infected mouse brains (Tsukuba1) was orally administered to 15, 20 and 25-day-old mice. Aβ-EGFP was also administered to 2-week- and 6-month-old Holstein cows. After administrations, intestines were analyzed histopathologically. The incorporating cells were identified using lectins (UEA-1 and WGA) or anti-villin antibody. PrP^{Sc} was identified using anti-prion protein antibody (P8 and T2).

Results and Discussion: Aβ-EGFP and PrP^{Sc} was incorporated into the cytoplasm of columnar epithelial cells (UEA-1-/WGA+) rather than M cells (UEA-1+/WGA-) in 15-day-old suckling mice. This uptake was, however, observed little in 20-day-old and not in 25-day-old weaned mice. The results suggest that Aβ-EGFP abundant in β-sheet structure can be used for the analysis of uptake of PrP^{Sc} in the intestine. Aβ-EGFP was also incorporated and accumulated in crypt patches of 2-week-old cow. The uptake through villin-positive columnar epithelial cells in a 6-month-old cow was much less that in 2-week-old cows. These results suggest that a certain specific uptake mechanism of PrP^{Sc} through the villous epithelium exists during suckling periods. The present study also suggests that the weaning period is important for the risk of transmission in BSE cases.

P3.13 Assessment of prion infection after fecundation from BSE-infected males

Authors
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Content
Vertical transmission of prion infection from the mother to the offspring is a source of natural contamination in sheep flocks infected with scrapie. Previous works have shown the existence of maternal transmission in animals infected with BSE (in cattle and in Tg-mice). High levels of PrP^{c} have been found in semen but the absence of PrP^{Sc} or infectivity in semen from scrapie-infected rams has been previously described while it is unknown if BSE can be transmitted from the BSE-infected male to the offspring. In this work, the possible paternal transmission from BSE infected males to their offspring is analysed. For this, the BoPrP-Tg110 mice line (overexpressing bovine PrP) has been used. Males were intraperitonelly inoculated with BSE and males were separately mated with healthy homologous females. The BSE infected mice were mated at several periods after inoculation (also with evident clinical signs), and were euthanized when the progression of the illness in males was marked. The offspring was analysed for the development of clinical signs due to neurological disease and euthanized when indicated by the age of the animals or by ethical reasons. When euthanized, tissues were harvested for further biochemical and histopathological analysis. Their brains were homogenised and tested biochemical and histopathologically and all the mice were scored negative by Western-blot and histopathology. The lack of transmission in this experiment using the BoPrP-Tg110 mice line propose that no infectivity accumulation is present in semen material or in those tissues interplaying in the fecundation process of the mouse model.
The results obtained in the model used suggest that the donor male should not be considered a high risk source of BSE-infection in the reproduction practises.

P3.14 No major change in the agent strain of variant CJD after secondary transmission via blood transfusion

Authors
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Content

Background: The identification of transmission of variant CJD by blood transfusion has prompted investigation to establish whether there has been any alteration in the vCJD agent following this route of secondary transmission. Any increase in virulence or host adaptation would require a reassessment of the risk analyses relating to the possibility of a significant secondary outbreak of vCJD. Since there are likely to be carriers of the vCJD agent in the population, there is a potential for further infection by routes such as blood transfusion or contaminated surgical instruments.

Objective: To observe differences between the transmission properties of vCJD and blood transfusion associated vCJD infection.

Methods: We inoculated both wild-type and transgenic mice with material from the first case of transfusion associated vCJD infection.

Results: The strain transmission properties of blood transfusion associated vCJD infection show remarkable similarities to the strain of vCJD associated with transmission from bovine spongiform encephalopathy (BSE).

Discussion: Although it has been hypothesized that adaptation of the BSE agent through secondary passage in humans may result in a greater risk of onward transmission due to an increased virulence of the agent for humans, our data presented here in two murine models suggest no significant alterations to transmission efficiency of the agent following human-to-human transmission of vCJD.

P3.15 Sporadic CJD Strain Classification by Transmission to Human Transgenic Mice

Authors
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Content

Background: The publication by Parchi et al (Classification of sporadic CJD based on molecular and phenotypic analysis of 300 subjects. Ann Neurol, 1999: 46(2) 224-33) is widely used to group sporadic CJD cases into six different subtypes.

Objective: Our goal was to determine whether these sCJD groups would show distinctive transmission properties to laboratory mice and therefore be described as sCJD strains, or whether the PrP_{Sc} type or codon 129 genotype showed dominance in the transmission results.

Methods: CNS material was prepared from typical cases of "Parchi" types and inoculated into transgenic mice expressing human prion protein with genotype variation at codon 129.

Results: Examination of the transmission properties (incubation period, lesion profile, and immunocytochemical and/or biochemical detection of PrP_{Sc}) has shown:
(1) Six types of sCJD (MM1, MM2, MV1, MV2, VV1, VV2) give different phenotypes on transmission to transgenic mice. (2) Data suggest that PrP_{Sc} type has a more dominant effect on the lesion profile than codon 129 genotype. (3) Material containing a dominance of type 2 valine/PrP_{Sc} is the most efficient at disease transmission.

P3.16 Intracerebrally-induced BSE in goats

Authors
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Content

Background: The BSE case reported in 2005 from a French goat highlighted a new potential human contamination risk from goat tissues. Many polymorphisms are described on the goat *PRNP* gene but specific alleles remain to be clearly correlated with TSE resistance. The genetic control of goat susceptibility to BSE is still unresolved.

Objective: This study is a first step to assess the BSE susceptibility of goats with different scrapie-susceptible *PRNP* genotypes.

Methods: Four Saanen goats from a scrapie-free herd (assessed by tonsil biopsies) were intracerebrally (IC) inoculated with the BSE strain. When the first animals reached the clinical stage, they were slaughtered and their tissues tested for disease-associated PrP (PrP_d) using IHC, WB and ELISA.

Results: Two goats (I142R154R211Q222S240/IRQQS and IRRQS/IRRQP) had clinical signs 20 and 21.5 months after inoculation, respectively. The 2 others (IRRQS/IRQQS and IRRQP/MRRQP) were slaughtered at 25 months of incubation. PrP_d was detected from all of them. The IRRQP/MRRQP goat had only slight PrP_d deposits in the thalamus. From the 3 others, PrP_d was detected in the central nervous system, peripheral nerves and in some muscle spindles. The labelling pattern of the spinal cord ependymal cells suggested a possible PrP_d release in the cerebro-spinal fluid. In the IRRQS/IRRQP goat, PrP_d was revealed in lymphoid organs such as lymph nodes, tonsils and Peyer's patches, but not the spleen.

Discussion: As previously reported, BSE can be easily induced in scrapie-susceptible goats following IC inoculation. Results from the IRRQP/MRRQP goat suggest a much longer incubation period. PrP_d deposits in peripheral organs showed that BSE is able to disseminate in goats as well as in sheep, through the nerve fibres and the lymph. Further studies are required for a better understanding of the genetic control of BSE susceptiblity in goats opening the door to a genetic selection allowing to protect human from this potential contamination risk.

P3.17 M129T mutation reduces the susceptibility of prion proteins to conversion induced by oxidative stress

Authors
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Content

The most characteristic finding of transmissible spongiform encephalopathies (TSEs) in animals, including the natural and experimental diseases of BSE, scrapie, CWD, and TME, is widespread spongiform degeneration of the central nervous system accompanied with reactive astrocytic gliosis and microglial activation. In addition, in human prion disease including kuru and sporadic Creutzfeldt-Jakob Disease (sCJD), the severe pathologic changes are observed in the cerebellum with loss of granular cells, loss of Purkinje cells, fusiform swelling of the proximal portion of Purkinje cell axons (torpedoes), and intense Bergmann radial gliosis. In the study of the transmission of BSE to various species of small rodents for development of well-suitable animal model, guinea pigs of Hartley strain inoculated intracerebrally with BSE showed high susceptibility. Incubation period of first passage was 366 dpi and this decreased to 296 dpi and 309 dpi at the second and third passages, respectively. Neuropathology showed mild to severe spongiform degeneration and gliosis in the cerebral cortex, thalamus and brainstem. In addition, the affected guinea pigs, especially 2nd or 3rd passage groups, had massive pathological changes in the cerebellum: cerebellar cortex revealed severe atrophy associated with Bergmann radial gliosis of the molecular layer and reduction in the width of the granular cell layer without spongiform change, and medulla showed severe spongiform degeneration with hypertrophic astrocytosis. Loss of Purkinje cell was not so prominent, but axonal torpedoes in granular cell layer and dendritic expansion were frequently observed. PrP immunostaining showed diffuse PrP deposition, and plaque-like or glial-type deposition associated with spongiform changes. In the cerebellum, intense PrP deposition was observed in the molecular layer and the granular cell layer. These lesions associated with atrophy of the cerebellar cortex, which were similar to the lesions of kuru or sCJD, have not been reported in animal prion diseases, although deposition of PrP_{res} and vacuolar degeneration in the cerebellum are observed.

P3.18 Evaluation of the possible transmission of prions to the two closely related teleost species, sea bream and sea bass

Authors

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Content

Background: Transmissible Spongiform Encephalopathies (TSEs) are a group of fatal neurodegenerative diseases, affecting both humans and animals. The cause of TSEs is thought to be the conversion of the normal, cellular prion protein, PrP_C, into its aberrant isoform, PrP_{Sc}. To date there is little known about the susceptibility of fish to prion infection, despite the fact that many PrP-like proteins have recently been identified in different fish species. Although meat and bone meal of mammalian origin has been banned from use in aquaculture for some years, the possibility of fish being infected through prion-contaminated feed can not be excluded.

Objective: The aim of the study was to evaluate the possible transmission of prion to fish after oral challenge.

Methods: Groups of sea bream (Sparus aurata) and sea bass (Dicentrarchus labrax) were force fed with scrapie-infected ovine or BSE-infected bovine brain homogenates, while similar control populations were fed with normal brain homogenates. At regular intervals after challenge, individuals from each group were sacrificed and their tissues were examined for any histopathological or immunohistochemical alterations.

Results: No behavioral signs of disease were observed in any of the challenged groups. We detected abnormal deposition of plaque-like aggregates in the brain tissues of sea bream, while no immunohistochemical or histopathological evidence of disease manifestation in sea bass was found. Individuals challenged with BSE-brain homogenates were more severely affected than those challenged with scrapie-brain homogenates.

Discussion: This study was conducted on two fish species with commercial value. Fish PrP proteins share a 40-45% homology with each other and a 10-15% homology with mammalian PrPs. The presence of abnormal deposits in fish brain after oral challenge with prion is reported for the first time. Further in vivo studies will be undertaken to determine the possible risk of transmissibility.

P3.19 PrP_{Sc} accumulation and BSE infectivity in the tongue and respiratory epithelium of terminally diseased cattle

Authors

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Content

Background: A number of recent experiments have revealed a unique distribution pattern of PrP_{res} and infectivity in BSE diseased cattle that differs from the distribution observed in other species. In this species, PrP_{Sc} and infectivity seems to be essentially restricted to the central and peripheral nervous system, while the lymphoid system except for the Peyer's patches of the distal ileum seems to be devoid of BSE infectivity.

Objective: To challenge these findings, we expanded the set of samples to be tested and at the same time applied novel highly sensitive detection methods in order to be able to detect even trace amounts of PrP_{Sc} and / or infectivity.

Results and Discussion: Using these highly sensitive methods, we were able to detect PrP_{Sc} and infectivity in samples that had been negative in all other assays before. Here we describe the detection of BSE infectivity and PrP_{Sc} in the lingual muscle as well as in the respiratory epithelium of terminally diseased clinical BSE field cases as well as in experimentally challenged cattle.

P3.20 Quali-quantitative evaluation of ileal peyer's patches innervation in scrapie-free or scrapie-affected sarda breed ovines

Authors

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Content

Although Peyer's patches (PPs) and the enteric nervous system (ENS) play a key role in early sheep scrapie pathogenesis, little is known on the kinetics of ENS plexuses colonization. This study was aimed at quali-quantitatively evaluating ileal PP innervation in 29 Sarda breed ovines (12 scrapie-free, 2 months-old lambs, 4 ARQ/ARQ, 4 ARR/ARQ and 4 ARR/ARR, respectively; 12 scrapie-free, 2-4 years-old sheep, 3 ARQ/ARQ, 7 ARR/ARQ and 2 ARR/ARR, respectively; 5 ARQ/ARQ scrapie-affected sheep). Terminal ileum was collected and processed for routine histology from all animals under study. Ileal PP innervation was immunohistochemically evaluated by means of an anti-PgP9.5 (pan-neuronal marker) polyclonal antibody (Ab) and of an anti-TH (sympathetic innervation marker) monoclonal Ab. Moreover, PrP_{Sc} immunohistochemistry was carried out by means of the F99/97.6.1 monoclonal Ab.

Quite a developed network of fibres was detected within PPs, almost exclusively located in the interfollicular lymphoid tissue and stromal component. Intrafollicular fibres could be very rarely observed, with no apparent differences in the innervation rate being found between scrapie-free and scrapie-affected sheep. In adult animals, both scrapie-free and scrapie-affected, nerve fibres could be detected close to the follicle-associated epithelium. Furthermore, the TH+ component was very limited.

In conclusion, no significant differences in ileal PP innervation seem to exist in relation to PrP genotype, age and PrP_{Sc} deposition within PP follicles.

Footnote: This work was carried out with research grants from the Italian Ministry for Education, University and Research (MIUR, PRIN 2006).

P3.21 The implementation of a novel prion protein (PrP^{Sc}) removal technology for solvent/detergent (S/D) treated human plasma (Octaplas®)

Authors

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Content

Background: All manufacturers of plasma-derived biopharmaceuticals are requested to perform appropriate prion safety evaluations of their product portfolio, and an improved safeguarding in terms of risk for prion transmission is promoted when possible from a technological point of view and feasible in terms of appropriateness and quality outcome.

Objectives: The aim of our studies was to evaluate the incorporation of a novel prion protein removal technology into the manufacturing process for Octaplas®, an S/D treated human plasma of biopharmaceutical quality. The PrP^{Sc} removal was achieved by a chromatographic step, utilising an affinity ligand selected for prion protein binding that was developed by the company PRDT (Pathogen Removal and Diagnostic Technologies Inc., USA).

Methods: A validated downscale model of the adapted manufacturing process was used as basis for all investigational studies. Exogenous spike materials derived from brains of hamsters infected with hamster-adapted scrapie 263K were used to investigate the PrP^{Sc} binding capacity of the resin. Standard Western blot assays were used for the detection and determination of PrP^{Sc} levels in the various samples.

Results: Our studies demonstrate that PrP^{Sc} binds rapidly and with very high affinity to the novel prion protein-affinity-resin. Based on the amount of PrP^{Sc} captured, as determined by Western blotting of both the product fractions and resin, a very high and robust binding capacity was demonstrated in this particular cell-free Octaplas® matrix. In the order of 6 log10 ID50 were bound per ml of resin.

Discussion: The incorporation of this new chromatographic technology to remove pathogenic prions, potentially present in plasma, during Octaplas® manufacturing has been shown to be both technologically possible and feasible. The robust, reproducible PrP^{Sc} binding demonstrated by the PRDT affinity resin will further improve the safety margin of Octaplas® in terms of prion diseases such as vCJD.

P3.22 Biochemical quality of solvent/detergent (S/D) treated human plasma (Octaplas®) after implementation of a novel prion protein (PrP^{Sc}) removal technology

Authors

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Content

Background: A new chromatographic step for the selective binding of PrP^{Sc} to an affinity ligand developed and optimised for PrP^{Sc} capture by the company PRDT (Pathogen Removal and Diagnostic Technologies Inc., US), was implemented into the manufacturing process of the S/D treated biopharmaceutical plasma Octaplas®.

Objective: The aim of this study was not only to evaluate the technical performance of the incorporated chromatographic step, but to demonstrate that the quality of Octaplas® is not impaired by the introduction of this novel technology at large scale routine manufacturing.

Methods: Scale-up batches Octaplas® with implemented ligand chromatography for specific PrP^{Sc} capture were manufactured by Octapharma PPGmbH, Austria. Octaplas® produced without the additional prion removal step were used as control samples. The biochemical quality was compared directly after manufacturing as well as after one year storage. All plasma samples were tested on global coagulation parameters, fibrinogen levels, and the activities of coagulation factors and protease inhibitors. In addition, markers of activated coagulation and fibrinolysis were measured and von Willebrand factor multimeric analyses were performed.

Results: The studies showed that Octaplas® produced with and without the ligand chromatography for selective PrP^{Sc} capture demonstrates an identical quality. In addition, extensive stability studies showed no significant changes in all parameters tested after one year storage.

Discussion: The above comprehensive biochemical investigation and stability studies confirmed that the ligand chromatography step under the developed conditions can be introduced into the Octaplas® manufacturing process as a mean to reduce potentially present PrP^{Sc}. The product after implementation of this novel technology has the same clinical safety and efficacy profile, except for the increased safety margin in terms of prion disease transmission such as variant Creutzfeldt-Jakob Disease.

P3.23 Conditional expression of PrP and its effect on transmissible spongiform encephalopathy

Authors

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Content

The expression of prion protein (PrP) has been proven to be essential for the development of transmissible spongiform encephalopathy (TSE). PrP knockout mice are completely resistant to TSE disease. We have developed and established a transgenic mouse model in which PrP expression can be controlled using the cre/lox system. Using this system we aim to determine in which cells and when PrP expression is vital to disease progression, both within the central nervous system (CNS) and also during neuroinvasion following peripheral challenge. Currently we are investigating the role of PrP expression in CNS neurones and peripheral myelinating glial cells (Schwann cells). Removal of PrP expression from myelinating glial cells revealed no effect on TSE neuroinvasion. This result was surprising as ~ 90 % of the PrP was lost from peripheral nerves including all glycosylated PrP species. This model is being investigated further to determine the cellular contribution to PrP glycoprofile and also the possible involvement of PrP in myelination or axon-glial interaction. Removal or PrP expression from neurones via a tamoxifen-inducible cre model revealed a ~ 50 % loss of PrP from whole brain. This is despite neurones only contributing to ~ 10 % of brain matter. Following intracerebral challenge we are currently observing a significant lengthening of incubation period, though these experiments are still in progress. Pathological examination of infected neuronal PrP knockout mice reveals much less PrP^{Sc} deposition and neuronal loss when compared to infected non-induced litter mates. These results indicate protection of neurones against disease effects.

P3.24 Rapidly progressive Jakob - Creutzfeldt disease in patients with Familial Mediterranean Fever

Authors

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Content

Background: Jakob-Creudtzfeldt disease (CJD) is the most common prion disease in human. One of the largest clusters of familial CJD (fCJD) exists in the North African Jews of Libyan and Tunisian origin. Familial Mediterranean fever (FMF) is an inflammatory disease characterized by episode of fever, abdominal pain and arthritis, and is also common in Libyan Jews.

Objective: Following the clinical observation of a virulent course of CJD in a patient with FMF we hypothesized that this pro-inflammatory condition may affect the course of CJD.

Methods: Patients were collected through the Israeli Register of Neurological Disease since 1963. A consecutive series of 372 CJD patients that were investigated clinically and genetically and diagnosed as CJD were included in our study, 236 of them with fCJD and 136 with sCJD. Following review of the patients files for the presence of FMF patients were divided into two groups: the first group included 3 patients with FMF-CJD co-morbidity and the second group included the 369 patients without FMF The two groups were compared for demographic and clinical parameters using the non parametric Mann Whitney U test.

Results: The 3 FMF patients had disease durations of 3.5, 1 and 2.5 months which was significantly shorter than in the non-FMF patients (median 5.6, p=0.02). Disease onset was also at an earlier age in the FMF patients (41,57and 54 years old) when compared to non-FMF patients (median 62.16, p=0.02).

Conclusions: Co-morbidity of FMF and CJD may lead to a younger age of onset and a shorter disease duration probably due to activation of pro- inflammatory factors determining the course of CJD. We suggest that the immune response to the pathologic isomer PrP_{Sc} is much more aggressive in FMF patients due to their reduced ability to suppress the immune cascade resulting in formation of IL 1 β which is suspected to mediate the main pathologic changes in CJD- the t of the brain tissue and the neuronal loss. This concept may suggest a potential therapeutic effect of anti inflammatory treatment in CJD.

P3.25 Transmission of BSE infection, in sheep, via blood transfusion – A model for vCJD

Authors

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Content

Background: It is well documented that sheep orally infected with BSE provide a suitable model to assess the potential of transfusion of blood components to transmit vCJD in man (Hunter et al., 2002, Houston et al., 2000). These studies have shown that infectivity can be efficiently transmitted following transfusion of whole blood and buffy coat. However, it is not known how infectivity is distributed among blood components used therapeutically in humans and how effective the current risk reduction measures are at removing infectivity in blood.

Objective: To determine which components of blood transmit infectivity following transfusion; To assess the effect of leucoreduction on endogenous infectivity associated with the BSE agent; To Identify the titre of infectivity associated with the blood samples using mouse bioassay.

Methods: Sheep, used as blood donors, were orally dosed with either bovine BSE-infected or uninfected bovine brain homogenate. Approximately 900ml was collected from donors at the late pre-clinical phase of infection. A unit of blood (approx 450 ml blood + 63ml anticoagulant) was prepared into components (red cells, plasma, buffy coat & platelets) using methods routinely employed for human blood by transfusion services. Paired components from the second unit were passed through human leucoreduction filters, prior to transfusion into recipients. A sub sample of all components transfused was also inoculated into transgenic mice over expressing ovine PrP.

Results: We have successfully conducted over 250 transfusions of these components. Our donor animals are nearing the clinical endpoint of disease and following post mortem will be subject to biochemical confirmation of TSE infection. We are awaiting the clinical outcome of recipient sheep and transgenic mice which were transfused and / or inoculated with the components prepared from donors. We will also collect whole blood from a proportion of primary recipients and perform further blood transfusions to secondary recipients. We are creating an extensive archive of blood samples from these sheep over the course of this 6 year study.

Discussion: The aim of these experiments is to determine qualitative and quantitative data on the changes in infectivity in blood and its clinically relevant components with time, as well as assessing the effect of leucodepletion of such products and the potential for secondary transmission by blood transfusion.

Department of Health, UK 007/0162

P3.26 Tracking prion infectivity in the blood of deer with chronic wasting disease

Authors
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Content
Background: The blood and saliva of deer infected with chronic wasting disease (CWD) contain infectious prions (Mathiason, et. al. Science 2006). Elucidation of the blood component(s) carrying prion infectivity would be valuable in understanding CWD prion trafficking and in focusing development of antemortem assays to detect prionemia.

Objective: The goal of these studies was to identify the blood components responsible for prionemia in CWD infection.

Methods: Two bioassay studies containing cohorts of n=4 CWD-naive white-tailed deer/cohort were conducted. Study A cohorts were inoculated intravenously with cellular vs. cell free components of blood from CWD+ deer. In Study B purified leukocyte subsets comprised of either CD21+ B cells, CD14+ monocytes or CD41/61+ platelets were assessed. Additional cohorts received whole blood from either CWD+ or CWD negative deer and served as controls for both studies. CWD infection status was monitored by immunohistochemistry (IHC) and western blotting (WB) for PrPCWD in tonsil biopsies collected at 0, 3, 6, 12, and 15 months post inoculation (mo. pi). At study termination (18 mo. pi, Study A) a wider array of lymphoid tisues and the brain were examined. Study B is ongoing (12 mo. pi).

Results: Study A: IHC and WB analysis of tonsil biopsies and terminal tissues revealed PrPCWD in 4/4 deer inoculated with the leukocyte + platelet fraction of blood from CWD+ donors. By contrast 0 of 4 deer receiving plasma from the same donors became CWD infected. Study B: Current results based on tonsil biopsies indicates PrPCWD detection in 2/4 deer receiving CD21+ B cells, 1/4 recipients of CD41/61+ platelets, and 0/4 CD14+ deer receiving monocytes. All 8 deer serving as positive controls became PrPCWD+ in tonsil biopsies between 6 and 12 mo pi while negative controls remained PrPCWD negative. Summary: Bioassays in the native host species demonstrate that blood-borne CWD infectivity is associated with B cells and platelets and not with cell-free plasma.

Discussion: We report for the first time association of infectious CWD prions with the cellular, B cell, and platelet enriched fractions and not with cell-free plasma fraction of blood from CWD+ deer. These results have bearing on the trafficking of CWD prions in deer and help direct efforts to develop an antemortem blood test to detect CWD in live cervids or other species.

P3.27 Potential of cell substrates used for production of biologics to propagate transmissible spongiform encephalopathy (TSE) agents

Authors
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Content
Background: TSE agents have contaminated a variety of products, including human-tissue-derived therapeutics and animal vaccines. Many biologics are prepared in cell cultures. Although most cultures studied resisted infection with TSE agents, a few were successfully infected. Susceptibility of cultured cells to TSE infections cannot be predicted from species or tissues of origin or by the level of expression of prion protein (PrP).

Objective: We are investigating susceptibility of several cell lines used or proposed for manufacture of various biologic products to propagate TSE agents.

Methods: We inoculated bacteria-free filtrates of 3 reference TSE agent inocula-brain suspensions containing agents of bovine spongiform encephalopathy (BSE), variant Creutzfeldt-Jakob disease (vCJD) or sporadic CJD-into several cell lines important or potentially important in the manufacture of biologic products.

Results: We studied these cell lines: Vero (African green monkey), CHO (Chinese hamster), MDCK (dog), Rab9 (rabbit), HEK-393 (human heteroploid), and WI-38 (human diploid). We also studied lines of human neuroblastoma-derived cells (SH-SY5Y) engineered to overexpress wild-type PrP and PrP isoforms having mutations associated with familial TSEs. Cells exposed to TSE agents were serially propagated for 30 passages and selected passages tested for TSE-associated PrP (PrPTSE) and for persistence of infectivity by intracerebral inoculation into TSE-susceptible transgenic mice and squirrel monkeys (BSE-exposed cells only). No PrPTSE was found in any exposed cells after 30 passages. Known susceptible cells exposed to a mouse-adapted human TSE agent as positive controls accumulated PrPTSE. No exposed cell line tested has transmitted TSE to mice or monkeys after more than a year of observation. We also exposed bovine cell cultures to BSE agent and passaged as for other cultures. BSE-exposed MDBK, EBTR and BT cultures were propagated for 30 passages; BCE C/D-1b and BL3.1 cultures are still under study (15 passages to date). No BSE-exposed bovine culture showed a cytopathic effect.

Discussion: We have found no evidence to date that any candidate cell substrate exposed to 3 TSE agents, including BSE agent, accumulated PrPTSE. Bioassays in susceptible animals are in progress.

P3.28 Early transcriptional changes in the brain of cattle orally infected with BSE

Authors
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Content
Bovine spongiform encephalopathy (BSE) is a fatal, transmissible, neurodegenerative disease of cattle. Between cattle it can be transmitted experimentally through the oral route and in this study brain tissue samples from animals at different time points post inoculation were analysed for changes in gene expression. The aims of this study were to understand the effect of prion pathogenesis on gene expression and to identify differentially regulated genes during the BSE progression.
The microarray analysis was carried out using Affymetrix Bovine Genome GeneChips, which contained probes for 24,128 sets of transcripts. The data were normalized and analyzed using the GeneSpring software. Clustering analysis showed that there is a correlation between the disease progression and expression patterns. The mRNA of 205 genes was found to be differentially regulated by ANOVA with a p-value cutoff being 0.05. Many of these genes encode proteins involved in immune response, apoptosis, cell adhesion, stress response and transcription. Among the genes differentially regulated, 13 of them have been reported to be associated with prion diseases or other neurodegenerative diseases in previous studies. To validate the micoarray data, quantitative reverse transcriptase-PCR (rtPCR) of six genes was carried out and the rtPCR showed similar profiles to those of the microarrays analysis.
This study also revealed a correlation between gene expression profiles and the progression of BSE in cattle. The highest degree of changes in gene expression occurred between the negative controls and the animals 21 months post incubation, suggesting that there are many pathogenic processes in the animal brain even prior to the detection of infectivity in the CNS of these orally dosed cattle. Moreover, the clustering analysis showed that it is possible to predict the infectious status of animals using the profiles of the 205 differentially regulated genes.

P3.29 Efficient prion transmission via the intranasal and aerosolic route in the absence of a functional immune system

Authors
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Content
Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases of humans and animals. The underlying infectious agent, the prion, was shown to accumulate not only in the central nervous system (CNS) but also in secondary lymphoid organs of affected hosts. Prions can colonize hosts by a variety of extracerebral routes, including parenteral injection, transdermal administration after skin scarification, and oral administration. Up to date prions were not largely considered to be transmissible by aerial routes. Here we have investigated the transmissibility potential of prions administered intranasally or by aerosols. Various transgenic mouse models expressing the cellular prion protein (PrP^c) in specific compartments or cells of the brain (e. g. exclusively in the CNS) were investigated to identify the cellular and molecular mechanism(s) of prion invasion via the intranasal or aerosolic route. In addition the importance of a functional immune system was assessed. Results of this study identify prion aerosols or prions administered intranasally as a startlingly efficacious pathway of prion transmission, and call for appropriate revisions of prion-related biosafety guidelines. Remarkably, prion transmission occurred in the absence of B- and T-cells pointing towards a direct transmission route after intranasal prion inoculation.

P3.30 The location of the glycosylphosphatidylinositol (GPI) anchor on PrP is critical for the propagation of prion infectivity

Authors
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Content
Background: The C-terminus of the prion protein has been suggested to play an important role in the interaction of PrP^c with PrP^{Sc}. Treatment of PrP^c with chemical compounds or antibodies directed to the far C-terminus inhibits several models of prion infection. Amino acid sequence comparison of rabbit PrP and mouse PrP revealed 87% sequence homology with the majority of the difference occurring at the far C-terminus around the GPI anchor attachment site. In silico prediction suggests that the GPI anchor attaches to rabbit PrP significantly upstream to that of mouse PrP and all other mammals investigated. Interestingly, rabbits are the only mammals known to be resistant to prion disease infection.

Objective: To determine the effect of amino acid sequence at the far C-terminus on the GPI anchor attachment site of PrP and ultimately the importance of the far C-terminus in the interaction of PrP^c with PrP^{Sc} in prion disease.

Methods: The mouse PrP (MoPrP) gene underwent mutagenesis to encode key elements of the rabbit GPI anchor attachment site (MoPrP-RbGPI), which will potentially shift the GPI anchor attachment site of MoPrP. This construct was then stably transfected into RK13 cells. To assess mutant PrP expression and localisation, western immunoblotting and immunofluorescence was used and cell lines were treated with PIPLC to determine if MoPrP-RbGPI is GPI anchored. Mass spectrometry was used to determine a change in the GPI anchor attachment site and to investigate whether transfected cells were capable of being infected, cells were inoculated with the mouse adapted human prion strain, M1000.

Results: MoPrP-RbGPI was found to be localised at the plasma membrane and attached via a GPI anchor. Initial mass spectrometry analysis indicates that the GPI anchor of MoPrP-RbGPI is located significantly N-terminal to that of MoPrP suggesting a loss of the far C-terminal residues. Furthermore, upon inoculation with M1000, cells expressing MoPrP-RbPrP were not capable of being infected and propagating protease resistant PrP.

Discussion: The probable loss of residues at the far C-terminus of MoPrP-RbGPI during processing and addition of the GPI anchor and the cells lack of ability to be infected, suggests that the far C-terminal residues play an important role in the interaction of PrP^c with PrP^{Sc} during infection.

P3.31 Photo-Fenton photocatalytic degradation of PrP adsorbed on metal powders

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Content
Background: Decontamination of non disposable surgical instruments potentially exposed to prion infected tissue is essential for the prevention of iCJD transmission from sCJD or gCJD carriers. TSE agents are highly resistant to conventional sterilization methods and exhibit high binding affinity to metal surfaces without losing their infectivity. Current effective protocols are hazardous to equipment, operators and the environment and therefore, they do not offer a solution for the routine reprocessing of surgical instruments.

Objective: The aim of this study is to apply a user-, instrument- and environmentally friendly oxidative method mediated by the photo-Fenton reagent, for the decontamination of surgical instruments.

Methods: For this purpose, two different metal powders, surgical stainless steel 316-L and titanium oxide (rutile) have been incubated with sheep and mice scrapie brain homogenates and treated with the Photo-Fenton reagent. Silver staining and immunoblotting have been performed to assess the efficiency of this treatment. Groups of C57BL/6J mice have been i.p. inoculated in order to asses the potential of the photocatalytic treatment *in vivo*.

Results: Immunoblotting of both metal powders after incubation with the brain homogenates and treatment with the photo-Fenton reagent demonstrated the potential of the method to completely degrade PrP immobilized on metal surfaces rapidly. Complete degradation of the total protein amount adsorbed on both types of powders requires, as expected, longer treatment under the same experimental conditions.

Discussion: According to our findings, homogeneous photocatalytic oxidation could be a powerful tool for disinfection of surgical instruments. The use of a low-cost catalytic system, combined with the simplicity of the necessary equipment, can offer alternative, economically reasonable, user- and environmentally friendly solutions to the routine processing of non-disposable surgical instruments.

P3.32 Ovine PrP genotype can influence lesion profile on primary transmission of classical scrapie to wild type mice

Authors
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Content
Background: Lesion profiles permit comparison of neuropathology between TSE isolates following passage to mice. Agent strain, the PrP genotype of the donor and the host are factors that influence the ability of a TSE isolate to transmit to a given host. Strain characterisation of classical scrapie in mice is generally considered to rely on sub-passage. The characteristics of primary transmission to mice have not been comprehensively reported.

Objective: The aim was to carry out a retrospective analysis of the influence of ovine PrP genotype on lesion profiles and immunohistochemistry patterns following transmission of classical scrapie to wild type mice. The results presented are the primary isolation data from the analysis of a large strain characterisation study.

Methods: 62 positive scrapie field cases were collected from individual farms between 1996 and 1999. A 10 % medulla homogenate was inoculated into RIII, C57bl and VM mice. Post mortem TSE diagnosis was confirmed histopathologically on haematoxylin and eosin (H&E) sections. Lesion profiles were assessed using standard methodology. For immunohistochemical analyses, samples were stained with rabbit polyclonal Rb486 antibody.

Results: Isolates with five or more clinically and H&E positive mice were analysed. With the exception of two, all derived from ARQ/ARQ or VRQ/VRQ ovine sources. Cluster analysis produced consistent lesion profiles in RIII and C57bl mice. Moreover we observed correlations between lesion profile clusters and the ovine PrP genotype, whilst immunohistochemical analysis indicated genotype-associated trends in PrP^{Sc} deposition pattern.

Discussion: Ovine PrP genotype is one of the factors that may influence both the lesion profile and the pattern of PrP^{Sc} deposition on primary transmission of classical scrapie to wild type mice. Analysis of sub-passage data will further elucidate the interplay between ovine PrP genotype and the manifestation of strains.

P3.33 New insights into early sequential PrP_{Sc} accumulation in scrapie infected mouse brain evidenced by the use of streptomycin

Authors
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Content
Prion disease symptoms develop always after a long period post infection and coincide with exponential PrP_{res} accumulation in the brain. During this long and silent incubation period, recent data have shown that blood transfusion could transmit the disease in animals as well as in humans; this demonstrates how much important it is to focus our knowledge on the early phase of the disease. Amplifying potentialities of streptomycin in the immunohistochemical (Strp-IHC) and biochemical (Strp-WB) detection of the abnormal prion protein (PrP_{Sc}) was established on different natural and experimental cases of transmissible spongiform encephalopathies (TSE).
Taking advantage of this property, we propose here to revisit the early post-infection period of experimental TSE using the C506M3 mouse transmission study, in which C57Bl/6 mice are either inoculated intra-cranially or intra-peritoneally. The weekly removed brains, from 7 to 63 days post intra-cranial inoculation were analysed using Strp-IHC. Besides, the weekly removed spleens, from 7 to 49 days post intra-cranial and intra-peritoneal inoculation were analysed using Strp-WB. In the brain, the Strp-IHC technique, allowed to detect a clear specific PrP_{Sc} deposition as early as 28 days post inoculation. The location of the first detected PrP_{Sc} deposits suggests a possible involvement of the cerebrospinal fluid in the early dissemination of the infectious agent. Because these deposits were made of fine particles transiently (detectable until 36 d.p.i.), the meaning of these newly accessible PrP_{Sc} deposits is discussed in relation to a possible nascent form of PrP_{Sc} molecules detected in situ for the first time. The aggregated PrP_{Sc} detected later resulted most likely from a peripheral re-circulation of the infectious agent as suggested by detectable PrP_{res} in the spleen as early as 7 days post-inoculation and whatever the inoculation route. This last observation raises the question of the physiopathological role of the lymphoid system in the early spread of prion infection. As well the infectious agent affinity for the lymphoid system appeared to be greater than for the brain. Altogether, these findings bring new data in our knowledge of the early stages after infection with prion agents.

P3.34 Biochemical and Bioassay Analysis of the Persistence of TSE Infectivity in soil

Authors
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Content
Background: Horizontal transfer of scrapie is known to occur in sheep although the route(s) of transmission are unclear. Both horizontal transmission in the absence of lambing and the absence of direct sheep to sheep contact have been demonstrated. There is accumulating evidence that prion infectivity can reside in the environment and particularly within soil leading to speculation that ingestion of soil bound prions may be one route of horizontal transmission. Furthermore, soil-bound prions are infectious in a rodent model using oral challenge (Johnson et al. 2007. Plos Pathog. 3:e93), a result that has potentially significant implications for the persistence and transmission of the TSE agent on infected pasture.

Objective: This project specifically addresses the interaction of PrP_{res} with a range of UK soil types within a laboratory setting.

Methods: We are using quantitative Western blot analysis of proteinase K resistant PrP_{Sc} to determine the persistence of such disease-associated prions within different soil types subjected to a range of environmental conditions. In the final phase of the study, the persistence of infectivity of soil-bound prions, both scrapie and BSE, will be determined using transgenic mouse bioassay. The study uses two TSE agents, namely classical scrapie and BSE, which should allow an assessment of the effects of TSE strain on the deposition and persistence of PrP_{Sc} and infectivity within soil.applied to soil from pasture likely to be contaminated with environmental scrapie from the VLA Ripley site.
We are in addition developing a high sensitivity assay for the detection of in situ PrP_{res} from pastures naturally contaminated with scrapie. Methods are being developed using spiked soil and are being.

Discussion: This study will enhance our knowledge of the importance of soil as a potential environmental reservoir of TSE infectivity. Furthermore, the development of an assay to monitor a validated marker of scrapie within environmental samples will provide a tool to aid in the management of the disease.

P3.35 A novel test procedure for evaluation of prion inactivation

Authors
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Content
Background: We have developed a method for the detection of contamination and remaining contamination after cleaning procedures of surgical instruments known as "radionuclide method". After mock contamination of instruments with radioactively labelled blood, the instruments are screened by a camera detecting radiation (gamma camera) before and after the cleaning. That provides a simple and non destructive way to localize and measure the remaining contamination both pin-pointedly and quantitatively, which is especially useful to study reprocessing of instruments with channels and cavities such as endoscopic instruments.

Objective and Methods: Pathological prion protein, the agent causing transmissible spongiform encephalopathies (TSE), was found in a number of other studies to bind tightly to surgical steel surfaces. This intensive binding of the prion protein to steel results in considerable problems to clean these surfaces, a fact relevant to the threat of iatrogenic transmission of e.g. Creutzfeld-Jacob Disease (CJD). In search of new chemical and physical decontamination procedures, we employed the radionuclide method in which purified pathological prion proteins are radioactively labelled and these are used as the test soil to contaminate model instruments such as steel wires.

Results and Discussion: After submitting the steel wires to cleaning and decontamination procedures, the remaining radioactivity will be determined and the wires will be used in a bioassay to evaluate a possible correlation between remaining radioactivity and survival time of the test animals. We will report the results of a pilot decontamination experiment using this method to examine enzymatic and alkaline detergents for their prion inactivation effect.

P3.36 Lack of Prion Accumulation in Lymphoid Tissues of Scrapie-affected Sheep with the AA136, QR171 Prion Protein Genotype

Authors
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Content
Background: Sheep scrapie is a transmissible spongiform encephalopathy which can be transmitted horizontally through the shedding of an infectious conformer (PrP_{Sc}) of the normal cellular prion protein (PrP_{C}). Genetics profoundly influence the susceptibility of sheep to scrapie. PrP_{C} amino-acid polymorphisms A136V, R154H, Q171R, and Q171H are predictive for relative susceptibility (V136, R154, Q171) or resistance (A136, H154, R171) to classical scrapie in natural settings and in experimental oral inoculation studies.

Objective: To compare the clinical course and PrP_{Sc} tissue distribution in sheep with high resistance and high susceptibility genotypes after inoculation of scrapie-affected sheep brain homogenate directly into the brain.

Methods: Five sheep each of genotype VRQ/VRQ, ARQ/ARQ(H), VRQ/ARQ, or ARQ/ARR were inoculated via intra-cerebral route with a 10% brain homogenate derived from a ARQ/ARQ sheep affected with scrapie. Sheep were euthanized in the terminal phase of scrapie development. Tissues collected at necropsy were examined by light microscopy, immunohistochemistry (IHC) and Western blot.

Results: All inoculated sheep succumbed to scrapie. Clinical signs, microscopic lesions, and Western blot profiles were uniform across genotypes and consistent with manifestations of classical scrapie. Mean survival time differences were associated with the 171 polymorphic site with VRQ/VRQ and ARQ/ARQ sheep surviving 18 and 19 months, whereas VRQ/ARR and ARQ/ARR survived 56 and 60 months, respectively.
Labeling of PrP_{Sc} by IHC revealed similar accumulations in central nervous system tissues across genotypes. Labeling of PrP_{Sc} in lymphoid tissue was consistently abundant in VRQ/VRQ, present in 4/5 ARQ/ARQ and VRQ/ARR, and totally absent in ARQ/ARR sheep.

Discussion: The results of the study demonstrate the susceptibility of sheep with the high resistance genotype ARQ/ARR to scrapie by the intra-cerebral inoculation route, with attendant PrP_{Sc} accumulation in CNS tissues, and concurrent lack of PrP_{Sc} in lymphoid tissue. Our data suggest that genetic resistance to scrapie is associated with failure of PrP_{Sc} to accumulate in lymphoid tissue, and not associated with permissiveness of CNS tissue to PrP_{Sc} amplification.

P3.37 Does the incidence of experimental BSE in sheep change with age?

Authors
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Content
Sheep/lambs were dosed orally in the age groups comprising 1-2 days, 14-21 days, 3 months, 6 months and over 12 months. They were dosed with 1.0g, 0.5g or 0.05g of BSE as well as a number of controls which were dosed with normal cow brain. There have been some intercurrent deaths. Results are based on immunohystochemical diagnosis of brain and lymphoid tissue from all sheep including clinical and intercurrent cases. The BG4 antibody was used and largely confirmed clinical assessment of putative disease-affected sheep. It is clear that the 14-21 day old lamb is most at risk from BSE by ingestion of disease. This is true for all three dosing regimes. Sheep of 3 months and over showed a very low disease incidence regardless of dose level.

P3.38 Tubulovesicular structures are present in an autopsy case of variant Creutzfeldt-Jakob disease

Authors
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Content
Background: Background: Tubulovesicular structures (TVS) are 27- 35 nm in diameter virus-like structures encountered in all transmissible spongiform encephalopathies (TSE) or prion diseases including Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease and fatal familial insomnia. Similar particles were also described in tissue cultures infected experimentally with the Creutzfeldt-Jakob disease (CJD) agent. In two cases variant CJD (vCJD) examined as brain biopsies, they were observed in single specimen, but have never so far been identified in autopsy samples.

Objective: To look for TVS in an autopsy brain tissue from a vCJD cases.

Methods: After routine preparations, we used thin-section electron microscopy.

Results: Here we described a TVS in autopsy sample of vCJD. By thin section electron microscopy, we observed a cluster of three neuronal processes containing TVS.

Discussion: This report shows that TVS may be found even under the suboptimal morphological conditions in brain autopsy tissue, and again stresses the need for a search for their true aetiological or pathogenic significance.

P3.39 Do innervation of germinal centre and contacts between FDC and nerve fibers be keys to understand the susceptibility difference between bovines and humans to the BSE agent?

Authors
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Content
Background: In regard to BSE and vCJD, the agent tropism for lymphoid tissues is completely different even if the infectious strain responsible and the way of inoculation are identical. During vCJD, the infectious agent crosses the digestive barrier and multiplies in lymphoid organs, before progressively reaching the brain. Indeed, in vCJD, it accumulates in the ileum, tonsils, spleen and appendix of infected individuals. In contrast, in cattle, the BSE agent has a low affinity for lymphoid tissues and mainly accumulates in the nervous system. During preclinical stages, infectivity, other than that in the peripheral nervous system or central nervous system, is confined in the distal ileum of orally infected cattle. So, it appears that, at least in the case of BSE and vCJD, host properties can influence the accumulation of the infectious agent in lymphoid organs.

Objective and Methods: In this study, we analysed by confocale microscopy the mucosal innervation and the interface between nerve fibres and FDC in bovine and human tonsils using a panel of antibodies. Since differences in the innervation of lymphoid organs depending on species and on age have been reported, we analysed two categories of bovines (calves less than 12 months old and bovines older than 24 months) and two categories of humans (patients less than 5 years old and patients older than 25 years).

Results: In both species, ways of innervation by-passing germinal centres could be postulated: nerve fibres are widely distributed in antigen/cell traffic area: the lamina propria, the interfollicular zone and the lymphoepithelial area. We pointed out that, only in tonsils of bovines older than 24 months, nerve fibres are observed to be in contact with FDC. In contrast, in human tonsils, no nerve fibres established contacts with FDC, whatever the age.

Discussion: Innervation of germinal centres can be said to be an age-dependent dynamic process in bovines. The weak innervation of the secondary lymphoid organs could thus be a rate-limiting step to neuroinvasion in humans. This species difference could influence the way of neuroinvasion and thus, the susceptibility of bovines and humans to the BSE agent.

P3.40 Transmission of TSEs by blood transfusion in sheep

Authors
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Content
Background: The emergence of variant Creutzfeld-Jakob disease (vCJD), the human form of bovine spongiform encephalopathy (BSE), led to concerns about the potential risk of iatrogenic transmission of disease by blood transfusion and the introduction of costly control measures to protect blood supplies.

Objective: To demonstrate for the first time that it was possible to transfer TSE infection by blood transfusion using the sheep as a model. Both BSE and natural scrapie were to be tested.

Methods: Donor sheep were inoculated with BSE or were naturally infected with scrapie. Blood was taken from these animals at various times during incubation period and transfused into uninfected susceptible recipient sheep.

Results: We have previously reported preliminary data demonstrating the successful transmission of BSE and natural scrapie by blood transfusion in sheep (Houston et al, 2000; Hunter et al 2002). The final results of this study, reported here, give unexpectedly high transmission rates, including from blood taken during the asymptomatic pre-clinical phase in the donor sheep. The high transmission rates and the relatively short and consistent incubation periods in clinically positive recipients suggest that infectivity titres in blood were quite substantial and/or that blood transfusion is a very efficient method of transmission.

Discussion: This experiment established for the first time the value of using sheep as a model for studying transmision of vCJD by blood products in humans and has resulted in a larger scale study currently underway (see McCutheon et al, Prion 2008). Project funded by UK Department of Health.

P3.41 DLC instrument coatings for reduced iatrogenic transmission of prion disease

Authors
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Content

Background: Transmissible spongiform encephalopathies (TSE) are a group of rare fatal neurodegenerative disorders that effect both animals (scrapie, BSE, CWD) and humans (CJD). The infectious agent is thought to be a protease resistant isoform (PrP_{Sc}) of the protease sensitive prion protein (PrP_C). Iatrogenic transmission of prion diseases has become a problem especially during neurosurgery due to the resistance of the infectious PrP_{Sc} to many chemical and enzymatic detergents and its strong adhesive properties to stainless steel. Diamond-like carbon (DLC) coatings have shown various useful properties in a number of areas of research including: hard wearing, smoothness, high refractive index and low friction. Small concentrations of elements can be doped into these coatings to alter the physico-chemical properties of the surface energies and therefore alter the level of tissue adhesion and ease of tissue removal.

Objective: To investigate the adhesion of protein and amyloid prion to doped DLC-coated surfaces compared to surgical stainless steel and the subsequent effect on the decontamination properties of four enzymatic cleaning chemistries.

Methods: 316 stainless steel and DLC-coated tokens, doped with 1%, 2%, 4% Al, B or N, were contaminated with 1 µl of 1 mg/ml ME7-infected brain homogenate and dual stained with Thioflavin T and SYPRO Ruby and then analysed using episcopic differential interference contrast/epifluorescence microscopy (EDIC/EF). These experiments were then repeated with the addition of a cleaning step using one of the four enzymatic cleaning chemistries prior to staining.

Results: Initial adhesion of tissue protein and amyloid prion prior to cleaning was reduced by ~15 fold on the doped DLC-coated tokens compared to that of the stainless steel. The enzymatic cleaning chemistries tested on the different surfaces showed various efficiency in removing either the general tissue protein or amyloid prion.

Discussion: DLC surfaces can significantly reduce the adhesion of both tissue protein and amyloid prion, related to their surface free energy properties, and are also easier to clean. DLC-coatings offer the potential for a new generation of surgical instruments to reduce iatrogenic transmission of prion disease.

P3.42 Improvement of a neural stem cell model for prion propagation

Authors
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Content

Background: The abnormal prion protein (PrP_{Sc}) plays a central role in the transmission of prion diseases. PrP_{Sc} is produced by conversion of the cellular isoform of the prion protein (PrP_C) which is essential for the pathogenesis of prion diseases. Accumulation of PrP_{Sc} in the central nervous system leads to neurodegeneration with neuronal cell death and gliosis. The cellular requirements for PrP_C conversion, strain specificity and propagation of PrP_{Sc} are still unclear.

Objective and Methods: To address these questions and investigate the molecular basis of prion diseases it is a necessity to find new cell culture models or to improve those already existing. We described that, after differentiation, neural stem cells (NSCs) from the central nervous system are able to convert the PrP_C into its pathologic isoform PrP_{Sc} in cell culture *(Milhavet et al. Stem Cells. 2006;24(10):2284-91)*. Thus, using this original model we have pursued our investigations and optimized culture conditions. We hypothesized that changing the culture conditions by modifying a given factor or a combination of factors could increase PrP_{Sc} production in NSCs.

Results: Our first results showed that the infection can be strongly influenced by supplements added to the culture medium. Those supplements enabled neuronal as well as astroglial differentiation of NSCs and could facilitate prion propagation and increase PrP_{Sc} production. NSC cultures from transgenic mice were also produced. NSCs from PrP knock-out mice were mainly isolated in order to establish cells expressing a heterologous prion protein with the long term objective to infect cells with prions from ovine and bovine origin. To progress towards this goal, these cells were first transduced with lentiviruses encoding $moPrP_C$. These experiments using engineered cells in combination with selected factors to modify differentiation along with additional data will efficiently add to our understanding of prion diseases and more importantly will allow us to develop more powerful models.

P3.43 Distribution and density of PGP 9.5 positive nerve fibers in the ileal Peyer's patch follicles of sheep are influenced by age and PrP genotype and not by scrapie infection

Authors
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Content

Background: Scrapie in sheep is the prototype of transmissible spongiform encephalopathies. In the natural disease, the uptake of the agent is most likely via the alimentary tract. The ileal Peyer's patch (IPP) in sheep is a large lymphoid organ, which reaches its peak development at 3 months of age, after which it gradually involutes. PrP_{Sc} is detected at an early, preclinical stage in the IPP and before any accumulation of PrP_{Sc} is detectable in the peripheral nervous system.

Objective: We wanted to investigate the density of nerve fibers in various tissue compartments (dome, follicle and interfollicular area) of the IPP and the correlation to age, PrP genotype and experimental scrapie infection.

Methods: Tissue sections from the IPP of 28 sheep orally inoculated with scrapie and 66 normal sheep at various ages (0 days-20 months old) were immunolabelled by PGP 9.5, a pan-neuronal marker. In a blinded study, the density of nerve fibers in tissue compartments was estimated. Sections from the scrapie sheep were immunolabelled with the PrP antibody L42. For statistical analyses the PROC MIXED procedure in SAS 9.1.3 was used with animal ID as a random effect.

Results: The PrP genotype and age were significant variables in explaining the variation in PGP labelling. Scrapie infection status did not contribute significantly to the observed variation. PrP genotype had an effect on the density of PGP positive nerve fibers in the dome, follicle and interfollicular areas. Sheep with PrP genotype VRQ/VRQ had the highest level of PGP positive nerve fibers in all these areas. Older sheep had significantly more fibers in the dome and younger sheep had significantly more fibers in the interfollicular areas.

Discussion: Peripheral neuroinvasion is necessary for the spread of PrP_{Sc} to the central nervous system. We found that the VRQ/VRQ sheep had more nerve fibers in all the examined tissue compartments of the IPP. Furthermore, young sheep possessed a higher density of nerve fibers in the interfollicular areas compared with older animals. Taken together these factors could contribute to the efficient dissemination of scrapie in VRQ/VRQ lambs. While experimental scrapie infection was found not to influence the density of tissue innervation, the study supports previous findings that PrP_{Sc} laden IPP follicles are innervated.

P3.44 Human transgenic mice inoculated with four different cases of sporadic CJD

Authors
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[1]ISS; [2]NCJDSU.

Content

Background: A cooperative study under NeuroPrion context renders human knock-in transgenic mice (HuTg) available to the scientific community for the characterization of a panel of sporadic Creutzfeldt-Jakob (sCJD).

Objective: To characterize prion strains associated with "typical" and "atypical" forms of sporadic CJD (sCJD) through inoculation of human samples into 3 transgenic lines expressing human PrP carrying homozygous methionine (M), homozygous valine (V), or heterozygous MV at the polymorphic codon 129.

Methods: One typical case of sCJD (MM at codon 129 with PrPTSE type 1, MM1) and three atypical cases (MV1, MV1/2 and MV2) were intracerebrally inoculated in MM, VV and MV HuTg mice. Clinical score, attack rate, time of survival, and PrP type were assessed for each mouse.

Results: Though the experiment is still running, each line of inoculated HuTg mice showed animals with clinical signs of prion disease. We observed:

1. The "typical" MM1 sCJD case easily transmitted to all lines of HuTg mice with remarkably similar survival times (from 327 to 372 days) and attack rates close to 100% in male mice. Significant longer survival times were registered for females.

2. The "atypical" sCJD MV1/2 and MV2 readily transmitted to VV transgenic mice with short survival time (less than 300 days) and 100% attack rate. MM transgenic mice were equally affected but showed an almost doubled survival time. Statistical significant differences were seen among sexes with female mice less susceptible or with longer incubation times than males when the inoculum was MV2 sCJD, the contrary happened with the MV1/2 sCJD "strain".

3. The "atypical" MV1 sCJD case has shorter survival time in MV HuTg male mice (~ 300 days, PrP positive), compare to females (about 50% survival at 500 days). MM and VV HuTg mice of both sexes are much more resistant (50% survival at about 400 days).

Discussion: We confirm that human transgenic mice are useful for the study of CJD. The longer survival times observed in the majority of female mice is similar to what has been reported in sporadic CJD, though it is still unclear the molecular mechanism for this difference in susceptibility.

P3.45 Comprehensive risk assessment of Chronic Wasting Disease (CWD) transmission to humans using non-human primates

Authors

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Content

Background: The rapid spread and high prevalence of CWD in North American cervids have raised concerns about a potential threat to human health. Experimental transmission of CWD into cattle, transgenic mice and non-human primates has been reported, and evidence is accumulating that skeletal muscles might harbour significant amount of prion infectivity. This is of great importance to consumers of venison, velvet and other cervid products, and may pose a risk to hunters while fielddressing deer, elk or moose.

Objective: We will assess the risk of primary CWD-transmission into humans through meat consumption or hunting.

Methods: Cynomolgus macaques (Macaca fascicularis) will be inoculated with brain and muscle homogenate of CWD whilte-tailed deer by intracerebral, per oral and dermal scarification. The risk of secondary CWD transmission via blood or blood-derived products will be assessed by blood transfusion of monkey-adapted CWD to naïve recipients. To characterize the CWD inocula used for monkey infection, biochemical methods, in vitro protein misfolding cyclic amplification (PMCA), in vivo mouse and hamster models and cell culture amplification systems will be applied. Potential strains in the original CWD preparation will be identified by hamster inoculation and FT-infrared spectroscopy of PrP_{Sc}.

Expected Results: The results of our risk assessment study will greatly contribute to policy decisions including monitoring of human blood products, CWD surveillance and CWD control in farmed and wild cervids.

P3.46 Confocal microscopy study about the cellular involvement in cerebellum from a natural Scrapie model

Authors

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Content

Astroglial proliferation associated with PrP_{Sc} deposition (preceding neuronal dysfunction) is widely described in TSEs. However, little is known about the real role that is played by glia in the pathogenesis of this neurodegenerative disease.

The aim of the present study is to determine whether PrP_{Sc} is located exclusively in neuronal or in both neuronal and glial cells present in the central nervous system from a natural Scrapie model. A portion of cerebellum from a total of ten Scrapie sheep from different flocks were sectioned with a vibratome, treated with formic acid and incubated with proteinase K. Following heat treatment, L42 and glial fibrillary acidic protein (GFAP) were applied as primary antibodies, prion and astrocytic specific markers, respectively. For visualization, a suitable mix of fluorochrome-conjugated secondary antibodies was used. Controls with secondary antibodies alone and belonging to control healthy animals were processed in the same manner. As determined by immunohistochemistry assessed by confocal microscopy prion protein deposits co-localize with glial cells in all samples analysed. These results suggest that these cells can sustain active prion propagation, agreeing with other authors who described similar findings in primary cell cultures and inoculated mice. Some controversy nevertheless persists about whether different TSE sources show differences in their tropism for different cell lineages in the brain of affected animals. The possible differences in processing of the PrP_{Sc} presented by different TSE agents in the natural host are discussed here.

P3.47 Three serial passages of BSE in sheep do not significantly affect discriminatory test results

Authors

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Content

Background: In the UK, during the 1980's, bovine spongiform encephalopathy (BSE) contaminated meat and bone meal was probably fed to sheep, raising concerns that BSE may have been transmitted to, and be re-cycling within, the national flock. A new form of human prion disease, variant Creutzfeldt-Jakob disease (vCJD) arose during the BSE epidemic, and human exposure to BSE-infected tissues has been implicated in vCJD's aetiology. The concern is that sheep BSE could possibly provide another source of BSE exposure to humans via sheep products. BSE is experimentally transmissible to sheep by parenteral inoculation or orally, and the resulting disease is clinically similar to natural scrapie. Immunological techniques; Western immunoblotting (WB) and immunohistochemistry (IHC), have been developed to distinguish scrapie from primary cases of experimental sheep BSE by the characteristics of their respective abnormal, disease-associated prion proteins (PrP_d). However, naturally transmissible BSE in sheep would now be several generations on from the original exposure, and therefore it is a possibility that serial passage of BSE from sheep to sheep might affect the discriminatory potential of these techniques.

Objective: This study compares the WB and IHC characteristics of PrP_d from brains of primary, secondary and tertiary experimental ovine BSE cases with those of cattle BSE and natural sheep scrapie.

Results and Conclusion: The IHC immunolabelling features were the same for each of the passages of BSE in sheep. Although the WB analyses of the ovine BSE samples showed a significant tendency towards the characteristics of scrapie molecular profiles as the passage number increased, discrimination between experimental sheep BSE and scrapie was still possible, by both methods, regardless of the route of challenge.

This study was funded by DEFRA, UK.

P3.48 Prion infection of mice transgenic for human APP

Authors

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[1]RKI, [2]PFIBR.

Content

Neuropathological, epidemiological, and experimental data suggest a potential interrelationship between Alzheimer´s disease and prion diseases. Most recently, proteolytic processing of amyloid precursor protein (APP) by β-secretase was suggested to be controlled by prion protein expression. Here, we characterized the prion-infection of Tg2576 mice, which overexpress the human APP Swe protein. The prion infection of Tg2576-mice led to an early death of the animals, which was preceded by a relatively short symptomatic stage. However, the disease-associated gliosis und deposition of misfolded prion protein PrP_{Sc} were identical in infected Tg2576-mice and non-transgenic littermate controls. To analyze the effect of prion infection on APP processing and generation of β-amyloid we determined cortical levels of soluble and fibrillar forms of β-amyloid (1-40) and (1-42) by ELISA. Insoluble formic acid (FA) extractable Aβ (1-42) levels were 10-fold higher in infected versus uninfected Tg2576 mice whereas other forms of Aβ were essentially unaffected by the prion-infection. Hence, the experimental model demonstrates that a prion infection of the CNS promotes selectively formation of FA-extractable Aβ(1-42) in Tg2576 mice.

P3.49 Proteomic profiling of PrP27-30 enriched preparations extracted from brain of hamsters with experimental scrapie

Authors

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Content

Background: Pathological PrP has a central role in the etiopathogenesis of transmissible spongiform encephalopathies (TSEs), but other factors are likely involved in the processes that lie beneath the multiplication of infectivity and the development of the disease in infected individuals. The identification of these factors might provide information on the molecular pathology of TSEs as well as on novel therapeutic targets for this still untreatable group of diseases.

Objective: Aim of this work is to identify protein factors that co-purify with the protease-resistant core of pathological PrP (PrP27-30) extracted from the brain of hamsters experimentally infected with the 263K strain of scrapie.

Methods: Protein profiling was performed on PrP27-30 enriched fractions extracted (Silvestrini M.C. et al. Nat. Med. 1997; 3(5): 521-525) from brains of Syrian golden hamsters, intracerebrally inoculated with the 263K strain of scrapie. Three complementary proteomic strategies were employed: two-dimensional electrophoresis mapping, in toto MALDI-TOF/TOF analysis, and in toto LC-MS/MS analysis with the high resolution FT-ICR mass spectrometer.

Results: Our study shows that various proteins are strictly associated with pathological PrP27-30. Major components are ferritin, calcium/calmodulin-dependent protein kinase type II alpha chain, apolipoprotein E, and tubulin. The highly sensitive FT-ICR mass spectrometer identified several other cellular components.

Discussion: The experimental approach of complementary proteomic strategies is particularly suitable for unraveling the protein composition of PrP27-30 aggregates, which appear to be highly complex and heterogeneous. Our results are the basis for subsequent experiments aimed to clarify the role of these proteins in the pathogenesis of TSE diseases.

P3.50 Distribution of infectivity in the oral tissues of VM mice and the potential risk of vCJD transmission through dentistry

Authors

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Content

Background: Ongoing concerns about the prevalence of variant Creutzfeldt-Jakob Disease (vCJD) in the UK population have heightened awareness of the iatrogenic transmission risks of the disease. Whilst the individual risk associated with dentistry may be very low, the extensive number of procedures carried out annually amplifies this significantly. There is limited data in this area to form the basis for accurate risk assessments.

Objective: To assess the relative levels of infectivity in oral tissues from a murine model following exposure to BSE-301V through the small intestine.

Methods: The study used a clinically relevant model of BSE-301V and VM indicator mice of known susceptibility to assess the potential for iatrogenic transmission of vCJD between humans. Infectious mouse brain homogenate was inoculated directly into the lumen of the small intestine in the region of the duodenum, to ensure no direct contamination of the oral tissues. The mice were killed at 3-weekly intervals over a 24 week time-course and a range of clinically relevant oral tissues (including lingual tonsil, dental pulp and gingival margin) were removed, processed and re-inoculated intracranially (i.c.) into indicator mice to observe for infectivity.

Results: The primary challenge proved to be a very efficient route of infection with a 100% attack-rate and a mean incubation to clinical disease of 157 ± 17 days (c.f. 120 days for the equivalent titre inoculated i.c.). Infectivity was observed in all oral and control tissues at a range of titres during both pre-clinical and clinical stages of the disease.

Discussion: This study demonstrates the spread of prion infectivity from the small intestine to the oral cavity in the mouse. The results demonstrate that a range of murine oral tissues contain variable amounts of infectivity. This supports the ongoing requirement for risk assessments looking at the potential for vCJD transmission via dental procedures and associated studies looking at the effectiveness of decontamination and re-use of dental instruments.

