Gut flora and poly(I:C) cooperate in the intestine to stimulate dendritic cells and protect neonate mice against an enteric infection by Cryptosporidium parvum

Sonia Lacroix Lamandé, Louis Lantier, Françoise Drouet, William Guesdon, Richard Lo-Man, Catherine Werts, Fabrice Laurent

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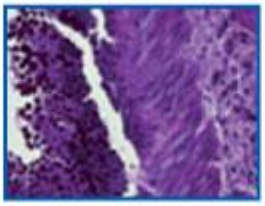
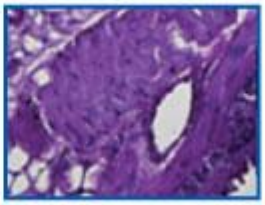
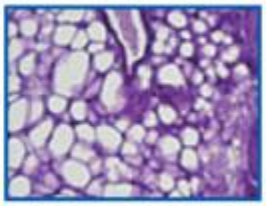

EMIG 2012 Abstract Book
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Invited Speakers - Biographies

IS1 - Dr. Jan Holmgren - *University of Goteborg, Sweden*

Dr. Jan Holmgren, MD, PhD is Professor of Medical Microbiology and Immunology at the University of Gothenburg, Sweden; he is also the Founding Director of the University of Gothenburg Vaccine Research Institute (GUVAX). He has published some 600 papers in microbiology, immunology and vaccinology, mainly on cholera and other enteric infections, mucosal immunity, and the development of mucosal vaccines.

IS2 – Prof. Luke O'Neill – *Trinity College Dublin, Ireland*

Professor Luke O'Neill was appointed to the Chair of Biochemistry at Trinity College Dublin in 2008, where he leads the Inflammation Research Group. He is also Academic Director of the Trinity Biomedical Sciences Institute. He has a PhD in Pharmacology from the University of London and carried out Post-Doctoral research at the Strangeways Laboratory in Cambridge. He has won numerous awards for his research, notably the Royal Irish Academy Medal for Biochemistry, The Irish Society for Immunology medal, the Royal Dublin Society/ Irish Times Boyle medal for Scientific Excellence and the Science Foundation Ireland Researcher of the Year Award 2009. He was elected a member of EMBO in 2005. He is a co-founder and director of Opsona Therapeutics. In 2008 he was appointed Chair of the Immunity and Infection panel of the European Research Council. His research is in the area of the molecular basis to inflammatory diseases, with a particular interest in pro-inflammatory cytokines and Toll-like receptors. He has published over 200 papers and reviews on his research, in journals such as Nature, Science, Cell, Nature Immunology, Nature Medicine, Nature Genetics and PNAS. He is on the editorial boards of 6 journals, including the Journal of Biological Chemistry and Trends in Immunology. He is also on the Board of Reviewing Editors for Science, covering Innate Immunity.

IS3 – Dr. Holm Uhlig – *University of Oxford, UK*

Dr. Holm Uhlig is a Senior Clinical Research Fellow in Paediatric Gastroenterology and Mucosal Immunology at the Translational Gastroenterology Unit, University of Oxford. He completed medical residency in pediatrics and pediatric gastroenterology at the University of Leipzig (Germany) and obtained a DPhil in mucosal immunology at the Sir William Dunn School of Pathology in Oxford (UK). Holm is interested in regulation of immune responses in the intestine. Current studies involve the genetic analysis of children with early onset of IBD and functional analysis of those defects.

IS4 – Prof. Allan Mowat – *University of Glasgow, Scotland*

Prof. Allan Mowat is Professor of Mucosal Immunology at the University of Glasgow and has been working on the regulation of immune responses in the intestine for more than 30 years. Current work in his laboratory focusses on the nature of macrophages and dendrtiic cells in the colon and small intestine, exploring how these cells contribute to maintaining local homeostasis and attempting to understand how the populations change in the context of inflammation or protective immunity.

IS5 – Prof. Denise Kelly – *The University of Aberdeen, Scotland*

Prof. Denise Kelly has a strong research interest in gut microbiology and immunology with 20 years post-doctoral experience. She obtained her BSc and PhD from Queens's University Belfast. Her first post-doctoral research appointment was with Nestle in Lausanne, Switzerland. She then moved to the Rowett Institute of Nutrition & Health, University of Aberdeen and is now Head of the Gut Immunology Group. Her main research interests are in gut microbiota and immunity and in the mechanisms of bacterial-induced inflammation and regulation. Recent achievements include appointment to the Editorial board of the US Journal Gut Microbes, UK partner in an EU-funded ITN project and the formation of a new Biotech company GT Biologics Ltd.

IS6 – Prof. Olivier Lantz – *Institut Curie Paris, France*

Prof. Olivier Lantz is an MD/PhD, heads the clinical immunology laboratory and is group leader in the Immunology department (INSERM U932) both at Institut Curie in Paris. His main scientific achievements relate to evolutionarily conserved invariant T cell subsets, NKT and MAIT cells. His group has contributed to most of what is currently known on MAIT cells. The group also studies basic questions regarding CD4 T-cell physiology and develops new mouse tumor models to study the interactions between the immune system and tumors that express nominal antigen.

IS7 – Prof. Catharina Svanborg – *Lund University, Sweden*

Early work in the Svanborg laboratory defined molecular mechanisms used by microbes to recognise and infect host cells, helping to define the field of ''Cellular microbiology''. In parallel, they identified innate immune response strategies defining susceptibility to UTI and how the host response quantitatively and qualitatively determines if infection will result in disease or asymptomatic bacterial carriage.In the UTI model, they have identified host defence dysfunctions that lead to pathology and chronic disease. Recent work on genetic determinants of human disease susceptibility have verified the human relevance of these observations. The group are currently exploring the genetic frameworks of susceptibility and virulence as well as mechanisms of mutual host-microbial adaptation in the urinary tract.

Since 1995, the Svanborg laboratory has also been pursuing the discovery of HAMLET; a human milk complex with broad tumoricidal activity. Their work has shown that the tumoricidal activity of HAMLET and the selectivity for tumour tissue is maintained in vivo. In a placebo-controlled clinical study, topical administration of HAMLET removed skin papillomas, without side effects. In patients with bladder cancer, HAMLET triggered rapid shedding of tumor cells into the urine and caused a reduction in tumor size. The findings identify HAMLET as a first member in a new and expanding family of lipid-bound partially unfolded proteins with novel structures and offer a highly interesting new tool in tumor biology. The group are currently defining the structure and mechanism of action of HAMLET and exploring in vivo effects in different cancer models.

IS8 – Prof. Andrea Cerutti – *Catalan Institute for Research and Advanced Studies. Spain*

Dr. Andrea Cerutti took his M.D. degree and completed residency training in hematology at the University of Padua (Italy). After postdoctoral training in immunology, he was appointed Assistant Professor and then Associate Professor of Pathology at Weill Medical College of Cornell University (New York). In 2009 he joined Mount Sinai School of Medicine (New York) as Professor of Medicine. In the same year, he also became ICREA Research Professor at IMIM-Hospital del Mar in Barcelona (Spain). He studies the regulation of antibody production and diversification in B cells.

IS9 – Prof. Per Brandtzaeg – *University of Oslo, Norway*

Prof. Per Brandtzaeg obtained his postgraduate training in microbiology, immunology and pathology at the MedicalCenter, Univ.of Alabamaat Birmingham, AL, USA, and received his PhD in immunology at the Univ.of Oslo. He is the former Head of the *Faculty Division, Rikshospitalet, Oslo University Hospital*, and the founder of the *Laboratory for Immunohistochemistry and Immunopathology* (LIIPAT), Department of Pathology, which is devoted to research on mucosal immunity and partner of the *Centre for Immune Regulation,*University of Oslo.

IS10 – Prof. Frits Koning – *Leiden University Medical Centre, The Netherlands*

Prof. Frits Koning is a Professor of Immunology at the Leiden University Medical Centre, Leiden, The Netherlands. He is an internationally recognized expert in the field of HLA-associated diseases, in particular celiac disease. He is the chairman of the Scientific Advisory Board of the Leiden Univesrity Medical Centre and CEO of the Dutch based Celiac Disease Consortium which performs basic and translational research in the area of celiac disease. He participates in national and international projects on CD. His group identified gluten peptides that are recognised by gluten-specific T cells isolated from the small intestine of CD patients. Moreover, he has elucidated the specificity of tissue transglutaminase, an enzyme that is involved in gluten toxicity. His group has developed an antibody-based test to screen foods for the presence of toxic gluten peptides and they are active in translational research aimed at the development of novel therapies for celiac disease patients. He authored over 160 peer reviewed papers. In 2010 he was awarded the Rank Prize for Nutrition (London,UK). In 2011 he received the Warren Prize for Excellence in Celiac Disease Research (La Jolla,USA).

IS11 – Prof. Fergus Shanahan – *University College Cork, Ireland*

Prof. Fergus Shanahan is Professor and Chair of the Department of Medicine at University College Cork, National University of Ireland, and Director of the *Alimentary Pharmabiotic Centre*, a research centre funded by Science Foundation Ireland which investigates host-microbe interactions in the gut. After medical residency in Dublin, he trained in clinical immunology at McMaster University,Canada and in gastroenterology at the University of California, Los Angeles. His interests are broad including most things that affect the human experience. He has published over 350 scientific articles and several books in mucosal immunology, IBD, and the gut microbiota

IS12 – Dr. Ed Lavelle – *Trinity College Dublin, Ireland*

Dr. Ed Lavelle graduated with a BSc in Microbiology from University College Galway in 1990 and a PhD in Immunology from the University of Plymouth in 1994. He carried out postdoctoral research at the University of Nottingham on nano and microparticles as vaccine adjuvants. This was followed by further postdoctoral positions at the Rowett Research Institute and Trinity College Dublin on vaccine adjuvants and immunomodulators. He was appointed as a lecturer in Immunology in 2004 and is a Principal Investigator in the School of Biochemistry and Immunology.

Oral Presentations

O01

Selective Depletion of Foxp3^{+R}egulatory T Cells Improves Adaptive Immunity and Suppresses Tumor Growth in Colitis-Associated Colon Cancer

Eva Pastille[1], Katrin Schumann[1], Diana Fleissner[1], Tim Sparwasser[2], Jan Buer[1], Astrid Westendorf[1]
[1]Institute of Medical Microbiology, University Hospital Essen, Essen, Germany, [2]Institute of Infection Immunology, TWINCORE, Centre for Experimental and Clinical Medicine, Hannover, Germany

Chronic inflammation severely increases the risk for cancer development as seen in patients with inflammatory bowel diseases. Although the exact mechanism of inflammation-associated tumor development remains to be shown, a role for the adaptive immune system has been implicated in colitis-associated cancer (CAC). In this context, the role of regulatory T cells (Tregs) is controversial as they act as potent suppressor of inflammation and thus can have protective effects in CAC, but simultaneously can prevent antitumor immunity. Therefore, we investigated the role of $CD4^+Foxp3^+$ Tregs in a mouse model of CAC. Blood and tissue specific $CD4^+Foxp3^+$ Tregs from CAC mice were compared to Tregs from healthy mice and mice with chronic colitis. We found a strong increase of $CD4^+Foxp3^+$ Tregs in the colonic tumors and the mesenteric lymph nodes of mice suffering from CAC. Tumor-infiltrating $CD4^+Foxp3^+$ Tregs were highly activated, suggested by the increased expression of CD25, CTLA-4 and IL-10. Moreover, they exhibited an increased suppressive effect on $CD4^+CD25^-$ responder T cell proliferation and TH1-cytokine production ex vivo. Mice expressing a diphtheria toxin (DT) receptor-enhanced fluorescent fusion protein under the control of the foxp3 gene locus (DEREG mice) allow the depletion of $Foxp3^+$ Tregs by DT injection. In vivo depletion of $CD4^+Foxp3^+$ Tregs during carcinogenesis in DEREG mice suppressed the tumor progression accompanied by an increase of $CD8^+$ INF-g producing effector cells. Our data suggest that selective, transient depletion of Tregs has the potential to evoke an efficient antitumor response and might have implications for therapeutic interventions in CAC patients.

O02

IL-28 Signaling in Intestinal Epithelial Cells Leads to a Revised Mucosal Wound Healing

Heike Dornhoff[1], Konstantin Fietkau[1], Sean Doyle[2], Jonas Mudter[1], Raja Atreya[1], Jürgen Siebler[1], Markus F. Neurath[1]
[1]University of Nuremberg/Erlangen I. Department of medicine, Erlangen, Germany, [2]ZymoGenetics, Inc., Seatle, USA

TypeIII Interferones like IL-28 were recently discovered and signal through a unique receptor consisting of the IL-28Rα and IL10Rβ Subunits which leads to phosphorylation of STAT1. This receptor has been demonstrated to be highly expressed on epithelial cells. The aim of this study was to determine the functional role of IL-28Rα expression on epithelial cells in the pathogenesis of experimental colitis.

Induction of colitis in mice by administration of DSS resulted in a significant upregulation of IL-28 expression in the gut. Treatment of primary intestinal epithelial cells with IL-28 leads to phosphorylation of p38 and STAT1 that are involved in cell cycle progression as well as in wound healing processes. Furthermore, analysis of the pathogenesis of DSS colitis demonstrated that IL-28Rα KO mice display a significant upregulation of the inflammation of the gut compared to WT mice. Furthermore, intestinal sections from these mice revealed a dramatic downregulation of proliferation of epithelial cells in the gut from KO mice, suggesting a delayed wound healing process. Additional wound healing experiments confirmed our findings regarding the decelerated wound closure after injury of the gut epithelium. Interestingly, topical administration of IL-28 onto the wound induces a better and faster closure of the lesion.

IL-28 directly affects intestinal epithelial cells, activating proliferation and aiding in wound healing in the DSS model of colitis. Thus, treatment with type III interferons may serve as a potential new therapeutic method to protect against chronic intestinal inflammation and promote wound healing processes in the context of colitis.

O03

MHC-II Expression on Intestinal Epithelial Cells Controls Local Lymphocyte Homeostasis

Simone Keck, Ed Palmer
University Hospital Basel, Department Biomedicine, Transplantation Immunology, 4031 Basel, Switzerland

The intestinal epithelium is a single-cell layer that tightly controls the interface between body and external environment. Although interactions between MHC-II expressing epithelial cells and naïve CD4+ T cells are critical for inducing regulatory T cells (Tregs) in both thymic and lung microenvironments, the role of MHC-II expression on intestinal epithelial cells (IEC) has not been defined. Using mice that specifically lack MHC-II expression in IEC (IEC MHC-II KO mice), we demonstrate a selective accumulation of CD4+ and CD8+ TCRα/β T cells in the lamina propria and intraepithelial lymphocyte compartment, while the TCRγ/δ T cell population remains unaffected. In addition, T cells from IEC MHC-II KO mice are hyperproliferative and express higher levels of activation markers. MHC-II deficient IEC also exhibit an altered phenotype characterized by lower expression of the coinhibitory molecules PD-L1 and ICOS-L, as well as lower amounts of tolerogenic cytokines including TGF-β and IL-10. Importantly, the altered mucosal environment appears to decrease the efficiency of Treg conversion in the mesenteric LN. Taken together, our findings indicate that MHC-II expression on IECs is important for maintaining local lymphocyte homeostasis and a normal cytokine milieu, and may have important implications for retaining the balance between tolerance versus autoimmunity.

O04

Reprogramming of Intestinal Epithelial Cells by Interleukin-22

Lydia Lebenheim[1], Melba Munoz[2], Markus Heimesaat[2], Martin Zeitz[1], Jörg-Dieter Schulzke[1], Michael Schumann[1]
[1]Dept. of Gastroenterology, Charité, Berlin, Germany, [2]Dept. of Microbiology, Berlin, Germany

Introduction: Interleukin-22 (IL-22) is known to elicit receptor-specific effects on epithelia. Since IL-22 has been associated with wound healing, its effects on the mucosal barrier have been classified as barrier-protective. However, further characterization of IL-22´s action on epithelia is missing so far.

Aims: To analyze effects elicited on intestinal epithelia by IL-22.

Methods: Transepithelial resistance (Rt) of intestinal epithelial cell lines (HT-29 and Caco-2) were determined. Paracellular passage of 400 Da biotin and integrity of structural tight junction (TJ) proteins were visualized by confocal microscopy. Epithelial apoptosis and wound healing capability were measured. Epithelial polarity was examined in 3-dimensional Caco-2 cysts, grown in matrigel. Ileal mucosae of IL-22 knock-out mice were studied for epithelial barrier using Ussing chambers.

Results: Rt was dose-dependently (1,10,100ng/ml) significantly reduced by IL-22 in Caco-2 and HT-29 cells - an effect being abolished by inhibitors of PI-3 kinase and MAP kinase, but not STAT inhibitors. Paracellular passage of biotin was elevated accordingly. Ileum explanted from IL-22-knock-out mice was protected against inflammation-associated defects of the epithelial barrier. IL-22´s positive effect on epithelial wound healing was confirmed in the HT-29 model. Moreover, epithelial apoptosis was modestly increased. ZO-1 and JAM-A localized to intracellular pools and not to TJs. Caco-2 matrigel cysts displayed an increase in multiple lumen, atypical mitoses and basal actin protrusions.

Conclusion: By reducing epithelial polarity, IL-22 reprograms intestinal epithelia to gain migratory properties and at the same time to lose barrier-like properties. This has implications not only for wound healing but also for inflammation-associated carcinogenesis.

O05

LysoDC Sample Antigens by Extending Dendrites through M Cell-Specific Transcellular Pores before Migrating in the Peyer's Patch Follicle

Hugues Lelouard, Johnny Bonnardel, Stéphane Meresse, Jean-Pierre Gorvel
Centre d'Immunologie de Marseille-Luminy, Marseille, France

Peyer's patches (PP) of the small intestine are antigen sampling and inductive sites for the establishment of the mucosal immunity. Luminal antigens are transported from the mucosal surface of PP to the subepithelial dome (SED) through the specialized epithelial M cells of the follicle-associated epithelium. Among the SED resident dendritic cells (DC), which are ideally situated for taking up these antigens, LysoDC express high levels of lysozyme and display a strong phagocytic activity. Here, we investigated the mechanisms by which LysoDC capture luminal antigens *in vivo* using two-photon microscopy on explants of PP from the lys-EGFP transgenic mice, in which LysoDC can be detected by the fluorescence of EGFP. We show that LysoDC extended dendrites through M cell-specific transcellular pores to reach the gut lumen. The M cell adhesion molecules JAM-A and EpCAM were recruited at the site of the transcellular migration. Transcellular dendrites scanned the M cell apical surface and the gut luminal content, and were able to take pathogenic bacteria and inert particles in the lumen before retracting back to the SED and migrating to the follicle region. These data describe a new sampling mechanism that occurs in PP and brings to light cooperation between M cells of the follicle-associated epithelium and DC of the subepithelial dome. It provides alternatives for the specific targeting of mucosal vaccines.

O06

The Cholinergic Anti-Inflammatory Pathway in the Gut Interacts with Resident Macrophages Independent of the Splenic T Cells

Gianluca Matteoli, Pedro J Gomez Pinilla, Martina Di Giovangiulio, Giovanna Farro, Andrea Nemethova, Guy E Boeckxstaens
University of Leuven, Translational Research Center for Gastrointestinal Disorders (TARGID), Leuven, Belgium

In sepsis, vagus nerve stimulation (VNS) exerts its anti-inflammatory effect via stimulation of adrenergic neurons in the prevertebral coeliac ganglion triggering splenic T cells to release acetylcholine. The latter dampens cytokines production by splenic macrophages via activation of α7 nicotinic receptors (α7nAChR) leading to increased survival. In the gut, we previously showed that VNS reduces intestinal inflammation, however to what extent the same pathway as described in sepsis is involved remains unclear. In the present study, the anti-inflammatory effect of VNS was evaluated on intestinal inflammation evoked by surgical manipulation, the key mechanism underlying postoperative ileus. As previously described, VNS reduced intestinal inflammation and improved intestinal transit. As in sepsis, this effect was dependent on α7nAChR, but not on the spleen or T cells, as shown in α7nAChR deficient mice, mice that underwent splenic denervation and Rag-2 KO mice. Using anterograde tracing we showed that the VN, most likely through synapses with cholinergic enteric neurons, exerts its anti-inflammatory effect by interacting with resident intestinal immune cells. In addition using chimera mice and sorting we revealed that α7nAChRs are carried mainly by resident macrophages in the intestinal wall. Our data clearly illustrate that the cholinergic (vagal) anti-inflammatory input to the gut largely differs from that involved in sepsis and does not involve splenic T cells. Instead, the vagus nerve synapses with myenteric cholinergic nerves interacting with intestinal macrophages carrying the α7nAChR. This insight is of great importance to develop new strategies to treat intestinal inflammatory conditions.

O07

Disconnect between LPS-Induced Cytokine mRNAs and Secreted Proteins in Dendritic Cells Tolerized by Intestinal Epithelia

John MacSharry[1], Julie Davies[1], Javier Garcia[1], Ailis Fagan[1], Conal Martin[1], Edith Hessel[1,2], Kevin Lee[1,2], Fergus Shanahan[1], Silvia Melgar[1], Ken Nally[1]
[1]University College Cork, Cork, Ireland, [2]II-CEDD GlaxoSmithKline, Stevenage, London, UK

In the gut microenvironment, dendritic cells (DCs) interact with epithelial cells (ECs) resulting in DCs that are hypo-responsive to Toll-like receptor (TLR) activation. However, molecular mechanisms underpinning this cross-talk and "tolerization" remain ill-defined. The aim of our study was to investigate the DC phenotype mediated by epithelia contact. Human monocyte-derived DCs were co-cultured with Caco-2 (ECs) and stimulated with LPS for 6 Hrs. Tolerized DC (DC-EC) phenotypes were confirmed by flow cytometry and ELISA. Initially, we investigated the ability of EC conditioned supernatants and EC derived exosomes to induce the DC-EC phenotype. However, neither treatment completely replicated the tolerization process suggesting cell-to-cell contact is required. Genome-wide mRNA expression analysis was performed on the non-tolerized and tolerized DCs +/- LPS. Gene Ontology (GO) enrichment analysis identified differing immune response characteristics following co-culture. EC co-culture increased CCR2, CXCL2, and CCL24 with a reduction in CCL2, CCL7 and IL-1b mRNA expression in DCs. Stimulation of the DC-EC cells with LPS resulted in differential cytokine production, with increased CXCL9, CXCL11 and reduced EGR1 expression. In terms of LPS responsiveness, there is a disconnect between LPS-induced cytokine mRNA and protein levels. DC-EC had reduced cytokine production (TNFa, IL-1b, IL-10, IL12p40 and IL-6) following LPS stimulation, however the corresponding mRNA transcripts were not reduced. These results demonstrate that tolerized dendritic cells still respond to TLR stimuli, such as LPS - in terms of induction of cytokine mRNAs - but the subsequent production of secreted cytokines is altered due to factors in the epithelia microenvironment.

O08

IRAK-M Deficiency Influences Colitis-Associated Cancer Development in Microbiota Independent Manner

Klara Klimesova[1], Miloslav Kverka[1], Zuzana Zakostelska[1], Tomas Hrncir[2], Pavel Rossmann[1], Martin Kostovcik[1], Jakub Mrazek[3], Helena Tlaskalova-Hogenova[1]
[1]Institute of Microbiology ASCR, Prague, Czech Republic, [2]Institute of Microbiology ASCR, Novy Hradek, Czech Republic, [3]Institute of Animal Physiology and Genetics ASCR, Prague, Czech Republic

Microbiota plays a crucial role in the transition of chronic intestinal inflammation to neoplasia. To investigate the impact of bacterial load on carcinogenesis, we induced colitis-associated cancer by azoxymethane/dextran sodium sulfate in conventional and antibiotic-treated mice, and investigated the underlying mechanisms. We found that antibiotic-treated wild-type mice had lower incidence and severity of tumors. Moreover, these mice have significantly altered gut microbiota composition and decreased β-glucuronidase activity, as compared with non-treated mice. Since negative regulation of Toll-like receptor (TLR) signaling is known to influence the development of colorectal cancer, we induced colitis-associated cancer in mice lacking key negative regulator of TLR signaling, Interleukin-1 receptor associated kinase-M (IRAK-M). IRAK-M-/- mice not only developed invasive tumors, but lowering microbiota burden by antibiotics did not rescue them from severe carcinogenesis phenotype. We conclude that gut microbiota promote carcinogenesis by increasing the exposure of gut epithelium to cancer causing substances and that microbiota restricted by antibiotics decrease the risk of cancer by IRAK-M dependent pathway.

O09

A Highly Diverse IgA Repertoire Requires a Complex Microbiota

Cornelia Lindner[1], Benjamin Wahl[1], Anna Smoczek[2], Sebastian Suerbaum[3], André Bleich[2], Oliver Pabst[1]
[1]Institute of Immunology, Hannover Medical School, Hannover, Germany, [2]Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Hannover, Germany, [3]Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Hannover, Germany

The intestinal mucosa is constantly exposed to microbial and dietary antigens all of which are thought to induce IgA secreting plasma cells. Considering the broad range of intestinal antigens, it is surprising that previous studies using classic sequencing approaches suggested a limited IgA repertoire diversity. Here we used 454 high-throughput sequencing to study the diversity of murine IgA^+ plasma cells from small intestinal samples. Interestingly, we found expanded and lowly frequent IgA sequences which together form a highly diverse polyclonal IgA repertoire with very little overlap between individual mice. Moreover, we showed that IgA repertoire diversity increases during aging in a dual process. On the one hand T cell-dependent but Peyer's patch-independent somatic mutations drive the diversification of expanded clones, and on the other hand new clones are introduced into the existing repertoire.

Furthermore, we investigated the role of microbiota in generating IgA diversity. Germ-free mice harbor only small numbers of intestinal IgA^+ plasma cells which have very few somatic mutations. Colonizing germ-free mice for four weeks with a complex murine flora resulted in the generation of normal numbers of intestinal IgA^+ plasma cells leading to an increased IgA repertoire diversity. However, these plasma cells showed only few somatic mutations. Interestingly, even though mono-colonization resulted in rapid induction of IgA^+ plasma cells the generated IgA repertoire is less diverse compared to mice colonized with a complex flora. Still, the overlap of the IgA repertoire between mono-colonized mice is negligible.

O10

Deciphering Microbiota-Driven Cell Signaling Modulation in the Human Gut Using a Functional Metagenomic Approach

Malgorzata Nepelska[1], Nicolas Lapaque[1], Alexandre Jamet[1], Tomas De Wouters[1], Antonietta Cultrone[1], Moez Rhimi[1], Florence Ledue[1], Julie Cadiou[1], Fabien Dumetz[1], Stanislas Dusko Ehrlich[1], Joël Doré[1], Emmanuelle Maguin[1], Maria Rescigno[2], Hervé M. Blottière[1]
[1]INRA UMR 1319 MICALIS, JOUY EN JOSAS, France, [2]European Institute of Oncology, Milan, Italy

The intestinal microbiota is a complex community, which exerts functions often associated with beneficial effects for its host. The microbiota complexity associated to the inability to culture most of these microbes lead to the development of new and powerful approaches namely metagenomics. To study interactions between intestinal epithelial cells (IECs) and commensal bacteria, a high throughput cell-based functional metagenomic approach was established .

Human stably transfected IECs bearing the luciferase reporter gene under the control of promoter of key genes or signaling pathways were used to screen metagenomic libraries bearing large DNA fragments (~40 kb) derived from human intestinal metagenome.

