Highly infectious prions generated by a single round of microplate-based protein misfolding cyclic amplification

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Prion Diseases in Animals

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P.146: Kinetics and cell association of chronic wasting disease prions shed in saliva and urine of white-tailed deer

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Chronic wasting disease, a transmissible spongiform encephalopathy (TSE) of deer, elk, and moose, is unique among prion diseases in its relatively efficient horizontal transmissibility. Recent studies have shown that excreta—saliva, urine, and feces—from CWD-positive cervids may play an important role in horizontal transmission of CWD, and although the precise onset of shedding in these excreta is unknown, it is thought to occur long before the onset of clinical symptoms. High levels of prion seeding activity have been demonstrated in excretory tissues of deer, including tongue, salivary glands, kidney, and urinary bladder, though the origin(s) and cellular nature of infectious prions in excreta is unknown. We hypothesized that excretory shedding of CWD prions in saliva and urine would coincide with the appearance of PrP^{d} appearance in peripheral lymphatic tissues, and that infectivity would associate with cellular preparations of these excreta. Following intracerebral inoculation of susceptible Tg[CerPrP] mice, we observed efficient transmission in saliva collected as early as 12 months post-exposure, coinciding with peripheral PrP^{d} appearance in tonsil biopsies; while urine collected at terminal disease was only minimally infectious in transgenic mice. We also found that acellular preparations of saliva, and cellular preparations of urine, were capable of transmitting CWD infection to transgenic Tg[CerPrP] mice with incubation periods similar to that of whole saliva or urine; saliva and urine from CWD-negative deer failed to induce prion disease in these mice. Infectious titers were determined for obex and bodily fluids, and were similar to those previously described. These findings extend our understanding of CWD shedding in white-tailed deer, and offer insight into the source and cellular associations of infectious CWD prions in excreta.

P.147: Gene expression profiling of brains from bovine spongiform encephalopathy (BSE)-infected cynomolgus macaques

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Background. Prion diseases are fatal neurodegenerative disorders whose pathogenesis mechanisms are not fully understood. In this context, the analysis of gene expression alterations occurring in prion-infected animals represents a powerful tool that may contribute to unravel the molecular basis of prion diseases and therefore discover novel potential targets for diagnosis and therapeutics. Here we present the first large-scale gene expression profiling of brains from BSE-infected cynomolgus macaques, which are an excellent model for human prion disorders.

Results. The study was conducted using the GeneChip® Rhesus Macaque Genome Array and revealed 301 transcripts with expression changes greater than twofold. Among these, the bioinformatics analysis identified 86 genes with known functions, most of which are involved in cellular development, cell death and survival, lipid homeostasis, and acute phase response signaling. RT-qPCR was performed on selected gene transcripts in order to validate the differential expression in infected animals versus controls. The results obtained with the microarray technology were confirmed and five genes were found to be highly regulated. In brief, *HBB* and *HBA2* were down-regulated in infected macaques, whereas *TTR*, *APOC1* and *SERPINA3* were up-regulated.

Conclusions. Some genes involved in oxygen or lipid transport and in innate immunity were found to be dysregulated in prion infected macaques. These genes are known to be involved in other neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Their protein products may become potential targets for both general diagnosis and therapeutic purposes of many neurodegenerative diseases.

P.148: L-BSE in genetically susceptible and resistant sheep: Changes in prion strain or phenotypic plasticity of the disease-associated prion protein?

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Sheep with prion protein (PrP) gene polymorphisms QQ171 and RQ171 were shown to be susceptible to the prion causing L-type bovine spongiform encephalopathy (L-BSE), although RQ171 sheep specifically propagated a distinctive prion molecular phenotype in their brains, characterized by a high molecular mass protease-resistant PrP (PrPres) fragment, distinct from L-BSE in QQ171 sheep.

Transmission studies were performed from QQ171 and RQ171 ovine L-BSE in transgenic mice expressing either bovine or ovine PrP and in C57Bl/6 wild type mice.

In both transgenic lines, ovine L-BSE transmitted similarly to cattle-derived L-BSE, with respect to survival periods, histopathology, biochemical features of PrPres in the brain, as well as splenotropism, clearly differing from ovine classical BSE or from scrapie strain CH1641. At the first passage, PrPres was found in the spleen of ovine PrP transgenic mice infected with RQ171 ovine L-BSE, the molecular phenotype is similar to the brain PrPres signature of RQ171 sheep used as inoculum. Both QQ171 and RQ171 transmitted a prion disease in wild-type mice, whereas we failed to transmit any disease from L-BSE in cattle or from L-BSE first passaged in hamsters or microcebes. Remarkably PrPres molecular features also showed a high apparent molecular mass in C57Bl/6, clearly distinct from BSE.

After passage in sheep, with either QQ171 or RQ171 genotype, the L-BSE agent maintained its specific strain properties in bovine and ovine transgenic mice, although striking PrPres molecular changes could be found in RQ171 sheep and in the spleen of ovine PrP transgenic mice.[1] In addition passage in sheep also allowed the L-BSE agent to propagate in wild type mice, with unexpected molecular features. These observations suggest either selection of prion strain or phenotypic plasticity of the disease-associated prion protein.

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P.149: Highly infectious prions generated by a single round of microplate-based protein misfolding cyclic amplification

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Introduction. Prions are transmissible agents responsible for incurable neurodegenerative diseases affecting both human and animals. The causal agent is of proteinaceous nature, resulting from the host normal prion protein PrP^{C} conformational transition from an alpha helix rich soluble state to beta sheet-enriched and aggregated structures termed PrP^{Sc}.

PMCA is a technique that emerged in the last decade as a valuable tool to amplify prions in a test tube. Here we report a number of improvements allowing efficient amplification of several prion strains in a single 48h round and in a microplate format. We then determined by bioassay in reporter mice the infectious titer of the PMCA-derived prions of one of the amplified scrapie strain.

Material and Methods. The detailed PCR microplate based mb-PMCA (miniaturized bead-PMCA) protocol was described.[1] 10% brain lysates of tg338, tg650, tg20 and tg7 transgenic mice over-expressing ovine, human, mouse and hamster PrP were prepared in PMCA buffer. Ovine prions (127S), mouse prions (139A, 22L, RML, Chandler, ME7, mouse-adapted BSE), hamster prions (263K, Sc237, HY and DY) and human prions (vCJD, sCJD VV1, VV2 and MV2 types) were used as seed for mb-PMCA reactions.

Results. We first report significant simplification and improvements of the PMCA technique, leading to high throughput and highly efficient amplification of PrP^{Sc} from several prion strains of different species (ovine, mice, hamster, human) in a single 48h round. We then demonstrate by end-point titration in reporter mice that this method restores an infectivity titer of 127S scrapie prion strain comparable to that of in vivo brain-derived prions, whatever the input dilution seeding the PMCA reaction.

Conclusion. The method developed here allows for the first time large-scale, fast, and reliable cell-free amplification of sub-infectious levels of prions from different species. The mb-PMCA format should help developing high-throughput prion assays for cognitive, diagnostic, and therapeutic applications. This simplified assay could be adapted to automated purposes and serve for urgently needed ante mortem diagnostic tests, by using bodily fluids containing small amounts of prion infectivity. Such assay is of paramount importance to reduce the transfusion risk in the human population, to identify asymptomatic carriers of variant