P3.51 Human and Animal Enterocytes as a model system to mimic the first entry barrier for prions in the body

Authors

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Content

The phenomenon that the transmission of TSEs from one species to another results in prolonged incubation times and survival is a major criterion for the "species barrier". This barrier is due to the variety of prion strains with their different infectivity. The barrier of an interspecies transmission is so strong that peripheral injection, oral transmission and sometimes even an intracerebral transmission with the TSE agent fails to develop TSE symptoms. Until today, BSE is the only known TSE that was transmitted to humans, causing the zoonotic disease vCJD. Recently, we found, that BSE-derived prions bound to and became internalized by human enterocytes via the 37 kDa/67 kDa laminin receptor LRP/LR (1), acting as a receptor for the cellular prion protein PrP^{c} (2) and infectious PrP^{Sc} (3). We investigate the species-specific entry barrier in the enterocyte cell system addressing the question whether different animal prion strains such as scrapie in sheep and chronic wasting disease (CWD) in cervids might have the potential to cause a new zoonotic disease. The role of LRP/LR in the binding and internalization processes is investigated by the use of anti-LRP specific antibodies such as W3 (4, 5) and scFv S18 (6). Moreover, we show cross-species binding and internalization studies on human and animal enterocytes such as FBJ (bovine), DWM-R (cervid), IPEC-J2 (porcine) and DOMI-1 (ovine primary enterocytes) with human and animal prions (7). Our data confirm a LRP/LR specific binding of BSE prions on human Caco-2/TC7 enterocytes. Furthermore we found a colocalization of prions from white-tail deer suffering from CWD and sheep Scrapie prions with the 37 kDa/67 kDa LRP/LR on the surface of human Caco-2/TC7 cells, suggesting that CWD and sheep scrapie might have the potential to cause a further zoonotic disease. With this in vitro cell system we mimic one of the first steps of prions entering the organism. The intestine might represent the crucial barrier for prions deciding upon the development of a zoonotic prion disease. (1) Morel et al. (2005) Am. J. Path. 167, 1033-1042. (2) Gauczynski et al. (2001) EMBO J. 20, 5863-5875. (3) Gauczynski et al. (2006) J. Infect. Dis. 194, 702-709. (4) Leucht et al. (2003) EMBO Rep. 4, 290-295. (5) Zuber et al. (2007) Prion, 1:, 207-212. (6) Zuber et al. (2008), Mol. Immunol. 45, 144-151. (7) Kolodziejczak et al., submitted.

P3.52 PrP^{Sc} detection and distribution in an Ojinegra sheep carrying an ARK haplotype

Authors

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Content

Background: The strategy for scrapie control / eradication in the EU has been mainly based on I) large-scale surveys for estimating the prevalence of the disease, II) implementation of Breeding Programmes for TSE resistance in sheep and III) complete depopulation of infected flocks or depopulation of all susceptible animals. Large-scale genotyping surveys in different countries have identified rare haplotypes, such as ARK. The influence of those rare haplotypes on scrapie susceptibility and pathogenesis remains unknown.

Objective: To describe the PrP^{Sc} distribution on a naturally scrapie infected sheep carrying an ARK haplotype.

Material and Methods: A scrapie outbreak was detected in a flock of the Ojinegra breed in the framework of the National TSE monitoring programme. In accordance with the EU regulations, all animals of the flock were genotyped (using primer extension analysis technique by a SnaPshot Multiplex Kit) and a partial culling of the flock was carried out. A 3 year old asymptomatic male with an undetermined genotype was selected for complete Open Reading Frame *PRNP* sequencing, showing finally ARK/ARQ genotype. The animal was euthanized and representative tissues were collected for immunohistochemical analysis using L42 antibody.

Results: Widespread deposition of PrP^{Sc} was detected mainly in lymphoreticular system (LRS), including the gut-associated lymphoid tissue, spleen, tonsils and peripheral lymphoid nodes. In the central nervous system, positive immunostaining was detected in lumbar, thoracic and cervical spinal cord, medulla oblongata and brainstem, but less prominent than in LRS.

Discussion: This result confirms that the ARK haplotype apparently does not confer resistance against scrapie and suggest that pathogenesis of scrapie in sheep carrying this haplotype is similar to those carrying ARQ haplotype with respect to the lymphatic spread of the PrP^{Sc}. These results should be considered when it comes to apply scrapie eradication and breeding programmes.

P3.53 Immunophenotyping and PrP_d detection in lymphoid tissues from sheep experimentally challenged with scrapie

Authors

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Content

Background: The involvement of the immune system has been established in natural scrapie and in animals experimentally inoculated via peripheral routes. However, there are few detailed in vivo studies which have focused on the immune system involvement in the natural host - the sheep.

Objective: To detect differences in the number of leucocytes in peripheral lymph nodes from sheep with varying levels of PrP_d deposition which were inoculated with scrapie.

Methods: Sixteen 6 month old Suffolk lambs (ARQ/ARQ) received 1 ml of 5 % clarified Suffolk ARQ/ARQ scrapie brain pool homogenate injected subcutaneously in the region draining the right prefemoral lymph node. At 18 months post inoculation, during the pre-clinical stage of infection, 11 of the animals were culled. The remaining five animals were left to progress to clinical scrapie. All tissues collected at post-mortem examination were subjected to immunohistochemistry (IHC) to detect the abnormal form of prion protein (PrP_d). On the basis of the intensity of PrP_d deposition (none, low and moderate) two lambs from each category were selected from the 18 month cull and in addition two animals with clinical disease were also included. Immune system cell markers: CD21, CD4, CD8, CD3, CD79 and gamma delta TCR were then detected using IHC in zinc salt fixed tissue from selected lymphoid tissues. The intensity of distribution of each marker was then scored from 1-5 (1 low and 5 high) in the medulla, cortex and follicles of the lymph nodes and white and red pulp of the spleen. The scores were analysed using Genstat statistical software.

Results: There was strong evidence of an increased number of CD21 cells present in the lymphoid follicles of animals with high levels of PrP_d deposition and had reached clinical disease. However, there were significantly less CD79 cells in follicles of these same animals. The numbers of T cells, either CD3, CD4, CD8 or gamma delta, was not found to be related to the level of PrP_d deposition for any of the tissues or specific areas of the tissues examined.

Discussion: Results from this study support previous findings which have shown that the CD21 cell populatin plays a central role in the pathogenesis of scrapie in ovine and murine models.

P3.54 PrP^{Sc} distribution in perfused placentas from suffolk sheep naturally infected with scrapie

Authors

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Content

Background: Lambing time is considered to be important for the natural transmission of scrapie. Although a range of reproductive tissues has been examined previously, further investigation is required to establish the amount of scrapie agent to which sheep are exposed during this time.

Objective: In this study we investigate the distribution of PrP^{Sc} in whole placentae and assess the PrP^{Sc} levels in comparison to brain.

Materials and Methods: Three Suffolk ewes from a flock with a high incidence of natural scrapie which showed no clinical signs were selected according to genotype and PrP_d status. Each ewe had two foetuses as a result of being mated with an ARQ/ARQ ram. Two ARQ/ARQ ewes, (one confirmed PrP_d positive by rectal biopsy and the other negative) and one ARQ/ARR resistant genotype were euthanased and the foetuses and placentae perfused with PBS/EDTA by cannulation of the middle uterine arteries and umbilical vessels. Each placenta was examined for PrP^{Sc} by western blot and immunohistochemistry. Comparison of the level of PrP^{Sc} signal between brain and placenta was assessed using densitometric analysis of western blots. Placentae from the ARQ/ARR ewe were used as negative controls.

Results: From the biopsy positive ARQ/ARQ ewe 100% positivity for PrP^{Sc} was observed throughout both placentae and also the levels of PrP^{Sc} signal were lower in both when compared with the brain of the ewe. The biopsy negative ARQ/ARQ and ARQ/ARR ewes were negative for PrP^{Sc} in both placentae and brain.

Discussion: This study showed that in the ewe which was PrP_d positive by rectal biopsy, the PrP^{Sc} was distributed throughout both placentae. Though the levels of PrP^{Sc} in placentae were lower when compared with brain, the amount of PrP^{Sc} positive tissue present suggests that the placenta may play a significant role in the dissemination of infection.

P3.55 Feline Spongiforme Encephalopathy in a cheetah from a German zoo: immunohistochemical and biochemical examinations

Authors

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Content

Background: The Feline Spongiform Encephalopathy (FSE) was first reported in United Kingdom (UK) in 1990 in domestic and in various species of wild cats which were kept in zoological gardens. FSE is probably caused by an accidential BSE infection of the animals by feeding them with infectious foodstuffs. The majority of cases has been found in the United Kingdom, but single cases were also diagnosed in felines in Switzerland, Norway, France and in Ireland.

Objective: Report on the first FSE case in a cheetah which was kept in a zoological garden in Germany.

Material and Methods: The nine-year-old female cheetah was born and spent her first years of life in a zoological garden in the Netherlands. It was then aquired by the Berlin Zoo. For its last two years it was exhibited in the Zoological Garden in Nürnberg. In 2007, over a period of eight weeks, the animal developed neurological symptoms like ataxia and progressive bilateral hindlimb lameness. The cheetah was eventually euthanised and necropsied because of animal welfare reasons.

Results and Discussion: The histopathology of the brain stem revealed a moderate spongiform encephalopathy. Due to the clear accumulation of PrP^{Sc}, detected by both immunohistochemistry and western blot, FSE was diagnosed. The analysis of the glycotyping and PK cleavage site patterns using a discriminatory immunoblot (FLI-Test) showed that PrP^{Sc} in this case clearly harboured BSE-like properties, which were distinct from those of scrapie. The immunohistochemical examination of formalin fixed tissues samples revealed a wide distribution of the PrP^{Sc} deposition in the central and peripheral nervous system as well as in the lymphoreticular system and in other tissues, suggesting an early and simultaneous haematogenous, lymphatic and nerval spread of the FSE prions in the body of the affected animal.

P3.56 Primary transmission of ovine prions of different PrP genotypes is associated with accumulation of distinct conformers of PrP^{Sc}

Authors

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Content

In order to begin to establish a more biochemical approach to ovine prion strain typing we have compared the molecular and transmission characteristics of ovine ARQ/ARQ and VRQ/VRQ scrapie isolates following primary passage in tg338 (VRQ) and tg59 (ARQ) ovine PrP transgenic mice and the conventional mouse lines C57BL/6, RIII and VM. We have found that these different genotypes of scrapie isolates display similar incubation periods of 350 days in conventional and tg59 mice. In contrast, facilitated transmission of sheep scrapie isolates occurred in tg338 mice with incubation times for VRQ/VRQ inocula reduced to 64 days, and to <210 days for ARQ/ARQ samples. Distinct genotype-specific lesion profiles were seen in the brains of conventional and tg59 prion-diseased mice, which was accompanied by the accumulation of significantly more PrP^{Sc}, following inoculation with ARQ/ARQ compared to VRQ/VRQ scrapie isolates. In contrast, the lesion profiles and quantities of PrP^{Sc} induced by the same inocula in tg338 mice were more similar than in the other mouse lines. Using a denaturant-dependent conformational stability assay we have found that the conformational stability of the accumulated PrP^{Sc} correlated with its glycoform profile. Transmission of ARQ homozygous scrapie isolates induced the accumulation of PrP^{Sc} characterised by a predominance of di-glycosylated PrP^{Sc} that was significantly more stable than that associated with the passage of VRQ homozygous inocula, which had similar levels of di- and mono-glycosylated PrP^{Sc}. These novel observations show that primary transmission of different genotypes of ovine prions is associated with the formation of different conformers of PrP^{Sc} with distinct molecular properties and provides a biochemical basis for ovine prion strain typing.

P3.57 Intestinal inflammation enhances prion susceptibility

Authors

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Content

Ingestion of prion-contaminated foodstuffs has led to prion transmission in humans (kuru and variant Creutzfeldt-Jakob disease), as well as cattle, mink, and cats. Dietary exposure to bovine spongiform encephalopathy (BSE)-contaminated beef is believed to be the cause of less than 200 human prion infections, although human exposure to the BSE agent was likely to have been massive. Thus the epidemiology of dietary prion infections suggests that genetic modifiers, and possibly exogenous cofactors, may play a crucial role in determining susceptibility to disease. However, only few cofactors influencing dietary prion susceptibility have been identified. Here we investigated whether bacterial colitis might represent one such cofactor. We found that a moderate bacterial colitis induced by an attenuated strain of *Salmonella enteritica typhimurium* more than doubles the susceptibility of mice to orally-induced prion infection, and accelerates clinical disease development after prion challenge. PrP^{c} upregulation occurred in intestines and mesenteric lymph nodes of mice with colitis, suggesting a mechanism for the impact of colitis onto prion pathogenesis. Therefore, moderate intestinal inflammation at the time of prion exposure may constitute a risk factor for prion infection.

P3.58 BSE transmission to lambs born from infected or contact ewes

Authors

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Content

Background: Scrapie transmission in infected sheep flocks essentially occurs during the perinatal period, through contamination of the environment by infected placentas and Scrapie agent excretion in milk, whereas in utero transmission has not been demonstrated.

Objective: Investigating the possibilities of vertical and horizontal transmission of BSE in sheep flocks, from incubating ewes to their own lambs or to contacts born simultaneously.

Methods: Susceptible ARQ/ARQ TSE free ewes were orally inoculated with ovine BSE (5g of brain homogenate). They were mated 6 months later at the same time than healthy ewes with susceptible rams. At birth of the first lamb from an infected ewe (11 months post inoculation), 5 infected and 5 healthy ewes were allowed to deliver in the same enclosure. Placenta cotyledons, colostrums, milk and blood were sampled. All lambs were weaned at 60 days and maintained as males and females groups until slaughtering at 23 months of age.

Results: Ewes were necropsied when 2 among them became clinical, 20 months pi. PrPBSE was detected in most lymphoid and nervous tissues from 4 out of the 5 inoculated ewes using ELISA, WB and IHC. Only 3 lambs from infected ewes were born alive, whereas they were 6 contacts born from healthy ewes. Two lambs developed BSE, from an infected ewe and a healthy one, respectively. Both were at similar stage of the infection, beginning of neuroinvasion, with a pattern of PrPBSE deposits characteristic of dissemination from the intestine tissues, suggesting an oral route of contamination during the perinatal period.

Discussion: Discussion. These results are pointing out the possibility of BSE vertical and horizontal transmission in sheep flocks, through routes of dissemination similar to the ones described for Scrapie. PrPBSE has neither been evidenced in placenta cotyledons nor in milk. However, retromammary lymph nodes from infected ewes were highly positive. Investigations on the infectious status of these tissues are underway using mouse bioassays.

Supported by EU contract QLRT-01309; Defra is acknowledged for supplying TSE free sheep; the animals were maintained at level 3 of bio-confinement by INRA-PFIE Tours.

P3.59 Detection of abnormal PrP (PrP_{TSE}) in cell substrates exposed to TSE agents

Authors

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Content

Title: Detection of abnormal PrP (PrP_{TSE}) in cell substrates exposed to TSE agents.

Background: Expression of normal PrP (PrP_C) is required for susceptibility to TSE infection and disease. PrP_C is expressed in several tissues, however PrP_{TSE} accumulates mainly in nervous and lymphoid tissues. PrP_{TSE} is often associated with TSE infectivity and has been proposed as the infectious agent of TSE or prion.

Objective: Priola et al. (Vorberg et al. JBC 2004) suggested that failure of TSE agents to infect many cell types might result from cells' inability to support formation of newly generated PrP_{TSE}. We are investigating ability of neural-derived and renal-derived cell lines to accumulate newly generated PrP_{TSE}.

Methods: We inoculated (i) bacteria-free filtrates of sporadic CJD brain extract into human neuroblastoma-derived cells (SHSY5Y) overexpressing a mutation found with familial TSE in humans (SHSY5YE200K) and (ii) 263K scrapie agent into hamster kidney-derived (BHK-21) cell lines. We tracked PrPTSE detected in exposed cells following serial passaging.

Results: Cells exposed to TSE agents were serially propagated, and selected passages tested for PrPTSE by Western blotting (WB). To increase sensitivity of detecting PrPTSE, we also assayed cell extracts by the protein misfolding cyclic amplification (PMCA) technique followed by WB of generated products (Saborio et al. Nature 2001). We optimized PMCA reaction conditions using 263K scrapie brain extracts with repeated additions of normal hamster brain suspension and sonications as described. In agreement with previous findings, we detected PrPTSE through 12 cycles of PMCA amplification. Interestingly, we repeatedly detected PrPTSE in dilutions of 263K scrapie hamster brain extracts through 10-3 by conventional WB and at the same dilution by PMCA. We have also initiated bioassays of selected PMCA products to compare detection of PrPTSE and infectivity. We found no PrPTSE in SHSY5Y cells after 30 passages. BHK cells exposed to 263K scrapie agent contained detectable PrPTSE in samples from passages 0 and 1 but not subsequent serial passages.

Discussion: BHK cells contained PrPTSE detectable by WB immediately after exposure to 263K scrapie agent and for a few days after initial passage but not in samples from later passages. The PrPTSE we detected was most likely a residue from the inoculum. Bioassays of exposed cells and PMCA products are in progress.

P3.60 Astrogliosis signaling and neuronal generation in brains of scrapie-infected mice

Authors

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Content

The transmissible spongiform encephalopathies (TSEs) including scrapie in sheep and in rodent models are characterized by pathological findings such as accumulation of pathogenic prion protein, neuroparenchymal vacuolation, neuronal loss, and astrogliosis in brains of affected animals. In this study, we investigated signaling pathway of astrogliosis and neuronal generation in brains of scrapie-infected mice. To determine the signaling pathway of astrogliosis in scrapie-infected brains, we investigated the expression levels and cellular localization of JAK-STAT signaling molecules and growth factors such as leukemia inhibitory factor (LIF) and ciliary neurotropic factor (CNTF) by Western blot analysis and immunohistochemistry. We found that expression levels of LIF and CNTF were increased in scrapie-infected brains, and phosphorylated (p)-JAK2, p-STAT1 (ser727, tyr701), p-STAT3 (tyr705) and GFAP were expressed strongly in scrapie-infected brains. Moreover, we found that p-STAT1 and p-STAT3 were found mainly in the nucleus in scrapie-infected brains. Immunohistochemically, p-STAT1 was colocalized with LIF, CNTF and p-JAK2 in many reactive astrocytes in scrapie-infected brains. In contrast, immunostaining for p-STAT3 was found in comparatively few astrocytes in limited regions; p-STAT3 staining merged with p-JAK2 in hippocampus sections of scrapie-infected brains. Next, to investigate the generation of neuron after scrapie-infection, we infused 5-bromo-22-deoxyuridine (BrdU), a DNA replication indicator, into both control and scrapie-infected mice. We found that the number of BrdU-labeled cells was observed in the striatum, hippocampus, and brain stem of scrapie-infected brains. Interestingly, increased BrdU-labeled cells were remarkably detected in the hippocampus of scrapie-infected mice compared to controls. We also found that BrdU positive cells were colocalized with the neuronal markers such as NeuN and MAP2, whereas BrdU staining was not merged with GFAP, an astrocytic marker. Taken together, our results suggest that scrapie-infection induces activation of JAK2-STAT1 signaling pathway and region-specific increase of neuronal generation.

Acknowledgements: This work was supported by the MRC program of MOST/KOSEF (R13-2005-022-01002).

P3.61 Protein citrullination in scrapie-infected mice: a possible role in pathogenesis

Authors

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Content

Peptidylarginine deiminases (PADs) are involved in protein citrullination (deimination) by the conversion of peptidylarginine to peptidylcitrulline in a calcium-dependent manner. Among the PADs group, PAD2 is widely distributed in various tissues and is the only type which is expressed in brain. To elucidate the involvement of protein citrullination by PAD2 in the pathogenesis of prion diseases, we examined the profiles of citrullinated proteins using the brains of scrapie-infected mice. We have found that, compared to controls, increased levels of various citrullinated proteins were detected in different brain sections of scrapie-infected mice. Supporting this data, expression level of PAD2 protein and its mRNA was significantly increased in scrapie-infected brains; the immunoreactivity was detected mainly in reactive astrocytes. In addition, enzymatic activity of PAD2 was increased during scrapie infection. Using 2-DE and MALDI-TOF mass spectrometry, various citrullinated proteins were identified in the brains of scrapie-infected including GFAP, myelin basic protein, and several glycolytic enzymes such as enolases and aldolases. We have found that the expression level of aldolase C was significantly increased in most of brain sections of scrapie-infected mice although its enzymatic activity did not changed compared to controls. The results for neuron-specific enolase showed slightly decreased expression levels in cerebellum and hippocampus of scrapie-infected mice. Its enzymatic activity was significantly decreased in hippocampus and cerebellum and increased in cerebral cortex of scrapie-infected mice. This study suggests that accumulated citrullinated proteins and abnormal activation of PAD2 may play a role in the pathogenesis.

[This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund)(KRF-2006-331-E00287).]

P3.62 Strain-dependent cellular markers of prion infection in cultured GT1-1 cells

Authors
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Content

Background: Prion strains differ profoundly in the way they interact with the host animal, which is reflected in variations in their brain tropism and neuropathological changes. At the cellular level, a number of molecular changes have been described in cell lines infected with the RML strain of scrapie. It was therefore hypothesized that prion strains may be characterized by signatures of biomarkers during infection.

Objective: We addressed the question whether different prion strains can cause different molecular changes in a cell line.

Methods: Prion-infected gondotropin-releasing hormone neuronal cells (GT1-1) were analyzed by Western blot for expression of insulin-like growth factor-1 receptor (IGF-1R) and SNARE proteins, which have shown changes after infection with the RML strain in neuroblastoma N2a (JBC 276:36110, 2001) and GT1-1 cells (JBC 280:1264, 2005), respectively. The expression of these molecules following infection with the RML strain was compared with that of 22L and Me7 strains at the same time after infection. Presence of PrP_{Sc} was studied by immunofluoresence and Western blot using the D13 antibody.

Results: GT1-1 cells showed large amounts of PrP_{Sc} in all cells after infection with the 22L strain, lesser after infection with the RML strain and after infection with the Me7 strain only some cells showed immunopositivity. Compared to RML infection, the levels of IGF-1R and syntaxin were increased after 22L infection, while the levels were decreased after Me7 infection. Synaptotagmin was instead decreased after RML, 22L and Me7 infection in comparison to non-infected control cells.

Discussion: These experiments show that different prion strains can induce differences in biomarker signatures in a cell line. Whether this reflects differences in intrinsic pathogenetic properties of the strains or in infectivity remains to be determined. (Supported by EC FP6-2004-F0OD-3-B-023183).

P3.63 Experimental transmission of chronic wasting disease (CWD) agent from imported elk in Korea to TgElk mice

Authors
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Content

Background: Chronic Wasting Disease (CWD), a transmissible neurodegenerative disease affecting cervids, is classified as one of the transmissible spongiform encephalopathies or prion diseases. CWD has been reported in at least 14 US states, 2 Canadian provinces and in imported elk in several farms in Korea.

Objective: The aim of this study was to document the entity of CWD agent through imported Canadian elk in Korea and to study the pathogenesis of CWD-affected TgElk mice.

Methods: The homozygous TgElk mice were infected with a CWD agent that is originated from first outbreak case in Korea by importing Canadian elk and then examined the incubation time of disease, neuropathological changes by immunohistochemical staining, the pattern(s) of PrP_{Sc} deposition and PrP_{Sc} Western blot profiles.

Results: Here, we found that TgElk mice infected with brain homogenate from CWD-affected elk showed shorter incubation time and more severe vacuolar degeneration than previous reports. In addition, the number of PrP_{Sc} deposition as a hallmark in prion diseases was significantly increased.

Discussion: These results suggested that homozygous TgElk mice efficiently transmitted CWD agents with a short incubation time can be used as a valuable research model of cervid prion as well as a reliable in vivo diagnostic tool.

P3.64 Identification of prion-specific gene expression changes using the neurotoxicant cuprizone as an experimental control

Authors
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Content

Background: The identification of genes expressed in response to prion infection may elucidate biomarkers for disease progression, agent replication, mechanisms of neuropathology and therapeutic targets. Several groups have sought to identify gene expression changes that are specific to prion disease, but have consistently identified expression profiles rife with neuroinflammatory changes. Cuprizone, a neurotoxicant, mimics the neuroinflammation observed in prion disease, causing both spongiform change and astrocytosis.

Objective: The use of cuprizone-treated animals as an experimental control during comparative expression profiling allows for the identification and removal of gene expression changes related to general neuroinflammation, with the remaining changes specific to prion disease.

Methods: Brain gene expression from C57Bl/6 mice infected with RML mouse-adapted scrapie agent and age-matched controls were profiled using Affymetrix gene arrays. Profiles of RNAs from pre-clinical and clinically infected animals were analyzed by robust multi-array analysis. A 0.4% cuprizone diet was utilized as a control treatment for comparative expression profiling.

Results: Of the 39,000 genes whose expression was measured, 128 transcripts were up-regulated at end-stage while 71 were down-regulated. Functional gene ontology was used to identify processes that changed in response to prion disease. The majority of the cellular processes identified related to the characteristic neuroinflammation known to occur during the progression of prion disease. Cuprizone treatment induced spongiosis as well as astroctye proliferation as indicated by glial fibrillary acidic protein (GFAP) transcriptional activation and immunohistochemistry.

Discussion: Through the use of cuprizone controls, we eliminated RNA changes related to neuroinflammation and have identified novel, neuronally-expressed RNAs that increase in abundance during prion infection.

P3.65 Cryo-immunogold EM for prions in the hippocampus

Authors
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Content

The subcellular distributions of PrP_C and PrP_{Sc} in the prion-infected brain, and the mechanisms and sites of PrP_{Sc} formation are still unclear. We aimed to identify sites containing high levels of PrP_{Sc} in hippocampal sections of mice infected with the RML prion strain, to obtain clues about how prion propagation occurs in this tissue.

We developed methods for comparing the distributions of PrP_C and PrP_{Sc} by cryo-immunogold EM, using two antibodies with different specificities and statistical analysis. We also performed immunofluorescence on semi-thin cryo-sections to look for co-localisations of prion protein with different structures. Antibodies were identified that recognised only PrP_C, and PrP_C plus PrP_{Sc}. At a late sub-clinical stage of prion infection, both PrP_C and PrP_{Sc} were detected principally on neuronal plasma membranes and on small vesicles in the neuropil. Quantification of the labeling in the stratum oriens showed that the labeling of PrP_C alone did not increase as a result of prion infection, whereas the labeling of PrP_C plus PrP_{Sc} was approximately six fold higher in the infected than the uninfected tissue. Clusters of gold labeling were found only in the infected tissue. The biggest increases in labeling density were found in small vesicles (24 fold) and on the plasma membranes (8 fold) of neurites measuring less than 250 nm across in the sections ("small" neurites). The vesicles resembled early endocytic or recycling vesicles. No evidence was found for an accumulation of PrP_{Sc} in neuronal lysosomes or late endosomes, although astrocytic lysosomes containing PrP were sometimes observed. No evidence of amyloid was found in the hippocampus at this stage. The relatively high abundance of PrP in "small" neurites, at the plasma membrane and in vesicles resembling early endocytic or recycling vesicles, suggests that these sites may be important for PrP_{Sc} formation or toxicity.

P3.66 Studies on the coinfection with scrapie and visna-maedi virus in ovine natural cases

Authors
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Content

Background: In natural scrapie PrP_{Sc} normally accumulate in nervous and lymphoid tissue but it is known that PrP_{Sc} can also be found in "no target" organs suffering from chronic inflammation in both natural and experimental conditions. Recently, PrP_{Sc} has also been found in "no target" organs without inflammatory evidence.

Objective: The main objective of this study is to describe the tissue distribution of PrP_{Sc} in sheep naturally coinfected with scrapie and visna-maedi virus (VMV).

Methods: We study four groups of animals, coinfected with scrapie and VMV (n=4), infected with scrapie or VMV (n=5 in both) and non infected sheep. This study is specially focused on two VMV target organs: lung and mammary gland. VMV infection was determined by ELISA and histopathology whereas scrapie lesions were studied by histopathology and presence of PrP_{Sc} by immunohistochemistry (mAb L42) and WB (mAb P4).

Results: Preliminary results indicate that PrP_{Sc} is detected in lungs and mammary glands of VMV infected sheep if there is presence of VMV-associated lesions, namely hyperplasia of lymphoid follicles and interstitial inflammatory reaction. So far, PrP_{Sc} has only been found in lymphoid follicles in both tissues and not all follicles showed presence of PrP_{Sc}. Pathologic prion protein has not been found in "no target" organs in any of the other group of animals.

Discussion: In the scrapie and VMV natural coinfected model, the development of chronic lesions associated to VMV, mostly lymphoid follicle formation, is a pre requisite for PrP_{Sc} deposition in "no target" organs such as lungs and mammary glands. Therefore, the VMV associated lesions can modify the known pathogenesis of scrapie disease.

P3.67 Pathogenesis of Chronic Wasting Disease in transgenic mice

Authors
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Content

Background: Chronic Wasting Disease (CWD) is a naturally occurring efficiently transmitted prion disease of cervids. Here we described studies of CWD multi-route susceptibility, tissue tropism, and pathogenesis using two lineages of cervid prion protein expressing transgenic mice (Tg[CerPrP]).

Objective: Our 3 objectives were: (1) to develop immunohistochemical (IHC) protocols for demonstration of PrP_{c} in Tg[CerPrP] mice; (2) to deterrmine the susceptibility of Tg[CerPrP] mice to CWD infection via multiple routes of inoculation; and (3) to develop tyramide signal amplification (TSA) strategies to enhance detection of PrP_{res} in CWD-exposed Tg[CerPrP] mice.

Methods: We used a combination of paraformaldehyde-lysine-periodate (PLP) fixation and the polyclonal antibody R505.5 to determine the distribution of PrP_{c} in two lineages of Tg[CerPrP] mice (1536 and 5037)1-3. Mice were inoculated via either the intracerebral (IC), intraperitoneal (IP), intravenous (IV), or oral (PO) route and PrP_{res} detected by amplified IHC.

Results: Cervid PrP_{c} was expressed in a wide array of tissues, including the nervous, lymphoid, gastrointestinal, and ndocrine systems. After inoculation 1536 and 5037 Tg[CerPrP] mice via either the IC, IV, or IP route (but not he PO route) PrP_{res} was detected by both traditional and TSA-based IHC protocols in a variety of nervous, ymphoid, hematopoietic, and gastrointestinal tissues, and for the first time in salivary gland.

Discussion: Using multiple IHC protocols, we have shown that two lineages of Tg[CerPrP] mice express PrP_{c} in a manner imilar to the native cervid species. Moreover, these mice are susceptible to CWD infection via multiple outes of infection. In addition to neural tissues, of particular interest was the demonstration of PrP_{res} in ancreas, salivary gland, and bone marrow. These findings confirm that in addition to infectivity bioasssy, the tility of Tg[CerPrP] mice extends to study of CWD prion shedding including salivary transfer, trafficking, and he endocrine dysfunction underlies the wasting syndrome.

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P3.68 Mission of deer and elk prions to bank vole

Authors
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Content

Background: Chronic Wasting disease (CWD) is a fatal prion disease of free-raging and captive deer and elk. During the course of infection, the pathological prion protein (PrP_{Sc}) progressively accumulates in nervous tissues and several other tissues. PrP_{Sc} is a useful marker for prion diseases, but its level in a given tissues does not necessarily correlated with the infectivity titer. To determine the infectivity levels of CWD affected tissues many studies were carried out in cervids. Recently, some animal models were transgenic mice expressing cervids prion protein have been demonstrated to be highly susceptible to CWD.

Objective: To investigate the transmissibility of CWD from deer and elk to bank vole (*Myodes glareolus, formely Clethrionomys glareolus*).

Methods: Three different CWD brain sources from deer and one from elk were used for transmission studies to bank vole by intracerebral route. The bank vole is a rodent species which proved to be very susceptible to some animal and human prions. The vole PrP gene is polymorphic at codon 109 codifying either Isoleucine or Methionine. Only Isoleucine homozygous line of voles (named Mg109II) was used for present study.

Results: Remarkably, all of the isolates tested transmitted efficiently, with close mean survival times ranging from 160 to 250 days. Second and third passage of all isolates gave much shorter incubation periods averaging 35 days post-infection. Neuropathological examination and neuroanatomical distribution of PrP_{Sc} analysis presented similar characteristics in all of the voles inoculated with CWD prions from deer and elk.

Discussion: These findings suggest that: 1) Mg109II voles are a promising animal model for bioassay of tissues from deer and elk affected with CWD; 2) CWD adapted strains in Mg109II voles could be interest for studying the biological properties of CWD strains in laboratory animals.

P3.69 Phophatidylinositol-glycan-phospholipase D is down-regulated in brain of a prion disease and associated with prion protein conversion

Authors
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Content

Abstract Background: The abnormal prion protein (PrP_{Sc}) plays vital role in the pathophysiology of prion diseases. PrP_{Sc} is formed from a glycosylphosphatidylinositol (GPI)-anchored prion protein (PrP_C) by posttranslational modification processes. Most of GPI-anchored proteins have been known to be cleaved by GPI phospholipases. Recently, it has been known that GPI-phospholipase D (GPI-PLD) is strictly specific enzyme for GPI anchors.

Objective: In order to investigation the involvement of GPI-PLD in the process of neurodegeneration in prion diseases.

Methods: We examined the expression of GPI-PLD mRNA and protein in the brain of a prion animal model (scrapie) and cerebrospinal fluid (CSF) of human Creuzfeldt-Jakob Disease (CJD) patients using reverse transcriptase polymerase chain reaction (RT-PCR) and Western blot analysis.

Results: We have found that the expression of GPI-PLD was dramatically down-regulated in scrapie brains, especially caveolin-enriched membrane fractions, compared with those of control brains. Interestingly, the decrease of GPI-PLD expression levels was started at the time when the PrP_{Sc} began to accumulate in the brains and its decrease was also observed in the CSF of CJD patients.

Discussion: Thus, these results suggest that the down-regulation of GPI-PLD protein may mediate the posttranslational conversion of the PrP_C into the scrapie isoform of PrP_{Sc} in the brains of scrapie-infected mice.

P3.70 Transmission characteristics of sCJD, vCJD, BSE and Scrapie in transgenic mice expressing human PrP

Authors
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Content

Many epidemiological and transmission studies suggested that BSE has originated the emergence of the new variant of Creutzfeldt-Jacob disease (vCJD) in humans. Polymorphism at residue 129 of the human PrP gene (*PRNP*) seems to influence the susceptibility to both sporadic and acquired human prion diseases. To date all vCJD patients studied are codon 129 methionine homozygous. In this study, transgenic mice expressing human PrP_C of MM129 and VV129 genotype were generated, under the murine PrP promoter in a murine PrP0/0 background. Intracerebral inoculations with sCJD, vCJD, BSE and classical Scrapie isolates were performed. Biochemical characteristics of PrP_{res}, lesion patterns and PrP_{res} depositions in brains were analyzed using western blot, histopathology and immunohystochemistry.

HuPrP-Tg340MM mice seemed to be highly susceptible to MM Type I sCJD showing short survival times. All the mice inoculated scored positive for PrP_{res}. These characteristics were maintained after second passage. These results would be consistent with a lack of transmission barrier to this isolate. HuPrP-Tg340MM mice also seemed to be susceptible to vCJD, according with a lack of a transmission barrier, but with significantly longer survival times than those of MM Type I sCJD. BSE appeared strikingly more difficult to transmit to the HuPrP-Tg340MM mice than the human isolates used. At first passage none of the HuPrP-Tg340MM mice inoculated with the BSE isolate scored positive for PrP_{res}. This transmission is probably restricted by the presence of a significantly transmission barrier. HuPrP-Tg340MM mice not inoculated or inoculated with healthy brains have similar survival times than the mice inoculated with the BSE isolate. These results could indicate that, probably, the incubation period of the BSE isolate used in this study exceed that of the lifespan of our mice model. HuPrP-Tg340MM mice also shown a considerable transmission barrier to the classical Scrapie isolate used. At first passage none of the mice inoculated scored positive for. Second passage is ongoing. The results obtained in this study could suggest that our human transgenic mice can be a useful model for the study of the transmission characteristics of the different TSE agents.

P3.71 A new mouse model for Cre-mediated expression of a dominant-negative PrP mutant

Authors
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Content

Prion diseases are fatal neurodegenerative disorders characterised by brain lesions and accumulation of a disease-associated protein, designated PrP_{Sc}. How prions proceed to damage neurons and whether all or only subsets of neurons have to be affected for the onset of the clinical disease is still unknown. The manifestation of clinical prion disease is characterised by motor dysfunctions, dementia and death. Recently, we have shown that loss of motor neurons in the spinal cord is a constant finding in different mouse models despite variable brain pathology suggesting that motor neurons in the brain or spinal cord are vulnerable cells for triggering the onset of clinical symptoms (Flechsig et al. Neuron, 27:399-408, 2000). To determine whether the protection of motor neurons against prion-induced dysfunctions is an approach for holding the disease at the sub-clinical level, we established a novel model for Cre-mediated expression of a dominant-negative PrP mutant (PrP Q167R) in the cells of interest. Mice were generated carrying a floxed lacZ marker gene and the coding sequence of the PrP mutant under control of the ubiquitin C promoter. Two Cre lines have been used to target either motor neurons in the spinal cord (Hb9-Cre) or various neurons of the brain and spinal cord (NFL-Cre). In the absence of Cre, lacZ is expressed in the neurons of the spinal cord and in different motor centers of the brain. The expression of PrP Q167R is found to be restricted either to motor neurons in the spinal cord or to most neurons of the mice upon breeding with Hb9-Cre or NFL-Cre mice. Of each group two lines expressing the highest levels of PrP Q167R were recently infected with mouse RML prions and are currently being monitored for any effects on incubation time and signs of clinical disease. Our transgenic model may open a new way to elucidate the role of motor neurons in the prion pathogenesis and to provide new insights into the pathophysiology of prion diseases.

P3.72 *PRNP* point mutations dictate the assembly state of disease related

Authors

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Content

Approximately 15% of human prion disease is associated with pathogenic autosomal dominant mutations in human prion protein gene (*PRNP*). Here we describe the transmission properties of human prions in transgenic mice expressing *PRNP* with pathogenic mutations P102L or E200K and methionine at polymorphic residue 129.While no spontaneous disease was apparent in aged mice of either transgenic line, primary transmission of E200K and classical CJD prions from 129 methionine homozygous patients resulted in clinical disease with equivalent efficiencies and short incubation periods in 200K transgenic mice. However, mismatch at residue 129 in the E200K prion inoculum produced a dramatically increased disease incubation period. In 102L transgenic mice short disease incubation periods were only observed with transmissions of P102L prions whereas classical CJD prions showed prolonged and variable incubation periods irrespective of the codon 129 genotype of the inoculum. Notably, analysis of glycoform ratios of PrP_{Sc} propagated in these mutant mice inoculated with classical CJD isolates resulted in uniform production of PrP_{Sc} with a predominance of both di- and mono-glycosylated PrP. This represents a significant change ($p < 0.0001$) in all 3 glycoforms compared to the glycoform ratios of the classical CJD inoculum in which the di-glycosylated PrP is characteristically the least abundant. This dramatic change in glycoform ratios is consistent with the hypothesis that *PRNP* point mutations can directly dictate the packing arrangement and therefore the stoichiometric ratio of the three PrP glycoforms in PrP_{Sc}. Remarkably, vCJD prions transmitted efficiently to both the 102L and 200K transgenic mice indicating that these mutations are compatible with propagation of the vCJD prion strain. Collectively these data indicate that *PRNP* P102L or E200K mutations have differing effects on prion propagation. These effects depend upon prion strain type and are strongly influenced by the codon 129 polymorphism.

P4.01 Prion Disease Surveillance in the United States

Authors
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Content

Background: Prion diseases are a family of rare progressive neurodegenerative disorders that affect both humans and animals. Creutzfeldt-Jakob disease (CJD) is the most common human prion disease and occurs worldwide at a rate of approximately 1 case per 1,000,000 persons.

Objective: To describe the incidence of CJD and the occurrence of variant CJD (vCJD) in the United States.

Methods: Analysis of death certificate data with CJD listed as a cause of death among US residents during 1979 through 2005 and deaths identified from other surveillance reporting mechanisms through 2007.

Results: During 1979 through 2005, 6627 deaths with CJD as a cause of death were reported in the United States, an average of approximately 245 deaths annually (range 172-304 deaths). The average annual age-adjusted incidence for CJD was 0.97 per 1,000,000 persons (95% CI=0.95-1.0). Most (62.0%) of the CJD deaths occurred among persons >65 years of age for an average annual incidence of 4.8 per 1,000,000 persons in this population. The median age of death was 68 years. The age-adjusted incidence for males was higher than that for females (1.05 and 0.92, respectively). Most deaths were among whites (94.6%), the age-adjusted incidence was significantly higher for whites than for blacks (1.04 and 0.40, respectively). In addition, three cases of vCJD were reported with deaths occurring in 2004 and 2006 (2 cases). Investigation provided strong evidence that two of these cases acquired their illnesses in the United Kingdom and the third case in Saudi Arabia.

Conclusion: The annual CJD incidence in the United States remained at approximately 1 per 1,000,000 persons. It is important to monitor the occurrence of thiese diseases to assess CJD trends, detect possible cases of vCJD, and identify novel prion diseases should they occur.

P4.02 Risk assessment of transmissible spongiform encephalopathies in European mouflon (Ovis gmelini musimon) from Germany

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Content

Background: TSEs represent a group of fatal neurodegenerative disorders in humans and several free-ranging and captive mammalian species. These include bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in North American cervids and scrapie in sheep and goats. Although scrapie can be transmitted both horizontally and vertically, the maternal transmission seems to be the prevailing route. Sequence analysis of the prion protein gene (*PRNP*) in sheep has shown that polymorphisms in this gene are related to susceptibility to scrapie. The European mouflon is an ancestor of the domestic sheep. Germany's free-ranging mouflon population is supposed to be the second largest in the world. A report of scrapie in mouflon from England and references of identical prion protein nucleotide sequences in mouflon and sheep indicate that mouflon are susceptible to TSE.

Objective: A survey was performed to examine the risk of the occurrence of TSE in mouflon in Germany, including a population screening for prion diseases and genotyping of *PRNP*.

Methods: All administrative districts in Germany were divided into four risk categories defined by three factors: the abundance of sheep, the number of hunted mouflon per year (as an index of population density), and the number of reported cases of scrapie in domestic sheep. Samples were taken from mouflon ewes and rams older than 18 months collected from hunting bags during the hunting seasons 2006/2007 and 2007/2008. In order to detect preclinical typical and atypical scrapie cases, samples were taken from brain stem, cerebellum and retropharyngeal lymph nodes. These were analysed by the IDEXX HerdChek BSE-scrapie ELISA. Brain tissue samples were used for DNA extraction and subsequent PCR amplification and sequencing to conduct prion protein genotyping.

Results: In total, 821 mouflon were sampled in both hunting seasons; the majority were females (n=549). Age distribution ranged from 18-24 months old (n=142), 2-4 years old (n=326) and 4-6 years old (n=257) to over 6 years old (n=96). 47 samples originated from road kills, mouflon found dead or emergency culled mouflon. No protease resistant prion protein (PrP_{res}) was detected in any of the 2447 examined brain and lymph node samples. The sequence analysis for the genotyping is ongoing and results are expected soon.

Discussion: At present, this study provides no evidence of the occurrence of TSEs in the German mouflon population. Nevertheless it cannot be entirely excluded that prion diseases may be present in the population at very low prevalence. These preliminary conclusions will be extended by the results of the *PRNP* genotyping and permit a more detailed risk assessment in mouflon for prion diseases.

P4.03 Surgery and risk of sporadic Creutzfeldt-Jakob disease in Denmark and Sweden: registry-based case-control studies

Authors
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Content
Background: Epidemiologic evidence of surgical transmission of sporadic Creutzfeldt-Jakob Disease (sCJD) remains controversial.

Objective: To identify associations between surgical history and CJD.

Methods: From Danish and Swedish registries we selected 167 definite and probable sCJD cases, with onset during 1987-2003; and 3,059 controls, 835 age-, sex-, and residence-matched, and 2,224 unmatched. Independent of case/control status, surgical histories were obtained from National Hospital Discharge Registries. Surgical procedures were categorized by body-system group, assigned to specific time-lag windows prior to onset of sCJD, and compared using logistic regression.

Results: A history of surgery involving all body systems, conducted 20 or more years before clinical sCJD onset, was more common in cases than both matched (odds ratio (OR) 2.44; 95% CI 1.46-4.07) and unmatched controls (OR 2.25; 95% CI 1.48-3.44). This observation was corroborated by a linear increase in risk per surgical discharge (OR 1.57 95% CI 1.13-2.18) and OR 1.50 95% CI 1.18-1.91), respectively.
Surgery on a range of body-systems were incriminated, including operations on peripheral vessels, digestive system and spleen, and female genital organs.
Conclusions: Following a long incubation period, a variety of major surgical procedures constitute a risk factor of sCJD, and a considerable number of sCJD cases may be conceived as health-care related, accidentally transmitted disorders.

P4.04 Risk of transmission of sporadic Creutzfeldt-Jakob disease in Denmark and Sweden quantified using an etiological classification of surgical procedures

Authors
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Content
Background: Surgical transmission of sporadic Creutzfeldt-Jakob Disease (sCJD) may exist.
Objectives: To identify associations of surgical history with sCJD, using a reported ad-hoc built etiological classification of surgical procedures (SP).

Objective: In order to investigation the involvement of GPI-PLD in the process of neurodegeneration in prion diseases.

Methods: From Danish and Swedish registries we selected 167 definite and probable sCJD cases, with onset during 1987-2003; and 3,059 controls, 835 age-, sex-, and residence-matched, and 2,224 unmatched. Surgical histories were obtained from National Hospital Discharge Registries. Following reported methods, SP were categorized by tissue and anatomic structures hypothetically contacted by surgical instruments and by putative transmission level –high, lower and lowest risk–, assigned to specific time-lag windows, prior to onset of sCJD, and compared using logistic regression.

Results: 5,990 SP associated with 3,876 discharges were studied. Results found in comparisons with both types of controls were similar. From comparison with matched controls statistically significant risk excess was seen: 1) at the >20 years window, for SP contacting blood vessels, peritoneum, "other tissues" and skeletal muscle, –OR 95%CI 4.54 (1.01-20.30), 2.38 (1.14-4.96), 2.26 (1.14-4.47) and 2.04 (1.06-3.92)– respectively and for SP with putatively lower risk, OR 95%CI 2.81 (1.62-4.88); 2) at 10-19 years time lag, for SP involving peripheral nerve and skeletal muscle, OR 95%CI 4.41 (1.17-16.64) and 1.58 (1.01-2.48) and; 3) at >1 years time lag, for procedures involving retina OR 95%CI 5.53 (1.08-28.34).

Conclusions: Surgery, acting with long incubation periods, constituted a risk factor for sCJD. Induction or latency periods might be lower for procedures involving peripheral nervous system than those involving peritoneum and peripheral veins. This classification might contribute to quantify effects masked by use of Body-system SP categories, for example for surgery of eye and adjacent structures.

P4.05 Prion inactivation efficacy of an alkaline cleaner is incomplete, but is superior to sodium hydroxide, and is comparable to sodium dodecyl sulfate

Authors
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Content
Abstract Background: A potential risk of prion transmission through blood-derived products poses a threat to industrial and healthcare facilities. Bioassay was mainly used to assess prion-inactivation efficacy, but its sensitivity may not be enough. Protein misfolding cyclic amplification (PMCA) technique,in which PrP_{Sc} can be amplified in vitro, was demonstrated to be faster and more sensitive to detect PrP_{Sc} than bioassay.

Objective: We evaluated the prion-inactivating efficacy of an alkaline cleaner, mip-PC-M (Ecolab, Japan), in comparison with sodium hydroxide (NaOH) or sodium dodecyl sulphate (SDS), using PMCA.

Materials and Methods: 1.0% (w/v) 263k-infected hamster brain homogenates (ScHg) was inactivated as follows, 1) mip-PC-M (containing 0.125 M NaOH) for 30 min at 70 oC, 2) 1N NaOH for 120 min at 25 oC, 3) 2N NaOH for 60 min at 25oC, or 4) 3.0% w/v SDS for 5 min at 100 oC. Samples were then diluted 1:99 in normal hamster brain homogenate (NHg), and 48 cycles (one round) of PMCA were carried out. Reaction products were diluted 1:9 in a fresh NHg at every round (a multi-round PMCA). Amplified products of each round were digested with proteinase K and PrP_{res} signals were detected by western blotting. A multi-round PMCA of 10-fold serial dilutions of ScHg was performed in parallel.

Results and Discussion: Regardless of disinfectants, PrP_{res} signals eventually appeared. Residual amount of PrP_{Sc} treated with mip-PC-M, SDS, 1 N or 2 N NaOH were estimated to be correspond to the amount of PrP_{Sc} in diluted 10% ScHg by the magnitude of 10-10, 10-10, 10-7 to 10-10, or 10-7 to 10-10 fold, respectively. These results showed that mip-PC-M could remove PrP_{Sc} at the same efficiency as SDS, with which bioassay showed complete inactivation of PrP_{Sc}, and more efficiently than 1 N or 2 N NaOH. Considering the amount of PrP_{Sc} in pooled plasma, if present, mip-PC-M can decontaminate prion sufficiently.

P4.06 Surveillance for BSE and CWD epidemic situation in Russia

Authors
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Content
Background: Diagnostic examinations of bovine brain samples for BSE signs have been carried out in the FGI "ARRIAH" since 1999. Totally 5,155 brain samples from risk group cattle from 72 regions of Russia have been tested by April 1, 2008. more than 2,000 of them were from cattle imported from BSE affected EU countries, mainly from Denmark, the Netherlands and Germany. Till 2005 samples were tested by histopathologic method, from 2005 – predominantly by ELISA using "TeSeE" kits (Bio-Rad). The prevention of CWD spread in deer population is a critical area of activity of Russian veterinary services. Deer are included in the list of animals subject to diagnostic tests for prion diseases. The domestic reindeer (Rangifer trandus) population in Russia in 2002 was 1.2 mln animals. The wild reindeer (Rangifer tarandus tarandus) population has increased during the last years and now amounts to 1.5 mln animals. Deer farming for velvet antlers production is developed in forest and forest-steppe zones. Two subspecies of red deer are mainly bred – Siberian maral (Cervus elaphus sibiricus) and Tian Shan wapiti (Cervus elaphus songaricus). An important problem is the restocking of the Sika deer (Cervis nippon) population. This deer subspecies was included in the Russian Red Book. In 1999 the import to Russia of reindeer, zoo and circus deer originating from the countries that are not free from BSE or fed by the feedstuff containing meat and bone meal from ruminants was prohibited. In recent years 119 brain samples were tested in the FGI "ARRIAH" for the presence of vacuoles and PrPCWD using histopathology and immunohistochemistry methods, 52 of them were from Siberian maral, 12 – from Reindeer, 34 – from Sika Deer, 20 – from Red Deer (Cervus elaphus), 1 – from Elks (Alces alces). In April 2008 brain samples from Reindeer with progressive neuropathology signs were received from the Yamalo-Nenetsky Autonomous Okrug. The tests resulted in the diagnosis of rabies. None of the testes samples showed vacuolization and accumulation of an abnormal form of prion protein.

P4.07 Medical procedures and sporadic Creutzlfedt-Jakob disease: analyses with the Japanese CJD surveillance.

Authors

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Content

Background: Up to the present, more than 400 patients with iatrogenic Creutzfeldt-Jakob disease (CJD) have been reported, and some case-control studies reported that medical procedures were possible risk factors for sporadic CJD (sCJD).

Objective: To elucidate association of medical procedures with sCJD.

Methods: *Surgeries and blood transfusion before the onset of sCJD.* We analyzed medical procedures in the patients registered by the CJD Surveillance Committee in Japan for recent 9 years. To estimate the risk of sCJD through the past surgery or blood transfusion, we performed a case-control study, using the patients with 'prion diseases denied' as a control group. Furthermore, the details were investigated for the sCJD patients who underwent neurosurgery or ophthalmic surgery at the same hospitals as other patients with any kinds of prion diseases had ever undergone neurosurgery or ophthalmic surgery. *Surgical procedures after the onset of sCJD.* We analyzed sCJD patients who underwent surgical procedures after the onset of sCJD, because they may cause secondary transmission of the disease through contaminated instruments.

Results: In a case-control study with 753 sCJD patients and 210 control subjects, we found no significant increase of risk for sCJD associated with surgeries or blood transfusion before the onset of sCJD. The neurosurgery or ophthalmic surgeries that sCJD patients underwent before onset had no obvious relationship with those previously performed in patients with any kind of prion diseases at the same hospital. After the onset of sCJD, 4.7% of the sCJD patients underwent surgeries, including neurosurgery in 0.8% and ophthalmic surgery in 1.9%.

Conclusions: Our results indicated that, in sCJD patients, there was no evidence of increased risk associated with the medical procedures, although we must be careful about the possibilities of secondary transmission through medical procedures.

P4.08 Temporal trend and geographical distribution of animal derived protein contamination of feedingstuff in Italy: a 7-year prevalence study

Authors

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Content

Background: Since 2001 a EU-wide ban on the feeding of processed animal protein (MBM) to all farmed species bred for food production has been enforced. So far few data about MBM contamination in Europe were made available. In Italy, the national control plan (PNAA) pursues different aims: to apply a constant control to ensure compliance with the EU ban through a targeted sampling, to monitor trends and to identify risk factors within a random sample of the Italian herds.

Objective: Aim of this work is to provide data on MBM contamination of feedingstuff in Italy between 2001 and 2007.

Methods: Data from PNAA were restricted to samples from dairy cattle farms where no specific targeting strategy was applied. Yearly prevalence rates (MBM-contaminated/ total samples) were obtained along with specific rates by region or geographical area and by type of feedingstuff (raw material vs compound feed). Risks were expressed in term of prevalence ratios. Upper 95% confidence limits of the prevalence (UCLs) by region were calculated even when all samples were negative.

Results: Between 2001 and 2007 15,650 samples were analysed from dairy farms. The prevalence showed a drop from 3.9% (CI95% 3.3-4.5) in 2001 to 0.05 % (0.001-0.3) in 2007. No difference was seen in risk of contamination by type of feedingstuff (prevalence ratio=1; 0.5-2). The risk was not evenly distributed over geographical areas: North showed a 6.6-fold (2.4-18.0) increased risk than Islands (i.e. Sicily plus Sardinia as reference category), while Centre and South showed, respectively, a risk of 4 (1.4-11.4) and 1.5 (0.5-4.9). The excess risks disappeared if restricting the calculation after 2003. Finally, for the whole period the UCLs of the individual regions ranged between 0.4 and 6.7%.

Discussion: The effectiveness of the total ban is shown by the abrupt fall in the prevalence trend after 2001. The results of continuing data analysis will be used to inform the on going risk based control campaign.

P4.09 Heterogeneity in the geographical distribution of scrapie: a first step to suggest risk factors

Authors

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Content

Background: The spatial study of a health event often shows a clustering pattern. The clustering may reflect a true difference in exposure to specific risk factors or a reflection of variations in population characteristics, in diagnosis and reporting.

Objectives: In order to provide epidemiological interpretations of the distribution of scrapie in Sardinia Region, in Italy, we managed data of scrapie outbreaks producing thematic maps and carrying out a statistical spatial analysis.

Methods: Incidence data have been calculated on a population of around 19,000 sheep-goat farms and around 150 outbreaks of classical scrapie observed over a 13-year period. After having produced descriptive maps different spatial techniques were applied in order to highlight the presence of one or more clusters of farm-level incidence.

Results: As a result of the preliminary exploratory analysis, it was made clear that the incidence distribution of the disease does not reflect the distribution of the population. Furthermore, the affected municipalities are characterised by highly heterogeneous incidence levels. This heterogeneity was confirmed by the Join Count Test. The mapping of global Bayesian estimates suggested a main clustering of high risk municipalities in the northern area of the Region. The use of local Bayesian estimates pointed out, on the other hand, the potential for 4 or 5 small aggregations of high risk municipalities in the southern part of the region as well.

Discussion: The main question to answer is why this heterogeneity involves a region which has almost half of the Italian sheep population and without evident differences in the management of the farms. Aspects like different intensity of surveillance between the municipal area, differences in the adopted control measures or in the duration of their application may act as confounders affecting the final geographical distribution of scrapie outbreaks.

P4.10 TSE Persistence in the Environment

Authors
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Content
Given the resistant nature of TSE agents, concern has long been raised about enduring infectivity in the environment. Sources of infectivity include diseased animal carcasses as well as bodily fluids from clinical or sub-clinical animals. If this infectivity persists and/or migrates within soil and soil-water then further cases of disease could ensue. In order to investigate this we are performing two large scale field experiments in which a series of 301V (mouse passaged BSE) spiked bovine heads have been interred within soil-filled lysimeters. The heads are serially exhumed and samples, collected from within and around the heads, analysed for the presence of PrP_{Sc} by western blot and for infectivity by bioassay. In parallel, boluses of infectivity have been placed within much larger lysimeters. Soil core samples are collected at various time points during the experiment and through analyses the aim is to detect the extent to which infectivity has disseminated. In both experiments two soil types have been used; a free-draining sandy loam soil and a water-retentive clay soil. Rainwater passing through the lysimeters is also being analysed. To date the year 1 and year 2 heads have been exhumed and core samples have been collected from the large bolus-containing lysimeters at 7 time points. A method for the extraction of PrP_{Sc} from soil has been developed and applied to the core samples, however no PrP_{Sc} has so far been detected. It was notable that whilst the year 1 and 2 bovine heads were largely decomposed, significant brain material remained within the brain cavity. Samples from both experiments have been extracted and bioassay experiments are underway.
Laboratory studies suggest that TSE infectivity may bind strongly to soil components and is therefore likely to persist in the environment for a considerable period of time. It is envisaged that data acquired can be used to build a predictive model of TSE behaviour in the environment and could influence policy and risk assessment.

P4.11 Scrapie Risk in Pakistani Sheep and Goats

Authors
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Content
Scrapie Risk in Pakistani Sheep and Goats.

Background: Scrapie of sheep and goats is a member of invariably fatal neurodegenerative disorders, commonly known as transmissible spongiform encephalopathies (TSEs). The putative causative agent of TSEs is aggregated conformer of host-encoded normal prion protein. Considering the threat of prion infectivity transmission that seems making animal trade well-planned among European and American states, molecular surveillance with special reference to scrapie susceptibility is being undertaken actively in most of the agricultural countries of the world for public health as well as animal welfare. Genetic selection against the disease occurrence is at present, the only tool for the eradication of scrapie, indicating the significance of prion protein gene sequencing studies.

Objective: The present work was carried out in 14 Pakistani sheep and goat breeds to determine whether they harbour defense against exposure to natural or arbitrary infection and also to reveal overall variability in the prion protein gene.

Methods: The coding region of the prion protein gene of 308 animals representing nine sheep breeds and 207 goats of five various breeds was PCR-amplified and sequenced in both directions using capillary gel electrophoresis separation and laser-based detection technology. The frequencies of scrapie-associated polymorphism and variability in the PrP gene were determined.

Results and Discussion: A total of 10 PrP polymorphisms were detected in goat and 7 in sheep. Among these polymorphisms six had no effect on protein sequence, while the remaining SNPs induced amino acid change in their respective wild type codons. The frequency of wild type alleles remained higher in both species and was found to be in the range of 0.62 (138c) in goats to 0.99 (154R) in goats and (101Q, 146N and 189Q) sheep. The prevalence of scrapie-associated polymorphisms ranged from 0.01 (154H) to 0.05 (143R) in goats and 0.04 (154H) to 0.15 (171H) in sheep. The frequency of most sheep scrapie-resistant 171R polymorphism was only 0.07. The most scrapie-resistant sheep breeds were crossbreds Hissardale and Pak-Karakul and goat breeds Pak-Angora and Teddy. The little prevalence of scrapie-resistant polymorphisms indicates that Pakistani sheep and goats are at risk of contracting disease in case of their inadvertent exposure to infectivity. These findings also suggest further epidemiological brain PrP profiling to provide true picture of prion diseases in Pakistan and to address possible concerns about future animal trade and public health.

P4.12 Ecological niche modelling for management of cwd in farmed cervids in alberta, canada

Authors
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Content
Background: Chronic Wasting Disease (CWD) has the potential to greatly affect the Canadian farmed cervid industry. Although research indicates it is unlikely for CWD to be transmissible to humans, the potential for this disease to become zoonotic is of great concern. Those who farm cervids need to mitigate transmission of the disease. However, specific mechanisms involved in the transmission of the disease remain unknown. Current work to identify geospatial characteristics which facilitate CWD transmission in wild cervid populations, allows us to quantify the CWD risk to farmed cervids from wild populations by geospatial analysis. GIS technology combined with information of potential risk factors for CWD transmission facilitate using newer techniques such as ecological niche modeling to develop better strategies for interrupting the potential for transmission between farmed and wild cervid populations.

Objective: An ecological niche model based on best known information of CWD transmission combined with GIS toolsets to analyze qualitative and quantitative data to (1) estimate the possible risk of CWD infected wildlife for transmitting the disease to farmed elk and deer, (2) identify areas of concern where more intensive testing and surveillance may be necessary.

Methods: Analysis will use Biomapper 3.2, developed by Dr. Alexandre Hirzel of the Department of Ecology and Evolution at the University of Lausanne, Switzerland, along with ArcView developed by ESRI. The ecological niche model will include information about CWD in the wild cervids and include geospatial data about farm locations and habitat characteristics. Data sources are provided for the study by collaborators, which include: the Canadian Food Inspection Agency (CFIA), Canadian Cooperative Wildlife Health Centre (CCWHC), Alberta Fish and Wildlife, and Alberta Agriculture and Rural Development.

Results adn Discussion: The preliminary results will be available at the time of the presentation.

P4.13 Initial Diagnoses Predict Survival Time in Human Prion Disease

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Content
Background: Human prion diseases demonstrate a variety of clinical presentations that commonly affect the timeliness and accuracy of diagnosis. Examining clinical and pathological characteristics of initial symptoms and diagnoses may aid in clinicopathological correlations.

Objective: To examine initial diagnoses of human prion diseases and their clinical characteristics.

Methods: Medical records of individuals with a clinical or pathological diagnosis of prion disease were btained from three medical centers. Clinical data were collected using a standardized instrument. iagnoses at the time of an individual's initial presentation to a health care professional were noted. ultinomial logistic and Cox proportional hazard regression models were used in data analyses.

Results: Eighty-four probable and definite cases of human prion disease were analyzed, 95% of which (n=80) were sCJD. Sixteen (19%) were diagnosed as a prion disease, 31 (37%) as a non-prion related dementia, 15 (18%) as a psychiatric disorder, 8 (9%) as stroke, and the remaining 14 (17%) were diagnosed as other medical conditions. Cases that were initially diagnosed as stroke or prion disease had the shortest survival times (89 and 107 days, respectively) and those diagnosed as a psychiatric or other medical illness had the longest survival times (269 and 138 days, respectively). The presence of myoclonus and depression at the initial evaluation predicted diagnoses of prion disease and psychiatric disorders respectively.

Discussion: The initial symptoms of prion diseases vary, which likely reflects the existence of distinct phenotypes as the onset of symptoms and initial diagnoses correlate with differences in survival times. These findings suggest that initial symptoms and diagnoses may be useful in clinicopathological correlations.
The relationship between initial symptoms and diagnoses of prion disease in respect to molecular subtypes will be the focus of future investigations.

P4.14 The application of the 1B2A rule to "no test" cattle – A risk assessment based on data from Great Britain

Authors
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Content

Background: The IB2A rule established under the auspices of Regulation 999/2001 is generally accepted as necessary to reduce risks to consumers, over and above that afforded by the removal of SRM alone (European Commission, 2001). A pragmatic decision, based upon discussion at the Scientific Steering Committee (SSC), assumed that the process of slaughtering, dressing and handling carcases would result in cross-contamination of carcases, particularly as a result of carcase splitting and exposure of spinal cord (SSC, 2001). Even in 2008 there remains little supporting data to quantify the extent of contamination and the scope of sequential dispersal along the slaughter line. VLA risk assessors were asked to consider the extent to which the application of the 1B2A rule might be protective to the food chain, and used as its baseline data for the slaughter and testing of British OTM cattle in 2006.

Objective: This risk assessment provides quantitative estimates of the protective impact of the "One Before and Two After" (1B2A) policy, whereby cattle that test postive for BSE when tested in the human food chain are removed from the food chain and destroyed. Carcases immediately prior to the postive carcase (1B) and two after (2A) are also destroyed, as a precaution against cross-contamination during the dressing process.

Methods: A stochastic quantitative model has been developed which includes consideration of both variability and uncertainty associated with parameter inputs and therefore the overall results. All outputs are given as Bovine Oral ID50 and no attempt is made to convert to human ID50 due to the additional uncertainties introduced by doing so.

Results and Discussion: The key finding from this risk assessment is that there is very little or no infectivity estimated to be present on the carcases of a 1B, 1A and 2A a "no test" carcase. The low prevalence of BSE, together with the low probability of a carcase testing as a "no test", results in a very low likelihood of a randomly selected "no test" carcase being infected with BSE. Assuming a constant prevalence at levels experienced in GB in 2006, this is estimated to be likely to occur once in 10 years.