High throughput screening led to the identification of bioactive metagenomic clones modulating key pathways in IEC. Sequencing, annotation and transposon mutagenesis allowed the identification of putative genes implicated. For one stimulatory clones derived from a Bacteroides-related strain, we identified 2 loci involved in NF-κB activation. Another clone, derived from Firmicutes, was selected for its stimulation of NF-κB, AP1 and TSLP reporter systems as well as IL-8 secretion. Biochemical characterization indicated that a small heat-resistant compound was secreted and transposon mutagenesis in an ABC transporter system abolished this effect. In a co-culture system, this clone was shown to indirectly activate dendritic cells through IEC stimulation and to further modulate T cell activity. Finally, in a new ex-vivo set up of organ culture, it protected the intestinal mucosa from the destructive effect of Salmonella. Our Functional Metagenomic approach allowed the identification of new bacterial genes involved in the cross-talk with gut epithelium.

O11

A Novel Subset of GM-CSF-Producing Innate Lymphoid Cells Links Splenic B Cell-Helper Neutrophils with Marginal Zone B Cells

Giuliana Magri[1], Linda Cassis[1], Irene Puga[1], Carolina Barra[1], Maurizio Gentile[1], Montserrat Cols[2], Sergi Serrano[1], Laura Comerma[1], Jose Antonio Lorente[1], Juan Ignacio Aróstegui[3], Manel Juan[3], Jordi Yagüe[3], Michio Myajima[4], Sidonia Fagarasan[4], Andrea Cerutti[1,2]
[1]Institut Municipal d'Investigació Mèdica-Hospital del Mar, Barcelona, Spain, [2]Mount Sinai School of medicine, New York, USA, [3]Hospital Clinic of Barcelona, Barcelona, Spain, [4]RIKEN Research Center for Allergy and Immunology, Yokohama, Japan, [5]Catalan institute for research and advanced studies, Barcelona, Spain

The peri-marginal zone (MZ) area of the spleen contains B cell helper neutrophils (N_{BH} cells) that stimulate antibody diversification and production by activating MZ B cells through the cytokines BAFF, APRIL and IL-21. Postnatal colonization of the spleen by N_{BH} cells involves signals from mucosal commensal bacteria. However, fetal spleens as well as spleens from germ-free mice or mice with defective signalling from microbial Toll-like receptors (TLRs) retain some N_{BH} cells, suggesting that N_{BH} cells also require developmentally regulated microbe-independent signals. We found that the peri-MZ of the spleen contains a novel subset of lymphoid tissue inducer (LTi) cells. Similar to the LTi cells that orchestrate the development of mucosal lymphoid organs, peri-MZ LTi cells lack common B and T cell markers, but express the surface NK-related molecules CD56 and NKp44, the IL-7 receptor CD127, the c-Kit receptor CD117, the nuclear retinoic acid orphan receptor γt (RORγt), and the cytokines lymphotoxin and IL-22. Of note, peri-MZ LTi cells also secrete the cytokines IL-8 and GM-CSF, which promote the survival, activation and possibly recruitment of N_{BH} cells. Furthermore, peri-MZ LTi cells release large amounts of the cytokine B cell-activating factor of the TNF family (BAFF), which promotes the survival of MZ B cells and their differentiation into IgM-secreting plasmablasts. By showing that splenic LTi cells cooperate with N_{BH} cells to induce IgM production as well as IgA class switching, our data identify a novel LTi cell-regulated pathway for the generation of antibody-inducing signals in the MZ of the spleen.

O12

SIRPα Expression Identifies Intestinal DC Subsets That Determine Mucosal T Cell Polarisation

Charlotte Scott, Calum Bain, Vuk Cerovic, Stephanie Houston, Aude Aumeunier, Simon Milling, Allan Mowat
University of Glasgow, Glasgow, UK

The intestinal immune system is continually challenged by a wide variety of antigens, including commensal bacteria, food proteins and invasive pathogens. It is essential to discriminate between the different types of antigens so that either protective immunity or tolerance is induced appropriately. These different responses are driven by distinct populations of $CD4^+$ T cells whose differentiation is determined by local dendritic cells (DC). However precise identification of the DC involved has been confused by the use of non-specific and overlapping markers that did not allow precise discrimination between DC and other myeloid cells in the intestine. Using rigorous gating strategies, we show here that intestinal DC can be subdivided based on the expression of CD103, CD11b, CD8α and signal regulatory protein α (SIRPα). Although SIRPα is expressed on all $CD11b^+$ DC and macrophages, SIRPα mutant mice, which lack the cytoplasmic signalling domain of the protein, have a selective defect in $CD103^+$ $CD11b^+$ DC in the small intestinal lamina propria (siLP) and MLN. This is associated with a profound decrease in IL17 producing $CD4^+$ T cells in the mucosa, but normal numbers of T_H1 cells and T_{reg}. Preliminary results suggest that SIRPα may control the survival of $CD103^+$ $CD11b^+$ DC rather than alter their development or migration. Together these findings indicate that SIRPα dependent $CD103^+$ $CD11b^+$ DC in the siLP may play a specific role in generating mucosal T_H17 cell responses.

O13

Tuft Cells: New Functions to Be Unraveled in the Intestinal Epithelium

François Gerbe[1,2], Johan van Es[3], Leila Makrini[1,2], Bénédicte Brulin[1,2], Georg Mellitzer[4], Sylvie Robine[5], Béatrice Romagnolo[6], Noah Shroyer[7], Jean-François Bourgaux[8], Christine Pignodel[8], Hans Clevers[3], Philippe Jay[1,2]
[1]Inserm, Montpellier, France, [2]CNRS, Montpellier, France, [3]Hubrecht Institute, Utrecht, The Netherlands, [4]IGBMC, Strasbourg, France, [5]Curie Institute, Paris, France, [6]Cochin Institute, Paris, France, [7]Children Hospital, Cincinnati, USA, [8]CHU, Nimes, France

The intestinal epithelium consists of a continuously renewed monolayer of cells, in which four well described differentiated cell types have been described to date: nutrient absorptive enterocytes and three secretory cell types known as enteroendocrine, Paneth, and goblet cells. Less well known is a minor population of epithelial cells called tuft (brush) cells, which have been described within several endoderm-derived epithelia for several decades. Such cells are characterized by a thick brush of long microvilli that project towards the lumen, but their function remains mysterious.

Using immunohistochemistry and genetic lineage tracing experiments, we defined a molecular signature allowing unambiguous identification of tuft cells and demonstrated their epithelial origin. This signature includes inflammation-associated proteins such as COX-1/2, the SOX9 transcription factor as well as the microtubule-linked kinase DCLK1, the later being previously thought to label a population of quiescent stem cells in intestinal crypts. We then analyzed the presence of tuft cells in several genetically engineered mouse models with altered intestinal cell differentiation. We showed that, whereas the ATOH1/MATH1 transcription factor is essential for tuft cells differentiation, Neurog3, SOX9, GFI1, and SPDEF are dispensable. These results indicate that tuft cells belong to the secretory lineages of the intestinal epithelium but are distinct from the enteroendocrine, Paneth, and goblet cell types, thus defining a new epithelial lineage. Finally, we showed that tuft cells are the main source of endogenous intestinal epithelium-produced opioids and, likely, prostanoids, suggesting important roles for these cells in inflammatory diseases and cancer.

O14

Integrin αvβ8-Mediated TGFβ Activation by Dendritic Cells Regulates Th2 Responses and Is Required for the Development of Chronic Gastrointestinal Parasite Infection

Joanna Klementowicz[1,2], John Worthington[1], Beata Czajkowska[1], Mark Travis[1]
[1]University of Manchester, Manchester, UK, [2]University of California, San Francisco, San Francisco, USA

The immune system must be rapidly activated in response to harmful pathogens to prevent overwhelming or chronic infection. An important world health problem is chronic infection with helminth parasites, which infect ~ 2.5 billion people in the developing world causing severe economic and health burdens. To develop chronicity, helminths must evade host Th2-type immune responses. However, the mechanisms underlying how helminths avoid host immunity during chronic infection are poorly understood.

An important immunoregulatory cytokine is TGFβ, which is produced by many cells but always as a latent complex that must be activated to function. How TGFβ regulates immune responses during parasitic infection and mechanisms that regulate TGFβ function upon infection are ill-defined. We now find that mice lacking expression of the TGFβ-activating integrin αvβ8 on dendritic cells are completely protected from chronic infection with the intestinal helminth Trichuris muris, an established model for the human parasite Trichuris trichura (which affects over 1 billion people world-wide). Protection is associated with an enhanced local Th2-type response early during infection, suggesting that DC-mediated TGFβ activation is important in suppressing protective responses during helminth infection. Interestingly, the protective mechanism does not appear to involve Foxp3+ Tregs, as depletion of these cells during infection does not alter worm burden.

Our data therefore suggests that the integrin-DC-TGFβ axis is important in dampening protective host immune responses against gut helminths, thus facilitating development of chronic infection. Such work will potentially lead to the identification of novel therapeutic targets aimed at preventing and treating these poorly managed diseases.

O15

Gut Flora and Poly(I:C) Cooperate in the Intestine to Stimulate Dendritic Cells and Protect Neonate Mice against an Enteric Infection by *Cryptosporidium parvum*

Sonia Lacroix-Lamandé[1], Louis Lantier[1], Françoise Drouet[1], William Guesdon[1], Richard Lo-Man[3], Catherine Werts[2], Fabrice Laurent[1]
[1]INRA UMR1282 ISP, Control and Immunology of Neonatal Enteric Disease group, 37380 Nouzilly, France, [2]Institut Pasteur, Biology and Genetics of Bacterial Cell Wall Group, 75724 Paris, France, [3]Institut Pasteur, Immune Regulation and vaccinology, 75724 Paris, France

Neonates are particularly prone to digestive and respiratory infections due to the immaturity of their immune systems. TLR-ligands are powerful stimulants of the innate immune system. In this study, we investigated the role of TLR3 stimulation in neonate mice to control Cryptosporidium parvum development in epithelial cells of the intestine. Following poly(I:C) administration, a rapid reduction in parasite burden was observed which further required the presence of TRIF signaling molecules and CD11c+ cells. However, the protective effect of poly(I:C) was significantly reduced when the gut flora of neonates was diminished by an antibiotic treatment. Therefore in addition to TRIF signaling, the mechanism of protection depends on the presence of additional signals from the commensal flora. Using several different deficient mouse models, we demonstrated that the presence of MyD88 signaling is necessary but the mechanism of protection is independent of TLR2, TLR4 and TLR9. This synergy between TRIF and Myd88 signaling allowed for a higher level of expression of type I IFN and IL-12p40; two cytokines necessary to the poly(I:C) induced protection. The observed decrease in parasite burden was independent of the presence of functional T cells but poly(I:C) administration was associated with NKp46+ cell activation and an increase in their intracellular IFNγ. IFNγ has previously been shown to play a key role in the elimination of C. parvum infected enterocytes. Overall these data demonstrate that the commensal flora provides the additional signals to poly(I:C) stimulation to induce strong dendritic cell activation and subsequent control of parasite replication.

O16

CX3CR1+ Macrophages Induce the Expansion of IL-22 Producing CD4+RORgt+CD3- Innate Lymphocytes Required for the Control of a *C. rodentium* Infection

Calin Manta[1], Katarina Radulovic[1], Valerio Rossini[1], Christian Riedel[2], Jan Niess[1]
[1]Univertity Clinic, Ulm, Germany, [2]University, Ulm, Germany

Introduction: Innate immune cells, such as innate lymphoid cells (ILC), intestinal epithelial cells (IEC), macrophages and granulocytes and provide a first line of defense to enteric pathogens.

Aims and Methods: To study the role of CX3CR1+ macrophages in host defence we infected CX3CR1-GFP animals with a red fluorescent Citrobacter rodentium mutant that was generated by integrating the plasmid p16S_PT5mRuby into a 16S locus of the bacterial chromosome.

Results: When CX3CR1-GFP animals are infected with C. rodentium CX3CR1-/- animals showed a delayed clearance of C. rodentium as compared to wt B6 animals as demonstrated by increased fecel counts, more sever histopathologic colitis scores, increased colon weight/colon length ratios and increased C. rodentium burden in liver, spleen and mesenteric lymph nodes (MLN). Red fluorescent C. rodentium are located within CX3CR1+ macrophages as shown by ex vivo confocal imaging. The delayed clearance of C. rodentium is associated with reduced numbers of IL-22 producing lymphoid-tissue inducer cells (LTi cells). The reduced IL-22 expression correlates with decreased expression of the antimicrobial peptides RegIIIβ and RegIIIγ. C. rodentium infection in RAG-/- x CX3CR1GFP/GFP is associated with increased macroscopic signs of C. rodentium-induced colitis, increased colon weight/colon length ratios and strong reduction of IL-22 producing cells in the colonic lamina propria. The depletion of CX3CR1+ cells by diphtheria toxin injection in CX3CR1-GFP x CD11c.DOG animals confirmed the role of CX3CR1+ macrophages in establishing IL-22 production by LTi cells.

Conclusion: The CX3CR1+ macrophage dependent regulation of IL-22 production may hence play an important defence mechanism to enteric pathogens.

O17

Re-Utilization of Peyers Patches´ Germinal Centers Explains How Gut IgA Responses Can Be Synchronized and Oligoclonal

Nils Lycke, Mats Bemark, Peter Bergqvist, Anneli Stensson
Univ. of Gothenburg, Gothenburg, Sweden

Whereas recent studies have pointed to multi-centered and diverse gut IgA responses to the microbiota, little information is available on IgA responses following oral immunization with T cell-dependent antigens. Here we have addressed how gut IgA responses develop using a novel approach where NP-hapten was conjugated to cholera toxin, which allowed us to follow, at the molecular level, the site of initiation, expansion, differentiation and distribution of a specific IgA B cell response. Clonal relationships and affinity maturation of specific IgA cells at gut inductive and effector sites were investigated. Unexpectedly, we found gut IgA B cell responses to be oligoclonal and dominated by high affinity maturation. Extensive lineage trees of gut NP-specific IgA cells were generated, revealing strong clonal relationships throughout the entire gut mucosal immune system. Thus, clonally related IgA cells were found in Peyer's patches, mesenteric lymph nodes and the small and large intestine, suggesting an effective expansion and selection process. This was achieved through synchronization of multiple PP hosting the same high affinity B cell clones. We show that a rapid distribution of primed B cells from one PP into existing germinal centers in multiple PP is the key to the effectiveness of gut IgA responses

O18

The Liver IgA Immune Response: A Business of B Cells and Dendritic Cells

Ludovic Moro-Sibilot, Bertrand Dubois, Dominique Kaiserlian
INSERM-U851, Lyon, France

IgA is the most abundant immunoglobulin of the body and plays a critical role in maintenance of intestinal homeostasis by regulating commensal bacteria and neutralizing pathogens without inducing overt tissue inflammation. B cell activation, IgA isotype switching and differentiation into plasma cells is initiated in mucosal-associated lymphoid tissues and involves cells and factors, also involved in oral tolerance. Capitalizing on our recent results that the liver, a site of T cell tolerance to intestinal antigens, is enriched in tolerogenic dendritic cells (DC) and crucial for oral tolerance (Goubier et al. Immunity 2008), we explored the role of the liver and hepatic dendritic cells in the IgA response. IgA plasma cells (PC) are i) highly enriched in the liver (70% of total liver PC) as compared to MLN, spleen and blood, ii) reduced by 10 fold in T cell-deficient mice, indicating that liver IgA PC differentiation is mostly T cell-dependant. Liver B cells, which account for 20-30% of liver leukocytes, contain a majority of IgD^+ follicular B cells and a minor fraction of B1 B cells. Both liver plasmacytoïd DC and conventional DC induce T-cell-dependent IgA class switch recombination of naïve IgD+ B cells under CD40 stimulation. Finally, in vivo studies revealed that activated Ag-specific B cells are found in the liver as early as day 5 after oral or cutaneous immunization with the cognate Ag together with adjuvant. Our data illustrate for the first time that the liver contains IgA-inducing DC and is a privileged site for the IgA response.

O19

Dietary Non-Digestible Oligosaccharides Suppress Mucosal T_h2 Responses through Suppression of Co-Stimulatory Molecule Expression by Lamina Propria DC

S. de Kivit[1], J. Kerperien[1], M.E. Morgan[1], S. Overbeek[1], B.C.A.M. van Esch[1,2], G.A. Hofman[1], L.M.J. Knippels[1,2], A.D. Kraneveld[1], L.E.M. Willemsen[1], J. Garssen[1,2]
[1]Pharmacology, Utrecht Institute Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands, [2]Danone Research - Centre for Specialised Nutrition, Wageningen, The Netherlands

Prebiotic galacto- and fructo-oligosaccharides (scGOS/lcFOS) resembling oligosaccharides in human milk have been reported to reduce the development of allergy through modulation of the intestinal microbiota and immune system. Recently, we have shown that dietary intervention with non-digestible oligosaccharides, in combination with Bifidobacterium breve M-16V (GF/Bb) suppresses mast cell degranulation via induction of galectin-9 secretion by the intestinal epithelium in a murine model for whey-induced cow's milk allergy. However, it is not known whether GF/Bb modulates antigen presenting cell and T cell responses in the intestinal mucosa. To this end, Balb/c mice fed a control or GF/Bb diet were orally sensitized to ovalbumin (OVA), followed by oral challenge with OVA and small intestinal lamina propria (LP) DC were collected for flow cytometric analysis. OVA-sensitized mice showed an acute allergic response upon intradermal OVA injection in the ear, which was suppressed by GF/Bb. This was paralleled by reduced CD40, CD80 and CD83 and increased CD103 expression by $CD11c^+MHC\text{-}II^+$ LP DC. Furthermore, $CD11c^+$ LP cells from OVA-sensitized mice fed GF/Bb produced less IL-4 compared to OVA-sensitized mice fed control diet upon oral OVA challenge, which was reflected by lower percentage of activated T_h2 ($CD4^+CD69^+GATA\text{-}3^+$) cells and increased T_{reg} ($CD4^+Foxp3^+$) cells in the LP. Hence, dietary intervention with GF/Bb suppresses LP DC activation and consequently the development of an allergic T_h2 type effector response in the small intestine. These data suggest that dietary supplementation with GF/Bb may be an effective strategy to induce antigen specific tolerance towards allergens in the intestinal mucosa.

O20

Overexpression of CXCL10/CXCR3 Axis in Intestinal Mucosa in Active Coeliac Disease

Constanza Bondar[1], Romina Araya[1], Ezequiel Rulli[1], Luciana Guzman[2], Eduardo Cueto Rua[2], Nestor Chopita[3], Fernando Chirdo[1]
[1]Laboratorio de Investigacion en el Sistema Inmune- LISIN. Facultad de Ciencias Exactas. UNLP, La Plata, Argentina, [2]Servicio de Gastroenterologia. Hospital de Niños, La Plata, Argentina, [3]Servicio de Gastroenterologia. Hospital San Martin, La Plata, Argentina

CXCR3/CXCL10 axis was pointed out as one of the most relevant pathways in attracting cells to inflamed tissues. CXCL10 interacts and activates CXCR3, which is expressed, among others cells, by T lymphocytes (particularly, CD4+Th1). CXCR3 also interacts with CXCL9 and CXCL11. Small intestine mucosa in active coeliac disease (CD) is characterised by a massive infiltration of CD4+Th1 cells.

The aim of this work was to assess the role of CXCL10/CXCR3 axis in CD pathogenesis. Intestinal biopsies and blood samples were taken from pediatric and adult patients under routine diagnostic procedure.

CXCL10 and CXCL11 mRNA levels, but not CXCL9, were significantly higher in biopsies from paediatric CD patients (n=12) compared to controls (n=10)($p=0,005$). These levels were consistent with an increased expression of γIFN ($p<0,0001$). Similar results were obtained in adult population. Analysis by immunofluorescence microscopy showed extremely high expression of CXCL10 in duodenal mucosa of CD patients. In part, CXCL10 producers were CD3+ T lymphocytes. By ELISA, serum concentration of CXCL10 was higher in pediatric CD patients (n=8) compared to controls (n=6)($p=0,04$).

CXCR3 mRNA showed no differences between coeliac and control biopsies. Otherwise, immunofluorescence analysis revealed a higher number of CXCR3+ cells in lamina propria of active CD patients (n=9) than controls (n=6)($p=0,012$).

In conclusion, we showed an increased production of CXCL10 in the small intestine of untreated CD patients and a higher number of CXCR3+ cells in intestinal lamina propria. These results suggest that a massive production of CXCL10 in active CD may mediate the recruitment and activation of CXCR3+ cells.

O21

Reduced Expression of Thymic Stromal Lymphopoietin in Coeliac Disease

Paolo Biancheri[1,2], Antonio Di Sabatino[1], Laura Brunetti[1], Alessandra Pasini[1], Paolo Giuffrida[1], Erika Mileti[3], Francesca Ammoscato[2], Maria Rescigno[3], Thomas T. MacDonald[2], Gino R. Corazza[1]
[1]First Department of Medicine, IRCCS Policlinico S. Matteo, University of Pavia, Pavia, Italy, [2]Centre for Immunology and Infectious Disease, Blizard Institute, Barts and the London School of Medicine and Dentistry, London, UK, [3]Department of Experimental Oncology, European Institute of Oncology, Milan, Italy

Background and Aims: Intestinal epithelial cells are an important source of thymic stromal lymphopoietin (TSLP), a cytokine which polarizes dendritic cells towards a tolerogenic phenotype. Mucosal dendritic cells display increased maturation and activation in untreated coeliac disease (CD). We therefore measured TSLP expression in CD mucosa, and evaluated the ex vivo effect of TSLP on pro-inflammatory cytokine production by CD biopsies.

Patients and Methods: Duodenal biopsies were collected from 8 untreated CD patients, 8 treated CD patients and 8 control subjects. TSLP was detected by immunoprecipitation followed by Western blotting and by confocal microscopy. Duodenal biopsies from 6 untreated CD patients were cultured ex vivo with or without recombinant human (rh)TSLP. Interferon (IFN)-gamma and interleukin (IL)-17A concentrations were measured in organ culture supernatants by ELISA.

Results: In vivo mucosal TSLP expression was significantly reduced in the duodenum of untreated CD patients compared to treated CD patients and controls, without differences between treated CD patients and controls. rhTSLP significantly inhibited both IFN-gamma and IL-17A production by untreated CD biopsies cultured ex vivo.

Conclusions: Defective mucosal TSLP expression may contribute to the pathogenesis of CD. TSLP inhibition of pro-inflammatory cytokine production by untreated CD biopsies cultured ex vivo may be mediated by the induction of tolerogenic dendritic cells.

O22

Single Cell Analysis Reveals Increased pSTAT1 and Altered Responses to Type 1 Interferons in CD4 T Cells from Non-Inflamed Intestinal Mucosa in IBD

Ed Giles[1], Theodore Sanders[1], Neil McCarthy[1], James Lindsay[1,2], Ian Sanderson[1,2], Tom MacDonald[1], Andy Stagg[1]
[1]Blizard Institute, Barts and the London School of Medicine and Dentistry, London, UK, [2]Digestive Diseases CAU, Barts and the London School of Medicine and Dentistry, London, UK

Background Control of T cell reactivity within the intestinal mucosa is poorly understood. T cell plasticity is not fully delineated by traditional cytokine measurement. Analysis of phosphorylated signalling proteins, including Signal Transduction and Activators of Transcription (STATs), offers a more dynamic picture. Therefore we used Phosflow analysis of pSTATs to define intestinal T-cell responsiveness in IBD at the single cell level.

Methods Endoscopic biopsies were obtained from IBD patients and controls. Lamina propria mononuclear cells were isolated and stimulated or not for 15 minutes with Type I Interferon (IFNα/β). The cells were labelled with phospho-specific antibodies (pSTAT1, 3, 5) and for transcription factors (T-Bet, RORγt, FoxP3) and analysed by flow cytometry.

Results Constitutive pSTAT1 was increased in CD4+ve T-cells from non-inflamed mucosa of IBD patients compared with controls (n=30 IBD, 16 control, p=0.03). In paired IBD samples, constitutive pSTAT1 was higher in non-inflamed than inflamed areas (n=18, p=0.02). IFNa treatment increased pSTAT1 expression but IBD-related differences were maintained. pSTAT1 was not associated with expression of the T_H1 transcription factor T-Bet. There were no differences in expression of pSTAT3, pSTAT5 or unphosphorylated STAT1 irrespective of IFNa stimulation. Compared with IFNα responses, IFNβ stimulated less pSTAT1 (n=13, p<0.001), and decreased pSTAT5 (p=0.05).

Conclusions Increased expression of pSTAT1 in CD4 from non-inflamed areas of IBD mucosa is not simply a measure of T_H1 cell phenotype, and may indicate a role for type 1 Interferon in restraining inflammation. Altered responsiveness to different Type I IFNs may ultimately contribute to the loss of immune homeostasis.

O23

Functional Consequences of a Novel IL-10 Receptor Alpha Mutation on Innate and Adaptive Immunity in Early-Onset Inflammatory Bowel Disease

Marieke van Leeuwen[1], Sharon Veenbergen[1], Rogier Kersseboom[2], Lilian de Ruiter[1], Rolien Raatgeep[1], Dicky Lindebergh-Kortleve[1], Gert-jan Driessen[3], Hankje Escher[1], Janneke Samsom[1]
[1]Dept. of Paediatric Gastroenterology, Erasmus Medical Centre- Sophia Children's Hospital, Rotterdam, The Netherlands, [2]Dept. of Clinical Genetics, Erasmus Medical Centre, Rotterdam, The Netherlands, [3]Dept. of Paediatric Infectious Disease and Immunology , Erasmus Medical Centre, Rotterdam, The Netherlands

Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the intestine driven by aberrant T-cell responses to intestinal microbiota. The immunosuppressive cytokine interleukin-10 (IL-10) plays a pivotal role in orchestrating intestinal immune homeostasis. Here, we describe a now 9-year old IBD patient with a novel homozygous frameshift mutation in the IL-10 receptor alpha chain. Disease presented in the first weeks of life with severe early-onset colitis and fistulising perianal disease; remission was achieved with thalidomide, intravenous immunoglobulin (IVIG) and colchicine. In contrast to previously reported mutations in early-onset IBD patients, the mutated form of the IL-10 receptor alpha was detectable on the cell surface of peripheral blood mononuclear cells (PBMCs) by flow-cytometry. However, lipopolysaccharide (LPS)-stimulated PBMCs failed to downregulate TNFa production in response to IL-10. Additionally, impaired IL-10-mediated signalling was observed as shown by deficient STAT3 (signal transducer and activator of transcription 3) phosphorylation in PBMCs. Despite normal co-stimulatory molecule expression, monocyte-derived dendritic cells released enhanced amounts of TNFa and IL-6 upon LPS stimulation. During treatment no significant differences were found in the frequency of circulating Foxp3+ regulatory T cells, T helper 1 (Th1) and Th17 cells in peripheral blood of the patient. However, IL-10 failed to control IFNγ and IL-17 production by activated CD4+ T cells in vitro. Taken together, our study describes the functional consequences of a novel IL-10 receptor mutation in immune cells and contributes to our understanding of how IL-10 controls both antigen presenting cells and effector T cells in human intestinal immune responses.