References: European Commission (2001) Commission Regulation (EC) No 1248/2001 of 22 June 2001 ammending Annexes III, X and XI to Regulation (EC) No 99/2001 of the European Parliament and of the Council as regards epidemio-surveillance and testing of transmissible spongiform encephalopathies. Official Journal of the European Communities No. L 173/12 of 27.6.2001. SSC (2001) Scientific report on stunning methods and BSE risks (the risk of dissemination of brain particles into the blood and carcass when applying certain stunning methods) (prepared by the TSE BSE and ad hoc group at its meeting of December 2001 and including the outcome of a public consultation via internet between 10 September and 26 October 2001).

P4.15 Plasma product recipients in the UK and the risk of variant Creutzfeldt-Jakob disease

Authors
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Content

Background: Variant Creutzfeldt-Jakob disease (vCJD) has been shown to be transmissible via blood component transfusion, with four instances of transfusion transmitted vCJD infection to date in the UK. This has resulted in significant public health consequences. Although worldwide there has been no known transmission of any type of CJD via plasma product transfusion, animal studies have shown that the risk of potential infection remains. This has resulted in concern by the UK health services of the potential risk of transmission of vCJD through plasma product transfusion.
In 2004 the UK CJD Incident Panel estimated the risk of vCJD in those who were treated with UK sourced pooled factor concentrates or antithrombin between 1980 and 2001. Three groups of individuals were described- high risk (recipients of one treatment with factor VIII, factor IX or antithrombin), medium risk (recipients of several infusions of intravenous immunoglobulin G or 4.5% albumin) of and low risk (recipients of intramuscular human normal immunoglobulin or 20% albumin).

Objective: To describe how many UK vCJD case are reported as having received plasma products and to attempt to ascertain the likelihood that these products might have been the route of vCJD transmission to these cases.

Methods: Routinely collected information by the UK National CJD Surveillance Unit (NCJDSU) of potential risk factors of all cases of vCJD referred to the unit, including data on transfusion and 'injection' histories, was collated. Information was obtained from interviews with relatives of cases and, where available, general practitioner and hospital records. Where possible, batch numbers of plasma products were compared with those from known vCJD cases and with each other.

Results: Of the 166 cases to date, it is known that 11 made 25 plasma donations, which were used to manufacture 178 plasma batches, prior to the UK importing plasma from abroad in 1999. Data will be provided on the number of UK vCJD cases who are known to have received plasma products by type and batch number, where available.

Discussion: A conclusion on the possible risk of transmission of vCJD by plasma products in the UK to date will be drawn.

P4.16 Effective Policy Interventions for Researchers

Authors
Ricketts, M.

Content

Background: Affecting policy change in government and industrial settings is the goal of public health. However, both have proven resistant to substantial change. Objectives: Propose a theory based approach to research agenda design because when ever change is required in social, structural, economic or regulatory structures, human health is best protected through the use of good science.Methods: Literature review and idea generation.Results: Learning theory has demonstrated that only the prepared can change their behavior. Recognition of the stage of change is essential for intervention. Discussion:Scientific research agendas will benefit from understanding the stage/adaptability of governments and organization by examing the regulatory capacity of the organizations targetted.

P4.17 Analysis of PrP_{Sc} deposits in lambs from scrapie-affected flocks. Preliminary results of a field study

Authors
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Content

Background: In 2005, the scrapie-monitoring programme was intensified in the EU, following the detection of the BSE agent in a goat. However, as no additional BSE case in small ruminants has been detected in 3 years, the EU has proposed a review of the monitoring programme, especially related with culling and repopulation policy in TSE affected flocks. In this case, if the national competent authority so decides, sheep and goats less than three months old are intended solely for slaughter.

Objective: The aim of this study was to determine whether PrP_{Sc} is detectable in lambs younger than 3 months old from scrapie outbreaks. Material and methods: Due to that in Spain lambs are slaughtered for human consumption weighting less than 30 kg (about 3-4 months old), a total of 300 lambs between 5 - 30 kg from 7 scrapie outbreaks were included in this study. Lambs from infected flocks were culled in a rendering plant and by random sampling, the following tissues were collected: ileum, mesenteric, retropharyngeal and pre-scapular lymph nodes. In addition, 90 animals less than 2 year old belonging to an experimental flock with high rate of scrapie were included in this study. In this case, nervous as well as lymphoid tissues were collected. Samples were fixed in 10% formaldehyde and processed for PrP_{Sc} detection by immunohistochemistry using L42 antibody.

Results: No PrP_{Sc} deposition was detected in any of the tissues studied from the lambs coming from the scrapie outbreaks. In the experimental flock, two animals were immunopositive at 12 months old and one at 21.

Discussion: It has been reported that PrP_{Sc} can be detected in lambs at 2-3 months of age in specific conditions (VRQ/VQR genotype and flocks with heavy rate of infection). However, there are very few reports about the time of PrP_{Sc} detection in lambs in field conditions, varying between 8-24 months of age. In agreement with these results, PrP_{Sc} is apparently undetectable in lamb younger than three months old.

P4.18 Risk Assessment of potential Transfusion Transmission of Variant Creutzfeldt-Jakob Disease (vCJD) in the United States

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Content

Background: Four probable transfusion-transmitted vCJD (TTvC) cases in the United Kingdom since 2003 suggest a potential risk of TTvC in the US associated with donors who might have had dietary exposure to the bovine spongiform encephalopathy agent during a visit in the UK or other countries in Europe for extended periods of time since 1980. Current US policy indefinitely defers donors who have a history of total stay in the UK for >3 months during 1980-1996 and other countries in Europe for >5 years since 1980.

Objectives: This study uses a probabilistic model to estimate the potential risk of TTvC in the US, and to evaluate the effectiveness of current US donor deferral policies in reducing the risk.

Methods: Because of uncertainty in estimating UK vCJD prevalence, the model used two different estimates of UK prevalence. The vCJD prevalence for US donors was estimated relative to the UK vCJD prevalences, and was adjusted by age group for susceptibility to the disease, donation rates, and frequency and duration of travel to the UK, and other countries in Europe since 1980.

Results: With the lower prevalence assumption the model estimated an annual mean of 1 vCJD transmission in every approximately 160 million blood transfusions; this result suggests that 1 case might be observed in 40 years. Using the higher prevalence assumption, the model estimated an annual mean of 1 vCJD transmission per 1.5 million blood transfusions. The model also estimated that the current donor deferral policy had reduced the US vCJD risk by more than 92%.

Discussion: Due to limited data and knowledge of vCJD, the model estimates are uncertain; however, model results indicate the risk of TTvC in the US is small. The results suggest that the current US donor deferral policy greatly reduces the risk of TTvC. This analysis identifies critical data gaps in understanding the risk of TTvC, and provides a tool to inform regulatory decision-making.

P4.19 Risk assessment of changes to BSE testing strategies for bovines in Ireland

Authors
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Content

The EU TSE Regulation (European Commission regulation No. 999/2001) requires all member states to carry out an annual programme for monitoring TSEs. The annual monitoring programme must include the testing of all healthy cattle aged over 30 months slaughtered for human consumption. The TSE Roadmap adopted by the Commission in July 2005 (and endorsed by both the European Parliament and the Council) states that amendments to certain TSE measures could be envisaged, including an increase in the age of healthy slaughtered animals for which testing is required. As a result an amendment to the TSE Regulation will allow member states to change their monitoring programme provided they can demonstrate an improvement in their epidemiological situation. The objective of this study is to assess the impact of changing the age of testing for healthy cattle in Ireland from over 30 months to over 42 months of age. The mechanistic model uses Monte Carlo simulation techniques to account for parameter uncertainty and variability and to calculate the number of expected BSE cases missed given changes in the monitoring system. The number of test positive animals missed for the proposed change in strategy is extremely low for both 2008 and 2009 with less than one test positive animal missed in the healthy slaughter stream. The model indicates the potential to change the testing strategy for healthy cattle in Ireland without compromising animal or human health.

P4.20 Variant Creutzfeldt-Jakob disease in France and the United Kingdom: evidence for the involvement of the same agent strain

Authors

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Content

Background: Variant Creutzfeldt-Jakob disease (vCJD) was first reported in the United-Kingdom (UK) in 1996. Since then the majority of cases have been observed in the UK where there was a major epidemic of bovine spongiform encephalopathy (BSE). France was the second country affected.

Objective: In order to address the hypothesis of the involvement of a common strain of agent, we have compared clinical, neuropathological and biochemical data on vCJD patients from both countries.

Methods: In France and in the UK, epidemiological and clinical data were obtained from the analysis of medical records and direct interview of the family of the patients using the same standardized questionnaire in both countries. When brain material was available, we performed, with similar methods and in two different reference laboratories, a comparative study of brain lesions and PrP_{res} glycoform ratios in both vCJD populations.

Results: Clinical data, genetic background, neuropathological and biochemical findings in patients in France and the UK were similar except for age at death. At the present time, blood transfusion is a risk factor identified only in the UK. Just one of the French vCJD cases had a history of extended residence in the UK.

Discussion: The close similarity between the cases of vCJD in France and the UK supports the hypothesis that a common strain of infectious agent is involved in both countries. Our results, together with the relative low level of indigenous BSE in France, suggest that the principal source of infection in France is attributable to imports from the UK containing BSE infectivity.

P4.21 Evaluation of Ozone to Inactivate the TSE agent, BSE-301V, using a Standardised Wire Implantation Model in VM Mice

Authors

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Content

Background: The development of inactivation methods for TSEs is an urgent requirement in relation to the potential for iatrogenic transmission of vCJD. The evaluation of the effectiveness of such methodologies requires a highly sensitive and specific assay or combination of assays. The bioassay remains the accepted approach to assess effectiveness; however, careful consideration of the TSE strain and host species is required to help ensure that the risks are appropriately evaluated with regard to vCJD transmission.

Aims: The project aims to assess the ability of a commercially available ozone sterilisation process (sold commercially as a means of surgical instrument sterilisation by TS03) to inactivate TSE agents, focusing on a model using the BSE-301V strain, designed to mimic the key features of possible vCJD transmission via contaminated surgical instruments.

Methods: BSE-301V infected mouse brain homogenate, previously titrated to 1x108.6 ID50 per gram, was dried onto the surface of surgical steel suture wires using a standardised process. Wires were processed in the ozone steriliser using different cycle lengths to assess efficacy; for autoclaving a 134oC, 18 min cycle was used. Wires were implanted i.c. into VM mice and monitored for clinical symptoms for up to 550 days.

Results: The wire-based titration series resulted in clinical symptoms observed in animals from groups across a 7-log dilution range; an approximate log linear relationship was observed across the dilution series, however, at dilutions below 10-4 transmission rates fell below 60%, suggesting that the useful range is between 104 – 10-5 dilutions. Data will be presented comparing the surface bound titration results with the equivalent in-solution titration series. The results from the ozone decontamination studies are ongoing but initial findings indicate that ozone may be more effective than autoclaving which achieved a 2-3 log drop in infectivity.

Conclusions: TMethods have been established to ensure a consistent exposure of wires to the decontamination process with no further manipulations of the carriers post processing. A titration series has been established for BSE-301V on surgical steel that
potentially covers a 4-5 log range and has been applied to determine the relative efficacy of both new and conventional sterilisation processes.

P4.22 Prevention measures for bse in chile

Authors

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Content

With the confirmation of the first native case of BSE in canada in 2003, and subsequently in USA, epidemiological surveillance systems for BSE in Chile have been intensified, because previously MBM from these countries had been imported. For this reason the control of food factories was increased and feeding that include mammal protein to ruminant was forbbiden, measures were taken to bring stability to the country system. In order to mitigate the external risk, animals and MBM imported from Canada and USA were located and identified, supposedly exposed farms that are under official control, furthermore a new animal monitoring system was begun. 32.500 samples were obtained corresponding to subpopulations: "clinic suspects", "emergency slaughter", "dead and fallen animals", "routine slaughter in slaughterhouses", "exposed farm to MBM" and animals without information for ELISA (TeSeE-BioRad® and IDEXX®), western blot (Prionics®) and immunohistochemistry (VMRD®) evaluation, this samples that were analyzed and compared with its correspondig controls. Since 2003 until now, all of analyzed bovine obex samples, 100% were tested negative to different immunodiagnostic accepted by OIE that have been caried out in Chile. This study was a part of a series of measures that have been performed in the country, allowing to implement immunodiagnostic methods that are accepted internationally for detecting to BSE in Chile. So, the country can be considered less vulnerable and more reliable in its animal health status in relationship to TSE.

P4.23 Screening of scrapie in chile

Authors
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Content
During some years the routine diagnosis was histopathology of CNS in suspicious animals. The epidemic of BSE in Europe has increased the need to carry out faster and accurate diagnostic methods based in PrP_{Sc} inmunodetection. The principal goal of this study pointed to carry out and compare specific diagnostic methods for scrapie in the country. Chile is a privileged country in terms of animal health, since no cases have been reported of transmissible spongiform encephalopathies, however, in order to keep it free of scrapie, since 1996 to 2002 started an active and passive surveillance programme of histopathological analysis of ovine brain samples, according to rules and procedures outlined by OIE. Since that year and referring to the monitoring programme in the country, another laboratory diagnostic test for the detection of ovine PrP_{Sc} were validated. Thus, in years 2002 begun the work with immunohistochemistry (VMRD®), western blot (Prionics®) and ELISA (TeSeE-BioRad®) with ovine obex and its corresponding controls, respectively. Then, in 2003 epidemiological surveillance was intensified in order to recover the quality of TSE free country, with thus 2500 of samples have been processed from all over the country, of which all have tested negative for scrapie. These results prove that Chile is a country in which scrapie has not been detected with internationally accepted methods.

P4.24 Atypical Scrapie in the Basque Country

Authors
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Content
Background: Atypical forms of scrapie appeared in most European countries after the spread of the use of more sensitive and sheep specific rapid tests. The characteristic pathologic prion protein involved in these cases was found in Norway in 1998 and is known as Nor98.

Objective: To report on the atypical scrapie cases appeared in the Basque Country from 2001 to 2008. Subjects and Methods: Slaughtered or found dead small ruminant tested in NEIKER with a screening rapid test within the TSE prevention and control program of the Basque Country. Positive samples are submitted to other rapid tests and to immunohistochemical analysis.

Results: The mean number of sheep analysed per year has been 680. Until March 2008, a total of 125 animals have been analysed. Five atypical scrapie cases have been found in this period. The two first cases (fallen stock) appeared on 2004, the third one was a slaughtered ewe tested in 2005 that was confirmed as Nor98 in the Norwegian National Veterinary Institute. The two more recent cases were detected both in 2008 and were, respectively, fallen stock and slaughtered for human consumption. Immunohistochemical analyses showed PrP_{Sc} deposits distributed throughout the brain without a defined distribution pattern. However, the cerebellum appears to be the most severely affected area in the majority of the cases. *PRNP* genotyping showed that three out of five animals were ARQ/ARQ and one ARR/ARQ. There is no genotypic data available at the moment for the remaining case. The protein pattern of PrP_{Sc} determined by immunoblot showed a weaker signal and a profile different from that found in classical scrapie cases, resembling that of the atypical scrapie PrP_{Sc}.

Discussion: The presentation of these cases seems to be random without any spatial or temporal pattern. The yearly incidence ranged from 0% to 2% depending on the year and the group of risk. These findings point towards an aetiology rather sporadic or a low effective transmission of the agent under natural conditions.

Acknowledgements: We gratefully acknowledge the help of Dr. S. Benestad who did the tests that confirmed the Nor98 type of one of our cases.

P4.25 Survey for chronic wasting disease in Finnish cervids

Authors
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Content
Background: As chronic wasting disease (CWD) continues to spread in North America, there is growing concern on the possible presence of CWD in the cervid populations in Europe. In Finland the cervid population consists of reindeer (Rangifer tarandus tarandus), moose (Alces alces), white-tailed deer (Odocoileus virginianus), roe deer (Capreolus capreolus), wild forest reindeer (Rangifer tarandus fennicus) and fallow deer (Dama dama). The size of the Finnish reindeer population is about 300 000 in summer. It is "semi-domesticated", mostly free ranging but corralled and supplementary fed in winter months, especially in the southern part of the reindeer herding area. The estimated wild cervid summer populations in Finland are: moose 100 000, roe deer 20 000, wild forest reindeer 2000, fallow deer 900 and a unique population of 40 000 white-tailed deer, which originates from few animals imported from Minnesota in 1930´s. The white-tailed deer is the main species affected by CWD in America.

Objective: The aim of study was to clarify if CWD or other TSE´s are present in semi-domesticated or wild cervids in Finland.

Methods: Heads of cervids over 18 months of age were collected with the help of hunters and veterinarians. Brainstem (obex) and retropharyngeal lymph nodes of reindeer (n=1406), white tailed deer (n=483), moose (n=110) and roe deer (n=27) were analysed in two rapid tests for TSE, Bio-Rad TeSeE® test and Prionics®Check WESTERN.

Results and Discussion: More than 2000 cervids were studied for the presence of TSE´s during the years 2003-2008 in Finland. Samples were analysed in two different TSE rapid tests with negative results. Although the amount of cervids included in this study represents a minor part of the cervid population in Finland, the results obtained so far indicate that the Finnish cervid population is free from CWD and other forms of TSE.

P4.26 Atypical scrapie found in two Icelandic sheep flocks - one secondary case detected

Authors
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Content
In Iceland we had active surveillance of scrapie since 1978, but no cases were detected among healthy slaughter until 2004, when rapid testing was implemented. Currently a few thousand sheep are tested each year, originating mostly from healthy slaughter, but to a minor extent from fallen stock as well as clinical suspects. Since 2004 a total of 18 scrapie flocks have been detected, the mean incidence being 4.5 cases/year. Two third of the cases were detected through passive surveillance and one third by active surveillance. Atypical scrapie has been detected in two sheep flocks in Iceland. The first case, age > 2 years, was detected in 2004 through active surveillance of healthy slaughter (confirmed as Nor98 by NVI, Norway). After culling of that first Icelandic Nor98 flock, brain samples from all adult sheep were tested by TeSeE Elisa (Bio-Rad) and one additional positive case was detected among the 335 sheep. It was confirmed as Nor98 by TeSeE Western Blot (Bio-Rad) (11-12 kD band). Both positive cases in this flock carried histidine at codon 154 of the PrP gene (index case, R/H; secondary case, H/H). The second atypical case in Iceland was detected in 2007. The seven year old sheep was tested because of clinical symptoms described as loss of balance. The flock was culled and all 131 samples tested negative for scrapie. Similar to the earlier Nor98 case, this sheep carried a PrP genotype found to be associated with Nor98 scrapie (154 H/H). In this study we compare PrP genotypes in the two Nor98 flocks to the ones of classical scrapie flocks detected in recent years. Genetic breeding against scrapie has not been used in Iceland except that commercial breeding rams carrying VRQ are excluded. According to an earlier breed survey of Icelandic sheep, VRQ is a risk genotype, while ARR was not found. AHQ was suggested as possibly protective in Icelandic sheep, but has since been detected in one classical scrapie case as well as in the three Nor98 cases.

P4.27 Comparative assessments of the risk of missing BSE test-positive cattle if the minimum age at testing in the healthy slaughter string were raised to 42 months

Authors
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Content
The main goal of this study was to investigate model uncertainty in risk assessment. This relates to un-quantified uncertainty due to assumptions, approximations and modelling techniques, as opposed to parameter uncertainty quantified in the model as calculated by probability distributions. It was decided to choose the following approach: carrying out a number of independent analyses of the same risk assessment problem. A risk question was posed and then each group conducted their own risk assessment without discussion with the other groups.. Each group used the same data. At the end of the process we will compare the models in terms of modelling approaches used, and the variability between results, which should, in theory, provide us with a rough measure of model variability. The risk question that was asked was: "How many BSE test positive animals would be missed if the testing age in the healthy-slaughter string were raised from 30 to 42 months?" Five independent groups developed models ranging from very simple stochastic with few parameters to highly complex mechanistic with many parameters to answer the risk question. Common data was based on BSE testing results and cattle demographics from the Netherlands was used by all groups. Standard assumptions for the age of infection, incubation period etc. were based on UK data. Preliminary results are comparable between the models however large differences in confidence intervals are noted. Further investigation should reveal the sources of the differences. The results suggest that it would be highly unlikely to miss a BSE test positive animal if the minimum BST testing age were raised to 42 months.

P4.28 Spatial analysis of BSE cases in the Netherlands

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Content
Background: Identifying case clustering patterns of infectious disease is important as such patterns can give clues about the transmission process. In many of the European countries affected by Bovine Spongiform Encephalopathy (BSE), clustering patterns of BSE cases have been observed. Most of these patterns have been interpreted in terms of specific heterogeneities in exposure of cattle to the BSE agent.

Objective: Research questions of this study are the following:
- Are the BSE cases in the Netherlands spatially clustered, and if so where are these clusters located?
- Can we relate any observed case clustering to one or more possible underlying heterogeneities?

Methods: We first tested whether clustering is present (global clustering test), and then detected and tested local clusters (Kulldorf scan test). Both the global and local clustering tests were performed in two ways: on the overall case data and on the case data for each birth cohort separately.

Results: We have found three spatial case clusters in the Dutch BSE epidemic. The clusters are geographically distinct and each cluster appears in a different birth cohort. When testing all birth cohorts together, only one significant cluster was detected. We have found that the 1996 cluster is significantly associated with one particular feed producer.

Discussion: We argue that the spatial clustering observed is most likely due to time-dependent heterogeneities in exposure related in part to cross-contamination risks differing between feed producers.

P4.29 Lymphotoxin-dependent prion replication in granulomas

Authors
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Content
Background: TBesides affecting the nervous system, prions frequently invade lymphoid organs and even sites of inflammatory lymphoneogenesis. Prion deposits typically colocalize with Mfge8+ follicular dendritic cells (FDCs), and are suppressed by FDC ablation.

Objective and Methods: Here we studied prion replication in granulomas, a very common form of chronic inflammation.

Results: Subcutaneous granulomas vigorously expressed PrP^c and LTβR, yet lacked all FDCs and did not express Mfge8. After intraperitoneal prion inoculation, *PRNP*+/+ granulomas, but neither *PRNP*o/o granulomas nor healthy *PRNP*+/+ skin, accumulated prion infectivity and disease-associated prion protein (PrP^{Sc}) long before clinical disease. Reciprocal bone marrow transfers between *PRNP*+/+ and *PRNP*o/o mice revealed that prion accumulation in granulomas depended on PrP^c-expressing radioresistant cells. Administration of lymphotoxin signaling antagonists drastically reduced prion infectivity of granulomas.

Discussion: These results identify granulomas as previously unrecognized, clinically silent reservoirs of prion infectivity, and uncover lymphotoxin-dependent prion replication in inflammatory stromal cells that are functionally and histogenetically distinct from FDCs.

P4.30 Comparative Country Case Study Analysis Using Relative Time Anchors to determine Policy Drivers for Bovine Spongiform Encephalopathy and Variant of Creutzfeldt-Jakob Disease Risk Management

Authors
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Content
Background: Bovine spongiform encephalopathy (BSE) emerged in the United Kingdom in the mid-1980s and eventually spread to other countries becoming a disease of global concern. Each BSE-affected country found their first domestic case at different points in time, faced different internal and external challenge levels and responded with different policy mixes. Two decades after BSE was first reported making sense of the different risk management responses by individual countries through time is convoluted due to the diverse country-specific contexts, changing scientific uncertainty, different compliance levels, various regulatory systems and import-export trading milieu.

Objective: To compare policy responses between BSE and non-BSE affected countries to key signal events.

Methods: Non-parametric statistic analyses were carried out for five different hypotheses designed to determine the impact of five signal events and their effect on inducing policy change. Twenty-five countries were evaluated for their response to external and internal challenge reduction.

Results: Five different hypotheses were tested for policy response and time to reduce external and internal challenge including: 1) Knowledge of the UK BSE outbreak as a policy driver (1986); 2) Linkage of BSE to the human form of the disease vCJD (1996); 3) A country's first imported BSE case; 4) A country's first reported domestic case; and 5) The peak of non-UK BSE cases (2001-2002).

Discussion: A semi-quantitative country comparison approach using signal events as time anchors revealed policy change occurred slowly despite knowledge of a new, emerging zoonotic disease. Imported BSE resulted in external challenge responses only. Many countries viewed BSE as a "foreign" problem and reacted only after domestic BSE was detected. Lengthy times to risk reduction measures despite knowledge of BSE signal events by many of the later-affected countries resulted in an extended outbreak of greater magnitude.

P4.31 A cell sorter specifically adapted to prion at the service of the Prion research community

Authors
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Content
The foundation Alliance Biosecure aims to sponsor research on the analysis, comprehension and management of risks for public health, linked to microbiological agents, in particular prions. Recognized as a Public Utility in December 2006 by the French Government, Alliance Biosecure promotes the discovery of technological solutions aimed at improving the detection, elimination and inactivation of these agents within biological products used in human therapy. Indeed biological safety regarding prion risk constitutes a major public health issue, as publicized by the report of a fourth human variant Creutzfeldt-Jakob Disease (vCJD) infection attributed to blood transfusion, in the UK. For this purpose, Alliance BioSecure Foundation is pleased to announce it's new Call for Grant Applications for research projects on biosafety related to prion diseases as well as diseases caused by other emerging pathogens, on themes selected by the Scientific Advisory Board. Of special note is the availability of a three-laser Influx® cell sorter, located in a BSL-3 containment facility (NeuroPrion CEA Research Platform, Fontenay-aux- Roses). This cell sorter was developed by Cytopeia (Seattle, WA, USA), with 12 detectors and a modified design suitable for Prion experiments, including specially adapted decontamination procedures. Here, we present several different experiments that illustrate the capabilities of this cell sorter. First, we show a method for assessment of instrument aerosol containment with bacteriophages. In second, we show with two foundation research projects related to prion its capacity to separate infected cell populations (from monkey blood and from cell culture lines) and to isolate cells (up to a rate of 50.000/s) as a function of cell surface marker expression (up to 10 markers simultaneously). Alliance Biosecure is proud to make available to scientific community this equipment, that will constitute a powerful tool to study & isolate rare cellular sub-populations in blood or in cell culture.

P4.32 Economic Impacts of Chronic Wasting Disease: Econometric Analysis of Hunter Response to CWD Spread and Prevalence

Authors
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Content
Chronic wasting disease (CWD) is present in the cervid populations in Alberta, Canada and its impact and management is a widely discussed topic. Alberta has been actively trying to manage the disease in wild deer herds utilizing three tools: 1) increased hunter harvest and testing, 2) decreasing herd density in CWD areas, and 3) removal of deer in the 10 km2 vicinity of positive CWD cases. These practices are controversial and their outcomes are poorly understood. Furthermore, decisions regarding management practices depend on the economic impact of CWD. This study, through analysis of effects on hunters, estimates the economic impacts of CWD and CWD management strategies. We surveyed resident hunters in Alberta and collected information on hunting activity, satisfaction with management practices, and perceptions of disease prevalence. Scenarios involving disease prevalence and management practices were presented and hunters were asked to indicate the location and number of trips they would take under those conditions. This is an innovative approach due to the data's spatial heterogeneity allowing discernment of hunting trip substitution patterns in response to changing conditions. By combining both the revealed preference and stated preference data, econometric models of hunters' behavioural responses were developed. In general, increases in CWD prevalence and reduction in herd size decreased the number of hunting trips while extra hunting licences did not significantly affect activity. The statistical results suggest that CWD, through prevalence and management, has an overall negative impact on hunting in Alberta. The availability of additional licences was not desirable and culling decreases hunting enjoyment. Interestingly, culling was introduced by the government because original efforts to increase hunter harvest through extra licenses were not successful. The modelling process also provides predictions of the economic impact of CWD spread and prevalence.

P4.33 Estimation and prediction for the BSE epidemic in Great Britain using a stochastic model

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Content
Background: In each country, authorities tried to control the BSE epidemic mainly by feed ban laws. Some questions remain such as the existence of infection through other ways than the artificial food, the efficiency of the main feed ban laws, the initial and the current intensity of the infection and the extinction time of the epidemic.

Objective: We aimed to estimate from the BSE epidemic in Great Britain modelled by a stochastic process, quantities such as the first feed ban law (1988) efficiency, the infection parameters, the distribution of the extinction time, and the number of cases during the first years (from 1982 to 1986).

Methods: We first built a multitype branching process with a time unit of one year in order to get rid off all seasonality effects. The types are the health states at each age. Then, assuming that the disease is rare at the initial time, and the infection is of Reed-Frost type, we derived from this model a limit model on the incidence of clinical cases as the initial size of the population increases to $infty$. We considered two age classes, the young animals (<1 year) and others and used a Bayesian methodology of estimation. We assumed a vertical transmission of 0.1 and modelled the incubation period by a Weibull distribution.

Results: The estimation of the infection parameters concerning the infected cattle food until 1988 were equivalent in the two age classes but around 400 times the infection parameter due to other ways of transmission. The first law (1988) seemed totally efficient. During the first years, the estimations of the number of cases per year from 1982 to 1986 were 0, 1, 2, 178, 545. The most probable extinction time is estimated in 2021. For each estimation, credibility intervals were given leading to a quite narrow credibility region of the epidemic.

Discussion: The reestimation of the initial data appeared necessary to avoid identifiability problems. A larger number of classes would be more interesting in order to check if the majority of cases concern the less than 3 years animals. The influence of the incubation distribution shoud be also tested using another distribution. The methodology could be used in other countries.

P4.34 Clinically Unrecognized Cases of Prion Disease in the National Alzheimer's Coordinating Center Database

Authors
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Content

Background: Analysis of brain tissue is necessary for confirmation of prion diseases such as Creutzfeldt-Jakob disease (CJD). Because of low autopsy rates and the existence of other, more widely reported dementing illnesses such as Alzheimer's disease (AD), it has been suggested that a substantial number of prion disease cases go unrecognized.

Objective: To investigate the possible occurrence of clinically unrecognized prion disease cases among patients with dementia who underwent autopsy.

Methods: The Minimum and Neuropathologic Datasets from the National Alzheimer's Coordinating Center (NACC) for 1984-2005 were reviewed. Patients included in the analysis had dementia and were coded as having either a clinical diagnosis of AD or a known suspected etiology other than a prion disease. All underwent autopsy, had available neuropathologic data, and belonged to a currently funded Alzheimer's Disease Center (ADC). For eligible patients with neuropathology indicative of prion disease, further information was collected from the reporting ADC to determine whether a prion disease was among the physician's clinical diagnoses or considered prior to autopsy.

Results: Of 6,040 patients that met the eligibility criteria, 24 autopsy-confirmed prion disease cases were initially identified. Further clinical information was collected for 21 of the 24 patients; two miscoded patients were subsequently excluded because neuropathology did not indicate a prion disease. Of the remaining 19 patients, a clinical prion disease diagnosis was considered for 12 patients but was potentially unrecognized for 7 patients (0.12% of the 6,040 patients). Most of these 7 patients had atypical manifestations.

Discussion: Discussion The proportion of dementia patients with clinically unrecognized but autopsy-confirmed prion disease was small (<0.17%). In addition to confirming clinically suspected cases, neuropathologic testing is useful to identify unsuspected, clinically atypical cases of prion disease.

P4.35 Risk management of bovine spongiform encephalopathy in the People's Republic of China

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Content

Background: China has experienced tremendous growth in recent years after the launch of a series of reforms in rural areas during the 1970s that transformed agriculture from a domestic planned to export market economy. With the growing economy, food safety in China is now an important domestic as well as an international concern. China has neither been assessed by the European Food Safety Authority (EFSA) nor the World Organisation for Animal Health for its geographical BSE risk (GBR) ranking. Despite being BSE-free, the fear of BSE agent entering the country has prompted the Chinese Government to establish bilateral negotiations with 37 World Trade Organization (WTO) members. China has promulgated many laws to enhance food safety including various BSE preventive measures and food safety policies to reduce external challenge risks of BSE.

Objective: To review the risk mitigation and management strategies for BSE introduced in China.

Methods: A comprehensive and structured survey was carried out for available literature published in peer review journals and books on risk management of BSE in China. Sources also included government reports, grey literature, internet web pages and newspaper databases to assess non-expert information on BSE risk mitigation strategies. The information was compiled to synthesize an overview of BSE risk management.

Results and Discussion: The study provides the background on China's agro-beef sector and comparisons have been drawn between China and its Special Administrative Regions (SAR) including Taiwan and Hong Kong to assess the comprehensiveness of the instituted BSE risk management measures. An attempt has been made to provide an account of the external and internal challenges faced by the country with regards to BSE. While China has implemented many effective BSE management policies some gaps, such as treatment of high risk materials still remain.

P4.36 Transport of the Pathogenic Prion Protein through Landfill Materials

Authors
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Content

Countermeasures to minimize the risk of TSE transmission often involve depopulation of infected herds. Large volumes of infected waste are generated by these responses to TSE threats and, thus, there is a significant need for safe, effective disposal of infected carcasses and other materials. Although landfilling and underground burial are attractive disposal options due to the large volumes of material generated, the risks associated with these options is unknown. Here, we report the results of a study evaluating the potential for transport of pathogenic prion protein (PrP^{T}_{SE}) derived from carcasses and associated wastes in a MSW landfill. Column tests were conducted to evaluate PrP_{TSE}transport in quartz sand, two fine-textured burial soils currently used in landfill practice, green waste residual (a potential burial material), and municipal solid waste (MSW). All experiments used PrP_{TSE}-enriched fractions prepared from the brains of clinically infected Syrian hamsters. Flow and transport models were used to assess migration of PrP_{TSE} in a typical landfill environment and to assess PrP_{TSE} concentrations that can be expected in a typical leachate collection system. The column tests demonstrated that PrP_{TSE} was retained strongly by quartz sand and the fine-textured burial soils. The column experiments conducted with both fresh and aged MSW as well as the green waste residual showed that PrP_{TSE} migrates through these materials. Burial of CWD-infected materials at MSW landfills is expected to provide secure containment of PrP_{TSE} provided reasonable burial strategies are used.

P4.37 Are we at risk of newly emerging TSEs in livestock?

Authors
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Content
The BSE epidemic is fading out in most of Europe and by now it is clear how BSE and classical scrapie can be controlled. Thus it would appear that TSEs in European livestock are no longer a problem. However, suggestions of lifting some of the rigorous control measures for BSE tend to be challenged by arguments on the risk of atypical forms of BSE. Further voices suggest that scrapie should be monitored better and the efficiency of the surveillance effort would lead to more relevant information if countries would apply the same specifications for surveillance of atypical scrapie for a limited time period. The question remains whether we really need to worry about those atypical TSEs. A lot of information is available for BSE, thanks to six years of active BSE surveillance in most of the EU and from the last 25 years of BSE knowledge worldwide. From these data we can quantify a maximum probability of new TSEs evolving into an epidemic problem under present conditions and estimate this to be small. Scrapie data is more sparse and far less complete than the BSE data, so a similar analysis does not yet lead to similarly optimistic conclusions with respect to TSEs in small ruminants. We place the risk of atypical TSEs and the costs of the present surveillance into perspective by comparing with other veterinary diseases and zoonoses which are presently emerging in the EU.

P4.38 Variations in the genotype profile and in the allele frequencies after 4 years from the start of the Italian Breeding Selection Plan for scrapie

Authors
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[1]CEA-IZSPLVA; [2]IZS Sardegna; [3]ISS Rome; [4]Ministry of Health.

Content
Background: Many studies helped in clarifying the epidemiology of scrapie and in identifying the risk factors favouring its spread such as e.g. movement of sheep, lambing management, flock size. However this knowledge hardly could be used to implement effective preventive measures. Only after the discovery of the linkage between classical scrapie disease risk and PrP genotype, new tools have been made available to control the disease: the potential for PrP genotyping and selective culling to control scrapie became the basis for a new strategy of handling the disease.

Objective: Aims of this work were to describe the Italian Breeding Selection Plan (IBSP) based on ram genotyping schemes and to provide preliminary results of its implementation.

Methods: Data were obtained from the central database of the IBSP. The differential enforcement of the plan by geographical area was assessed by calculating rates (per 1000) of involvement of local flocks. The genotype profile and allele frequencies of ram lambs were calculated by breed, geographical area and birth cohort. Variations of the frequencies in successive crops of ram lambs were monitored between 2004 and 2007.

Results: So far 15 out of 21 regions have launched their own breeding plan showing a wide range of the proportion of involved flocks and of the number of rams tested. Over 36,000 rams were genotyped throughout Italy. An overall increase in the resistant genotypes (ARR/ARR shift from 19.3% to 21.1) and in the ARR haplotype frequency (from 41.2% to 42.7) is noticeable when comparing the ram lambs tested till 2005 with those tested in the last two years. Sarda and Biellese breeds, the most tested ones, show the best results.

Discussion: After 4 years from its implementation the IBSP shows promising results. That is relevant for Italy where scrapie is circulating in its large sheep population. Differences in the plan implementation between breeds and geographical areas are evident and need to be dealt with.

P4.39 Is amyloid detection on surgical stainless steel a sufficient marker for removal of prion infectivity?

Authors
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Content
Background: Prions are unique infectious agents which have been shown to be transmitted iatrogenically through contaminated surfaces. The standard method for direct validation of new procedure has historically been rodent bioassay. This method requires a certain number of animals and the duration generally exceeded one year. We developed and adapted a protocol for the contamination of stainless steel wires adapted to different animal models infected with specific strain (scrapie strain 263K or BSE strain 6PB1) and validated original decontamination procedures.In addition to such bioassay and cell models, it is critical to urgently develop a method for the detection of protein contamination on surgical instruments and other surfaces. Recently, light microscopy and episcopic differential interference contrast/epifluorescence (EDIC/EF) techniques have been developed which incorporate sensitive fluorescent dyes SYPRO Ruby and Thioflavin T, for the detection of very low levels of protein and amyloid contamination respectively on surgical metal surfaces.

Objective: To compare EDIC method to bioassay validated with 263K model. To study by EDIC technique the resistance of 263K when spiked in various healthy brain sources and compare with pattern of the protein detected in western-blot before and after treatments.

Methods: Stainless steel wires were exposed to 263K strain spiked in various brain homogenates, including rodent and non-rodent sources. The effect of various decontamination procedures on general protein (Sypro identification) and amyloid forms (Thioflavin T labelling) was assessed and compared to standard in vivo (bioassay) and in vitro (western-blot) methods.

Results: We were not able to establish a direct relationship between EDIC/EF technique and both standard methods. There appears to be no immediately discernable correlation between the prion removal and the reduction of infectiosity titre evaluated in bioassay after treatments. Moreover, there was not direct evidence of role of surrounding brain matrices in spike experiments.

Conclusions: The lack of correlation between homogenates and the reproducibility of the results would suggest a possible lack of homogeneity within samples instead of a specific result due to the mammalian species. The lack of correlation between infectiosity levels in animal assays and detection of amyloid forms by EDIC would tend to confirm recent suggestions that PrP_{Sc}-associated amyloid would only partially represent the infectious agent and raises question as to its reliability as a marker for prion disease.

P4.40 Role of Humic Acid on the Adsorption of Prions to Clay

Authors
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Content
Soil may contribute to the horizontal transmission of the prion diseases sheep scrapie and chronic wasting disease of deer, elk and moose by serving as an environmental reservoir for the infectious agent. We previously demonstrated that the disease-associated form of the prion protein (PrP_{TSE}) binds to soil particles and that the interaction of PrP_{TSE} with the clay mineral, montmorillonite (Mte), is remarkably avid. Here, we investigate the effect of natural organic carbon on the interaction of PrP_{TSE} with soil particles by studying its sorption to humic acid-montmorillonite (HA-Mte) complexes. HA-Mte complexes have a lower affinity for PrP_{TSE} than Mte alone and the binding capacity of Mte for the protein decreased with increasing HA content. We previously established that PrP_{TSE} desorbed from Mte surfaces is cleaved at an N-terminal site. Prion protein desorbed from HA-Mte complexes were similarly cleaved exhibiting a lower molecular mass than the starting material. Extraction of humic acid from HA-Mte complexes after PrP_{TSE} sorption suggests that PrP_{TSE} associates primarily with Mte surfaces and that organic carbon blocks PrP_{TSE} binding sites on Mte. Sorption of prions to Mte prior to oral exposure dramatically enhances disease transmission.

P4.41 Low Density Lipoprotein Levels Predict Survival Time in Sporadic Creutzfeldt-Jakob Disease

Authors

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Content

Background: Lipids have been implicated in the conversion of PrP_C to PrP_{Sc} and post-mortem brain studies have shown that PrP_{Sc} binds to VLDL and LDL lipoproteins. We are not aware of any studies that have examined serum lipid levels and survival time in sCJD patients.

Objective: To examine associations between serum lipid levels and survival time in sCJD patients

Methods: Medical records of individuals with a clinical or pathological diagnosis of sCJD were obtained from three medical centers. Clinical data were collected using a standardized instrument. Mean serum lipid levels obtained one year prior to the onset of illness and throughout its duration were divided into quartiles for statistical analyses using Cox regression proportional hazard and chi-square analyses.

Results: Sixteen cases of probable (n=5) and definite sCJD cases (n=11) with a mean age at illness onset of 64 years and mean survival time of 264 day were included in this study. Seven cases had two or more serum lipid tests, drawn an average of 27 days into the course of illness. The mean total cholesterol level was 186mg/dL, mean LDL level was 111mg/dL, and mean HDL level was 47mg/dL. Survival time was inversely related to LDL levels when controlling for age at illness onset, history of coronary artery disease or stroke, and the use of statin medications. No significant associations were detected between survival time and HDL levels. Preliminary analyses of *PRNP* codon 129 genotypes and PrP_{Sc} types failed to demonstrate significant differences in lipid levels and survival time.

Discussion: Prior studies have reported that LDL and VLDL bind to PrP_{Sc} and are sites of PrP_{Sc}conversion. Since PrP_{Sc} precipitates further transformation of PrP_C, the inverse relationship of survival time and LDL levels may be in turn correlated with levels of PrP_{Sc} and/or neurotoxicity. Further studies are needed to investigate this phenomenon.

P4.42 Assessing the impact of a revision of the BSE Monitoring regime in some European Union Member States

Authors

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Content

Background: The European Food Safety Authority (EFSA) is the responsible body in the European Union for Risk Assessment regarding food and feed safety. In the context of the declining BSE epidemic in cattle and following the strategic document "The TSE Roadmap", in January 2008 the European Commission asked EFSA to provide an assessment on the existence of an additional risk to human and animal health, compared with the situation at the time, following the implementation of a revised BSE monitoring regime in the old 15 European Union Member States (EU15).

Objective: The EFSA was invited to consider age options between 30 and 60 months (with 6 months intervals) for BSE testing of healthy slaughtered cattle and between 24 months to 60 months (with 6 months intervals) for testing of at risk cattle in EU15 and compare the different scenarios. In addition, in March 2008, Belgium asked EFSA to extend the assessment to healthy slaughtered cattle up to 84 months of age and to healthy slaughtered cattle born after 01/01/2004.

Methods: Based on the data collected on BSE active and passive surveillance in EU15 during the period 2001 – 2007, a model has been created to provide an estimate of the number of detectable BSE cases per age and target group in future years in EU15 under different scenarios. The model remains valid in the context of the continuation of the feed ban in EU. The assessment considers the ability of a monitoring system to detect: i) a new trend in the epidemiology of BSE, ii) an increase in incidence of atypical BSE and iii) a possible emergence of new TSEs.

Results: The study assesses the different scenarios and provides the number of estimated BSE cases that would be missed when increasing the age limit for active BSE surveillance in cattle. Further considerations about the sensitivity of a BSE surveillance system and atypical BSE are included.

Discussion: The main parameters to be considered when setting up a TSE monitoring system in cattle are discussed.

P4.43 Quantitative Risk Assessment of Developing Variant Creutzfeldt-Jakob Disease (vCJD) From Human Islet Cell Transplantation Procedures

Authors

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Content

Background: A recently improved method for successfully transplanting human islet cells into adult patients with Type 1 diabetes can significantly reduce the effects of the disorder. The medical treatment is called the Edmonton Protocol. Concerns have been raised that the method used for islet cell isolation may potentially put recipients, who may undergo multiple rounds of islet cell infusions over time, at an increased risk for iatrogenic transmission of variant Creutzfeldt-Jakob disease (vCJD).

Objective: To estimate the risk of iatrogenic transmission of vCJD during islet cell transplantation procedures using a quantitative risk assessment.

Methods: Monte Carlo Simulation (MCS) as a tool for Scenario Analysis was used to create a model. Different steps of the protocol and enzyme manufacturing process were examined. Factors that either amplified or reduced the overall risk were identified and included in the final model which estimates, as the endpoint, the potential risk of infection per islet cell treatment. MCS Scenario Analysis was conducted for minimum, most likely and maximum values as a range for each of the input variables. Appropriate probability distributions were assigned to the input variables to generate probability distributions for model outputs. The method was chosen because of its capability to model low probability situations.

Results: The result of the risk assessment estimates that the potential risk of exposure to disease-causing prions per islet cell procedure is at one in one hundred million (1.12x10-8). The 95% confidence interval around the uncertainty of this result is below de minimis risk levels (CI: 3x10-9, 1x10-6).

Discussion: The prevalence of Type 1 diabetes worldwide and increased incidence in some vulnerable populations make assessing the level of iatrogenic vCJD risk from islet cell transplant procedures of immense importance. The results suggest the probability of vCJD transmission by this exposure route is negligible.

P4.44 Study of decontamination methods applied on reusable materials in the manufacturing process of plasma-derived products

Authors

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Content

Transmissible spongiform encephalopathies (TSE) are fatal neurodegenerative diseases of humans and animals. The underlying infectious agent, commonly called prion, accumulates not only in the central nervous system (CNS) but also in peripheral lymphoid organs, which then could contribute to potential infectivity of bodily fluids, including blood. Indeed, in vivo experimental assays and epidemiological data show that blood does harbour low levels of TSE infectivity. Nonetheless, prions have never been detected in human plasma, so their presence therein remains theoretical.

In spite of this, LFB, a producer of plasma-derived medicinal products, is committed to the evaluation of prion removal and decontamination of surfaces in contact with plasma or its derivatives during manufacturing. The unique biochemical properties of prions pose both challenges and opportunities for its clearance and decontamination. Prion particles can be inactivated by unfolding of their native protein structure. Various conditions of decontamination, including nature and concentration of the decontaminant, exposure time and temperature were studied on different matrices and are described here. Practical goals we are attempting to achieve are effective and complete decontamination of reusable materials while maintaining that the safety of the operators is ensured.

P5.01 The Proteomic Identification of the Prion Protein Membrane Interactome

Authors
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Content
The prion protein is suggested to be multifunctional protein involved in several pivotal cellular processes. However, the biological role of PrP^c is strongly depended on its expression level. Under physiological conditions prion protein promotes neuroprotection and it is engaged in the regulation of the cell death, the protection against oxidative stress, the copper binding and the modulation of few signal transduction pathways known to promote cell survival. On the other side, PrP overproduction sensitizes cells to apoptotic stimuli. However, the proteins, in particular membrane, modulated by different levels of PrP^c expression causing the observed anti- and pro-apoptotic phenotypes are largely unknown.
In these proteomic studies we aim to identify membrane proteins, that either directly or indirectly contribute to the observed pro-apoptotic phenotype of cells overexpressing cellular prion protein. The PrP^c membrane interactome was investigated in HEK 293 cell line and an overexpression of cellular prion protein was achieved by using conditional Tet-off system, which permits simultaneous and high co-expression of the gene of interest and the reporter gene in response to the absence of doxycycline in the cell culture medium. The membrane fractions of wild type and transfected cells were separated using the 2-dimensional technique, 16-BAC/SDS-PAGE. This method is in particular designed for the efficient investigation of hydrophobic and highly insoluble proteins. Afterwards, the quantitative analysis of proteins expression patterns were carried out. The identification of PrP^c membrane interactome might provide a deeper insight into the mechanism underlying prion pathology.

P5.02 Prion Protein-deficient Cattle are Resistant to Prion Disease

Authors
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Content
Background: Transmissible spongiform encephalopathies (TSEs) or prion diseases are caused by the propagation of a misfolded form (PrP^d) of the normal cellular prion protein, PrP^c. Disruption of PrP^c expression in the mouse results in resistance to PrP-propagation and disease. However, the impact of the ablation of PrP^c function in a natural host species of prion diseases is unknown. Recently, we have reported the generation and characterization of PrP^c-deficient cattle (PrP-/-) produced by a sequential gene targeting system.

Objective: In this study we wanted to determine whether PrP-/-cattle are susceptible or resistant to prion diseases.

Methods: Five *PRNP*+/+ wild-type and five *PRNP*-/- cattle were inoculated intracerebrally with a 10% brain homogenate derived from a bovine infected with a cattle-adapted TME isolate. Six other cattle (three each *PRNP*+/+ and *PRNP*-/-) were inoculated with normal brain material.

Results: *PRNP*-/- cattle inoculated intracerebrally with a cattle-adapted transmissible mink encephalopathy (TME) isolate (i) do not replicate abnormal PrP^d in their central nervous system (CNS) for at least 23 months post inoculation (MPI) and (ii) are clinically normal at least 27 MPI. In contrast, all five PRNP+/+ cattle inoculated with TME were euthanized with clinical signs within 18 MPI and contained abnormal PrP^d in their CNS tissues.

Discussion: Our results for the first time determine that PrP^c is a critical component in the pathogenesis of TSE disease of a natural host.

P5.03 Comparative analysis of prion protein isoforms in human CSF and serum by using a panel of prion protein antibodies

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Content
The cellular prion protein (PrP^c) is a cell surface, glycosylphosphatidylinositol-anchored protein that is abundantly expressed in neurons. Despite its pathologic role in transmissible spongioform encephalopathies PrP^c is highly conserved in evolution and occurs as a ubiquitous glycoprotein in three different isoforms: unglycosylated, monoglycosylated and diglycosylated PrP^c. Sporadic CJD cases are currently subclassified according to the methionine/valine polymorphism at codon 129 of the *PRNP*. In our study we investigated human body fluids (cerebrospinal fluid and serum) from three different sCJD subtypes (MM, MV, VV) by SDS-PAGE and Western Blotting.
To detect subtype-specific PrP^c isoforms or PrP^c-processing fragments we used a panel of different PrP-antibodies raised against a broad spectrum of epitopes on PrP^c. We analyzed the PrP banding-patterns (glycosylation status and cleavage fragments of PrP^c) of each sCJD-subtype in comparison to control patients without any dementia diseases. Almost every antibody shows a characteristic banding-pattern, which is also body fluid dependent. CSF and serum differs in their PrP-glycosylation-status. Moreover, some antibodies also discriminate between CSF-PrP and serum-PrP, indicating different PrP-structures in both fluids. Additionally, we could detect variations in the glycoforms composition between sCJD-subtypes. In conclusion, our study provides valuable new insights in the exploration of the PrP^c-biology and in the establishment of diagnostic assays for PrP-diseases in humans.

P5.04 Polyanions and their influence on the cellular prion protein

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Content
Background: In nowadays very much the attention is spared to the problem of the treatment of prion infections. Most efforts are concentrated on the search of preparations which would block occurance or reduced the level of already present PrP^{Sc}. At the same time, very little attention is spared to problem of prophylaxis of the prion infections. Although work in this direction is rather active, taking into considiration a creation of transgenic cattle in which the gene of *PRNP* is knocked-out. But still it is unknown, how exactly will react an organism on absence of such important protein as PrP^c. That is why we consider, that more perspective direction is a search of preparations which would reduce the level of PrP^c, in the same time depriving PrP^{Sc} of substrate for replication. These preparations can be applied in risky herds, with suspicious or sick animals were founded, for the reduction of risk of prion infections origin among the healthy individuals.

Objective: That is why the purpose of our work was a search of preparations which would reduce the level of PrP^c in the organs of the prion-replicating system of laboratory animals.

Methods: Work was done on laboratory rats treated with heparine and pentosan polisulfate (PPS). After decapitation in the proper organs were probed: general maintenance of PrP^c, type of expression of its izoforms, maintenance of zinc and copper, activity of superoxidedismutase, evaluation of DNA damage, ELISA test of PrP^c interaction with pentosan polisulfate.

Results: It is set that heparine and PPS is able to reduce the level of PrP^c in the organs of the prion-replicating system; unlike heparine, it is shown that PPS that he operates in far less concentrations and does not influence on the state of the antioxidant system. It is also shown that action of PPS on PrP^c proportionally depends on the concentration of preparation. In the case of heparine such dependence was not discovered. It is set that both preparations do not influence on genomic DNA, which is proved by the absence of doblestrain DNA breaks. Taking into consideration this results, we decided to check up a possibility of interaction of PPS with PrP^c.

Discussion: It is set an inhibiting action of PPS and heparine on PrP^c. Also was probed a nature of PPS/PrP^c interaction and mapped functional groups, accountable for interaction of these molecules.

P5.05 A large ribonucleoprotein particle induced by cytoplasmic PrP shares striking similarities with an RNA granule known as the chromatoïd body

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Content

Background: Although the prion protein (PrP) is mainly targeted to and expressed at the plasma membrane, PrP has also been detected in the cytosol of neuronal and non-neuronal cells. Moreover, the observation that PrP N-terminal signal peptide is particularly inefficient has brought speculations concerning a possible function of the protein in the cytosol. Hypoxia induces the expression of a cytosolic splice variant of the prion protein, indicating a possible regulation of cytosolic PrP in particular conditions. Previously, we have shown that cytosolic PrP co-aggregates with poly(A)+ RNA to form a ribonucleoparticle. Here, we have further investigated the relationship between cytosolic PrP and RNA.

Objective: To determine the nature of the ribonucleoparticle in cells expressing PrP in the cytoplasm.

Methods: Neuronal and non-neuronal cultured cells were transfected with a construct encoding a cytosolic form of PrP, termed cyPrP. The distribution of RNA molecules and proteins was determined by fluorescence in situ hybridization with specific probes and by immunofluorescence, respectively. Association of different proteins was examined by co-immunoprecipitation.

Results: Here, we show that cells expressing cyPrP display a large juxtanuclear cytoplasmic RNA organelle surrounded by a cage of vimentin protein. Importantly, the assembly of this RNA organelle is independent of cyPrP aggregation. Components of the organelle fall into three classes: (1) mRNAs; (2) proteins, including the RNAseIII family polymerase Dicer, decapping enzyme Dcp1a, DEAD-box RNA helicase DDX6, and small nuclear ribonucleoprotein-associated proteins SmB/B'/N; (3) and non-coding RNAs, including rRNA 5S, tRNAs, U1 small nuclear RNA, and microRNAs. This composition is similar to RNA granules or chromatoid bodies from germ cells, or planarian stem cells and neurons, which are large ribonucleoprotein complexes predicted to function in posttranscriptional gene regulation. The domain of PrP encompassing residues 30 to 49 is essential for the formation of the RNA particle.

Discussion: Our findings underscore an unexpected function for cytosolic PrP, assembling a large RNA processing center similar to chromatoïd bodies. The function of this RNA particle and its possible involvement in prion diseases will be the focus of future investigations.

P5.06 Divalent Metals Stabilize Cellular Prion Proteins and Alter the Rate of Proteinase-K Dependent Limited Proteolysis

Authors
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Content

Background: The key biochemical event in the pathogenesis of prion diseases is the conversion of normal cellular prion proteins (PrP^{c}) to the proteinase K (PK) resistant, abnormal form (PrP^{sc}); however, the cellular mechanisms underlying the conversion remain enigmatic. Binding of divalent cations such as copper to the octapeptide repeat regions of PrP has been shown to be important for the stability of the protein. Nevertheless, the roles of other divalent cations in the normal processing of cellular PrP^{c} are not well understood.

Objective: In the present study, we examined the role of transition metals (Mn2+ and Cu2+) on PrP^{c} expression and degradation in cell culture and brain slice models.

Methods: Neuronal cells expressing mouse prion proteins with a genetically altered novel epitope (mAb 3F4) and brain slices were exposed to Mn2+ and Cu2+ over 24hr. Levels of PrP^{c} protein and mRNA were measured. Limited proteolysis, mRNA stability, proteasomal activity and pulse-chase experiments were conducted.

Results: Metal treatment increased PrP^{c} levels in both cytosolic and membrane-rich fractions in a time-dependent manner. However, metal treatment neither increased PrP mRNA transcripts nor altered the mRNA stability, indicating that Mn may act at the post-translation and/or degradation levels of PrP^{c}. Additionally, metal exposure did not alter the proteasomal or lysosomal degradation pathway. Pulse-chase analysis showed that the PrP^{c} turnover rate was significantly decreased with metal treatment. Limited digestion with PK also revealed that metal treatment decreased the digestion rate of PrP^{c}.

Discussion: Collectively, these data suggest that certain divalent metals can alter the normal processing of PrP^{c}, resulting in the accumulation of PrP^{c} with altered susceptibility to PK. (supported by DoD/MHRP grant W81XWH-05-10239).

P5.07 PrPc controls neurotransmitter catabolism in neuronal cells through TNFa signaling

Authors
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Content

Background: We have previously documented that the cellular prion protein PrP_C acts as a cell surface receptor able to instruct downstream signal transduction events upon antibody-mediated ligation. In mature bioaminergic neuronal cells, PrP_C fulfils some neuronal-specific function that is sustained by its association with the membrane protein caveolin and the Fyn tyrosine kinase within a platform located on neuritic extensions. Our initial studies allowed us to propose that PrP_C may take part to the homeostasis of neuronal functions.

Objective: The aim of the present study was to shed further light on the signaling events through which PrP_C takes part to the homeostasis of neuronal cells.

Methods: Our investigation model is the 1C11 cell line, endowed with the capacity to acquire the overall functions of either serotonergic (1C115-HT) or noradrenergic (1C11NE) neurons. We monitored the impact of antibody-mediated PrP_C ligation on TNFa shedding (ELISA), and on the accumulation of serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine (NE) degradation products (HPLC).

Results: Antibody-mediated ligation of PrP_C at the surface of mature 1C115-HT or 1C11NE neuronal cells induces the shedding and accummulation of TNFa in the cell milieu. We show that the PrP_C-dependent TNFa release is relayed by the Fyn kinase and the metalloproteinase TACE (TNFa Converting Enzyme). TNFa acts in turn as a second message signal and elicits 5-HT and NE degradation into 5-Hydroxy-Indole-Acetic Acid (5-HIAA) and 3-Methoxy-4-Hydroxy-Mandelic acid (VMA) in 1C115-HT and 1C11NE cells, respectively.

Discussion: Our data establish for the first time a functional link between PrP_C signaling and neurotransmitter metabolism in bioaminergic neuronal cells. Our study defines TNFa as a downstream target of PrP_C signaling and an intrinsic modulator of neurotransmitter-associated functions. Distorsion of such PrP_C control on TNFa release and neurotransmitter metabolism by PrP_{Sc} may account for elevated levels of TNFa and dysfunctions of the 5-HT and NE systems in TSEs.

P5.08 CREB-dependent gene regulation by prion protein: impact on MMP-9 and beta-dystroglycan

Authors
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ontent

While PrP_C plays a key role in Transmissible Spongiform Encephalopathy pathogenesis, the physiological function of the protein remains poorly understood. Taking advantage of the 1C11 neuroectodermal cell line, endowed with the capacity to differentiate into either 1C115-HT serotonergic of 1C11NE noradrenergic neuronal cells, we have previously assigned a signaling function to PrP_C. Here, we decided to build upon our previous findings and go further into the characterization of PrP_C-dependent signaling cascades. We used antibody-mediated PrP_C ligation as a means to mimic the interaction with an extracellular ligand and instruct signaling events. We identify the cAMP responsive element binding protein (CREB) transcription factor as a target of PrP_C signaling downstream from the MAPK ERK1/2, in 1C11 precursor cells and their 1C115-HT and 1C11NE neuronal progenies. In response to PrP_C ligation, CREB triggers Egr-1 and c-fos transcription, two immediate early genes that relay CREB''s role in cell survival and proliferation as well as in neuronal plasticity. Furthermore, in 1C115-HT and 1C11NE exclusively, we draw a link between the PrP_C-dependent CREB recruitment and the matrix metalloproteinases (MMPs) system with a transcriptional regulation of MMP-9 and its inhibitor TIMP-1, which play pivotal roles in neuronal pathophysiology through extracellular matrix remodeling. At last, we show that the PrP_C-dependent control of MMP-9 impacts on the processing of the transmembrane protein, beta-dystroglycan. Taken together, our data constitute the prime evidence that PrP_C stimulation can switch on a transcription factor and define molecular mechanisms that likely mirror PrP_C ubiquitous contribution to cytoprotection as well as its role in neuronal plasticity.

P5.09 A proteomic study to investigate the role of the prion protein in the cellular defence against oxidative stress.

Authors
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Content

Background: Several functions have been attributed to the cellular prion protein, PrP_C, amongst which its anti-oxidant role has rapidly been gaining interest in the recent years. We and others have previously shown, that PrP_C expressing cells, of neuroblastoma or epithelial origin, seem to exhibit a higher overall viability towards paraquat toxicity than cells expressing basal or low levels of the protein [Dupiereux et al, 2007; Senator et al, 2004]. The mechanism of this PrP_C-mediated protection is, however, unknown.

Objective: Our objective was to investigate, at a proteomic level, by which potential mechanism PrP_C could protect neuroblastoma cells against paraquat induced oxidative damage.

Methods: Human neuroblastoma cell lines were untransfected (SH-SY5Y) or transfected with a murine PrP_C construct (wtPrP) to over-express PrP_C. The experimental strategy for the proteomic study consisted in highlighting certain target(s) of paraquat toxicity and then proceeding to study their protein levels in relation with PrP_C expression. Protein lysates of experimental conditions were separated by 2DLC based chromatofocusing and reverse phase, and by 1DLC-GE based reverse phase chromatography coupled with lava purple stained SDS-PAGE, for complementary data mining. Differential peaks between the paraquat untreated controls and the paraquat treated cells were analysed using LTQ orbitrap tandem mass spectrometry (MS/MS).

Results: A total of 26 differential chromatographic peaks, from the 2DLC and 1DLC-GE techniques, were analysed for identifying the potential target(s) of paraquat toxicity. Among the candidates highlighted, we studied the implications of PARP-1 and peroxiredoxin-1 in PrP_C-mediated anti-oxidant defence against paraquat toxicity.

Discussion: An interesting aspect of our study has been the detection, by 2DLC and 1DLC-GE coupled to MS/MS, of several candidates that could participate in PrP_C-mediated protection against paraquat induced oxidative stress. Although, it was out of our scope to investigate each of these candidates in the present study, it presents an interesting perspective for future studies. We have, however, shown the implication of one such candidate: PARP-1. Complimentary tests will be necessary in the future to confirm the actual interaction of this candidate with PrP_C.

P5.10 Developmental role of the prion protein in the nervous system

Authors
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Content

Background: The cellular form of the prion protein (PrP^c) in mammals is expressed in all tissues, mostly in the central nervous system (CNS). During development, its expression in the CNS increases then it remains at plateau in hippocampus and olfactory bulbs, whereas in other brain regions it decreases with age. PrP^c is mainly expressed on fiber tracts and on the surface of elongating axons, and with age its expression shifts to the synaptic boutons. This latter characteristic of the protein points out at possible functions for PrP^c in that compartment.

Objective: Aim of this work is to identify the neurodevelopmental expression pattern of PrP^c in the CNS. The anatomical distribution of PrP^c might also be indicative of the possible functions of PrP, and consequently of possible dys functions in prion-infected individuals.

Methods: We investigated PrP^c localization both at protein and at mRNA level by using immunolabelling and in situ hybridization techniques. We stained slices of mouse brains collected from early embryonic to adult life.

Results: PrP^c results expressed during neurodevelopment in brain structures belonging to the thalamo-limbic system, with localization in specific white matter fiber tracts. Then with age its expression increases in other brain regions as expected, and stabilizes at adult life.

Discussion: Thalamo-limbic system regulates circadian, autonomic and hormonal functions, and stress response behaviors. Animals, where PrP^c may not carry out its physiological functions, either in its absence (knock-out mice) or in its abnormal and pathogenic form (prion-infected animals), show behavioral and biological dysfunctions. These alterations can be related to disruptions of the thalamo-limbic neuro-circuitry. Here we show that during neurodevelopment PrP^c is abundantly expressed in neuronal structures belonging to this neural system. These findings can point out a role for PrP^c in the correct development, structuring and functioning of this complex neural system.

P5.11 Investigating the biophysical and neurotoxic properties of PrP peptides modelled on C-terminal fragments generated from endogenous cleavage

Authors
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Content

Background: Full length mature PrP^c (23-231) can undergo proteolytic cleavage before residue 112 or 111 (alpha cleavage), generating N1 and C1 fragments. It can also be cleaved around residue 90 (beta cleavage), generating N2 and C2 fragments. Beta cleavage is associated with reactive oxygen species (ROS). The C2 fragment appears increased in post-mortem CJD brains and correlates with pathogenesis in scrapie-infected mice, and accumulation of PrP^{sc} in infected cells. In Gerstmann-Sträussler-Scheinker (GSS) syndrome, abnormal PrP conformers commonly undergo further proteolysis resulting in both N- and C-terminal truncation yielding a fragment approximating residues 90-145. Alpha cleavage interrupts a potentially amyloidogenic and neurotoxic domain based on previous 106-126 synthetic peptide studies. It is therefore of importance to understand the potential role of PrP processing in prion disease.

Objective: To investigate implications of endogenous PrP processing by evaluating fundamental biophysical and neurotoxic properties of synthetic PrP fragments modeled on alpha and beta cleavage, of a truncated PrP isoform found in familial GSS (Y145stop mutation).

Methods: Synthetic prion peptides (based on human PrP sequence and equivalent endogenous processing) 112-144 and 111-144 (alpha cleavage) and 90-144 (beta cleavage) were characterised using a number of biophysical techniques and an established cell viability assay.

Results: Fluorometric cell viability assays showed 90-144 is toxic to human SH-SY5Y neurons, whereas 111-144 and 112-144 are not. This toxicity was not a simple consequence of increased aggregability as 112-144 and 111-144 aggregate faster than 90-144. Altered production of ROS was not different between the peptides, and therefore does not account for toxicity also. Further studies however, added greater complexity, as incubation of these synthetic human peptides with murine cell cultures demonstrated that both 111-144 and 90-144 were toxic, whereas 112-144 remained non-toxic.

Conclusion: Overall, our results support the view that increased C-terminal beta cleavage of PrP^c appears detrimental to human neurons. Investigations so far have not revealed the biophysical basis or mechanism of toxicity, but studies to date show this does not simply correlate with peptide aggregability, Cu2+ binding, or production of ROS. In addition, work thus far suggests endogenous cleavage cleavage may have differing effects depending on the specific mammalian species context.

P5.12 PrP isoforms and their effect on susceptibility to prion disease

Authors
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Content

PrP^c usually exists as a membrane attached glycoprotein but minor isoforms can span the membrane or they can be released as soluble PrP. During posttranslational modification of the protein specific cleavage of full length PrP occurs between codons 112 and 113 of ovine PrP. This cleavage event results in two fragments of 9kDa and 18kDa, called N1 and C1. The function of these additional isoforms is unknown. Truncated isoforms have been found in cell culture models, ovine milk, epididymal fluid of male sheep and in human and ruminant brain. When the prion protein is cleaved it reduces the amount of available PrP. The reduction in the availability of PrP may have an effect on convertibility of PrP^c to PrP^{sc} or amount of PrP^c recycling in the normal cell. We propose that the quantitiy of full length PrP is important in the conversion of PrP^c to PrP^{sc}. Our aim is to show an association between cleavage ratios of full length PrP and the C1 isoform and susceptibility to scrapie disease. This project will investigate (i) The effect of PrP gene polymorphisms on the ratio between full length PrP^c and the C1 isoform. (ii) The effect of age on the ratio between full length PrP^c and the C1 isoform. Using monoclonal antibody 6H4 we analysed full length PrP and C1 cleavage ratios in four regions of the sheep brain. Two of these areas show significant reduction in the amount of C1. Quantification of the signal intensities were carried out using densitometry. A range of genotypes have been analysed although there does not appear to be any significant differences between these groups. Animals of varying age groups were also tested for differences with some groups showing a significant reduction in the amount of C1.

P5.13 Modulated Intracellular Release of Copper Ions Leads To Depletion of PrP

Authors
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Content

Background: PrP^c is known to be a copper binding protein and copper is known to play an important role in the aetiology of prion diseases. Recent studies have shown that metal bis(thiosemicarbazonato) complexes provide a method for direct intracellular delivery of copper (Donnelly PS, et al, JBC, 2008). These complexes can cross the blood brain barrier and are engineered to be either stable (atsm) or susceptible (gtsm) to intracellular reduction and release of metal. We examined the outcome of treating neuronal cells with two such copper complexing ligands to monitor their effects on the prion protein.

Objective: To determine the effects of the metal-complexing ligands, Cu (gtsm) and Cu (atsm) on the prion protein in neuronal cell models.

Methods: The neuronal cells were set up in 24 well plate format at equal cell density. The compounds were dissolved in DMSO and serial dilutions were made in complete media. The various dilutions were added to the cells, which were then incubated at 37ºC for selected time periods. Following treatment, the cells were collected and lysed for western blot analysis. The ICSM-18 antibody was used to detect PrP to identify differences between wildtype and treated samples.

Results: Treatment of neuronal cell lines with Cu (gtsm) leads to depletion of PrP in a dose-dependent manner. In contrast, the cells treated with the stable complex Cu (atsm), displayed no detectable decrease in PrP as the copper was not bioavailable. MTS assays confirmed that low concentrations of these compounds in the nanomolar range had no effect on cell viability.

Discussion: Direct intracellular release of copper from the ligand leads to a decrease in PrP levels in both human and mouse neuronal cell lines. The results support that the Cu (gtsm) compound is a modulator of PrP expression and is possibly a potential therapeutic approach for the treatment of prion diseases.

P5.14 Characterization of prion protein-enriched domains, isolated from rat cerebellar granule cells

Authors
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Content

Background and Aim: A powerful strategy for elucidating the physiological function of PrP^c that remains largely unknown, would be to identify other cellular proteins with which PrP^c interacts. Over the years, a number of candidates have been identified as potential PrP-binding partners, using co-immunoprecipitation, cross-linking and other methods but the physiological relevance of the proposed interactions, remains uncertain. In cerebellar granule cells, PrP^c is preferentially localized in lipids rafts and in particular, in a specific sub-types domain called *Prion Domain* [1]. The aim of present investigation, is to characterize proteome and lipidome of Prion Domain, purified by immunoprecipitation, in order to explain the PrP^c physiological role.

Methods: Granule cells, obtained from the cerebella of 8-day-old Sprague-Dawley rats, were prepared as described [1]. Detergent resistant fractions (DRM) and the immunoprecipitation were performed, with minor modification, according to Palestini [2]. Proteins in DRM, immunoprecipitates (IP) and corresponding supernatants, were separated by EF/WB and analyzed by µLC-ESI-MS/MS for identification.
Radioactive-lipids were separated by HPTLC and analyzed by radiochromatoscanner.

Results and Discussion: In the immunoprecipitate obtained from DRM, some proteins were co-immunoprecipitated with PrP^c and by µLC-ESI-MS/MS, were identified proteins implicated in neuronal growth and survival, cellular adhesion, secretion, cell shape. Finally, the lipid composition of *Prion Domain* was assessed. Regarding the pattern of glycerolphospholipids, the content of phosphatidylcholine increase significantly from the homogenate to the IP with concomitantly decrease in phosphatidylethanolamine and phosphatidylinositol. The percentage of plasma-choline and -ethanolamine was similar in homogenate and DRM, but in IP, pPC increase (+30 %) and pPE decrease (-40 %). References

(1) Botto et al, 2004.
(2) Palestini et al., 2000.

P5.15 A raft- and clathrin-dependent pathway regulates PrP^c internalization in epithelial FRT cells

Authors
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Content

Background: The cellular prion protein (PrP^c) plays a key role in the pathogenesis of Transmissible Spongiform Encephalopathies in which the protein undergoes post-translational conversion to the infectious form (PrP^{sc}). Although endocytosis appears to be required for this conversion, the mechanism of PrP^c internalization is still debated, as caveolae/raft- and clathrin-dependent processes have all been involved. Objectives: We have investigated the mechanism of PrP^c endocytosis in Fischer Rat Thyroid (FRT) cells, which lack caveolin-1 and caveolae, and in FRT/caveolin-1 cells which form functional caveolae. Methods: Using a combined approach of immunofluorescence (examined by confocal microscopy), electron microscopy, biochemistry and siRNA technique we have followed and characterized the internalization of mouse transfected PrP^c in FRT cells and analysed the involvement of lipid rafts and of the molecular factors known to regulate different endocytic pathways (eg., Cdc-42, Eps15, Dynamin and Clathrin). Results and discussion: We show that PrP^c internalization requires activated Cdc-42 and is sensitive to cholesterol depletion but not to caveolin expression suggesting a role for rafts but not for caveolae in PrP^c endocytosis. PrP^c internalization is also affected by knock down of clathrin and by the expression of dominant negative Eps15 and Dynamin 2 mutants, indicating the involvement of a clathrin-dependent pathway. Notably, PrP^c co-immunoprecipitates with clathrin on the cell surface and remains associated with detergent-insoluble microdomains during internalization suggesting cooperation between the raft- and clathrin-dependent pathways.

P5.16 The relationship between the prion protein and Ca2+ homeostasis in cerebellar granule cells

Authors
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Content

The prion protein (PrP^c) is a constitutive glycoprotein anchored to the cell membrane, which is most abundantly expressed in the central nervous system. An abnormal isoform (PrP^{sc}) of the protein is the main, or sole, component of prions, the infectious particles causing fatal neurodegenerative disorders, named transmissible spongiform encephalopathies (TSE), or prion diseases, which affect humans and animals. Despite the several biological roles attributed to the protein, ranging from protection against oxidative insults to neuronal adhesion and cell differentiation and survival, its physiological function remains unknown. Experimental evidence in PrP^c-deprived, or prion-infected, cell cultures and in cell model systems has suggested a role of PrP^c in the control of Ca2+ fluxes. Ca2+ is the most important carrier of cellular signals, governing key functions of the cell, among which secretion, gene transcription and cell survival. We have examined the possible influence of PrP^c on Ca2+ homeostasis by analyzing local Ca2+ fluctuations in primary cultures of cerebellar granule cells (CGC), which are the target of prions in some TSE forms. To this end, we have generated CGC cultures from isogenic wild type (WT), PrP-knockout (KO) or PrP-overexpressing (OE) mice, and analyzed the entry of Ca2+ from the extracellular space, after infecting CGCs with a viral vector encoding for the Ca2+-sensitive probe aequorin specifically targeted to the inner leaflet of the plasma membrane. Our preliminary experiments show that, under the used conditions, Ca2+ entry is mediated exclusively by store-operated Ca2+ channels (SOCCs), and that the degree of PrP^c expression in these cells highly influences both the peak of Ca2+ accumulation in the plasma membrane inner microdomains, and the kinetic of Ca2+ extrusion from the cytosol. To rationalize these observations at the molecular level, we have carried out Western blots of the different cells, which suggest that WT and PrP-OE CGC express higher amounts of plasma membrane Ca2+-ATPases with respect to PrP-KO cells. Current experiments are carried out to determine, by Western blot and RT-PCR, if PrP^c influences also the expression of the Ca2+ pumps of the endoplasmic reticulum (ER), of the molecular system mediating the store-operated Ca2+ -entry, and of Ca2+ -binding proteins. To have a complete picture of the relationship between PrP^c and CGC Ca2+ homeostasis, future directions of this study include the analysis of Ca2+ movements in the ER and mitochondrial matrix.

P5.17 Analysis Of Murine CNS Proteomes From PrP0/0, PrPp101L, PrPG3 AND WT Mice

Authors
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Content

Background: The prion protein (PrP) is fundamental to TSE disease biology and its conversion from the normal, cellular form (PrP^{c}) to a detergent insoluble, protease resistant isoform (PrP^{sc}) appears to be a pre-requisite for disease progression. Aggregates that accumulate in TSE disease are strongly immunopositive for PrP^{sc}, suggesting that PrP^{sc} aggregation may be responsible for neurodegeneration via a 'gain of function' mechanism. However, in some TSE cases, extensive pathology can exist in the absence of detectable levels of PrP^{sc}. The role of PrP^{sc} in neuropathogenesis is therefore unclear and an alternate hypothesis suggests that loss of PrP^{c} function during disease progression could be responsible for neurodegeneration. We hypothesise that PrP^{c} may function as a neuroprotective molecule and believe that mutations in the *PRNP* gene could initiate pathological disease due to impaired functioning of PrP^{c}. The normal biological role of PrP^{c} is still unclear and mice devoid of PrP^{c} (PrP0/0) were developed in order to address this point. These mice show subtle defects in synaptic transmission, mitochondrial function and circadian rhythm and an initial, collaborative, microarray based pilot study of wild type (WT) versus PrP0/0 mice uncovered several intriguing differences between them.

Objective: To confirm and build on preliminary microarray data and to define more specifically the temporal molecular changes in PrP0/0 mice and establish whether mutant PrP, with a reduced neuroprotective function, can invoke similar changes.

Methods: Soluble mouse brain proteins have been subjected to isoelectric focusing, separated by SDS PAGE and visualised using fluorescent and/or silver staining techniques. Gel images have been digitised and comparative analyses has been performed using Progenesis SameSpots analysis software.

Results: Using comparative proteomic analysis we have identified several protein changes occurring in brain tissue taken from WT, PrP0/0, PrP101LL & PrPG3 mice at 400 and 700days old. We are currently in the process of validating these results and comparing the changes with those observed in the ongoing microarray study. Ultimately, we hope to gain an insight into the influence that PrP^{c} can have in normal cellular function and begin to define a role for PrP^{c} in neuroprotection during ageing.

P5.18 Heat shock protein 90 (Hsp90) modifies the conformation of a copper-induced recombinant prion protein polymer in a nucleotide-dependent manner

Authors
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Content

The cellular prion protein (PrP^{c}) is a copper-binding cell membrane glycoprotein with unknown functions. The posttranslational conformational change of the protein into a pathogenic isoform called PrP^{sc} is involved in the development of prion diseases. We have previously reported that either heat shock protein 90 (Hsp90) or its endoplasmic reticulum homolog protein Grp94 modifies the conformation of a copper-loaded recombinant prion protein (rPrP) in a nucleotide-dependent manner. To uncover molecular mechanisms underlying the Hsp90/Grp94-assisted PrP-conformational modification, we performed sucrose density gradient centrifugation analysis as well as limited trypsin digestion analysis in which conformation-modified (e.g. unfolded, denatured) rPrP is digested with trypsin at such a low dose that rPrP of a native conformation is not chopped up. The following findings were revealed: 1) recombinant mouse Hsp90 (rHsp90) formed a stable complex with naked rPrP; 2) rHsp90 modified the conformation of naked rPrP to a trypsin-sensitive form in the absence of nucleotides; 3) Copper (II) induced polymerization of rPrP into a high molecular weight polymer (>440 kDa); 4) rHsp90 formed a stable complex with the copper-induced rPrP polymer; 5) rHsp90 modified the conformation of polymerized rPrP to trypsin-sensitive form in the presence of nucleotides such as ATP, ADP, nonhydrolyzable ATP analog, AMP-PNP, but not in the presence of AMP. These findings suggest that Hsp90/Grp94 might control the PrP^{c} conformation, which are necessary for its physiological function or degradation process in the cells.

P5.19 The 37 kDa/67 kDa Laminin Receptor as a key player in neurodegenerative diseases

Authors
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Content

The non-integrin 37 kDa/67 kDa laminin receptor (LRP/LR) (i) plays an important role in cell adhesion, cancer progression and metastasis (1), (ii) operates as a receptor for viruses such as alphaviridae, dengue virus and AAV and (iii) acts as the receptor for the cellular prion protein (PrP^{c}) (2) and infectious prions (3). LRP/LR colocalizes with PrP^{c} on the cell surface (2) and in the perinuclear compartment (4). The following tools targeting LRP/LR might represent alternative therapeutics for the treatment of prion disorders. (i) Antibodies directed against LRP/LR include polyclonal antibodies (W3) and single chain antibodies (scFvs S18). W3 cures neuronal cells from scrapie (5), interferes significantly with peripheral PrP^{sc} propagation and prolongs the survival time in scrapie infected mice (6). scFv S18 also inhibits significantly peripheral PrP^{sc} propagation after passive immunotransfer (7) or microinjection of recombinant AAV (8). (ii) siRNAs directed against LRP mRNA reduce PrP^{sc} propagation in neuronal cells (5). Microinjection of recombinant lentiviral vectors expressing anti-LRP siRNAs into mouse brains resulted in downregulation of LR in the brain and significantly prolongs incubation times in Scrapie infected mice (9). (iii) The LRP decoy mutant LRP102-295::FLAG reduces PrP^{sc} propagation in neuronal cells (10). Transgenic animals ectopically expressing this mutant in the brain revealed a significant prolongation of incubation time plus survival (11). Human enterocytes (Caco-2/TC7 cells) - the major cell population of the intestinal epithelium – may represent the first entry barrier for prions in the human body. BSE-prions become rapidly internalized by Caco-2/TC7 cells dependent on LRP/LR (12), confirming that BSE caused the zoonotic disease vCJD. CWD and sheep scrapie prions colocalize with LRP/LR on the surface of Caco-2/TC7 cells suggesting that both CWD and sheep scrapie might have the potential to cause further zoonotic diseases (13).

PrP^{c} has a protective effect on the development of Alzheimer's disease by blocking the β-secretase and inhibiting the formation of the Aβ-peptide (14). We proved a specific interaction between LRP/LR and APP and are further investigating a possible role of LRP/LR in the development of Alzheimer's Disease. (1) Zuber et al., (2008) J. Mol. Biol. 378, 530-539 (2) Gauczynski et al. (2001) EMBO J. 20, 5863-5875. (3) Gauczynski et al., (2006) J. Infect. Dis. 194, 702-709. (4) Nikles et al. (2008) BBA-Molecular Basis of Disease 1782, 335-340 (5) Leucht et al. (2003) EMBO rep 4, 290-295. (6) Zuber et al., (2007) Prion, 1, 207-212 (7) Zuber et al., (2008) Mol. Immunol. 45, 144-151 (8) Zuber et al., J. Gen. Virol., in press (9) Ludewigs et al., submitted (10) Vana & Weiss (2006) J. Mol. Biol. 358, 57-66. (11) Ludewigs et al., submitted (12) Morel et al. (2005) Am. J. Path. 167, 1033-1042. (13) Kolodziejczak et al., submitted. (14) Parkin et al., (2007) PNAS, 104, 11062-11067.

P5.20 Altered processing of the cellular prion protein in murine neuroblastoma cells after exposure to chronic hypoxia

Authors
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Content

The cellular prion protein (PrP^{c}) is a glycosyl-phosphatidylinositol (GPI) anchored membrane-bound protein that is expressed in many cells, especially neurons. PrP^{c} has an unspecified biological role, whereas the scrapie isoform (PrP^{sc}) is the causative agent of prion diseases, such as bovine spongiform encephalopathy. It has been shown that the prevalence of developing neurodegenerative disease is significantly increased after a stroke or ischemic episode. During these episodes oxygen levels are greatly reduced, subjecting the brain to hypoxic conditions. A distinct connection has been determined between the presence of PrP^{c} and the severity of the damage of an ischemic episode. Mice lacking the PrP gene display a greater area of necrotic damage after an ischemic episode, than in wild type mice. This suggests that PrP^{c} may have a potential neuroprotective role in the cellular response to hypoxia. To investigate this potential neuroprotective role of PrP^{c}, the level of endogenous PrP^{c} was established in murine neuroblastoma N2a cells, after exposure to chronic hypoxic conditions (1% 02), and compared with normal (normoxic) conditions (21% 02). Through Western blotting it was observed that after 24 hours of exposure to chronic hypoxia, full length PrP^{c} levels were significantly reduced in cell lysates ($p < 0.01$) with a simultaneous significant increase of soluble PrP^{c} in the media ($p < 0.05$), in comparison to normoxic cells. An MTT assay showed the viability of the N2a cells, after exposure to these conditions, was not affected. An increase in the hypoxic marker, glucose transporter-1 (GLUT-1), was also observed. Exposing N2a cells infected with PrP^{sc} to chronic hypoxia, showed no change in the generation of proteinase K resistant material. This shedding event of PrP^{c} into the media, after exposure to 24 hours of chronic hypoxia, could be a key event in the potential neuroprotective role of PrP^{c}, providing further links between PrP^{c} and hypoxia.

P5.21 Manifold prions in human brains and blood cells

Authors

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Content

The agents that cause transmissible spongiform encephalopathy are known as pathological prion proteins (PrP_{Sc}) which mainly accumulate in the central nervous and lymphoreticular system, and infectivity was also found in blood reported by cases of vCJD caused by blood transfusion. The molecular mechanisms leading to PrP_{Sc} induced transformation are poorly understood; however PrP_{Sc} is the result from conversion of the host encoded cellular prion protein (PrP_C) accompanied by a conformational change. PrP_C in brains and tissues are highly heterogeneous as results of expression and of presented glycoprotein profiles reflecting differences in the ratios of the di-, mono- and non-glycosylated protein signal intensities. We suggest that distinct PrP_C isoforms may be converted facile while others change cumbersome to PrP_{Sc}. However the basis proving this idea is to identify these various PrP isoforms within protein overlays of whole cell lysates. With a set of antibodies we quantitatively analysed human PrP_C derived from different brain regions and from blood cells as thrombocytes by densitometry to a single centrifugation step. We identified high and low soluble PrP_C isoforms with variable protein patterns. Using N-terminal binding antibodies the diglycosylated isoforms of high soluble PrP dominated in brain regions cerebellum, cortex and hippocampus, while nonglycosylated PrP of thalamus, pons and medulla oblongata resulted in highest immunoreactivity in the sediments. Brain PrP at the size of monoglycosylated proteins were intensely labelled in supernatants and pellets using C-terminal binding antibodies. In the case of PrP derived from thrombocytes the protein profiles moved in favour of a dominance of the diglycosylated PrP bands. The identification of further PrP profiles as silent prions in whole cell preparations supports the idea of the existence of manifold prions which may lie dormant awaiting a change in the state of the host.

P5.22 A role for the cellular prion protein in neuronal zinc metabolism

Authors

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Content

Whilst an exact physiological function remains elusive for the cellular isoform of the prion protein (PrP_C), an involvement in metal homeostasis has been proposed. To date, this work has centred on copper metabolism, evaluating roles on binding and uptake of the ion. However, there is a possible role for PrP_C in neuronal zinc (Zn) homeostasis. Zn has been shown to induce endocytosis of the protein; a process that is dependent on the octapeptide repeats in PrP_C. Furthermore, an increase in Zn-bound PrP_C has been shown in affinity purified material from prion-infected mice.

In order to evaluate whether PrP_C is involved in neuronal Zn uptake, two Zn-sensitive fluorochromes were used. Zinpyr-1 staining indicated that cells exposed to increasing Zn concentrations in their media showed significant time- and dose- dependent increases in Zn-associated fluorescence; an effect which was enhanced in cells expressing PrP_C. Newport Green measurements determined that the rate of Zn uptake was also enhanced in the presence of PrP_C. Knockdown of endogenous PrP expression by siRNA reduced Zn uptake. Significantly, when the endocytosis of PrP_C was inhibited, Zn uptake could still be measured suggesting that an alternative mechanism was responsible for the increase in intracellular Zn. Further studies to determine this mechanism are being investigated. These data demonstrate a role for PrP_C in the cellular uptake of Zn, potentially demonstrating a novel physiological function for the protein.

P5.23 Non-fibrillar form of PrP_C Central Domain peptide promotes degeneration in *PRNP* knockout cerebellar neurons

Authors

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Content

Central domain (CD) of the cellular prion protein (PrP_C) is constituted by two subdomains, called charge cluster (CC) and hydrophobic region (HR). Although physiological functions of PrP_C remain enigmatic, huge number of studies have confirmed a principal role of the CD of these protein in apoptosis and cell survival. In the last years, several mutant mice models, such as N-terminal truncated overexpressing mice devoid of PrPc like DF35, DCD or DCR lines, aimed to clarify the implications of the CD and its adjacent regions in PrPc-mediated but "non prionic" neuronal degeneration processes. Moreover, in vitro experiments using domain-mimicking peptides (e.g. PrP 106-126), despite of their controversy regarding toxicity, infectivity or dependence of PrP_C to exert its effects, have let us to explore crucial topics, like the aggregation and biochemical properties of these domains. In the present study we aimed to link the study of prion peptides (using new approaches in chemical synthesis) with the function of the protein. Consequently, we show that CD peptide, as CC peptide are unable to form amyloid-like fibrils, however, CD peptide promotes a strong increase of cleaved caspase-3 levels, rather than CC one. In addition, HR peptide formed high amounts of amyloid fibrils in a short period of time, promoting neuronal cell death independently of PrP_C. Further experiments would be of interest in order to evaluate the plausible ligand-related mechanism whom CD exert cerebellar granule neuron apoptosis independently of PrP_C presence and amyloid aggregation.

P5.24 Exosome-mediated shedding of the prion protein

Authors

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Content

Exosome-mediated shedding of the prion protein Lotta Wik, Mikael Klingeborn and Tommy Linné Department of Biomedical Sciences and Veterinary Public Health Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, Biomedical Centre, Box 588, SE-751 23 Uppsala, Sweden Determining the intra- and intercellular trafficking and processing of PrP_C, is important in discerning its normal physiological function and the mechanism of conversion to disease-associated isoforms. PrP_C is bound to the cell membrane via a glycosylphosphatidylinositol (GPI) anchor but secreted forms of PrP_C have been identified. In the cell medium two distinct PrP populations could be found. The major fraction was released by proteolytic cleavage near the GPI anchor. A minor fraction released in association with exosomes was characterized by western immunoblotting with exosome-specific markers, phospholipase assays, and electron microscopic analysis. Less than 1% of the released PrP was exosome-associated. The role of cell-to-cell transport of PrP_C by exosomes is studied further.

P5.25 Neuroprotective activity of untranslocated prion protein

Authors
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Content
A cytosolic form of the prion protein (PrP) has been proposed to play a key pathogenic role in prion diseases. However, the consequence of cytosolic PrP localization on neuronal viability is unclear, having either cytotoxic or anti-apoptotic effects been reported in different studies. The cellular mechanism by which PrP would appear in the cytosol of neurons is also debated, since either retrograde transport of misfolded PrP molecules from the endoplasmic reticulum or abortive cotranslational translocation during PrP biosynthesis have been involved. In this study, we have investigated the consequence of mild proteasome inhibition on appearance of cytosolic forms of PrP in neurons cultured from different mouse brain regions. We found that when the proteasome is inhibited, an untranslocated form of cytosolic PrP (SP-PrP) accumulates in cortical and hippocampal cells. SP-PrP was also detected in cerebellar granule neurons when these cells were treated with a cyclopeptolide that interferes with the correct insertion of the signal peptide into the translocon. Accumulation of SP-PrP was not toxic, rather, it was associated with increased neuronal survival. These results reinforce the conclusion that cytosolic PrP is not toxic to neurons, and argue that cytosolic targeting of PrP during biosynthesis could serve a physiological protective function in specific neuronal populations.

P5.26 Role of glypican-1 in prion protein metabolism

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Content
Background: Whilst many aspects of prion disease have been studied exhaustively controversy remains regarding the precise subcellular site of PrP conversion. After synthesis, PrP^{c} resides in cholesterol-rich domains on the cell surface called lipid rafts, and is subsequently endocytosed by a variety of mechanisms. Both lipid rafts and the endocytic pathway have been implicated as sites for PrP^{sc} formation. Hence, cellular proteins that interact with PrP^{c} and direct its trafficking are likely to play a role in disease by bringing PrP^{c} into favourable environments for conversion. Whilst PrP^{c}'s association with rafts is due, in part, to its glycosyl-phosphatidylinositol (GPI) anchor, the N-terminal domain of PrP^{c} also contains raft targeting determinants. In addition to binding copper and zinc ions, the N-terminus of PrP^{c} is capable of binding heparan sulphate and its derivatives, which in a cellular context would be a constituent of heparan sulphate proteoglycans (HSPGs). Signifcantly, HSPGs have been implicated as receptors for PrP^{sc} and as co-factors for PrP conversion.

Objective: To investigate the relationship between HSPGs and lipid raft association of PrP.

Methods: Studies were initially performed using SH-SY5Y human neuroblastoma cells expressing PrP^{c} or a transmembrane-anchored mutant of PrP (PrP-CTM) where lipid raft association occurs only via its N-terminal domain. Raft association of the PrP constructs after various treatments (heparin or phosphatidylinositol-specific phospholipase C treatment or glypican-1 siRNA) was assessed by sucrose density gradient centrifugation. Endocytosis experiments were performed using a standard biotinylation protocol. N2a mouse neuroblastoma cells chronically infected with PrP^{sc} (ScN2a cells) were treated with glypican-1 siRNA to assess its role in prion conversion. Cell lysate samples from all experiments were analysed by SDS-PAGE and western blotting.

Results: The lipid raft association of PrP-CTM was abrogated by addition of exogenous heparin or treatment of cells with phosphatidylinositol-specific phospholipase C. This suggested that a GPI-anchored HSPG may interact with PrP in lipid rafts. Glypican-1 is the major neuronal GPI-anchored HSPG. Using siRNA directed against glypican-1 we show that raft association of PrP-CTM is significantly reduced, implying that glypican-1 is a lipid raft binding partner of PrP. Basal endocytosis of PrP^{c} was enhanced in glypican-1 depleted cells suggesting glypican-1 may inhibit PrP^{c}'s translocation from lipid rafts into clathrin-coated pits under normal circumstances. Most significantly, siRNA against glypican-1 in ScN2a cells significantly reduced the amount of PrP^{res} in these cells, implying that glypican-1 is a novel co-factor in prion conversion.

Discussion: Our data show that glypican-1 constitutes a novel raft targeting determinant for PrP^{c}. Furthermore, our results argue that glypican-1 is involved in prion conversion, at least in infected cultured cells. Further work will clarify the role of glypican-1 in prion protein conversion and eventually may lead to new disease intervention strategies.

P5.27 Developmental features of cellular prion protein (PRP^{c}) in the human brainstem, cerebellum and peripheral organs

Authors
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Content
Background: In a previous study we showed the developmental feature of PrP^{c} expression in the human forebrain (Adle-Biassette et al. JNEN 2006;65:698-706).

Objective: In this study, the developmental pattern of PrP^{c} expression in the brain stem, the cerebellum and peripheral organs are reported. We also examined the influence of codon 129 polymorphism of the *PRNP* gene which influences the susceptibility and the phenotype of prion diseases.

Methods: Using immunhistochemistry and double immunofluorescence, PrP^{c} expression was detected from the embryonic stage to the end of gestation.

Results: Developing axonal tracts in the brain stem were intensively labelled during the first and second trimester, earlier than in the forebrain. Obvious neuronal PrP^{c} expression was detected from 16th week of gestation (GW) in the brainstem and 30 GW in the cerebellum. Synapses expressed PrP^{c} at increasing levels throughout synaptogenesis. PrP^{c} expression was detected in various neuronal and glial cells as reported previously. In addition, PrP^{c} was expressed in different organs, peripheral nerves and ganglion cells, various epithelial, mesenchymal and immune cells. Western blot analysis showed increasing maturation of various PrP^{c} isoforms ranging from 35 to 27 kDa during development. The *PRNP* codon 129 genotype distribution was 39% for methionine-methionine, 30.5% for methionine-valine and 30.5% for valine-valine.

Conclusion: PrP^{c} expression in the brain followed the caudo-rostral and ventro-dorsal development of the central nervous system and was detected earlier in the brain stem elongating axonal tracts and postmitotic neurons than in the forebrain or the cerebellum. The pattern of expression of PrP^{c} was not influenced by codon 129 polymorphism of the *PRNP* gene in the 20 fetus genotyped.

P5.28 Antibodies to prion protein effective against colon cancer

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Content

Background: The purpose of the cellular prion protein, PrP_C, remains unclear despite many years of research and several proposed roles. A putative function for PrP_C in apoptosis has been implicated. Indeed, in the gastric cell line AGS, it has been shown that PrP_C slowed down apoptosis with suppression of ROS and upregulation of Bcl-2. Increased PrP_C expression in gastric adenocarcinomas correlated with histopathological differentiation and tumour progression. Additionally, silencing of PrP_C sensitized breast carcinoma cells to TRAIL-mediated cell death.

Objective: PrP_C expression was examined in colon cancer cell lines in order to extend the correlation of PrP_C level with tumour aggressiveness to other tumour types. Furthermore, it was proposed that the inhibition of PrP_C in PrP_C-dependent tumours would lead to reduced growth and greater susceptibility to cytotoxic agents. Inhibition of PrP_C using potent antibodies was investigated with MTT assays, with the intention of increasing apoptosis and reducing cell proliferation in colon cancers. Human colon tumour xenografts in nude mice were treated with antibodies to PrP_C to examine the in vivo effects of PrP_C antibodies.

Methods: Antibodies to PrP_C were both generated in house (2A5) and sourced commercially. BAR and SAF antibodies were obtained from Sapphire Biosciences whereas 3F4 and 6D11 antibodies were from Covance. Recombinant PrP_C was purchased from Jena. Human colon cancer cell lines HT-29, HCT116, SW480 and CaCO2 (ATCC) were examined for PrP_C level by a commercially available ELISA kit (SPI Bio). Cell lines were chosen to reflect differences in tumour grade and metastatic potential. Cells were maintained in RPMI1640 (Sigma) containing 10% heat inactivated fetal calf serum at 37°C in a humidified atmosphere of 5% CO2 and 95% air. Drug sensitivity was evaluated by MTT assay. Cells were plated in 96-well plates (Nunc, Milan, Italy) at a density of 5000 cells/well. After 24 hr, the medium was replaced with fresh growth medium containing 5% FCS and various concentrations of drugs with or without antibody to PrP. After 72 hr of growth in the presence of drugs, cells were assayed for viability. Briefly, MTT reagent (final concentration 500 mg/ml) was added to each well and 4 hr later adherent cells were lysed with 100 mL methyl sulfoxide per well. The absorbance of the formazan product was measured with a Molecular Devices plate reader at a wavelength of 490 nm. Each drug or antibody concentration that produced 50% inhibition of growth (IC50) was estimated by using the relative survival curves. Relative inhibitory rate of cell growth by different concentrations of drug or antibody was calculated according to the following formula: R = (A2-A1)/A2, where R is relative inhibitory rate of cell growth by drug or antibody, A1 is the absorbance value of cells in the presence of drug or antibody for 72 hr and A2 is the absorbance value of control cells without treatment. Each study was performed in triplicate and repeated multiple times. Mouse immunoglobulin was used as a control.
In vivo experiments were conducted under Australian Ethics Committee approval and guidelines. Nude mice were injected subcutaneously with 3 x 106 HCT116 human colon cancer cells. After 1 week mice were treated twice per week for two weeks with irinotecan (40 mg/kg, i.p.) and or anti-PrP_C antibody (9 mg/kg, i.v.). Tumour size was measured thrice weekly with by three independent diameters and tumour volume calculated.

Results: Human colon cancer cell lines HT-29, HCT116, SW480 and CaCO2 were maintained under identical conditions and PrP_C levels assessed using a SPI Bio ELISA kit employing recombinant human PrP_C as standard. A correlation was observed between tumour grade and metastatic potential and the PrP_C level, as had previously been observed in gastric and breast cancers. The effect of various antibodies to PrP_C was investigated in MTT assays on the HCT116 cell line. Antibodies were shown to have varying degrees of anti-proliferative activity with 3F4 and 6D11 essentially inactive, compared to highly active BAR221 and BAR236 antibodies. Surprisingly, BAR221 and BAR236 antibodies were particularly potent and, compared to controls, afforded >90% reduction in proliferation at 10 mg/mL. For antibodies with a similar species cross-reactivity, anti-proliferative effectiveness correlated closely with the reported scrapie-curing potential in scrapie infected N2a neuroblastoma cells. Combination treatments of cytotoxic drug and antibody were also examined. Antibody 2A5 reduced the IC50 for irinotecan, 5FU, cisplatin and doxorubicin to varying degrees. Little enhancement was seen for the combination of doxorubicin and antibody over doxorubicin alone, however use of the antibody in combination with irinotecan lowered the IC50 by over 2-fold. In combination treatments of 2A5 antibody and irinotecan in HT-29, HCT116, SW480 and CaCO2 colon cell lines, reduction in irinotecan IC50 correlated with PrP_C expression, except for HT-29. Irinotecan IC50 was reduced by 2A5 antibody at 50 ug/mL by 1.3X, 2.1X and 3.8X in SW480, HCT116 and CaCO2 cell lines respectively, relative to irinotecan alone. Remarkably, in an in vivo nude mouse model bearing human HCT116 xenografts, tumour growth was inhibited by treatment with PrP_C antibody. At 9 mg/kg antibody tumour growth was approximately 50% of the control animals 15 days after the start of treatment. Additionally, use of antibody and irinotecan in combination was consistently more efficacious than irinotecan alone.

Discussion: The relationship between tumour stage and progression and PrP_C expression has been extended to include colon cancer. This work has substantiated the claim that PrP_C serves as a cancer marker and also a potential therapeutic target. Antibodies to PrP_C were shown in vitro, even at low concentrations, to be effective at reducing cell proliferation in human colon cancer cell lines. Antibody potency correlated with the ability of the antibody to remove scrapie infection from infected mouse neuroblastoma cells. This work is the first report of anti-cancer effectiveness of a PrP_C antibody.
Antibodies were also effective as components of combination therapy in MTT assays with irinotecan, 5FU and cisplatin, but not doxorubicin. Surprisingly, the highest PrP_C expressing cell line, CaCO2 was also most effectively inhibited by antibody. It could have been expected that this would be the hardest cell line to treat as more antibody would be required to overcome the greater level of PrP_C. This result suggests that aggressive tumours that overexpress PrP_C are using PrP_C as a mechanism for growth and proliferation. In an animal model bearing human HCT116 colon xenografts, treatment with PrP_C antibody alone reduced tumour growth by as much as 50% of controls. Use of PrP_C antibody in combination therapy with irinotecan was also effective with the combination group tumours consistently smaller on average than those treated
with only irinotecan. This is the first reported in vivo treatment of cancer by suppressing PrP_C and represents a potential breakthrough as a novel approach to cancer therapy.

P6.01 Positive correlation between relative mRNA expression of *PRNP* and SPRN in cerebral and cerebellar cortex of sheep

Authors
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Content
Background: The exact pathogenesis of prion diseases is still far from elucidated. Therefore, research on *PRNP*, but also on other genes and proteins with a potential effect on TSE pathogenesis, is essential. Because of its expression in brain tissue, its remarkable sequence homology with the hydrophobic region of *PRNP* and its PrP-like effects in functional experiments on *PRNP*o/o mice, *SPRN* is an interesting new member of the *PRNP* family.

Objective: The purpose of this study was to investigate the relative expression levels of *SPRN* and *PRNP* in sheep cerebrum and cerebellum and to assess the mutual relationship between these expression levels.

Methods: Relative expression levels of *SPRN* and *PRNP* were determined by quantitative real-time PCR on 45 cerebral cortex and 47 cerebellar cortex samples and normalized using 3 reference genes (SDHA, RPL13A and ACTB).

Results: The *PRNP* mRNA expression level was significantly higher ($p < 0.05$) in cerebellum than in cerebrum, while no significant difference was detected for *SPRN* between these tissues. The expression level varied clearly more for *SPRN* than for *PRNP*, and the variation was larger in cerebrum than in cerebellum for both genes. Remarkably, the expression levels of *PRNP* and *SPRN* showed a highly significant positive correlation in both cerebrum ($p < 0.0001$) and cerebellum ($p < 0.001$).

Discussion: The correlation between the relative mRNA expression levels of *PRNP* and *SPRN* might indicate co-regulation between these genes. Further investigation on the causal nature of this correlation may provide new insights into prion pathogenesis.

P6.02 Mutations at codon 178, 200-129, and 232 contributed to the inherited prion diseases in Korean patients

Authors
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Content
Polymorphisms of human prion protein gene (*PRNP*) contribute to the genetic determinants of Creutzfeldt-Jakob disease (CJD). Numerous polymorphisms in the promoter regions as well as ORF of *PRNP* were investigated. Interestingly, greater than 90% of Koreans, Chinese and Japanese carried homozygote 129MM codon. In Korea, polymorphisms have not been comprehensively studied, except codon 129 and 219 in *PRNP* among Korean CJD cases. Although polymorphisms at 129 and 219 played an important role in the susceptibility to sporadic CJD, patients with other polymorphisms in *PRNP* exhibited critical distinctions of clinical symptoms. In this study, to identify other polymorphisms apart from 129 and 219 and to understand their implications in the clinical progressions of the disease among Korean, the genetic analyses of *PRNP* were carried out among probable CJD patients in comparison with the results from magnetic resonance imaging (MRI) and electroencephalogram (EEG). The molecular analysis revealed that three polymorphisms at codon D178N, E200K and M232R in heterozygosity. Patients with D178N and M232R mutations had 129MM codon, where patient with E200K mutation showed 129MV heterozygosity. They all revealed strong 14-3-3 positive signal. Patient with D178N mutation at age of 67 showed progressive gait disturbance and dysarthria was in process. Patient with E200K coupled to the 129MV at age of 58 reveled gait disturbance, dysarthria, agitation and ataxic gat, and progressed rapidly to death, 3 months from the first onset. Patient with M232R mutation showed rapidly progressive memory decline and gait disturbance, and died within 1 year after admission. Despite differences in ethnicity, the clinical and pathological outcomes were similar to the respective mutations around the world, except absence of insomnia in D178N subject.

P6.03 Cathepsin D SNP associated with increased risk of variant Creutzfeldt-Jakob disease

Authors
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Content
Background: Variant Creutzfeldt-Jakob disease (vCJD) originally resulted from the consumption of foodstuffs contaminated by bovine spongiform encephalopathy (BSE) material, with 163 confirmed cases in the UK to date. Many thousands are likely to have been exposed to dietary infection and so it is important (for surveillance, epidemic modelling, public health, and understanding pathogenesis) to identify genetic factors that may affect individual susceptibility to infection.

Objective: This study looked at vCJD patients, for a polymorphism in the cathepsin D gene (refSNP ID: rs17571) previously examined in Alzheimer''s disease (AD).

Methods: Blood samples taken from 110 vCJD patients were tested for the C-T base change, and genotype data were compared with published frequencies for a control population using multiple logistic regression.

Results: There was a significant excess of the cathepsin D polymorphism TT genotype in the vCJD cohort compared to controls. The TT genotype was found to have a 9.75 fold increase in risk of vCJD compared to the CT genotype and a 10.92 fold increase compared to the CC genotype.

Discussion: This mutation event has been observed to alter the protease activity of the cathepsin D protein and has been linked to an increase in amyloid beta plaque formation in AD. vCJD neuropathology is characterised by the presence of amyloid plaques, formed from the prion protein, and therefore alterations in the amyloid processing activity of cathepsin D may affect the neuropathogenesis of this disease. (This study has been published in BMC Medical Genetics 2008, 9:31)

P6.04 The PrPc-like Shadoo protein in Humans and Sheep: common missense and insertion/deletion polymorphisms affect the hydrophobic domain of ovine *SPRN*

Authors
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Content
Background: Despite the importance of the *PRNP* gene variations in modulating prion diseases in humans and animals, other factors may also play a role in disease pathogenesis. For example, not all animals of the "susceptible" *PRNP* genotype such as ARQ/ARQ sheep develop disease in an exposed setting. Recently, the *SPRN* gene encoding the Shadoo protein (Sho) has been identified as a new member of the prion superprotein family. Sho has neuroprotective properties and is down-regulated in prion-infected CNS tissue.

Objective: We assessed *SPRN* polymorphisms in human and sheep populations, as a prelude to determining a role in disease pathogenesis.

Methods: Using an available human *SPRN* sequence and by cloning a prototype ovine *SPRN* gene, *SPRN* coding sequences were PCR amplified from 93 and 107 humans and sheep, respectively. Sheep breeds examined included the Cheviot genetic background associated with the genesis of multiple scrapie strains.

Results: Paralleling the case for human and ovine *PRNP*, where sheep exhibit high frequency polymorphisms for V136A, R154H and Q171R versus a single common M129V polymorphism in humans, we failed to detect high frequency polymorphisms in human *SPRN*. In contrast, ovine *SPRN* exhibited >6 allelic types, including 4 missense mutations and insertion/deletion variations within the Sho protein hydrophobic domain. Some alleles reached frequencies of up to 20%.

Discussion: Most genetic variations observed in sheep *SPRN* affect the hydrophobic domain of the Sho protein, which is conserved in other species, and is conserved in PrP. Since refolding of the PrP hydrophobic domain and prion replication are closely associated events, the chemical and biological properties of Sho protein variants in healthy and diseased animals warrant investigation. Furthermore, the diversity in ovine (but not human) *PRNP* and *SPRN* sequences suggest that selection pressure may work to maintain multiple variants of each protein within sheep populations.

P6.05 Identification of a heritable polymorphism in the bovine prion gene associated with genetic transmissible spongiform encephalopathy: evidence of heritable BSE

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Content

Background: Bovine spongiform encephalopathy (BSE) is a transmissible spongiform encephalopathy (TSE) of cattle. Classical BSE is associated with ingestion of BSE-contaminated feedstuffs. H- and L-type BSE, collectively known as atypical BSE, differ from classical BSE by displaying a different disease phenotype and they have not been linked to the consumption of contaminated feed. Interestingly, the 2006 US H-type atypical BSE animal had a polymorphism at codon 211 of the bovine prion gene resulting in a glutamic acid to lysine substitution (E211K). This substitution is analogous to the most prevalent form of heritable TSE in humans, and it is considered to have caused BSE in the 2006 US atypical BSE animal.

Objective and Methods: In order to determine if this amino acid change is a heritable trait in cattle, we sequenced using stand DNA sequencing the prion alleles of the only known offspring of this animal, a 2-year-old heifer.

Results: Sequence analysis revealed that both the 2006 US atypical BSE animal and its 2-year-old heifer were heterozygous at bovine prion gene nucleotides 631 through 633 for GAA (glutamic acid) and AAA (lysine). Both animals carry the E211K polymorphism, indicating that the allele is heritable and may persist within the cattle population.

Discussion: This is the first evidence that the E211K polymorphism is a germline polymorphism, not a somatic mutation, indicating BSE may be transmitted genetically in cattle. This also provides the first evidence that all 3 etiologic forms of TSEs (spontaneous, hereditary, and infectious) are present in a non-human species. Atypical BSE arising as both genetic and spontaneous disease, in the context of reports that at least some forms of atypical BSE can convert to classical BSE in mice, suggests a cattle origin for classical BSE.

P6.06 Genetic susceptibility of goats to Nor98: AHQ as risk factor

Authors
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Content

Background: Atypical scrapie, first reported in sheep in Norway in 1998, is now widely recognized in small ruminants across Europe. The majority were detected in sheep, whereas atypical cases in goats were reported from France, Spain, Switzerland and Italy. Prion protein gene (*PRNP*) polymorphisms are involved in modulating sheep susceptibility to atypical scrapie, with the alleles AHQ and AF141RQ strongly associated with the occurrence of the disease. The AHQ allele has also been detected in Nor98-affected goats but data that would permit a statistical association between Nor98 and this allele are still lacking.

Objective: To carry out a case-control study to test for association of such allele with susceptibility to Nor98.

Methods: Blood from eight positive goats belonging to Italian Nor98 scrapie outbreaks and 246 negative herdmates were collected. Direct sequencing of the ORF of the *PRNP* gene was performed. A chi-square test was applied to look for associations between alleles and Nor98 scrapie status.

Results: Four haplotypes were identified in the positive goats: A136R154Q171Q222S240; A136R154Q171Q222P240; A136H154 Q171Q222S240 corresponding to the sheep AHQ; A136H154Q171K222S240, described here for the first time. Only the frequencies of these four alleles were considered in the control group. The AHQ allele was found to be associated with Nor98 cases.

Discussion: This is the first case-control study on genetic susceptibility to Nor98 in goats and it demonstrates that the AHQ allele is a risk factor for this disease. European policy on atypical scrapie outbreaks management does not take into account direct measures based on genetics; nevertheless in ovine Nor98 outbreaks, selective culling of animals carrying the alleles AHQ and AF141RQ could be an effective measure to decrease the risk of atypical scrapie without heavy loss of animals. In light of our data, a control strategy targeting AHQ, or the mutation H154, may be suggested also for goat Nor98 scrapie outbreaks.

P6.07 Polymorphisms in the HSP90AA1 5'flanking region could affect scrapie incubation period

Authors
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Content

Scrapie is a prion disease affecting sheep and goats. Although susceptibility to scrapie is mainly controlled by point mutations at the *PRNP* locus, several studies argue in favour of other genes contributing to resistance/susceptibility to this disease. Prion diseases seem to be associated with events of protein misfolding in which molecular chaperones provide the first line of defence and probably function at the earliest stages of disease pathogenesis. In this regard, the hypothesis that the gene coding for the Hsp90a (*HSP90AA1*) could be a good functional and positional candidate modulating the response to scrapie in sheep has been investigated. In the present work, we compare several polymorphisms affecting different putative regulatory elements in the *HSP90AA1* gene that seems to be associated with scrapie incubation period in two independent protocols, one from Spain and one from France. The first data set was obtained from six flocks of sheep, conserved for research purposes by the prion research centre of the University of Zaragoza and concerns 80 ARQ/ARQ Rasa Aragonesa sheep. The second data set was obtained from one naturally scrapie infected flock maintained at the Langlade experimental farm and concerns 68 ARQ/VRQ Romanov sheep produced by one sire. As a result it seems that two nucleotide substitutions located at positions -660 and -528 from the transcription start site could be associated with scrapie incubation period. It should be noted, these analyses are still in progress and further studies should be performed in order to check the significance of these results.

P6.08 Modulators of prion disease: the *SPRN* gene is an excellent candidate

Authors

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Content

The mammalian *SPRN* gene encodes the shadoo protein (Sho) which has strong homology to the *PRNP* gene encoding prion protein PrP. Recently it was shown that shadoo has similar neuroprotective activity to PrP protein and that shadoo expression is altered during prion disease (Watts et al., EMBO J. 26, 4038-4050). This suggests a role in neurodegeneration for the *SPRN* gene, involving either similar or novel mechanisms compared to the *PRNP* gene. Genetic variants of the *PRNP* gene are associated with the modulation of susceptibility and neurodegeneration of prion diseases. However, not all disease outbreaks can be fully explained by *PRNP* genetics, implying the action of a second modulator gene. The *SPRN* gene is an excellent candidate for this role. To explore whether the *SPRN* gene contributes to the phenotypic variation and control of prion diseases we investigated the genetic variability of the *SPRN* coding region in species that are hosts of prion diseases. *SPRN* gene sequences were obtained from over 600 samples from more than ten species in the orders ruminants and rodents. We report here the sequences of more than 20 different Sho proteins. Single amino acid substitutions and amino acid deletions/insertions were common in sheep, but less common in cattle and goats. No polymorphisms were found in laboratory mouse strains. An alanine-rich sequence with high homology to a hydrophobic segment in PrP protein which is proposed to have multiple functions and amyloidogenic characteristics was present in all Sho sequences. This core sequence showed variation in the number of alanine residues making it a special target for disease association studies in sheep. Case–control groups of TSE outbreaks were studied for association of the newly detected *SPRN* gene polymorphisms with disease susceptibility.

P6.09 Familial prion disease in a family with a rare truncating mutation in *PRNP* (Q160X): clinical, pathological, and biochemical features

Authors

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Content

Background: : The clinical and pathologic phenotype of familial prion disease due to *PRNP* mutations can be remarkably variable. Of particular interest is the relationship between the location and type of mutation and the phenotype.

Objective: To report a family with a rare *PRNP* mutation (Q160X) and distinct clinical and neuropathologic phenotypes.

Methods: The proband was evaluated in a longitudinal study of aging and dementia, as had her mother approximately 10 years earlier. Both underwent standardized neurological and neuropsychological evaluation and consented to autopsy at the time of death. After death, both underwent extensive neuropathologic evaluation including standard histology, immunohistochemistry, electron microscopy, and biochemical analysis.

Results: The proband presented at age 42 years with a three year history of progressive short term memory impairment. Neuropsychological testing revealed severely impaired memory testing, and preserved attention, and construction. She was diagnosed with Alzheimer's disease. She died at age 47 years. Microscopic evaluation revealed extensive limbic and neocortical neurofibrillary tangle formation and neuritic plaques consistent with a Braak stage of VI. The neurofibrillary tangle pathology was immunopositive with multiple tau antibodies (PHF-1. Alz 50, AT8, RD3, RD4), and electron microscopy revealed paired helical filaments. However, the neuritic plaques were immunonegative for Aβ, while immunostaining for PrP was positive. The mother of the proband had presented with a similar clinical picture, and subsequent re-evaluation of her brain tissue confirmed similar tau and PrP immunostaining findings. Genetic analysis revealed that both proband and mother had a rare *PRNP* mutation (Q160X) that should result in the production of truncated PrP. This was confirmed on western blot analysis.

Discussion: We report a family with a rare *PRNP* mutation and a clinical and pathological picture remarkably similar to that observed in Alzheimer's disease. There are phenotypic similarities to another reported *PRNP* mutation leading to production of truncated PrP (Y145Z), including severe neurofibrillary tangle formation. However, we did not observe a similar severity of amyloid angiopathy or the deposition of full length PrP as reported in that mutation.

P6.10 E200K genetic Creutzfeldt-Jakob disease clinically mimicking variant CJD

Authors

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Content

Background: Variant Creutzfeldt-Jakob disease (vCJD) originally resulted from the consumption of foodstuffs contaminated by bovine spongiform encephalopathy (BSE) material and should be strictly distinguished from other forms of human prion disease.

Objective: To report a case with mutation in the prion protein gene (*PRNP*) clinically mimicking vCJD.

Methods: Clinical examination and follow-up, MRI examination, full length analysis of the *PRNP*.

Results: A 54-years-old Hungarian woman who has not travelled abroad, developed behavioural change, anxiety and aggressivity. This was followed by dystonic movement in the right and soon in the left upper extremity. Some weeks later ataxia, scanning dysarthria and prominent myoclonus in the limbs, startle response, along with progressive cognitive decline was observed. She died in a state of akinetic mutism approximately 8 months after the first complaints. There was no history of iatrogenic exposure to prions. Routine laboratory examinations including CSF analysis were in a normal range. Examination of 14-3-3 was not performed. A family history of neuropsychiatric disorder was lacking. EEG did not show typical periodic sharp wave complexes, however MRI examination revealed the pulvinar sign. *PRNP* analysis revealed MM homozygosity at codon 129 together with the E200K mutation.

Discussion: This report underpins the importance of the examination of *PRNP* when neuropathology cannot be performed. Only the demonstration of E200K mutation distinguished this case from vCJD.

Conclusion: When the genetic examination is lacking, caution is warranted when diagnosing probable variant CJD.

P6.11 A genome scan for classical BSE susceptibility and/or resistance in European Holstein cattle

Authors

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Content

Background: Genetic susceptibility to transmissible spongiform encephalopathies has been observed in many species including mice, humans and sheep. Evidence of bovine alleles located on several chromosomes that correlate with classical BSE resistance/susceptibility has previously been reported.

Objective: The objective of this study was to perform a genome scan to test for genetic associations with classical BSE susceptibility in European Holstein cattle.

Methods: Assays for 3072 genome wide single nucleotide polymorphisms (SNPs) including 17 haplotype tagging SNPs (htSNPs) within the prion gene (*PRNP*) locus were genotyped across two sample sets of Holstein cattle: a multiple family sample set consisting of 156 families (n=336) and the large family sample set consisting paternal half sibs from 5 sires (n=481). Both sample sets include BSE positive and control animals.

Results: In the multiple family sample set 31 SNPs located at 27 chromosomal regions were associated with disease susceptibility ($p<0.01$). Of these associations 20 SNPs on 11 chromosomes had a $p<0.005$. A total of 8 SNPs on 7 chromosomes (BTA 4, 10, 14, 15, 21, 25, 28) had a $p<0.001$ of which 3 SNPs (BTA 4, 14, 21) had a $p<0.0005$. In the large family sample set, 60 SNPs located at 24 chromosomal regions were associated with disease susceptibility ($p<0.01$). Of these associations 23 SNPs on 16 chromosomes had a $p<0.005$. A total of 7 SNPs on 5 chromosomes (BTA 3, 6, 16, 27, 27) had a $p<0.001$ of which 5 SNPs on 4 chromosomes (BTA 6, 16, 17, 27) had a $p<0.0005$.

Discussion: Although there is some concordance across the two sample sets at the loci with lower p-values, the differences observed in the significant associations may be a reflection of a decreased diversity in the family sample set due to limited sire numbers as compared to the multiple family sample set. This data has not been corrected for multiple testing and therefore all SNP associations should be considered putative. Preliminary analyses indicate that SNP alleles associated with classical BSE susceptibility in this study are located on chromosomes previously reported to be associated with classical BSE susceptibility in addition to several new chromosomes. These results, while they are preliminary, are an important step towards determining the relationship between cattle genotypes and BSE susceptibility.

P6.12 Genetic variability of the *PRNP* gene in wild ruminants from Italy and Scotland

Authors

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Content

Background: *PRNP* genetics play a relevant role in determining relative susceptibility to TSEs. The presence of leucine at codon 132 in American elk *PRNP* has been associated with longer CWD incubation periods. Mule deer carrying phenylalanine at codon 225 were underrepresented in the CWD infected animals. Although there is so far no evidence that TSEs occur in European wild ruminants, *PRNP* genetic variability has not been thoroughly investigated and *PRNP* characterization in wild species, occasionally sharing grazes with sheep and goats, is still incomplete.

Objective: In this study we describe nucleotide sequence variation in the *PRNP* locus of red deer, chamois and roe deer.

Methods: Red deer samples were collected from Italy (n=191) and Scotland (n=132). Chamois (n=203) and roe deer (n=189) came all from Italy. DNA amplicons corresponding to the complete *PRNP* open reading frame (ORF) were sequenced. Genetic distances and dendrograms were computed using MEGA and PHYLIP packages.

Results: A total of nine single nucleotide polymorphisms (SNPs) were identified in red deer at codons 15, 21, 59, 78, 79, 98, 136, 168 and 226. These polymorphisms gave rise to 10 haplotypes. One silent mutation was detected in chamois at codon 119 and no SNPs were found in roe deer. Sequence alignment and phylogenetic analysis showed a higher similarity between red deer and roe deer and between sheep, goat and chamois. Frequency differences between Italian and Scottish red deer were found and confirmed by genetic distance analysis.

Discussion: This study confirms that red deer *PRNP* carries several polymorphic sites, corresponding to different variants of the mature PrP, while genetic variability in roe deer and chamois proved to be very low. *PRNP* seems to be highly conserved particularly in Italian roe deer. The *PRNP* analysis of European wild ruminants and the comparison with the *PRNP* of North American cervids may give preliminary data on their susceptibility to CWD and other TSEs.

P6.13 Analysis of *PRNP* gene noncoding regions for association with BSE infection in Italian cattle

Authors

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Content

Background: Two insertion/deletion (indel) polymorphisms of the prion protein gene (23bp in the promoter region and 12bp in intron I) have been associated with bovine spongiform encephalopathy (BSE) susceptibility in cattle. These polymorphisms are thought to influence *PRNP* expression level thus playing an important role in BSE incubation time and/or susceptibility. Previous reports have compared BSE-affected and healthy cattle whereas studies comparing BSE cattle with their age-cohorts are still lacking.

Objective: Study 1: to investigate *PRNP* variability outside the ORF in Italian BSE-affected cattle (n=56) and in a representative sample of healthy control animals (n=56). Study 2: to compare the frequencies of 23bp and 12bp indel polymorphisms in BSE-affected cattle (n=21) and animals from their age-cohorts (n=640).

Methods: A DNA region of 5.2 kb comprising the putative promoter region, exon 1, intron I and exon 2, has been amplified. PCRs flanking the 23bp and the 12 bp indels were performed using a dye-labeled primer. Amplicons were analyzed by capillary electrophoresis. "Chi square" analysis comparing allele and genotype frequencies in the BSE-affected group versus the negative sample groups has been carried out.

Results: Study 1: 56 *PRNP* polymorphisms have been identified. Among these variants 49 SNPs were found, two MNPs and five single or multiple base indels. Statistical analysis showed no significant difference between BSE-affected and healthy cattle at the 23bp and 12bp indel polymorphisms. Frequency differences at any of the detected nucleotide polymorphisms have been evaluated. Study 2: screening of indel polymorphisms is ongoing and results will be presented in the poster.

Discussion: The present work provides novel data about variations and distribution of the bovine *PRNP* gene polymorphisms in Italian BSE cases and control animals. So far, based on results of study 1, we cannot confirm association between indel polymorphisms and BSE in Italian cattle.

P6.14 The AT137RQ and ARQK176 PrP alleles protect sheep from natural scrapie

Authors

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Content

Background: Susceptibility of sheep to scrapie is under the control of the host's genotype at the PrP gene. PrP polymorphisms at codon 136, 154 and 171, combined in the five main alleles VRQ, ARQ, AHQ, ARH and ARR, have been identified as the main determinants of the susceptibility/resistance of sheep to scrapie. Observations deriving from experimental challenge of sheep carrying mutations on the ARQ allele, suggest that alleles other than the ARR may have a protective effect against scrapie and BSE.

Objective: To investigate the effect of polymorphisms of the ARQ allele on the susceptibility of sheep to natural scrapie.

Methods: A multi-flock study was carried out. Outbreaks enrolled were large flocks of Sarda breed, with classical scrapie and a high scrapie prevalence rate. PrP genotyping at the three codons, was carried out on 5386 sheep from all flocks. Subsequently PrP genotype of all scrapie cases (n=154) and a subset of ARQ/ARQ negative sheep (n=378) was determined by sequencing analysis. Odds ratio (OR) was calculated to compare the risk of scrapie between ARQ/ARQ animals carrying additional variations and ARQ wt/ ARQwt flock mates.

Results: The OR estimates showed that the probability to test positive for scrapie was significantly lower in sheep carrying any additional polymorphism than in ARQwt/ARQwt sheep. In particular, a statistically significant OR=0 was observed for the AT 137RQ/XXX and ARQK176 /XXX genotypes in 5 and 3 out of 5 flocks examined, respectively. For the AF 141 RQ/XXX genotype the OR was lower than 1 indicating a protective role also of this allele.

Discussion: This is the first study revealing ovine PrP alleles other than the ARR that exhibit significant protection against natural scrapie. If future investigations will demonstrate the protective effect of the AT 137RQ and ARQK176 alleles in different countries, breeding programmes against scrapie could take advantage on the existence of additional resistant alleles.

P6.15 Creutzfeldt Jakob disease in a patient with the mutation V203I in the prion protein gene

Authors

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Content

Background: Transmissible Spongiform Enchephalopaties (TSE) are a group of invariably fatal neurodegenerative diseases, of humans and animals. The most common TSE in humans is Creutzfeldt Jakob disease (CJD), which may be of sporadic, genetic or iatrogenic origin. The genetic form of CJD is linked to mutations in the human prion protein gene (*PRNP*) which are transmitted in an autosomal dominant fashion. V203I is a very rare mutation related to Creutzfeldt-Jakob disease.

Objective: To describe a case of a probable CJD (WHO, 2003) linked to the V203I mutation, and lacking a family history of neurologic disease.

Patient and Methods: A 76-year-old woman was referred to the Italian National Register of the CJD and related disorders, because of a rapidly progressive cognitive decline and behavioral disturbances. Few days after the onset, she also showed visual hallucinations, ataxia, pyramidal signs, myoclonus and one month later, akinetic mutism. The family history was negative for neurological disorders. The EEG became typical 1 months after the onset. The protein 14-3-3 test was positive. The brain MRI showed atrophy and hyperintense signals in the basal ganglia on T2-weighted images. Death occurred 2 months after onset. The case was further studied by sequence of prion protein gene (*PRNP*), biochemical characterization of the protease K resistant-prion protein (PrPTSE) and neuropathologic investigations.

Results: Genetic analysis of the *PRNP* revealed the rare mutation at codon 203 and homozygosity for methionine at codon 129. Western blot analysis showed the presence of PrPTSE type 1. The cerebellum and the cerebral cortex showed a synaptic pattern of PrP_{Sc} deposition.

Discussion: This is the second case-report describing the clinico-pathological features associated to the rare V203I mutation. Our findings underline and confirm the importance of carrying out genetic analysis in patients with the clinical suspect of CJD even in the absence of a positive family history.

P6.16 Analysis of polymorphisms in *PRNP* 5' UTR region in sporadic Creutzfeldt-Jakob subtypes

Authors
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Content
Background: The primary sequence of the coding region of the prion protein gene (*PRNP*) plays a central role in both susceptibility and phenotypic expression of Creutzfeldt-Jakob disease (CJD), but significant evidence indicates that other genetic factors are also involved. Quantitative-trait-locus studies of incubation periods in infected mice demonstrated a positive region close to *PRNP*, but independent of its coding sequence. The study of several polymorphisms upstream of *PRNP* coding region has shown to date a positive association between sporadic CJD (sCJD) and *PRNP* 1368, or *PRNP* 12533 although the results have been inconsistent among the different studies (Mead et al 2002, Mc Cormack et al 2002, Croes et al 2004, Vollmert et al 2006, Bratosiewicz-Wasik et al.2007).

Objective: To investigate the role of different *PRNP* haplotypes and of single SNPs in modulating susceptibility and phenotypic expression in sCJD.

Methods: We screened 260 autopsy confirmed sCJD and 200 controls for 9 SNPs in the *PRNP* locus for possible positive associations with the disease. The data were analyzed either considering the SNPs as single markers or as haplotypes. Furthermore, the sCJD population was analyzed either as a single group or in subtypes according to the molecular and neuropathological classification of the most common variants.