O24

Dysregulated Dendritic Cell Function in Ulcerative Colitis is Partially Restored by *Lactobacillus plantarum* Extracellular Encypted Peptide

Elizabeth Mann[1], David Bernardo[1], Borja Sanchez[2], Hafid Al-Hassi[1], Simon Peake[1,3], Jonathan Landy[1,3], Maria Urdaci[4], Aberlardo Margolles[2], Stella Knight[1]
[1]Imperial College London, Harrow, UK, [2]Instituto de Productos Lacteos de Asturias-CSIC, Asturias, Spain, [3]St. Mark's Hospital, Harrow, UK, [4]Universite de Bordeaux, Bordeaux, France

Background: Gut dendritic cells (DC) are pivotal in the induction of immune tolerance. The aryl hydrocarbon receptor (AhR) regulates intestinal inflammation, but gut DC had yet to be characterised for AhR expression. We aimed to identify changes in gut DC underlying the dysregulated immune response in patients with ulcerative colitis (UC). We also aimed to condition these DC with a peptide (STp) secreted from probiotic strain Lactobacillus plantarum which we recently identified in the colon of healthy individuals but not UC patients.

Methods: Human DC from healthy controls and patients with active UC were isolated from colonic biopsies, conditioned for 24 hours +/- STp, and characterised by flow cytometry.

Results: Human gut DC expressed AhR; expression was reduced in UC patients. Few DC expressed Toll-like receptors (TLRs) 2 and 4 in healthy controls, but expression was greatly enhanced in UC. In contrast, expression of DC activation markers CD80 and CD83 was restricted on gut DC from UC patients. STp conditioning reduced TLR2 and TLR4 expression on gut DC from UC patients, but increased expression of activation markers CD80 and CD83. However, STp did not alter expression of AhR on gut DC.

Conclusions: We demonstrate for the first time that human gut DC express AhR, likely to contribute towards their role in mucosal immune tolerance. The enhanced TLR expression and reduced AhR/activation marker expression indicate a dysregulated DC function in UC. These results suggest independent roles for AhR and STp in normal gut homeostasis, that are missing in UC.

O25

The Human CD3-Specific Antibody Otelixizumab Down-Regulates the Inflammatory Response in Inflammatory Bowel Disease *In Vitro* in an IL-10-Dependent Manner

Anna Vossenkämper[1], Christian Hundsrucker[2], Lisa Das[3], Thomas MacDonald[1]
[1]*Centre for Immunology and Infectious Disease, Barts and The London School of Medicine and Dentistry, Blizard Institute, London, UK,* [2]*Institute for Functional Genomics, Computational Diagnostics Group, University of Regensburg, Regensburg, Germany,* [3]*Centre for Digestive Diseases, Barts and London School of Medicine and Dentistry, London, UK*

Inflammatory bowel disease (IBD) is largely mediated by T cells. Treatment of IBD with anti-CD3 antibodies has so far been unsuccessful. This study aimed to evaluate the anti-inflammatory properties of the Fc-engineered anti-CD3 antibody otelixizumab in intestinal samples from Crohn's disease (CD) and ulcerative colitis (UC) patients in vitro. Otelixizumab did not induce proliferation of mucosal T cells from control or CD and UC tissues and did not impair the viability of lamina propria mononuclear cells (LPMCs). Mucosal explants as well as LPMCs from IBD patients showed reduced production of IFN-γ and IL-17 when treated with otelixizumab. Otelixizumab reduced the phosphorylation status of a variety of receptor tyrosine kinases and signaling molecules involved in T cell-receptor and MAP-kinase signaling in inflamed mucosa. CD LPMCs cultured with otelixizumab showed reduced T-box transcription factor T-bet expression. CD biopsies and LPMCs showed increased p-STAT3 expression and IL-10 production when cultured with otelixizumab. Otelixizumab's anti-inflammatory effect was depended on IL-10 since neutralisation of IL-10 prevented down-regulation of IFN-γ production. This work suggests that otelixizumab may induce immune deviation in activated T cells by delivering a signal that activates the STAT3 pathway. Otelixizumab might therefore be a new candidate in the treatment of IBD.

O26

Nasal Immunization: Characterization of Primary Activation of Antigen-Specific T Cells

Gennaro Prota, Elena Pettini, Annalisa Ciabattini, Fabio Fiorino, Gianni Pozzi, Donata Medaglini
University of Siena, Siena, Italy

Priming of T cells is a key event in vaccination, since it bears a decisive influence on the type and magnitude of the immune response. Using the model antigen ovalbumin (OVA) plus CpG oligodeoxynucleotides as adjuvant, we have deeply studied and characterized the nasal route of immunization. Primary activation and dissemination of antigen-specific T cells was analyzed in vivo after adoptive transfer of OVA-specific transgenic $CD4^+$ T cells. Clonal expansion of transgenic T cells was evaluated in draining (cervical and mediastinal) and distal (iliac) lymph nodes and in the spleen at different time points (0, 48, 60, 72, 96, 108 and 120 hours after immunization). Proliferating T cells were detected in lymph nodes draining the upper respiratory tract (cervical) 60 hours after immunization while in distal lymph nodes and in the spleen they appeared 96 hours after the inoculum. Differently, if the nasal inoculum was mainly drained in the lower respiratory tract (mediastinal lymph node), a very rapid activation of OVA-specific T cells was observed in the spleen (60 hours). The progressive up-regulation of CD44 and CD62L and down-regulation of CD45RB in the progeny within draining lymph nodes indicated that dividing T cells were activated and capable of disseminating in other lymphoid organs.Priming of T cells following nasal immunization was compared with the one induced after vaginal and subcutaneous routes, and results were analysed by applying mathematical models. Different prime-boost schedules were also tested to identify optimal strategies for boosting nasally primed T-cells.

O27

MHC Class II Restricted H-2d Recognition of the Influenza M2e-Peptide Reveals CD4 T Cell Mediated Protection Independent of Humoral Immunity

Dubravka Grdic-Eliasson[1], Karin Schön[1], Xavier Saelens[2], Walter Fiers[2], Nils Lycke[1]
[1]Biomedicine, Gothenburg, Sweden, [2]VIB Molecular Virology Unit, Ghent, Belgium

Few mucosal vaccines have successfully been launched on the market. This is partly because of the lack of potent mucosal vaccine adjuvants. We have generated a targeted fusion protein based on the non-toxic mucosal adjuvant CTA1-DD, harboring tandem repeats of the conserved matrix protein 2 ectodomain (M2e) of influenza A virus. This mucosal influenza vaccine candidate, CTA1-3M2e-DD, was found to confer strong protective immunity against a lethal challenge infection with live influenza virus in mice. Although anti-M2e IgG antibodies correlated with protection in Balb/c mice, this correlation was less prominent in congenic Balb/b mice. These mice exhibited poor resistance to the challenge infection despite comparable anti-M2e IgG antibody titers. Hence, we hypothesized that CD4 T cell recognition of the H-2d-restricted M2e epitope conferred a better level of protection than specific serum antibody levels alone. Next we investigated the protection against live influenza in the complete absence of B cells. Remarkably, CTA-3M2e-DD immunized B cell deficient mice exhibited higher resistance against influenza compared to unimmunized controls. Using M2e-specific tetramers we were able to monitor specific T cells that recognized M2e. Interestingly, most of the M2e-specific T cells following immunization and influenza challenge were located in the lung of infected mice and recall response to M2e in challenged mice was dominated by IL-17A. In addition, IL-17A knockout mice immunized with CTA1-3M2e-DD prior to influenza challenge were less resistant against influenza than immunized wild-type controls, clearly highlighting the importance of CD4 T cells for protection against influenza infections.

Poster Presentations

P001

B Cells and CD4$^-$ CD8$^-$ T Cells Are Increased in Number in the Duodenal Mucosa of Children with Celiac Disease

Henrik Toft-Hansen[1,2], Hardee J. Sabir[1,2], Torben Barington[1,2], Steffen Husby[1]
[1]*Hans Christian Andersen Children's Hospital at Odense University Hospital, University of Southern Denmark, Odense, Denmark,* [2]*Dept. of Clinical Immunology, Odense University Hospital, University of Southern Denmark, Odense, Denmark*

Intestinal mucosa inflammation is a hallmark of celiac disease (CD), where the duodenal mucosa is infiltrated by lymphocytes. The pathogenesis is known to involve gluten-specific CD4$^+$ T cells, but other lymphocyte populations may also play a role. Using flow cytometry, we quantified lymphocyte populations in blood samples and duodenal biopsies from 14 children with CD (previously un-diagnosed) and compared to biopsies from 13 children, who did not have CD. There were no statistically significant differences in the blood samples for any of the lymphocyte populations. In the biopsies, we did not find any difference in the number of CD4$^+$ or CD8$^+$ T cells, CD4$^+$ CD8$^+$ (double-positive) T cells, NK cells or NKT cells, whereas the number of B cells (CD3$^-$ CD19$^+$) and the number of CD3$^+$ CD4$^-$ CD8$^-$ (double-negative) T cells were significantly elevated 6-7 fold in children with CD. The observed increase in B cells is likely different from the previously reported increase in plasma cells, since we measured CD19$^+$ cells. The increased number of B cells may reflect a role of B cells as antigen presenting cells in CD pathology. The increase in number of CD4$^-$ CD8$^-$ T cells likely reflects an increase in gamma/delta T cells, although this was not verified. We intend to correlate these results with gene expression results obtained from parallel biopsies to study genes involved in intestinal mucosa inflammation.

P002

Increased Protein Production of IL-15 in Adult Coeliac Disease Patients

Celia Escudero-Hernández[1], Sara Vallejo-Díez[1], Beatriz Maríinez-Abad[1], Enrique Montalvillo[1], Manuel Marugán[3], Luis Fernández-Salazar[3], José Antonio Garrote[2], Eduardo Arranz[1]
[1]*Universidad de Valladolid, Valladolid, Spain,* [2]*Hospital Universitario Rio Hortega, Valladolid, Spain,* [3]*Hospital Clinico Univeristario, Valladolid, Spain*

Introduction: An increased expression of the specific receptor of interleukin 15 (IL15-Ra) has been characterized in coeliac active patients but not in healthy individuals. Furthermore, the gluten ingestion in both groups gives rise to the expression of IL-15. Therefore, the study of this cytokine and its receptor could be interesting targets to better understand this disease.

Patients and methods: Western blot has been used to determine the expression of IL-15 and IL-15Ra using b-actin as a reference. The samples were protein extracts of duodenal biopsies from coeliac patients (n=13: 4 children, 5 adults, 1 adult in gluten-free diet and 3 refractive coeliac patients), non-coeliac pathologic controls (n=10) and apparently healthy individuals (n=10).We also used flow cytometry to study the cell level expression of IL-15 and IL-15Ra, extracellular and intracellular, using again duodenal biopsies from coeliac patients (n=5), non-coeliac pathologic controls (n=4) and apparently healthy individuals (n=5).

Results: Western blot analysis resulted in a significant increase of IL-15 in adult coeliac patients when compared to pathological ($p<0.1$) or healthy controls ($p<0.05$). What is more, IL-15 was significantly higher in all the groups but coeliac children ($p<0.05$). Moreover, the significant band of IL-15 western blot coincides with a band of IL-15Ra western blot; although IL-15Ra western blot did not show any significant differences.

On the other hand, flow cytometry shows the presence of intracellular IL-15 in lymphocytes but not IL-15Ra for both groups intraepithelial and lamina propria lymphocytes.

P003

DC-SIGN/HLA-DR+ Cells Increased in Coeliac Duodenum

Enrique Montalvillo[1], Juan Jesús García-Vallejo[3], Beatriz Martínez-Abad[1], Sara Vallejo-Diez[1], Celia Escudero-Hernández[1], Jose Antonio Garrote[1,2], Eduardo Arranz[1]
[1]University of Valladolid/I.B.G.M. Immunology Lab., Valladolid, Spain, [2]Hospital Universitario Rio Hortega, Valladolid, Spain, [3]Dpt. Cell Biology & Immunology. VU University Medical Center., Amsterdam, The Netherlands

We aimed to determine the DC-SIGN expression by immunofluorescence in duodenal biopsies from coeliac patients and controls.

We obtained endoscopic duodenal biopsies from 3 active Coeliac Disease (aCD), 3 Gluten Free Diet (GFD) and 3 Healthy Controls (HC). The samples were frozen in liquid nitrogen. After cutting 8µm tissue sections, slides were dried overnight.

The staining was done adding rabbit DC-Sign anti-human, labelled with α-rabbit AlexaFluor 488 and mouse HLA-DR anti-human or mouse CD4 anti-human, labelled with α-mouse AlexaFluor 647 for 1h at room temperature. We used the corresponding isotypes as controls. Slides were mounted using Vinol mounting medium with DAPIand viewed/analysed in a Leica DM6000 microscope.

DC-SIGN was found to be most highly expressed in the lamina propria cells of aCD patients. In these patients, we have observed that all the DC-SIGN+ cells are HLA-DR+. On the contrary, both HC and GFD show less DC-SIGN/HLA-DR+ cells. Also these samples show some HLA-DR+ cells that do not express DC-SIGN.

In aCD CD4+ T cell infiltration occurs in both the lamina propria and the epithelium. The T cells infiltration is accompanied with an increase in both the number of DC-SIGN+ cells and the expression level of DC-SIGN on HLA-DR+ cells in the lamina propria. This appears to be specific of aCD, as HC do not show CD4+ T cell infiltration and/or increased presence of DC-SIGN+. GFDpatients also show a lower CD4+ T cell infiltrate and DC-SIGN expression. This decreased depends on the time elapsed since the start of the gluten-free diet.

P004

Defective Tight Junctions in Refractory Celiac Disease

Michael Schumann, Sarah Kamel, Mare-Luise Pahlitzsch, Lydia Lebenheim, Martin Zeitz, Jörg-Dieter Schulzke, Severin Daum
Dept. of Gastroenterology, Charité, Berlin, Germany

Introduction: Celiac disease refractory to a gluten-free diet occurs in a small percentage of celiacs. An epithelial barrier defect is known to be an integral part of celiac pathophysiology. However, the mucosa in refractory celiac disease underlies a constant inflammatory process, which has not been addressed in this condition so far.

Aims: To investigate the tight junction-associated barrier in refractory celiac disease functionally and structurally.

Methods: Duodenal mucosae from endoscopy were selected: 9 celiac disease patients, 6 patients on gluten-free diet (GFD), 7 refractory celiac disease (RCD) patients (2x RCD type I, 5x RCD type II) and 9 control individuals. To analyze epithelial barrier function epithelial electrical resistance (Re) was determined in Ussing chambers by one-path impedance analysis. Western blotting and immunofluorescence confocal microscopy were applied for expression level and protein localization of claudin-2,-3,-4,-5. Filter-grown Caco-2 cells were studied for claudin endocytosis.

Results: Re was similarily reduced in celiac and RCD patients (celiacs: 13±2, RCD 15±1, controls 25±2 W×cm2) while patients on a GFD showed almost an Re-normalization (21±3 W×cm2). Western blotting and immunofluorescence confocal microscopy revealed a normal expression of claudin-4 in celiac disease but a downregulation in RCD, presumably by two mechanisms, reduced protein expression and increased claudin endocytosis, as revealed by experiments using the inhibitor dynasore on Caco-2 cells. Furthermore, the tightening claudin-5 is downregulated and the pore-forming claudin-2 is upregulated in RCD and celiac disease.

Conclusion: The epithelial barrier defect in RCD reflects structural tight junction alterations partially similar and partially different to celiac disease.

P005

Bile Acid Receptor-Activation Enhance LPS-Induced Inflammatory Responses of Human Monocytes

Kaia Mobraten, Tarjei Haugbro, Charlotte Ramstad Kleiveland, Lene Therese Olsen Hult, Tor Lea
University of Life Sciences, Department of Chemistry, Biotechnology and Food Science, Group of Molecular Cell Biology, Aas, Norway

In the discussion concerning the onset of metabolic inflammation one hypothesis is that during the feeding/fasting cycle, a pulsatile systemic inflammatory response will occur. This inflammatory response is likely a result of increased intestinal permeability postprandially, which couples uptake of nutrients with inflammatory molecules from the luminal microflora. After a meal, plasma levels of both bile acids and lipopolysaccharide (LPS) from gram-negative bacteria will increase and exceed the concentration threshold necessary to activate peripheral blood monocytes.

Bile acids are recognized as signalling molecules, mediating their effects both through nuclear receptor FXR and the cell surface receptor TGR5. Recent studies carried out in mice report that TGR5 have immunomodulating properties. We here report co-stimulatory effects of LPS and TGR5-activation in human monocytes. Treatment of human monocytes with LPS alone elicited production of the cytokines IL-6 and IL-8. Simultaneous treatment of human monocytes with LPS and the TGR5-agonist betulinic acid resulted in potently increased cytokine production compared to LPS alone. This synergistic effect of LPS and betulinic acid together, was also evident in the activation of the NF-κB signalling pathway.A low-grade inflammatory response like this will probably not be harmful because the inflammation will cease during fasting. However, overfeeding may result in a build-up of inflammatory responses, both systemically and in metabolic tissues.

These results may be important for the understanding of bile acids in inflammation and their functions as signalling molecules, in general.

P006

Regulation of Antibody Diversification and Production by Neutrophils

Irene Puga[1], Montserrat Cols[2], Carolina M Barra[1], Linda Cassis[1], Maurizio Gentile[1], Bing He[2], Meimei Shan[2], Alejo Chorny[2], Sergi Serrano[1], José Antonio Lorente[1], Juan Ignacio Aróstegui[3], Jordi Yagüe[3], Manel Juan[3], Vassilios Lougaris[4], Alessandro Plebani[4], Jean Donadieu[5], Jean-Laurent Casanova[6], Charlotte Cunningham-Rundles[2], Kang Chen[2], Andrea Cerutti[1,7]
[1]IMIM (Hospital del Mar Research Institute), Barcelona, Spain, [2]Mount Sinai School of Medicine, New York, USA, [3]Hospital Clínic de Barcelona, Barcelona, Spain, [4]Brescia University, Brescia, Italy, [5]Hopital Trousseau, Paris, France, [6]The Rockefeller University, New York, USA, [7]Catalan Institute for Research and Advanced Studies, Barcelona, Spain

Neutrophils are generally viewed as non-specific innate immune cells involved in phagocytosis, killing and inflammation. Neutrophils utilize immunoglobulins (Igs) to clear antigen, but their role in Ig production is unknown. Here we identified neutrophils around the marginal zone (MZ) of the spleen, a B cell area specialized in T-independent Ig responses to circulating antigen. Neutrophils colonized peri-MZ areas after post-natal mucosal colonization by microbes and enhanced their B-helper function upon receiving reprogramming signals from splenic sinusoidal endothelial cells, including IL-10. Splenic neutrophils induced Ig class switching, somatic hypermutation, and production by activating MZ B cells through a mechanism involving the cytokines BAFF, APRIL, and IL-21. Neutropenic patients had fewer and hypomutated MZ B cells and less preimmune Igs to T-independent antigens, which indicates that neutrophils generate an innate layer of antimicrobial Ig defense by interacting with MZ B cells.

P007

Intestinal IL-6 Controls Immune Inflammation in Human Ulcerative Colitis and Induces a Pro-Inflammatory Skin Homing Phenotype in Dendritic Cells and T-Cells They Stimulate

David Bernardo[1], Sara Vallejo-Díez[2], Elizabeth R Mann[1], Hafid O Al-Hassi[1], Beatriz Martínez-Abad[2], Enrique Montalvillo[2], Cheng T Tee[1], Aravinth U Murugananthan[1], Henar Núñez[3], Simon TC Peake[1], Jonathan Landy[1], Ailsa L Hart[4], Luis Fernández-Salazar[5], Jose A Garrote[6], Eduardo Arranz[2], Stella C Knight[1]
[1]Antigen Presentation Research Group. Imperial College London, Northwick Park & St Mark´s Campus, Harrow, UK, [2]Mucosal Immunology Lab. Department of Paediatrics & Immunology. Universidad de Valladolid, IBGM-CSIC, Valladolid, Spain, [3]Service of Gastroenterology. Hospital Universitario Rio Hortega., Valladolid, Spain, [4]Department of Gastroenterology. St Mark´s Hospital. North West London Hospitals NHS Trust, Harrow, UK, [5]Gastroenterology service. Hospital Clínico Universitario de Valladolid., Valladolid, Spain, [6]Genetics and Molecular Biology Lab. Service of Clinical Laboratory. Hospital Universitario Río Hortega, Valladolid, Spain

Background. Dendritic cells (DC) control the type and site of immune responses. Ulcerative colitis (UC) is considered a TH2 disease mediated by IL-13 where up to 1/3 of the patients can develop extra-intestinal manifestations. We hypothesised that local homeostatic factors are masked by ongoing inflammation in inflamed areas of the gut.

Methods: Colonic biopsies from inflamed and non-inflamed areas of UC patients were cultured in vitro. Cytokine secretion in culture supernatants was determined. Cell-free supernatants were used to condition human blood enriched DC from healthy volunteers. Phenotype and function of DC was determined by flow cytometry and mixed leukocyte reactions respectively.

Results: Levels of IL-13 in the culture supernatants were below the detection limit in most cases and the cytokine profile suggested a mixed profile rather than a TH2 cytokine profile. IL-6 was the predominant cytokine found in inflamed areas from UC patients and its concentration correlated with the Mayo endoscopic score for severity of disease. DC conditioned with non-inflamed areas from UC patients acquired a regulatory phenotype with decreased stimulatory capacity. However, DC conditioned with inflamed areas acquired a pro-inflammatory phenotype, increased expression of skin homing CCR8, did not decrease their stimulatory capacity for T-cells and primed them with the skin-homing CLA molecule in an IL-6 dependent mechanism.

Conclusion: Our results highlight the role of IL-6 in UC, question the concept of UC as a TH2 disease, the relevance of IL-13 in its aetiology and provide a molecular explanation for the development of extra-intestinal manifestations in IBD patients.

P008

Epithelial Layer Damage Primes the Recognition of Bacterial Products by Human Intestinal Lamina Propria Cells

Felix Lasitschka, Sonja Schwarz, Thomas Giese, Antje Heidtmann, Timea Sziksai, Sabine Wentrup, Antonia Schrödter, Mohammed Al-Saeedi, Christine Leowardi, Stefan Meuer, Jutta Schröder-Braunstein
Universitätsklinikum Heidelberg, Heidelberg, Baden-Württemberg, Germany

Human resident intestinal lamina propria cells express only low levels of pattern recognition receptors (PRR). Their mode of activation in the case of intestinal infections therefore remains obscure. Here, we investigated the interrelationship between epithelial layer damage, bacterial stimuli and inflammatory response of colonic mucosal cells using an organ culture model.

Punches of intact intestinal mucosa or injured mucosa denuded of epithelial cells were cultured in the presence or absence of E.coli lysates. After 12h, cytokine and surface receptor expression were determined in mucosal tissue as well as in leukocytes emigrated from the injured tissue.

Intact mucosa constitutively released no or only low amounts of IL-1beta, TNF-alpha IFN-gamma, and IL-22 into the organ culture supernatant. Exposure to E.coli lysates did not affect production of these cyokines. In contrast, injured mucosa denuded of epithelial cells released detectable levels of IL-22, which were further increased in the presence of bacterial lysates. Secretion of IL-1beta, TNF-alpha, and IFN-gamma by the injured mucosa was low /undetectable in the absence but clearly induced in the presence of E.coli lysates. Mucosal expression of CD14, TLR2, and CD86 was up-regulated following epithelial layer damage. It was also increased in mononuclear cells emigrated from the damaged tissue when compared to resting mononuclear mucosal cells. Exposure to E.coli lysates did not significantly further enhance expression of these receptors.

In conclusion, epithelial layer damage is required for the recognition of bacterial products by human lamina propria cells. It may promote recognition of bacterial products by up-regulating PRR in these cells.

P009

Pro-Inflammatory Vδ2+ Anti-Microbial T-cells Populate the Human Intestinal Mucosa but are Depleted by Therapy in Crohn's Disease

Neil McCarthy[1], Charlotte Hedin[1], Anna Vossenkämper[1], Shaumick Bhattacharjee[1], Sabrina Brown[1], Edward Giles[1], Theodore Sanders[1], Kevin Whelan[2], Thomas MacDonald[1], James Lindsay[3], Andrew Stagg[1]
[1]Centre for Immunology and Infectious Disease, The Blizard Institute, Bart's and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK, [2]Nutritional Sciences Division, King's College London, London, UK, [3]Digestive Diseases Clinical Academic Unit, Bart's and The London School of Medicine, The Royal London Hospital, London, UK

Vγ9Vδ2+ (Vδ2)T-cells are anti-microbial lymphocytes found only in higher primates and whose role in human intestinal immunity is unknown. Primate Vδ2T-cells expand and accumulate in mucosal tissues following microbial activation, and human Vδ2T-cells produce pro-inflammatory IFNγ, TNFα and IL-17A in response to bacterial species that colonize the gut. We hypothesized that Vδ2T-cells contribute to mucosal inflammation in Crohn's disease (CD). Intestinal biopsies and peripheral blood were analyzed by flow-cytometry in CD patients and healthy controls. Cell suspensions were stimulated with microbial phosphoantigen (HDMAPP) and IL-2 to determine Vδ2T-cell phenotype, cytokine production and proliferative potential. Circulating Vδ2T-cells displayed a 'multi-potent' tissue-homing profile, but expression of gut-homing integrin α4β7 predominated among these cells. Blood Vδ2T-cells proliferated and up-regulated α4β7 upon activation with HDMAPP and IL-2 in vitro, consistent with increased mucosal recruitment. Accordingly, Vδ2T-cells were detected in human colonic lamina propria using confocal microscopy, and intestinal Vδ2T-cells expressed high levels of epithelium-associated integrin CD103. Intestinal Vδ2T-cells proliferated, up-regulated CD70 and HLA-DR, and produced copious IFNγ and TNFα upon exposure to HDMAPP and IL-2 in vitro, indicating potential for pro-inflammatory responses to the gut microbiota. However, Vδ2T-cells were selectively lost from the blood and mucosa of CD patients receiving azathioprine therapy, and physiological doses of azathioprine blocked the proliferation of intestinal Vδ2T-cells in vitro. These data demonstrate that circulating human Vδ2T-cells display enhanced gut-homing potential upon microbial activation and can populate the intestinal mucosa. Human intestinal Vδ2T-cells may contribute to pro-inflammatory responses against the gut microbiota but are depleted by therapy in CD.

P010

Protective Role of a Non-Commmensal Methanotroph Bacterium in a Mouse Model of Colitis

Charlotte R. Kleiveland[1], Lene T. Olsen Hult[1], Signe Spetalen[3], Morten Bj. Jacobsen[1,2], Tor Lea[1]
[1]Molecular Cell Biology Group, Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Aas, Norway, [2]Ostfold Hospital Trust, Fredrikstad, Norway, [3]Oslo University Hospital, The Norwegian Radium Hospital, Oslo, Norway

Inflammatory bowel disease (IBD) is believed to be due to dysregulated interactions between environmental, immunological and bacterial factors in genetically susceptible individuals. In this context the significance of microbial stimuli from the gut flora has been highlighted as important regulators of mucosal homeostasis.

We have conducted a pilot experiment in a dextran sulfate sodium (DSS)-model of IBD. C57BL/6 mice were given either a standard diet (SD) or a standard diet containing a bacterial meal consisting mainly of a non-commensal methanotroph bacterium, for 7 days before being exposed to 3% DSS in the drinking water.

Inclusion of the bacterial meal in SD lead to a significantly reduced inflammation examined both by colon length, histological scoring and effects on a variety of parameters known to be involved in pathogenesis of IBD such as SAA3, S100A9 and MPO. In the lamina propria gene expression studies revealed strongly decreased levels of all inflammatory cytokines, including IL-6 and IL-17, and the transcription factors characteristic of the different T-effector cells, including Treg. However, the depth, number of cells and the amount of proliferating cells in colon crypts were significantly increased in mice receiving bacterial meal compared to SD. These results show that mice receiving bacterial meal are less prone to developing inflammation after DSS-exposure, and that this could be caused by increased renewal of the colon epithelium due to increased crypt cell proliferation. Our data point to the importance of microbial stimuli in epithelial growth regulation and its significance in the prevention of intestinal inflammation.