Results and Discussion: We detected the whole spectrum of haplotype diversity in the sCJD group as awhole and in each of the different subtypes as well, thus excluding the possibility of a genetic association between sCJD and a single *PRNP* haplotype. Although we found an increase in frequency of the *PRNP* 1368 SNP in the sCJD group compared to controls, the data was not statistically significant once it was corrected for the effect of codon 129. In contrast, we found a significant different distribution of the 12553 SNP between sCJDMM1 and sCJDMM1+2. This is the first observation of a specific association between a *PRNP* SNP and sCJD subtypes. A similar result, however, was demonstrated in cattle (Clawson et al 2008).

P6.17 Differential Gene Expression in Caudal Medulla Tissues between healthy Cattle and Cattle Orally Infected with Bovine Spongiform Encephalopathy

Authors
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Content
The identification of variations in gene expression in response to Bovine Spongiform Encephalopathy (BSE) may help in the discovery of biomarkers for disease progression, therapeutic targets and elucidate the mechanisms of neuropathology and agent replication. In this study, we compared the genes that are differentially expressed in the caudal medulla tissues of control (not BSE infected) and treated (infected orally with BSE material) animals at 12 and 45 months post infection using cDNA microarrays containing 24,000 oligonucleotide probes. The data were analyzed using Gene Sifter software package (VizX Labs, Seattle, WA). There were 805 and 337 genes found to be differentially expressed between control animals and animals 12 and 45 month post-infection respectively. Genes identified from both experiments were considered to be the genes which may be associated with BSE disease. In total, we identified 167 in common between both time points many of which have been previously reported in others transmissible spongiform encephalopathies (TSEs). In addition a number of novel genes were identified which have not been associated previously with TSE diseases. Gene ontology (GO) analysis of these genes revealed that 83 genes could be involved in the biological process such as: cellular metabolic process, cell communication, regulation of biological process and cell adhesion. The KEGG pathway database was used for linking genes with changed expression to biological systems by the processes of pathway mapping. Using this approach, we identified genes related with: Neuroactive ligand-receptor interaction pathway; cell adhesion; MAPK signaling pathway; Neurodegenerative disorder; SNARE interactions in vesicular transport pathway; TGF beta signaling pathway and Complement and coagulation cascades pathways. The differentially expressed genes identified in this study are currently being validated using quantitative RT-PCR.

P6.18 Distribution of genotypes of PrP gene at Codon 136 and 171 in Makueii sheep breed

Authors
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Content
Makui as one of the common and native sheep breeds are widespread livestock in the Middle East, particularly Iran, and are used for both meat and wool. The susceptibility of sheep to scrapie is influenced by genotypes of the prion protein (PrP) gene, so for the first time, this study was done to assess status of different PrP genotypes at codon 136 and 171in Makui breeds. Blood DNA samples of 60 randomly selected animals were extracted and examined using allele specific PCR amplification. The frequency of V allele (26.67%) and VV genotype (14%) at codon 136 was significantly lower then allele A and AA and AV genotypes. At codon 171, frequency of allele H (1.67%) was significantly lower then Q and R but no significant difference was found between alleles Q and R. The frequencies of QR and QQ genotypes were the same (36.67%) and significantly higher then RR and RH. None of QH or HH genotypes were found. Although status of alleles and genotypes is not so far from some reported breeds but the frequency of susceptible alleles is higher then famous breeds like as Hampshire, it motivate an additional study to find out susceptibly of these genotypes to natural or experimental scrapie and/or put emphasis on planning breeding controls to eradicate susceptible genotypes.

P6.19 HECTD2, an E3 ubiquitin ligase, is associated with prion disease incubation time in mice and susceptibility to vCJD and Kuru

Authors
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Content
Prion diseases or transmissible spongiform encephalopathies are characterised by a prolonged incubation period, variation in which is determined by many factors including host genetic background. Although the prion protein gene is the main genetic determinant it is now clear that other genes are also important. Here we use a heterogeneous stock of mice to identify Hectd2, an E3 ubiquitin ligase, as a putative quantitative trait gene for prion disease incubation time in mice and establish an association between HECTD2 haplotypes and susceptibility to vCJD and Kuru but not sporadic CJD. We also show a genotype associated differential expression of Hectd2 mRNA in mouse brains and human lymphocytes and a significant up-regulation of transcript in mice at the terminal stage of prion disease. Although the substrate of HECTD2 is unknown, these data highlight the importance of proteosome directed protein degradation in neurodegeneration. This is the first demonstration of a mouse quantitative trait gene that also influences susceptibility to two acquired human prion diseases. vCJD is still a major public health concern especially with the risk of iatrogenic transmission through blood and surgical instruments therefore the identification of susceptibility factors is key to estimating individual risk and providing new therapeutic targets.

P7.01 Characterization of polish atypical bovine spongiform encephalopathy including transmission studies in transgenic mice

Authors

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Content

Background: Bovine spongiform encephalopathy emerged as a new disease in the UK in 1985 and then spread to Europe and to other continents. Uniform characteristics of the causative agent, based mostly on histopathological studies performed in the UK suggested that a single strain was responsible for the epidemic. In 2004 researchers in France and Italy detected two novel unusual glycoprofiles of the prion protein in BSE cases from older cattle. These new types were described as H-type and L-type BSE, according to their higher (H) or lower (L) molecular mass of the unglycosylated fraction of the prion protein. Since then more atypical cases of both types were recorded in Europe and North America. The first case of BSE in Poland was diagnosed in May, 2002. Until March 2008, 61 BSE cases were confirmed including 9 cases of atypical BSE.

Objective: The aim of the study was to characterise atypical BSE cases in comparison to classical BSE (C-type) taking into account epidemiological data, geographic distribution, animal age at detection and to evaluate the glycoprofiles of PK resistant prion protein (PrPres) after transmission to transgenic mice expressing bovine PrP (Tg bovXV mice).

Methods: Epidemiological data for all BSE cases was reviewed and a comparative analysis of C-type and atypical BSE was undertaken. Glycoprofile analysis with a panel of monoclonal antibodies was used to discrimate between both forms.

Results and Discussion: Analysis of the dynamics of C-type BSE in Poland showed a constant rise from 2002 (3 cases) to 2005 (18 cases) with a sharp drop in 2006 (8 cases). This is in contrast to atypical BSE where besides 2002 and 2003 (1 and no cases reported respectively), 2 cases were recorded each year in the four consecutive years. While C-type BSE was dominant in healthy slaughtered animals, atypical BSE was almost equally found in healthy slaughered and risk group animals. The mean age of C-type and atypical BSE was 7 yrs and 11 yrs respectively. While the majority of atypical cases was located in Eastern Poland (67%), C-type BSE was present equally in Eastern and Central parts (40% and 42% respectively). Transmission studies in mice confirmed the abberant glycoprofiles of both L-type and H-type variants proving the existence of all three distinct strains of BSE in the cattle population in Poland. Since the prevalence of atypical BSE in cows in Poland is relatively high (15%) as compared to other countries, and since the use of meat and bone meal could not be confirmed in the majority of BSE cases, one can speculate that BSE in Poland is not only due to the use of contaminated concentrates but it can also exist as a sporadic disease.

P7.02 atypical scrapie in the 1980s (not a new emergent tse?)

Authors

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Content

Background: Over the last few years, with increasing frequency, there have been a number of cases of different forms of the TSE disease scrapie being isolated throughout the UK and Europe. Of major concern is the fact that these "atypical" forms of scrapie, such as the 1998 case in Norway (Nor98), appear to affect genotypes of sheep previously regarded as resistant to classical scrapie.

Objective: Work undertaken during the last year in the Neuropathogenesis Division of The Roslin Institute has taken advantage of an exceptional archive of sheep brain tissues to determine whether these atypical cases are actually of a new and emerging form of TSE, or whether their long presence in sheep flocks is only now being isolated due to the vastly increased and improved levels of surveillance currently in use. The archive importantly dates back to the 1960s and contains around 2000 samples from sheep of various ages from throughout the NPU's Cheviot and Suffolk flocks as well as from a range of sites across the UK.

Methods: The archive search uses techniques such as genotyping (to identify samples that were at the most risk from atypical forms of scrapie) and the Biorad TeSeE detection kit (which is able to detect atypical forms of scrapie), before employing Western blotting and Immunohistochemistry to firmly establish the atypical TSE in a specific sample.

Results: A number of cases have already been isolated in the archive including one from a Scottish flock in 1989 ("Nor98-like sheep scrapie in the United Kingdom in 1989" Bruce, M. E., et al. Veterinary Record 2007).

Discussion: This study aims to provide an indication as to the frequency of occurrence of atypical scrapie in the UK at dates earlier than those currently published. The presence of a Nor98-like form isolated in 1989 already lends weight to the hypothesis that atypical scrapie has been present for longer than previously thought. Results to date will be reported upon.

P7.03 Experimental challenge of cattle with german atypical bse isolates

Authors

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Content

Background: After the detection of two novel BSE forms designated H-type and L-type atypical BSE the question of the pathogenesis and the agent distribution of these two types in cattle was fully open. From initial studies of the brain pathology, it was already known that the anatomical distribution of L-type BSE differs from that of the classical type where the obex region in the brainstem always displays the highest PrP_{Sc} concentrations. In contrast in L-type BSE cases, the thalamus and frontal cortex regions showed the highest levels of the pathological prion protein, while the obex region was only weakly involved.

Objective: To address this issue, we performed intracranial inoculations of cattle (five and six per group) using 10% brainstem homogenates of the two German H- and L-type atypical BSE isolates.

Methods: The animals were inoculated under narcosis and then kept in a free-ranging stable under appropriate biosafety conditions afterwards. Three animals per group were killed and sectioned in the preclinical stage and the remaining animals were kept until they developed clinical symptoms. The animals were examined for behavioural changes every eight weeks throughout the experiment following a protocol that had been established during the oral BSE pathogenesis study with classical BSE.

Results and Discussion: All animals of both groups developed clinical symptoms and had to be euthanized within 16 months. The clinical picture differed from that of classical BSE, as the earliest signs of illness were loss of body weight and depression. However, the animals later developed hind limb ataxia and hyperesthesia predominantly at the head. Analysis of brain samples from these animals confirmed the BSE infection and the atypical Western blot profile was maintained in all animals. Samples from these animals are now being examined to analyse the pathogenesis and agent distribution in these novel BSE types.

P7.04 Variability in pathological phenotypes suggests the existence of natural sheep scrapie strains

Authors

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Content

Background: Different clinico-pathological phenotypes are observed in mice following challenge with uncharacterised ovine scrapie material. It is not known, however, whether the variety of murine scrapie strains so far identified is representative of strain diversity in the natural sheep host or of adaptation of the infectious agent after serial passage in different mouse lines.

Objective: Determine the presence or absence of phenotypic variability in natural scrapie from a variety of geographical sources and a single *PRNP* genotype.

Materials and Methods: Brain samples from 18 clinically affected, ARQ/ARQ sheep from 13 different flocks and 4 countries were examined by immunohistochemistry (IHC) for disease-associated PrP (PrP_d) by an agreed protocol between the participating laboratories. Each laboratory examined their own cases and one of them examined all of them in a blind trial, in which neither the flock nor the country of origin were known.

Results: On the basis of the PrP_d profile and neuroanatomical distribution, sheep were allocated into five groups: Group 1 (5 Scottish and 5 Italian cases) was characterized by prominent extracellular PrP_d deposits associated with glial cells. Group 2 (3 Spanish and 1 Dutch) exhibited marked intracellular deposits and also coalescing aggregates in the neuropil. Intraneuronal and intraglial PrP_d were also conspicuous in animals of group 3 (1 Spanish and 1 Dutch), but these showed diffuse particulate PrP_d in the neuropil and abundant stellate deposits. Groups 4 (one Spanish) and 5 (one Dutch) showed vascular amyloid plaques but differed markedly in the amount of intracellular PrP_d. The different participating laboratories had made the same allocation of sheep to the different groups as in the blind trial.

Discussion: Detailed immunohistochemical examination provides a useful means of characterizing pathological phenotypes, which can be harmonized between different laboratories. The variability of such phenotypes between sheep of the same PrP genotype suggests the existence of different naturally occurring strains of sheep scrapie. Studies are in progress to correlate the pathological findings with the biochemical PrP profiles on the same samples and to investigate the possible effect of other PrP polymorphisms in the phenotypic diversity.

P7.05 Transmission of atypical bse in humanized mouse models

Authors

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Content

Background: Atypical BSE cases have been discovered in several continents since 2004; they include the L-type (also named BASE), the H-type, and the first reported case of naturally occurring BSE with mutated bovine *PRNP* (termed BSE-M). The public health risks posed by atypical BSE are largely undefined.

Objective: To investigate these atypical BSE types in terms of their transmissibility and phenotypes in humanized mice with various *PRNP* genotypes.

Methods: Transgenic mice expressing human PrP-129M, PrP-129MV, or PrP-129V were or will be inoculated with several classical (C-type) and atypical (L-, H-,or M-type) BSE isolates, and the transmission rate, incubation time, characteristics and distribution of PrP_{Sc}, symptoms, and histopathology were or will be examined and compared.

Results: Sixty percent of BASE-inoculated transgenic (Tg) mice expressing human PrP-129M became infected with minimal spongiosis and an average incubation time of 20-22 months, whereas none of the C-type BSE-inoculated mice developed prion disease after more than 2 years. Protease-resistant PrP_{Sc} in BASE-infected humanized Tg mouse brains was biochemically different from bovine BASE or sCJD. PrP_{Sc} was detected in the spleen of 22% of BASE-infected humanized PrP-129M mice, but not in those infected with sCJD. Transmission studies with the H- and M-type BSE isolates are ongoing.

Discussion: Our results demonstrate that BASE is more virulent than classical BSE and have a lymphotropic phenotype in humanized mice. Our progress on secondary transmission of BASE and primary transmissions of BSE-H and BSE-M in humanized Tg mice will also be presented. Supported by NINDS NS052319, NIA AG14359, NIH AI 77774 and Charles S. Britton Fund.

P7.06 Phenotypic similarity of transmissible mink encephalopathy in cattle and l-type bovine spongiform encephalopathy in a transgenic mouse model

Authors

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Content

Transmissible mink encephalopathy (TME) is a food-borne transmissible spongiform encephalopathy (TSE) of ranch-raised mink. Although contamination of feed with scrapie-infected sheep parts has been proposed as the cause of TME, the origin of the disease remains elusive. To further investigate the hypothesis of an infection with a ruminant TSE, a comparative study was performed in an ovine transgenic mouse line (TgOvPrP4) inoculated with bovine-passaged TME isolate and 3 distinct isolates, typical, H-type and L-type of bovine spongiform encephalopathy (BSE). Survival periods, brain lesion profiles, disease-associated prion protein brain distribution and biochemical properties of protease-resistant prion protein, in first and second passages study, were analysed. Transgenic mice were susceptible to infection with bovine-passaged TME, typical BSE and L-type BSE but not with H-type BSE. The complete analysis of the three successfully transmitted TSE isolates provided experimental evidence that this TME agent is distinct from typical BSE but has phenotypic similarities to L-type BSE in TgOvPrP4 mice. Thus the present study allowed to collect a body of arguments in favour of the hypothesis that L-type BSE appears as a more likely candidate for a bovine source of TME infection than typical BSE.

P7.07 A novel prion disease affecting subjects with three prion protein codon 129 genotypes:could it be the sporadic form of gertsmann-straussler-scheinker disease?

Authors
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Content
A novel human prion disease affecting subjects with the three prion protein codon 129 genotypes: Could it be the sporadic form of Gerstmann-Sträussler-Scheinker disease? Pierluigi Gambetti, Qingzhong Kong, Wenquan Zou National Prion Disease Pathology Surveillance Center, Case Western Reserve University, Cleveland OH, 44106 USA.

Background: While Creutzfeldt-Jakob disease (CJD) and Fatal Insomnia have both sporadic and genetic forms, and the phenotypes of the sporadic forms overlap with those of the genetic forms, no sporadic form has ever been reported for Gerstmann-Sträussler-Scheinker disease (GSS). We previously described 11 cases affected by a novel prion protein (PrP) disease characterized by an abnormal PrP that was about 60 fold less protease resistant than that of sporadic CJD (sCJD) and on immunoblot generated a distinct ladder-like profile. The type of the spongiform degeneration and the PrP immunostaining pattern characterized by the presence of immature micro plaques were also different from those of the common human prion diseases. All affected subjects where homozygous for valine at codon 129 (VV) and had no mutation in the PrP gene.

Methods: We have searched the archives of our Prion Surveillance Center and looked prospectively for such cases, and we have re-examined their histopathology, PrP immunohistochemistry and abnormal PrP characteristics.

Results: We now report that a disease overall similar to that reported in the VV subjects also affects subjects that are methionine/valine heterozygous (MV) and methionine homozygous (MM) at codon 129 and have no PrP gene mutation. The abnormal PrP isoforms associated with the MV and MM cases generate electrophoretic profiles very similar to that of the VV cases but the new PrP isoforms show increasing levels of protease-resistance. The histological lesions and the PrP immunohistochemical patterns, although overall similar, are apparently distinguishable by the prominence and maturity of the prion plaques.

Discussion: We propose 1) that the new disease including VV, MV and MM cases is the long sought sporadic form of GSS based on the resemblance of the disease phenotype and PrP characteristics; 2) that the genotype at codon 129 modifies the phenotype and the PrP characteristics of this new disease as it does in sCJD. This new prion disease expands the spectrum of human prion diseases; its detailed study may bring to light new aspects of prion strains, pathogenesis and PrP conversion mechanisms in prion diseases. (Supported by NIA AG-14359, NINDS NS052319, CDC UR8/CCU515004 and Charles S. Britton Fund).

Topic: Emerging Strains.

P7.08 Accessibility of a critical prion protein region involved in strain recognition and its implication for detection of a group of unique prions

Authors
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Content
Background: Human prion diseases including Creutzfeldt-Jakob Disease (CJD) are typified by the presence in the brain of proteinase K (PK) resistant PrP fragments PrP 27-30 or PrP7-8 detectable by 3F4, a monoclonal antibody (mAb) to PrP109-112. Using the mAb1E4, which recognizes PrP97-108, we identified a PK-resistant PrP fragment called PrP*20 in normal human brains and ladder-like PrP fragments ranging from 7kDa to 30 kDa in a newly identified prion disease, termed protease-sensitive prionopathy (PSPr). Yet, all of these PK-resistant fragments detected by 1E4, were virtually undetectable with 3F4 in the absence of enriching procedures, although they contain the 3F4 epitope.

Objective: To investigate the molecular mechanisms underlying the difference in accessibility, within this critical PrP region, between the newly-identified abnormal PrP species and the typical PrP27-30.

Methods: PK-resistant PrP is isolated from normal brains and brains with PSPr. The N-terminal starting sites, GPI anchor, and glycosylation of the isolated PrP species are studied using one/two-dimensional gel electrophoresis, antibody mapping, N-terminal sequencing and deglycosylation with various enzymes.

Results: The accessibility of the two adjacent epitopes of the 1E4 and 3F4 antibodies between PrP residues 97 and 112 is differently regulated by their neighboring N-terminal sequence. Compared to PrP27-30 from sCJD, PK-resistant PrP fragments from normal brains and from PSPr have different N-terminal starting sites and glycosylation.

Discussion: The N-terminal region of PrP27-30 including PrP_{Sc} type 1 and 2, starting at residues 82 and 97, respectively, is involved not only in the structural transition of prion formation but also in the determination of prion strains. Remarkably, both 3F4 and 1E4 epitopes encompassing residues 97-112 are located in this critical region. Our study reveals that the PK-resistant fragments detected preferentially by 1E4 represent a group of novel prion strains that are different from the typical PrP27-30 widely present in various human prion diseases identified thus far. Accordingly, in order for novel forms of prion diseases not to be overlooked, we recommend that in addition to 3F4, other antibodies against different PrP domains should be used in the routine diagnosis of prion diseases. (Thanks to Dr. Pierluigi Gambetti's critical comments. Supported by CJD Foundation, NIH AG-14359, CDC UR8/CCU515004 and Charles S. Britton Fund).

P7.09 Biochemical screening for identification of atypical bse in belgium, 1999-present

Authors
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Content

Background: Recently two atypical forms of BSE have been described. Western blot analyses showed that, in comparison to the classic BSE (C-type), they are demonstrable by a higher or lower molecular weight of the unglycosylated PrP_{res}. They were thus named H-type and L-type BSE (L-type is also called BASE). In addition they show a lower proportion of diglycosylated PrP_{res} than C-type. These emerging types represent different strains of BSE. They show unique incubation periods and histological lesions. Such types have been described on different continents. Indeed they might correspond to "sporadic" forms of BSE. In 2004 we already described one L-type in Belgium.

Objective: We retrospectively analysed the bovines at least 7-year-old in the Belgian archive of BSE-diagnosed cattle in order to determine the prevalence of the two types of atypical BSE in Belgium.

Methods: We analysed homogenates from 39 bovines of 93 months old in median (min: 84, max: 181 months). The most recent one was diagnosed in 2006. We used Western blot with a panel of anti-PrP antibodies (Ab). They detect different regions of the PrP protein, from N-terminal to C-terminal: 12B2, 9A2, Sha31, SAF84, 94B4. Their combination is aimed at an efficient typing diagnostic. We detected bound Ab with SuperSignal West Dura (Pierce) and analysed PrP_{res} signals with an image-analysis software (Quantity One, Bio-Rad).

Results: The results are still under analysis. We will detail the most crucial characteristics for typing PrP_{res}. These include 1) the apparent molecular mass of the un-, mono- and diglycosylated bands, 2) the binding affinity to the five Ab (e.g. 12B2 for H-type), 3) the presence of a fourth (unglycosylated) band and 4) the glycoprofile based on the relative proportions of the visible bands.

Discussion: The emergence of atypical types of BSE is partially due to a better knowledge of prion strains and more efficient diagnostic techniques. As the area in the brain where the PrP_{res} is deposited can differ drastically between the types, it is essential to ascertain that the sampling techniques and analyses are adapted to these new types. As these new strains seem more virulent than classic types, they represent one of the next challenges in the field of prions.

P7.10 Two unusual bovine spongiform encephalopathy (bse) cases detected in great britain (gb)

Authors
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Content

Background: BSE was first identified in GB in 1986 and was subsequently detected in many other countries, world-wide. A decade after the start of the bovine epidemic the first cases of new variant Creutzfeldt-Jakob disease (vCJD) in humans were linked to probable ingestion of BSE infected tissue, highlighting a new zoonotic disease. An abnormal protease-resistant protein (PrP_{res}) in a diseased subject, derived from a post translational change of a normal host cellular membrane protein (PrP_c), is a reliable disease marker for the whole group of neurodegenerative transmissible spongiform encepalopathies (TSEs). Immunology-based techniques, such as Western immunoblotting, have previously indicated that BSE cases all give a uniform molecular profile for PrP_{res}. Periodic lesion profiling of the spongiform change through out different brain regions of infected mice and cattle has also indicated a single agent for BSE. However, in 2001 rapid testing for PrP_{res} was introduced for the active surveillance of ruminants within Europe and approximately 40 BSE cases have now been recognised that differ in their molecular profiles from those typically found. These unusual BSE cases have been detected in several European countries, and in Japan and the USA. At present the cases appear as two distinct types based on the molecular mass of the unglycosylated PrP_{res} protein band relative to that of classical BSE. One type is of a higher molecular mass (H-type) and the other shows a lower molecular mass (L-type). Transmission studies in mice have shown that both H-type and L-type BSE have biological characteristics that are different from those of the classical BSE agent.

Objective and Results: This presentation shows the molecular profiles of the first two cases of H-type BSE detected in GB in comparison to those obtained for classical BSE, and scrapie in sheep from GB, and a control H-type BSE case from France. The *PRNP* ORF of the two GB atypical BSE cases were found not to contain polymorphic amino acids such as the E211K polymorphism identified in an atypical BSE case from the USA.

Conclusions: Therefore, our results support previous reports that H-type BSE is not caused by a novel germ-line mutation in the prion gene coding region. It has recently been reported that polymorphisms in the prion gene promoter region that could influence classical BSE susceptibility are not applicable to other TSEs in cattle. The genotype of these two polymorphic indels in the two GB H-type BSE cases differed from each other, with one being homozygous for insertions at both the 12 and 23 bp indels, and the other was homozygous for deletions at both indels. The genetic data support the findings of other researchers that these indels do not contribute to the susceptibility of atypical BSE.

This study was funded by DEFRA, UK.

P7.11 Biological and biochemical characterization of l-Type bse prion detected in japanese beef cattle.

Authors

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Content

A case of L-type atypical bovine spongiform encephalopathy (L-BSE) was detected in 14-year-old Japanese Black beef cattle (BSE/JP24) in Japan. To clarify the biological and biochemical properties of the BSE/JP24 prion, we performed a transmission study with wild-type mice and bovinized transgenic mice (TgBoPrP). The BSE/JP24 prion was transmitted to TgBoPrP mice, and the incubation periods were found to be 199.7 ± 3.4 days, which was shorter than those with classical BSE (C-BSE) (223.5 ± 13.5 days). Further, when C-BSE was transmitted to wild-type mice, the incubation period was 400 days whereas BSE/JP24 inoculated mice showed no clinical signs up to 530 days. Severe vacuolation and a widespread and uniform distribution of PrP_{Sc} were observed in the brain of BSE/JP24-affected TgBoPrP mice. The molecular weight and glycoform ratio of PrP_{Sc} in BSE/JP24 were different from those in C-BSE, and PrP_{Sc} in BSE/JP24 exhibited weaker proteinase K resistance than that in C-BSE. These findings revealed that the BSE/JP24 prion has distinct biological and biochemical properties in C-BSE. Interestingly, shorter incubation period was observed at the subsequent passage of the BSE/JP24 prion to TgBoPrP mice (152.2 ± 3.1 days). This result implies that BSE/JP24 prion has newly emerged and has not completely adapted to cattle species.

P7.12 Co-existence of classical and atypical scrapie strains in a sheep from an italian outbreak

Authors

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Content

Background: The Nor98 is an atypical scrapie strain characterized by a PrP_{Sc} with a molecular pattern and a distribution in the brain different than in classical scrapie. Genetic studies demonstrated that atypical scrapie affects frequently PrP genotypes considered resistant for classical scrapie. In Italy 63 atypical cases, 54 sheep and nine goats, were identified so far.

Objective: To describe the molecular features and distribution pattern of PrP_{Sc}, and the genetic analysis of the *PRNP* gene in a scrapie-positive sheep from an Italian outbreak.

Methods: The sheep came from an outbreak where the index case and other four cases resulted affected by classical scrapie. Western Blot (WB), Immunohistochemistry (IHC) and Istopathological (HP) analyses were carried out on the brain, the sub-mandibular lymph node and the tongue. A complete sequencing of the *PRNP* gene was also carried out.

Results: IHC revealed the simultaneous presence in the brain of pathological features characteristic of Nor98 and classical scrapie. This was confirmed by WB: PrP_{res} fragments characteristic of Nor98 and scrapie were simultaneously present in all areas investigated, although in different proportions, with Nor98 being more abundant in the cortex and classical scrapie in the brainstem. The lymph node showed the presence of PrP_{Sc} with a molecular pattern referable only to classical scrapie, while the tongue resulted negative. Genetic analysis showed the following genotype: A136L141R154Q171/A136F141R154Q171.

Discussion: Our results suggest either the co-existence of Nor98 and classical scrapie in this sheep or the presence of a new scrapie phenotype. The presence of classical scrapie in the outbreak and the genotype of the animal support the first event, which might be explained by the different genetic and cellular targets of the two strains. The bioassay analysis in bank voles and Tg338 mice, at the moment in progress, will help to confirm this hypothesis.

P7.13 Ph variations affect pk stability of guanidine-treated bse and base-associated PrP

Authors

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Content

Background: The agent responsible for Bovine Spongiform Encephalopathy may exist in at least 3 different forms referred to as C-Type, H-Type and L-Type based on electrophoretic mobility and glycoprofiling of PrP_{Sc}. Passages in conventional mice and inoculation in cows has shown diversity in bovine prion strains in terms of incubation period and lesion profile strengthening the hypotesis that, as for human TSE, each prion conformer carries the specific information that determine its own distinct biological properties.

Objective: We investigated biochemical and physicochemical properties of PrP_{Sc} associated to classical BSE (C-type) and BASE (L-type) Italian isolates in order to unravel differences in their molecular structure.

Methods: Aliquots of BSE and BASE terminally diseased brain homogenates were incubated with increasing concentration of GdnHCl at different pH values followed by limited proteolysis with PK. Then samples were precipitated with methanol and subjected to Western blot with a set of MAbs.

Results and Discussion: Treatment with 2.5 M GdnHCl pH 7.6 led to a significant increase in PK sensitivity of L-type PrP compared to C-type that showed resistance to PK up to 3.5 M GdnHCl. Interestingly, when GdnHCl solutions at pH 3.5 were used an increased stability to PK of both C-type and L-type PrP was observed as they were digested at GdnHCl concentration of 4.5 M and 4.0 M respectively. These data indicate the possibility of discriminating C-type and L-type PrP on the base of their distinct stability to PK-induced proteolysis following exposure to guanidine. They also suggest the existence of differences in their conformational properties that seem to be affected by pH variations. Assessing the extent of such a biochemical diversity may provide hints to understand how differences in the molecular structure of C-type and L-type PrP are related to the distinct pathological phenotype of BSE and BASE.

P7.14 Splenic replication of scjd but not cwd prions in a "humanized"mouse model system

Authors

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Content

Prion diseases or transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases affecting humans and a large variety of animals. Ethical constraints prevent most functional analyses of neuroimmunological aspects of TSEs in humans. By combining transgenesis and xenotransplantation, we have created an accurate animal model circumventing such constraints (1). CD34+ hematopoietic stem cells derived from umbilical cord blood of healthy human newborns are transferred into immuno-compromised mice. By this we have established a human immune system in the mouse, which can be tested for its capability to replicate human prions in vivo. Here we tested whether peripherally administered prions derived from a human sCJD brain homogenate (129MM) replicate in the spleens of CgRag2-/- mice, reconstituted with umbilical cord blood (129MM). As early as 60 - 90 dpi we could detect Prp_{Sc} in spleen but not in mesenteric lymph node homogenates. In contrast, two different brain homogenates derived from a white tail deer and an elk did not lead to splenic PrP_{Sc} deposition in reconstituted CgRag2-/- mice (129MM). Therefore, we conclude that humanized CgRag2-/- mice (129MM) enable splenic prion replication of sCJD (129MM) but not of CWD prions.

References: Traggiai et al., SCIENCE 2004.

P7.15 Inter-species transmission of CWD into hamsters: Evidence for a new strain?

Authors
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Content
Chronic wasting disease (CWD), a prion disease of cervids, is maintained in populations via lateral transmission. Shed disease agent has the potential to contaminate exposed environs for a substantial period of time. Since eradication of CWD in the environment is unlikely, the potential for interspecies transmission of CWD is of great concern. Based on the geographic separation of affected cervid populations as well as polymoprhisms in the primary sequence of the prion protein (PrP), it is likely that there is more than one strain of CWD. We experimentally transmitted the CWD agent from Wisconsin white-tailed deer into Syrian golden hamsters. Obex homogenates (10% w/v) from hunter-harvested CWD-positive white-tailed deer (of known PrP genotypes) were inoculated intracerebrally into hamsters. Although clinical symptoms were not observed on first passage, all hamsters had detectable levels of proteinase K resistant prion proteins (PrPTSE). In a separate experiment, concentration of the CWD agent prior to inoculation resulted in all hamsters presenting with clinical disease. Clinical presentation was unique compared to other hamster-passaged TSE agents (hyper, 263K, drowsy) and was maintained upon second passage. These outcomes are different than observed when CWD agent from western white-tailed deer (i.e., from Colorado/Wyoming) are passaged into hamsters suggesting that CWD agent is different in the two populations. Studies are currently underway characterizing the biochemical properties of the PrPTSE of the hamster-adapted CWD agent. Different strains of CWD agent may have different biological properties including different species barriers.

P7.16 White matter kuru plaques in atypical sporadic Creutzfeldt-Jakob disease: study of three cases

Authors
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Content
Background: The presence of kuru plaques in the cerebellum is the hallmark of the subtype of the sporadic Creutzfeldt-Jakob disease (sCJD) associated with methionine (M)/valine (V) heterozygosity at codon 129 of the prion protein (PrP) gene and with scrapie PrP (PrP_{Sc}) type 2 which we identify as sCJDMV2. Some reports have shown the same plaques in a few patients belonging to the sCJDMM1/MV1 subtype. To our knowledge, kuru plaques are commonly detected only in the brain grey matter.

Objective: To carry out a comparative study of the phenotypical and molecular features of three patients affected by sCJD with presence of kuru plaques in the white matter.

Methods: The 129 genotype of the three subjects was determined, and genotypically homologous sCJD cases were screened by one- and two-dimensional high-resolution western blotting, and conformational stability immunoassay to determine the biochemical and biophysical characteristics of the PrP_{Sc}. Histological, immunohistochemical examinations and clinical data analysis were also carried out.

Results: All three subjects are MM homozygous at codon 129. Mean age at death is 55.7±3.1 years (range: 53- 60 years); mean disease duration is 10±2.9 months (range: 6-13 months). Kuru plaques are detectable in the white matter of the cerebellum. The distinctive electrophoretic profile of the PrP_{Sc} recovered from the cortical and sub-cortical brain regions of these three cases is the presence of two PK-resistant unglycosylated PrP_{Sc} fragments in addition to PrP27-30. The fragment with the fastest mobility co-migrates with the unglycosylated PrP_{Sc} isoform type 1, whereas the slowest migrating PrP_{Sc} fragment is ~ 0.5 kDa higher and is often the best represented.

Discussion: The discovery of a novel human PrP_{Sc} strain and of a distinct clinical and pathological subtype of sCJDMM1 widen the spectrum of human prion diseases and, as in naturally occurring prion diseases of animals, force us to take into account an increasing number of novel human PrP strains.
(Supported by, NIH AG-14359, CDC UR8/CCU515004 and Charles S. Britton Found).

P7.17 Chronic wasting disease (CWD) susceptibility of several north american rodents that are sympatric with Cervid CWD epidemics

Authors
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Content
ChronChronic wasting disease (CWD) is a contagious transmissible spongiform encephalopathy (TSE) of cervids; CWD epidemics are currently occurring in free-ranging cervid populations at a number of locations in the United States and Canada. We intracerebrally challenged four species of native North American rodents that inhabit locations undergoing cervid CWD epidemics. The species were: deer mice (*Peromyscus maniculatus*), white-footed mice (*P. leucopus*), meadow voles (*Microtus pennsylvanicus*), and red-backed voles (*Myodes gapperi*). The inocula were prepared from the brains of hunter-harvested white-tailed deer (*Odocoileus virginianus*) from Wisconsin that tested positive for CWD. Meadow voles proved to be most susceptible, with the earliest presentation of disease signs after about 7 months and with 100% mortality by 374 days. Immunoblotting and immunohistochemistry confirmed the presence of PrP_{res} in the brains of all challenged meadow voles. The disease progression in red-backed voles, which are closely related to the European bank vole M. glareolus that have been demonstrated to be sensitive to a number of TSEs, was slower than in meadow voles. Deer mice did not exhibit disease signs until nearly 1.5 years post-inoculation, but appear to be exhibiting a high degree of disease penetrance. After an incubation period of similar length, white-footed mice have still not exhibited any signs of disease. Second passage experiments now underway show significant shortening of incubation periods. Meadow voles in particular appear to be interesting lab models for CWD. These rodents scavenge carrion, and are an important food source for many predator species. Furthermore, these rodents enter human and domestic livestock food chains by accidental inclusion in grain and forage. Further investigation of these species as potential hosts, bridge species, and reservoirs of CWD is required.

P8.01 A new bacterial model system to study PrP-like DNA-induced protein amyloidogenesis

Authors
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Content

Background: Non-pathogenic proteins can be suitable model systems to study amyloid proteinopathies, including prion diseases, because the molecular principles underlying protein amyloidogenesis are universal (1). Thus yeast Sup35p, Ure2p, Rnq1p and HET-s have substantially contributed to our current knowledge on prion structure and propagation (2). However, we were still lacking of a bacterial intracellular model system for amyloidogenesis. We have worked out the molecular basis for the switch enabling a bacterial protein (RepA) as a plasmid replication initiator (3): dissociation of RepA dimers is coupled to a conformational change in the N-terminal Winged-Helix domain (WH1), which transforms part of its alfa-helical structure into β-strands through a metastable folding intermediate (4,5). Binding to specific plasmid dsDNA sequences triggers RepA structural remodelling (6,7).

Results and Discussion: We have recently found a protein stretch in RepA-WH1 with the potential to aggregate through β-strands (8). Mutations in a single residue of the motif result in enhanced or reduced WH1 aggregation. Combining these mutants with binding to short dsDNA oligonucleotides, including the natural targets for WH1 in full length RepA (3-7), it is possible to modulate the formation of diverse amyloid assemblies (8). A synthetic peptide including the amyloidogenic motif assembles into cross-β fibres (1,8). The extent to which each DNA sequence transforms WH1 structure (6,7) inversely correlates with the order of the resulting amyloid assemblies. DNA is not part of the fibres, thus acting as a real inducer, rather than as a structural component, of the aggregates (8). Our studies provide important insights into the way nucleic acids could promote mammalian PrP replication (9). We have also found that di- and tetra-sulphonated derivatives of the indigo stain compete with WH1 binding to dsDNA, thus abolishing fibre assembly (10). Attempts to modulate the assembly of WH1-GFP amyloids in E. coli and S. cerevisiae are underway.

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P8.02 Potential for Cross-Species CWD Transmission Assessed by PMCA

Authors
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Content

Background and Objective: Chronic wasting disease (CWD) of cervids is a transmissible spongiform encephalopathy (TSE) of increasing prevalence in North America. Whether non-cervid species may become naturally infected is not known. We previously reported efficient in vitro amplification of CWD PrP_{RES} (PrPCWD) in Tg(cerPrP)1536 mice by PMCA.
In an effort to predict species susceptibility to CWD and gain insight into CWD transmission and propagation, we are using PMCA to assess whether CWD prions can be propagated in brain homogenates from species anticipated to encounter CWD in nature, e.g. coyotes, mustelids, felids, field mice, prairie voles and others.

Methods: Normal brain homogenates (NBH) from target non-cervid species were spiked with CWD-positive deer brain homogenate in 96-well plates and subjected to PMCA (30 min. incubation/40 s pulse sonication, 48 hr, Misonix 3000). For serial PMCA, aliquots from each reaction mixture were transferred into fresh NBH and the protocol repeated. PrP_{res} was detected by immunoblotting after PK digestion of control and test samples using antibodies proven to detect the target species PrP.

Results: We demonstrate that cervid PrPCWD can convert PrP_C from several non-cervid species to PrP_{RES} in vitro. Successful amplification was indicated by the generation of newly formed protease-resistant bands in samples seeded with PrPCWD with concomitant digestion of PrP_C in non-PrPCWD-seeded samples. Interestingly, degree of sequence homology between deer and target species *PRNP* was not a predictor of successful amplification.

Discussion: Brain homogenates from several non-cervid species expected to encounter CWD in nature support amplification of PrPCWD in vitro. We are currently assessing the in vivo susceptibility of selected non-cervid species and the infectivity of in vitro generated PrPCWD in these species. These results suggest that PMCA may offer insight into the CWD host range and the species barrier.
Supported by contract N0125491 from NIH, NIAID and by grant D07Z0-072 from the Morris Animal Foundation.

P8.03 Effect of sporadic Creutzfeldt-Jakob disease prion protein type and *PRNP* codon 129 genotype in an in vitro prion protein conversion assay

Authors

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Content

Background: Sporadic Creutzfeldt-Jakob disease (sCJD) is phenotypically heterogeneous, occurring in a series of subtypes, each characterised by a distinct clinico-pathological phenotype and currently classified according to the patients *PRNP* codon 129 genotype (MM, MV, VV) and the protease resistant prion protein isoform present in the brain (PrP_{res} type 1 or type 2). The majority of individuals that have at least one *PRNP*-129M allele have a predominantly type 1 PrP_{res} and conversely the majority of individuals that have at least one *PRNP*-129V allele have a predominantly type 2 PrP_{res}.

Objective: To use protein misfolding cyclic amplification (PMCA) to model PrP_{res} conversion and to test the *PRNP* codon 129 substrate specificity of PrP_{res} from each of the major subtypes of sCJD.

Methods: Seeds for PMCA reactions were prepared from autopsy human brain specimens from each of the sCJD subtypes (MM1, MV1, VV1, MM2c, MM2t, MV2, VV2). The substrates for PMCA were prepared from humanised transgenic mice homozygous for *PRNP*-129M (HuMM) or *PRNP*-129V (HuVV) or these mice crossed to produce heterozygotes (HuMV). A single round of PMCA (48 cycles of sonication and incubation) was carried out and the degree of PrP_{res} amplification assessed by Western blotting.

Results: sCJD MM1 and MM2 amplified in HuMM and HuMV substrate whereas sCJD VV1 and VV2 amplified in HuVV substrate. The sCJD MV1 behaved similarly to the MM1, amplifying in the HuMM substrate and the MV2 behaved similarly to the VV2, amplifying in the HuVV substrate. The PrP_{res} type of the seed was maintained in the amplified reaction product.

Discussion: Modelling prion protein conversion *in vitro* using PMCA shows a high degree of biochemical fidelity with the PrP_{res} type of the seed faithfully reproduced in the PMCA reaction product. The results suggest a key role for compatiblity of codon 129 of seed and substrate in determining conversion efficiency and further strengthen the observational association between the *PRNP*-129M allele and type 1 PrP_{res}, and the *PRNP*-129V allele and type 2 PrP_{res}.

P8.04 Structure of PrP^{C} and its interaction with PrP^{Sc} on the lipid membrane

Authors

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Content

Background: The conversion of the cellular prion protein (PrP^{C}) into the disease-associated PrP^{Sc} is the primary event of prion diseases. This conversion process might take place either at the membrane surface or in its close proximity (1, 2). PrP^{C} is attached to special plasma domains on the exterior cell surface by its glycosyl phosphatidyl inositol (GPI-) anchor, which is linked posttranslationally to its C-terminus.

Objective: We study the structure of the membrane-anchored PrP^{C} in interaction with PrP^{Sc} and the structural transition process at the membrane or in its close proximity.

Methods: Posttranslationally modified PrP^{C} was purified from transgenic CHO-cells (3, 4). The thermodynamics of the insertion of soluble CHO-PrP^{C} into model membranes, mimicking the natural rafts domains, were studied by surface plasmon resonance (SPR) using the Biacore instrument (5). The structure of membrane-immobilized CHO-PrP^{C} was analyzed in the total reflection mode, using the FTIR method (6).

Results and Discussion: Purified, soluble PrP^{C} was inserted into model bilayer bound on a chip surface, and the thermodynamics and kinetics of this insertion were analyzed quantitatively. We could observe high specificity and affinity of PrP^{C} to the model membrane with a KD of about 10-8 M. The FTIR studies showed that the native-membrane anchoring induced formation of intermolecular β-sheets between PrP^{C} molecules. Such a transition is not observed in solution and is membrane specific. In order to simulate the infection process PrP^{Sc} particles were added to the solution in contact with PrP^{C}-covered membrane. We could observe that PrP^{Sc} binds quite stably to immobilized PrP^{C}. In a control experiment with a PrP^{C}-free lipid bilayer a little or no binding was observed. We conclude that as a first step of the conversion PrP^{Sc} particles bind to PrP^{C} which is anchored on the lipid surface.

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P8.05 Dimerization-induced Aggregation of Prion Protein is a Prerequisite for Amyloid Fibril Formation

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Content

Background: The conversion of PrP_C into an aggregated infectious PrP_{Sc} isoform is the central molecular event in prion diseases. However, in the absence of PrP_{Sc}, the exact mechanism that triggers this conversion is still under debate.

Objective: To test if dimerization of recombinant PrP_C would induce a conformational transformation of the protein into aggregated forms To determine the potential capacity of the aggregates for subsequent formation of the amyloid fibrils.

Methods: We have developed a conversion system using a recombinant prion protein with a potential self-dimerizing capability (FvPrP) in the presence of the dimerizing AP20187 ligand. The mechanism of FvPrP aggregation and the resulting amyloid fibril formation were characterized using biochemical and biophysical methods such as circular dichroism (CD), analytical centrifugation, light absorbance at 340 nm, Thioflavin T (ThT) assay, fluorescence and electronic microscopy.

Results: The incubation of FvPrP with AP20187 resulted in the protein conversion to a β-sheet-rich structure. As judged by circular dichroism (CD), this β-sheet conformation possessed a higher thermal stability (Tm = 79.84ºC) compared to the control proteins (Tm = 70.15ºC), suggesting the formation of stable PrP aggregates. In control experiments, addition of a monovalent ligand (FK506) did not change the structure of FvPrP. The dimerization-induced aggregation of PrP was confirmed by turbidity and centrifugation. We report that the addition of AP20187 increased the absorbance (O.D. at 340 nm) of FvPrP up to 25-fold. Furthermore, analytical centrifugation studies demonstrated that dimerization of FvPrP stimulated a conformational switch to the protein aggregated forms. In addition to β-sheet aggregates, the oligomeric form of the protein was characterized by electron microscopy as well as its affinity for amyloid-specific dye, thioflavin T (ThT), providing preliminary evidence of the involvement of PrP dimerization in the formation of *in vitro* amyloid fibrils.

Discussion: Together, these results suggest that in the absence of PrP_{Sc}, PrP_C dimerization is a central mechanism of intermolecular β-sheet aggregation and subsequent amyloid fibril formation.

P8.06 Cell division modulates prion accumulation and clearance in cultured cells

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Content

Background and Objective: The phenotypic effect of prions is influenced by the physical properties of the prion strain as well as their level of accumulation within the cell. In mammalian cell culture, the interplay between the processes of de novo prion formation, catabolism, cell division and horizontal cell-to-cell transmission determines the extent of prion accumulation. Understanding this dynamic allows the analytical modeling of protein-based infectivity and heritability.

Methods: We quantitatively measured the competing effects of prion formation, catabolism, cell division and horizontal cell-to-cell transmission in mouse neuroblastoma (N2a) cells and propose a concordant reaction mechanism to explain the kinetics of prion propagation in culture.

Results: and **Discussion:** The process of cell division leads to a predictable reduction in steady-state prion levels within the cell, but does not result in complete clearance. Scrapie-infected N2a cells were able to accumulate different steady-state levels of prions, dictated in part by the cell-division rate. We observed that the primary mode of prion transmission in N2a cells was from mother cells to daughter cells, not horizontally from cell to cell. These kinetic results were quantitatively modeled based on a mechanism that assumes that a subpopulation of prions is capable of self-catalysis and the levels of this subpopulation reach saturation in fully infected cells. Our findings also indicate that the apparent efficacy of antiprion compounds in culture is influenced strongly by the growth phase of the target cells. Our results suggest that compared to immortal cell lines, stationary cultures are a more relevant model for evaluating the potential effectiveness of antiprion compounds.

P8.07 Optimal incubation period depends on sonication conditions in order to increase the efficiency of protein misfolding cyclic amplification

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Content

Background: Protein misfolding cyclic amplification (PMCA) is a method, in which PrP_{Sc} can be amplified in vitro by repeating cycles of sonication and incubation. Although PMCA was demonstrated to be faster and more sensitive than bioassay, it still takes many days to detect a minute amount of PrP_{Sc}. To apply PMCA to screening tests or process validations of blood-derived products, the total reaction period should be shortened.

Objective: Focusing on sonication (PrP_{Sc} breakage) and incubation (PrP_{Sc} growing) conditions in PMCA, we aimed to increase the efficiency of PrP_{res} propagation and to minimize the total reaction periods to detect minute amounts of PrP_{Sc}.

Materials and Methods: 0.002% (w/v) Scrapie strain, 263k, infected hamster brain homogenate (ScHg) was prepared by diluting 10% (w/v) ScHg in normal hamster brain homogenate (NHg). 0.002% ScHg was propagated by automated PMCA machine (Elecon Science, Japan), with various sonication and incubation periods conditions for up to 24hr. All the samples were digested with proteinase K and an amplification factor was estimated by Western blotting. 100-Fold serial dilution of 10% ScHg were subjected to multi-round PMCA, in which propagated products were diluted 1:99 in fresh NHg at every round.

Results and Discussion: Incubation (PrP_{Sc} growing) condition depended on sonication (PrP_{Sc} breakage) condition for optimal PrP_{Sc} propagation in PMCA. 14 Min, or 2 min incubation periods were most efficient when 4 x 5 sec or 1 x 5 sec bursts sonication were applied, respectively. An amplification factor reached about 300 fold at 12 hr under either condition. PrP_{Sc} in 108-fold dilution of 10% ScHg was detected within 3 rounds (36hr) of PMCA. By optimizing physical conditions of PMCA, we could shorten the total reaction periods of multi-round PMCA considerably.

P8.08 The use of serial protein misfolding cyclic amplifcation (sPMCA) to detect surrogate markers for chronic wasting disease in surface water and soil

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Content

Background: Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy of deer and elk. Research has indicated that CWD is transmitted horizontally, and that both blood and saliva can transmit disease. Environmental exposure to pens where infected animals have been kept has resulted in disease transmission to experimental deer. However, examination of environmental components such as soil and water for prions has been hampered by sensitivity limitations of conventional western blotting and inoculation limitations of bioassays.

Objective: In this study we evaluated the ability of PMCA to detect PrP_{res} in environmental samples such as water and soil.

Methods: Serial protein misfolding cyclic amplification (sPMCA) was used to detect prion-infected brain homogenate spiked into soil and water to determine detection limits of this assay in environmental samples. We next evaluated surface water from a CWD endemic region of Colorado and soil from a ranch populated by confirmed CWD-positive animals for PrP_{res}, the misfolded proteinase-resistant protein associated with prion disease, by sPMCA.

Results: The PrP_{res} detection limit for water after 4 rounds of PMCA was 1:1.6 x 106 and 1:3200 for soil. PrP_{res} was detected in the majority of surface water samples collected at several time points and in a ranch soil sample from a heavily used wallow area.

Discussion: Our data suggests that sPMCA can be a useful tool in evaluating water and soil for PrP_{res} in CWD-endemic areas.

P8.09 Towards imaging prion replication and spread

Authors
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Content

Background: Prion infections involve the spread of PrP_{res} within and between cells. Cellular mechanisms mediating the initiation, propagation, and intercellular spread of PrP_{res} are poorly understood. Among protein misfolding diseases, only prion diseases are capable of transmission between hosts under natural conditions.

Objective: Our objective is to determine the mechanisms by which prions replicate and spread between host cells using imaging-based approaches. We are also investigating factors that contribute to the unique transmissibility of prion diseases, focusing on the role of GPI-anchored membrane association. Highlights of other RML work can be presented.

Methods: We have developed IDEAL-labeling, a novel technique that allows rapid and specific fluorescent labeling of cell-surface proteins containing the tetracysteine (TC) tag. We have created several TC-tagged PrP_C constructs. We are also expressing the amyloid domains from various amyloidogenic proteins, such as Sup35NM, as GPI-anchored fluorescent protein fusions in neuroblastoma cells.

Results: We show that IDEAL-labeled TC-tagged PrP_C molecules are converted to fluorescent PrP_{res} in live cells. Chase studies with anti-prion compounds post IDEAL-labeling have provided insight into their mechanisms of action. We also show inducible formation of self-propagating Sup35NM aggregates in cells expressing GPI-anchored Sup35NM-GFP. Aggregate formation can be initiated by treatment with pre-formed recombinant Sup35NM fibrils or co-culture with cells producing aggregated Sup35NM-mCherry-GPI. These events have been visualized by live cell imaging.

Discussion: With the advent of IDEAL-labeling and TC-tagged PrP_C, we have created the tools necessary for the first ever experiments aimed at imaging the propagation and intercellular spread of newly formed PrP_{res} in live cells. We have visualized related processes in a new model system utilizing a GPI-anchored version of another amyloidogenic protein. Our data advance our understanding of mechanisms by which GPI-anchored protein aggregates form and spread between cells and support the concept that GPI-anchoring modulates the transmissibility of amyloidogenic proteins. This provides a potential explanation why other protein misfolding diseases are not efficiently transmissible.

P8.10 Characterization of prion infectivity released by cultured cells.

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Content

Background: The mechanisms by which prions are transferred from one cell to another are poorly understood. While cell culture assays provided indications that cell-to-cell transmission of infection could occur through direct cell contacts, prion-infected cultured cells are also known to secrete infectivity into their environment supporting the possibility that prion dissemination could occur by other means than direct cell-to-cell contacts. Recently, we purified cell-free forms of prions from the conditioned media of cultured cells infected with a sheep prion strain and showed that infectivity was associated with small microvesicles from endosomal origin, known as exosomes.

Objective: In this study, we will determine the levels of prion infectivity released by cultured cells infected by various prion strains and will investigate the biochemical features of the associated abnormal PrP.

Methods: Conditioned media from different cell lines (including RK13 and N2a) persistently infected with various prion strains (from sheep and mouse scrapie) are being collected and the amount of prion infectivity is quantified by Scrapie Cell Assay. Infectivity associated with microvesicles (e.g. exosomes) is determined after fractionation of the infectious culture media on sucrose gradients. Biochemical characteristics of the different abnormal PrP forms (PrP_{res}, PK-sensitive PrP_{Sc}...) present in the infected cells, in the culture media and in the purified microvesicles are investigated in parallel.

Results: Although the majority of infectivity was found to be associated with the cells, infected cells do release prions in their extracellular milieu. PK-resistant PrP (PrP_{res}) can be easily detected in the crude conditionned media as well as in the pelletable (100,000 Xg) material. Further purification indicates that fractions containing cell-derived microvesicles are positive for PrP_{res}. Biochemical characteristics of released abnormal PrP will be presented.

Discussion: Our ongoing work with cells infected with different prion strains should provide a comprehensive picture of the cellular processes leading to secretion of prions. It should reveal if the efficiency of release varies with the prion strain and may give insights on the more infectious forms of abnormal PrP.

P8.11 A novel resistance-linked ovine PrP variant and its equivalent mouse variant modulate the in vitro cell-free conversion of rPrP to PrP_{res}

Authors
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Content

Prion diseases are associated with the conversion of the normal cellular prion protein, PrP_C, to the abnormal disease associated PrP_{Sc}. This conversion can be mimicked in vitro using the cell-free conversion assay. This assay can be modified to use bacterial recombinant PrP as a substrate and mimic the in vivo transmission characteristics of rodent scrapie. Here we demonstrate that the assay replicates the ovine polymorphism barriers of scrapie transmission. In addition, the recently identified ovine PrP variant ARL168Q, which is associated with increased survival of sheep to experimental BSE, modulates the cell-free conversion of ovine recombinant PrP to PrP_{res} by 3 different types of PrP_{Sc}, reducing conversion efficiencies to levels similar to the ovine resistance-associated ARR variant. Also, the equivalent variant in mice (L164) is resistant to conversion by 87V scrapie. Together these results suggest a significant role for this position and/or amino acid in conversion.

P8.12 Conformational stability and infectivity of protease-resistant prion protein derived from the Chandler strain

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Content

Background and Objective: Although the major component of prion is believed to be the oligomer of PrP_{Sc}, little information is available concerning regions of PrP_{Sc} molecule that affect prion infectivity. During the course of analyzing PrP_{Sc} derived from various prion strains, we found that PrP_{Sc} derived from the Chandler strain showed an unique property in the conformational stability assay, and this property appeared useful for studying a relation between regions of PrP_{Sc} molecule and prion infectivity. Thus, we analyzed PrP_{Sc} derived from the Chandler strain in detail and analyzed infectivities of the N-terminal denatured and/or truncated PK-resistant PrP.

Methods: The conformation stability assay was carried out as follows: brain homogenates were treated with 0 to 4 M GdnHCl at 37C for 1 h. After adjusting the GdnHCl concentration to 0.4 M, samples were digested with 10 μg/ml PK at 37C for 30 min. Finally, PrP_{Sc} was detected by immunobloting using a panel of anti-PrP antibodies. Bioassays were carried out using ICRslc mice.

Results and Discussion: The N-terminal region of PrP_{Sc} derived from the Chandler strain showed a region-specific stability against GdnHCl treatment. The region approximately from amino acid (aa) 80 to aa 140 began to be denatured by the treatment with 1.5 M GdnHCl. Within this, the region approximately between aa 80 and aa 90 was denatured almost completely with 2 M GdnHCl. Furthermore, the region approximately from aa 90 to aa 140 was denatured completely with 3 M GdnHCl. However, the region from about aa 140 to the C-terminus was extremely resistant to the GdnHCl treatment. This property was not observed in PrP_{Sc} derived from other prion strains. Denaturation of the region approximately from aa 80 to aa 140 by 3M GdnHCl significantly prolonged the incubation periods (207 ± 25 days) compared to untreated control (159 ± 12 days), although denaturation of the region approximately from aa 80 to around aa 90 by up to 2 M GdnHCl showed a marginal effect on the infectivity. More strikingly, denaturation and removal of the region approximately from aa 80 to around aa 140 nearly abolished the infectivity. This suggests that the conformation of the region between aa 90 to aa 140 of PrP_{Sc} molecule of the Chandler strain is tightly associated with the prion infectivity.

P8.13 Influence of prion sequence in small ruminants on conversion of the prionprotein *in vitro*

Authors
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Content

Background: The prion diseases are a group of neurodegenerative disorders that include Creutzfeldt-Jakob disease (CJD) in humans, BSE in cattle and scrapie in sheeps. According to the protein-only theory abnormally folded prion protein, PrP_{Sc}, is the main component of this infectious agent. which derives from a cellular progenitor, PrP_{C}, by a conformational change. In contrast to PrP_{C}, PrP_{Sc} is only partially digested by Proteinase K and tends to form large aggregates. The transition from PrP_{C} to PrP_{Sc} is called conversion and needs the direct interaction between both isoforms. The efficiency of this conversion is determined by prion strain characteristics and the functional similarity of the amino acid sequences of PrP_{C} and PrP_{Sc}. Polymorphisms of the allele encoding ovine prion protein at codons 136, 154 and 171 are known to influence the scrapie susceptibility in sheep. Sheep expressing the A136R154R171 allele are nearly resistant to scrapie, whereas the A136R154Q171 haplotype conveys a high susceptibility to scrapie.

Objective: Resistance of small ruminants to scrapie is associated with single polymorphisms at several positions in the prion gene. The polymorphisms even display dominant-negative effects on prion replication. These effects were evaluated with various mutants of recombinant full length prion protein in a cell-free conversion assay.

Methods: Ovine prion alleles were generated via PCR, expressed in E. coli and purified over a N-terminal His-tag using a Ni-NTA-column according to standard procedures. Cell-free conversion is carried out by mixing recombinant prion protein with PrP_{Sc} seeds in an appropriate buffer. Detection of newly converted PrP_{res} fragments is done by immunoblotting and incubation with mab P4.

Results and Discussion: A set of prion constructs was created by introducing mutations at codon 154 and 171 of the ovine prion protein and analyzed in the cell-free conversion assay. The study shows that all mutated prion variants are inconvertible by Me7 seeds and demonstrates that both polymorphic aminoacid residues determine the propensity of the ovine prion protein for conversion and replication. To investigate this dominant-negative effect at the protein level, resistant and susceptible alleles were incubated simultaneously in the cell-free conversion assay. The co-incubation resulted in a significant reduced total amount of newly generated PrP_{res} in comparison to conversion of the susceptible allele alone. In summary the results of the cell-free conversion experiments demonstrate the impact of single aminoacids on prion conversion and replication.

P8.14 Generation of new prion species by in vitro prion replication

Authors

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Content

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative disorders affecting both humans and animals. TSEs can be of genetic, sporadic or infectious origin. The infectious agent associated with TSEs, termed prion, appears to consist of a single protein which is an abnormal conformer (PrP_{Sc}) of a natural host protein (PrP_C) that propagates by converting host PrP_C into a replica of itself. One of the characteristics of prions is their ability to infect some species and not others and this phenomenon is known as the transmission barrier. In general, the transmission barrier is expressed by an incomplete attack rate and long incubation times which become shorter after serial inoculation passages. The absence of natural TSE cases and/or failed experimental transmissions has suggested that certain species could be resistant for prion diseases. Unfortunately, the molecular basis of the transmission barrier phenomenon is currently unknown and we cannot predict the degree of a species barrier simply by comparing the prion proteins from two species. We have conducted a series of experiments using the Protein Misfolding Cyclic Amplification (PMCA) technique that mimics in vitro some of the fundamental steps involved in prion replication in vivo albeit with accelerated kinetics. We have used this method to efficiently replicate a variety of prion strains from, among others, mice, hamsters, bank voles, deer, cattle, sheep and humans. The in vitro generated PrP_{res} possess key prion features, i.e. they are infectious in vivo and maintain their strain specificity. We are using the PMCA technique to generate infectious PrP_{res} from species hitherto considered to be resistant to prion disease. The correlation between in vivodata and our in vitro results suggest that PMCA is a valuable tool for studying the strength of the transmission barriers between diverse species and for evaluating the potential risks of the newly generated prion species to humans and animals.

P8.15 White matter kuru plaques in atypical sporadic Creutzfeldt-Jakob disease: study of three cases

Authors

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Content

Background: Mouse bioassays remain the gold standard for the proof of infectivity, strain typing and titre estimation in ruminant TSE research. An alternative approach employing ex vivo cell-based assays is now however a real possibility. The first tissue culture to support the propagation of PrP_{Sc} directly from ovine scrapie was developed by modifying a rabbit kidney cell line to express the ovine PrP VRQ allele (Rov9 cell line, Vilette et al 2001).

Aims and Objective: The primary aim of this project is to identify and compare cell lines directly permissive to ovine scrapie.

Methods: Rov9 cells were compared with subclones previously found to be permissive to infection with SSBP/1 and 127S scrapie as assessed by the scrapie cell assay (SCA). The PrP_C expression level and subcellular location was compared between the most permissive subclones and the original Rov9 cell line using Western blot and confocal microscopy. RK13 cells expressing four other ovine PrP alleles (ARR, AHQ, AF141RQ and AL141RQ) were also assessed. These cell lines and the Rov9 subclones were challenged with 20 field cases of scrapie from sheep with a range of PrP genotypes, and de novo infection assessed by the SCA. Finally, titre estimates were obtained for several scrapie samples, one of which has been previously titred in mice, using the most permissive subclones and the SCA.

Results: Subcloning the Rov9 cells has generated several clones that are at least 100 times more sensitive to infection with ovine scrapie. All the subclones and the parental Rov9 cells were permissive to infection with 5 out of 20 scrapie field cases tested. Of these 5, 4 were from VRQ/VRQ sheep and one was from a VRQ/ARR sheep. The level of PrP_C expression was found to be slightly elevated in the most permissive subclones and the proportion of cells expressing PrP_C was also increased. To date no infection has been seen in the cell lines expressing ovine PrP_C other than the VRQ allele. Titre estimation obtained using the most permissive cell lines compared favourably with that obtained by mouse bioassay.

Discussion: The SCA is a useful tool for screening large numbers of subclones for permissiveness to infection with scrapie. We have increased the sensitivity of the Rov9 cell line by subcloning but have not altered the subset of scrapie field cases to which the cells are permissive. This work was funded by DEFRA, UK.

P8.16 A theoretical size-structured model of prion nucleated polymerization

Authors

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Content

Background: Abnormal aggregated prion protein (PrP_{Sc}) replicates itself by a still poorly characterized autocatalytic process. Some mathematical models of prion multiplication have been developed to test which mechanisms best fit experimental data. However, in all these models of seeded polymerization, each size of aggregates (from small oligomers to large fibrils) has the same behaviour in matter of polymerization ability and pathological properties. Recent experimental analysis of relation between biological properties and size distribution of aggregates contradicts this uniform behavior of aggregates. Furthermore, bimodal distribution of the polymer size seems more likely to occur within the real process (Silveira, Nature 2005) than the unimodal one predicted by the models.

Objective: The goal of the present study is to better understand the replicative mechanism of PrP_{Sc} leading to the unexpected experimental size distribution of prion aggregates in brain, and to investigate the potential implication of this size distribution in the strain phenomenon.

Methods: We have generalized Masel and coauthors' model of prion replication to take into account aggregate size-dependent properties by considering non-constant parameters.

Results and Discussion: We show how the existing model can be adapted to recover the bimodal size distribution of PrP_{Sc} aggregates. Notably, we found that a reasonable bell-shaped extension rate induces bimodality, that is in accordance with experiments. In this non-constant framework, we mathematically investigated the influences of parameters (such as the fragmentation rate, the locus or the width of the extension rate) on the PrP_{Sc} size distribution. Our study suggests that PrP_{Sc} aggregate size distribution could be a signature of a strain in a given host and a constraint during the adaption mechanism of the species barrier overcoming, that open experimental perspectives for prion strain investigation.

P8.17 Immune responses to peptides from the C-terminal part of prion protein

Authors

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Content

Peptide-based vaccine strategies offer an attractive approach in the attempt to overcome self-tolerance. In our previous studies we obtained strong humoral responses to peptide P1 from the C-terminal part of human prion protein (PrP) bound to KLH (KLH-P1214-226). We were interested if P1 alone is able to elicit an immune response as well. In this study, immunogenicity of human- and mouse-derived prion peptides was investigated. For binding to MHC I and II, peptides of 8-10 amino acid and 12-28 amino acid residues, respectively, are optimal. In order to have 20mer peptides, human P1 and mouse P1 analog were N- and C-terminally elongated, respectively. In the experiments performed, all six peptides under investigation, i.e. human 13mer P1 (P1214-226), 20mer P1-N (P1207-226) and P1-C (P1214-233) and the corresponding mouse analogs, were used without binding to any carrier molecule. To better understand the influence of antigen structure on immune responses, two systems were set up. In particular, wild type mice were immunized with peptides and T cell proliferation and antibody production was assessed. In addition, cellular immune responses of human- and mouse-derived prion peptides was explored in a human in vitro system. These results give further insights in the structural requirement for immunogenicity of prion peptides and elucidate the involvement of T- and B-lymphocytes in an antigen-specific immune response.

P8.18 Characterisation of prion strain replication in organotypic brain slices

Authors
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Content
The occurrence of multiple prion strains in genetically identical hosts is believed to reflect conformational variability of PrP_{Sc}, a disease-associated, aggregated isoform of the cellular prion protein, PrP_C. Here we combine the prion organotypic slice culture assay (POSCA1, 2) with luminescent conjugated polymers (LCPs3) to characterize the biology of different mouse-adapted prion strains. The POSCA propagates prions to detectable levels within three weeks. LCPs possess fluorescent properties and bind specifically to b-sheet rich aggregates (e.g. PrP_{Sc}; Ab4). The flexibility of their conjugated side groups (e.g. polythiophene acetic acid (PTAA)) enables LCPs to emit different light spectra according to the structure they bind to. This allows for discrimination of different prion strains on the basis of the tertiary/quaternary structure of the associated aggregates. Our preliminary results show that diverse prion strains display (1) distinct deposition patterns in genetically homogenous organotypic cerebellar slices. Moreover, (2) aggregates can be distinguished by different morphologies, dynamics of replication and emission spectra after PTAA staining. This suggests (1) the contribution of different cell types to replication/transport and (2) strain variability at the molecular level. Additionally, prion-induced neurodegeneration may occur in a strain-dependent manner.

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P8.19 Tetracysteine tags as a tool for following prion protein conformational change

Authors
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Content
Conformational change of prion protein is intimately involved in transmissible spongiform encephalopathies. Prion protein is able to convert from the native, predominantly alpha into the beta pathological form. Conformational transition is typically monitored by fluorescent dyes that bind to amyloids, most commonly used among them is Thioflavin T. We decided to investigate the potential of inserted tetracysteine tag (CCPGCC) into the different parts of the structure of prion protein, so that we could detect conformational changes with biarsenical molecules. Biarsenical compounds such as FlAsH-EDT2 are membrane-permeant molecules based on fluorophores like fluorescein. Rigid spacing between both arsenate atoms enables molecules to bind with high affinity and specificity to the tetracysteine motif introduced into proteins. It has previously been shown that fluorescence of FlAsH in complex with protein is dependent on conformation of the protein. The aim of our study was to determine whether biarsenical reagents could be used to follow prion protein conformational change. PrP mutants with tetracysteine tag inserted at different sites were expresed in E.coli, purified and refolded on Ni-NTA columns. Only mutant in which we replaced 4 amino acids with cysteines in alfa helix 1 could not be refolded, most likely because cysteines interfered with formation of correct disulfide. We found that the presence of tetracysteine tag in correctly folded mutants did not interfere with fibrillization. FlAsH reagent could distinguish between native and fibrillized form of tetracysteine mutants of mouse recombinant prion protein, with tetracysteine tag inserted at C-terminal, N-terminal and in the loop between helixes 2 and 3. Insertion of tetracysteine tag at the suitable position could be a useful tool for monitoring prion protein conformational change. Because biarsenical molecules easily cross cell membrane and can be used on live cells, we could follow conformational change *ex vivo*.

P8.20 New strategy of prion infection to study sensitivity and permissivity of cell lines

Authors
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Content
Background: Considering the prion risk for iatrogenic transmission via medical devices linked to the distribution of PrP for variant Creutzfeldt-Jakob disease (vCJD), alternative methods compatible with thermosensitive devices have been proposed. The standard method for direct validation of new procedure has historically been rodent bioassay. We developed and adapted a protocol for the contamination of stainless steel wires adapted to different animal models infected with specific strain (scrapie strain 263K or BSE strain 6PB1). This reproducible method requires a certain number of animals and the duration generally exceeds one year. Further cell systems permissive to prion replication have been investigated in the last few years to develop sensitive and rapid cell-based assay as for the standard scrapie cell assay with potential applications of pre-screening investigations of decontamination of medical devices, the evaluation of biological products (e.g. blood) and new therapeutic compounds. However they are restricted to a very limited number experimental and mouse-adapted scrapie strains with often a range of sensitivity lower than bioassay.

Objective: To develop, optimize and validate various cell models that can sensitively and quantitatively detect various strains of prion infectivity with sensitivity at least equivalent to bioassay.

Methods: We developed and adapted a surface-cell assay based on in vivo methods: cells were exposed to the infectious samples adsorbed on various specific surfaces, including wires and polydispersed matrices. The cells were cultured and assayed in western-blot technique for the presence of prion replication. Otherwise, further adapted analysis by cytometry, cell sorting strategy and confocal microscopy have been developed to investigate prion replication at the cellular level and to better understand the cell-to-cell spreading of prions.

Results and Conclusions: Different wire cell-based assays will be presented and compared to other specific surfaces. Ongoing work is currently leading to the development of more robust and pertinent cell models of prion infections: upstream with new experimental strategies to explore the mechanisms of cellular permissivity and resistance to prion strains; downstream in validating the cell models with decontamination procedures already evaluated in bioassay.

P8.21 Generation of a new prion disease by de novo formation of PrP_{Sc} in vitro

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Content
Background: Prions are the proteinaceous infectious agents responsible for Transmissible Spongiform Encephalopathies (TSEs). Compelling evidence support the hypothesis that prions are composed exclusively by a misfolded version of the prion protein (PrP_{Sc}) that replicates in the body in the absence of nucleic acids by inducing the misfolding of the cellular prion protein (PrP_C). The most common form of prion disease is sporadic which likely originate in a low frequency event of spontaneous misfolding to generate the first PrP_{Sc} particle that then propagates as in the infectious form of the disease.

Objective: The main goal of this study was to mimic sporadic disease by attempting de novo generation of infectious PrP_{Sc} in vitro.

Methods: and **Results:** For this purpose we used a modified version of the protein misfolding cyclic amplification (PMCA) procedure and showed that PrP_{Sc} can be generated in the absence of pre-existing PrP_{Sc} in three different animal species at a variable rate. De novo generated PrP_{Sc} was infectious when inoculated to wild type hamsters producing a new disease with unique clinical, neuropathological and biochemical features.

Discussion: Our results represent a strong evidence to support the prion hypothesis and provide a simple model to study the mechanism of sporadic prion disease. The findings also suggest that the universe of possible prions is not restricted to those currently known, but that likely many new forms of infectious protein foldings may be produced, resulting in completely novel diseases.

P8.22 Endogenous N-terminal trimming of PrP^{Sc} occurs at a fairly variable level depending on the infected host cell

Authors

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Content

Cell cultures permissive to infection provide useful models in which biochemical events associated with prion replication can be studied. Rov (1) and Mov (2) are cell lines that express the ovine PrP and allow an efficient propagation of natural scrapie agent. Primary cultured cerebellar granule neurons (CGN) established from ovine PrP transgenic mice tg338 were also shown to be highly susceptible to prion infection (3).

Under protease K (PK) treatment, PrP^{Sc} is N-proximally truncated at around amino acid residue 90. This cleavage promotes the formation of the so-called C2 fragment, which corresponds to the protease-resistant core of PrP^{Sc}. The present study was aimed to examine the endogenous N-terminal processing of PrP^{Sc} in the above mentioned and other cell models, i.e. prior PK digestion. Using a combination of approaches (IMAC-Cu++ chromatography, sedimentation, proteolytic digestion and epitope mapping with a panel of anti-PrP antibodies), we showed that in both Rov and Mov cell lines infected with 127S scrapie strain, PrP^{Sc} essentially accumulated as N-terminal truncated species. In contrast, PrP^{Sc} was mainly full length in CGN primary cultures and in the brain of tg338 mice infected by 127S prion. Moreover, in CAD neuronal cells, a newly developed cell line system permissive to mouse prions (4), both full length and N-terminally truncated forms of PrP^{Sc} were found to accumulate, with a predominance of the truncated species. The proteolytic processing of PrP^{Sc} in Rov and CAD cells was reversibly inhibited by ammonium chloride and leupeptin, arguing that lysosomal proteases are involved in this event. Collectively, the above data support the view that the N-terminal trimming of PrP^{Sc} may strikingly differ depending on the cell type in which abnormally folded prion protein is formed and trafficked. They also suggest that the lysosomal pathway that is involved in the storage and clearance of PrP^{Sc} may be more marginally implicated in prion-infected post-mitotic neurons in the brain than in immortalized cells.

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P8.23 Peptide ligands that target the cooperative folding network in the prion protein stimulate PrP^{Sc} formation

Authors

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Content

Background: Despite substantial progress in understanding prion replication in vitro, the mechanisms and factors implicated in PrP^{Sc} formation in vivo are still unknown. Several pieces of evidence imply the presence of an unidentified cellular cofactor for successful prion replication. Although it is well known that PrP^{C} binds to different molecules *in vivo*, none of these molecules have been shown yet to catalyze prion replication.

Objective: Identification of domains in PrP with propensity to bind factors that alter PrP folding and rationally design peptides that target this domain and may enhance PrP misfolding in vitro.

Methods: Using a computational approach that localizes binding sites on proteins based on their ability to propagate binding energy, we have identified a potential such domain in the human prion protein (HuPrP). Considering the known tridimensional structure of PrP we designed peptide ligands that target this domain. The binding of these peptides and its effect on the conformation of recombinant HuPrP was studied by NMR. The influence of this interaction on PrP misfolding was assessed in vitro using an assay based on the conversion of recombinant PrP and the protein misfolding cyclic amplification technology.

Results: Three peptides were designed to act as an innocuous PrP binder (P1), PrP^{C} misfolding inhibitor (P2) and a promoter of PrP misfolding and prion replication (P3). All the peptides specifically bind to HuPrP at physiological conditions according to NMR binding experiments, with P2 having the highest affinity. The NMR studies showed also that the interaction of the peptides with PrP induce changes in the conformation of the protein. In vitro studies shown that P3 stimulate conversion of HuPrP into a PK-resistant fragment, reminiscent of PrP^{Sc}, whereas the other peptides had no effect. Moreover, P3 enhances seeded PrP^{Sc} formation using the standard PMCA assay in whole brain homogenate. Currently we are testing infectivity of the PrP^{Sc}-like forms induced by interaction with the P3 peptide and assessing the effect of the peptide in the development of prion disease in vivo.

Discussion: Our findings suggest that PrP^{C} specific binders can have a significant effect on PrP^{C} to PrP^{Sc} conversion, providing additional support to the idea that PrP^{Sc} formation is modulated in vivo by to date unknown specific cellular factors. The identification of PrP domains susceptible to be conformationally altered by binder partners and the generation of peptides capable to modulate PrP^{Sc} formation may contribute to understand the structural requirements of PrP^{Sc} replication.

P8.24 In vitro prion strain interference

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Content

Background: Prion diseases are fatal neurodegenerative disorders animals including humans. Prion strains can interfere with each other in vivo. For example, inoculation of hamsters with the drowsy (DY) strain of hamster-adapted transmissible mink encephalopathy (TME) prior to superinfection with the hyper (HY) strain of TME can result in the extension of HY TME incubation period or completely block HY TME from causing disease. The ability of the DY TME agent to interfere with the HY TME agent in vivo correlates with an accumulation of DY PrP_{Sc} and results in a reduction of HY PrP_{Sc}. This suggests that the two strains are either competing for a limiting host factor (e.g. PrP_C) or PrP_{Sc} from the two strains are directly interacting. An in vitro method of strain interference would facilitate characterizing the mechanism of strain interference.

Objective: In this study we used the protein misfolding cyclic amplification (PMCA) technique to investigate prion strain interference in vitro using the HY and DY TME agents.

Methods: The PMCA technique is designed to mimic some of the fundamental steps involved in PrP_{Sc} conversion in vivo. In brief, a mixture of PrP_{Sc} seed and excess PrP_C is subjected to several incubation and sonication cycles, where newly formed PrP_{Sc} catalyzes the formation of more PrP_{Sc}. We used HY or DY TME agent-infected hamster brain homogenates as the PrP_{Sc} source and uninfected hamster brain homogenate as the PrP_C source. We mixed HY TME brain homogenate with increasing amounts of DY TME brain homogenate, to determine if the DY TME agent could interfere with HY PrP_{Sc} accumulation as determined by Western Blot.

Results: PMCA using either HY or DY TME as the seed resulted in amplified PrP_{Sc} that is biochemically similar to the original PrP_{Sc} and maintain the in vivo strain replication kinetics. PMCA interference determined that HY PrP_{Sc} is present in the amplified product when DY PrP_{Sc} is in ten fold excess compared to HY PrP_{Sc}.
However, when DY PrP_{Sc} is greater than 100 fold in excess to HY PrP_{Sc}, Western blot analysis indicates the amplified product is DY PrP_{Sc}.

Conclusions: These results indicate that a greater than 100 fold excess of DY PrP_{Sc} compared to HY PrP_{Sc} can interfere with HY amplification.

P8.25 Protein Misfolding Cyclic Amplification (PMCA) using ovine and bovine species

Authors
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Content

Polymorphisms in the prion protein (host and donor PrP genotype) are known to influence TSE susceptibility and transmissibility *in vivo*. These modulating effects on the underlying molecular conversion of prion proteins can be readily assessed in vitro. The so-called VRQ allelic variant of sheep PrP is generally associated with high susceptibility to sheep scrapie. The so-called ARR allelic variant is in general considered to encode lowered susceptibility for scrapie, although this might be questioned for atypical and/or sheep derived BSE. In vitro tests assessing the (potential) species/polymorphism barriers support these findings on the molecular level and they can measure virtually every species barrier. The protein misfolding cyclic amplification (PMCA) is one of the techniques to allow the *in vitro* generation of protease-resistant prion protein (PrP_{res}) using only a small seed of disease-associated prion protein (PrP_{Sc}) to convert normal cellular prion protein (PrP_C) in brain homogenates. We used the PMCA to study species/polymorphism barriers in sheep genetic variants using natural scrapie scrapie and BSE sources but we also used materials from experimentally infected animals (like BSE passages in sheep and scrapie in ARR/ARR sheep). The results largely confirmed the observed specificities using Gdn based conversion systems. Some new data on assessed species/polymorphism barriers will be presented. One of the "problems" using PMCA is the need for negative brain homogenates as PrP_C source preferentially from PrP homozygote genotypes. Some of these negative animals are hard or unable to get and not all variants are available in transgenic mice. In addition, measuring species barriers to humans would require negative human brains which are very restrictive. Here we present our efforts to replace brain homogenates as a source of PrP_C by alternatives like cell lines expressing different PrP variants/genotypes. In addition we will present a selection of efforts in optimizing the *in vitro* amplification of prions.

P8.26 In Vitro Strain Adaptation Of CWD Prions Using Serial Protein Misfolding Cyclic Amplification

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Content

Background: There are numerous reports supporting evidence of in vitro generation of infectious hamster prions using serial protein misfolding cyclic amplification (sPMCA) and the use of sPMCA to generate PrP_{res} from mouse-adapted scrapie (PrP_{Sc}) and CWD prions (PrPCWD) but the infectivity has never been shown. Inoculation of animals with varying strains has been used to show efficiency of transmission into new hosts as well as strain adaptation. Conformational stability assays, among other biochemical and biophysical prion property assays, have been developed to investigate transmissible structural differences among different prion strains.

Objective: To assess the infectivity of *de novo* PrPCWD generated by sPMCA upon inoculation and to investigate host adaptation and strain differences between amplified (sPMCA) D10, mouse-passaged D10 (CWD strain), and serial mouse-passaged D10.

Methods: Generate *de novo* PrPCWD using serial protein misfolding cyclic amplification (sPMCA). Inoculate amplified material and passage D10 (CWD strain) into transgenic 1536 mice (Tg(cerPrP)1536) and evaluate strain adaptation of all material using a CSA.

Results: Using serial protein misfolding cyclic amplification (sPMCA), we observed linear amplification over two logs of D10 dilutions after amplification of the D10 strain of CWD prions. We generated *de novo* PrPCWD using PMCA and report that sPMCA sustained CWD prion amplification and was infectious upon inoculation into Tg(cerPrP)1536 mice. We report that inoculation of amplified D10 or serial-passaged D10 caused terminal disease 80 days earlier than the original D10 strain inoculum. We further assessed strain differences using a conformational stability assay and found that amplified D10 and serial-passaged D10 were significantly different than that of the original D10 strain. We characterized sPMCA using a 1:1000 D10 strain dilution into Tg(cerPrP)1536 mouse normal brain homogenate (NBH) substrate at each round for seven rounds with a conformational stability assay.

Discussion: These findings suggest that sPMCA can adapt and amplify prion strains in vitro more rapidly and as proficiently than by mouse-passaged strain adaptation *in vivo*.

P8.27 Strain-specific role of RNAs in prion in vitro conversion

Authors
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Content

The prevalent hypothesis in prion diseases proposes that the infectious agent is mainly or exclusively composed of a misfolded version of the cellular prion protein. Several lines of evidence suggest that other co-factors are also required for prion replication. PrP binds to polyanions such as RNAs and glycosaminoglycans and recently RNAs were shown to promote and induce the conversion of PrP_C into PrP_{res} in vitro. In the present study, we used the serial automated Protein Misfolding Cyclic Amplification (saPMCA) to investigate in more detail the role of RNAs in PrP_C to PrP_{res} conversion.
We found that RNAse treatment inhibits converting activity of two scrapie strains, 22L and ME7, while it has little if any effect in the case of the RML strain. These data suggest a strain-specific role for RNAs in TSE agent propagation, and maybe also in the infection process, which is currently under investigation using the Scrapie Cell Assay (SCA).

P8.28 Prion infected cells sorting to develop robust cell models infectable by various prion strains

Authors

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Content

Background: Considering the iatrogenic risk linked to the variant Creutzfeldt-Jakob disease (vCJD), the need of a cellular model for vCJD is critical. Currently, only a limited number of cell models infected by certain prion strains have been described and there is no cell line durably infectable by human Prions. Cellular susceptibility to specific Prion strains remains largely unknown and no marker of this susceptibility has been identified. However, by using infected cell lines, we have previously identified modification of immunoreactivity of specific membrane proteins, linked to the infection.

Objectives: Based on these preliminary results, the first aim of this study is to evaluate cells sorting strategy to purify infected cells from permissive global cell population after infection with different strains of Prion. The second aim is to adapt cell sorting strategy to improve the unique cell system reported so far as infectable by rodent-adapted BSE agent. The third aim is to test adapted cell sorting strategy to several human cell lines exposed to vCJD agent.

Methods: SN56 and GT1 cell lines as susceptible standardized models, and transgenic RK13 cell line which expressed Bank-vole PrP as susceptible model of BSE bank-vole strain have been used. Different human cell lines derived from several embryologic origins have been exposed to vCJD strain. Specific cell sorting strategy has been developed with Alliance Biosecure Fundation Influx cell sorter. Phenotypic characterizations of cell sorted have been studied by biochemistry, transcriptomic and confocal microscopy approaches.

Results and Discussion: Our cells sorting strategy has allowed us to purify murine infected cells from global cell population after infection. Therefore, we identified populations in cell cultures, such as cells that replicate Prion at different levels. These differences were maintained during many cell passages. Thus, we demonstrated the presence of a balance between infected and uninfected clones in permissive cell lines. Consequently, our cell sorting approach allowed us to enrich the susceptible cell subpopulations. Phenotypic and transcriptomic characterisations of subpopulations in murine cellular models are currently under progress. Moreover, adaptation of our cell sorting strategy to RK13 cell line infected by BSE bank vole strain and to human cell lines with human Prion strains are currently ongoing. This original approach of infected cell sorting should provide a new tool to investigate Prion replication and Prion susceptibility at the cellular level.

P8.29 The kinetic of prion accumulation in splenic cell populations of mice

Authors

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Content

In most transmissible spongiform encephalopathies (TSE) prions accumulate in the lymphoreticular system (LRS) long before they are detectable in the central nervous system. A good body of evidence shows that hematopoietic and stromal cells play fundamental roles for the spread of prions in the LRS and may be a prerequisite for neuroinvasion. The contribution of different cell types to the accumulation and spread of prions in the LRS, however, is not well understood. A comprehensive study to compare prion titres in candidate cells has not been performed to date due to prohibitively large numbers of animals required. We now utilised the Scrapie cell assay (SCA), an in-vitro infectivity assay to quantify the rate of prion propagation in representative splenic cell populations from 129 Sv x C57BL/6 mice in a time interval from 7 to 60 days post inoculation (dpi). Lymphocytes (B and T cells) and antigen-presenting cells, i.e. dendritic cells (DC) and macrophages (M$)) were isolated from splenocytes by sequential magnetic sorting (MACS separation). Cell homogenates were then prepared for each cell type with a mechanical homogeniser (Ribolyzer) in presence of protease inhibitors followed by the determination of prion titres in prion-susceptible cells. The sensitivity of the assay was assessed with serial dilutions of titred RML brain homogenate (9.3 log LD50 units/g brain). A 10-9 dilution of RML resulted in 7 positive wells out of 24, corresponding to a sensitivity of at least 2 LD50 unit equivalents or Tissue culture infectious units (TCIU)/ml. Infectious titres at time points as early as 7 dpi were similar for all cell types studied (20 to 45 tissue culture infectious units (TCIU)/Mio cells) and increased about 20-fold in DC and M$ by 60 dpi. In comparison a 10 fold increase in infectious titres was observed in lymphocytes (B and T cells). Notably, in prion protein knockout mice (*PRNP*o/o), i.e. in absence of prion replication, prion titres of about 1 TCIU/Mio cells were detected in DC and M$ after 3 dpi, whereas infectivity was not detectable in B and T cells, indicating that antigen-presenting cells may have an important role in the transport or sequestration of the infectious agent

P8.30 Characterization of RML prions

Authors

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Content

The speed and convenience of the scrapie cell assay has allowed us to assess a range of conditions that influence the infectivity of RML prions in 10% brain homogenates. We have assessed (1) the effects of prolonged incubation at varying temperatures, (2) the distribution of infective material between pellet and supernatant after centrifugation, (3) the potential increase in infective titre by sonication treatments, either through fragmentation and/or PrP_C-recruitment, (4) the effect of freeze-thaw procedures, (5) the impact of high concentrations of reducing agents and (6) the effect of phospholipid additives on the efficiency of infection. Importantly, we have also elucidated the relationship between the duration of exposure of cells to the RML agent and the extent of infection, i.e. the time required for transmission of the prion agent. We find that RML infectivity is stable to prolonged incubation and to freeze-thaw procedures, but is increased significantly by interaction with cationic detergents. Surprisingly, it is unaffected by concentrations of DTT that would reduce the disulphide bridge in the PrP polypeptide chain. Exposure of cells to RML prions for 1, 6, 16 and 72 hours all led to the same level of infection of cells demonstrating that the process is rapid.

P9.01 Physiological expression of a cellular prion receptor on simian blood cells as detected by FACS analysis and quantitative PCR: laminin receptor 1 (LamR-1)

Authors
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Content
The LamR-1 (67 kDa laminin receptor and its 37kDa precursor molecule) mediates extravasation of leukocytes, acts as a cellular receptor for prions and a number of viruses, and enhances the metastatic potential of tumour cells. Here, we examined the physiological LamR-1 expression in a non-human primate model for variant Creutzfeldt-Jakob disease, cynomolgus monkey (Macaca fascicularis), since its role in this animal model is so far completely unknown. Blood samples were collected from non-infected female cynomolgus monkeys over several years (6 – 10 years of age) and were analyzed for LamR-1 expression by multi-colour FACS analysis and mRNA expression by quantitative PCR (qPCR). Real-time PCR was performed using three different endogenous controls (Beta-2 microglobulin, Glucose-6-phosphate dehydrogenase and TAT-binding protein) and the comparative Ct method (ddCT) for relative quantification using an ABI PRISM 7900. Lymphocyte blood cell subsets were examined by four-colour FACS analyses using a commercially available monoclonal antibody (clone 3F252, US Biologicals) directed against human LamR-1. Absolute counts were determined by a two-platform method. Fourteen per cent (460 PBLs/µl blood, median) of peripheral blood lymphocytes were found to be LamR-1-positive (range: 1 – 40 %, 10 – 2000 PBLs/µl blood). The highest values of up to 40% were found among non-healthy animals, for instance suffering from severe inflammations. All three main lymphocyte subsets (T-, B- and NK-cells) expressed LamR-1. Seasonal variations were not observed, but an age-dependent decrease occurred in both relative and absolute numbers. mRNA levels were inversely correlated with absolute LamR-1+ cell counts indicating a regulatory feed-back mechanism. We established two reliable routine protocols to quantify LamR-1 expression in simian cells and will apply these techniques to examine its role in a simian variant Creutzfeldt-Jakob-disease animal model.

P9.02 Alteration in retinal function and morphology in cattle and sheep with TSEs

Authors
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Content
Background: The retina, a highly organized rostral extension of the brain with well-characterized cellular phenotypes, is well suited for studying alterations of cellular morphology and synaptic connectivity in the CNS. Abnormal prion protein (PrP_{Sc}) accumulates in the retina of natural and non-natural hosts affected with prion diseases, but the effect of its accumulation has not been well characterized.

Objective: We used immunohistochemistry and electroretinography to investigate the effect of PrP_{Sc} accumulation on the morphology and function of retinal neurons in a sheep with scrapie and cattle inoculated with transmissible mink encephalopathy (TME).

Methods: To assess the effects of PrP_{Sc} accumulation on retinal morphology we used antibodies directed against proteins expressed in retinal ganglion cells (MAP-2ab), rod bipolar cells (PKC-alpha, VGLUT1), Müller glia (glutamine synthetase), and activated Müller glia (GFAP). We used electroretinography to assess retinal function in animals inoculated with scrapie (sheep) and transmissible mink encephalopathy (cattle).

Results: Our results demonstrate that PrP_{Sc} accumulation affects retinal cellular morphology in both sheep and cattle affected with TSEs. Further we report significant changes in electroretinograms of both clinically affected (sheep, cattle) and preclinical (cattle) animals.

Discussion: Our results demonstrate the value of the retina as a tissue for better understanding the pathogenesis of TSEs and a target tissue for the development of new diagnostic approaches.

P9.03 Human platelets as a substrate source for the *in vitro* amplification of the abnormal prion protein (PrP_{Sc}) associated with variant Creutzfeldt-Jakob disease

Authors
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Content
Background: Four recent cases of transfusion related transmission of variant Creutzfeldt-Jakob disease (vCJD) infectivity highlight the need to develop a highly sensitive and specific screening test to detect infectivity in the blood of asymptomatic infected individuals. Protein misfolding cyclic amplification (PMCA), a method for the amplification of minute amounts of disease associated abnormal prion protein (PrP_{Sc}) to readily detectable levels, could be incoporated into such a test provided a suitable substrate source for routine use in human PMCA reactions can be found.

Objective: To evaluate the use of human platelets as a substrate source in human PMCA reactions.

Methods: We have evaluated human platelets as a PMCA substrate using brain tissue from individuals with vCJD and sporadic CJD (sCJD) subtypes MV2 and VV2 as the seed source. The effects of seed/substrate prion protein gene (*PRNP*) codon 129 genotype compatibility on amplification, freeze/thaw on a substrate[11]s ability to support amplification and the degree of amplification acheived by serial PMCA (sPMCA) were investigated.

Results: Seed/substrate *PRNP* codon 129 compatibility had a major influence on PrP_{Sc} amplification efficiency. Individual substrates, of the same *PRNP* codon 129, could be pooled and stored frozen for use in subsequent PMCA reactions. A consistent 10-fold increase in PrP_{Sc} detection sensitivity was acheived following each round of sPMCA, resulting in a 10,000-fold increase in detection sensitivity after four rounds with no evidence of de novo PrP_{Sc} production detected in the unseeded substrate.

Discussion: Providing issues of seed/substrate *PRNP* codon 129 compatibility are addressed human platelets are a good, readily available, renewable substrate source for use in human PMCA experiments. The need to carry out multiple rounds of sPMCA to acheive maximum PrP_{Sc} amplification means that PMCA is more suited for use in a confirmatory test rather than a rapid high-throughput screening assay.

P9.04 IMPY and CLINDE as imaging probes candidates for the detection of amyloid deposits and Benzodiazepine receptors in prion diseases

Authors

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Content

Background: Imaging abnormal protein deposits and inflammatory reactions is a potential tool for studying the development of experimentally induced neuropathologies. It also represents a promising approach for the ante-mortem diagnostic of these diseases. Co-localisation of chronic inflammatory responses and amyloid deposits have been reported in Alzheimer disease (AD) and Transmissible spongiform encephalopathies (Eikelenboom et al., 2002).

Objective: In this study, we examined the potentials of two ligands as imaging probes: the rouge congo derivative b-amyloid ligand IMPY (6-iodo-2-(4-dimetylamino-) phenyl-imidazo [1,2-a]pyridine) and CLINDE, a peripheral benzodiazepine receptor (PBR) ligand.

Methods: Labelling by tested probes of prion deposits and activated microglia were investigated at the terminal phase of the disease (160 days dpi) in brain samples from C57BL/6J mice intracerebrally infected with Scrapie strain C506M3. The binding to amyloid deposits and activated microglia were evaluated by intravenous injection, 1 hour prior sacrifices, with [125I]IMPY or [125I]CLINDE. Labelled areas were detected through direct autoradiography of 20 µm thickness frozen sections of brains from infected mice or negative controls.

Results: PrP_{Sc} deposits and IMPY targeting signals were detected in the thalamus, hippocampus and cortex of scrapie infected mice as compared to negative controls. However, a high background level of the IMPY labelling assay was observed. The same brain areas were targeted by CLINDE in scrapie infected mice with a higher signal intensity and a lower background. This was confirmed by the quantitative measurement of radioactivity (Bq/mg) in section areas, which indicated statistically significant differences between infected and control samples.

Conclusion: Quality of the CLINDE signal and its co-localization with PrP_{Sc} in the same mouse brain areas suggest that it could represent an interesting candidate for the targeting of lesions associated to prion diseases using in vivo imaging techniques.

Acknowledgements: This study was supported by grants from Association France Alzheimer and European DiMI.

P9.05 The role of CSF proteins as early diagnostic markers for sporadic CJD

Authors

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Content

Background: Sporadic CJD (sCJD) can be difficult to diagnose in the early stages. A number of reports have suggested that in the early stages of sCJD CSF 14-3-3 may not be detectable, and that CSF tau protein may be more sensitive than CSF 14-3-3.

Objective: In this study we compared the diagnostic value of CSF protein 14-3-3, s100b and tau in the early stages of sCJD (defined as being within 6 weeks of symptom onset).

Methods: 3 groups of patients were compared: patients with probable or neuropathologically confirmed sCJD with CSF taken within 6 weeks of onset (""sCJD<6 week"" group; n = 47); patients with CSF taken within 6 weeks of disease onset but with a diagnosis other than CJD (""non-CJD <6 week"" group; n = 21); patients with neuropathologically proven sCJD where CSF was taken later than 6 weeks after onset (""sCJD > 6 week"" group; n = 216). The sensitivity and specificity of different combinations of neuronal proteins was ascertained. We reviewed the codon 129 status and PrP type of patients in the ""sCJD<6 week"" and ""sCJD > 6 week"" groups.

Results: The sensitivities of all 3 markers were similar and ranged from 96 to 98%. The sensitivity of these markers was greater in the ""sCJD < 6 week group"" than in the ""sCJD > 6 week group"". Tau protein had the greatest specificity (82%). S-100b had a high sensitivity but low specificity. Combining S-100b with either an elevated CSF tau protein or a positive CSF 14-3-3 did not improve sensitivity. Combining CSF S-100b with 14-3-3 did improve specificity. 1 patient in the ""sCJD < 6 week"" group was homozygous for valine at codon 129. The remaining 30 patients were all homozygous for methionine. All patients in this group tested showed PrP type 1 (n=21). In the ""sCJD > 6 week group"" there was greater heterogeneity in codon 129 distribution (56% of patients being MM, 27% MV and 17% VV) and prion protein isotype (50% type 1, 37% type 2, 13% types 1 & 2).

Discussion: CSF protein markers are highly sensitive in the early stages of sCJD, with tau having the greatest specificity and efficiency. Combining 14-3-3 and S100b gave a specificity which is similar to that of tau. Our findings indicate that CSF protein markers are effective tests in the early stages of sCJD. This is likely to be related to the fact that most patients presenting within 6 weeks of disease onset were of the MM1 subtype.

P9.06 Experimental Blood Test of Scrapie-Infected Hamster

Authors

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Content

Background: Transmissible spongiform encephalopathy (TSE) is a transmissible neurodegenerative disease. Expansion of diseases by secondary infection through several hypothetical routes is of significant concern. Appearance of victims by secondary infection of vCJD through transfusion in the UK indicated the real presence of these hypothetical routes. As a result, expansion of vCJD through blood among the general population became an actual concern. The lack of presymptomatic test means that when and to what extent infection and expansion of TSE including vCJD among the population will occur cannot be presumed.

Materials and Methods: Eight week old male hamsters were inoculated i.c. by scrapie infected hamster brain homogenate (sc-). Hamsters of the same age were inoculated with uninfected healthy hamster brain homogenate and referred to as mock infected (mc-) animals. Once per every two weeks blood was collected through post-orbital sinus with ACD containing EDTA as the anti-coagulant. Collected blood was separated into plasma and cellular fractions. Cellular fractions were washed with 0.25M NaCl. Prepared plasma and cellular fractions were processed to PK treatment (50 µg/ml) and inactivated by boiling with 3% SDS and 50 mM DTT. These preparations were served to acidic SDS precipitation method to detect the PrP_{res} using 3F4.

Results: 3F4-reactive proteins were observed as early as 2 to 6 weeks after the scrapie inoculation in the first trial. As Mw of these 3F4-reactive proteins were relatively low, most of them seemed to be degraded partial molecules. In the secondary trial, no signal of 3F4-reactive proteins was observed in either plasma or 0.25M NaCl wash.

Discussion: Acidic SDS precipitation with highly sensitive chemiluminescence detection method used here has the potential to become the presymptomatic blood test of TSE-infected individuals because 3F4-reactive proteins were observed as early as 2 to 6 weeks in the first trial. However, the two trials did not show equal results. Consequently, it was suggested that the method must be modified in crucial points including the amount of test specimens.

Acknowledgments: We would like to thank Dr. Yukita Sato at the Biomedical Research Center of Nihon University and Ms. Ryoko Toyoshima and Ms. Juri Kono, graduate students at Nihon University, for their skilful technical assistance.

P9.07 Amyloid b-Structure analysis by Laser-Raman and infrared spectroscopy

Authors

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Content

Background: Transmissible spongiform encephalopathies (TSEs), are a group of deadly neurodegenerative diseases caused by a family of unusual pathogenic prion proteins. Conformational changes of the cellular prion protein (PrP) play a key role in the pathogenesis of TSEs or prion diseases and are widely considered to be fundamentally involved in their etiology. A major goal for both medical and veterinary research is the development of a rapid, in vivo, highly sensitive test that could identify a TSE during the preclinical period of the disease [1]. The presence of proteinase-resistant prion protein, PrP_{Sc}, appears to be an accurate marker of infectivity [2]. The tests for PrP_{Sc} detection developed up to date have been widely shown to have high sensitivity in the central nervous system and peripheral lymphoid tissue, but seem not to be efficient in blood [3].

Objective: Routine tests reveal unknown strains of prions, and therefore researchers remain concerned that if prion variants continue to emerge as they have, some of them may escape detection by existing tests. Consequently, new tests that do not involve antibodies are desirable.

Methods and Results: Raman and infrared spectroscopies have proven here to be suitable techniques to analyse a key membranous fraction of blood containing PrP_{Sc}. In this fraction, a significant increase in b-sheets has been correlated with the TSE worsening in naturally scrapie-infected animals in comparison with healthy controls. It should be noted that, since b-sheets as predominant structure is a common feature in all PrP_{Sc}, Raman and infrared spectroscopies solve the inconvenience of unknown prion strains escaping detection by current immunological tests. Some examples are shown here in relation to detection of b-sheet structure in TSEs and in brain structural changes resulting from oxidative stress generated by the administration of certain drugs.

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P9.08 The Mini-Mental State Examination (MMSE) in sporadic Creutzfeldt-Jakob disease

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Content

Background: Creutzfeldt-Jakob disease (sCJD) is a fatal neurodegenerative disease with rapid progression. According to diagnostic criteria, dementia is an essential symptom for diagnosis (besides a broad range of neurological symptoms). The codon 129 polymorphism of the *PRNP* gene (genotypes either MM, MV or VV) is known to influence the clinical presentation of sCJD.

Objective: To evaluate MMSE scores in patients with sporadic CJD at the point of clinical diagnosis in respective to their codon 129 genotype.

Methods: Mini-Mental State Examination (MMSE) as a well-established test to detect dementia (maximum 30 points, 25-27 points mild cognitive impairment, <25 points suspect of dementia). All patients have known genotype and were diagnosed as probable sCJD according to established diagnostic criteria or by neuropathological confirmation (until now 7 out of 25).

Results: According to MMSE dementia was found in 20 out of 25 patients, mid cognitive impairment was found in 3 patients and no dementia was found in 2 patients. In respective to the *PRNP* codon 129 polymorphism the median MMSE-scores were 16 in MM, 22 in MV and 20 in VV. All patients showing normal MMSE-score at the point of clinical diagnosis were homozygote for valine but developed dementia during course of the disease. Additionally, these patients showed highly suggestive findings in CSF and MRI. The median duration between onset of symptoms and diagnosis was 90 days in MM, 201 days in MV and 122 days in VV.

Discussion: Although dementia is essential for the diagnosis of sCJD, atypical courses without detectable dementia are known. These patients correspond mainly to abnormal presenting subtypes MV and VV. Nevertheless, in patients with rapid decline CJD should be taken into account although dementia might not (yet) be present. Thus, diagnostic criteria are less valuable in early stages and atypical genotypes. Additional diagnostic tests such as CSF, MRI or EEG could support the diagnosis in these cases.

P9.09 Establishment of Standardization of 14-3-3 protein assay as a Diagnostic Tool in Creutzfeldt-Jakob disease patients' CSF

Authors
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Content

Background: Both of PSD observed on an EEG and the presence of 14-3-3 protein in the CSF, are included in the diagnostic criteria for Creutzfeldt-Jakob disease (CJD)supplied by the WHO. But, the judgment of 14-3-3 protein of CSF is occasionally obscure.

Objective: So, we tried to establish the standardization method of 14-3-3 protein assay and clarify the cut-off data of 14-3-3 protein in Western blots method. We searched for the most suitable isoform of 14-3-3 protein in 14-3-3 protein assays, and the most sensitive antibody among four antibodies of 14-3-3 protein assay kits. Therefore, we established the standardization method of 14-3-3 protein assay and the clarification of the cut-off data of 14-3-3 protein in semi-quantitative Western blots method.

Subjects and Methods: We checked all isoform of 14-3-3 protein among 112 CJD patients and 98 patients with other disease (AD, CVD, Pick's disease, Parkinson''s disease, CBD, Huntington disease, Wernicke encephalopathy, limbic encephalopathy, PCD/LEMS, MELAS and ALS). We compare for the characterization of four antibodies (Sc-639, 18641, 18647, K0203-3 antibodies). We performed the semi-quantitative analysis of gamma-isoform of 14-3-3 protein in Western blot methods by LAS 3000 system that was capable of producing a digital image from the luminescence.

Results and Discussion: We clarified that the best isoform was the gamma-isoform of 14-3-3 protein in Western blot methods and the most suitable for the semi-quantitative analysis of 14-3-3 protein. We showed that the repeatability of the detection of 14-3-3 protein used by the antibody of gamma-isoform of 14-3-3 protein had high quality, and our system was able to show the semi-quantitative analysis of Western blots method.

Our method was the utilization of the results to previous data, and our method was covered with the shortcoming of the previous research.

P9.10 The useful application of rapid diagnostic screening system of heart-type fatty acid binding protein in CSF of CJD patients as a quick bed-side diagnostic tool

Authors
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Content

Background: Prion diseases are neurodegenerative diseases which include Creutzfeldt-Jakob disease (CJD) in humans. Recently, some drugs have been reported as anti-prion agents and proposed as immediately applicable treatment for CJD. Therefore, it is very important to diagnose the patient as CJD accurately and quickly. But, it takes one or two week to check the assays of 14-3-3 protein of CSF or tau protein.

Objective: We seek for new diagnostic marker of CJD patients as accurately and quickly as possible.

Subjects and Methods: To compare CJD patients with healthy subjects by 2D-PAGE, we identified new biochemical markers as heart-type fatty acid binding protein (H-FABP). We evaluated ELISA of H-FABP and the accuracy of Rapicheck® H-FABP of CSF for the patients with rapid dementia or sub-acute cerebellular ataxia (n = 199). And the brain section in CJD patients was performed in the immunostaining of H-FABP.

Results and Discussion: The patients with rapid dementia or sub-acute cerebellular ataxia (n = 199) were divided into CJD patients (n=112), and Alzheimer's disease (n = 54), CVD (n = 7), Pick's disease (n = 1), Parkinson's disease(n = 5), corticobasal degeneration (n = 2), Huntington's disease (n = 1), fronto-temporal dementia (n = 1), PSP (n = 3), Wernicke's encephalopathy (n = 2), limbic encephalopathy (n = 3), and amyotrophic lateral sclerosis (n = 3). Rapicheck H-FABP is based on the technology of one-step immunochromatography, and Rapicheck® H-FABP was able to check the results within 15min. We analyzed H-FABP of CJD's CSF by ELISA method of H-FABP and Rapicheck® H-FABP. The sensitivity and specificity was 80.36% and 73.2%.

We identified positive staining in the vascular endothelial cell, the neurons in cerebral cortex and the astrocyte.

Conclusion: Rapicheck® H-FABP of CSF was usefulness to diagnose patients as CJD quickly, and in the future this kit was used frequently.

P9.11 Kinetics of plasma, plasma fractions and white blood cells infectivity in mice orally exposed to scrapie.

Authors
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Content

Background: Blood infectivity has been evidenced in various TSE models. The support of this infectivity and its duration are still controversial.

Objective: The infectious status of plasma, plasma fractions and blood leucocytes was evaluated during the presymptomatic and clinical phases of mice orally inoculated with a short incubation Scrapie strain.

Methods: Transgenic mice overexpressing the VRQ allele of the sheep *PRNP* gene (Tg338) were orally exposed through a food pellet soaked with 5 mg of mouse brain homogenate infected with the rapid strain PG127S. Five groups of 15 mice were killed sequentially and mesenteric lymph nodes (MLN), spleen, brain and blood were sampled. PrP_{Sc} tissue content was investigated using ELISA, WB and IHC. Concentrated plasma was fractionated with sucrose gradient sedimentation and fractions were evaluated for their immunoglobulin (M, G1 and G2) and PrP contents. Infectivity of white blood cells, plasma, and plasma fractions was tested at each time point by mouse bioassay.

Results: About 30 to 50 % of mice showed detectable quantity of PrP_{Sc} in their MLN (from day 33), spleen (from d75) and brain (from d105). Pooled white blood cells were highly infectious during the presymptomatic phase, with a peak at mid incubation time on d75 (6/6, 77d+10). Plasma infectivity was also detected from d33 to d141 with a maximum on d75 (6/6, 119d+18), and was shown to be strongly linked to the detection of PrP_{Sc} in spleen and MLN. Although all the plasmatic PrP was found in the top fractions of a 5-40% step sucrose gradient, infectivity was evidenced both in the PrP and IgM fractions, but was absent in high molecular weight fractions.

Discussion: This model of oral exposition in mice expressing the sheep *PRNP* gene confirms the infectivity of blood cells and plasma during the preclinical period. The methodology will be adapted to sheep infected with Scrapie and/or BSE in order to detect the biochemical support of plasma infectivity at early stages of the infectious process.

P9.12 Subtype-specific pattern of 14-3-3 isoforms in sporadic Creutzfeldt-Jakob disease

Authors
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Content
Background: Molecular disease subtypes determine the value of diagnostic tests, age at onset and prognosis in sporadic CJD. These subtypes are defined by a combination of polymorphism at codon 129 (MM, MV, VV) and prionprotein type (1 or 2) resulting in six subtypes. Clinical CJD diagnosis is highly supported by detection of proteins 14-3-3 in CSF with sensitivity of more than 90%, however levels of 14-3-3 vary across subtypes (Sanchez-Juan et al. 2007, Gmitterova et al. 2008). We aimed to demonstrate, if these differences might be explained by distinct 14-3-3 isoform pattern in various molecular disease subtypes.

Objective: To evaluate the pattern of 14-3-3 isoforms in different subtypes of sporadic CJD.

Results: Dot Blots show high specifity for isoform specific antibodies (IBL). Five isoforms (gamma, eta, epsilon, zeta, sigma) are similarly expressed in all subtypes with minor changes in intensity. 14-3-3-beta is detectable in CSF in all subtypes except VV2. Heterogeneous results were found for 14-3-3-tau: it is mainly detectable in MM1 patients whereas not in MV1, MV2 and VV1 subtype. In MM2 and VV2 it is detectable in about half of the patients investigated.

Discussion: The role of 14-3-3 is assumed as marker of neuronal destruction but also involvement in disease-specific pathways cannot be ruled out. 14-3-3 isoforms are variably detectable in sporadic CJD subtypes. Especially for 14-3-3 tau a very heterogenous pattern within all subtypes was found. Isoforms eta and sigma are not detectable in CSF of our sCJD patients. The varying pattern of 14-3-3 isoforms within the subtypes of CJD might reflect different pathogenetic processes or locations. Further studies will evaluate whether a subtype-dependent allocation of 14-3-3 isoforms in CJD is present and how it is part of the pathogenetic processes.

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P9.13 Differential immunoprecipitation: new method for separate molecular characterization of multiple PrP_{res} pattern isolates

Authors
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Content
The specific biochemical marker of transmissible spongiform encephalopathies (TSEs) detected by Western Blot analysis (WB) is a partial protease-resistant fragment (PrP_{res}) of the disease-associated prion protein (PrP_d). Molecular characterization of PrP_{res} is an important criteria for classification of TSEs (Parchi et al., 1999). PrP_{res} typically exhibits a typical 3-band pattern between 30-18 kDa representing bi, mono and nonglycosylated forms of the protein. Recently, some TSEs in ruminants were described that showed a more complex biochemical signature, such as Nor98-like isolates in sheep or goats (Arsac et al., 2007) and atypical H-type BSE in cattle (Biacabe et al., 2007). In this study, H-type BSE which showed two different cleavage products of PrP_{res} named PrP_{res} #1 and PrP_{res} #2 is analysed. The first fragments family consists on a protein of 19 kDa (for the nonglycosylated band) which is labelled by all mAbs raised against sequences of the bovine PrP protein comprised between AA 62 and 236. On the other hand, PrP_{res} #2 presents a molecular weight of 14 kDa in its unglycosylated form and is labelled with mAbs that recognize the sequences of bovine PrP protein comprised between AA 175 and 236. This second family consists on an unusually described C-terminally cleaved PrP_{res} fragment. These differences of immuno-labelling between the two forms are used in our DIp method. DIp used superparamagnetic polystyrene beads coated with different mAbs to enrich the samples in the C-terminally cleaved form of PrP_{res} (PrP_{res} #2), by depletion of the other form of PrP_{res} (PrP_{res} #1) with N-terminal antibodies. Beads coated with a C-terminal mAb then permit to specifically capture PrP_{res} #2. This allows to characterize separately the two forms of PrP_{res} in atypical H type of BSE, and this method could further be applied to other TSEs showing complex molecular features.

P9.14 PRP_d immunorreactivity in the cerebellum of atypical scrapie cases

Authors
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Content
Background: Scrapie is a Transmissible Spongiform Encephalopathies (TSE) in small ruminants where a new variant, Nor 98, of Scrapie cases with atypical features were identified (Benestad et al 2003). Further similar cases have been reported in several European countries as Germany, Sweden, Ireland and Portugal. (Buschmann et al. 2004, De Bosschere et al. 2004, Gavier-Widen et al. 2004, Lühken et al. 2004, Madec et al. 2004, Onnasch et al. 2004, Orge et al. 2004). In classical Scrapie the typical target areas obex and cerebellum shows different lesion profile and the dorsal motor nucleus of the vagus nerve (DMNV) is always affected, in comparison with atypical cases where the nucleus of the spinal tract of the trigeminal nerve (SNT) and cerebellum displays conspicuous immunoreactivity. In addition, the PrP gene polymorphisms of the *PRNP* gene influence on their susceptibility to Scrapie, while atypical Scrapie cases tend to be detected in sheep carrying the ARR or AHQ alleles, classical cases used to be detected in those cases with the ARQ and VRQ allele.

Objective: Since 2001 the Spanish National Reference Laboratory carried out a the TSE Surveillance Program according to the UE rules (R. CE 999/2001), and more than 80 atypical cases have been identified by discriminatory and confirmatory methods. The aim of this study is to analyse the distribution of PrP_d by immunohistochemistry in those cases where cerebellum fixed-tissue were available (n=23) in spanish atypical cases and the genotype frequency of these isolates.

Methods: Twenty-six cerebellum of atypical cases were fixed in formalin 10%. Paraffin-embedded sections were cut at 4µm; histology (H&E) and immuno-histochemicals (IHQ) techniques were performed using 2G11 (Institut Pourquier) and P4 (r-biopharm) antibodies. The sections were pre-treated with formic acid and hydrated autoclaved.
Sample Processing: Blood was collected in EDTA-treated vacutainer alicuoting tubes. DNA extraction, PCR and Microsequencing were performed by Genesis Platforms and multipipettors from Tecan. SNaPshot: Specific designed primers for classical mutations at codons 136, 154 and 171. Two new primers were designed to improve detection of Lys-171 polymorphism. SNaPshot reagents and reactions analysed by AbiPrism 3730xl sequencers were from AppliedBiosystems.

Results: PrP_d immunorreactivity at cerebellum were anañyzed in 23 cases. The classical histological changes, neuropil and intra-neuronal vacuolization were not observed, while a fine-puntacte immunolabelling in the trigeminal area could be detected in 8 cases. According to the of PrP_d immunoreactivity in the cerebellum, a wide variety and diversity of patterns were observed. PrP_d deposits were identified in the molecular layer (n=8), in the granular layer (n=5) and in both of them (n=10). The immunolabelling was a intense synaptic staining showing a fine-puntacte, fine-granular and/or granular-like. Three cases showed a remarkable granular dot-like staining in the granular layer, in comparision with the other cases. In the white matter of the cerebellum, most of the cases showed small deposits around cells of micro-glial type.
Atypical cases examined in this study show the allele ARQ in homozygous (n=6) and in heterozygous (n=13) with AHQ, ARR and ARH; and the allele ARR in homozygous (n=1) and in heterozygous (n=1) with AHQ.

Discussion: Ours results showed that the cerebellum, one of the hallmark of the atypical Scrapie cases, showed a diversity patterns of PrP_d immuno-staining, as other atypical cases detected in other UE countries. The genotype of 18 cases showed a susceptible type, and two of them are of the resistant type, although we have found different genotypes, no correlation have been detected between alleles polymorphirms and its lesion profile.

P9.15 Detection of proteinase K resistant prion protein in human urine

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Content
Recent concern about the possible secondary spread of vCJD through blood transfusion and blood products has highlighted the need for a sensitive test for the identification of PrPTSE in clinical specimens collected in a non-invasive way. In addition, a more accurate estimate of the prevalence of pre-clinical vCJD in the population may be possible if there were a screening test that could be applied to easily available material such as urine. As a step towards this goal, the detection of putative PrPTSE in the urine of CJD patients has been improved, based on proteinase K digestion of samples and Western blotting. In its present form, the test uses concentrated urine as a starting material. After proteolytic treatment followed by electrophoresis and Western blotting, membranes are incubated with an anti-PrP antibody conjugated directly with horseradish peroxidase. The results of this study demonstrate the presence of proteinase K resistant bands in urine from some sCJD and vCJD patients, but also in disease control patients.

P9.16 Detection of prions in blood from asymptomatic scrapie sheep by Epitope Protection technology

Authors
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Content
Background: Amorfix Life Sciences has developed the EP-TSE™ blood test to diagnose scrapie in sheep using Epitope Protection technology. The assay detects aggregated PrP_{Sc} in a plasma sample by blocking the large excess of PrP_C by incubation with short-lived and highly reactive chemicals. PrP molecules within prion particles are "protected" from chemical modification, and can then be detected by our ultrasensitive immunoassay following sample disaggregation.

Objective: 1) Differentiate scrapie sheep from uninfected control sheep using the EP-TSE™ test.
2) Measure the sensitivity and specificity of the EP-TSE™ test.

Methods: Several unblinded and blinded panels from various independent sources were tested using the EP-TSE™ test. The panels consisted of plasma samples collected from control sheep and scrapie sheep, at various stages of disease, with a variety of different genotypes and collected from several locations in North America and Europe.

Results and **Discussion:** The EP-TSE™ test differentiates scrapie-infected sheep from normal controls for AA, AV and VV genotypes and for both naturally and experimentally infected animals. Asymptomatic scrapie-infected lambs as young as 17 months of age were detected by the Amorfix EP-TSE™ test. The test can also identify infected lambs before the animals test positive by IHC tests of lymphoreticular tissue. Studies to detect atypical scrapie and BSE in sheep are ongoing.

P9.17 Development of Rapid Pre-Mortem tests for TSE's in Sheep

Authors
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Content
Background: At present there are no reliable tests for the live-animal diagnosis of TSEs. Such tests would represent a significant advance in the study and management of these diseases and as such would contribute to reducing and eventually eradicating TSEs from the GB national sheep flock. In the absence of other appropriate surrogate markers, live animal TSE tests would require exquisitely sensitive detection of the very low levels of PrP_{Sc} present in readily available biological matrices such as blood.

Objective: With respect to published rapid methods for TSE diagnosis, the development of a pre-mortem TSE assay will require a more efficient method for the concentration of PrP_{Sc} from large volumes of a biological matrix, coupled with an increase in the analytical sensitivity for PrP_{Sc} detection. The objective was to optimise a mineral precipitation method to recover prion from large volumes, which could be applied to different blood fractions taken from sheep.

Methods: We have screened a number of potential PrP-binding compounds and found one (silicon dioxide) that was capable of efficient PrP extraction from large volumes of buffer and was compatible with Western blot analysis of the resulting extract. During the course of the study, blood samples from 12 VRQ/VRQ lambs exposed to scrapie from the VLA Ripley flock have been taken from 3 months of age until the clinical stages of disease, together with age/genotype matched controls taken from the ADAS ARSU sheep flock. These samples have been processed and archived as both buffy coat and plasma fractions.

Results: The introduction of a silicon dioxide precipitation step into the immunological detection of PrP_{Sc} increased detection sensitivity by up to 1600-fold. The developed method was applicable to PrP_{Sc} -spiked buffy coat and plasma fractions of ovine blood after removal of endogenous PrP_C by thermolysin digestion. Analysis of blood samples from scrapie exposed animals up until clinical disease is currently under way.

P9.18 First Report of Classical Scrapie in Portugal, including co-existence with atypical scrapie in the same flock

Authors

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Content

Background: Since the implementation of a surveillance plan for scrapie in small ruminants by the European Union in all member states in 2002 (according to EU regulation 999/2001 and subsequent alterations), a total of 331.407 small ruminants (69.763 risk population and 261.644 healthy slaughter) have been tested by the BIORAD rapid test in Portugal, until March 2008. A total of 277 small ruminants (276 sheep and 1 goat) were affected with atypical scrapie and were identified all over the country (except in Madeira and Azores). Despite this extended TSE surveillance, no classical scrapie has been diagnosed in portuguese sheep until this year.

Objective: This work describes the first cases of classical scrapie in Portuguese sheep detected in three flocks within the same region of the country, highlighting the coexistence of atypical scrapie in one flock.

Material and Methods: All the classical scrapie cases were detected by the screening test *TeSeEâ BIORAD* on brainstem samples and confirmed by histopathology, immunohistochemistry and *TeSeE BIORAD Western Blotä*. To distinguish between BSE and scrapie the samples were submitted to the Discriminatory Test for strain typing (*CEA/BIORAD*). The *PRNP* genotype (136/141/154/171) was determined by PCR and automated cycle sequencing. All the cohorts (n=266) of the first affected flock were culled and samples from brainstem, cerebellum, tonsil and retropharyngeal lymph nodes were collected and screened by the *TeSeEâ BIORAD*.

Results: Under the Active Surveillance Plan it was detected a total of nine Portuguese sheep showing classical scrapie phenotype (two slaughtered for human consumption and seven fallen stock). Seven sheep were from the same flock in which one atypical case was also confirmed. The other two cases belong to two different flocks. The histopathology, PrP_{res} distribution and electrophoretic profile will be presented as well as the *PRNP* genotypes. The preliminary results regarding the cohort's analysis will also be revealed.

Discussion: The first three outbreaks of classical scrapie were identified in the same region (center east) of Portugal, whereas the atypical scrapie cases have been diagnosed scattered all over the country, but mainly in the South, a region with a larger sheep population. Despite the effectiveness of the sampling of all healthy small ruminants over 18 months slaughtered for human consumption, the collection of fallen stock has been only consistently performed in the southern part of the country. The number of sampled dead small ruminants in the northern half of the country was remarkably low until last September, when the collection system for these animals has started systematically. This issue could possibly explain the identification of these classical cases within the fallen stock. Nevertheless, knowledge on the epidemiology of the disease, identifying risk factors for the introduction of scrapie infection and the risk of the disease occurrence in the Portuguese sheep is fundamental. Our results also demonstrate that more than one TSE strain could coexist within in the same flock as previously reported by other authors.

P9.19 Detection of classical and atypical BSE (BASE) and scrapie prion strains by prion protein motif-grafted antibodies

Authors

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Content

Background: The generation of PrP motif-grafted monoclonal antibodies (IgG 89-112; IgG 136-158) distinguishing among PrP_C and PrP_{Sc} conformations was previously published (1). Such reagents were demonstrated to specifically immunoprecipitate infectious fractions of PrP_{Sc} from brain tissue of humans and rodents affected by prion diseases but not PrP_C.

Aim: To evaluate the reactivity of these antibodies with scrapie, Nor98, BSE and BASE prions of naturally infected animals.

Methods: 12 scrapie and 12 Nor98 cases from sheep and goats, 4 BSE and 3 BASE cases from cattle were selected. CNS of healthy animals was used as negative control. Two immunoprecipitation (ip.) methods were employed. In case of strong intensity signal at confirmatory methods ip. was carried out as described (1). In feeble cases ip. was preceded by an ultracentrifugation step to enrich the sample for PrP_{Sc} and analysis was also made in duplicate with and without ip. step (*Wb and Wb ip.* respectively). Scrapie and Nor98 cases with very weak signals were serially diluted to compare methods' sensitivities. PrP was immunoblotted by P4 or 6H4 antibodies.

Results: The antibodies immunoprecipitated specific PrP bands from undigested and pK-digested brain samples in all cases examined. The bands reproduced the distinctive patterns of the four different prion strains assayed, indicating strain-specific binding of the antibodies. No PrP was detected by ip. in negative controls. In weak cases examined by *Wb* and *Wb* ip. Nor98 cases were better detected when the ip. step was added, as confirmed by dilutions.

Conclusions: This study confirms the intrinsic specificity and affinity of two independent regions of PrP sequence for epitopes found on disease-associated PrP conformers encountered in animal and human TSEs. These molecules may find broad application in prion diagnostic, structural and basic research studies.

References: 1) Moroncini G. et al. (2004) PNAS USA 101-28: 10404-9.

Grant: ARS Cooperative Agreement 5836254F217.

P9.20 Comparison of labeling abilities and bioactivity of three PrP_{Sc}-specific, recombinant antibody fragments

Authors

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Content

Prion diseases are invariably fatal neurodegenerative diseases in humans and animals. Replication of the prion agent involves conversion of normal host PrP_C to the disease-associated conformation PrP_{Sc}. The cell biology of PrP_{Sc} is still largely unknown, mainly due to the lack of specific detection reagents in live of fixed cells, or in tissues.

Here, we generated and expressed PrP_{Sc}-specific binding polypeptides for investigating the cell biology of PrP_{Sc}. Three PrP_{Sc}-specific mABs were chosen for cloning of complementarity determining regions into scFv fragments. The PrP_{Sc} specificity of the mABs was determined by immunoprecipitation. The mABs consisted of mAB 15B3 (Korth et al., 1997 Nature 390:74) and two novel monoclonal antibodies. All mAbs were cloned as scFv fragments both as EGFP fusion proteins behind an N-terminal IgGk-signal sequence for expression in mammalian cells and as scFv fragments expressed in E. coli. Although in immunoprecipitation all (full length) mAbs displayed PrP_{Sc} specificity, staining patterns of PrP_{Sc} -specific scFv-EGFP in of ScN2a cells was different. This may be due to different epitopes; however, we were not able to map these conformation-specific epitopes to a specific surface-exposed domain. Antiprion activity of these fragments in ScN2a cells was also evaluated, and compared to other PrP_C or PrP_C/ PrP_{Sc} targeting polypeptides. We conclude that PrP_{Sc} is a conformationally and/or multimerically heterogenous population of PrP molecules that is not an easy antiprion target in vivo. These PrP_{Sc}-specific scFvs are of particular interest for developing sensitive blood-tests and for investigating PrP conversion in infected cells.

P9.21 Diffusion, and its Increase with Age, is Reduced in the Putamen of Healthy Carriers of the E200K Prion Mutation

Authors

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Content

Background: We previously reported in a smaller sample that brain diffusion changes could be detected in healthy carriers of the E200K mutation that causes familial CJD. Here we investigated the differential aging effects in such carriers, compared to their non-carrier healthy relatives, and searched for other areas that show such trends.

Objective: To investigate all cerebral areas that show differential aging between mutation-negative and mutation-positive healthy subjects.

Methods: Healthy subjects (by history and neurological examination) were recruited from relatives of fCJD patients. We studied 60 subjects from the affected families, 30 positive (C+) and 30 negative (C-) for the mutation; the two samples were matched for age and gender, and the overall age was 50 ± 9 (range 31-72). MRI was performed on a 1.5T GE Signa, and brain diffusion was quantified as Apparent Diffusion Coefficient (ADC, μ2/s) from Diffusion-Weighted Imaging (DWI) at b=1000 s/m2. ADC data were analyzed by SPM5, using p<.005, k>5. To compare relevance of regions with highly disparate size, the area of significant clusters was normalized by literature averages of structure volume.

Results: The putamen primarily, and the caudate and thalamus less, showed reduced diffusion in C+. Elevated diffusion was found primarily in frontal, temporal and parietal cortex, and also in the cerebellum. Significant interaction between aging and genotype was again found primarily in the putamen (lower increase with age), and in the caudate and neocortex (higher increase with age). Age regressions indicated that putaminal and thalamic diffusion increases by ~ 3-4 μ2/s/yr in the C- subjects, but decreases by ~ 1 μ2/s/yr in mutation carriers.

Discussion: These data confirm previous analyses that suggested a diffusion reduction in the putamen of healthy subjects carrying the E200K mutation, decades prior to disease onset. Further, the current data suggest that diffusion elevations can also be detected in cortex and cerebellum long before disease onset. Finally, these changes appear linear from age 30-70, without a precipitous decline around age 60, when disease onset is common, suggesting a continuous process, possibly related to PrP_{Sc} accumulation.

P9.22 The Effect of Apolipoprotein μ4 Allele on Cerebrospinal Fluid Markers in Creutzfeldt-Jakob Disease

Authors

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Content

Background: The clinical diagnostics of Creutzfeldt-Jakob disease (CJD) can be supported by the analysis of various cerebrospinal fluid (CSF) markers such as 14-3-3 protein, tau protein, neuron-specific enolase (NSE), S100 protein or β-amyloid 1-42 (Aβ1-42). The presence of the Apolipoprotein μ4 allele (APOE) is known as an important risk factor for Alzheimer disease. For the distribution of APOE phenotypes in Creutzfeldt-Jakob disease inconsistent reports have been published. The APOE μ4 allele is associated with more reduced cerebrospinal fluid levels of Aβ1-42 in Alzheimer disease. The influence of APOE μ4 allele on the selected CSF markers is not known.