P011

Impact of Dietary EPA and DHA on Colitis Prevention in a Mouse Model of IBD

Viral Brahmbhatt[1], Manuel Oliveira[1], Viktoria Bastic Schmid[1], Cecil Pace-Asciak[2], Jalil Benyacoub[1], Nabil Bosco[1]
[1]*Nestlé Research Center, Lausanne, Switzerland,* [2]*The hospital for sick children, Toronto, Canada*

Although the immunoregulatory effects of omega-3 fatty acids have been well characterized in vitro, their role in intestinal inflammation is controversial. The aim of this study was to assess the impact of dietary fish oil intake on the development of colitis and investigate the effects on eicosanoid metabolism in a mouse model of inflammatory bowel disease (IBD). Rag-2 deficient mice were fed fish oil (omega-3 fatty acids enriched) for 4 weeks before colitis induction by adoptive transfer of naïve $CD4^+CD45RB^{high}$ T cells and maintained in the same diet for 4 additional weeks. Onset of colitis development was monitored by colonoscopy and further confirmed by immunological and histological examinations. Eicosanoid metabolites were measured by HPLC-MS/MS in colonic samples of control and experimental animals. No significant reduction in IBD scores was observed between fish oil or control diet fed mice. However, lipidomic analyses of colonic tissue clearly show significant reduction of proinflammatory lipid metabolites in fish oil fed mice compared to control. Further investigations on the time course of development of inflammation would be needed to better determine the potential benefit of dietary fish oil on colitis.

P012

The Transcription Factor NFATc2 Promotes IL-6 Dependent Colitis Associated Colorectal Cancer

Katharina Gerlach[1], Hans A. Lehr[2], Alexei Nikolaev[4], Thomas Gerlach[1], Raja Atreya[1], Stefan Rose-John[3], Markus F. Neurath[1], Benno Weigmann[1]
[1]*University Erlangen Department of I.Medical Clinic, Erlangen, Germany,* [2]*Universite Lausanne Institut Universitaire de Pathologie, Lausanne, Switzerland,* [3]*University Kiel Institute of Biochemistry, Kiel, Germany,* [4]*University Mainz Institute of Molecular Medicine, Mainz, Germany*

NFATc2 (Nuclear factor of activated T-cells) belongs to a transcription factor family regulating Th2 activation, cytokine production the cell cycle and the cell growth implying a role in cancer development. The transcription factor family members play an important role in the activation and function of T-cells. Furthermore NFATc2 has an influence on the pro-tumorigenic cytokine IL-6. Therefore we examined the role of NFATc2 in the establishment of colon cancer mediated by IL-6 expression.

Methods: NFATc2ko mice were treated with AOM/DSS to induce colon cancer. Miniendoscopic analysis has been done to monitor the number and size of the tumors. Intracellular IL-6 immunohistochemistry staining was analysed in sections from normal and tumor tissue. Cytokine measurements were done by ELISA and RT-PCR.

Results: In our experimental colitis-associated cancer model NFATc2ko mice do not develop colorectal tumors, in contrast to wildtype. The protection of colonic tumor progression was associated with increased apoptosis rate in the lamina propria of NFATc2ko mice and significantly increased levels of IL-6 in the serum and the colon. Further analyzing IL-6 involvement in the colon cancer model, the application of hyper IL-6, a fusion protein of IL-6 and soluble IL-6-receptor, to the NFATc2ko mice abrogated the protective effect and restored the tumor development approving the pro-tumorigenic effect of IL-6.

Conclusion: The findings point out the important role of NFATc2 in the development of colorectal tumors as NFATc2 has a tumorpromotive effect in the experimental cancer model. This is mainly caused by the regulation of the critical pro-inflammatory cytokine IL-6

P013

Nod2 Deficiency is Associated with Increased Mucosal Regulatory Response Abrogated by Antibiotics Treatment

Antonello Amendola, Alessia Butera, Massimo Sanchez, Monica Boirivant
Istituto Superiore di Sanità, Roma, Italy

Background: It has been reported that Peyer's patches (PPs) of Nod2 KO mice show, as a consequence of the presence of ileal microbiota, an increased intestinal permeability and increased tissue content of IFN-γ and TNF-α (Gut 2010;59:207), yet the mice do not show intestinal inflammation. Moreover, Nod2 variants in healthy Crohn's Disease relatives associate with increased intestinal permeability. Nod2-deficient mice display also an increased load of commensal microbiota, and altered microbial composition. Humans carrying NOD2 variants (SNP13) also have significantly increased loads of Bacteroidetes and Firmicutes. Therefore, Nod2 mutation is not sufficient "per se" to establish inflammatory lesions both in humans and in animal models.

Aim: We evaluated the effect of NOD2 on the severity of TNBS colitis and the effect of induced dysbiosis. To this purpose we induced TNBS colitis in NOD2 KO and WT mice previously treated or not with Ampicillin for 3 weeks after weaning.

Results: NOD2 KO mice show, when compared with WT, a significant less body weight reduction after TNBS colitis induction associated with less severe inflammatory lesions. Protection from TNBS colitis was associated to the presence of LAP positive regulatory lamina propria mononuclear cells. WT mice treated with ampicillin did not show variation in TNBS colitis when compared with untreated mice. NOD2 KO mice treated with ampicillin showed a severe TNBS colitis comparable to the one observed in ampicillin treated/untreated WT mice.

Conclusions: NOD2 KO mice show reduced susceptibility to TNBS colitis that is abrogated by antibiotic treatment.

P014

Does Interleukin IL-13 Have a Role in Sustaining the Mucosal Pro-Inflammatory Immune Response in Ulcerative Colitis and in Promoting Intestinal Fibrogenesis in Crohn's Disease?

Francesca Ammoscato[1], Paolo Biancheri[1,2], Antonio Di Sabatino[2], Ijeoma Joe-Njoku[1], Laura Rovedatti[2], Gino R. Corazza[2], Thomas T. MacDonald[1]
[1]*Centre for Immunology and Infectious Disease, Blizard Institute, Barts and the London School of Medicine and Dentistry, London, UK,* [2]*First Department of Medicine, Fondazione IRCCS Policlinico S. Matteo, University of Pavia, Pavia, Italy*

Background and Aims. Interleukin (IL)-13 is produced mostly by Th2 cells and appears to be involved in animal models of gut fibrosis. It has also been suggested that IL-13 is over-expressed in ulcerative colitis (UC). We have therefore investigated IL-13 production in the mucosa and muscle layers of patients with UC or Crohn's disease (CD).

Patients and Methods. Biopsies and lamina propria mononuclear cells (LPMCs) from inflamed colon of 11 CD, 9 UC and 15 control patients were cultured ex vivo or with anti-CD3/CD28-antibodies. IL-13, IL-17A and interferon (IFN)-gamma production was measured by ELISA. Strictured and non-strictured muscle layer explants from 6 CD patients were cultured ex vivo and IL-13 and collagen production measured.

Results. IL-13 production did not differ between CD, UC and control biopsies although IFN-gamma and IL-17A were significantly higher in CD and UC than in controls. Anti-CD3/CD28-stimulated LPMCs showed a small increase in IL-13 production without significant differences between the groups. Collagen production was higher in strictured CD, but IL-13 did not differ between strictured and non-strictured CD explants.

Conclusions. Our findings showing that IL-13 production by both inflamed UC mucosa and strictured CD explants was low do not support a prominent role for this cytokine in mediating mucosal inflammation in UC and intestinal fibrogenesis in CD.

P015

Interaction between Glycans and the Immune System: Do Glycans Play a Role in Crohn's Disease Pathogenesis?

Liran Baram[1], Sarit Cohen-Kedar[1], Hofit Elad[1], Lior Spektor[1], Zamir Halpern[2], Hanan Guzner-Gur[3], Iris Dotan[1,2]
[1]Mucosal Immunology Laboratory, The research center for digestive tract and liver diseases, Tel-Aviv Surasky medical center, Sackler Faculty of Medicine Tel-Aviv University, Tel-Aviv, Israel, [2]Department of Gastroenterology and Liver Disease, Tel-Aviv Surasky medical center, Sackler Faculty of Medicine Tel-Aviv University, Tel-Aviv, Israel, [3]Internal Medicine B, Tel-Aviv Surasky medical center, Sackler Faculty of Medicine Tel-Aviv University, Tel-Aviv, Israel

Introduction: Crohn's disease (CD) is characterized by loss of tolerance towards intestinal microorganisms, reflected by serologic responses towards yeast-characteristic glycans such as mannan and β-glucans.

Objective: To explore glycan-induced immune responses and their correlation with intestinal inflammation.

Methods: Peripheral blood mononuclear cells (PBMCs), isolated from CD and normal control patients, were stimulated by glycans or heat killed (HK) yeasts. Mucosal cells were freshly isolated from surgical specimens. Expression of β-glucan receptor- Dectin1, and mannose receptor- MR, cytokine secretion, and signaling pathways were assessed using flow cytometry, immunofluorescence, and ELISA. Mucosal biopsies were obtained from CD patients (inflamed/ non-inflamed) and controls. Dectin1 (CLEC7A) and MR (MRC1) mRNA levels were assessed by real time quantitative PCR.

Results: Mannan and β-glucans induced significantly higher pro-inflammatory cytokine secretion (pg/ml) by CD vs. normal PBMCs ($p<0.05$): IL-1β [laminarin: 284 vs. 56, mannan: 701 vs. 279]; IL-6 [laminarin: 2903 vs. 1075, mannan: 5645 vs. 2856]. Significant inhibition of glycan-induced cytokine secretion was observed using syk inhibitor. HK C. albicans induced higher TNF-α, while HK S. cerevisiae induced lower IL-10 secretion by CD vs. normal PBMCs. Dectin1 was expressed by intestinal macrophages and epithelial cells. Mucosal Dectin1 and MR expression was higher in inflamed CD vs. controls.

Conclusions: Glycans are capable of stimulating immune responses. Glycan receptors are expressed by peripheral and mucosal immune cells and are enhanced in intestinal inflammation. Response to β-glucans and mannan is syk mediated. CD is characterized by hyperresponsiveness towards yeast-characteristic glycans. Thus, glycans may have a role in intestinal inflammation.

P016

Crotoxin from C*rotalus durissus terrificus* is Able to Down-Modulate Th17 Response in the Acute Intestinal Inflammation in Mice

Caroline Almeida[1,2], Thiago Patente[3], Patrícia Clissa[2], Jacqueline Jacysyn[4], Eliana Faquim[2]
[1]São Paulo University, São Paulo, São Paulo, Brazil, [2]Immunopathology Laboratory, Butantan Institute, São Paulo, São Paulo, Brazil, [3]School of Medicine of São Paulo University - LIM25, São Paulo, São Paulo, Brazil, [4]School of Medicine of São Paulo University - LIM62, São Paulo, São Paulo, Brazil

Crohn's disease is classically regarded as a Th1 mediated inflammatory disorder. Recently, it was verified that the Th17 cells are also involved in inflammatory intestinal response. The crotoxin (CTX) is the main component of the Crotalus durissus terrificus rattlesnake venom and has immunosuppressive action. Here, we investigated the effect of CTX on 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice. After 18 hours of the TNBS-colitis induction, mice were treated with CTX or PBS and sacrificed 4 days after induction. The colon was separated in parts for cytokines measurement, extraction of mononuclear lamina propria (MLP) cells for flow cytometry and for histological analysis. The cytokines expression was also analyzed in mRNA obtained from mesenteric lymph nodes (MLN) cells. Clinical and histological scores showed that the CTX-treatment decreased the disease progression. High secretion of IL-6, TNF-α, IFN-γ and IL-17 was verified in cell supernatants from TNBS-mice and the RT-PCR confirmed these data. In contrast, CTX treatment decreased the cytokine secretion in TNBS-induced colitis mice. Furthermore, the CTX administration also induced higher IL-10 and TGF-β secretion when compared with the TNBS-group. The TNBS colitis mice showed higher percentage of CD4+ROR-γt+ and CD4+T-bet+ cells compared with the CTX treated TNBS-induced colitis group. These results suggest an immunomodulatory role for CTX in Th17/Th1 response observed in acute intestinal inflammation induced by TNBS.

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P017

Triggering Receptor Expressed on Myeloid Cells 1 (TREM-1) Activation Increases the Production of Pro-Inflammatory Cytokines by Inflammatory Bowel Disease Mucosa

Francesca Ammoscato[1], Paolo Biancheri[1,2], Aneta Kucik[1], Iona M. Bell[1], Renata Curciarello[1], George Boundouki[1], Laurens Kruidenier[4], Thomas T. MacDonald[1]
[1]Centre for Immunology and Infectious Disease,Blizard Institute, Barts and the London School of Medicine and Dentistry, London, UK, [2]Academic Surgical Unit, Centre for Digestive Diseases, Blizard Institute, Barts and the London School of Medicine and Dentistry, London, UK, [3]First Department of Medicine, IRCCS Policlinico S. Matteo, University of Pavia, Pavia, Italy, [4]Immuno-Inflammation Centre of Excellence in Drug Discovery, GlaxoSmithKline, Stevenage, UK

Background & Aim: Intestinal macrophages play an important role in the pathogenesis of inflammatory bowel disease (IBD). Inflamed IBD mucosa contains high numbers of $CD33^{+}CD68^{+}$ macrophages overexpressing Triggering receptor expressed on myeloid cells 1 (TREM-1). After comparing the percentage of TREM-1-expressing macrophages in IBD versus control mucosa, we explored the ex vivo effects of a TREM-1 activating antibody on the intestinal immune response in IBD.

Material & Methods: Lamina propria mononuclear cells (LPMCs) were isolated from the inflamed colon of 11 IBD patients (5 ulcerative colitis and 6 Crohn's disease), and from normal colon of 8 control subjects, and the expression of CD68, CD33 and TREM-1 was analysed by flow cytometry. IBD biopsies from inflamed mucosa were cultured ex vivo with or without an activating anti-TREM-1 monoclonal antibody, and the production of interleukin (IL)-1beta, IL-6 and IL-8 was determined by ELISA.

Results: The percentage of mucosal $CD33^{+}CD68^{+}$ macrophages was significantly higher both in Crohn's disease and ulcerative colitis in comparison to control subjects. Compared to control subjects, TREM-1 expression by mucosal macrophages was significantly higher both in ulcerative colitis and in Crohn's disease compared to control subjects. TREM-1 activation significantly increased IL-1beta, IL-6 and IL-8 production by IBD biopsies cultured ex vivo.

Conclusions: TREM-1 is overexpressed on macrophages in IBD mucosa, and its activation amplifies the production of pro-inflammatory cytokines. Further studies using chromatin immunoprecipitation assays are under way in order to establish whether TREM-1 overexpression in IBD may derive from epigenetic changes.

P018

Analysing the Role of Mucosal Mast Cells Inducing Colitis-Associated Colorectal Cancer

Benno Weigmann[1], Michael Stassen[1,2], Christian Taube[1,2], Markus Neurath[1]
[1]I.Med.Clinic, University Hospital, Erlangen, Germany, [2]Department of Immunology, Johannes Gutenberg University, Mainz, Germany

Introduction: Colorectal cancer is one of the most malignancies. However, the molecular pathogenesis of colorectal cancer is poorly understood. In order to investigate the functional role of mast cells, which play a more prominent role in immunological processes, we used a previously established murine colon carcinoma model (DSS/Azoxymethan) with mast cell deficient mice.

Methods: Accordingly, mice were treated with AOM followed by three consecutive cycles of orally administrated dextran sulfate sodium (DSS) over a period of 7 days. To monitor tumorigenesis in mice in vivo, we used our mini-endoscopic system.

Results: By using this system together with methylene blue staining, we were able to detect aberrant crypt foci in DSS plus AOM-treated wild-type mice at early time point before macroscopically visible lesions were seen. First visible lesions associated with inflammation appeared in wt mice around day 45, which were followed by the development of more and growing tumors until day 90. In contrast, mast cell deficient mice are protected against tumor development and although they showed colitis-similar symptoms. The possibility, that mast cells play a tumor-promoting role in the development of colon tumors led us to perform a screening of the expression of involved cytokines in colons and tumors of treated mice vs untreated mice. Even in long term study, a marginal increase of the tumor prevalence concerning mast cell deficient mice could be observed.

Discussion/Conclusion: Our data contribute extensively the understanding of mast cells in colitis-associated colon cancer and encourage of rethinking the role of mast cells in colitis-associated colorectal cancer.

P019

The Human CD14^{+}HLA-DR^{dim} Macrophage Subset Dominate the Inflamed Mucosa of Crohn's Disease Patients

Susanne Thiesen[1,2], Sabina Janciauskiene[3], Heli Uronen-Hansson[2], William Agace[2], Carl-Magnus Högerkorp[1], Pieter Spee[1], Katarina Håkansson[1], Olof Grip[2]
[1]Novo Nordisk, Copenhagen, Denmark, [2]Lund University, Lund, Sweden, [3]Hannover Medical School, Hannover, Germany

Background and aims: Intestinal macrophages play an essential role in maintaining homeostasis. However, little is known about these cells, their precursors and their role in disease, which prompted us to characterize the inflammatory CD14^{+} cell populations in intestinal mucosa in relation to their peripheral blood monocytic counterparts in patients with Crohn's disease (CD).

Methods: Blood samples and lamina propria (LP) cells from inflamed and un-inflamed mucosa were obtained from CD patients and controls and analysed by flow cytometry. Monocytes from healthy donors were used to assess migration, cytokine and matrix metalloproteinase (MMP) release.

Results: Three distinct HLA-DR^{+} expressing subsets were found among the LP CD14^{+} macrophages. When compared to un-inflamed, the inflamed mucosa contained a predominant increase in proportions of the CD14^{+}HLA-DR^{dim} cellular subset. This subset resembled the classical CD14^{++}CD16^{-} blood monocytes with low CD16 expression and high secretion of TNF-α, IL-1β, IL-6, MMP-1 and MMP-9. Furthermore, the classical CD14++CD16- monocytes migrated towards CCL2 and where diminished in the blood of the patients with active CD.

Conclusions: In the inflamed mucosa of CD patients the CD14^{+}HLA-DR^{dim} is the most dominant subset among LP CD14^{+} cells. This population resembles the classical pro-inflammatory blood monocytes and suggests that the CD14^{++}CD16^{-} cells are the precursors to the intestinal CD14^{+}HLA-DR^{dim} subset. Understanding the mechanisms regulating intestinal macrophage subpopulations during homeostasis and inflammation will increase the knowledge needed for future therapy in CD.

P020

Profiling of Systemic Cytokines in Patients with Quiescent and Active Ulcerative Colitis

Áine Balfe[1,4], Gráinne Lennon[1,4], Aonghus Lavelle[1,4], Neil Docherty[2], J Calvin Coffey[3], Desmond Winter[1,4], P Ronan O'Connell[1,4]
[1]School of Medicine and Medical Science, University College Dublin, Dublin, Ireland, [2]Department of Physiology, Trinity College Dublin, Dublin, Ireland, [3]Graduate Medical School, University of Limerick, Limerick, Ireland, [4]Centre for Colorectal Disease, St. Vincent's University Hospital, Dublin, Ireland

Introduction: Cytokines are mediators of the mucosal healing in Ulcerative Colitis (UC), but also have a role to play in the augmentation and perpetuation of inflammation. The aim of this study was to profile a panel of cytokines (IL-2,-4,-5,-6,-8,-10,-13, GM-CSF, TNFα and IFNγ) in both healthy controls and patients with UC.

Methods: Plasma was harvested from blood of 20 patients (10 quiescent, 10 acute). Fourteen healthy volunteers acted as controls. The systemic cytokine levels were determined by Quantibody® Human Th1/Th2 cytokine array kit.

Results: No significant differences were observed in the cytokine profiles between health and quiescence. Detection of pro-inflammatory cytokines (IL-2,-5,-6,-8,-13, TNFα and IFNγ) was more frequent in the acute cohort than healthy controls ($p<0.05$). Of these, IL-5,-6,-8, and -13 were more frequently detected in acute patients than their quiescent counterparts ($p<0.05$). Importantly the anti-inflammatory cytokine IL-10 was more frequently detected in the acute patients than their quiescent counterparts ($p<0.05$). A significant elevation in concentration of IL-8 and IL-10 was found within the acute group compared with healthy and quiescent UC groups ($p<0.05$). Furthermore, an increase in IL-6 and IL-13 was observed between the acute and quiescent cohorts ($p<0.05$).

Conclusion: The cytokine profiles within patients with quiescent disease closely resemble those of the healthy control subjects. Acute UC can be characterised by a distinct alteration in both pro and anti-inflammatory cytokines. The data presented here provide a profile of changes in key, disease associated cytokines and reflect the systemic manifestations of a disrupted immune homeostasis in acute UC.

P021

Minocycline Inhibits MAP Kinase Signaling Pathways in Stimulated Intestinal Epithelial Cells

Natividad Garrido-Mesa[1], Francesca Algieri[1], Maria Elena Rodriguez-Cabezas[1], Monica Comalada[2], Antonio Zarzuelo[1], Alba Rodriguez-Nogales[1], Julio Galvez[1]
[1]CIBERehd Department of Pharmacology, University of Granada, Granada, Spain, [2]Macrophage Biology Group, Institute for Research in Biomedicine (IRB), Barcelona, Spain

The immunomodulatory properties of the minocycline, a tetracycline antibiotic, contribute to its intestine anti-inflammatory properties in experimental colitis. The major biological responses to inflammatory cytokine in intestinal epithelial cells are mediated through multiple signaling pathways, being one of the major effectors mitogen-activated protein (MAP) kinases. The aim of this study was to investigate the effects of this antibiotic on these signaling pathways involved in the immune response mediated by the intestine epithelium. Caco-2 cells were incubated for 2 hours with minocycline (10-50 µg/ml) and stimulated with IL-1β (10 ng/ml) for another 20 minutes (western blot) or 24 h (IL-8 assay). For western blotting, cells were harvested and lysated. Western blots were performed with protein extracts to analyze phosphorylated or total forms of p38 MAP kinase, p42/44 ERK or SAPK/JNK. For IL-8 measurement the culture supernatants were collected and evaluated by ELISA. The results revealed that minocycline inhibited dose-dependently IL-8 production, and this effect was associated with a reduced phosphorylation of the three MAP kinases evaluated. In conclusion, minocycline is able to down-regulate IL-8 production in IL-1β stimulated Caco-2 cells through inhibition of MAP kinase pathways, and this could contribute to the reported immunomodulatory effects ascribed to this antibiotic.

P022

The Intestinal Epithelium, a Crucial Target for TNF-Induced Toxicity

Filip Van Hauwermeiren[1,2], Roosmarijn E. Vandenbroucke[1,2], Claude Libert[1,2], Riet Declercke[1,2]
[1]University Ghent, Ghent, Belgium, Belgium, [2]VIB, Flanders, Belgium, Belgium

Tumor necrosis factor (TNF) was first discovered for its potent anti-tumor potential, but it is also a powerful activator of the immune system and a well-known target for treatment of autoimmune diseases. Injection of a high dose TNF induces a systemic inflammatory response syndrome (SIRS) characterized by extensive damage to multiple organs and other shock symptoms. The molecular mechanisms leading to TNF-induced toxicity remain unclear and are the topic of our recent research. We show that the induction of both TNF-induced toxicity and anti-tumor effects is TNFR1-dependent. Additionally, we provide evidence that the intestinal epithelial cells (IECs) are an early target of TNF-induced toxicity, which is sufficient to induce lethality. Consequently, we investigated the molecular mechanisms in the IECs leading to TNF-induced toxicity. Unexpectedly, we found that the well-known TNF-induced damage and apoptosis in IECs is not a major determinant of TNF-induced toxicity. Rather, we propose a role for inflammation induced mucus depletion in goblet cells and induction of intestinal permeability mediated by the intestinal flora. In conclusion, we identified TNFR1-driven inflammation in the intestinal epithelium as a crucial mechanism for TNF-induced systemic toxicity.

P023

In Vitro Comparison of Commensal, Probiotic and Pathogenic Strains of Enterococcus faecalis

Trine Eker Christoffersen[1,2], Charlotte Kleiveland[2], Lene Olsen[2], Morten Jacobsen[3,2], Tor Lea[2]
[1]Østfold University College, Halden, Norway, [2]Norwegian University of Life Sciences, Aas, Norway, [3]Ostfold Hospital Trust, Fredrikstad, Norway

In vivo studies have provided evidence that microorganisms have important roles in immunological, digestive and respiratory functions, providing health benefits on the host. Several in vitro methods have been advised for screening microbes for potential health effects. The objective of this study was to employ such in vitro methodology to characterize commensal, pathogenic and probiotic strains of Enterococcus faecalis. It was expected that the commercial product marketed as a probiotic, Symbioflor-1 (Symbiopharm, Germany), would perform better in at least one of the tests employed. Tolerance towards low pH and viability after exposure to human gastric and duodenal juices was assayed. Symbioflor-1 was most susceptible to these treatments, and also exhibited the lowest adhesion capacity to intestinal epithelial cells (IECs) and mucus. Competitive binding studies using heparin indicated that glycosaminoglycans might be involved in the adhesion to IECs, but also that differences in these putative bacteria-host interactions do not cause the relative low adhesion capacity of Symbioflor-1. Maturation of dendritic cells after exposure to bacteria was assayed as an indication of an immunomodulatory effect. All strains induced a moderate elevation of the DC maturation markers CD83 and CD86, however no strain specific differences were detected. We are now studying how these strains affect the differentiation of macrophages to either M1 or M2, to further investigate how Symbioflor-1 may confer its probiotic properties. So far, Symbioflor-1 have not exhibited probiotic properties in the in vitro tests employed, emphasizing that human clinical trials are the definite tool for establishing probiotic status.

P024

Lymphocytes Regulate Intestinal Microbial Composition in the Zebrafish

Sylvia Brugman[1,4], Merlijn Witte[1,4], Kerstin Schneeberger[1], Tom van den Bogert[1], Jos Boekhorst[5], Patrick van Zon[2], Edward Nieuwenhuis[1]
[1]Wilhelmina Children's Hospital UMCU, Laboratory of Mucosal Immunology, Utrecht, The Netherlands, [2]Wilhelmina Children's Hospital UMCU, Department Medical Genetics, Utrecht, The Netherlands, [3]Wageningen University, Department of Microbiology, Wageningen, The Netherlands, [4]Hubrecht institute for Stem Cell research, Utrecht, The Netherlands, [5]NIZO, Wageningen, The Netherlands

In the last decades progress has been made in understanding intestinal microbial influences on the host immune system. However, little is known about how the host shapes this intestinal microbial community. Recent studies suggest that the environment pre-select species that are able to colonize and that, alongside, site-specific host factors determine the relative abundance of the different bacterial taxa. These host factors likely include cells and mediators of the mucosal immune system. Dissection of the innate and adaptive immune signalling pathways in microbial host interaction has proven difficult, due to the extensive crosstalk. In order to specifically address the contribution of the adaptive immune system during bacterial colonization, we made use of the zebrafish.

Zebrafish develop ex-utero and until 4 weeks after fertilization no mature adaptive immune responses are present. In these first four weeks the zebrafish solely rely on innate immunity. This unique feature allows investigation into the effects of adaptive immune development on intestinal homeostasis over time. In this study, we show that T-lymphocytes are important regulators of the intestinal microbial composition of zebrafish. Lack of mature T lymphocytes in Rag1-/- zebrafish leads to an overgrowth of (pathogenic) Vibrio parahaemolyticus. In adoptive transfer studies we show that T lymphocytes help to activate the innate immune system by inducing epithelial CXCL8. Overall, zebrafish enable detailed study of the influence of adaptive immunity in bacterial-host interaction.

P025

Influence of Oxygen Concentration on TLR Function of Caco-2 Cells

Sabrina Klein, Konrad A. Bode, Klaus Heeg
Heidelberg University Hospital, Department of Infectious Diseases, Heidelberg, Germany

Intestinal epithelial cells (IECs) line the front to microbial components in the gut lumen. Their close contact to various immune cells as well as their barrier function gives them a central role in maintaining immunological homeostasis in the gut.

While ambient air comprises about 20% oxygen, oxygen levels in tissues are far below this. As many cellular and immune functions are influenced by oxygen concentration, it may also be of importance in IECs.

Here, the colon epithelial cell line Caco-2 was investigated under a physiological oxygen concentration of 1%. Proliferation was not significantly diminished compared to conventionally cultured Caco-2 cells and no effect on the viabiliy could be observed. Expression of the differentiation marker alcaline phosphatase-1 was even higher under low oxygen. Transepithelial electrical resistance (TEER) was initially higher under 1% O2, but reached equal levels over time.

Interestingly, reduction of the oxygen concentration resulted in an alteration of the pattern of TLR-induced cytokine and chemokine secretion. Furthermore, decreased oxygen concentration had a direct effect on TLR expression itself.

Understanding the influence of the local oxygen pressure on the physiology of the cells could contribute to a better understanding of the complex immunological network in the intestine.

P026

TLR10 Mediates the Inflammatory Response to *Listeria monocytogenes* Infection in Intestinal Epithelial Cells

Tim Regan[1,2], Ken Nally[1], Fergus Shanahan[1], John MacSharry[1], Elizabeth Brint[1,2]
[1]*Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland,* [2]*Dept. of Pathology, University College Cork, Cork, Ireland*

Pathogen Recognition Receptors (PRRs) of the innate immune system are the body's earliest responding sentinels to infection. L. monocytogenes is a food-borne gram positive bacterium which can cause septicaemia and meningitis. Although Intestinal Epithelial Cells (IECs) represent the initial point of entry utilised by L. monocytogenes for infection, the immune response to L. monocytogenes in these cells has been poorly characterised to-date. The aim of this study was to determine which PRRs are involved in mediating the immune response to L. monocytogenes in IECs. We performed a PRR siRNA library screen of 50 PRRs in the HT-29 epithelial cell line. This included Toll-Like-Receptors (TLR) 1-10, 22 NOD-Like-Receptors (NLR) and 15 C-type-Lectin-Receptors (CLR). Cells were infected with L. monocytogenes and inflammatory gene induction was measured by qRT-PCR. Significant reductions in CCL-20 and IL-8 induction following infection were seen following silencing of TLR1, TLR2 and NLRP2 while an increase in induction of these genes was seen following silencing of NLRP7 and NLRP10. The most significant effect was seen after silencing of TLR10, resulting in 4 fold reduced IL-8 and 4 fold reduced CCL-20 induction. A stable knockdown of TLR10 by lentivirus has been generated in HT-29s which display similar results upon infection. These data indicate a key role for TLR10 in epithelial sensing of pathogenic infection and moreover have potentially identified L. monocytogenes as a specific ligand for the orphan receptor TLR10.

P027

Intestinal Epithelial Cell Barrier Function and Transcriptional Response to *Candida albicans* Challenge

Michael Böhringer[1], Daniela Albrecht-Eckardt[2], Oliver Kurzai[1]
[1]*Septomics Research Centre, Friedrich Schiller University and Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany,* [2]*BioControl GmbH, Jena, Germany*

Intestinal epithelial cells (IEC) form a tight barrier to the gut lumen. Tight junction proteins regulate the paracellular permeability of the intestinal barrier, which can be modulated by microorganisms and other stimuli. The polymorphic fungus Candida albicans has the capacity of traversing this barrier and establishing systemic disease within the host. Here we investigated the response of IEC on infection with and the role of barrier integrity for invasion by C. albicans.

Caco-2 derived C2BBe1 IEC were cultivated on permeable cell culture inserts to establish polarized monolayers. Infection of IEC with wild-type C. albicans led to a transient increase of transepithelial electric resistance (TEER) before subsequent barrier disruption with high cytotoxicity levels.

To determine the role of filamentation and active penetration, IEC were infected with non-filamentous C. albicans mutants. Here, TEER increased until reaching a two-fold level which continued to the end of the experiment. Cytotoxicity remained at the level of non-inoculated control.

To examine the molecular basis of barrier disruption in wild-type infected IEC we analysed expression levels of junctional proteins. All of the junctional transmembrane proteins tested showed a time-dependent decrease in wild-type, but also in mutant inoculated IEC, albeit to a lower extent.

Moreover, we investigated the transcriptional response of IEC monolayers to C. albicans infection by microarray. KEGG pathway enrichment analysis revealed an overrepresentation of MAPK and TLR signaling pathways, but only minor overall differences between wild-type and mutant infections.

Differentially regulated targets, possibly explaining phenotypic differences between wild-type and mutant infections will be analysed further.

P028

Human Buccal Epithelium Acquires Microbial Hyporesponsiveness at Birth, a Role for Secretory Leukocyte Protease Inhibitor (SLPI).

C.L. Menckeberg[1], J. Hol[1,3], L. de Ruiter[1,2], H.C. Raatgeep[1], Y. Simons-Oosterhuis[1], P.E. van Lierop[1], M. Groeneweg[1,4], G. Kraal[5], B.E. Elink-Schuurman[2], J.C. de Jongste[2], E.E.S. Nieuwenhuis[1], J.N. Samsom[1]
[1]*Erasmus Medical Center, Rotterdam, The Netherlands,* [2]*Sophia's Childrens Hospital, Rotterdam, The Netherlands,* [3]*University Hospital Ghent, Ghent, Belgium,* [4]*Maasstad Hospital, Rotterdam, The Netherlands,* [5]*VU Medical Center, Amsterdam, The Netherlands*

The oral cavity is rapidly colonized at birth after which the buccal mucosal epithelium is continuously exposed to harmless microbial stimuli. Previously, we have reported that in healthy children, buccal epithelial cells are unresponsive to microbial stimulation while buccal epithelial cells from pediatric Crohn's disease patients spontaneously secrete chemokines. We therefore questioned whether repetitive interaction with microbial stimuli determines that primary buccal epithelial cells from healthy individuals become hyporesponsive to TLR stimulation. Thereto, buccal epithelial cells were collected directly after birth and in later stages of life. Primary neonatal buccal epithelial cells were found to spontaneously produce CXCL-8 and were highly responsive to microbial stimuli. Within the first weeks of life these epithelial cells attained a state of hyporesponsiveness to TLR triggering, associated with sustained cytoplasmic levels of IκBα. In contrast, activated neonatal buccal epithelial cells displayed degradation of IκB. Secretory leukocyte protease inhibitor (SLPI) is a negative regulator of NF-κB-mediated activation in the cytoplasm and the nucleus. Using the colonic epithelial cell line Caco-2, we found that microbial induction of hyporesponsiveness elicited SLPI expression in epithelial cells. This led us to determine SLPI expression in hyporesponsive primary buccal epithelial cells. Indeed, hyporesponsive adult epithelium exhibited SLPI mRNA and nuclear expression of SLPI whereas activated neonatal buccal cells did not contain nuclear SLPI expression. In sum, human primary buccal epithelium acquires microbial hyporesponsiveness in the first weeks after birth which correlated with decreased degradation of IκB and accumulation of the regulatory protein SLPI in the nucleus.

P029

Differences in Early-Life Environment Influence the Microbiota and Immunometabolic Profile in Later Life, and the Physiological Response to *Bifidobacteria lactis* NCC2818

Marie Lewis[1], Claire Merrifield[2], Bernard Berger[3], Sandrine Claus[2], Jake Pearce[2], Olivier Cloarec[4], Swantje Duncker[2], Silke Heinzmann[2], Marc-Emmanuel Dumas[2], Sunil Kochhar[3], Serg Rezzi[3], Annick Mercenier[3], Jeremy Nicholson[2], Mick Bailey[1], Elaine Holmes[2]
[1]University of Bristol, Bristol, UK, [2]Imperial College London, London, UK, [3]Nestle Research Center, Lausanne, Switzerland, [4]Korrigan Sciences Ltd, Maidenhead, UK

Birth and weaning are critical points in development of the ability of the mucosal immune system to discriminate between 'harmless' and 'dangerous' antigens. This study uses a novel, systems approach to study the relationships between the intestinal microbiota, metabolism and the mucosal immune system in neonates, and the response to nutritional intervention around weaning.

We used 16S pyrosequencing to show that the composition of the microbiota, and urinary metabolic profile by ^{1}H NMR, in 5 week old piglets was influenced by the environment during the first day of life. Specific metabolites were associated either directly to the diet, e.g betaine, or to alterations in gut microbial fermentation, e.g p-cresol glucuronide, phenylacetylglycine and 2-hydroxybutyrate. Administration of the human probiotic Bifidobacterium lactis resulted in further changes in the metabolic profile which were independent of the earlier, persistent effects due to environmental differences during the first day of life. Probiotic intervention also resulted in significant changes in production of cytokines and immunoglobulins by mucosal tissues, and these immunological effects were correlated with levels of urinary sarcosine, betaine, lactate and alanine. Interestingly, changes in composition of the microbiota were insufficient to explain the effect of probiotic on immunological function, suggesting that the probiotic either acted directly on the immune system, or altered the activity of the gut microbiota rather than its composition.

We suggest that analysis of urinary metabolites in human patients may provide a means of assessing the status of the mucosal immune system and its interactions with the intestinal microbiota.

P030

Knockdown of the Autophagy Protein ATG16L1 in Intestinal Epithelial Cells Favour the Adherent-Invasive *Escherichia coli* (AIEC), HM605, to Replicate Resulting in a Reduction in Barrier Integrity

Trevor Darby, Conal Martin, David Clarke, Fergus Shanahan, Ken Nally, Silvia Melgar
Alimentary Pharmabiotic Centre, Cork, Ireland

Adherent and invasive Escherichia coli (AIEC) are commonly found in lesions of Crohn's Disease (CD) patients. These bacteria adhere, invade and replicate within intestinal epithelial cells and macrophages suggesting AIEC may play a role in the immunopathology of CD. ATG16L1 is an important member of the autophagosome complex and is a known CD susceptibility gene.

In the present study, we sought to determine the role of ATG16L1 in AIEC infection. Intestinal epithelia lacking expression of ATG16L1 were generated using lentiviral transfection, polarized and infected with the AIEC strain HM605. We studied the effect of ATG16L1 knockdown (ATG16L1KO) on the kinetics of bacterial invasion, trans-epithelial electrical resistance (TEER), MAPK signalling and autophagy response.

HM605 infected ATG16L1KO cells had higher bacteria invasion in comparison to Non Target (NT) infected cells, at 7 and 21 hours post infection (p.i.). Enhanced MAPK signalling was observed in ATG16LKO as early as 3 hours p.i.. ATG16L1KO AIEC infected cells displayed a reduced autophagy response at 7 and 21 hours p.i. with decreased expression of the autophagy proteins; beclin 1, ATG5, ATG12, ATG7 and the LC3 complex. Furthermore, ATG16L1KO infected cells displayed a higher reduction in TEER compared to NT at 21 hours p.i.

Our results demonstrate an increased susceptibility to HM605 replication in ATG16L1 deficient cells which results in reduced barrier integrity and autophagosome formation. This data suggests AIEC in association with a deficiency in ATG16L1 expression contributes to impaired barrier function and pathogenesis of Crohn's disease.

This work was supported by Science Foundation Ireland (SFI).

P031

Cultivation in the Presence of Host Epithelial Cells Modifies the Surface Proteome and Promotes Immunosuppressive Functions in *Lactobacillus casei*

Kaarina Lähteenmäki, Minna Tiittanen, Ilja Ritamo, Joni Keto, Johanna Haiko, Pirjo Wacklin, Janne Nikkilä, Annika Kotovuori, Leena Valmu, Jukka Partanen, Jaana Mättö
Finnish Red Cross Blood Service, Helsinki, Finland

Lactobacillus strains are studied for their beneficial effects on human health. As their effects are thought to take place during colonization of the intestine, we determined functional properties and surface protein profiles of Lactobacillus in conditions that simulate interaction of bacteria with intestinal epithelium. We cultivated Lactobacillus casei ATCC334 in the presence of human intestinal HT-29 epithelial cells and, for comparison, in standard MRS-broth. We then compared effects of bacteria in subsequent co-culture with human peripheral blood mononuclear cells (PBMC). L. casei cultivated with epithelial cells promoted generation of regulatory T cells (CD4+CD25+FoxP3+) more efficiently, and induced more of the anti-inflammatory IL-10 and less of the pro-inflammatory TNF-alpha than bacteria from MRS broth. This suggests that cultivation with epithelial cells promotes an immunosuppressive phenotype in L. casei.

We extracted surface proteomes of L. casei from both cultivation conditions by labelling intact bacteria with biotin and collecting biotin-linked proteins with streptavidin-coated magnetic beads. A gel-based mass spectrometric analysis showed that epithelial cells significantly affected the surface proteome, as approximately half of the bacterial proteins detected after cultivation with epithelial cells were specific to this condition. A comparative quantitative mass spectrometric analysis showed that 25 proteins in bacteria from epithelial cells had a statistically significant change in expression level. We are currently analysing the roles of the surface proteins with major quantitative changes in the immunosuppressive functions. Our work suggests that simulation of bacterial contact with intestinal epithelium is a valuable tool for identification and production of Lactobacillus proteins for therapeutic use.

P032

Development of an Improved *In Vitro* Model of Human Intestinal Follicle Associated Epithelia to Study Cellular and Molecular Interactions of Candida albicans with M Cells

Carolina López Alayón, Sandrine Albac, Marc Sautour, Frédéric Dalle
Agroécologie UMR 1347 Agrosup, INRA, Université de Bourgogne. Pôle Microbiologie environnementale et risques sanitaires MERS, Dijon, France

Candida albicans is a commensal inhabitant of the human mucosa causing harmful invasive infections in immuno-compromised patients, taking origin mainly from the gastro-intestinal tract. A better understanding of the mechanisms by which C. albicans interacts with the intestinal mucosa will improve our knowledge of the physiopathology of disseminated candidiasis. *C. albicans* can grow upon mucosal surfaces in both yeast and hyphal forms, the transition from the yeast to the hyphal form playing a key role in its virulence. Mucosal immunity contributes to both commensalism and pathogenicity of the fungus, possibly through presentation of C. albicans antigens to the underlying organized lymphoid structures via transcytosis, mediated by the specialized epithelial M cells. With this aim, we developed an in vitro model of the human intestinal Follicle Associated Epithelium (FAE) where enterocytes of the Caco-2 cell line in close contact with mucosal lymphocytes differentiate in M cells.

Studying adherence, invasion and translocation of C. albicans across co-cultures suggest that C. albicans interacts differentially with M cells / enterocytes co-cultures as compared to monolayers of Caco-2 cells alone. The uptake mechanism allowing C. albicans to translocate across the co-culture model is under investigation. Moreover, the respective contribution of the yeast and hyphal forms to this process will be studied using KO mutants of C. albicans unable to produce hyphae. Finally the cytokine production resulting from C. albicans and M cells / Caco-2 co-cultures interaction will be studied.

P033

Targeted Endoscopic Sampling of the Normal Human Intestinal Mucosa Reveals Cross-Sectional and Longitudinal Alterations in Bacterial Community Structure

Aonghus Lavelle[1,5], Grainne Lennon[1,5], Aine Balfe[1,5], Neil Docherty[2], J Calvin Coffey[4], Hugh Mulcahy[1,5], Glenn Doherty[1,5], Orla O'Sullivan[1,5], Desmond Winter[1,5], P Ronan O'Connell[1,5]
[1]School of Medicine and Medical Sciences, University College Dublin, Dublin, Ireland, [2]Department of Physiology, School of Medicine, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland, [3]Teagasc Food Research Centre, Fermoy, Ireland, [4]Graduate Entry Medical School, University of Limerick, Limerick, Ireland, [5]Centre for Colorectal Disease, Saint Vincent's University Hospital, Dublin, Ireland

Background: Intestinal immune homeostasis in rodent models relies on mutualism at the host-microbial interface. Disruption of mutualism as a result of alterations in the structure of the intestinal microbiota (dysbiosis) is implicated in the pathogenesis of inflammatory bowel disease (IBD). The present study used targeted endoscopic sampling and analysis to identify topographical differences in bacterial community structure in the normal human large intestine.

Materials and Methods: A microbiological protected specimen brush was used to sample superficial mucus from the caecum and rectum in healthy patients presenting for colonoscopy (n=9). Adjacent biopsy samples were acquired and DNA was extracted from all samples. Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis was used to assess similarity and diversity indices of the different communities. An additional patient was sampled for 454 pyrosequencing to determine bacterial composition.

Results: T-RFLP analysis showed that profiles are individualized and cluster by technique of acquisition and not location (P<.001).. Deep sequencing confirmed clustering by technique and showed a longitudinal increase in bacteroidetes and decrease in firmicutes in brush samples.

Conclusion: The data reveal a segregation of bacterial communities between the superficial and deep regions of the mucus gel layer and suggest the existence of longitudinal differences in the ratios between the two main phyla of the intestinal microbiota. The techniques described may allow for more accurate determination of species gradients and niches in health, alterations in which may play a role in disrupted mucosal immune homeostasis in IBD

P034

Modulation of Heat Shock Proteins and TLR4 Mediated Inflammatory Signals by *Lactobacillus amylovorus* in Intestinal Cells Infected with Enterotoxigenic *E. coli*

Alberto Finamore, Marianna Roselli, Barbara Guantario, Ambra Imbinto, Elena Mengheri
National Research Institute For Food and Nutrition (INRAN, Roma, Italy

HSPs are typically intracellular proteins responding to several stimuli. Recently, extracellular HSP72 and HSP90 have been implicated in pathological events. They associate with Toll-like receptor (TLR)-4 and appear to be crucial for TLR4 induced pro-inflammatory signal. Probiotic bacteria, such as lactobacilli and bifidobacteria, exert beneficial effects for the host, including prevention of inflammatory diseases. Recent studies have shown a reduction of TLR-4 mediated induction of NFkB signalling by probiotics.

We investigated intestinal stress response to a pathogen and whether Lactobacillus amylovorus could inhibit this response. We analysed the expression of intracellular and extracellular HSP72 and HSP90, as well as TLR4, NFkB and pro-inflammatory cytokine expression in Caco-2/TC7 cells grown on Transwell filters, treated either alone or simultaneously with Lactobacillus amylovorus DSM16698 ($5x10^7$ CFU/mL) and enteroxigenic E. coli (ETEC)-K88 ($5x10^6$ CFU/mL), for 2.5 hours. ETEC induced an up-regulation of HSP72, HSP90, TLR-4 and NFkB. L. amylovorus unaffected the expression of these proteins when added alone to the cells, and was able to inhibit the ETEC induced up-regulation. An increase in IL-8 and IL-1β secretion was found in infected cells, and was abolished when the cells were co-treated with ETEC and L. amylovorus.

In conclusion our data show that L. amylovorus is able to prevent the ETEC induced up-regulation of HSP72 and HSP90. This finding correlates with the downregulation of TLR4 signaling mediated by L. amylovorus

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P035

Role of Bacteria and the Mucus System in Intestinal Tumorigenesis

Tiziana Schioppa, Elena Zagato, Giuseppe Penna, Silvia Guglietta, Maria Rescigno
European Insitute of Oncology, Milan, Italy

Colorectal cancer (CRC) is one of the major plagues in Western Europe and is a disease affecting a site, the gut, where the study of immune phenomena is particularly interesting. The $Apc^{Min/+}$ mouse is widely used as a model for human familial adenomatous polyposis (FAP), a genetic CRC predisposing syndrome. However, these animals develop adenomas in the small intestine rather than in the colon.

$Apc^{Min/+}$ mice show progressive accumulation of adenomas in the small intestine, a gradual depletion in circulating adaptive immune populations and an expansion of the myeloid compartment. We observe that B cells in young $Apc^{Min/+}$ mice are functional and responsive, since they are as able as littermates to develop Salmonella-specific IgG and IgA after oral vaccination. Interestingly, the response in $Apc^{Min/+}$ mice is IgA-skewed while specific-IgG are lower in abundance compared to littermate mice.

This is possibly explained by the differential Salmonella distribution in $Apc^{Min/+}$ mice compared to littermates upon acute infection: in $Apc^{Min/+}$ mice bacterial load in the mesentheric lymph nodes is higher compared to control animals. Moreover we observe a preferential localization of Salmonella at the level of tumor lesions compared to the apparently healthy intestine of $Apc^{Min/+}$ mice or the intestine of littermates.

Preliminary data show that mucus composition in $Apc^{Min/+}$ mice is altered already at young age. We hypothesize a role for mucus in mediating the interaction bacteria-epithelium in the $Apc^{Min/+}$ model. We will perform gene expression profile for mucin genes and IHC for the mucus to address its characteristics in our system.

P036

Comparison of the Immunomodulatory Effects of *Escherichia coli* Nissle 1917 and *Enterococcus faecalis* UGRA10 in Intestine Epithelial Cells and Macrophages

Francesca Algieri[1], Alba Rodriguez-Nogales[1], Natividad Garrido-Mesa[1], Maria Elena Rodriguez-Cabezas[1], Monica Comalada[2], Antonio Zarzuelo[1], Eva Valdivia[3], Mercedes Maqueda[3], Manuel Martinez-Bueno[3], Julio Galvez[1]
[1]CIBERehd Department of Pharmacology, University of Granada, Granada, Spain, [2]Macrophage Biology Group, Institute for Research in Biomedicine (IRB), Barcelona, Spain, [3]Departmento of Microbiology, Granada, Spain

Probiotics have been proposed to display immunomodulatory properties. The aim of the present study was to compare the in vitro effects of two probiotics, *Escherichia coli* Nissle 1917 and *Enterococcus faecalis* UGRA10, in two different cell types involved in the immune response: HT-29 cells (epithelial cells) and RAW 264.7 cells (macrophages). Cells were incubated for 3 hours with each probiotic 10exp8 CFU/ ml. Then, they were stimulated, or not, with LPS (10 µg/ml) for 24 hours. Afterwads, the supernatants were collected and cytokine production (IL-8 in HT-29, and IL-1β or TNFα in RAW 264.7) was determined by ELISA. Also, nitrite levels were determined by the Griess assay. The results revealed that the incubation of both cell types with *E.coli* Nissle 1917 promoted an increased production of all the mediators studied when compared with those cells without probiotic incubation. However, *E. faecalis* UGRA10 did not modify IL-8 production in HT-29 cells, whereas it increased the production of IL-1β and TNFα in macrophages, without affecting nitrite levels. Typically, the increase production observed after probiotic treatment was lower than that obtained for the LPS control. When cells were incubated with each probiotic before the LPS-stimulation, the production of IL-8, TNFα or nitrites, but not IL-1β, was decreased. Of note, the incubation of the macrophage cell line RAW 264.7 with *E. faecalis* UGRA10 completely abolished the increased generation of nitrites induced with LPS. Both probiotics show immunomodulatory properties, but they do not display the same profile in the two different cell types studied.

P037

A Cell Culture System to Study the Immunomodulatory Effects of Bifidobacteria

Siva Gowri Pathmanathan, Michelle McConnell, Blair Lawley, Margaret Baird, Gerald Tannock
Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand

A complex mixture of commensal bacteria populates the human gut. The mechanisms behind the immune hypo-responsiveness of intestinal epithelial cells to these commensals are not fully understood. Kelly et al. (2004), using an intestinal epithelial cell line (Caco-2), detected an anti-inflammatory mechanism activated by a commensal bacterium (Bacteroides thetaiotaomicron). We have established a model system using the human intestinal epithelial cell-line HT29 to detect immunoregulatory effects of Bifidobacterium strains. B. thetaiotaomicron ATCC 29148 strain served as a positive control (Kelly et al., 2004). HT-29 cells were stimulated with the synthetic analog of viral dsRNA, polyinosinic-polycytidylic acid [Poly(I:C)], for 3 hours with or without the addition of freeze-dried bifidobacterial cells. RT-qPCR was used to quantify expression of the interleukin-8 (IL-8) gene, which is commonly used as a pro-inflammatory indicator. Poly(I:C) induced up-regulation of the IL-8 gene by at least 5 fold. We have tested 15 Bifidobacterium cultures using this model and found that regulation of IL-8 gene expression is dependent on strain and whether or not the bacterial preparations had been heat-inactivated.

P038

Intestinal Epithelial Cells Respond to Glycans through Dectin-1 and Dectin-2

Sarit Cohen-Kedar[1], Liran Baram[1], Hofit Elad[1], Hanan Guzner-Gur[3,4], Iris Dotan[1,2]
[1]Mucosal Immunology Laboratory, The Research Center for Digestive Tract and Liver Diseases, Tel-Aviv Sourasky medical center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, [2]Department of Gastroenterology and Liver Diseases Tel Aviv Sourasky medical center, Tel Aviv University, Tel Aviv, Israel, [3]Internal Medicine B Tel Aviv Sourasky medical center, Tel Aviv, Israel, [4]Sackler Faculty ofMedicine, Tel Aviv University, Tel Aviv, Israel

Introduction: Antibodies directed against glycans are associated with Crohn's disease and may represent loss of tolerance towards microorganisms. Most serologic responses are directed against glycans which are major building blocks of microorganisms' cell walls. Intestinal epithelial cells (IECs), may participate in intestinal immune responses. The potential role of microorganisms, specifically their cell wall glycans, in modulating intestinal immune responses and the cells recognizing them are still unclear.

Aim: To assess whether glycans affect IECs and define the mechanism.

Methods: Freshly isolated IECs were generated from surgical specimens. Dectin-1 (the major β-glucan receptor) and Dectin-2 (mannan receptor) expression was detected using flow cytometry, Western-blot and immunofluorescence. Human IEC lines were stimulated with glycans (curdlan, zymosan and mannan) with or without spleen-tyrosine-kinase (Syk) inhibition. Cytokine secretion was assessed using ELISA.

Results: Dectin-1 and Dectin-2 expression by IECs, so far reported on myeloid lineage cells only, was detected. Curdlan and zymosan (Dectin-1 ligands), and mannan (Dectin-2 ligand) induced IL-8 secretion by IEC lines (35, 20 and 2 ng/ml, respectively). This was an up to 50 fold increased IL-8 secretion compared to no treatment. Syk (downstream Dectin-1/2 activation signaling mediator) inhibition was associated with approximately 50% decrease in IL-8 secretion.

Conclusions: Human IECs express the glycan receptors Dectin-1 and Dectin-2. IECs respond to glycans by IL-8 secretion in a Syk-dependent manner, suggesting a signaling pathway similar to that observed in monocytes. Elucidation of the mechanism underlying IECs response to glycans may contribute to better understanding of IBD immunopathogenesis.