Objective: The aim of the study was to analyse if a correlation between the values of CSF markers and the APOE phenotypes in Creutzfeldt-Jakob disease exists and therefore may improve the diagnosis.

Methods: The following CSF markers were investigated: 14-3-3 protein, tau-protein, NSE, S100 protein, Aβ 1-42 and Aβ1-40. Concerning theses markers we focused our interest on the subgroups: Alzheimer disease, sporadic CJD, fatal familial insomnia, genetic CJD and CJD with additional typical neuropathologic changes for Alzheimer disease and the correlation with APOE phenotypes.

Results: We present the influence of the different APOE phenotypes on the measured CSF markers with a variable sensitivity in the listed subgroups.

Discussion: Our study provides valuable new insights regarding the effects of Apolipoprotein μ4 allele on cerebrospinal fluid markers in prion diseases and Alzheimer disease. It might be useful in the improvement of the clinical diagnosis.

P9.23 Proteolytic removal of PrP^c in plasma with preservation of endogenous TSE infectivity

Authors
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Content

Proteolytic removal of PrP^c in plasma with preservation of endogenous TSE infectivity
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Background: There is great interest in diagnosis of TSE infections from plasma. Low concentrations of TSE infectivity are present in plasma but no demonstration of PrP^{res}. PrP^c can be visualized by Western blot and measured by immunoassays. We estimate that the plasma concentration of PrP^c is at least 100,000-fold greater than PrP^{res} (J Virol Meth (2008) 149, 251-259). A major obstacle to plasma-based diagnosis will be differentiation of PrP^{res} from contaminating PrP^c. Little is known about the physical properties of either PrP^{res} or PrP^c in plasma and plasma itself is a challenging matrix for proteolysis. We report here our investigations of the proteolytic sensitivities of endogenous plasma-associated PrP^c and, since endogenous PrP^{res} could not be detected, endogenous plasma infectivity. Infectivity measurements were by limiting dilution titration.

Objective: The objectives of this study were 1. To establish optimal conditions for proteolytic discrimination of PrP^{res} in plasma by proteinase K (PK) digestion and 2. To characterize plasma-associated PrP^c.

Methods: The kinetics of endogenous PrP^c digestion were obtained for various digestion regimes and concentrations of PK, by analysis for the presence of PrP^c using affinity concentration and immunoassay. Four digestion conditions were selected for titration using the limiting dilution method. PrP^c concentrated from hamster and human plasma by affinity, was deglycosylated and analyzed by Western blot using six monoclonal antibodies.

Results: For all conditions selected for titration (except controls), PrP^c was digested to below the limit of detection. Preliminary results indicate that infectivity (and presumably PrP^{res}) was digested at higher concentrations of PK. PrP^c from human and hamster plasma is primarily diglycosylated. Deglycosylation results in several PrP^c bands by SDS-PAGE. Using various PrP markers and a panel of antibodies, we were able to assign most of the deglycosylated PrP bands.

Discussion: There is a relatively wide range of PK concentrations in which most PrP^c is digested and PrP^{res} is not affected. As a consequence it may be possible to use PK digestion to discriminate PrP^{res} from PrP^c in a prion diagnostic test. This investigation has also revealed previously unknown information on the molecular characteristics of plasma PrP^c.

P9.24 In vitro amplification of chronic wasting disease in elk

Authors
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Content

Chronic wasting disease(CWD) of cervids is a transmissible spongiform encephalopathy(TSE). TSE pathogenesis is associated with refolding of the normal prion protein, PrP^c, into a partially protease-resistant isomer termed PrP^{Sc}. Soto and colleagues greatly extended the process and power of in vitro PrP^{res} amplification in developing protein misfolding cyclic amplification(PMCA). In PMCA, normal brain homogenates(NBH) supply PrP^c, which upon addition of infected brain homogenate is refold into protease-resistant isoform, PrP^{res}. To enhance sensitivity of CWD prion(PrPCWD) detection in elk, we established in vitro serial PMCA modeled after Soto et al. Here we report amplification using CWD-negative brain homogenate from cervid PrP transgenic mice[Tg(elkPrP)]. The serial PMCA- diluting amplified material into fresh Tg(elkPrP) NBH for each successive round – resulted in a yield of 109 fold after just 5 rounds. The magnitude of PrPCWD conversion obtained with serial PMCA may make it possible in vitro detection of PrPCWD in retropharyngeal lymph node and brain of infected animals.

P9.25 Real-time Immuno-PCR Method for Detecting Low Level Prion Protein in Food and Processed Materials

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Content

Background: Prion diseases are a group of progressive neurodegerative disorders known as transmissible spongiform encephalopathies (TSEs), especially Creutzfeldt-Jakob disease (CJD) in human. Transmission of prion by oral route is not fully understood yet, however, infected patients or animals were characterized by accumulation of PrP^{Sc} in their skeletal muscles in a low level. The Western blot or ELISA, commonly used to detect PrP, are not sufficient for detecting a very low level of PrP in food and processed materials contaminated by PrP^{Sc} –containing skeletal muscles. The more precise and more improved tools for detecting PrP are required.

Objective: Real-time immuno-Polymerase chain reaction (IPCR) method has been developed to improve the sensitivity of PrP detection compared to the current tools.

Methods: Taking advantage of the system, we expressed and purified recombinant bovine (recBo) PrP, and selected several peptides from PrP of animals for immunization of goat to make polyclonal antibodies. Using recBoPrP and polyclonal antibodies, IPCR has been performed in real-time.

Results: Limit of detection using recPrP was about 100 pg at least 103-fold higher sensitive than ELISA, though the sensitivity was decreased ten times in case of PrP^{Sc}. Dilution factor in PrP^{Sc} in inoculated brain homogenates was 10-4 because of insolubility of the protein, however, PrP^c in normal brain homogenates was 10-5.

Discussion: Our results showed at least 10 2 -fold high sensitivity to PrP than conventional detection method of Western blot and ELISA. However, for application to routine detection for PrP^{Sc}, it is necessary to study using various biological specimens containing PrP^{Sc} in various conditions for confirmation and validation of real-time IPCR.

P9.26 Detection and imaging of prion particles

Authors
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Content

Background: The agents of prion diseases are composed primarily of the pathogenic isoform of the prion protein designated PrP^{Sc}, which is generated by a conformational change of the cellular isoform PrP^c. In contrast to its cellular isoform, the pathogenic isoform PrP^{Sc} forms insoluble aggregates. Hitherto approved prion tests use the Proteinase K-resistance of PrP^{Sc} as a marker for the disease. Because of varying portions of PK-resistant PrP^{Sc} [2; 3] we developed a test, which does not rely on PK-resistance.

Objective: Processing of test samples without Proteinase K digestion - Detection and counting of single prion particles - Development of the test system for early diagnostic.

Methods: We use Surface-FIDA [1]. Partially purified prion particles are labelled by two different antibodies with different fluorescence labels and were concentrated on a chip surface by capture antibodies. Upgrading the Fluorescence-Correlation-Spectrometer (FCS) allows us imaging of the single prion particles.

Results and Discussion: In the past we are able to distinguish Scrapie-infected hamster as well as BSE-infected cattle in the clinical stage from a control group [1]. Preliminary data showed that we are able to detect disease associated PrP-aggregates in the cerebrospinal fluid of BSE-afflicted cattle. In the recent study we adapted the application to Scrapie in sheep. We optimized the assay further in respect to image single PrP-aggregates from Scrapie-infected sheep. [1] Birkmann et al., (2007) Counting of single prion particles bound to a capture-antibody surface (Surface-FIDA), *J. Vet. Microbiol.*, 123, 294-304. [2] Birkmann *et al.*, (2006) Detection of prion particles in samples of BSE and Scrapie by Fluorescence Correlation Spectroscopy without Proteinase K digestion. *Biol Chem.*, 387, 95-102. [3] Safar, J. et al. (1998). Eight prion strains have PrP^{Sc} molecules with different conformations. *Nat Med.* 10, 1157-1165.

P9.27 Neuroimmune contacts between peripheral nervous system and follicular dendritic cells in ovine lymph organs.

Authors
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Content

Background: Scrapie is a lethal disease affecting sheep, goat and moufflon. In sheep, th PrP genotype is a major susceptibility factor in the development of clinical scrapie. The causative agent, called prion (PrP_{Sc}) enters the organism mainly by the oral route and rapidly invades the mucosal associated lymphoid tissues. It is now clearly established that follicular dendritic cells (FDCs), stromal-differentiated cells present in the germinal center of the lymphoid follicles are greatly implicated in the retention and replication of PrP_{Sc} after scrapie infection. However, how scrapie agent spread from the FDCs to the peripheral nervous system remains unclear.

Objective: In this study, we examined the topography of the peripheral nervous system in ovine tonsils and their draining lymph nodes in order to identify potential sites for neuroinvasion by pathogen agents.

Material and Methods: Palatine, pharyngeal tonsils and medial retropharyngeal lymph nodes were removed from sheep 6 to 8 month old and cryosections were processed for immunofluorescence. FDCs were stained with FDC-B1 antibody. Nerve fibers were identify with specific polyclonal antibodies directed against L, M and H neurofilaments. All samples were observed with confocal microscope.

Results: PMCA FDC-B1 immunostaining localized the FDCs network in the light zone of the germinal centre of the lymphoid follicles. Whatever analysed organs, the M neurofilaments were the most abundant in the lymphoid compartment. On the other hand, H and L neurofilaments were mostly localized in the connective tissue. The M neurofilaments surrounded the FDCs network and were probably located in the mantle zone. This pattern of innervation was particularly developed in palatine tonsils. A combined approach of image and spectral analyses allowed us to confirm the presence of some contacts between FDCs and M neurofilaments.

Discussion: Our results support the hypothesis of a possible transfer of prion protein directly from FDCs, resident cells of the germinal centre, to the nervous system via M neurofilaments.

P9.28 A rapid dual staining procedure for the quantitative discrimination of protein and prion amyloid deposits in infected brain tissue contaminating surgical stainless steel.

Authors
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Content

Background: Transmissible spongiform encephalopathies (TSE) are fatal neurodegenerative diseases often associated with the accumulation of amyloid plaques in the brain and other tissues. Variant Creutzfeldt-Jakob disease (vCJD) is an acquired TSE with a relatively long asymptomatic incubation period, which can potentially be iatrogenically transmitted. This has prompted the need for sensitive and rapid methods of detection of the pathology indicator, the protease-resistant prion protein (PrP_{Sc}), in tissues and on surgical instruments deemed at risk of being contaminated.

Objective: To discriminate between common tissue proteins and amyloid-rich aggregates such as those formed by the abnormal prion in tissues and on surgical instruments.

Methods: We developed a thioflavine T/SYPRO Ruby dual staining procedure, used in combination with episcopic differential interference contrast/epifluorescence (EDIC/EF) microscopy for rapid scanning of instrument surfaces. This method was assessed by double-blind studies using various proportions of ME7-infected brain mixed with normal brain homogenate applied on surgical stainless steel tokens and instruments.

Results: We observed a ME7 concentration-related characteristic thioflavin T signal on contaminated surgical stainless steel surfaces, as assessed by image analysis. The detection limit of this direct observation technique proved at least 100-fold better than the classic Western blot using the same prepared samples.

Discussion: The improvement in detection threshold is likely to be greater considering the need for swabbing the instrument surface, which might hinder the sensitivity of any such assay since PrP_{Sc} tends to bind strongly to surfaces, particularly if these are serrated or pitted. Furthermore the proteinase K digestion step prior to Western blot may also reduce the amount of PrP_{Sc} detected. Our new sensitive, in situ microscopy procedure is rapid and can be applied directly to instrument surfaces to assess protein contamination and provide a quantitative measurement of amyloid content. This advance will be important for routine determination of instrument cleanliness and also validation of decontamination protocols and equipment.

P9.29 Comparison of in-vitro and in-vivo methods for the detection of hamster 263K scrapie in human red-cell concentrate

Authors
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Content

The *in vivo* bioassay is widely regarded as the "gold standard" method for the detection of the infectious form of the prio-protein. However, due to the long-incubation periods and the cost of these assays, the majority of research is conducted using a variety of less-sensitive *in vitro* methods. The HPA is currently assessing whether these assays can be directly correlated to the *in vivo* bioassay. This is particularly relevant as such biochemical assays are widely used to assess/screen prion-removal and decontamination technologies. This study will measure hamster 263K scrapie in human red-cell concentrate using the in vivo bioassay and compare the results against two *in vitro* methods; the widely used Western Blot assay, which relies on detection of the protease resistant fragment PrP27-30 as a marker for infectivity and the conformation dependent immunoassay (CDI) which measures the differential binding of specific C and N-terminal anti-PrP monoclonal antibodies depending on the extent of denaturation of the molecule. There is little data in the literature on using the CDI-assay to detect hamster 263K scrapie. The study will also attempt to compare the use of these methods in the detection of BSE-301V. This evaluation aims to measure how the dirfferent TSE agents and detection methods compare in terms of their ability to validate prion-removal or decontamination methods in relation to reducing the risks of iatrogenic vCJD

P9.30 White Matter Lesions in Patients with Creutzfeldt-Jakob Disease and the Affects on Clinical Presentations and Survival Times Considering Phenotypic Variability

Authors

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Content

Background: Creutzfeldt-Jakob disease is a neurodegenerative, invariably fatal disease frequently seen in patients between 60 and 70 years of age. The disease is characterized by clinically progressive dementia with a broad spectrum of neurological symptoms such as ataxia, myoclonus, visual symptoms and pyramidal signs. The histopathologic changes associated with signal hyperintensities in MRI contribute to diagnose CJD and can help to differentiate the various subtypes due to codon 129 polymorphism. Thus MRI plays an important part in the in vivo CJD diagnosis. Some atypical presentations of phenotypes can still lead to problems in diagnosing CJD.

Objective: To ascertain if white matter lesions in patients with probable or definite Creutzfeldt-Jakob disease modify the clinical presentation and survival time.

Methods: Two neuroradiologists, blinded to diagnosis, retrospectively evaluated FLAIR images from 50 patients with probable or definite CJD that additionally showed white matter lesions and control subjects without white matter lesions.

Results: Clinical presentations and survival times where evaluated between these groups. The CJD patients with white matter lesions displayed increased longevity compared to control group. However these results showed no statistical significance (p=0,346). Further investigation shows clinical presentations of the different phenotypes.

P9.31 Generation and properties of all-PrP and PrP^{Sc}-specific monoclonal antibodies

Authors

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Content

Transmissible spongiform encephalopathies (TSE) are a group of invariably fatal neurological disorders in animal and humans. Diagnosis of this group of diseases is usually performed by brain biopsy or post mortem due to the lack of reliable ante mortem tests for TSE in living individuals. This is due to the fact that the infectious agent causing TSE is the pathological isoform (PrP^{Sc}) of an endogenous cellular protein (PrP^{C}) that escapes current detection methods. Therefore, the current diagnosis of TSE is mainly performed using Proteinase K, which degrades the cellular form PrP^{C} but leaves PrP^{Sc} that is then detected by universal, all-PrP recognizing antibodies. To improve diagnosis of TSE, antibodies specific for the pathological isoform PrP^{Sc} would be advantageous. We have used sodium phosphotungstic acid (NaPTA)-precipitated antigen to generate both monoclonal antibodies universally recognizing all-PrP or exclusively PrP^{Sc}. This immunogen was shown to contain infectivity, representing the most relevant characteristic of scrapie. Resulting antibodies were used in ScN2a cells to clear PrP^{Sc}, validated by bioassays. Furthermore, one antibody and derivatives thereof have been used for therapeutic studies in inoculated animals. In addition to conventional all-PrP recognizing antibodies, we also generated two monoclonal antibodies specifically detecting PrP^{Sc} but not the cellular isoform PrP^{C}, as determined by immunoprecipitation experiments. Testing for species-specificity of these PrP^{Sc}-specific antibodies revealed a broad pattern of specific recognition of PrP^{Sc} in experimental mouse scrapie, CWD and CJD. These antibodies are currently applied for studies in the cell biology of PrP^{Sc} as well as diagnostic tools in ante mortem prion tests. lothar.stitz@fli.bund.de

P9.32 Different PrP^{Sc} accumulation patterns in goat brain. Should the official method of sampling be reconsidered?

Authors

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Content

Background and Objective: Scrapie is a progressive fatal neurodegenerative disease affecting both sheep and goats. Its diagnosis is officially based on the detection of the abnormal prion protein (PrP^{Sc}) in the medulla oblongata at the level of the obex. The objective of this study was to assess the distribution of PrP^{Sc} in the brain of goats raised in a flock with high incidence of scrapie and to evaluate the impact on current sampling and testing techniques.

Methods: The brains of 86 culled goats, older than 12 months of age, from a scrapie-affected flock were studied. Samples were collected from medulla oblongata, pons, cerebellum, midbrain at the level of superior colliculus, hypothalamus at the level of optic chiasm, and frontal lobe of cerebrum, to study the distribution of PrP^{Sc}. In total 489 samples were examined by ELISA "BSE Platelia, Bio-Rad" and an immunochromatography assay "Prionics-Check® PrioSTRIP SR". Positive results were confirmed by western blot (WB) using mAbs 6H4 and P4, and/or immunohistochemistry (IHC) using m'b 2G11. Genomic DNA was extracted from brain tissue and the DNA sequences of the PrP gene entire ORF were determined after PCR amplification and sequencing.

Results and Discussion: Both ELISA and immunochromatography assays revealed the presence of PrP^{Sc} in 48 samples from 15 goats. PrP^{Sc} detection revealed different accumulation patterns in the CNS regions with the highest frequency of deposition found in the obex and the hypothalamus. Interestingly, in 3 goats, PrP^{Sc} was not found in the obex but only in the cerebrum and/or hypothalamus. In these goats, PrP^{Sc} was confirmed by IHC. Finally, WB analyses showed a molecular profile of PrP^{Sc} similar to that observed in atypical scrapie. These results reveal that sampling of the hypothalamus in addition to the obex will increase the frequency of PrP^{Sc} detection in goat TSE surveillance schemes. PrP polymorphisms were identified at codons 154 (R/H), 171 (Q/H), 222 (Q/K) and 240 (P/S) with R154Q171Q222 allele having the highest frequency in both scrapie-affected and healthy goats. Three of the PrP^{Sc} positive goats carried the H154Q171 allele indicating a possible association to host susceptibility similar to that reported in atypical scrapie cases in sheep. Finally the allelic variant encoding lysine at position 222 was found only in healthy goats and could be associated with resistance.

P9.33 The differential of sporadic Jakob-Creutzfeldt disease (sCJD)

Authors
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Content
Background: sCJD diagnosis can be difficult and time-consuming. Earlier diagnosis allows caregivers to concentrate on patient care, and may allow for sooner potential treatment. Recognizing the early differential diagnosis of sCJD patients may speed the diagnostic process.

Objective: To determine which medical specialties see sCJD patients prior to diagnosis and to identify the common early misdiagnoses.

Design and Methods: Between 8/2001 and 8/2007, 976 potential prion cases were referred to our center.
Subjects missing records from more than two visits were deemed insufficient for this study and those subjects were excluded. Of 163 definite sCJD cases, 97 had records deemed sufficient for this study. Records were reviewed retrospectively to determine all the diagnoses considered before a diagnosis of CJD was considered the single most likely diagnosis. To simplify data analysis, these 180 misdiagnoses were first classified into 40 different conditions and then further classified into16 general diagnostic categories. Patient data were entered into and queried from a secure clinical relational database.

Results: Upon retrospectively reviewing the 97 sCJD cases we identified a total of 373 unique diagnoses considered in this cohort. The five most common diagnostic categories (of 16) were: neurodegenerative (13%; n=49), autoimmune (13%; n=48), infectious (11%; n=41) toxic-metabolic (11%; n=40), and unknown dementia (10%; n=37). The most common first specialist seen were neurologists (74%), ophthalmologists (7%), psychiatrists (5%), cardiologist (4%) otolaryngologist (2%) and orthopedists (2%). The most common types of physicians seen prior to evaluation at our center were neurologists (42%), internist (23%), unknown (7%), family physicians (6%), ophthalmologists (4%) psychiatrists (3%), cardiologists (3%), pulmonologists (2%), and psychiatrists (2%).

Discussion: CJD is often confused with other conditions, particularly early in the disease course. Despite a neurologist being the first specialist to see most sCJD patients, there were many misdiagnoses. About 25% of patients were first seen by a non-neurology specialist. Further education of neurologists and these other specialists about early signs of sCJD may improve earlier diagnosis. Earlier diagnosis is critical for improving the effectiveness of potential treatments and reducing the risk of iatrogenic transmission.

P9.34 Prion Protein Analysis in Slovak Genetic CJD Cases

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Content
Creutzfeldt-Jakob disease (CJD) in Slovakia is characterized by unique accumulation of genetic cases (gCJD) associated with mutation E200K of prion protein gene (*PRNP*).
The objective of this study was to analyze a cohort of slovak gCJD cases. To identify their abnormal prion protein (PrP_{Sc}) types according to the predominant protease-resistant core fragment size and subtypes characterized by a ratio of three glycoforms as well as the disease phenotype. Western blot analysis of the PrP_{Sc} types in the CNS has been performed in a 48 gCJD cases. The distribution of M129V polymorphism was 36 homozygotes methionine/methionine and 12 heterozygotes methionine/valine.
Presented work provides data about prion protein types and it's association with codon 129 polymorphism, phenotype, duration of the disease and age of patients with genetic CJD, the most frequent form of CJD in Slovakia (75%). Achieved data have been compared with our findings in sporadic CJD as well as with published data. Work was supported by project of the Ministry of Health of the Slovak Republic, No. 2005/33-SZU-11.

P9.35 A rapid light microscopy technique for sensitive detection of prion infectivity in cell models

Authors
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Content
Background: Transmissible spongiform encephalopathies (TSE) are fatal neurodegenerative diseases often associated with the accumulation of protease-resistant amyloid prion (PrP_{Sc}) in the brain and other tissues. There is a need for sensitive and rapid methods to assess the potential infectivity of suspect tissues. Cell infectivity assays offer a cheap, rapid and ethical alternative to other current assays. We have recently applied our Episcopic Differential Interference Contrast/Epi-Fluorescence (EDIC/EF) microscopy technique, in combination with thioflavin T, to quantitatively assess prion amyloid deposition on surgical instruments, and shown this to be at least 100-fold more sensitive than Western blot detection.

Objective: To use EDIC/EF to follow in situ amyloid prion accumulation in a permissive stem cell infectivity model.

Methods: A neuroblastoma cell line (N2a) and primary Neural Stem Cell (NSC) cultures were infected with various prion strains, and prion accumulation was assessed in situ using EDIC/EF, and compared with classic Western blot detection.

Results: Observations suggest that increased fluorescence characteristic of amyloid aggregates can be observed in cultured cells after successful prion infection in less than 12 days, similar to Western blot.

Discussion: The assay remains to be calibrated to assess the detection limits in cells, and correlate the amount of detected fluorescence with the amount of protease-resistant prion detected using classic Western bloting following partial Proteinase K digestion. In addition to being faster and more ethically acceptable, this rapid detection technique may prove more sensitive, more reliable and more cost effective than current immunochemical methods.

P9.36 The TSE Sample Archive at VLA - review and prioritisation of contents

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Content
A review of the costs and output of the TSE Archive, and two associated sheep flocks was carried out in recent months by Defra & VLA. Throughout the BSE epidemic and in subsequent years, a diverse range of samples from cattle and sheep has been collected, stored and supplied to researchers. However, costs and storage capacity dictate a requirement to identify important samples for retention, and those that might be sent for disposal. The Archive has written to NeuroPrion members seeking scientific opinion on this matter.

P9.37 Sporadic Creutzfeldt-Jakob Disease in the Basque Country: Coexistence of prion protein strains in the same and different brain regions

Authors

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Content

Background: The description of the BSE agent and the demonstration of its transmissibility to humans (vCJD) led to the implementation of an active TSE surveillance program in the EU.

Objective: To report on the frequency and molecular profile of the prion protein in the TSE cases from the Basque Country.

Subjects: All cases for which family authorization for autopsy was granted during the period 2001 and December 2007. A total of 51 cases clinically suspect of TSE were submited to NEIKER-Tecnalia for testing.

Materials and Methods: Prion protein characterization was performed by immunoblot with 3F4 mAb of nine brain regions: cortex (occipital, parietal, frontal and temporal), striatum, caudate nucleus, thalamus, cerebellum and brain stem. On one sample from each individual a 129 codon testing was also performed.

Results: Forty one out of 51 suspect cases were confirmed to suffer from prion disease. Among them, 37 came from the Basque Country, while the remaining 4 came from surrounding provinces. The final diagnostic for the Basque cases were sporadic CJD (33), FFI (3) and fCJD (1). Genotypic frequencies of polymorphism 129 for sCJD were 60.6% MM, 15.2%MV and 24.2% VV. Familiar cases showed a regular prion protein pattern, while sCJD cases revealed a higher variability not only on prion protein type but also on its distribution through the different brain regions analysed. We found that 48.5% of sCJD were of type 1, 24.2% presented type 2A and 27.3% showed the coexistence of the aforementioned types of protein within the same or in different brain region. Considering codon 129 genotype as a breakdown variable we found that among MM cases 60.0% were of type 1 and 40.0% showed the coexistence of both types; MV cases showed a predominance of type 1 (80.0%) versus type 2A (20.0%) and VV individuals were predominantly of type 2A (87.5%) and a minority presented both types of prion protein (12.5%).

Discussion: The Basque Country presents one of the highest annual incidences of confirmed sCJD in Spain and in Europe (1.87 per million inhabitants per annum). It also suffers the highest frequency of FFI (43.12%). Regarding regional pattern distribution, our results show a predominance of PrP_{Sc} type 1 in sCJD. Surprisingly, a high frequency of cases with both types of prion protein was observed, indicating that the coexistence of two different molecular patterns in the same individual is more common than expected.

P9.38 Analysis of monoclonal antibodies for detection of the prion protein in goats bearing different *PRNP* gene polymorphisms

Authors

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Content

Background: Several monoclonal antibodies (mAbs) targeting different PrP regions have been developed for scrapie diagnosis. They can be generally grouped in mAbs directed against: the N-terminus, the core or the C-terminus of the protein. PrP is determined by the *PRNP* gene, which is highly polymorphic in goats, with polymorphisms encoding amino acid substitutions at protein level: G37V, T110P, G127S, L133Q, M137I, I142M, H143R, R154H, P168Q, Q222K and S240P are the main substitutions identified in goat *PRNP*. None of the mAbs used for scrapie diagnosis in goats have been developed for specific PrP detection in this species.

Objective: To evaluate by Western Blot (WB) the capability of different mAbs to detect the PrP from goats bearing different *PRNP* polymorphisms and thus to assess their reliability for scrapie diagnosis and surveillance in goats.

Methods: Samples of nervous tissue from four scrapie positive and 28 negative goats with different genotypes were collected. The PrP_{Sc} and PrP_{C}, derived from affected and unaffected goats respectively, were analysed by WB with a panel of mAbs, recognising different epitopes within the PrP sequence: SAF32, P4, 8G8, 9A2, 12F10, BAR 224, SAF70, SAF84 and F99/97.6.1. To determine analytical sensitivity, serial dilutions of the samples were prepared.

Results: In positive cases, PrP_{Sc} was detected by all the mAbs but with the following decreasing sensitivity: P4 and 9A2 > BAR224 and SAF70 > SAF84 and F99/97.6.1 > 12F10 and 8G8. SAF32 failed to detect all the positive samples, probably for the loss of the epitope after the proteolytic digestion. Immunoblot analyses on PrP_{C} from the negative goats revealed a different molecular pattern by using the various mAbs.

Discussion: Our study revealed that all the examined mAbs are suitable for PrP detection in goats, with P4 and 9A2 showing the highest sensitivity. The different polymorphisms in the *PRNP* of samples do not appear to affect the PrP detection by the different mAbs.

P9.39 TSE active surveillance in small ruminants: how much can we trust rapid tests?

Authors

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Content

Background: TSE active surveillance in EU in sheep and goats is based on a screening procedure using rapid tests specific for ovine and caprine animals, approved according to the Commission Regulation (EC) 21/2008.

Objective: Aim of this study was to evaluate the performances of three approved TSE rapid tests, verifying, in field conditions, their suitability for the detection of affected animals in the early preclinical stage of Scrapie.

Methods: One hundred and thirty-eight Scrapie positive brainstems were selected to evaluate the sensitivity (Se) of rapid tests Bio-Rad TeSeE Sheep/Goat (A), IDEXX HerdChek BSE-Scrapie Antigen Test Kit EIA (B) and Prionics Check Western Small Ruminant (C). This sample size was obtained testing 970 asymptomatic sheep (belonging to 23 flocks, with age over 18 months, various genotypes and breeds) by the three rapid tests; positive results were confirmed by Western Blotting (WB). Each sample was divided into five aliquots to be available for the three rapid tests, the confirmatory and discriminatory WB analyses. To evaluate the precision (inter and intra-lab agreement) of the tests, 40 samples (8 positive and 32 negative) were selected and the Cohen's k index was calculated. Furthermore the analytical sensitivity of the diagnostic systems on classical Scrapie cases was assessed.

Results: Test A detected 133 positive samples out of 138 (Se=96.4%, 95%CI: 91.7-98.8), test B137 (Se=99.3%, 95%CI: 96.0-100) and test C 128 (Se=92.7%, 95%CI: 87.1-96.5). The only case of atypical Scrapie was not detected by test C. The precision was good for all the rapid tests; however only test B presented k=1 (95%CI: 0.69-1). Tests A and B showed higher analytical sensitivity than test C.

Discussion: Our findings indicate that only the appliance of rapid tests with more comprehensive and higher standards than have previously been accepted for TSE active surveillance can guarantee the detection of the true prevalence of Scrapie infection in field.

P9.40 In vitro prion strain interference

Authors

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Content

Compared to conventional mice, transgenic PrP mouse models offer the possibility of bioassays of improved efficiency for the detection, differentiation and diagnosis of ruminant TSE agents. Preparation and challenge of transgenic mice expressing different PrPs would allow comparison of the transmission characteristics of a range of ruminant TSE agents and identification of susceptible models. The purpose of the study was to generate and characterise Tg lines overexpressing sheep, bovine and kudu PrPs and assess the lines for susceptibility in TSE challenge studies compared to wild-type mice. Tg mice were produced by pronuclear microinjection of FVB/N fertilised oocytes. PrP transgenes based on the half-genomic vector were prepared to produce mice expressing sheep and kudu PrPs, whereas a 60 kb transgene derived from a BAC bovine genomic library clone was used to generate Tg(BoPrP) mice. Tg progeny were bred with PrP null mice to transfer transgene expression to an ablated mouse PrP background. Southern hybridisation and Western blotting were used to determine transgene copy number and to estimate expression levels in brain and peripheral tissues, respectively. In TSE challenges, Tg(OvPrPAHQ) mice were highly susceptible to atypical scrapie and produced reduced incubation periods compared to classical scrapie. Tg(BoPrP) lines showed greater susceptibility and reduced incubation periods to BSE and scrapie challenge compared to wild-type mice. Western blot detection of PrP_{res} was used to confirm prion disease in clinically-affected mice. Tg lines overexpressing PrPs have been generated and several lines have been bred stably to transgene homozygosity. In TSE challenges, the findings so far indicate high susceptibility and reduced incubation periods in several Tg lines compared to wild-type controls. Completion of the transmission studies and biochemical and neuropathological analyses will confirm the suitability of the Tg lines produced as improved bioassay models.

P9.41 Comparison of WHO vCJD brain and spleen homogenates for levels of infectivity in C57BLK6 mice

Authors

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Content

The National Institute for Biological Standards and Control (NIBSC) supply standards and reference materials to laboratories testing for blood borne pathogens. Under the auspices of the World Health Organisation it was determined that there was a need for reference materials for human TSE research and diagnosis. The CJD Resource Centre at NIBSC holds the WHO CJD reference materials. These materials have been assessed through collaborative studies with participants from private and public institutions and in-house by western blotting and DELFIA assays. Relative levels of PrP_{res} in vCJD brain and spleen homogenates are known. However a comparison of actual levels of infectivity in these tissues has not previously been determined. C57BLK6 mice were inoculated by intracranial (IC) and interperitineal (IP) routes using serial ½ log dilutions to gain an accurate ID50. Brain and blood was collected from animals and disease confirmed by western blot analysis of brain homogenates.
The experiment has been completed and the results will be presented.

P9.42 Expression, purification and characterization of a prion protein-specific recombinant antibody

Authors

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Content

Background: Antibodies against prion protein can antagonize infections even when those antibodies are directed towards PrP^{c} rather than PrP^{sc}. However, active immunization with PrP has not been possible due to host tolerance to endogenous PrP^{c} and such immunizations have not produced antibody titers sufficiently high to be of utility in the treatment of prion diseases. While administration of monoclonal antibodies passively might be an approach to interfere with prion disease progression, the poor diffusion of large antibodies through the blood-brain barrier might render that approach ineffective. However, recombinant antibodies of the single chain variable fragment (ScFv) type are small enough to possibly circumvent this problem. In addition, diagnostic recombinant antibodies can be produced in systems devoid of PrP (yeast, bacteria, plants), Thereby abolishing the copurification of contaminating antigen during antibody production.

Objective: Polymenidou and Aguzzi have generated several hybridoma cell lines that produce monoclonal antibodies against specified epitopes in the N- and C-terminal portions of PrP. Some of these epitopes are repeated 4-5 fold in mammalian PrP species, and enable cooperative binding. Our objectives were to generate recombinant antibodies of the single chain variable format (ScFv) from these hybridoma cell lines and characterize their properties.

Methods: Using phage display techniques, we have recently generated ScFvs from several of these hybridoma cell lines and determined the sequences of the complementarity determining regions (CDRs) of these ScFvs.

Results and Discussion: Selected ScFvs, recognizing the N- and C-terminal portions of the mouse prion protein have been expressed in E. coli, purified from inclusion bodies and refolded to generate functional molecules. The properties of one ScFv recognizing the N-terminal portion has been characterized and our results indicate that their affinity for the target antigen is comparable to that of the parent monoclonal antibody. We have also generated co-crystals of this ScFv with its peptide epitope. Our findings from these studies will be presented and discussed within the context of possible development of novel preventive/therapeutic strategies.

P9.43 Utilising ion mobility mass spectrometry as a diagnostic tool for differentiating conformational variants of prion proteins.

Authors
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Content
Protein misfolding diseases have been proposed to be initiated by a change in protein shape. A new mass spectrometry technique (termed ion mobility) has recently been developed that is able to yield information regarding molecular shape.
This may be able to provide an insight into the conformational changes of prion proteins by measuring the change in cross section of the protein on misfolding. Measurement of misfolded protein would aid diagnosis and promote understanding of the disease propagation. All experiments were carried out on a Synapt HDMS System (Waters, Manchester, UK) which has a hybrid quadrupole/ion mobility/orthogonal acceleration time-of-flight (oa-TOF) geometry. Ions are accumulated in a trap travelling wave (T-Wave) device and periodically released into the ion mobility separation (IMS) T-Wave where they separate according to their mobility. The ions are then propelled through a transfer T-Wave to the oa-TOF for mass analysis. Ion arrival time distributions are recorded by synchronization of the ao-TOF with the gated release of ions into the IMS T-Wave. Recombinant prion samples of Syrian hamster prion protein, SHa PrP(23-231) and SHa PrP(90-231), were expressed in both predominately a-helix and misfolded predominately β-sheet forms. Circular dichroism experiments were carried out in order to confirm the nature of the secondary structure of the prion protein. Estimations of the rotationally averaged cross sections of the proteins were made by reference to standards of known cross section at varying physiologically relevant pH. The ability to differentiate between the alpha and beta forms of the PrP (90-231) has been demonstrated at acidic pH (similar to conditions experienced in the endosome). Our measurements using purified recombinant proteins approaching biologically relevant concentrations (pmol/µL) indicate the powerful nature of this technique as a diagnostic tool. Optimisation studies using differing mobility gases are underway with preliminary results suggest that conformation in the gas phase is independent of the buffer gas present. Binding of copper ions to recombinant PrP^{c} (23-231) in the octarepeat unit has been proposed with suggestions that a conformational change may occur as a result. Our IM mass spectrometry results indicate cooperative binding up to 4 copper ions per monomeric unit. Measurements have been obtained for the metal-free and metal-bound PrP^{c} 9+ to 23+ charge states and their rotationally averaged relative cross-sections calculated. Initial experiments suggest that little conformational change is observed upon metal binding using nitrogen as the mobility buffer gas. The study has been extended to include the binding of other metal cations to PrP^{c} (23-231) analysed in the presence of alternate mobility buffer gases, revealing little conformational change observed upon metal binding.

P9.44 Multiplex approaches for biochemical TSE strain characterisation and differentiation

Authors
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Content
Background: Current biochemical transmissible spongiform encephalopathy (TSE) strain typing methods are largely reliant on assessment of Western blot (WB) PrP_{res} banding patterns. WB has proven useful for distinguishing experimental BSE from sheep and classical scrapie but there may be limitations when applied to differentiation of BSE in sheep from certain unusual scrapie strains.

Objective: Development of multiplex immunoassay techniques to identify, characterise and differentiate TSE strains.

Methods: Studies focus on the development and application of established multiplexing technology, to provide simultaneous (fluorescence based) microwell immunoassay (Bio-Plex array reader, Bio-Rad) or WB detection (Typhoon Trio Variable Mode Imager) of multiple target epitopes in a single sample aliquot. This approach involves use of a panel of antibodies, which target comprehensive epitope coverage in critical regions of the PrP N-terminus, enabling profiling of strain associated differences in N-terminus of PK truncated PrP_{Sc}.

Results: Preliminary findings indicate that use of an antibody diplex enabled the relative extent of PrP_{Sc} truncation by PK to be estimated by simultaneous determination of two distinct N-terminal PrP_{res} epitopes. Apparent differences in proportions of these epitopes present in scrapie and BSE PrP_{res} demonstrated the potential of this approach for strain discrimination.

Discussion: Multiplexed assays provide strong possibilities for disease screening and confirmation of TSE strain type, for monitoring the emergence of unusual or atypical strains and for increasing knowledge of the occurrence of TSE variants in the sheep population. Within the limitations of epitope overlap, increasing the degree of multiplexing may provide for the possibility of detecting and differentiating a more comprehensive range of strains than is viable using a battery of monoplex assays.

P9.45 Application of a rapid chromatographic immunoassay to draw the Brain PrP_{Sc} Distribution Curve (BPDC) of confirmed classical scrapie cases

Authors

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Content

Background: Active surveillance for scrapie is mainly performed by rapid diagnostic tests on the basis of post-mortem immunological detection of resistant prion protein (PrP_{Sc}) in caudal brain stem samples. The Prionics®-Check PrioSTRIP is a rapid chromatographic immunoassay kit to diagnose bovine spongiform encephalopathy (BSE) approved by the European Union in 2004. Prionics®-Check PrioSTRIP SR is the revised format of the kit to diagnose TSE in small ruminants. The Prionics®-Check PrioSTRIP SR is approved in Switzerland and submitted for registration in Europe.

Objective: The aim of this study was to assess the performance of the Prionics®-Check PrioSTRIP SR, for classical scrapie diagnosis, and also its ability to detect PrP_{Sc} in different brain areas. The results of the rapid test were correlated to immunohistochemical detection of PrP_{Sc} as one of the "gold standards" for post-mortem TSE confirmation.

Methods: A chromatographic immunoassay, Prionics®-Check PrioSTRIP SR was used on freshly homogenised brain tissue and immunohistochemistry was carried out on formalin fixed paraffin embedded brain tissue using L42antibody against PrP_{Sc}. The levels of PrP_{Sc} in the different brain areas were plotted to give the brain PrP_{Sc} distribution curve (BPDC).

Results and Discussion: BPDC obtained from classical scrapie cases showed a wide distribution of PrP_{Sc} throughout the brain, with a major involvement of the brain stem and cerebellar structures. PrP_{Sc} deposition on the different cortical lobes was more variable. Differences in the curves could be linked to the disease stage of each case, i.e. animals at a more advanced clinical stages had widespread PrP_{Sc} deposition while in preclinical stage cases PrP_{Sc} deposition was restricted to the caudal brain stem. The Prionics®-Check PrioSTRIP SR assessment of PrP_{Sc} correlated well with the immunohystochemical results, indicating that the Prionics®-Check PrioSTRIP SR can be used for the detection of classical scrapie in various areas of sheep brain.

P9.46 Ante-mortem biomarkers of prion disease

Authors

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Content

Definitive TSE diagnosis is currently limited to post-mortem assays for protease-resistance prion protein. By utilizing mass spectrometry based protein profiling and bioinformatics we are developing an ante-mortem diagnostic test using surrogate markers of prion disease. Syrian Golden Hamsters and mice were experimentally infected with either the 263K strain of hamster-adapted Scrapie or the RML strain of mouse-adapted scrapie. Age matched animals served as controls. Cerebrospinal fluid and sera was collected from individual infected and control animals throughout the time course of disease progression. Proteomic profiling of trypsinized hamster cerebrospinal fluid was carried out on a matrix assisted laser desorption/ionization - Fourier transform mass spectrometer utilizing a novel pulse-sequence for the analysis of multiple masses simultaneously. Mouse sera was fractionated using two dimensions of chromatographical separation with the first being lectin affinity and the second being hydrophobicity. Proteins were detected by electrospray ionization time of flight mass spectrometry and subsequently identified by sequencing using coupled MS/MS. Following mass spectrometry, protein profiles were displayed using software developed in house to illustrate differentially expressed features for subsequent validation. Machine-learning algorithms are employed to classify spectra according to disease state. Using a "leave-one-sample-out" cross validation, we have been able to detect disease state better than chance suggesting the validity of proteins as prion disease biomarkers.

P9.47 Evaluation of the diagnostic performance of the Prionics®-Check WESTERN for detection of PrPCJD in brain tissues of Creutzfeldt-Jakob Disease cases

Authors

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Content

Background: Transplantation of human tissues harbours the risk of CJD transmission. Several routes of transmission were reported in the past, one of which is via corneal graft transplants from cadaver-derived human corneal tissue. Estimated epidemiological risk of transmission is relatively low and could be effectively managed through better donor screening.

Objective: Presymptomatic and potentially infective cases could be excluded only after testing of donor´s brain tissue for the presence of the pathogenic isoform of the prion protein, PrPCJD. As of January 2007, brain tissue of all corneal graft donors in the Czech Republic is obligatory tested by Western blot analysis performed in the Czech National Reference Laboratory for Human Prion Diseases at the Department of Pathology and Molecular Medicine of Thomayer´s Teaching Hospital in Prague.

Methods: The Prionics®-Check WESTERN is designed for the in vitro detection of TSE-related PrP_{Sc} and has been validated and approved as rapid test for the BSE testing program in cattle and scrapie in small ruminants which are set up in accordance with Regulation (EC) No 999/2001. Previously, we have assessed the feasibility of the Prionics®-Check WESTERN for the detection of PrPCJD in post mortem cerebral tissue of vCJD patients. To that purpose analytical sensitivity was determined by direct analysis of vCJD brain homogenates obtained from the National Institute of Biological Standards and Controls (NIBSC) using the Prionics®-Check WESTERN.

Results and Discussion: To assess the diagnostic performance of the Prionics ®-Check WESTERN on human brain tissue, we now propose a retrospective, anonymous evaluation study of 650 brain tissue samples that were previously tested negative for CJD and 15 brain tissue samples from confirmed CJD cases positive by immunohistochemistry, histology and Western blot analysis. Diagnostic sensitivity and specificity of the Prionics ® -Check WESTERN on these human tissue samples will be presented.

P9.48 Molecular characterization of low molecular mass C-terminal fragments in different Creutzfeldt-Jakob disease subtypes

Authors

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Content

Background: In sporadic Creutzfeldt-Jakob disease (sCJD) the clinical variability has not been fully explained by molecular studies relating two major types of PrP27-30 with unglycosylated peptides of 21 (type 1) and 19 kDa (type 2) and the amino acid methionine or valine at position 129. In a previous work, by using two-dimensional immunoblot we identified distinct N-terminal truncated forms of prion protein in different sCJD subtypes.

Objective: In the present study, we searched on low molecular mass PrP_{Sc} fragments (below 10kDa) which might correlate with the phenotypic variability observed in different sCJD molecular subtypes.

Methods: Brain homogenates of sCJD subjects were separated by mono- and two-dimentional electrophoresis and immunoblotted by using anti-PrP antibodies directed to N- and C-terminus epitopes.

Results and Discussion: We biochemically characterized by mono- and two-dimensional analyses novel C-terminal PK-resistant fragments migrating at ~5.5 kDa and with an isoelectric point around 4. These fragments were found in almost all different sCJD subtypes with minor variabilities among subjects. These data show the presence of multiple PrP_{Sc} conformations in sCJD and, in addition, shed new light on the correlation between sCJD phenotypes and diseaseassociated PrP molecules.

P9.49 Blood storage affects detection of cellular prion protein on lymphocyte sub-populations and on circulating dendritic cells

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Content

Background: We hypothesize that prion infection may lead to expression of misfolded prion protein (PrP) on a small sub-population of distinct blood cells to the level which can allow its detection by conformation sensitive antibodies using flow cytometry. Due to scarcity of CJD patients the studies of PrP expression may involve shipment of blood samples from several collaborating centers.

Objective: Our study was aimed on defining best conditions for shipment of blood and on examining the effect of different conditions on the level of nonspecific binding of PrPtse specific antibody V5B2.

Methods: The effect of anticoagulant and storage temperature on the binding of PrP antibodies to selected populations of blood leukocytes was studied. Blood of healthy donors (n=10) was collected in standard EDTA and citrate vacutainer tubes. The blood was split into three parts. First was analyzed within 4 hours of blood collection, the other two were stored overnight at different temperatures (R.T., 4oC) before analysis. The analysis of PrP expression was accomplished using quantitative multicolor flow cytometry utilizing R-phycoerythrin conjugated monoclonal antibodies AG4 (binds to PrP 31-51), AH6 (PrP 159-175) and V5B2 (PrP 214-225). Studied cell populations were circulating dendritic cells which were defined as lineage markers negative (CD3, CD14, CD19, CD20, CD56 - all FITC) and HLA-DR (PE DY647 conjugate) positive cells. The second antibody panel utilized CD3 (PerCP), CD19 (APC) and CD56 (FITC) to define T-cell, B-cell and NK-cell population of lymphocytes, respectively.

Results: Significant effect of studied factors on the detection of PrP^{c} was found. Generally, the storage of blood led to decrease of PrP^{c} expression, which was more pronounced at R.T. and with AG4 detection. The extent of decrease was cell population specific ranging from almost complete loss to negligible difference.
Differences in nonspecific binding of V5B2 were not detected.

Conclusions: Both the choice of anticoagulant and storage temperature influences the detection of PrP on sub-populations of blood cells. If shipment of blood samples can not be avoided, than anticoagulation with EDTA and temperature 4oC are conditions of choice. Changes introduced by storage of blood did not introduce artifactual binding of V5B2 suggesting the significance of its eventual binding to cells in CJD blood.

P9.50 An overview of differential protein expression profiling as an alternative approach to diagnosis of TSE disease

Authors
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Content

Background: Current diagnostic tests for the detection of TSE disease are based on the detection of the only definitive disease marker, the protein PrP_{Sc} which in practice is often limited to tissue samples at post-mortem. There is however an urgent need for new diagnostic tools which could be applied in the live animal at pre-clinical stages of TSE infection to identify affected individuals.

Objective: Our approach examines differential protein expression using surface enhanced laser desorption and ionisation time of flight mass spectrometry (SELDI-TOF). Spectral output from all proteins selectively captured from individual samples, are compared as "profiles" in groups of infected and non-infected animals. Differential protein expression between groups is thus highlighted and statistically significant protein "peaks" used to construct a panel of disease specific markers.

Methods: Experiments using a well characterised scrapie murine model displaying severe pathology in the hippocampus were carried out. In an initial experiment brain tissue samples from infected and uninfected groups of mice at the terminal stage (~250dpi) of disease (hippocampus area) were examined. A similar temporal study followed in which brain tissue samples were taken at regular intervals throughout the course of disease. Samples were processed on two ProteinChip array surfaces (CM10 and Q10) and read on a ProteinChip reader (SELDI-TOF). Individual proteins were also isolated and indentified from brain tissue using SELDI in conjunction with a tandem mass spectrometer. Further work was carried out which examined differential protein expression profiles in the CSF of BSE infected non-human primates. CSF samples from six animals infected with BSE were taken at regular intervals throughout the course of disease. These samples were spotted on Q10, CM10 and IMAC arrays and the protein profiles compared with uninfected control CSF samples.

Results: Many differences in protein expression were observed which were statistically significant, some at early time points in disease in both the brain tissue samples and CSF. Three proteins were identified by mass spectrometry from murine brain tissue samples and verified in tissue sections by immunohistochemistry.

Discussion: From our results we have shown that this approach has diagnostic potential for application in TSE disease. We are now extending our studies to include blood plasma samples from ovine models of TSE disease.

P9.51 Evolution of the protocols for the evaluation of TSE rapid tests in the EU

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Content

Background: Scientific advice in the form of evaluation protocols from the former Scientific Steering Committee (SSC) earlier and currently from the European Food Safety Authority (EFSA) sets up the basis for the official approval by the European Commission of rapid tests employed for the monitoring of TSE in ruminants in the European Union (EU). Up to 2007 there have been several updates of these protocols led by scientific expert review.

Objective: The aim of the study is to analyse the evolution over time of the evaluation protocols for post-mortem BSE rapid tests in bovine animals, post-mortem TSE rapid tests in ovine and caprine animals and ante-mortem TSE rapid tests in ruminants to be officially approved for their use in the EU.

Methods: Past and present evaluation protocols for TSE rapid tests to be employed in the EU are reviewed and compared. When identified, both scientific and technical aspects leading to the update of those protocols are assessed.

Results: The results of the review show the evolution over time of the evaluation protocols. Changes to and updates of such protocols are highlighted, including the reasoning behind and tailoring this for the different types of tests, diseases and species.

Discussion: The improvements achieved with the evolution of the protocols, as well as the work ahead in order to further improve them, are discussed.

P9.52 Development of a sensitive sandwich ELISA for the detection of prions using oligomer-specific antibodies

Authors
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Content

Development of a Sensitive Sandwich ELISA for the Detection of Prions Using Oligomer-specific Antibodies Taylor William, Jones Daryl, Smith Martin, English Kate, Catchpole Brian, Bate Clive, Hawke Simon, Williams Alun & Tayebi Mourad*. *Correspondence should be addressed to Dr Mourad Tayebi (mtayebi@rvc.ac.uk) Department of Pathology & Infectious diseases, The Royal Veterinary College Hawkshead Lane, Hatfield, Hertfordshire, AL9 7TA, United Kingdom.

Background: Currently there is no method for detecting PrP_{Sc} in the pre-clinical phase, when humans or animals harbouring the illness may be infectious to others 1. However several groups have made attempts to increase sensitivity by developing reagents that specifically bind PrP_{Sc} as diagnostic tools, including anti-PrP monoclonal antibodies (mAbs) 2,3. Here, we describe the development of a screening assay in a Sandwich format using monoclonal antibodies raised against the native forms of the abnormally-folded prion protein. These antibodies were used subsequently to immuno-detect oligomeric forms in tissues from animals infected with prions.

Objective: To develop a novel sandwich ELISA assay to immunodetect oligomers of PrP_{Sc} in scrapie-infected tissues using oligomer-specific anti-prion monoclonal antibodies with the view of using this assay format as a platform for the detection of prions in blood.

Methods: The monoclonal antibodies produced against native PrP_{Sc} were used to immuno-capture PrP_{Sc} in prion-infected tissues and neuroblastoma cell lines. The novel Sandwich ELISA was performed using these anti-prion antibodies sequentially in different combinations as either immuno-capture or immuno-detection antibody to bind PrP_{C} and/or PrP_{Sc} in tissues. Western blot analysis was also performed using prion-infected tissues in order to assess the sensitivity limit of our novel Sandwich ELISA using our newly produced antibodies.

Results: Sandwich ELISA results have demonstrated that our monoclonal antibodies were effective in detecting PrP_{Sc} in prion-infected tissues. Furthermore, our antibodies were able to only detect the oligomeric forms of PrP_{Sc} in these tissues. Work is currently underway to assess whether these antibodies will be effective in detecting the prion oligomers in blood of prion-infected mice.

Discussion: Using mAbs raised in other projects against native PrP_{Sc}, we have developed a highly sensitive and specific sandwich ELISA able to immunodetect oligomeric forms of PrP_{Sc}. Oligomers are believed to be the neurotoxic infectious particle involved in synapse damage, leading to neurodegeneration 4. Although scarcely distributed in early phase of the disease, oligomers can be detected in tissues of infected animals. Furthermore, having the ability to detect these oligomeric forms in tissues opens the prospect of using the same mAbs to detect oligomers in blood and plasma of prion-infected patients.

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P9.53 Association of csf 14-3-3 protein and neopterin for cjd diagnosis

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Content

Background: The detection of the protein 14-3-3 in the cerebrospinal-fluid (CSF) is presently the main tool for the biological *ante-mortem* diagnosis of CJD, included in the WHO criteria for probable sporadic CJD diagnosis. False positive CSF 14-3-3 detections are observed in several conditions, including noticeably infectious encephalitis, recent stroke, epileptic fit, neoplasia and paraneoplasia.

Objective: The improvement of the biological diagnosis is of a main interest for the ante-mortem diagnosis of Creuztfeldt-Jakob disease. We describe here the use of 14-3-3 western-blotting in combination with neopterin quantitation in the CSF for CJD diagnosis. Neopterin is a pteridine derivative present in body fluids, and a marker for inflammation in human CSF.

Methods: Neopterin was assayed by HPLC. Elevated levels result noticeably from immune system activation as in malignant diseases and infections.

Results: This retrospective analysis included 332 patients initially referred to us for 14-3-3 detection between 2005 and 2007. Only patients with definite clinical diagnosis were considered. Among them, 57 were negative for CSF 14-3-3 detection by western blotting, 31 had a weak signal, and 244 were positive for 14-3-3 detection.
In all definite sporadic CJD (n=61), the neopterin was less than 10 nM, and 14-3-3 was positive in 93%; the 14-3-3 was doubtful in two additional cases. Correlation with codon 129 genotype will be presented. Among probable CJD (n=66), one patient had a neopterin value at 25 nM, 14-3-3 being positive in 95%. Among possible CJD (n=6) two patients had neopterin value superior to 20 nM, one patient has a neopterin at 68 nM, and 67% were positive for 14-3-3. Of note, twenty-six patients patients positive for 14-3-3 had CSF neopterin concentrations higher than 20 nM (median : 35 nM; range 20.4-137 nM), the definite clinical diagnosis was neoplasia in 7 cases, encephalitis and meningitis in 5 cases, other diagnosis including metabolic and vascular diseases.

Discussion: In conclusion, CSF neopterin level higher than 20 nM may be a useful indicator for suspecting CSF 14-3-3 accumulation unrelated to CJD. Correlated to the protein level in CSF, it may be a useful tool for biological diagnosis of CJD.

P9.54 immunoglobulin m but not immunoglobulin G detection during the course of experimental prion disease in mice

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Content

Background: Native prions have generally failed to stimulate an immune response in experimental animal models and only few antibodies had been produced that recognised any form of PrP 1,2. In this study, we show that long-term peripheral delivery of PrP_{Sc}-Dynabeads 3 to mice led to persistent production of anti-prion antibody of the IgM isotype but had also an effect in delaying the course of the disease.

Objective: To assess whether active immunisation using native prions using a novel method of immunisation would lead to a delay in prion disease onset. Further, to measure the dominant antibody isotypic response initiated by the immunisation process.

Methods: RML-infected brain homogenate was adsorbed to immunomagnetic particles (called PrP-Dynabeads) 3 then subsequently used for the active immunisation experiments in mice. Groups of mice were injected once every two weeks via intraperitoneal route with PrP-Dynabeads. Animals were monitored daily for clinical symptoms of scrapie.
The antibody response as well as prion replication in the mice immunised with PrP-Dynabead was assessed using ELISA and Western blot.

Results: Antibody titers were determined by serial dilutions of sera. To determine the correlation between antibody levels and duration of disease incubation time, the mice were bled and serum was added to the ELISA plates. Interestingly, antibody levels were not dramatically reduced prior to onset of clinical signs of disease, indicating that although anti-sera increased the incubation period probably through peripheral reduction of PrP_{Sc} accumulation, they did not completely alter or prevent neuroinvasion and disease establishment in the brain that has led to the death of all animals.
Screening of sera from all mice that have received PrP-Dynabead led to the production of high titres of anti-prion antibodies. These antibodies were of the IgM isotype and did not undergo isotype switch towards IgG antibodies even after long term repeated antigen delivery.

Discussion: Immunisation of mice with PrP-Dynabeads led to chronic secretion of anti-prion antibodies in vivo. The antibody response during the active immunisation process was of the IgM isotype and of high affinity. More importantly, PrP-Dynabeads led to a significant increase in the incubation period.

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P9.55 Prionins which convert PrP form a label for sensitive detection of TSE in blood

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Content
The understanding of the pathology of prion diseases has been heightened by the discovery of the absolute requirement for cellular PrP and the conversion of PrP to PrP_{Sc}, a reaction catalysed by a host-specific factor. Concommittantly, the concept of self-aggregated altered PrP molecules becoming pathogenic and infectious and subsisting to neurodegeneration in TSE was shown to be incorrect. It is reasonable to suggest that the converting factor, not aggregated PrP_{Sc}, is the neurodegenerating factor in TSE. Nevertheless, untill now, because PrP_{Sc} was the only identified constituent in prions, it was widely accepted as the presumed cause of TSE. Given the transmissibility of the disease through blood of infected animals and humans, PrP_{Sc} is suspected to occur, albeit in minute amounts, in the blood of these subjects. A simple assay for the detection of such small amounts of PrP_{Sc}, especially in humans, is needed. Earlier we demonstrated instant conversion of native PrP to PrP_{res} solely by the addition of a synthetic peptide identical to a conserved domain in bovine and ovine prionins (converting peptide). Prionins are alternatively encoded in and expressed from the PrP coding region in a TSE specific manner. The reaction and the products produced showed features similar to those of the pathogenic conversion of PrP in TSE, and we suggested that prionins are the PrP converting factor/factors *in vivo*. Here, using anti-human and anti-bovine prionin monoclonal antibodies, we show that the converting peptide, after *in vitro* conversion of PrP, remains bound to converted PrP and forms a tag on the latter. This opens the possibility for a specific and highly sensitive ELISA to rapidly identify the pathogenic conformer of PrP in blood. Note that this assay needs no cycling and no seeding material. The possibility of high throughput makes this ELISA ideal for large scale screening for BSE contamination in humans.

P9.56 Evaluation the sensitivity of candidate vCJD diagnostic tests

Authors
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Content
Diagnostic tests intended for screening blood for the presence of lethal pathogens are controlled by the EC Medical Devices Directive which ensures that the test is fit for purpose. These tests are considered to be high risk and fall within annex IIA of the directive allowing very strict regulation. Evaluation of Annex IIA tests can require the use of a common technical specification (CTS) to determine a protocol for evaluating performance. It has been recognised that tests for vCJD should come under these regulations. Currently there are very limited quantities of blood component samples from vCJD clinical patients and none from individuals thought to be at risk (recipients of blood components from a donor that later developed CJD). In the absence of sufficient human samples it is likely that tests for variant CJD will be evaluated using samples from animals naturally or experimentally infected with TSEs. To ensure that tests have the sensitivity required to detect infectivity in blood assays initial evaluation will be carried out using diluted tissue homogenates. NIBSC has a bank of materials that have been collected and prepared specifically for test evaluation. These include blinded panels of human tissues spiked into pooled human plasma and plasma from animals known to be negative for TSEs and confirmed to be clinically positive. Panel distribution is controlled by the CJD RC steering committee. Examples of studies used to evaluate test performance will be presented.

P9.57 Recombinant PrP based methods for the detection of PrP_{Sc} by replication in vitro

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Content
Background: The determination of prion infection from blood samples requires the detection of minute quantities of PrP_{Sc}. Protein Misfolding Cyclic Amplification (PMCA) is a technique which can amplify small amounts of seed PrP_{Sc} to a level detectable by conventional methods. We have previously shown that PMCA of whole mouse blood can detect infection in pre- and post-symptomatic animals. Although amplification or replication of PrP_{Sc} is likely to be a key component of a blood-based diagnostic assay the many technical limitations of PMCA mean that it is unlikely to be of practical benefit in the development of rapid diagnostic assays needed. Alternative methods for the amplification or replication of PrP_{Sc} from blood are required that utilise simpler substrate materials than PMCA, ideally synthetic peptides or recombinant PrP.

Objective: To develop novel amplification and diagnostic methodologies for the detection of prion infection in blood. In particular, to investigate the use of recombinant PrP as a substrate for in vitro replication or amplification of PrP_{Sc}. To use the methods developed for investigations of prion propagation, and peripheral pathogenesis with the aim of achieving a quantitative description of blood-borne infectivity throughout the incubation period of prion infection.

Methods: Recombinant PrP was used as the substrate in a variety of different amplification methodologies. Various approaches were used to destabilise the native fold of PrP and hence predispose the polypeptide to conversion. Reactions were initiated with normal or RML-infected brain homogenates spiked into whole blood. Recruitment of recombinant PrP was monitored by a battery of methods; thiazole fluorescence, recruitment of fluorescently tagged peptides, western blotting and SCEPA (Scrapie Cell End Point Assay).

Results and Conclusions: Recombinant PrP can be used as a substrate for in vitro amplification reactions and we have shown that seeded polymerisation reactions are capable of discriminating between normal and infected brain homogenates. However, although infected tissue homogenates can initiate a conformational change in recombinant PrP leading to the formation of amyloid fibrils, this is not associated with the production of infectivity.
These novel approaches give us the ability to rapidly and sensitively distinguish between normal and scrapie infected samples and provide sophisticated tools for the study of seeded fibrillisation and its relationship to the replication of infectivity.

P9.58 Regulation of PrP_{res} glycosylation in sporadic Creutzfeldt-Jakob disease

Authors

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Content

Background: The glycoprofile of pathological prion protein (PrP_{res}) is widely used as a diagnosis marker in Creutzfeldt-Jakob disease (CJD) and is thought to vary in a strain-specific manner. However, that the same glycoprofile of PrP_{res} accumulates in the whole brain of one individual has not been demonstrated.

Objective: We aimed to determine whether and how PrP_{res} glycosylation is regulated in the brain of patients with sporadic or variant Creutzfeldt-Jakob disease.

Methods: PrP_{res} glycoprofiles in various brain regions from 134 patients with sporadic or variant CJD were analyzed with regard to the genotype at codon 129 of *PRNP* and the Western blot type of PrP_{res}.

Results: We showed that i) the regional distribution of PrP_{res} glycoforms within one individual was heterogeneous in sporadic but not in variant CJD patients; ii) PrP sequence, Western blot type of PrP_{res} and cerebral topography significantly regulated PrP_{res} glycoforms ratio; iii) in some molecular subtypes of CJD patients classified as sporadic cases, the glycoprofile of thalamic PrP_{res} was undistinguishable from that observed in variant CJD.

Conclusions: The present work evidences regulations that lead to highly significant variations of PrP_{res} pattern between brain regions in sporadic CJD patients, involving host genotype and Western blot type of PrP_{res}, and may contribute to the specific brain targeting of prion strains, such as vCJD in the thalamus, and have direct implications for the diagnosis of the different forms of CJD.

P9.59 Immuno real time PCR for detection of PrPCWD in chronic wasting disease affected elk(cervus elaphus nelsoni) in Republic of Korea

Authors

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Content

Chronic wasting disease (CWD) has been recognized as an important prion disease in native North American deer and Rocky mountain elks. CWD was first detected in imported elks from Canada in 2001 in Republic of Korea. Additional cases were also found in elks in 2004 and 2005. Immuno-real time PCR is an extremely sensitive detection methods which combines the specificity of antibody detection and the sensitivity of PCR. We have established an immuno-PCR exploiting real-time PCR technology, in order to improve this immunodetection method and make quantification possible. To illustrate the advantages of immuno-PCR, we have compared it with a conventional ELISA technique in experiments aimed detecting the resistant form of prion protein in elk brain extracts. The detection limits is also improved in immuno-real time PCR(1pg/ml) comparing with ELISA results(7~15ng/ml). This technique could be a choice for post-mortem diagnostic methods in infected elks.