P039

Low Frequency of CD11c$^+$CD103$^+$ Dendritic Cells in the Neonatal Intestine Is Responsible for the Sensitivity to *Cryptosporidium parvum* Infection

Louis Lantier[1], Sonia Lacroix-lamandé[1], Coralie Metton[1], Catherine Werts[2], Yves Le Vern[3], Françoise Drouet[1], Fabrice Laurent[1]
[1]INRA UMR1282 ISP, Control and Immunology of Neonatal Enteric Disease group, 37380 Nouzilly, France, [2]Institut Pasteur, Biology and Genetics of Bacterial Cell Wall Group, 75724 Paris, France, [3]INRA UMR1282 ISP, Flow cytometry platform, 37380 Nouzilly, France

The mucosal immune system in all mammalian is still under development at birth and neonates are vulnerable to digestive and respiratory infections. Cryptosporidium parvum is a protozoan parasite that develops exclusively in the epithelial cells of the intestine and causes profuse diarrhea in children and immunocompromised patients worldwide. Preliminary studies revealed that the innate immune system is sufficient to control the acute phase of this infection. We first investigated the presence of different subsets of mononuclear phagocytes in the small intestine of neonatal mice and observed markedly lower frequencies of CD11c$^+$ cells compared to adults. C. parvum infection in the intestinal mucosa of neonates did not affect the colonization dynamics of the CD11c$^+$ CX3CR1$^+$ subset however, a rapid and marked increase in the recruitment of CD11c$^+$ CD103$^+$ DC occurred. A transient depletion of CD11c$^+$ cells in the neonates within the first few days of infection led to increased parasite growth. The infection was then controlled by the generation of new CD103$^+$ CD11c$^+$ DC in the next 72h. A fast and effective control of parasite development was achieved either by increasing the number of intestinal CD11c$^+$ CD103$^+$ DC by FLT3-L administration or by activating these cells with TLR-ligands. The mechanisms by which these DC are recruited and how they control the parasite development in epithelial cells is under investigation. Overall, these results highlight the importance of CD103$^+$ intestinal DC in the innate control of intestinal pathogens and the importance of developing safe strategies that will favour their recruitment.

P040

The Role of P2X7 Receptors in Human Lamina Propria Mononuclear Cells (LPMC)

Serin Helena Schiessling[1,2], Änne Schröder[2], Felix Lasitschka[3,2], Thomas Giese[2], Stefan Meuer[2], Jutta Schröder-Braunstein[2]
[1]University of Heidelberg, Department of Surgery, Heidelberg, Baden-Württemberg, Germany, [2]University of Heidelberg, Institute of Immunology, Heidelberg, Baden-Württemberg, Germany, [3]University of Heidelberg, Institute of Pathology, Heidelberg, Baden-Württemberg, Germany

P2X7 receptors (P2X7R) belong to the family of purinergic receptors and are known to be expressed on peripheral blood mononuclear cells (PBMC). They are activated by increased ATP-levels, as measured during active inflammation. In response, downstream signalling is coupled to pro-inflammatory cascades including formation of the NALP3-inflammasome and release of mature IL-1β.

This study aimes to determine the presence and regulation of P2X7R activity in human lamina propria mononuclear cells (LPMC) and its impact on inflammation. Up to now, the exact pathway of inflammational activation in LPMC is unclear, particulary as LPMC do not have pattern recognition receptors and the role of danger signals has not been investigated so far in the human system. We explored the gene and protein expression of P2X7 receptors in LPMC in comparison to peripheral blood mononuclear cells (PBMC) employing flow cytometry, and rtPCR analysis. Furthermore the cellular expression pattern of P2X7R was assessed by immunohistochemistry and immunofluorescence in tissue samples of healthy mucosa and IBD tissue samples. Functionally, the effect of ATP stimulation on gene expression and IL-1β release by LPMC vs. PBMC was assessed. Moreover, employing an organ culture model of intestinal inflammation we analysed the effect of a P2X receptor antagonist on inflammatory gene transcription in resident lamina propria cells.

P041

Induction of Intestinal Tolerance by Antigen-Targeting of DEC-205 on Gut Dendritic Cells

Munisch Wadwa[1], Karsten Mahnke[2], Jürgen Büning[3], Astrid M. Westendorf[1]
[1]University Hospital Essen, Institute of Medical Microbiology, Essen, Germany, [2]University of Heidelberg, Department of Dermatology, Heidelberg, Germany, [3]University Hospitel Schleswig-Holstein, Lübeck, Germany

Crohn´s Disease and Ulcerative Colitis are two major types of inflammatory bowel disease. The loss of tolerance towards commensal gut bacteria and food antigens seems to be an initial event for the manifestation of disease. Promising players for the induction of tolerance in the intestine are dendritic cells (DCs) which express the antigen receptor DEC-205 on their surfaces. In the present study we analysed the potential of DEC-205 for the induction of tolerance in the intestine. Therefore, an MHC class II restricted epitope of Influenza hemagglutinin (HA110-120) was cross-linked to anti-DEC-205 antibody (anti-DEC-HA) and used for in vitro and in vivo applications. We report that DEC-205-mediated antigen-delivery leads to the presentation of HA not only by splenic DCs but also by DCs from mesenteric lymph nodes and the lamina propria. Furthermore, HA-specific CD4+ T cells turned anergic after in vivo activation via anti-DEC-HA since they did not proliferate and produced considerably lower amounts of pro-inflammatory cytokines like IL-17, IFN-gamma and TNF-alpha after re-stimulation in vitro. For the application of DEC-HA in intestinal inflammation we established a new antigen-specific colitis mouse model in which the transfer of Th1-polarized HA-specific CD4+ T cells into VILLIN-HA transgenic mice leads to the manifestation of severe ileitis. In further studies, the potential of DEC-205 for the inhibition or therapy of intestinal inflammation will be validated in this model. In summary we demonstrated so far that antigen targeting via DEC-205 under steady state conditions represents a potential tool for the induction of tolerance in the intestine.

P042

Crosstalk between Colonic Epithelial Cells and Macrophages

Maja Kristek, Arman Rahman, Christine E. Loscher
Dublin City University, Dublin, Ireland

Macrophages are the most abundant mononuclear phagocytes in the intestine. Unlike other macrophage populations, intestinal macrophages seem to be in a state of partial activation showing no response to inflammatory stimuli, no production of pro-inflammatory cytokines and low expression levels of innate response receptors. Intestinal macrophages are thought to derive from circulating blood monocytes, which are fully responsive to inflammatory stimuli. Therefore it is probable that the intestinal environment itself is responsible for altering the phenotype of these macrophages upon arrival in the gut.

In order to explore whether the intestinal environment can influence macrophage phenotype and function J774A.1 macrophages were conditioned with supernatants from the murine colonic epithelial cell line, CMT-93. Conditioning of J774 cells with CMT-93 media altered the expression levels of innate response receptors and cytokine production. Furthermore, conditioning with CMT-93 media modulated the macrophage response to Toll-like receptor stimuli. They acquired more of a regulatory phenotype with the down-regulation of CD80, CD40 and MHC class II expression and IL-12p40 production, along with increased production of TNF-α.

This data implicates intestinal epithelial cells as one of the factors that can change and shape macrophage response, therefore it is important to investigate this crosstalk further in order to understand its role in the maintenance of intestinal homeostasis.

P043

Microbiota/Host Crosstalk Biomarkers: Regulatory Response of Human Intestinal Dendritic Cells Exposed to *Lactobacillus plantarum* Extracellular Encrypted Peptide

David Bernardo[1], Borja Sánchez[2], Hafid O. Al-Hassi[1], Arancha Hevia[2], Elizabeth R. Mann[1], Simon T. C. Peake[1,3], Jonathan Landy[1,3], Emma Ronde[1], Maria C. Urdaci[4], Abelardo Margolles[2], Stella C. Knight[1]
[1]Imperial College London, Harrow, UK, [2]Instituto de Productos Lácteos de Asturias-CSIC, Asturias, Spain, [3]St Mark´s Hospital, Harrow, UK, [4]Université de Bordeaux, Bordeaux, France

The human gastrointestinal tract hosts a huge variety of commensal microbiota and diverse pathogens; at this site, a balance between immunity and immune tolerance is required. Intestinal dendritic cells (DC) control the mechanisms of immune response/tolerance in the gut. Extracellular proteins of bacteria are currently being characterised as potential mediators between commensal bacteria and the human host. We hypothesized that the dialogue between intestinal bacteria and DC is partially mediated by the secretion of soluble bacterial compounds (including proteins). To that end, we used a model of Lactobacillus plantarum (the lactic-acid-producing bacterium with the largest genome) and human DC.

We have identified a single peptide secreted by L plantarum and encoded within protein D1, which lacks cleavage sites for the major intestinal proteases and is characterised by the abundance of serine and threonine residues within its sequence (STp peptide). STp was found in the colonic microenvironment from healthy controls although was absent in that from patients suffering from ulcerative colitis (a form of inflammatory bowel disease) suggesting a potential role as a biomarker of gut homeostasis. When studied in vitro, STp expanded the ongoing production of regulatory IL-10 in human intestinal DC from healthy controls. STp-primed DC also induced an immunoregulatory cytokine profile and skin-homing profile on stimulated T-cells suggesting diversion of effector T-cells from the intestinal tissue.

Our data suggests that the molecular dialogue between intestinal bacteria and DC may be mediated by immunomodulatory peptides, encoded in larger extracellular proteins, secreted by commensal bacteria.

P044

CX_3CR1^+ Cells that Have Phagocytosed CFP-OVA^+*E.coli* Are Located Close to $DsRed^+$ CD4 T Cells

Valerio Rossini[1], Katarina Radulovic[1], Calin Manta[1], Daria Zhurina[2], Christian Riedel[2], Jan Hendrik NIess[1]
[1]Internal Medicine I, Uniklinik Ulm, Ulm, Baden Württemberg, Germany, [2]Department of Microbiology and Biotechnology, Ulm University, Ulm, Baden Württemberg, Germany

Background: CX_3CR1^+ cells in the colon are a heterogeneous cell population consisting of $F4/80^{hi}CD11b^+$ and $F4/80^{low}CD103^-$ $CD11b^+$ cells.

Methods: To study the role of CX_3CR1^+ cells in mucosal immune responses we generated CFP-OVA^+*E.coli* (*E.coli* constitutively expressing the cyan fluorescent protein (CFP) gene linked with the gene for chicken ovalbumin protein (OVA)). After the colonization and reconstitution of $CX3CR1^{+/GFP}$-$RAG^{-/-}$ hosts with CFP-OVA^+*E.coli* and OT-II/DsRed T cells colonic tissues were analyzed by *ex vivo* 3D confocal microscopy.

Results: CX_3CR1^+ cells in the colonic lamina propria are a heterogeneous cell population that can be distinguished by F4/80 and CD11c expression. Ex vivo confocal 3D imaging demonstrated that CX_3CR1^+ cells from $CX_3CR1^{+/GFP}$ and $CX_3CR1^{+/GFP}$-$RAG^{-/-}$ mice extend processes into the intestinal epithelium.

Fourteen days after adoptive cell transfer $DsRed^+$T cells into $CX_3CR1^{+/GFP}$-$RAG^{-/-}$ hosts $DsRed^+$CD4 T cells formed aggregates with CX_3CR1^+cells. After colonization of $CX_3CR1^{+/GFP}$-$RAG^{-/-}$ mice with CFP-OVA^+*E.coli* CX_3CR1^+ cells phagocytosed CFP-OVA^+*E.coli* and activated OT-II cells as indicated by IFN-γ production. CX_3CR1^+ cells (that have phagocytosed CFP-OVA^+*E.coli)* are located close with $DsRed^+$/OT-II cells as indicated by ex vivo 3D imaging. The activation of $DsRed^+$/OT-II cells by CX_3CR1^+ cells in $CX_3CR1^{+/GFP}$-$RAG^{-/-}$ hosts colonized with CFP-OVA^+*E.coli* resulted in the development of colitis as indicated by progressive body weight loss and histopathological colitis signs.

Conclusion: After the colonic colonization of $CX_3CR1^{+/GFP}$-$RAG^{-/-}$ hosts with CFP-OVA^+*E.coli* the reconstitution with $DsRed^+$/OT-II T cells resulted in the development of colitis. The phagocytosis luminal antigens by CX_3CR1^+ cells and their interactions with CD4 T cells may regulated the integrity of the mucosal immune system.

P045

Synthesis of Retinoic Acid by Human Intestinal Antigen Presenting Cell Populations in Health and Inflammatory Bowel Disease

Theodore Sanders[1], Neil McCarthy[1], Edward Giles[1], James Lindsay[2], Andrew Stagg[1]
[1]Centre for Immunology & Infectious Disease, The Blizard Institute, London, UK, [2]Digestive Diseases Clinical Academic Unit, Barts & The London School of Medicine & Dentistry, London, UK

Introduction: Retinoic acid (RA) contributes to intestinal T-cell responses, inducing gut-tropism and enhancing regulatory or effector activity depending on concentration and context. RA is generated from retinol by sequential action of retinol dehydrogenases (RDH) and retinaldehyde dehydrogenases (RALDH). In the mouse, RALDH expression is confined to CD103+ intestinal dendritic cells (DC) and is downregulated in models of inflammatory bowel disease (IBD) which may contribute to disease. Here we examine RA generation in human intestinal antigen presenting cell (APC) populations in health and IBD.

Methods: Cells were extracted from intestinal biopsies and analyzed by flow cytometry. APC subsets were obtained by immunomagnetic or FACS sorting. Enzyme expression was measured by quantitative RT-PCR and RALDH activity assessed by Aldefluor assay. Stimulatory capacity was assessed by co-culture with naive allogeneic CD4+ T-cells.

Results: Both RALDH and RDH were detected in all myeloid APC populations studied: CD103+ and CD103- DC identified as HLA-DR+CD11c+Lin- cells and in HLA-DR+CD11c+Lin+ monocyte-like cells (DR+lin+ cells). However, RT-PCR suggested involvement of different RALDH isoforms (RALDH2 in DC, RALDH1 in DR+lin+ cells). RALDH activity was significantly enhanced in DR+Lin+ cells from non-inflamed IBD compared with healthy tissue ($p=0.017$) with a similar trend observed in both DC subsets. Naive T cell stimulation was enhanced in CD103+ APCs.

Discussion: In divergence from mouse data, multiple human intestinal APC have RA-generating capacity. Ability to activate naive T-cells is more restricted, indicating that RA from some APC may modulate previously primed T-cells within the mucosa. Altered RA synthesis may contribute to IBD.

P046

Intestinal Inflammation Regulates Retinoic Acid-Dependent Imprinting of Gut Tropism by Human Dendritic Cells Independently of RALDH Expression

Theodore Sanders[1], Neil McCarthy[1], Edward Giles[1], James Lindsay[2], Andrew Stagg[1]
[1]Centre for Immunology & Infectious Disease, The Blizard Institute, London, UK, [2]Digestive Diseases Clinical Academic Unit, Barts & The London School of Medicine & Dentistry, London, UK

Introduction: In mice, expression of retinaldehyde dehydrogenases (RALDHs) by CD103+ intestinal dendritic cells (DC) enables them to generate of retinoic acid (RA) and induce the 'gut homing receptor' α4β7 integrin. In mouse models of inflammatory bowel disease (IBD), this RALDH expression is reduced but little is known about human intestinal RA. Here, we tested whether factors in the human intestine regulate RA generation and activity.

Methods: Conditioned media (CM) were generated by culture of intestinal biopsies. DC were differentiated from monocytes with or without CM. Enzymes expression was assessed by quantitative RT-PCR and RALDH activity determined by Aldefluor assay. Induction of α4β7 on T- cells was measured by flow cytometry.

Results: Upregulation of α4β7 on T cells activated in the presence of DC was RA-dependent. The DC possessed retinal-inhibitable Aldefluor activity and expressed both alcohol dehydrogenase (RDH10) and RALDH (RALDH1,2,3) enzymes required for RA generation. CM significantly suppressed Aldefluor activity ($p<0.0001$) irrespective of tissue sources (healthy and inflamed or non-inflamed IBD), an effect that was partially reversed by a prostaglandin E2 (PGE2) EP-2 receptor antagonist. Although DC differentiated in inflamed or non-inflamed IBD CM had similar RALDH activity, the former induced significantly higher ($p<0.05$) levels of α4β7 expression.

Conclusions: Factors, including PGE2, generated in the human intestinal mucosa limit RALDH activity in DC and may thereby impact upon DC generation of RA. In inflammation, intestinal mediators can influence the imprinting of gut tropism independently of effects on RALDH activity. Manipulation of RA availability may offer new therapeutic options in IBD.

P047

Macrophage Immune Response to Intestinal Bacteria Clearly Differentiates between Gram-Positive Strains

Judith Rodriguez-Ruiz[1], Fernanda Velhote[2], Oscar Reina[3], David Rossell[3], Julio Galvez[4], Jordi Xaus[4], Monica Comalada[1,4]
[1]Macrophage Biology Group, Institute for Research in Biomedicine (IRB), Barcelona, Spain, [2]Pharmacology Department, University of Sao Paulo (USP), Sao Paulo, Brazil, [3]Biostatistics and Bioinformatics Unit, Institute for Research in Biomedicine (IRB), Barcelona, Spain, [4]CIBERehd, Pharmacology Department, University of Granada, Granada, Spain

The intestinal microbiota plays a crucial role in the maturation of the neonatal immune system and in the development of the immune tolerance. The activation of macrophages is dependent on the detection of microbial associated molecular patterns by pattern recognition receptors, of which the family of TLRs are the most studied. However, relatively little is known about the capacity of these cells to discriminate between similar bacterial strains, and thus to induce the required response in each situation.

The objective of this work is to evaluate the immune and genetic response of macrophages to different intestinal Gram-positive bacterial strains belonging to the phyla Firmicutes.

Bone marrow-derived macrophages were stimulated with $5x10^7$ cfu/ml of heat-inactivated bacteria. RNA was extracted to perform full mice genome expression by Affymetrix array analysis and confirmed by Real Time-PCR. Moreover, supernatants of the cultures were analyzed for cytokine expression by Cytometric Bead Assay.

Gram-positive bacteria induce a genetic macrophage response that significantly modifies less than 3% of the total gene diversity. Moreover, almost 70% of the modified probes are common to all bacterial strains tested. Nevertheless, each bacterial strain modifies a particular repertoire of genes that suggests a differential recognition. Supporting it, the immune response induced by each bacterial strain (measured as TLR expression and cytokine secretion, nitric oxide production, etc...) also allows to differentiate them. Therefore, different intestinal strains can induce diverse gene responses in macrophages despite its close phylogeny suggesting the fine-tuned differential bacterial recognition system evolved in these cells.

P048

A Direct Interaction with Dendritic Cells but Not with Epithelial Cells Is Required for the Oral Adjuvant Activity of Cholera Toxin

Tobias Gustafsson[1,2], Yeu-Jiann Hua[1,2], Madelene Dahlgren[3], Bengt Johansson-Lindbom[3], Megan Livingston[1,2], Ulf Yrlid[1,2]
[1]Dept. of Microbiology & Immunology, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden, [2]Swedish Foundation for Strategic Research - The Mucosal Immunobiology and Vaccine Center (MIVAC), Gothenburg, Sweden, [3]Immunology Section, Lund University, Lund, Sweden

While new effective vaccines against mucosal pathogens are needed, the number of mucosal adjuvants for human use is very limited. Although cholera toxin (CT) is a potent mucosal adjuvant in rodents, the mechanism underlying its adjuvant activity is not known. We explored the cellular interactions required for oral adjuvant activity using mice deficient for the beta-1,4-N-acetylgalactosaminyltransferase (GM2/GD2 synthase) gene, which lack all complex gangliosides, including ganglioside GM1, the only known receptor for CT. Chimeric mice that lacked GM1 on non-hematopoietic cells still mounted sufficient antibody responses to OVA fed together with CT. In contrast, mice deficient for GM1 expression on hematopoietic cells generated no intestinal anti-OVA IgA and had reduced serum titers of OVA-specific IgG. In addition, feeding OVA and CT to these chimeric mice resulted in reduced proliferation and impaired expression of surface markers PD-1 and CXCR5 following adoptive transfer of OVA-specific $CD4^+$ T cells (OT-II), indicating impaired T follicular helper cell development. To determine whether CT acts directly on dendritic cells (DCs), we used mixed bone marrow chimeric mice in which the presence or absence of GM1-expressing $CD11c^+$ cells was controlled by the injection of diphtheria toxin. In these mice, the activation of OT-II T cells was significantly decreased, indicating a requirement for DC-expressed GM1. In summary, our results show a complete dependence on direct stimulation of dendritic cells for the adjuvant activity of CT. These results therefore have important implications for the generation of novel oral adjuvants.

P049

A Unique Adjuvant Mechanism for Targeting of Follicular Dendritic Cells and Up-Regulating Germinal Center-Promoting Genes which Dramatically Lowers the Requirement for Classical Dendritic Cells

Johan Mattsson[1], Tobias Gustafsson[1], Madelene Dahlgren[2], Anneli Stensson[1], Bengt Johansson-Lindbom[2], Ulf Yrlid[1], Nils Lycke[1]
[1]Gothenburg University, Gothenburg, Sweden, [2]Lund University, Lund, Sweden

The cholera toxin derived CTA1-DD adjuvant was found to exert a unique targeting and activating function on follicular dendritic cells (FDCs). The effect was dependent on the ADP-ribosylating function of CTA1, which stimulated the up-regulation of several germinal center (GC) promoting genes, including IL-1β, IL-6, ICAM-1, VCAM-1, Baff, IL-15, Cr2 and LTβR in targeted FDCs. The consequence of this up-regulation was associated with greatly pronounced expansion and frequencies of germinal centers and enhanced antibody responses following immunization. Although CTA1-DD was originally designed to target B cells, we now know it will target FDC and be taken up by classical dendritic cells (DCs). Therefore, we investigated whether the adjuvant function was dependent on classical DCs using the well-established CD11c-DTR-model. To this end DC depletion of mice was performed once and intravenous immunizations with the CTA1-DD adjuvant were tested. We found that although DC-depletion was significant we observed a comparatively strong antigen-specific serum response and germinal centers were observed in the spleen. The adjuvant function was independent of B cells, as chimeras with MHC class II deficient B cells still exhibited strong enhancement. While follicular helper T cells were limited in DC-depleted mice, we conclude that CTA1-DD adjuvant partly compensates for the requirement of DC-dependent CD4 T cell priming for enhanced antibody responses.

P050

Cholera Toxin Adjuvant Works Independently of $CD11c^{+}CD8a^{+}$ DCs and IL-12

Nils Lycke, Johan Mattsson, Ulf Yrlid
Univ. of Gothenburg, Gothenburg, Sweden

The mechanism of action of most adjuvants is incompletely understood. Cholera toxin (CT) is perhaps the strongest mucosal adjuvant we know of today. For long it has been associated with skewing of CD4 T cell responses towards Th2-differentiation because of an impaired IL-12 production in innate cells. However, we can now show that the adjuvant effect is completely independent of IL-12 and Th1-differentiation of CD4 T cells occurs even in the absence of IL-12, as seen in IL-12-deficient mice. While CT accumulates in the marginal zone (MZ) following administration and is trapped in marginal zone macrophages (MZMØ) these cells are dispensable for the adjuvant function. Rather CD11c dendritic cells (DC) bind CT adjuvant and migrate, while maturing, into the T cell zone, causing effective priming of the T cells, a mechanism which is so powerful that it eliminates the ability to prime another immune response for nearly 10 days. Whereas, CD11c CD8a cells are instantaneously lost the CD11b CD11c population is responsible for priming of the CD4 T cell and CT-induced resistance against additional priming can be restored by transfer of isolated CD11c cells. Hence, CT mediates its adjuvant function primarily through $CD11c^{+}CD11b^{+}$ $CD8a^{-}$ DCs, as shown in mice, CD11cGsa-, generated to lack the target protein Gsa in the DC population through Cre-Lox technology.

P051

Immunization with Functionalized Calcium Phosphate Nanoparticles Induces Potent Mucosal T Cell Responses against Influenza Virus Infection in Mice

Torben Knuschke[1], Viktoriya Sokolova[2], Olga Rotan[2], Peter Staeheli[3], Matthias Epple[2], Jan Buer[1], Astrid M. Westendorf[1]
[1]Institute of Medical Microbiology, Essen, Germany, [2]Institute of Inorganic Chemistry and Center for Nanointegration, Essen, Germany, [3]Department of Virology, Freiburg, Germany

The ability of vaccines to induce T cell responses is crucial for preventing diseases caused by viruses or bacteria. Nanoparticles are considered as an efficient tool for inducing potent immune responses. Recently, we developed biodegradable calcium phosphate nanoparticles (CaP NPs) that serve as carriers of immunoactive Toll-like receptor 9 ligand (CpG) combined with a viral antigen from the influenza A virus hemagglutinin (HA) to dendritic cells (DCs). In the present study, we examined the in vivo efficiency of HA/CpG-functionalized CaP NPs as a vaccination system against influenza virus infection. Functionalized CaP NPs were efficiently taken up by DCs in vitro and in vivo, and this uptake resulted in DC maturation by increasing the surface expression of CD80 and CD86, which allows the processing and presentation of viral antigens by MHC class I and II. Intraperitonal and intranasal immunization with HA/CpG-functionalized CaP NPs induced efficient, influenza-specific $CD4^+$ and $CD8^+$ T cell responses with increased secretion of IFN-γ. This potent protective response was accompanied by the migration of HA-specific T cells to the lung-draining lymph nodes and by elevated viral clearance after immunized mice were infected with influenza virus. Taken together, these results indicate that functionalized CaP NPs prime cellular immunity by immunization much more efficiently than immunization with soluble factors. This study demonstrates the great potential of CaP NPs as a novel vaccination tool that offers substantial flexibility for several infection models.

P052

The Pertussis Toxin Derived S1-DD Vaccine Adjuvant Functions as a Mucosal Adjuvant and Induces a Mixed Th1/Th2 Response

Linda Fahlén-Yrlid, Lena Ekman, Hilda Bergroth, Jan-Olof Andersson, Nils Lycke
The Mucosal Immunology and Vaccine center (MIVAC), Department of Microbiology and Immunology, Institute of Biomedicine, Gothenburg University, Gothenburg, Sweden

Pertussis Toxin (PT) is a well-known microbial component with immunoadjuvant effects that belongs to the A-B toxin family, in which the B component is responsible for specific binding to target cells and the A subunit expresses enzymatic activity. The A subunit of PT, S1, catalyzes the ADP-ribosylation of the a-subunit of a subset of heterotrimeric G-proteins. Since PT can virtually bind to and affect any mammalian cell via the B component the mechanism by which PT promotes the adjuvant effect has not been completely understood. Our previous work established that a fusion protein derived from cholera toxin, CTA1-DD, exerted powerful adjuvant effects. To test whether the S1 enzyme could replace CTA1 in the fusion protein with retained adjuvant function we constructed the S1-DD fusion protein. Surprisingly, following intranasal administration we found that S1-DD equaled CTA1-DD in its adjuvant efficacy, despite that CTA1 and S1 act through two completely different G-proteins; Gs and Gi, respectively. Mechanistic studies will follow comparing the two systems. We hope to unravel common denominators that may help explain the adjuvant function of these toxin-derived adjuvants.

P053

Food-Grade Bacterial Vectors as Novel Tuberculosis Vaccines

Geir Mathiesen[1], Charlotte R. Kleiveland[1], Lene Th. Olsen Hult[1], Ranja Dimitrijevic[2], Lasse Fredriksen[1], Tor Lea[1], Vincent G.H. Eijsink[1]
[1]*Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (UMB), Ås, Norway,* [2]*Innovation center of the Faculty of Chemistry, Belgrade, Serbia*

About one third of the world's population is infected by *Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis(TB). New vaccines with simplicity, safety and low cost, and effective against all forms of TB are urgently needed. Microbial vaccine delivery vehicles have the potential to become the cheapest and safest option for vaccination, because they are needle-free, and easy to produce and distribute. We work on developing lactobacilli, which are natural inhabitants of the human gastrointestinal tract with interesting immunomodulatory properties, as producer and delivery vector of such vaccines.

Different plasmids for directing and anchoring Ag85B-ESAT6, a fusion of two major Mtb antigens, to surface exposed locations in *Lactobacillus plantarum* have been constructed. Concomitantly, we have been exploring the use of dendritic cell (DC) binding peptides for DC targeting and co-expression of surface-exposed Invasin, which has adjuvant properties and may induce translocation of the microbe over mucosal M-cells to Peyer's patches.

These advanced versatile tools for surface-display in lactobacilli have been successfully used for production and controlled precision anchoring of antigens and Invasin, as confirmed by flow-cytometry and fluorescence microscopy. Experiments with monocytic cell lines showed that Invasin-displaying bacteria induced activation of NF-κB. i.e. a expected and desired proinflammatory response. Immunization studies with *Lactobacillus*-displayed Ag85B-ESAT6 in mice are currently in progress. Future work will include studies with other TB antigens, as well as rotavirus antigens.

P054

Immune Responses to Model Antigen Elicited by Immunization Via Conjunctiva Associated Lymphoid Tissue

Aleksandra Inic-Kanada[1,2], Sandra Belij[1], Marijana Stojanovic[1,2], Jacqueline Montanaro[1], Simone Schlacher[1], Emilija Marinkovic[1,2], Ivana Stojicevic[1,2], Elisabeth Stein[1], Nora Bintner[1], Talin Barisani-Asenbauer[1]
[1]*Medical University Vienna, ISPTM, OCUVAC - Centre of Ocular Inflammation and Infection, Vienna, Austria,* [2]*Institute of Virology, Vaccines and Sera – TORLAK, Belgrade, Serbia*

Infections of the ocular surface cause several blinding disorders that affect the conjunctiva (e.g. trachoma). As parenteral immunization routes do not induce significant, antigen-specific SIgA or protective immunity in mucosal tissues, we assessed the efficacy of immunization via ocular mucosa and conjunctiva-associated lymphoid tissue (CALT).

BALB/c and C57BL/6 mice were immunized via conjunctiva with tetanus toxoid (TTd) as a model antigen. 100 µg/mouse of TTd was applied onto conjunctiva of each eye, together with merthiolate-inactivated B. pertussis, which served as adjuvant. Control mice were immunized subcutaneously with 100 µg/mouse of TTd. Three immunizations were performed at 2-week intervals.

We found TTd-specific IgG and IgA in tears and sera of both mice strains, in addition to IgG positive TTd-specific cells. There was strong correlation between the amount of TTd-specific antibodies in sera and the presence of TTd-specific B cells in draining lymph nodes. B. pertussis enhanced IgG and IgA immune responses in both mouse strains. T cell activation (increase in CD25 expression and in percentages of $CD4^+$, $CD8^+$ and $CD3^+$ cells) and B cell activation (increase in percentages of $CD19^+$ $CD25^+$ cells) occurred in all mice, but mice immunized with TTd in combination with B. pertussis had the strongest responses.

Immunization via conjunctiva induced TTd-specific local and systemic immune responses. The strongest immune responses developed in mice that received TTd together with B. pertussis. We plan to explore the efficacy of novel bacterial adjuvants, such as bacterial ghosts, using the conjunctival route to elicit protective mucosal immunity.

P055

Oral Immunotherapy for Cow´s Milk Allergy Using a Mouse Model of Food Allergy

Guillermo Docena, Paola Smaldini, Lucia Orsini, Renata Curciarello, Angela Candreva, Carlos Fossati, Silvana Petruccelli
University of La Plata, La Plata, Argentina

Food allergy is caused by a breakdown in immunologic tolerance and there is no approved therapy for this pathology. We aimed to induce oral tolerance to cow`s milk proteins (CMP) using CMP and cross-reactive soy proteins (SP) in a food allergy mouse model.

Balb/c mice were gavaged with CMP (CMP Tol) or SP (PS Tol) prior to oral sensitization with CMP and cholera toxin, and then orally challenged with CMP. Immune response in vitro (serum IgE, IgG1, IgG2a and IgA level; IL-5, IL-13 and IFN-γ secretion by splenocytes and Foxp3 T cells in the intestinal mucosa) and in vivo assays (clinical score and cutaneous tests) evaluated.

A lower medium clinical score was achieved in tolerized mice, as compared to sensitized mice, with a reduction in mast cell reactivity in tolerized mice. Immunological changes include decreased specific IgE (1,57±0,2 vs 1,06±0.3 SP Tol; 0,52±0,1 CMP Tol) and IgG1, decreased IL-5 and IL-13 at the protein and mRNA level and induction of regulatory Tcells in lamina propria, Peyer´s patchs and mesenteric lymph nodes in tolerized mice. IgG2a and IgA levels were not modified.

In conclusion, oral immunotherapy based on cross-reactivity between CMP and SP can be used to down-modulate the allergic specific immune response in CMP-sensitized mice. This approach could provide a new therapeutic intervention for CMP food allergy.

P056

A Distinct Cytokine and Chemokine Profile at the Genital Mucosa Is Associated with HIV-1 Protection among HIV Exposed Seronegative Commercial Sex Workers

Julie Lajoie[1], Jen Juno[1], Adam Burgener[1], Charles Wachihi[5], Frank Plummer[1,2], Josuha Kimani[1,5], Blake Ball[1,4], Keith Fowke[1,3]
[1]Department of medical microbiology, University of Manitoba, Winnipeg, MB, Canada, [2]Health Canada Agency, Winnipeg, MB, Canada, [3]Department of Community Health Sciences, University of Manitoba, Winnipeg, MB, Canada, [4]University of Nairobi, Nairobi, Kenya, [5]Kenyan AIDS Control Program, Nairobi, Kenya

The predominance of HIV-1 sexual transmission requires a greater understanding of the interaction between HIV-1 and the mucosal immune system. The study of HIV-1 exposed seronegative (HESN) individuals serves as a model to identify the correlates of protection and to aid in microbicide development. Twenty-two cytokines/chemokines were analyzed at the systemic and mucosal compartments in 57 HESN, 51 HIV-1 negative, and 67 HIV-1 infected commercial sex workers from Nairobi, Kenya. HESN had significantly lower expression of MIG, IP-10 and IL-1a in their genital mucosa compared to controls. HESN cytokine expression also distinctly correlates with mucosal antiproteases, suggesting that HESN have a unique pattern of mucosal chemokine/cytokine expression, which may result in reduced trafficking at the mucosa. These data support the Immune Quiescence model of protection, whereby lower T cell activation/recruitment at the mucosal compartment reduces HIV-1 target cell numbers and is an important component of natural protection from HIV-1.

P057

Influence of the Sex Work on the Female Genital Tract

Julie Lajoie[1], Josuha Kimani[1,2], Makubo Kimani[2], F Nyamiobo[2], Rupert Kaul[4], Frank A Plummer[1,3], Keith Fowke[1]
[1]University of Manitoba, Winnipeg,MB, Canada, [2]Kenyan AIDS Control Program, Nairobi, Kenya, [3]Public Health Agency of Canada, Winnipeg, MB, Canada, [4]University of Toronto, Toronto, On, Canada

Sexual activities leading to discharge of sperm are known to activate the female genital tract (FGT) immune system. Compared to low risk women, female sex worker (FSW) are exposed to a wide variety of sperm and we think that their mucosal immunity is than more activated.

Cervico vaginal lavage (CVL) and cervico mononuclear cells (CMC) from 120 HIV- FSW and 44 HIV- non-FSW women were analyzed for the presence of 22 chemokines/cytokines by Milliplex and by cytometry (CCR5, CXCR3, HLA-DR, CD69, Live dead, CD16, CD4, CD56, CD8, CD3) respectively.

In non-FSW, we observed higher level of MIP-3a, ITAC, MIG, IL-1a, IL-1b, IL-1Ra, Il-6, IL-8, IL-10, IP-10, MDC, MIP-1a, MIP-1b, MCP-1 and TNF-a compared to the level observed in the FSW, while the level of IL-15 and sIL-2Ra were higher in FSW. We observed that FSW had a higher frequency of CD3-CD56dim, whereas low risk women have higher level of CD4+, CD4+CCR5+, CD8+, CD8+CCR5+ and CD8+CD69+ cells.

These results show an important difference in the mucosal milieu of the FGT due to sex work. Surprisingly, non-FSW women have a more activated milieu than the FSW. Rather, this data supports an alternative hypotheses that: a) constant exposition to allo-antigens lead to immune quiescence that has been previously described in HIV exposed seronegative individuals; b) sexworker with high levels of mucosal immune activation are more susceptible to HIV infection and an enrichment of those with low immune activation occurs over time.

P058

Role of Toll-Like Receptors in the Recognition of Probiotics by Monocyte-Derived Dendritic Cells

Beatriz Martínez-Abad[1], Jose Anotino Garrote[2,1], Sara Vallejo-Diez[1], Enrique Montalvillo[1], Celia Escudero-Hernández[1], David Bernardo[3], Enrique Vázquez[4], Ricardo Rueda[4], Eduardo Arranz[1]
[1]Mucosal Immunology Lab. Pediatrics and Immunology Dpt. University of Valladolid. IBGM-CSIC, Valladolid, Spain, [2]Hospital Universitario Río Hortega, Valladolid, Spain, [3]Antigen Presentation Research Group. Imperial College London, St. Mark's and Northwick Park Hospital, London, UK, [4]Discovery Technology Department, Abbott Nutrition R&D, Granada, Spain

To determine the recognition of probiotics and pathogen bacteria by TLRs in monocyte-derived dendritic cells through a PCR array.

Monocytes were cultured with GM-CSF and IL-4 for 5 days. Escherichia coli, Salmonella typhimurium and Clostridium perfringens were used as positive bacterial control. Four species of probiotics were used from the lactobacillus genus: L1, L2, L3 and L4; and two strains from Bifidobacterium genus: B1 and B2. Bacteria were collected in the exponential phase of growth and monocyte-derived dendritic cells (moDCS) were stimulated in a ratio 50 bacteria: 1 moDC for 3 hours. Stimulated moDCs were harvested and RNAs extracted. moDCs were obtained from the collection of six blood donors. We studied the TLR pathway performing the RT2 Profiles TM PCR Array Human Toll-Like Receptor Signalling Pathway. Changes in the transcriptional expression of ACTB were estimated by the $\Delta\Delta$CT method using basal condition as reference.

The expression of TLR2 and molecules from the TLR signalling pathway was triggered by E. coli, S. typhimurium and L3. With the remainding probiotics, we observed a lower expression of TLR pathway molecules such as MyD88, IRAK2, MAPK and NFKB. Among probiotics, Bifidobacteria induced the lowest expression in our assay. CXCL10 and IFNB1 expression was decreased in a greater degree by Bifidobacteria. L3 produced an increase of IL10 and CSF3 expression which are involved in regulation. There are genes whose expression were up-regulated in all culture conditions such as IL1B, IL8, PTGS2, TNF, MAP2K3, CSF2 and IL1A. However, IFNG expression is specific for positive controls.

P059

Protective Effects of Dietary EPA and DHA on Ischemia-Reperfusion Induced Intestinal Stress

Viral Brahmbhatt[1], Manuel Oliveira[1], Muriel Briand[1], Genevieve Perruisseau[1], Viktoria Bastic Schmid[1], Frederic Destaillats[1], Cecil Pace-Asciak[2], Jalil Benyacoub[1], Nabil Bosco[1]
[1]Nestlé Research Center, Lausanne, Switzerland, [2]The hospital for sick children, Toronto, Canada

The immunoregulatory effects of dietary omega-3 fatty acids are still not fully understood. The aim of this study was to determine whether dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in rats limits intestinal ischemia-reperfusion (IR) injury. To test this, rats were fed either control or EPA/DHA supplemented diet for 3 weeks following which they underwent either a sham or an IR protocol. A significant reduction in mucosal damage was observed after EPA/DHA supplemented diet as reflected by maintenance of total protein content. To address the underlying mechanisms of protection, we measured parameters of oxidative stress, intestinal and serological cytokines and intestinal eicosanoids. Interestingly, EPA/DHA fed animals displayed a higher activity of oxidative stress enzyme machinery i.e. superoxide dismutase and catalase in addition to a reduction in total nitrate/nitrite content. While no changes in cytokines were observed, eicosanoid analyses of intestinal tissue revealed an increase in metabolites of the 12-lipoxygenase pathway following IR. Further, IR in EPA/DHA fed animals was accompanied by a significant increase of 17,18-epoxyeicosatetraenoic acid, 8-Iso prostaglandin $F_{3\alpha}$ and thromboxane B_3, by more than 12-, 6-, 3- fold, respectively. Thus, the data indicate that EPA/DHA supplementation may be able to reduce early intestinal IR injury through anti-oxidative and anti-inflammatory mechanisms.

P060

Immunomodulatory Effects Exerted by *Lactobacillus salivarius* Strains on Immune Cells

Ludovica F. Butto[1,2], Silvia Melgar[1], Fergus Shanahan[1], Ken Nally[1], Paul W. O'Toole[1,2]
[1]Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland, [2]Department of Microbiology, University College Cork, Cork, Ireland

Background: Lactobacilli are found in many different environments such as plants, fermented foods, and in human and animals as commensals. It is well established that commensal bacteria interact with the immune system and modulate the host response in a different manner to pathogenic bacteria. In this study, the immunostimulatory effect of Lactobacillus salivarius strains on a variety of immune cells was investigated by measuring cytokine secretion.

Results: Thirty-three L. salivarius strains were evaluated for their capacity to modulate release of pro- and anti-inflammatory cytokines in human and murine immune cells in vitro and ex vivo. The data indicated that the induction of immune responses in mammalian cells was remarkably bacterial strain-dependent. In addition, bacterial strains of the same species may elicit different or opposite patterns of cytokine production under the same conditions.

Conclusion: Results from this study will facilitate selecting strains to investigate and identify effector molecules with pro- and anti-inflammatory properties in ex-vivo and in vivo models.

P061

Developmentally Regulated Inflammatory Response of the Newborn Pig Intestine

Mette Viberg Østergaard, Per T. Sangild, Stine B. Bering
Department of Human Nutrition, University of Copenhagen, Frederiksberg, Denmark

Background: The immature human intestine is characterized by an excessive inflammatory response to microbial antigens from immature epithelial cells. This may be central to necrotizing enterocolitis (NEC), an acute inflammatory condition in preterm infants. We hypothesized that this mechanism explains high NEC sensitivity in pigs born around 90% gestation (day 105, term day 115), where preterm pigs first become viable. We tested our hypothesis ex vivo using intestinal explants derived from newborn preterm pigs of advancing gestational age.

Method: Thirty pigs were delivered by cesarean section at 86% (d100; n=4), 92% (d105; n=9), 96% (d110; n=11), and 100% (d115; n=6) of gestation. Proximal jejunum explants were incubated for 24 hrs with or without LPS (10 ng/ml), flagellin (10 ng/ml), CpG oligonuclotide (0.1 µM) or MDP (1 µg/ml). IL-6, IL-8, and TNF-α concentrations in explant culture supernatants were quantified by ELISA.

Results: Basal IL-6 secretion was higher at d100 compared with all other gestational ages (3-14-fold; $p<0.001$), whereas basal IL-8 secretion was higher at d110 compared with all other gestational ages (1.5-2-fold; $p<0.05$). Basal TNF-α secretion did not differ between groups. LPS and MDP induced IL-8 secretion at all gestational ages (1.5-2-fold; $p<0.05$), whereas LPS induced IL-6 only in explants from d105 and d115 of gestation (2-6-fold; $p<0.001$). TNF-α was not induced by any of the ligands.

Conclusions: Basal and induced intestinal cytokine secretion is developmentally regulated and generally higher in preterm versus term pigs. The age-related cytokine response does show clear correlation with the gestational age of high NEC sensitivity.

P062

Protection of Cow's Milk Allergy (CMA) by Non Digestible Oligosaccharides in Mice is Associated with Altered Mucosal Immune Polarization and Abrogated by IL10 and TGFbeta Neutralization

JoAnn Kerperien[1], S. de Kivit[1], B.C.A.M. van Esch[1,2], G.A. Hofman[1], H.J.G. van de Kant[1], L. Boon[3], A. Rijnierse[1,2], A. Hartog[1,2], L.M.J Knippels[1,2], J. Garssen[1,2], L.E.M. Knippels[1]
[1]Utrecht University, Utrecht, The Netherlands, [2]Danone Research, Wageningen, The Netherlands, [3]Bioceros BV, Utrecht, The Netherlands

We determined effects of dietary intervention with shortchain galacto-(scGOS), longchain fructo-(lcFOS) and/or pectin derived acidic-(pAOS) oligosaccharides on the mucosal immune response and assess the contribution of IL10 and TGFbeta in the protective effect of the diet in CMA.

Mice were fed control or 1% scGOS/lcFOS/pAOS (9:1:2) diet prior to and during oral whey sensitization (5*, once a week). In a second study mice were injected 24h prior to each sensitization with anti-IL10R, anti-TGFβ or isotype antibodies. The acute allergic skin response, anaphylaxis, immunoglobulin's and mMCP1, intestinal cytokine and transcription factor expression (qPCR/IHC) were determined and MLN were characterized.

scGOS/lcFOS/pAOS reduced the acute allergic skin response over 50% and abrogated anaphylaxis compared to whey sensitized mice fed control diet. In the middle ileum of whey sensitized mice mRNA expression of Treg, TGFbeta, IL10 and Th2 type IL-4 and Gata3 was enhanced compared to sham sensitized mice (qPCR/IHC). Furthermore, scGOS/lcFOS/pAOS enhanced the expression of IFNgamma, Tbet, and IL17 mRNA compared to control diet in sensitized mice ($p<0.05$). The reduction of the allergic skin response in CMA mice fed scGOS/lcFOS/pAOS was reversed by both IL10 and TGFbeta neutralizing antibodies.

Dietary scGOS/lcFOS/pAOS can probably enhance intestinal Treg, Th1, Th2 and Th17 type immune activation in association with reduced allergic effector response in CMA, important will be the balance. IL10 and TGFbeta may be of key importance for the protective effect of the diet. Because of these promising results (qPCR/IHC) functionally will be assessed in the near future using intestinal lamina propria isolation (FACS).

P063

Perinatal Exposure to Low Doses of the Food Endocrine Disruptor Bisphenol A Impairs Oral Tolerance and Immune Sensitization to Food Antigens in Offspring Rats at Adulthood

Sandrine Ménard, Viorica Braniste, Mathilde Leveque, Corinne Lencina, Manon Naturel, Lara Moussa, Soraya Sekkal, Cherryl Karkat, Eric Gaultier, Vassilia Theodorou, Eric Houdeau
INRA Toxalim Neuro-gastroenterology and nutrition, Toulouse, France

Background: The food contaminant and xenoestrogen Bisphenol A (BPA) used in food packaging impacts gut barrier functions after perinatal exposure. This work aimed to address the consequences of perinatal exposition to low doses of BPA on intestinal immune homeostasis at adulthood.

Material and methods: Gravid and breast feeding rats were treated per os from the 15th day of gravidity to pups weaning with or without BPA [0.5, 5 or 50µg/kg/d]. Paracellular and transcellular jejunal permeability, as well as oral tolerance and sensitization to ovalbumin (OVA) were addressed on female adult offsprings aged of 45 days.

Results: Perinatal treatment with BPA decreases jejunal paracellular (0.5 and 50µg/kg/d) and transcellular (5µg/kg/d) permeability. Furthermore, perinatal treatment with 5 and 50µg/kg/d of BPA increased anti-OVA IgG titers following an oral tolerance protocol. Anti-OVA IgG response was increased at 5µg/kg/d when performing a sensitization. Enhanced humoral response in rats treated with 5µg/kg/d was associated with a higher secretion of IFNγ by spleen in sensitized rats and by mesenteric lymph nodes in tolerized rats. Finally, a 40% increase of colonic MPO activity (i.e. neutrophil infiltration) was observed in 5µg/kg/d BPA treated rats and orally challenged with OVA.

Conclusion: Perinatal exposure to low doses of BPA decreased jejunal paracellular and transcellular permeability and impaired oral tolerance and sensitization to dietary antigens at adulthood. BPA treatment during perinatal period affects intestinal homeostasis in a nonlinear dose-response relationship. These results suggest that perinatal period is a critical window for BPA exposure that may trigger food intolerance in later life.

P064

Combined Therapy of Oral Tolerance and Probiotic Treatment on Donor Mice Affects the Experimental Graft versus Host Disease Development

Ana Carolina Mercadante[1,2], Suelen Martins Perobelli[2,4], Ana Paula Gregório Alves[1,2], Triciana Gonçalves Silva[2,4], Anderson Miyoshi[3], Ana Maria Caetano Faria[3], Adriana Bonomo[2,4]
[1]National Cancer Institute, Rio de Janeiro/RJ, Brazil, [2]Oswaldo Cruz Institute, Rio de Janeiro/RJ, Brazil, [3]Federal University of Minas Gerais, Belo Horizonte/MG, Brazil, [4]Federal University of Rio de Janeiro, Rio de Janeiro/RJ, Brazil

The major limitation of allogenic hematopoietic stem cell transplantation (aHSCT) is the graft-versus-host disease (GVHD), an immune syndrome caused by donor T cells and characterized by injury affecting the gastrointestinal tract, liver and skin. The current therapy for GVHD is based on immunosupressants, which is non-specific. Here we propose an alternative therapy based on oral tolerance induction and probiotic treatment of the donor. To test the efficiency of this combined therapy, experimental acute GVHD was induced using a semiallogeneic HSCT experimental model. Prior to transplantation donor mice were tolerized by oral administration of recipient cell extract (gavage) and ad libitum doses of Lactococcus lactis. Treatment of donor animals with the combined therapy ameliorated the clinical and pathological manifestations of acute GVHD and survival remained at 100% for over one year after sHSCT. Four days after the sHSCT, spleen and lymph nodes analysis of transplanted mice revealed a higher frequency of regulatory T cell, suggesting that these cells may be responsible for the protection observed, although depletion of Treg from donor spleen did not abolish the protection. It is known that GVHD is closely related with a benefic effect named graft versus leukemia effect (GVL). To test if the GVL is maintained, tumor cells were inject at the moment of HSCT and surprisingly the GVL effect was maintained. Our results show that combined therapy using L. lactis and recipient proteins prevent aGVHD and preserves the GVL effect opening new possibilities to treat human patients. The mechanism of tolerance is under investigation.

P065

Effect of IRF-1 Polymorphisms on the Immunology of the Female Genital Tract

Aida Sivro[1], Julie Lajoie[1], Ruey Chyi Su[1], Joshua Kimani[1,3], Keith Fowke[1], Francis A. Plummer[1,2], Terry B. Ball[1,2]
[1]Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada, [2]National Microbiology Laboratory, Winnipeg, Manitoba, Canada, [3]Kenyan AIDS Control Program, Nairobi, Kenya

Background/Rationale: Mucosal tissues are the primary sites of natural HIV infection and a major reservoir of HIV replication. Previous work has shown association of three polymorphisms in Interferon Regulatory factor -1 (IRF-1) with decreased susceptibility to HIV-1 infection and a reduced likelihood of seroconversion. Peripheral blood mononuclear cells from patients with protective IRF-1 genotypes exhibited significantly lower basal IRF-1 expression and reduced responsiveness to IFN-γ stimulation. IRF-1 is an important immune regulator and individuals with protective IRF-1 genotypes have been shown to regulate the level of immune activation systemically by controlling the IRF-1 protein levels. A detailed understanding of the role IRF-1 polymorphisms might be playing at the female genital tract (FGT), remains to be determined.

Methods: A total of 22 cytokines/chemokines were analyzed at the systemic and mucosal compartments in commercial sex workers from Nairobi, Kenya with different IRF-1 polymorphisms.

Results: Preliminary analysis shows differences in systemic and mucosal cytokine/chemokine expression in individuals with different IRF-1 genotypes. Individuals with protective IRF-1 polymorphisms have significantly higher expression of IL-2, sIL2Ra, IL-15 and IFNg in their genital mucosa compared to individuals with non-protective polymorphisms.

Conclusion: Our data shows that individuals with protective IRF-1 polymorphisms have unique pattern of cytokine expression which may contribute to decreased susceptibility to HIV-1 infection. It is important to fully characterize the effect of IRF-1 polymorphisms at mucosal tissues as this will further the understanding of the mechanisms of HIV resistance and could potentially be used in HIV prevention and treatment.

P066

G-CSF Treated Granulocytes Promotes Gut, Skin and Liver Protection against the Graft Versus Host Disease and Maintains the Graft Versus Leukemia Effect

Suelen Martins Perobelli[1], Ana Carolina Mercadante[2], Ana Paula Gregório Alves[2], Triciana Gonçalves Silva[1], Alberto Nóbrega[1], Adriana César Bonomo[1,3]
[1]Federal University of Rio de Janeiro, Rio de Janeiro/ Rio de Janeiro, Brazil, [2]National Institute of Cancer, Rio de Janeiro/ Rio de Janeiro, Brazil, [3]Oswaldo Cruz Institute, Rio de Janeiro/ Rio de Janeiro, Brazil

Allogeneic hematopoietic stem cell transplantation (HSCT) is used to treat a series of hematological diseases. While the presence of T cells mediates the graft-versus-leukemia (GVL) effect and improves engraftment, it is also responsible for the graft-versus-host disease (GVHD), which is the main barrier of HSCT. Patients transplanted with cells from G-CSF treated donors show an unexpected low rate of acute GVHD given the high numbers of T cells present. We have previously shown that blood from G-CSF treated donors have high numbers of inhibitory granulocytes. Experimentally these granulocytes are able to prevent aGVHD in a semi-allogeneic mouse model. Here, our goal was to study the mechanism of action of these G-granulocytes. Spleens from 5 day G-CSF treated donors together with control bone marrow cells (G-B6) were injected into irradiated F1(B6XBALB/c) with survival rate of 100%, against 10% in non-G-B6 transplants. This protection dependes on Gr1+ cells. G-B6 had milder pathological and clinical manifestations in gastrointestinal mucosa, skin and liver. On the other hand, splenic T cells from 9 month old G-B6 chimeras are perfectly competent and able to reject third party A/J skin grafts while sparing F1 skin in a transfer assay, indicating that the tolerance is specific. Finally, the anti-tumoral effect in G-B6 quimeras is as potent as the B6 control chimera and this effect was mantained for more than 20 weeks after HSCT. So, the treatment with G-CSF reduces GVHD while keeping GVL effect opening a promising road in the treatment of human aGVHD.

P067

A Mucosal Tolerance-Inducing Vector for Treatment of Autoimmune Diseases

Charlotta Hansson[1], Annemarie Hasselberg[1], Karin Schön[1], Irina Kalbina[2], Sören Andersson[2], Åke Strid[2], Nils Lycke[1]
[1]*Biomedicine, Gothenburg, Sweden,* [2]*Science and Technology, Örebro, Sweden*

We have developed a fusion protein that serves as a platform for tolerizing peptide-specific CD4+ T cells. The CTA1(R7K)-X-DD fusion protein consists of the A1 subunit of cholera toxin that is mutated and thus unable to ADP-ribosylate. This moiety is linked to a dimer of a Staphylococcus areus protein A fragment. Tolerance is observed to peptides incorporated in the fusion protein after intranasal immunization.