P9.60 Comparing Italian and U.S. confirmatory BSE protocols developed for the detection of classical, high and low molecular weight BSE cases

Authors

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Content

Background: Three distinct forms of bovine spongiform encephalopathy (BSE) have been identified to date: Classical (C), Low (L) or High (H) type BSE. At present in Italy out of 141 confirmed BSE cases 3 have been ascribed to a L-type variant, named BASE. In the U.S. 3 BSE cases, one of C-BSE (in a cow imported from Canada) and 2 H-type cases (both U.S. born) have been reported.

Objective: Aim of this study was to compare the Italian and U.S. immunohistochemical (IHC) and Western blot (WB) confirmatory BSE protocols.

Methods: Serial obex sections from the U.S. and an Italian C-BSE case, and from a H-type U.S. case and a L-type Italian (BASE) case were tested by both the Italian and U.S. IHC and WB methods. The Italian IHC protocol to detect pathological prion protein (PrP_{Sc}) consists in a 25 minutes formic acid treatment of tissue sections followed by hydrated autoclaving at 121° C. PrP_{Sc} is detected by primary antibody F99/97.6.1 and the reaction visualized by the avidin-biotin-peroxidase complex. The U.S. IHC procedure uses formic acid treatment for 5 minutes, demasking in decloaking chamber and automated staining on the Ventana NexES carousel. F99/97.6.1 is applied as primary antibody and alkaline phosphatase as detection reagent. The U.S. confirmatory WB protocol resembles the OIE-recommended PrP_{Sc} enrichment method; the Italian one uses 10% Sarkosyl homogenisation and ultracentrifugation steps. Both WB methods use the PrP-specific 6H4 antibody as primary antibody.

P10.01 Dynamics based drug design (DBDD) to regulate the prion's pathogenic conversion process

Authors

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Content

Background: Prion proteins are key molecules in transmissible spongiform encephalopathies (TSEs). Although the precise mechanism of the conformational conversion process from the cellular form (PrP_C) to the scrapie form (PrP_{Sc}) is still unknown, it may be generally composed of two stages, i.e. stage 1: unfolding of PrP_C and stage 2: nucleation dependent generic folding to PrP_{Sc}. Therefore it may be feasible to design a small compound which can stabilize the PrP_C conformation and regulate the reaction process at stage 1.

Objective: We aimed at developing a general strategy for designing a small compound, termed "chemical chaperon", which can selectively stabilize the PrP_C conformation thereby inhibiting its pathogenic conversion process.

Methods: We conducted in silico screening to find compounds that fitted into a 'pocket' created by residues undergoing the conformational rearrangements between the native- and the sparsely populated high energy states (PrP*) elucidated by Carr-Purcell Meiboom-Gill relaxation dispersion method (NMR), and directly bind to those residues. Hit compounds were tested by ex vivo and in vivo screening, and if effective, they were subjected to determination of the complex structure and further lead optimization processes. The cyclic process between (1) structure determination, (2) in silico design, (3) organic synthesis, and (4) bioassay, was repeated recursively, and termed DBDD.

Results: More than hundred compounds were tested in a TSE-infected cell culture model, and more than twenty compounds including, 2-pyrrolidin-1-yl-N-[4-[4-(2-pyrrolidin-1-yl-acetylamino)-benzyl]-phenyl]-acetamide, termed GN8, efficiently reduced PrP_{Sc}. Subsequently, administration of GN8 was found to prolong the survival of TSE-infected mice. Heteronucler NMR and computer simulation showed that the specific binding sites are the A-S2 loop (N159) and the region from helix B (V189, T192 and K194) to B-C loop (E196), indicating that the intercalation of these distant regions termed 'Hot Spots' hampers the pathogenic conversion process.

Discussion: Dynamics Based Drug Discovery (DBDD) strategy demonstrated here focusing on the hot spot of PrP_C will open the way to the development of novel anti-prion drugs.

P10.02 Increase of monoamine oxidase-B activity in the brain of scrapie-infected hamsters

Authors

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Content

In the present study, the purpose is to determine activities of monoamine oxidases (MAO) in the brain of 263K scrapie-infected hamsters during the development of this experimental prion disease. Indeed, MAO activity modifications which have already been related in aging and neurodegenerations is suspected to be involved in the neuron loss process by elevated hydrogen peroxide formation. Monoamine oxidase type A (MAO-A) and B (MAO-B) activities were followed in the brain at different stages of the disease. MAO-A activity did not change significantly during the evolution of the disease. However, concerning the MAO-B activity, a significant increase was observed from 50 days post infection and through the course of the disease and reached 42,9 ± 5.3 % at its ultimate stage. Regarding these results, MAO-B could be a potential therapeutic target then we have performed a pre-clinical treatment with irreversible (Selegiline or L-deprenyl™) or and reversible (MS-9510) MAO-B inhibitors used alone or in association with an anti-scrapie drug such as MS-8209, an amphotericin B derivative.

Our results show that none of the MAO-B inhibitors used was able to delay the onset of the disease. Neither these MAO-B inhibitors nor R-NMDA inhibitors (MK-801) can enhance the effects of MS-8209. The present findings clearly indicate a significant increase of cerebral MAO-B activity in scrapie-infected hamsters. Furthermore, inhibitors of MAO-B do not have any curative or palliative effect on this experimental model indicating that the raise of this activity is probably more a consequence rather than a causal event of the neurodegenerative process.

P10.03 Cyclodextrins and prion disorders

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Content
Background: Prion diseases or Transmissible Spongiform Encephalopathies (TSEs) are a group of fatal neurodegenerative diseases of humans and animals. The nature of the infectious agent and the pathogenic mechanisms of prion disease are not yet fully understood, research suggests however that a build up of the abnormal form (PrP_{Sc}) of the endogenous host prion protein (PrP_C) is responsible for TSE's. Despite years of research, no cure is available for TSEs. Our group has identified that cyclodextrins (CD) can clear PrP_{Sc} in infected cell culture.a Their action in TSEs is investigated further here.

Objective: To investigate the action of Cyclodextrins in Prion Disorders.

Methods: Compounds were tested for antiprion activity by culturing the neuroblastoma cell line (N2a) infected with 22L (N2a22L20) in the presence of the compounds employed for up to 3 weeks. Cells were then lysed and examined for PrP_{Sc} using SDS-PAGE and western blotting techniques.

Results: We have identified that CDs have the ability to clear PrP_{Sc} in cell culture. A concentration of 500μM β-Cyclodextrin was seen to give clearance of PrP_{Sc} comparable to that of Congo red after two weeks, and has a half-maximal inhibitory concentration (IC50) of 75μM. Here further investigation has yielded second generation compounds which can clear PrP_{Sc} from cell culture at a concentration of 10μM in two weeks.

Discussion: The Cyclodextrins are macrocyclic, non-reducing maltooligosaccharides made from alfa-1,4 linked glucose units, and are used as complexing agents to increase drug delivery, and solubility. The CD's antiprion action may be attributed to their ability to modify the location of the PrP molecules in the lipid raft domains of the cell. Here, the CDs have been examined for antiprion activity and it is shown that simple modification of the structure has dramatic effects on the ability of CDs to clear PrP_{Sc} in cell culture. The mechanism of action Cyclodextrins in prion disease is currently under investigation by our group.

References: A. M. Prior, S. Lehmann, M. S. Sy, B. Molloy, and H. E. M. McMahon.
J. virol, Oct. 2007, p. 11195–11207 Vol. 81, No. 20

P10.04 Asymptomatic carriers of CJD-specific mutation and Doxycycline :Could be the drug useful in the prophylaxis of genetic CJD?

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Content
Asymptomatic carriers of CJD-specific mutation and Doxycycline : could be the drug useful in the porphylaxis of genetic CJD? Mitrová Eva National Reference centre for Prion Diseases, Research base of Slovak Madical University, Bratislava, Slovakia.

Background: Increasing number of studies provide evidence on the efficacy of Doxycycline to prolonge survival time in CJD patients and incubation period in experimental scrapie. It was suggested that Doxycycline might interact with pathological prion protein and interfere with prion amyloid formation. Hypothetically, Doxycycline may be even more beneficial if applied before the onset of the disease, in asymptomatic carriers of CJD-specific mutation. Probably it may significantly prolong the preclinical stage and postpone or prevent the clinical onset of genetic CJD (gCJD).

Objective: Slovakia is characterized by geographic clustering of gCJD cases with the world wide occurring, most frequent *PRNP* mutation E200K. Up to now 137 definite gCJDE200K have been verified. Out of 438 tested relatives, 36 % are asymptomatic carriers of this mutation. They represent the „genetic CJD-risk group" in Slovak population. The penetrance of this mutation is 59%. There is no preclinical test for the differentiation of carriers developing the disease. Up to now, the number of Slovak „healthy„ carriers is 157, their age distribution is 45.5 % under 50 years and 54.5 % over 50 years. Considering preliminary results achieved by Doxycyline in CJD patients or scrapie infected animals, prophylactic administration of this drug to carefully selected carriers should be considered.

Discussion: Asymptomatic carriers of CJD-specific mutation are at present the only CJD-risk group where preventive measures (pharmaceutical, molecular genetic) as soon as would be available - could be applied. The aim of this contribution is to open a discussion concerning a prophylactic administration of Doxycycline to asymptomatic carriers of the mutation E200K. Questions concerning the age for starting the treatment in individual carriers (considering the confirmed anticipation in affected families), the dosis and intervals between individual Doxycycline administration cycles, side effects, ethical issues etc. should be discussed. For countries with known asymptomatic carriers of CJD-specific mutation a common project for prophylactic administration of Doxycyline is suggested. Supported by Slovak Ministry of health, project No.: 2005/33-SZU-11.

P10.05 A variety of anti-prion compounds discovered by an in silico screening based on PrP^C structure: a correlation between anti-prion activity and binding affinity

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Content

Background: Transmissible spongiform encephalopathies (TSEs) are fatal and untreatable neurodegenerative diseases that include Creutzfeldt-Jakob disease in human and scrapie and bovine spongiform encephalopathy in animal. Conversion from the cellular form (PrP^C) to the scrapie form (PrP^{Sc}) is a key event in the pathogenesis of all TSEs, which are associated with the accumulation of the PrP^{Sc} in the brain of human and animal.

Objective: It may be possible to intervene in this conversion process by introducing a chemical chaperon which is capable of stabilizing the PrP^C conformation. To create the lead compound for prion disease treatment, we searched for such chemical chaperons.

Methods: To discover chemical chaperons, we performed in silico screening based on the PrP^C structure. The lead-like subset of the ZINC database and AutoDock were used for docking simulations. To evaluate the anti-prion activities of the selected compounds by the calculated docked energy, we conducted ex vivo screening with the mouse neuronal cell line stably infected with Fukuoka-1 strain. To analyze the direct interaction between the PrP^C and selected compounds, surface plasmon resonance was measured using the BIAcore system.

Results and Discussion: We selected more than 200 compounds that were the high ranks in in silico screening and commercial available and conducted ex vivo screening. 24 compounds with diverse chemical structures were found to significantly inhibit the PrP^{Sc} production in the cell cultures. These compounds would be very useful as lead compounds for prion disease treatment. Moreover, this unbiased broad spectrum of effective to non-effective compounds in ex vivo screening would provide valuable information for the tuning of program parameter in in silico screening. Surface plasmon resonance studies revealed that the binding affinities of most compounds roughly correlated with their anti-prion activities. On the other hand, there were some compounds with low affinities and high anti-prion activities. Existence of such compounds indicates involvement of some modulation factors for the anti-prion activity other than the direct binding to PrP^C. The categorization of these diverse compounds would facilitate the anti-prion drug discovery and understanding the pathogenic conversion mechanism.

P10.06 Scrapie Affected Mice Survive Longer With Parenteral Pentosan Polysulphate

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Content

Background: We have shown that pentosan polysulphate (PPS), a heparin analogue with anti-coagulant, -thrombotic, -inflammatory and -viral activity, increases survival time, and reduces susceptibility, if given parenterally early in rodent scrapie and BSE pathogenesis. The later in disease progression the drug delivery is initiated the smaller the increase in survival time: efficacy is also TSE model dependent. Some vCJD affected patients have received intraventriculocerebral PPS because highly charged drugs are unlikely to cross the blood brain barrier. Survival was extended compared with non-PPS treated individuals but brain atrophy progressed.

Objective: To test potentially clinically relevant interventions in TSE models.

Methods: Recently we have established a low dose, 100% incidence, intravenous model of ME7 in C57BL mice. Incubation period, survival time, TSE incidence, neuropathology, PrP^{Sc} deposition, and residual infectivity in long term survivors were analysed after PPS was given subcutaneously or intravenously at intervals from TSE exposure.

Results: Early intervention gave very prolonged survival as expected. However not all infectivity was cleared, with some animals living more than 100% longer than controls having evidence of TSE infection. Animals only given PPS parenterally within the clinical phase survived longer than untreated controls and exhibited reduced neuropathology in specific brain areas.

Discussion: PPS treatment soon after exposure increased survival time, novelly with subcutaneous delivery, but infectivity had not been completely cleared. The kinetics of infection in this model and how drug impact this require further study. Treatment later after exposure reduced efficacy, however proof of concept was established that parenteral PPS, starting even after symptoms appear, can increase survival time. Such indirect evidence that PPS can enter the CNS may be due to the loss of blood brain barrier integrity late in disease. Transient depletion of BBB to deliver PPS from the periphery to the CNS of patients with vCJD would obviate the complications of intraventricular catheterisation and might increase drug distribution into parenchyma thus slowing or preventing the progression of brain atrophy.

P10.07 Cholesterol and prion disorders

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Content
Cholesterol and prion disorders. Brendan Molloy1, Hilary E. M. McMahon1 UCD1 School of Biomolecular and Biomedical Science, Ardmore House, University College Dublin, Dublin 4.

Background: During prion infection abnormal misfolding of the endogenous glycosylphosphatidyl inositol (GPI) anchored prion protein (PrP_C) is thought to occur in 'lipid or cholesterol raft' domains producing the scrapie isoform PrP_{Sc}. It has been shown that removal of cholesterol using methyl beta cyclodextrin (MβCD)a or inhibition of cholesterol esterificationb clears scrapie from infected cell lines. This finding prompted further investigation into the significance of cholesterol for maintenance of scrapie infection.

Objective: To investigate the antiprion effect of a range of known modulators of cholesterol synthesis and transport.

Methods: The neuroblastoma cell line (N2a) infected with the scrapie strain 22L was treated with a range of cholesterol manipulating compounds at the time of passage over a two week period. Cells were then lysed at different time points and examined for PrP_{Sc} using SDS-PAGE and western blotting using standard techniques.

Results: After two weeks of treatment PrP_{Sc} was reduced to undetectable levels in infected cell lines that were treated with compounds modifying cholesterol content and type i.e. esterified or free cholesterol.

Discussion: Removing cholesterol is known to disrupt lipid rafts preventing co-localisation and conversion of PrP_C to PrP_{Sc}. Altering cholesterol levels affects the plasma membrane increasing membrane stiffness, this would affect protein interactions and possibly the access of PrP_{Sc} to PrP_C. Abnormal cholesterol metabolism could affect intracellular protein transport, retarding normal maturation and trafficking of PrP_C.

Reference: aPrior M, Lehmann S, Sy M S, Molloy B, and McMahon H E. 2007. J Virol. 81(20):11195-207.bPani A, Norfo C, Abete C, Mulas C, Putzolu M, Laconi S, Orrù CD, Cannas MD, Vascellari S, La Colla P, Dessì S. 2007. Antimicrob. Agents Chemother. 51(11):4141-7.

P10.08 Is Immune System Really Blind To Prion Infection?: A Potential Role For CD4+ Regulatory T Cells on an anti-PrP response and prion progression.

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Content
Background: Due to the strong tolerance to endogenous PrP, immune system seems to be blind to prion infection as no specific immune response develops in the lymphoid organs of wild-type mice. However, preliminary results indicate that infection with 139A strain is accompanied by decreased T and B cell responses to the main PrP class II-restricted peptide. This project is based on the hypothesis that the accumulation of PrP_{Sc} in the periphery could lead to the stimulation of PrP-specific CD4+CD25+Foxp3 regulatory T cells (Tregs) as described in other chronic infectious diseases. Yet it is not clear whether these cells could influence the course of infection and if their elimination could be beneficial to the host.

Objective: We tested whether PrP-specific Tregs were implicated in the control of an anti-PrP immune response in 139A-infected mice and might influence the progression of experimental scrapie.

Methods: The frequency of IFNgamma-secreting CD4+ precursor T cells in response to the main T cell epitope was measured by ELISPOT in healthy or infected mice after in vivo or in vitro removal of Tregs by anti-CD25 mAb treatment. Treg accumulation was quantified by flow cytometry in blood or lymph nodes during the course of infection and their suppressive function was assessed on proliferation to anti-CD3 mAb or PrP peptide.

Results: In vivo and in vitro removal of CD4+CD25+ Tregs restored the PrP-specific T cell response to the main CD4+ PrP T cell epitope in infected mice. However, the differences in Treg counts and suppressive activity between healthy and infected mice were low. Injection of anti-CD25 mAb into mice at 14-day-intervals during the first 10 weeks following 139A infection, increased PrP_{Sc} accumulation in the spleen.

Discussion: These preliminary data point to a possible negative interaction between PrP_{Sc} accumulation and the development of a specific immune response to PrP. They indicate that the immune system is not blind to prions and suggest for the first time a role for Tregs. Moreover, the increased accumulation of PrP_{Sc} induced by chronic elimination of CD25+ T cells after prion infection suggests that PrP-specific effector T cells might emerge in the absence of Tregs.

P10.09 Heparan Mimetics Oligosaccharides for Anti-prion Therapies

Authors
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Content
No therapy is actually available for prion diseases. Heparan sulfate bind PrP and play an active role in the PrP catabolic pathway, for this reasons, we thought that new heparan mimetics (HMs) initially developed for their ability to stimulate tissue repair would represent good candidates for the development of a PrP-targeted therapeutic against prion diseases. Here, we report the synthesis, structure characterisation and structure-activity study concerning the relationship between the anti-prion activity of HMs oligosaccharides, their degree of sulfation and the influence of the hydrophobic cores (phenylalanine derivatives). In order to do that, five synthetic HMs oligosaccharides, different in their degree of sulfation but of same molecular weight (ie. 15 glucose units), were tested for their capacities to inhibit the replication of PrP_{res} in chronically infected cells (ScSN56). It is very interesting to point out that small HMs oligosaccharides were as efficient as the complete molecules to inhibit PrP_{res} accumulation only if the degree of sulfation was around or equivalent to 1. This activity was enhanced by introducing hydrophobic moieties. In conclusion, our data suggest that the reduction of the molecular size of these drugs (HMs oligosaccharides) and hydrophobicity increase their capacities to cross the brain blood barrier, which is crucial for the treatment of neurodegenerative disorders.

P10.10 Knockdown of Mouse *PRNP* in N2a Cells by Introducing microRNA

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Content

Background: To delay the onset of prion diseases as one of the therapeutic approaches, reduction of the amount of cellular prion protein is required. Although knockout strategies have been applied to reduce the gene expression, these are not practically applicable for therapeutic issues. The use of RNAi was recently shown to result in high and specific inhibition of targeted gene expression.

Objective: Attempts were made to access RNAi technology as a new therapeutic tool for prion diseases. Before *in vivo* study, optimal conditions for down-regulation of *PRNP* by miRNA were investigated in N2a cell culture model.

Methods: Two siRNAs targeting *PRNP* were designed and transfected into N2a cells. The cells were harvested at each time point and the down-regulation pattern was determined by Western blot. Pre-miRNAs targeting the same sequences of the siRNAs were designed by an algorithm. The pre-miRNAs were cloned into a vector by single or chaining manner and were introduced into N2a cells. The efficacy of down-regulation through the miRNAs was analyzed.

Results: Each siRNA at concentration of 100nM was introduced into N2a cells and the time-dependent inhibition was analyzed. The expression was the most highly inhibited at 48h after transfection by 94.9% with siRNA1 and 96.6% with siRNA2 considering that of non-siRNA treated cells as 0%. The expression of prion protein was suppressed by 10.6%, 18.7% and 77.5% by miRNA1, miRNA2 and the chained miRNA1-2, respectively.

Discussion: The decreasing pattern of down-regulation after 48h might be based on that transfection efficiency depends on cell types and the siRNA effect only lasts for a period of time. When miRNA1 and 2 targeting different site of *PRNP* are expressed in clusters in long primary transcripts, the inhibition was more efficient than an independent manner. Further in vivo studies could be performed based on these results using appropriate miRNA delivery system such as lentiviral vector.

Acknowledgments: This study was supported by BK21 Program for Veterinary Science, KRF 2006-005-J502901 and the Research Institute of Veterinary Science, Seoul National University, Korea.

P10.11 Molecular dynamics simulation of the interaction between an anti-prion compound GN8 and cellular prion protein

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Content

Background: Prion diseases are considered to be primarily caused by the conversion of prion protein(PrP) from the normal cellular form (PrP_C) into the abnormal scrapie-type isoform (PrP_{Sc}). Recently, Kuwata and his colleagues found a low-molecular-weight compound GN8, which suppress propagation of PrP_{Sc}. GN8 is expected to have a good potentiality as a lead compound. Thus optimization of GN8 structure according to SAR is in progress in our laboratory.

Objective: Although GN8 acts as a chemical chaperon, details of its interaction with PrP_C are still unknown. The proposed putative binding mode was obtained from the docking simulation, its dynamical behaviors are still unclear. The aim of research is to contribute to the optimization of GN8 by elucidating the dynamical behavior of the interaction between GN8 and PrP_C by using the molecular dynamical simulation.

Methods: To research the binding state of GN8, we performed molecular dynamics(MD) simulation using full-atom model. GN8 and PrP_C which are placed at the mutually distant positions, and immersed in a truncated octahedron box with ^9000 water molecules. After the position of GN8 was restrained during the stage of the equilibrium run, sampling simulation without any restraints was performed at 300K in 10ns. 15 structures were prepared initially to remove the initial configuration dependencies.

Results and Discussion: As a result, GN8 dissociated from PrP_C in five runs. In the 10 runs GN8 bound to PrP_C, but its binding mode was different from the putative one. In three runs one side of GN8 was buried in the cavity of the putative binding mode, but the other side exhibited very large fluctuation. In the rest runs GN8 kept binding to C terminus side in the helix B of the PrP_C. In this way the binding mode was dynamically switching in a time dependent manner. Results of this research suggested that the interaction of GN8 with PrP_C is dynamically fluctuating and may be quite different than the picture represented by putative single binding mode. Thus we need clarify further the interaction between GN8 and PrP_C to characterize the accurate binding mode, in order for promoting the anti-prion drug optimization.

P10.12 The 5'Untranslated Region of the prion protein gene as a potential target for therapy

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Content

Background: A critical event in the pathogenesis of prion diseases is the post-translational conversion of the cellular prion protein (PrP_C) into the disease-associated misfolded isoform (PrP_{Sc}). As both PrP_C and PrP_{Sc} are required for pathogenesis and infection they are targets for therapeutic strategies. To date, however, there is no cure for prion diseases.

Objective: Recently modulation of expression via the 5'Untranslated Region (5'UTR) in the mRNA of the human prion protein gene (*PRNP*) was observed. In our studies we focus on a further characterization of translational regulation conferred by this region as a potential approach for therapy. The observation that PrP null mice are protected against disease and show almost normal development and behaviour underlines the rationale for this approach.

Methods: We employed a luciferase reporter assay to detect translational effects via the PrP5'UTR. In this assay the 5'UTR of the *PRNP* gene was cloned upstream a gene for luciferase regulating its translation. A second construct comprising the 5'UTR of the gene for Alzheimer's precursor protein (APP) served as a control for monitoring effects via the PrP5'UTR. For the APP5'UTR an atypical iron responsive element (IRE) has been identified and expression is modulated by intracellular iron levels. SH-SY5Y cells stably transfected with PrP5'UTR and APP5'UTR luciferase-constructs were treated with the iron chelator deferoxamine (DF) and levels of luciferase expression assessed by detection of luminescence signals.

Results and Discussion: Treatment with the iron-chelating agent deferoxamine resulted in decreased luminescence signals by ca. 20% for both APP and PrP5'UTR conferred translation. The similar translational regulation by treatment with DF for the PrP5'UTR compared to APP5'UTR indicates the presence of an iron-responsive-type element and confirms that expression via the PrP5'UTR can be modulated by exogenous interference.

P10.13 Correlation between Global Thalamic Atrophy and Survival of CJD Patients

Authors
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Content

Background: Diffusion abnormalities in the basal ganglia and thalamus are commonly found in CJD and have been proposed as a diagnostic marker. Their relationship to clinical course and symptomatology is unknown.

Objective: To investigate prediction of survival from anatomically defined diffusion values obtained early in the course of the disease.

Methods: 22 patients (17 fCJD with the E200K mutation, 5 sCJD) were examined early in the course of the disease (3.6±2.4 mos from onset). MRI was performed on a 1.5T GE Signa, and brain diffusion was quantified as Apparent Diffusion Coefficient (ADC, μ2/s) from Diffusion-Weighted Imaging (DWI) at b=1000 s/m2. ADC values were thresholded at <1250 to avoid CSF contamination. Volumes of interest (VOIs) were defined for the caudate nucleus (CN), putamen (Put), globus pallidus (GP) and thalamus (Thal) by FIRST (FSL4), and within those VOIs we extracted the ADC values, averaged bilaterally.

Results: There were no significant differences on any of the measures used here between fCJD and sCJD patients. The age of the patients was 60±7, their CNS score was 11±5 and their MMS 23±5; they survived 4.5±4 months after this visit. In univariate regressions, age, duration of disease, neurological CNS score and MMS did not correlate significantly with survival. Significant negative correlations with survival were obtained for ADC in the Put (r=-.49, p<.05) and Thal (r=-.55, p<.008), but not in CN or GP. A multiple stepwise regression was conducted using age, CNS, MMS, Put and Thal ADC, and total brain volume. Only thalamic ADC and MMS were retained, for a total r=-.78 ($F_{2,12}$=9.34, p<.004). Total brain volume was marginally significant (r=-.40, p<.07).

Discussion: The thalamus is heterogenously affected in CJD, showing both elevated and reduced diffusion in different subdivisions and during different stages of the disease. In the current study, elevated ADC, indicating subtle loss of tissue, was associated with shorter survival. Thalamic ADC values > ~775 predicted very short survival. The regression was similar in fCJD and sCJD patients. These data require replication, and are also limited by using global thalamic values derived by an automatic VOI technique. They suggest, however, that elevated thalamic diffusion may have prognostic implications.

P10.14 Pharmacovigilance clinical studies with P-Capt® prion capture filter in human volunteers

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Content

Background: The P-Capt® prion capture filter is the first CE marked product to demonstrate adequate TSE infectivity reduction from human leucodepleted red cell concentrates (RCC) in both brain spike (exogenous) and blood borne (endogenous) infectivity models.

Aims: Two post market pharmacovigilance clinical studies on human volunteers were conducted to assess the effect of filtration of units of human leucodepleted RCC with the P-Capt® filter on; a) red cell recovery and b) neoantigenicity of re-infused units. Both studies were single centre, randomised, open label studies.

Methods: In the red cell recovery study, 29 subjects had one unit of whole blood removed using a whole blood CPD/SAGM collection set. Following leucodepletion and processing, either SAGM or autologous plasma was added to the packed red cell. Each unit was randomly assigned to one of the following cohorts; Cohort 1 – P-Capt filtered blood at Day 1 with 28 day storage in plasma, Cohort 2 – No P-Capt filtered blood with 28 day storage in plasma, Cohort 3 – P-Capt filtered blood at Day 1 with 42 day storage in SAGM and Cohort 4 – No P-Capt filtered blood with 42 day storage in SAGM.

At the end of the storage period, an aliquot of the stored blood was labelled with 51Cr and reinfused back into the subject for evaluation of red cell recovery. In the neoantigenicity study, 14 subjects were stratified by blood type and had one unit of whole blood removed using a whole blood CPD/SAGM collection set. After processing and leucodepletion, the unit was randomly assigned to be stored in either autologous plasma (28 days) or SAGM (42 days), followed by filtration at day 1 using the P-Capt® filter. Evaluation for changes in red cell antigens (D, C, c, E, e, K, k, Fya, Fyb, S, s) band 3, CD47 and reactivity with a panel of normal plasma occurred at the end of storage before the RCC was re-infused to the donor. Subjects were then evaluated at 6 weeks post infusion (Day 70 for plasma storage and Day 84 for SAGM storage) by direct antiglobulin testing and antibody screening.

Results: In the red cell recovery study, data demonstrated that; 85.7% of the subjects in the P-Capt® 28 day group (red cells stored in plasma) and 100% of the subjects in the P-Capt® 42 day group (red cells stored in SAGM) had red cell recoveries of > 75% at 24 hours. There were also no significant differences between the P-Capt ®and no P-Capt® groups in percent recovery at 24 hours. In the neoantigenicity study, data demonstrated the lack of changes in the results of red cell antigens, band 3, CD47, direct antiglobulin testing and antibody screening for red cell antigens 6 weeks after infusion of autologous prion filtered red cells.

Conclusion: The use of the P-Capt® prion filter demonstrated no indication of the development of neoantigenicity and red cell recovery rates were within established guidelines following filtration and re-infusion of autologous red cells in normal volunteers.

P10.15 Creation of a transgenic mouse expressing a T cell receptor directed against an epitope of the prion protein

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[1]Inserm.

Content

Background: Background: Because PrP_{Sc} results from an infectious conversion of PrP_C, a host-encoded self protein, prions are not immunogenic and do not spontaneously elicit immune responses in infected hosts. Notwithstanding this difficulty, several groups are trying to develop immune-based therapies against prion diseases and other neurodegenerative diseases, by forcing the immune system to overcome tolerance, or by passively transferring lymphocytes or antibodies raised under non tolerogenic conditions, against the pathogenic proteins.

Objective: Major difficulties on the way to efficient and safe vaccines against neurodegenerative diseases are due to the fact that we still do not know 1) how immune effectors (antibodies and/or T cells) fight neuronal death, 2) whether microglia and astrocytes contribute positively or negatively to therapy and should therefore be mobilized and 3) whether it is possible to conceive efficient vaccines without deleterious autoimmune secondary effects against brain self proteins.

Methods: The creation of transgenic (tg) mice artificially expressing a T cell repertoire predominantly directed against PrP will be of major help for exploring the above issues. To this aim, the rearranged alfa and β chains of a CD4+ T cell TCR reactive against PrP, have been cloned and are currently microinjected into fertilized eggs.

Results: A first founder expressing the β chain alone was isolated and was bred on a PrP+ and a PrP-null background. Offspring at 2nd and 3rd generation are currently analyzed for phenotype and function. Our data show that the transgenized rearranged β chain (Vβ5+) dominates the CD4+ T cell rearrangements, both on a PrP+ and a PrP-null background and that, most importantly, this single β chain pairing with stochastically rearranged a chains promotes in PrP-null mice only, a CD4+ T cell repertoire predominantly directed against PrP.

Discussion: On the basis of these data, we are initiating adoptive Tg CD4+ T cell transfers (polarized Th1 or Th2) into healthy and infected (139A) recipients in order to find out what type of CD4+ T cells is most therapeutic and whether there is a risk that adoptive T cell immunotherapy may generate autoimmune complications.

P10.16 Migration of mesenchymal stem cells to brain lesions of prion disease

Authors
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Content

Background: Recently, we reported that intraventricular administration of anti-PrP monoclonal antibodies (mAbs) partly antagonizes the disease progression in murine scrapie model, even when administration was initiated after clinical onset of the disease. The result also suggested that an improvement of mAb delivery may enhance the effect. Bone marrow-derived mesenchymal stem cells (MSCs) are reported to migrate to brain lesions in the experimental models of ischemia, brain tumor, and neurodegenerative diseases, and ameliorate functional deficits. Thus we examined whether MSCs migrate to brain lesions of prion diseases.

Methods: Immortalized human MSCs (hMSCs) that stably expressed LacZ gene were used. The hMSCs were transplanted into the left hippocampus or thalamus of mice inoculated with the Obihiro or Chandler strain at 120 days post infection (dpi). Cryosections of brains were prepared and hMSCs were detected by IFA using anti-b-galactosidase (b-gal) antibodies.

Results and Discussion: Two days after transplantation, the b-gal-positive hMSCs became detectable in the contralateral hippocampus and thalamus of mice infected with prion. The number of b-gal-positive hMSCs in the contralateral side increased gradually up to 3 weeks after the transplantation. In contrast, the hMSCs were hardly detected in the contralateral hippocampus and thalamus of mock-infected mice. Interestingly, migration of hMSCs to hypothalamus differed between prion strains; hMSCs migrated to the hypothalamus of mice infected with the Obihiro strain but not well migrated to the hypothalamus of mice infected with the Chandler strain. This tendency was consistent with a severity of the PrP_{Sc} deposition and/or spongiform changes at the hypothalamus. In addition, hMSCs migrated to the brain lesions of mice infected with prion even via intravenous injection. The hMSCs produced various trophic factors such as NGF, BDNF, CTNF, NT3, NT4/5, and VEGF in the brains of mice infected with prion, and could differentiate into cells of the neural lineage such as MAP2-, GFAP-, or CNPase-positive cells. These results suggest that MSCs act as cellular vectors for delivery of therapeutic genes to the brain lesions of prion diseases and may be useful for the regeneration of degenerated neuronal tissues.

P10.17 A transdominant-negativ 37kDa/67kDa laminin receptor mutant as therapeutic tool for the treatment of prion diseases

Authors
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Content

Prion diseases are a group of fatal neurodegenerative diseases. The 37kDa/67kDa laminin receptor (LRP/LR) has been identified as the cell surface receptor for cellular 1 and infectious prions 2. Recently, we showed that a N-terminally truncated LRP mutant encompassing the extracellular domain of the LRP/LR (LRP102-295::FLAG) is secreted into the extracellular space and may act in a transdominant negative manner as a decoy by trapping PrP molecules 3. In order to investigate the therapeutic potential of the LRP102-295::FLAG mutant in vivo with respect to a possible delay or prevention of prion disease, transgenic animals were generated ectopically expressing LRP102-295::FLAG in the brain. The plasmid encoding LRP102-295::FLAG was microinjected into pronuclei of mouse zygotes followed by transfer into oviducts of pseudopregnant female NMRI mice. The offspring were routinely screened by PCR to identify transgenic animals. Subsequently, the expression of the LRP102-295::FLAG protein was analyzed by western blotting of homogenates of cortical and cerebellar origin. Three hemizygous transgenic mouse lines were established, showing expression of LRP102-295::FLAG in cortex and cerebellum. Hemizygous transgenic mice were intracerebrally inoculated with brain homogenates from scrapie infected animals (RML) and monitored for incubation time and survival. The incubation time plus survival of transgenic scrapie infected mice, expressing the LRP-mutant in the brain, was significantly prolonged compared to scrapie infected wildtype mice.
Thus, the LRP102-295::FLAG mutant represents a alternative tool to develop an anti TSE therapy.

(1) Gauczynski et al., EMBO J. 2001, 20, 5863-75
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(3) Vana & Weiss, J. Mol. Biol. 2006, 358(1), 57-66

P10.18 Microinjection of lentiviral vectors expressing siRNAs directed against laminin receptor precursor mRNA prolongs incubation time in scrapie-infected mice

Authors

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Content

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases, which include Scrapie in sheep, BSE in cattle, CWD in cervids and CJD in humans. Prions, the causative agents of TSEs, are known to interact with the cellular prion protein (PrP_C) by inducing conformational changes. It has been demonstrated that the 37 kDa/67 kDa laminin receptor (LRP/ LR) acts as the cell surface receptor for both PrP_C 1 and the infectious prions 2,3. Additionally, heparan sulfate proteoglycans (HSPGs) were identified as co-factors/co-receptors for PrP_C 4. Furthermore, it has been shown that LRP/LR is essential for PrP_{Sc} propagation in neuronal cells 5. The accumulation of PrP_{Sc} in scrapie-infected neuronal cells (N2aSc +) has been prevented by transfection with small interfering (si) RNAs specific for the LRP mRNA 5. Vector-based application of siRNAs circumvents the transient effect of downregulation of gene expression and allows persistent suppression. Employment of HIV-based lentiviral vectors expressing siRNAs directed against defined regions of the LRP mRNA as a delivery system resulted in reduction of both PrP_{res} and LRP levels in scrapie-infected neuronal cells 6. To further enlighten the role of LRP/LR in prion diseases, intracerebral injection of recombinant lentiviral particles expressing LRP-specific siRNAs into mice was performed. Western Blot analysis of brain tissue showed a downregulation of the 67kDa LR. To study the effect of LRP/LR downregulation on the incubation time and survival during scrapie infection, mice were injected intracerebrally with lentiviral particles expressing siRNAs directed against LRP mRNA following scrapie inoculation via the same route. Western blot analysis of brain homogenates of the scrapie infected mice showed a significant downregulation of LRP- and PrP_{Sc} levels, respectively 6. A significant prolongation of incubation times were detected, demonstrating that LRP/LR plays a crucial role in the progression of TSEs. Therefore, LRP/LR represents an alternative target for the development of prion disease therapeutics.

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(6) Ludewigs et al., submitted

P10.19 Cannabinoids for therapy of prion infections?

Authors

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Content

tThe endocannabinoid system is involved in the pathogenesis of several neurodegenerative diseases like Alzheimer´s disease, Huntington´s disease and multiple sclerosis. Cannabinoid receptor CB1 was described to be expressed on neurons in the brain and was reported to exert neuroprotective functions. Cannabinoid receptor CB2 is thought to be preferentially expressed on immune cells suggesting a possible role in inflammatory reactions. In prion-infected brain tissue we could show that CB2 is exclusively expressed on activated astrocytes, no CB2-positive microglia cells were detectable. CB2 expression was strongly increased during disease development. In terminally diseased brain tissue high CB2 expression was found in hippocampus, thalamus and medulla oblongata; moderate expression was found in cortex, cerebellum and midbrain. CB2 expression was undetectable in uninfected control brains. In infected brain tissue CB1-expression was detected on astrocytes as well as on neurons. Neuronal CB1-expression was also detected in uninfected control brains. To test the therapeutic potential of a cannabionoid receptor agonist we treated scrapie-infected mice with different dosages of Dronabinol (THC). Dronabinol-treated mice showed slightly but not significantly prolonged survival times. Accumulation of PrP_{Sc} was unchanged in treated compared to untreated control mice.

P10.20 Historical controls for ethical clinical trials in human prion diseases

Authors

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Content

Background: No therapy is available for human prion diseases and few compounds are being tested in large clinical studies. Two trials in Creutzfeldt Jacob Disease (CJD) are actually registered at the web site http://clinicaltrials.gov: the CJD Quinacrine Study (sponsored by the National Institute on Aging [USA]), a randomized trial, and the PRION-1: Quinacrine for Human Prion Disease (sponsored by the Medical Research Council [UK]), a partially randomized patient preference trial.
The PRION-1 trial experience highlighted that many patients have a strong preference for receiving the drugs immediately, while other patients have a strong preference for not receiving the drugs.

Objective: We aim to suggest an ethical approach useful to properly assess clinical efficacy of treatments for human prion diseases.

Methodological Issues: Patients (or his/her parents/guardian) might prefer to decide for treatment or no treatment rather than being randomized. In general, the uncertainty principle, often used to justify the randomised clinical trials and to recruit patients, may be unfair to patients. Moreover, effective therapies for prion diseases are not yet available, and preclinical data do not encourage any planning for a trial. Since prion diseases are very rare, rapidly progressive and invariably fatal, their natural course and management will likely not change in time and in space. Thus, historically controlled trials should be considered a valid and ethical alternative to randomised trials, especially if the proposed treatment would have a high a priori probability to be effective. Even if randomized controlled trials are preferred as ethical and efficient to assess efficacy, historical controls may shorten study duration by enrolling a small calibration randomized control group to combine with the historical one. Therefore, it is urgent to collect detailed data on untreated patients (historical controls) in a well-planned protocol, with clinician agreement both within and between countries about inclusion and exclusion criteria, phases and timing of disease assessment, cognitive scales, neurological deterioration indexes, quality of life items, and instrumental and laboratory findings to be used, with the aim that the same structured framework will be adopted in future trials.

Expected Results: In human prion diseases, historical controlled trials might be a valid alternative to randomized clinical trial.

P10.21 In vitro prion strain interference

Authors

Zuber, C.[1]; Rey, C. [1]; Rupprecht, W.[1]; Pace, C.[2]; Mitteregger, G.[2]; Schuhmann, N.[3]; Hallek, M.[3]; Büning, H.[3]; Kretzschmar, H.[2]; Weiss, S.[1]

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Content

Transmissible spongiform encephalopathies (TSEs) are a group of incurable neurodegenerative diseases, which occur both in humans (Creutzfeldt-Jakob disease) and animals (scrapie in sheep, BSE in cattle, chronic wasting disease in deer and elk). A hallmark of prion diseases is the accumulation of the abnormal partially protease resistant isoform (PrP_{Sc}) of the cellular prion protein (PrP_C) followed by spongiform changes in the CNS and neuronal death (for review (1, 2)). The 37kDa/67kDa laminin receptor (LRP/LR) acts as the cell surface receptor for the cellular prion protein (3) (review (4)) and the infectious prion protein (PrP_{Sc}) (5). A LRP/LR specific polyclonal antibody (W3) abolished PrP_{Sc} propagation in scrapie infected neuroblastoma cells (6) and reduced peripheral PrP_{Sc} propagation in scrapie infected mice (7), demonstrating that the disruption of the LRP-PrP interaction is a relevant strategy to treat prion diseases. We developed single chain antibodies directed against LRP/LR alternative tools for therapeutic approaches in prion diseases. Selection by phage display resulted in two scFvs termed N3 and S18 (8). Since passive immunotransfer of scFv S18 into scrapie infected mice was not sufficient in prolonging the survival (8), we developed recombinant AAV encoding for scFv to achieve permanent delivery (9). After stereotactical injection of the rAAV encoding for scFv into the hippocampus of mice, expression of the scFvs in the brain was verified.

For a therapeutic approach mice were intracerebrally injected with rAAV followed by scrapie infection. Although we observed a significant reduction of the PrP_{Sc} level by approx. 60% in the spleen, the incubation times survival were not significantly prolonged (9). Future alternative delivery systems are currently developed, including scFv secreting muscle cells. Encapsulation of muscle cells is currently reported for clinical trials in parkinsons disease, providing therefore also for prion disease therapy a promising approach.

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(4) Vana et al., Inf. Disorders - Drug Targets, in press
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(6) Leucht et al., EMBO rep (2003) 4, 290-295.
(7) Zuber et al., Prion, (2007)1;3, 207-212
(8) Zuber et al., Mol. Immunol., (2008), 45, 144-151.
(9) Zuber et al., J. Gen. Virol., in press.

P10.22 Pravastatin oral treatment prolongs survival time of scrapie-infected mice

Authors

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[1]ISS.

Content

Background: Statins are potent inhibitors for HMG-CoA reductase in the cholesterol biosynthesis pathway. They are either lipophilic (i.e. *simvastatin*) or hydrophilic (i.e. *pravastatin*) compounds, considered mainly for long-term treatment of hyper-cholesterolemic individuals. Beneficial effects of statins are not exclusively related to their lipid-lowering effects. They also possess a cholesterol-independent action, the so-called pleiotropic effect (e.g. *antiinflammatory and antioxidative*). Two recent independent studies revealed that in scrapie intracerebrally (ic) infected mice, treatment with simvastatin delayed disease symptoms and significantly increased survival. Little is known about brain effects of statins. It has been reported that pravastatin (PRV) alters the transbilayer distribution of cholesterol in synaptic plasma membranes of chronically treated mice and that PRV oral treatment in mice results in measurable levels in the brain. PRV, enters cells via an ATP-dependent transporter of anion-transporting polypeptide (i.e. *rodents: Oatp; humans: OATP*) family.

Objective: To test whether PRV is effective in increasing the survival times in mice with experimental scrapie.

Materials and Methods: PRV sodium-salt was a generous gift from Bristol-Myers Squibb, USA. Treated (n=13) and control (n=10) female C57BL mice were injected ic with 1% 139A scrapie strain. PRV was administered from the time of scrapie inoculation in the drinking water at a dose of 200 mg/kg of body-weight per day. Water consumption was monitored twice-weekly and drug concentration was adjusted of as required. Control animals received tap water without PRV.

Results: PRV-treatment delayed the loss of motor function and prolonged significantly survival times (194 vs. 177 days; Mann Whitney Test, p=0.0001) of scrapie infected mice. No difference in terms of brain deposition and glycotype pattern of PrP_{Sc} was observed between treated and control groups.

Discussion: Hydrophilic statins may have a role in the treatment of prion diseases. Though it is likely that their anti-prion effect is mediated by the modification of cholesterol in neuronal cells, the precise mechanism of action requires further experimental work.

P10.23 The Search for Novel Biomarkers of Prion Disease Using Surface Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometry (SELDI-TOF-MS)

Authors

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Content

Background: A definitive diagnosis of prion disease relies upon neuropathological data usually obtained at autopsy. The identification of surrogate biomarkers, or combinations of markers in body fluids may enable pre-clinical detection of disease.

Objective: To identify changes in the protein expression of serum and plasma in scrapie infected sheep, and to explore their potential as diagnostic markers of infection.

Methods: Protein profiling using SELDI-TOF-MS provides a sensitive, high-throughput methodology for protein biomarker discovery to analyze serum samples from scrapie infected sheep. Samples were collected at several time points throughout the pre-clinical and clinical stages of disease. Bio-Rad''s proprietary Equalizer™ bead technology was used to reduce the dynamic range of proteins present in the blood. Serum was mixed with a bead-based library of all possible hexamer peptides. Low abundance proteins were enriched by binding to their high affinity ligands on the beads, highly abundant proteins rapidly saturate their ligands and excess is removed by washing. Proteins were eluted from these beads and fractionated prior to analysis using SELDI-TOF-MS. Statistically significant differentially expressed proteins were selected and a list of potential candidate biomarkers determined based on their expression in multiple individuals, and the potential for detection pre-clinically. Identification of some of these biomarkers using fractionation, 2D gel electrophoresis followed by proteolytic digestion and peptide mapping is underway.

Discussion: Reliable biomarkers to screen for prion infection pre-clinically, and to detect contaminated blood and biological products, are extremely important in averting risk to human health. The cross-species utility of candidate biomarkers identified in these studies will be tested, as will their usefulness in diagnosis of other neurodegenerative diseases.

P10.24 Blocking calcineurin activity as a novel therapeutic approach for Prion disorders

Authors

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Content

Background: Prion diseases are fatal neurodegenerative disorders affecting mammals, characterized by a long pre-symptomatic phase followed by rapid and progressive clinical phase. Although rare in humans, the unconventional infectious nature of the disease raises the potential for an epidemic. Therefore, development of efficient therapeutic interventions is crucial. While some compounds have been shown to delay disease progression, they are effective only when administered early during pre-symptomatic phase, limiting their therapeutic potential.

Objective: Our main goal in this project is to develop a clinically relevant therapeutic approach against prion disorders. Compelling evidence has implicated the calcium-dependent phosphatase Calcineurin (CaN) in key cellular pathways controlling both synaptic plasticity and neuronal apoptosis. We have previously shown that exposure of cells to infectious prions result in a rapid and sustained increase in intracellular calcium, leading to hyperactivation of CaN. Here we report a novel therapeutic approach aimed to down-regulate the CaN activity and thereby restore the signaling impairment and decrease disease severity and increase survival.

Methods: C57BL/6 mice were inoculated with RML intra-cerebrally and let them to develop prion disease. The infected mice were treated intra-peritoneally at the symptomatic phase with CaN inhibitor or vehicle as a control. The disease progression was measured by progressive motor impairment and the animals were let to die with the disease. Brains tissues were harvested for biochemical and histological analysis.

Results: Our data show that administration of a CaN inhibitor during symptomatic phase of the disease result in a significant delay of disease progression and increase in survival time compared to vehicle treated controls.

Discussion: Currently, there is no therapy available against prion disorders. The self-propagating misfolding process that features prion diseases amplifies the abnormally folded prion in a logarithmic scale making it difficult for development of an efficient therapy at the symptomatic phase. Our approach focuses on a novel molecular target down-stream of the misfolding process and aims to prevent the signaling pathways leading to synaptic alterations and neuronal death. Our data suggests that down regulation of CaN activity may be a promising target for prion disease therapy.

P10.25 Gene Therapy for Prion Disease

Authors
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Content

Background: PrP^c knockout mice are resistant to prion disease and antibodies targeting PrP^c or PrP^{Sc} inhibit prion replication *in vitro*. Passive transfer of antibodies suppresses peripheral prion replication *in vivo*, however the blood brain barrier diminishes their therapeutic effect in the CNS. Lentiviral vectors can transducer non-dividing cells, such as neurons, providing long-term gene expression and stable integration into the host genome. Lentiviral vectors expressing short hairpin RNA (shRNA) have been shown to efficiently knockdown genes both in vitro and *in vivo*.

Objective: (1) Design and clone several novel shRNA sequences targeting PrP^c.
(2) Test PrP^c knockdown efficiency both in vitro and *in vivo*.
(3) Test the efficacy of PrP^c knockdown on prion disease progression both in vitro and *in vivo*.
(4) Test novel methods of anti- PrP^c antibody delivery to the CNS.

Methods: shRNA sequences were cloned into an HIV-1 based lentiviral vector which allows co-expression of GFP and shRNA. Viral particles were produced by transient transfection of 293T cells. To date, 8 *Prn-p* sequences have been targeted, several exhibiting cross species activity. Cell lines, including GT1-7 and 293T, were used to assess PrP^c knockdown by RT-PCR, Western blotting and immunohistochemistry. Stably Fukuoka prion-infected GT1-7 cells and BALB/c mice inoculated with Fukuoka strain prions have been treated with lentiviral vector and antibodies.

Results and Discussion: shRNA sequences targeting PrP^c were successfully cloned into the lentiviral vector and verified by sequencing. PrP^c knockdown was demonstrated both at mRNA and protein levels *in vitro*. Assessment of efficacy against prion disease progression *in vivo* is ongoing. Lentiviral vectors may be useful for inhibiting prion disease progression, providing sufficient shRNA expression can be achieved throughout the brain.

P10.26 Development of embryonic stem cell therapy strategy for the treatment of prion diseases

Authors
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Content

Background: Prion diseases are fatal neurodegenerative disorders for which there are currently no effective treatments. Importantly, in Human, the diagnosis of prion disease is still difficult and often leaves a short therapeutic window after the appearance of the first clinical signs. As serious damages to the brain have already occurred before clinical symptoms manifest, an ideal therapeutic strategy must target not only the formation of toxic aggregates, but also the neuronal loss. The most efficient strategy would therefore be achieved by combining gene therapy with cell therapy.

Objective: By taking advantage of prion "resistant" polymorphisms Q171R and E219K that naturally exist in sheep and humans, respectively, we proposed embryonic stem (ES) cell therapy strategy combined with gene therapy. In order to orchestrate a brain repair with prion resistant cells, our specific objective was to genetically modify ES cells by introducing "dominant negative" PrP mutants using lentiviral vectors, before their transplantation in scrapie infected mouse brain.

Methods: We first set up the differentiation protocol by using a "neural" differentiation medium, in which the murine ES cells formed embryoid bodies (EBs) enriched in neural precursors. Optimised conditions for gene delivery in EBs using FIV derived lentivirus have also been set up.

Results and Discussion: We succeeded in transducing the EBs with the lentivirus carrying the dominant negative PrP mutants. To test the feasibility of this graft approach, C57bl/6 mice have been infected with prions and transduced EBs have then been injected into different area of the mice brain using a stereotaxic frame. We are now assessing the effect of the transplantation on the development of the disease. However tumours also appear in some of the grafted mice. We then decided to modify our protocol of neural differentiation as well as the protocol of lentiviral transduction.

P10.27 In vitro prion strain interference

Authors
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Content

While not required for conversion of PrP, the N-terminal domain of the prion protein, including the octarepeat region and two polybasic sequences, is in part responsible for interactions with inhibitors of PrP^{res} formation. Pentosan polysulfate, a highly sulfated carbohydrate used therapeutically for the treatment of CJD, has been demonstrated to bind to these regions, causing cross-linking of the protein and ultimately becoming internalized along with PrP. The objective of this work is to characterize the structural changes that occur within the intrinsically disordered N-terminus of PrP upon binding to pentosan polysulfate (PPS) in order to gain an understanding of the role this domain plays in the mechanism of PrP^{res} inhibition. Three-dimensional NMR techniques were applied to the complex formed between the 15N, 13C labeled construct consisting of residues 23-106 of recombinant Syrian hamster PrP and the inhibitor PPS. 15N-HSQC data demonstrated that binding to PPS causes dramatic chemical shift changes in 50% of the amide crosspeaks, suggesting a large interaction surface between the two molecules, while retaining the narrow chemical shift dispersion characteristic of a disordered protein. Backbone and sidechain chemical shifts for approximately 70% of the peptide in complex with PPS were assigned, despite the limited dispersion and extensive overlap of abundant glycine and proline resonances. Octarepeat residues displayed identical chemical shifts, indicating that they adopt a symmetrical arrangement. NOE data collected on the complex showed that binding to PPS stabilizes turns within the octarepeat region, and allowed the structure calculation of this poorly understood yet biologically relevant portion of the prion protein.

P10.28 Evaluation of new anti-Prion chemical compounds

Authors
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Content

Background: Since Prion contamination can occur by blood transfusion, there is still need for therapeutics. Moreover, since Prion diseases share common features with other proteopathies, validated treatments might be of use in other diseases, such as Alzheimer's disease. Nevertheless, although many therapeutics strategies have been tested, both in animal and for clinical compassional treatments in human, none of them presents significant role neither in Prion prevention nor in Prion treatment.

Objective: Since new therapeutics approaches must be defined, we propose to identify new compounds, with high anti-Prion activity : both cellular toxicity and PrP^{res} inhibition ability will be tested, and potent candidates will be further characterized and validated *in vivo*. After evaluating both lethal doses (LD50) and inhibiting concentrations (IC50), mechanistic studies will be done.

Methods: We developped a high-throughput screening test, dot-blot based, with the use of murine cells infected by different Scrapie strains. The ICSN chemical library includes 3.000 pure compounds, with known chemical structures. After validation using known PrP^{res} inhibitors, the compounds of the ICSN library are tested, first on Chandler-infected SN56, and then on 22L-infected GT1-7 cells. Mechanisms of action of the most potent inhibitors are investigated using confocal microscopy, flow cytometry and biochemical techniques.

Results and Discussion: Eight compounds presented a high Prion inhibition and a low toxicity, for two different cell types and two separate Scrapie strains. These eight molecules, which have never been identified in the Prion field before, file in two separate chemical classes. Preliminary data allow to propose an unpublished mechanism for some of these compounds. These novel compounds will be tested in vivo, using conventional mice, infected by different Prion strains: they may constitute novel anti-Prion compounds.

P10.29 Development of novel antibody binders effective at inhibiting PrP^{Sc} replication in prion-permissive neuroblastoma cell lines

Authors
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Content

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Background: The development of effective therapy for prion diseases is of major importance for public health in case there is an explosion of a vCJD epidemic. The antibody-based therapy appears to be the method of choice as has been demonstrated by the use of these molecules, directed against specific prion epitopes, against chronically infected susceptible Neuroblastoma (N2a) cells 1,2.

Objective: 1) To characterise a panel of novel anti-prion antibodies effective at inhibiting PrP^{Sc} replication in prion-permissive neuroblastoma cell lines.
2) To assess the potential neurotoxic effects of these novel antibodies on neuroblastoma cell lines.

Methods: PrP^{Sc} inhibition studies were conducted using prion-permissive neuroblastoma cells (ScN2a cells). Following cell lysis and proteinase K (PK) digestion, lysates were used to assess PrP^{Sc} replication by Sandwich ELISA and Western Blotting. Neurotoxic effects of the PrP^{Sc} binders were also assessed using N2a cells with varying concentrations of PrP^{Sc} binders. The cells were fixed and permebealised then subsequently assessed for apoptosis using TUNNEL staining by flow cytometry.

Results: A significant reduction in PrP^{Sc} replication was observed in all treatment groups using our novel PrP^{Sc} binders. Furthermore, the PrP^{Sc} binders were effective in reducing prion load in cell lines as assessed on cell lysates after 24 hours treatment. An isotype mached control did not load to similar observations and had no effects on PrP^{Sc}.
Prion replication was not seen to recur following treatment termination in either the 3 day or 6 day recurrence periods, indicating that our PrP^{Sc} binders lead to indefinite depletion of PrP^{Sc} in prion-permissive cell lines. Some but not all of the novel PrP^{Sc} binders lead to apoptosis in N2a cells, and work is currently underway to selectively isolate non-causing apoptosis PrP^{Sc} binders that will subsequently be used *in vivo*.

Discussion: Antibody based therapy has gained a lot of momentum following the observation that they were able to delay peripheral prion disease in mice3. In this study we show that our novel PrP^{Sc} binders are effective in permanently curing chronically prion-infected ScN2a cell lines. Our PrP^{Sc} binders are also able to cross the blood-brain barrier and could prove to be very effective in curing prion disease *in vivo*.

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P10.30 In vitro prion strain interference

Authors
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Content

Background: We have previously shown that monoclonal antibodies 1,2 were effective in curing peripheral animal prion disease. When animals were inoculated peripherally (directly into the peritoneal cavity) with infectious prions then treated subsequently with antibodies, they all survived throughout their life span. In contrast, when animals were inoculated centrally with infectious prions then treated subsequently with antibodies, they all succumbed to disease at the expected onset. These monoclonal antibodies are relatively large molecules and cannot therefore cross the blood brain barrier.

Objective: • To characterise a panel of novel PrP^{Sc} binders using various molecular biology and immunological assays
• Assess transmigration of these PrP^{Sc} binders across an artificial blood brain barrier

Methods: Brain microvascular endothelial cells were used in vitro to assess transmigration of novel PrP^{Sc} binders. The ability of PrP^{Sc} binders to traverse the in vitro blood-brain barrier was assessed using standard ELISA.

Results: Here, we show that our novel PrP^{Sc} binders efficiently cross the blood-barrier in vitro as measured by ELISA. Anti-prion IgG and IgM as well drugs that are known to bind PrP^{Sc} were unable to cross the artificial blood-brain barrier. More importantly, these PrP^{Sc} binders have shown greater diffusion into the brain parenchyma as demonstrated with immunohistochemistry.

Discussion: Despite their ability to inhibit prion replication and delay disease in vivo, antibodies are not efficient in crossing the blood-brain barrier 1. Our PrP^{Sc} binders transmigrate efficiently across an artificial blood-brain barrier in contrast with other PrP binding molecules. Work is currently underway to assess whether the PrP^{Sc} binders are able to cross the blood-brain barrier in vivo and delay disease.

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P10.31 Defining the use of monoclonal antibody immunotherapy in Prion

Authors
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Content

Background: Prion diseases are infectious neurodegenerative diseases associated with brain accumulation of abnormal prion protein (PrP_{Sc}) derived from its normal cellular precursor (PrP_C). The observation that vCJD is transmissible by blood transfusion has highlighted the urgency for a treatment. We previously showed that anti-PrP monoclonal antibodies (mAbs) ICSM18 and ICSM35, injected at 4mg/week intraperitoneally (ip) prevented mice from developing scrapie. We wish to humanise ICSM18 and 35 for use as post exposure prophylaxis to prevent those accidentally infected with prions from developing clinical disease, as well as devising a strategy to treat patients with CJD. Prior to human trails, we are carrying on an extensive study to optimise the therapeutic dose and route of administration of the mAbs at different stages of infection and investigate side effects.

Objective: To study therapeutic efficacy of ICSM18 (IgG1 to epitope 143-153) and ICSM35 (IgG2b to epitope 93-105) at lower doses than those reported.

Methods: RML prion-infected FVB/N mice at post infection day 30 (PID30) were injected ip biweekly with doses of 1mg and 0.25mg/week of ICSM18, ICSM35, isotype control mAbs or PBS. Brain and splenic PrP_{Sc} accumulations were quantified by western blotting and histology, and sera IgG by ELISA.

Results: Despite similar levels of circulating IgG in ICSM18 and ICSM35 treated mice, ICSM18 was more effective in extending survival time. Infected mice treated with PBS or isotype control mAb survived to PID189-202 and had splenic and brain PrP_{Sc} accumulation. In contrast at PID430, there was a 56% and 10% survival in mice treated with 1mg and 0.25mg/ week of ICSM18, and 50% survival in mice treated with 1mg/week of ICSM35. Splenic PrP_{Sc} accumulation was inhibited at PID69 in ICSM18 1mg/week treated mice, and was maintained up to when the mice were culled due to scrapie. A slower peripheral clearance of PrP_{Sc} was seen in ICSM35 1mg/week treated mice with little inhibition at PID69, but a complete clearance by the time the mice developed scrapie and were culled.

Discussion: Passive immunisation of ICSM18 and 35 at 1mg/week is effective in extending life of 56% of ICSM18 and 50% of ICSM35 treated mice by 230 days. The improved survival time observed with ICSM18 may be due to its ability of clearing peripheral PrP_{Sc} earlier than ICSM35, thus delaying neuroinvasion. Half life of the mAbs will be tested in vivo to help determine the optimum therapeutic dose.

P10.32 Structural and thermodynamic characterisation of the interactions of PrP_C with potential therapeutic compounds

Authors
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Content

Background: Prion diseases are caused by the conversion of the cellular prion protein, PrP_C, into a misfolded, aggregation-prone form termed PrP_{Sc}, although the mechanism for conversion remains largely unknown. PrP_C is essential for disease formation but it is not essential for normal brain function. The stabilisation of PrP_C against conformational change is therefore a good therapeutic target in Prion diseases, for which there is currently no cure and limited therapeutic intervention available.

Objective: The primary requirement for therapeutic stabilisation is the binding of any potential therapeutic compounds to PrP_C. We have developed a series of biophysical assays to directly detect interactions between recombinant prion protein and small molecule compounds, and we have validated these assays using a selection of putative therapeutic compounds identified from published literature.

Methods: We have applied a number of orthogonal methodologies to detect compound-PrP interactions, including Isothermal Titration Calorimetry (ITC), Analytical Ultracentrifugation (AUC) and Nuclear Magnetic Resonance (NMR).

Results: We find that for compounds which interact specifically with PrP with a high affinity, the interaction is detectable using each of the techniques we have employed. Weaker and/or non-saturable interactions are more likely to show equivocal responses in these assays, and/or a differential response across the different assays.

Discussion: We show that a number of compounds which show treatment efficacy in cellular and in in vivo models of prion disease interact specifically with PrP, and accordingly their efficacy is likely to be mediated directly by that interaction. There are a number of putative anti-prion compounds which have been identified using cellular models of prion disease for which we do not find evidence of direct binding to PrP in our comprehensive series of assays; we deduce that the anti-prion effects of these compounds are mediated by proteins other than PrP. Our methods are optimal for identifying compounds which interact with PrP with affinities significantly greater than mM. Such interactions are required for therapeutic stabilisation of PrP_C against prion disease formation.

P10.33 Lithium enhances clearance of prions by induction of autophagy

Authors

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Content

Background: It has been shown that lithium induces autophagy in an mTOR (mammalian target of rapamycin) independent manner, thereby enhancing the clearance of mutant huntingtin and alfa-synucleins. Recently we could show that the tyrosine kinase inhibitor imatinib is activating autophagy and reducing the pathological prion protein (PrP_{Sc}).

Objective: In this work we were analyzing the effect of lithium on the amount of PrP_{Sc}. Thereby, direct correlation of induction of autophagy and reduction of PrP_{Sc} was investigated.

Methods: The effects of lithium on both PrP_{Sc} levels and induction of autophagy was analyzed in cell culture, mainly by Western blot analysis, confocal laser microscopy and flow cytometric analysis.

Results: We show here for the first time that lithium significantly reduces the amount of PrP_{Sc} by inducing autophagy. Treatment of prion-infected cells with 3-methyladenine (3-MA), a potent inhibitor of autophagy, counteracted the anti-prion effect of lithium, demonstrating that induction of autophagy mediates degradation of disease-associated PrP_{Sc}. Cells co-treated with lithium and rapamycin, a drug widely used to induce autophagy, showed an additive effect on the clearance of PrP_{Sc} compared to treatment with either drug alone. In addition, we provide evidence that the ability to reduce PrP_{Sc} and to induce autophagy is common for diverse lithium compounds, not only for the drug lithium chloride, usually administered in clinical therapy. Furthermore, we show that besides reduction of PrP_{Sc} aggregates by lithium-induced autophagy, lithium apparently mediates reduced levels of PrP_{C}. This may result in limiting the substrate available for conversion of PrP_{C} into PrP_{Sc}, contributing indirectly to the degradation of PrP_{Sc} by lithium-induced autophagy.

Discussion: Further in vitro and in vivo experiments are required to elucidate the exact function of autophagy in prion propagation and infectivity and will be needed to further validate whether autophagy plays a general role in prion disease scenarios. Goal of our future studies is also to reveal whether drug-induced autophagy can be used as a novel experimental avenue for therapy against prion diseases.

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