In order to understand the underlying mechanisms of action we have incorporated two well established model epitopes; either Ovalbumin(323-339) or E-alpha(52-68). Both constructs lead to impaired T cell proliferation, increased IL-10 production and reduced IL-17 and IFNy levels. These models are used to investigate phenotype, activation and localization of the antigen presenting cell as well as the peptide-specific T cell population.

Furthermore, tolerance has been achieved in several murine models for autoimmune disease, such as the collagen induced arthritis (CIA) model. This effect cannot be attained through oral administration, most likely due to degradation in the gut. Hence, we transfected Arabidopsis plant with a plasmid encoding our tolerogen. Transfected plants hold promise as bioreactors but might also provide a good delivery system for oral tolerization. Indeed, upon feeding mice with Arabidopsis expressing the fusion protein, we observed no or significantly reduced symptoms. More experiments are required in order to understand the mechanisms behind this.

In summary, we have created a tolerance platform that could be used to treat several autoimmune diseases as well as answer questions regarding tolerance induction at the mucosal surface.

P068

The Vagus Nerve a New Player in Intestinal Immune Tolerance

Martina Di Giovangiulio, Pedro July Pinilla-Gomez, Giovanna Farro, Andrea Nemethova, Kathleen Lambaerts, Gianluca Matteoli, Guy Boeckstaens
Translational Research Center for Gastrointestinal Disorders (TARGID) KULeuven, Leuven, Belgium

To maintain intestinal homeostasis, the immune system has evolved redundant regulatory strategies. These mechanisms are able to avoid exaggerated immune reactions against harmless antigens such as microflora and food antigens present in the gut lumen. Recently, cross-talk between immune and nervous system has been shown to play a role for immune regulation. In particular the parasympathetic nervous system, via the vagus nerve (VN), has been proposed to play a crucial role in the regulation of the immune response through the cholinergic anti-inflammatory pathway (CAIP). Stimulation of the VN induces the release of acetylcholine that dampens activation of immune cells by interacting with the alpha-7 nicotinic acetylcholine receptors (α7AChR). We provided evidence that VN stimulation reduces intestinal inflammation triggered by abdominal surgery suggesting vagus-mediated modulation of the intestinal immune system. Based on these findings, we hypothesize that the vagal innervation of the gastrointestinal tract plays a major role in the intestinal immune homeostasis. Interestingly, we found that vagotomised (VXG) mice failed to develop tolerance to orally delivered ovalbumin (OVA sham 0,4±0,05 vs OVA VXG 1,2±0,3; $p<0,01$, n= 9). A significant lower conversion of OVA specific naive T cells into FoxP3 T regulatory cells is present in VXG mice upon OVA gavage (Sham 31,6% vs VXG 20,7%, $P<0.01$). In line with these findings, α7nAChR deficient mice showed a significant loss of oral tolerance (OVA WT 0,7±0,05 vs OVA KO 1,9±0,3; $p<0,01$, n= 9). In conclusion we have evidences suggesting that VN has a potent and selective regulatory role on intestinal immune homeostasis.

P069

Type I Interferon Gene Expression by Non-Mucosal Mononuclear Phagocytes Requires Poising by the Commensal Microbiota

Stephanie Ganal[1,3], Stephanie Sanos[1], Carsten Kallfass[1], Caroline Johner[2], Carsten Kirschning[4], Peter Staeheli[1], Andreas Diefenbach[1]
[1]IMMH, University of Freiburg, Freiburg, Germany, [2]Max-Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany, [3]Spemann Graduate School of Biology and Medicine, Freiburg, Germany, [4]Institute of Medical Microbiology, University of Essen, Essen, Germany

Mononuclear phagocytes are an important component of an innate immune system perceived as a system ready to react upon encounter of pathogens. We could show that mononuclear phagocytes residing in non-mucosal lymphoid organs of germ-free mice fail to produce type I interferons (IFN-I) in response to microbial stimulation. Consequently, NK cell priming and anti-viral immunity are severely compromised in germ-free mice. Pathogen-inducible expression of a set of inflammatory response genes, including the various IFN-I genes, require poising by signals from the commensal microbiota. While signaling downstream of various pattern recognition receptors and nuclear translocation of NF-kB p65 and IRF3 were normal in mononuclear phagocytes of germ-free mice, binding to their respective cytokine promoters was impaired, which correlated with the absence of the activating histone mark H3K4 trimethylation. Our data reveal a previously unrecognized role for postnatally colonizing microbiota in the introduction of chromatin level changes in the mononuclear phagocyte system, thereby poising expression of central inflammatory genes to initiate a powerful systemic immune response during viral infection.

P070

Differential Regulation of Mucosal Tolerance in the Small and Large Intestine

Sharon Veenbergen, M. Fleur du Pré, Lisette A van Berkel, Ytje Simons-Oosterhuis, Janneke N Samsom
Erasmus Medical Center – Sophia Children's Hospital, Rotterdam, The Netherlands

Chronic inflammation in the gastrointestinal tract, as seen in Crohn's disease, arises from a failure to maintain tolerance to harmless luminal antigens. However, location of inflammation varies amongst patients suggesting that small and large intestine have different susceptibility to inflammation. In this study, we investigated whether distinct locally adapted regulatory mechanisms maintain tolerance in small and large intestine. Administration of the protein antigen ovalbumin to the colon led to antigen-specific T cell proliferation in the iliac lymph nodes (ILN), while antigen administration via the oral route exclusively elicited a T-cell response in the mesenteric lymph nodes (MLN). Interestingly, gut-derived $CD103^+$ DCs in the MLN expressed higher mRNA levels of retinal dehydrogenase 2, a vitamin A-converting enzyme important for Foxp3 induction, whereas $CD103^+$ DC from the ILN showed enhanced levels of the immunosuppressive cytokine IL-10. Of particular importance, both routes of antigen application induced mucosal tolerance via enhanced differentiation of suppressive regulatory T cells, however, de novo induction of Foxp3-expressing T cells was more pronounced after oral antigen administration. Our results show that tolerance induction in different intestinal compartments is mediated by phenotypically distinct immune cells, suggesting that the mucosal immune system utilizes locally adapted regulatory mechanisms to prevent mucosal inflammation.

P071

The Early Activation Marker CD69 Regulates the Intestinal Inflammation by Affecting Migration of CD4 T Cells

Katarina Radulovic, Calin Manta, Valerio Rossini, Jan-Hendrik Niess
University Hospital Ulm, Ulm, Baden-Württenberg, Germany

Background: Regulation of lymphocyte migration is crucial for normal functioning of immune system. Here, we report that the surface receptor CD69, a type II membrane glycoprotein, regulates the migration of CD4 T cells.

Methods: The expression of chemokine-ligand genes in the intestinal tissues of non-treated and dextran sodium sulphate (DSS) treated B6 and CD69-deficient mice was analyzed by quantitative real time PCR (qRT-PCR).

Results: The expression of CCL-1, CCL-19 and CXCL-10 chemokine genes is increased in mesenteric lymph nodes, small intestinal and colonic tissues of non-treated $CD69^{-/-}$ compared to B6 mice. Micro-array analyzes of CD4 T cells showed increased expression of chemokine-related genes in the absence of CD69. In vitro, $CD69^{-/-}$ CD4 T cells showed increased chemotaxis toward CCL-1 and CXCL-10 compared to B6 cells. In vivo, the transfer of equal numbers of $CD69^{-/-}$ and B6 CD4 T cells into the B6 hosts resulted in the migration of higher number of CD69-deficient cells into the intestinal tissues. Administration of DSS in the drinking water induced severe colonic inflammation in $CD69^{-/-}$ mice associated with accelerated body weight loss and elevated levels of CCL-1, CCL-19 and CXCL-10 gene transcripts in the colonic tissue. Three days after the DSS administration $CD69^{-/-}$ mice had significantly higher number of CD4 T cells in the colonic lamina propria compare to B6 animals.

Conclusions: In the absence of CD69, CD4 T cells express increased levels of chemokine ligands and receptors and migrate more efficiently to the intestinal tissues inducing the severe intestinal inflammation after the DSS treatment.

P072

Detection of Immune Responses in Mucosal T-cells in Kenya

Hannah M. Cheeseman[1], Onyango J. I. Obila[2], Simon Ogola[2], Philip J. Bergin[1], Dilbinder K. Gill[1], Harriet H. Park[3], Jacquelyn Nyange[2], Dagna Laufer[3], Pat Fast[3], Bashir Farah[2], Jill Gilmour[1], Omu Anzala[2]
[1]International AIDS Vaccine Initiative Human Immunology Laboratory, Imperial College London, London, UK, [2]Kenya AIDS Vaccine Initiative, Nairobi, Kenya, [3]International AIDS Vaccine Initiative, New York, USA

We, and others, have previously shown that antigen-specific T-cell populations isolated from peripheral blood and gut mucosa are functionally different, indicating that assessment of mucosally-targeted vaccines should be made using mucosal cells. Immune responses also differ with race and geographical location, therefore these studies should be performed in the target population.

Methods optimized at the IAVI Human Immunology Laboratory (HIL) in London were modified and applied at the Kenya AIDS Vaccine Initiative (KAVI), Nairobi. We isolated mucosal mononuclear cells (MMC) from gastro-intestinal biopsies, and analysed reactivity upon peptide stimulation using 10-colour multi-parametric intracellular cytokine staining (ICS) flow cytometry, to distinguish antigen-specific responses.

A panel of 15 PBMC specimens with a range of CMV responses was used to demonstrate equivalence between methods at HIL and KAVI. In addition, 31 volunteers were recruited, biopsied, and their MMC and PBMC isolated and stained with the 10-colour panel. PBMC were also assessed using IFN-g ELISpot for direct comparison of pp65-CMV responses. From these 31 volunteers, 11 satisfied our pass/fail criteria. Of these 11 volunteers, 8 PBMC and 5 MMC samples demonstrated a CMV response (CD4 and/or CD8) by ICS, while all 11 responded by ELISpot (PBMC; mean 723 SFU/million; range 95-1440 SFU/million). PBMC ELISpot CMV responses significantly correlated with ICS CMV responses; $r^2 = 0.62$.

This study demonstrates that it is feasible to collect biopsies and perform multi-parameter ICS in Kenya, generating baseline data and methods that may be used to assess antigen-specific T-cell responses in future vaccine trials.

P073

Expansion of Peripheral and Gastrointestinal CD8+ T-cells for Investigation of Functional T-cell Responses in HIV-1-Infected and Seronegative Individuals

Hannah M. Cheeseman[1], Peter Hayes[1], Jakub Kopycinski[1], Alan Steel[4], Peter Kelleher[2,3], Jill Gilmour[1], Dilbinder K. Gill[1]
[1]International AIDS Vaccine Initiative Human Immunology Laboratory, Imperial College, London, UK, [2]Immunology Section, Imperial College, London, UK, [3]HIV/Genitourinary Medicine Directorate, Chelsea and Westminster NHS Trust, London, UK, [4]Gastroenterology Unit, Chelsea and Westminster NHS Trust, London, UK

CD4+ T-cells from gastrointestinal-associated lymphoid tissue (GALT) are massively depleted during primary HIV-1 infection. Consequently, the assessment of HIV-1 vaccine-induced immune responses at this site may be an essential component of future clinical trials, which has been restricted by difficulties in isolating sufficient numbers of T-cells from the gut.

We describe a method for expansion of GALT CD8+ T-cells using bi-specific antibodies and their subsequent application to intracellular cytokine staining, ELISpot and functional virus inhibition assays. Mucosal Mononuclear Cells (MMC) were isolated by disaggregation of endoscopic biopsies along with parallel PBMC. Mean ex vivo MMC numbers increased 3.7-fold following culture with CD3/4 bispecific antibody and IL2. Post-expansion CD8 T-cells were, on average, 82**%** of the total CD3+ cell population and maintained 80% viability. The memory phenotypes of CD8+ T-cells expanded from PBMC and MMC were significantly different; expanded PBMC contained a higher percentage of naïve and central effector memory T-cells while expanded MMC contained a higher percentage of effector memory T-cells.

Expanded CD8+ T-cells demonstrated equivalence between PBMC and GALT compartments (n=15) in their ability to inhibit in vitro replication of clade B HIV-IIIB. However, ELISpot responses to IIIB-matched peptide pools did not correlate between these compartments, differing in both magnitude and breadth of response.

These data show that the functional profiles and anti-viral capacities of peripheral and gastrointestinal CD8+ T-cells may differ. A complete profile of vaccine candidate immunogenicity should include peripheral and mucosal sampling to inform on vaccine design and prioritisation of vaccine candidates.

P074

Environment and Antibiotics Have a Transient Effect on Immune Regulation in Early Life and a Sustained Effect on Total CD4 T Cells in the Neonatal Intestine

Zoe Christoforidou[1], Marie Lewis[1], Charlotte Inman[2], Denise Kelly[3], Chris Stokes[1], Mick Bailey[1]
[1]University of Bristol, Bristol, UK, [2]University of Birmingham, Birmingham, UK, [3]University of Aberdeen, Aberdeen, UK

Disruption of gut microbiota in early childhood, either by changes in the environment or by antibiotic treatment, has been linked to allergic disease. Mechanisms are difficult to study in human infants, but similarities in physiology and nutritional requirements make neonatal piglets good animal models.

We used fluorescence immunohistology to investigate $CD4^+CD25^+$ $FoxP3^+$ regulatory T-cells (Tregs) in the intestinal lamina propria of 28-day-old and 56-day-old piglets. All piglets spent their first 24-hours with the sow, either on an extensive or intensive farm. At 24-hours old, a subset of piglets from each litter was transferred into a high-hygiene isolator, where half were treated daily with antibiotics. After 28 days, isolator piglets from an intensive farm had fewer $CD4^+CD25^+FoxP3^+$ T-cells as a proportion of the total $CD4^+$ staining than their siblings which stayed with the mother. In contrast, there was no effect on the proportion of Tregs of transferring piglets from the extensive farm into the isolator. Antibiotic-treatment significantly reduced Treg/CD4 ratio only in the extensive farm/isolator piglets. By 56 days differences in Treg/CD4 ratio were no longer detectable in any of the groups. However, differences in total number of CD4 T cells were sustained.

Our results suggest that both an isolator environment and antibiotic treatment may predispose to allergy by changing the number of Tregs and the number of total CD4 T cells in the neonate intestinal mucosa. Spending the first 24-hours in an extensive farm seems to protect against the isolator effect, but not the combined effect of isolator and antibiotics.

P075

CD4 TH17 Cytokine Secretion is Not Depleted in the Blood or Colon of HIV-1 Patients on ART

Louise Greathead[1], Hannah Cheeseman[1], Dilbinder Gill[1], Robert Goldin[1], Alan Steel[0,2], Mark Nelson[0,2], Brian Gazzard[0,2], Peter Kelleher[1]
[1]*Imperial College, London, UK,* [2]*Chelsea and Westminster Hospital NHS Trust, London, UK*

The gastrointestinal tract (GIT) is a major site of CD4 T cell depletion and viral replication during HIV-1 infection. The hallmark of progressive disease is CD4 T cell loss accompanied by systemic immune activation. Preferential depletion of the TH17 subset within the mucosa has been linked to markers of microbial translocation and immune activation. However, there is conflicting data on whether TH17 levels are restored in the GIT once ART commences. This study examined TH17-associated cytokine expression in matched mucosal and peripheral blood mononuclear cells (MMC's, PBMC's) from 8 HIV-1 patients on ART (median 3 years treatment), 8 HIV seronegative controls and 7 patients with Crohn's disease (CD), a mucosal inflammatory disease control. There was no correlation observed between CD4 percentages in PBMC and MMC in HIV-1 and CD patients in contrast to that observed in controls. HIV-1 patients had significantly lower CD4 percentages in both MMC's and PBMC's (p=0.002 and 0.004) compared to controls and CD patients. Expression of cellular activation markers CD38 and HLA-DR were increased on CD4 T cells in the HIV-1 group compared to controls (p=0.04). Secretion of IFN-g and TNF-a in PBMC and MMC were similar across all groups. There was also no significant difference in the expression of TH17-associated cytokines (IL-17A, IL-22, IL-17F) in CD4 T cells in the MMC or PBMC. Our findings indicate that the capacity of CD4 T cells isolated from blood and colonic tissue to secrete TH-17 cytokines in HIV patients on ART is similar to controls and CD patients.

P076

Distribution of $CD4^{+}CD25^{high}CD127^{low/neg}$ $FOXP3^{+}$ Regulatory T Cells in Gastrointestinal Biopsies of Several Gut Regions in Humans

Martin Tauschmann[1], Barbara Prietl[1], Gerlies Bock[1], Christine Neuper[1], Michael Wolf[1], Winfried Graninger[2], Gregor Gorkiewicz[4], Patrizia Kump[3], Christoph Högenauer[3], Thomas Pieber[1]
[1]*Department of Internal Medicine, Divison of Endocrinology and Metabolism, Medical University of Graz, Graz, Austria,*
[2]*Department of Internal Medicine, Division of Rheumatology and Immunology, Medical University of Graz, Graz, Austria,*
[3]*Department of Internal Medicine, Division of Gastroenterology and Hepatology, Medical University of Graz, Graz, Austria,* [4]*Institute of Pathology, Medical University of Graz, Graz, Austria*

The gut immune system is involved in development of several autoimmune-mediated diseases like inflammatory bowel disease or diabetes type 1. In this context $CD4^{+}CD25^{high}CD127^{low/neg}FOXP3^{+}$ regulatory T cells (Treg) are of particular interest due to their critical role in immune homeostasis. Data on Treg frequencies in the intestinal mucosa throughout human gut are sparse.

Gastroduodenoscopy and Colonoscopy was performed in 13 healthy volunteers (mean± SD: 25.35±4.73 years) and biopsies were drawn from several gut regions including gastric antrum and corpus, duodenum, terminal ileum, caecum, ascending and descending colon. The frequencies of $CD8^{+}$-, $CD4^{+}$- and regulatory-T-cells in the gastrointestinal mucosa were assessed by multi-parameter FACS-analysis.

The relative abundance of $CD8^{+}$ T-lymphocytes was higher in upper gastrointestinal tract with highest levels in corpus, compared to descending colon (39.19%±14.11% vs. 13.11%±5.18%, p<0.001), whereas $CD4^{+}$ lymphocytes were predominant found in terminal ileum, caecum and colon (descending colon 42.64%±7.08% vs. corpus 21.00%±11.71%, P<0.001). The relative abundance of Treg was highest in the regions caecum and colon (caecum 4.57%±2.00%vs. corpus 2.26%±1.34%, p=0.002).

Our study provides a systemic assessment of T cells including Treg in gastrointestinal mucosa of healthy humans. The distribution of T cells in gastrointestinal mucosa varies according to the gut region suggestive of a correlation to the function of the respective organ location. It seems that in gastric mucosa a more cytotoxic milieu is prevalent, whereas in the lower intestinal tract a tolerogenic milieu can be found. Therapeutic implications for an increase in gastrointestinal Treg by immune modulating agents needs to be further investigated.

P077

Modulation of Established Systemic KLH-Specific T- and B-Cell Responses in Humans by Oral Antigen

Arwed Hostmann[1,2], Tim Meyer[1], Kerstin Kapp[4], Jochen Maul[1], Rainer Duchmann[3], Martin Zeitz[1], Reiner Ullrich[1]
[1]Medical clinic for gastroenterology, infectiology and rheumatology, CBF, Charité, Berlin, Germany, [2]Clinical research center for hair and skin science, Charité, Berlin, Germany, [3]Medical clinic, Hospital Zum Heiligen Geist, Frankfurt a. M., Germany, [4]MOLOGEN AG,, Berlin, Germany

We investigated whether oral antigen can modulate existing T- and B-cell responses in humans. Healthy volunteers were immunized with keyhole limpet hemocyanin (KLH) until a DTH developed. The oral group (n=8) but not the controls (n=7) then received 5 mg KLH/d p.o. for 10 days. Ten days later both groups were re-immunized with KLH. PBMC were isolated at defined time intervals and KLH-specific T cells were identified by the expression of CD154 after in vitro restimulation with KLH and anti-CD28. The proportions of KLH-specific CD4 T cells expressing IFN-γ, IL-4, TNF-α, IL-10, IL-2, IL-17, CLA and integrin beta7 as well as KLH-induced proliferation of CD4 T cells were analyzed by flow cytometry. KLH-specific serum IgG, IgA and IgM as well as IgG subclasses were measured by ELISA.

KLH-specific proliferation and KLH-specific CD4 T cells were quantitatively similar in the oral group and controls. However, compared to controls KLH-specific IL-17-producers were reduced after ten days of oral KLH. Prior to the first re-immunization at day 34, the proportion of KLH-specific CD4 T cells expressing CLA, IFN-γ and IL-2 was reduced; the proportion of IL-10-producing CD4 T cells was increased at days 34 and 14 days after booster immunization in the oral group. Oral KLH did not affect the proportion of KLH-specific CD4 T cells expressing IL-4, TNF-α and integrin beta7 and did not lead to significant changes in KLH-specific serum antibodies during the study period.Our findings suggest that oral antigen can modulate pre-existing systemic T cell rather than B cell responses.

P078

Skewing of SAG Mediated Therapy for a Predominant Th1 During Visceral Leishmaniasis on Triggering CD2 Epitope

Sukrat Sinha[1], Shanthy Sundaram[1], Sanjiva Bimal[1,2]
[1]Centre for Biotechnology, University of Allahabad, Allahabad, U.P, India, [2]Division of Immunology, Rajendra Memorial Research Institute of Medical Sciences, Patna, Bihar, India

Visceral leishmaniasis is a macrophage associated disorder which is linked with a profound decrease in the immunotherapeutic potential of the infected subjects leading to a marked reduction in the CD4 linked Th1 protective immune response. It greatly affects the liver leading to abnormal levels of SGPT and SGOT. Also the patients suffering from VL have been reported to be coinfected with Hepatitis C during some circumstances. Simultaneously the patients in Bihar are showing unresponsiveness towards SAG which is still a first line of drug in many countries around the world against Visceral Leishmaniasis.

It has been found in the present set of studies that stimulation of CD2 co receptor along with along with therapeutic dose of SAG has led to the enhancement in the release of IFN-gamma which leads to the release of TNF-alpha and activates the macrophages. An increase in the NO mediated killing further observed by the activated macrophages leading to the reduction in the parasitic load. The results indicate that enhancing the immune potential of a VL patient will help in the better response of Sodium Antimony Gluconate which is the first line of drug against VL in many countries.

P079

MAIT Cells: α,β -Chain Interrelation upon Interaction with MR1

Rangsima Reantragoon[1], Lars Kjer-Nielsen[1], Onisha Patel[2], Zhenjun Chen[1], Alexandra J. Corbett[1], Patricia T. Illing[1], Mugdha Bhati[2], Lyudmila Kostenko[1], Mandvi Bharadwaj[1], Bronwyn Meehan[1], Ted H. Hansen[3], Dale I. Godfrey[1], Jamie Rossjohn[2,4], James McCluskey[1]
[1]University of Melbourne, Parkville, Victoria, Australia, [2]Monash University, Clayton, Victoria, Australia, [3]Washington University School of Medicine, St.Louis, Missouri, USA, [4]Cardiff University School of Medicine, Heath Park, Cardiff, Wales, UK

MAIT (Mucosal-associated invariant T cells) are a subpopulation of T cells that are known to be conserved in evolution. Activation of these cells is restricted to the HLA class Ib molecule, MR1 (MHC-related protein). However, the molecular mechanisms and the nature of the TCR which orchestrate antigen-MR1 recognition are yet to be determined. Alanine-scan mutagenesis revealed key residues in the invariant α-chain necessary for MR1-Ag recognition. These residues are necessary for recognition of different bacteria and are conserved across different animal species. The results provide a basis for the highly conserved residues in the Vα and Jα regions. Further characterization of primary human MAIT cells using an anti-Vα7.2 antibody revealed the different patterns of MAIT TCR usage. These findings provide a striking comparison to the iNKT receptor, in that the MAIT TCR, a component of the adaptive immune system, has evolved innate recognition characteristics.

P080

Characterising the Intestinal Epithelial Epimmunome: Cell-Surface Proteomic Profiling Identifies T Cell Regulators

Mahima Swamy[1,2], Thomas Bock[3], Yasmin Köller[1], Philip East[1], Bernd Wollscheid[3], Adrian Hayday[1,2]
[1]London Research Institute, Cancer Research UK, London, UK, [2]King's College School of Medicine, London, UK, [3]NCCR Neuro Center for Proteomics, ETH Zurich, Zurich, Switzerland

The epithelial cells lining the gut are subject to constant microbial and non-microbial challenges, which alter the cell-surface repertoire and secreted molecules that these cells express. These molecules serve as indicators of the state of the epithelium to the intra-epithelial lymphocytes (IEL) that patrol these surfaces, and are collectively termed 'the epimmunome'. Using the recently described Cell Surface Capturing (CSC) workflow for selective targeting, enrichment and identification of N-glycosylated proteins we have characterized the cell-surface proteome of intestinal epithelial cells, both in resting and in Toll-like Receptor (TLR)-stimulated states. We then compared these quantitative datasets with data from non-stimulatory cells and with expression data from IEL to define epithelial surface molecules that are capable of instructing and triggering IEL responses. We were able to confirm the JAML-CAR axis in activating intestinal IEL, and further, we identified ICOS-ICOSL as a new axis of communication between IEL and IEC. Thus, the identification of unknown molecules upregulated on peripheral epithelial cells by various stresses should be instructive in understanding immune responses to oral and/or skin challenges.

P081

Regulatory CD25+FoxP3+ T Cells in the Intestinal Mucosa of Patients with Inflammatory Bowel Disease. Corticoids Treatment Effect

Anna Carrasco[1], Fernando Fernandez-Bañares[1], Merce Rosinach[1], Carme Loras[1], Montserrat Aceituno[1], Elisabet Pedrosa[2], Josep Manye[2], Violeta Loren[2], JosepMaria Viver[1], Maria Esteve[1]
[1]*Hospital Universitari Mútua Terrassa. Fundació per a la Recerca Mútua Terrassa. CIBERehd, Terrassa, Spain,* [2]*Institut de Recerca en Ciències de la Salut Germans Trias i Pujol, Badalona, Spain*

Increase of regulatory CD3+CD4+CD25+FoxP3+ T cells (Treg) in peripheral blood (PBMC) was associated to good response to rescue treatments in steroid-refractory Inflammatory Bowel Disease (IBD). Aim: To analyze Treg number in PBMC and intestinal mucosa in IBD and the effect of corticosteroid treatment. Methods: 8 healthy controls, 6 Ulcerative Colitis (UC) and 31 Crohn's disease (CD) were included. The majority of CD patients were included at diagnosis (early CD). Samples were taken from patients with active CD and after 3-5 weeks of corticosteroids. In steroid-dependant and refractory patients samples were taken after rescue treatment. FoxP3 was analysed by flow cytometry (FCM) and immunohistochemistry (IHQ). Results: Median[IQ]. No differences were found in Treg between IBD and controls in PBMC by FCM. Significant increase of Treg was detected in intestinal mucosa of UC (10.89 [8.33-11.52]) compared to controls (5.62 [3.27-7.40]), (p=0.006). An increase in Treg was detected by IHQ both in CD 42,7 (12,1-133,9) and UC 45,3 (42,0-68,1) compared to controls 11,0 (2,8-38,4) (p=0.000 and 0.001). Corticosteroids caused a decrease of Treg in PBMC (Pre: 4.74 [2.22-10.11]; Post: 3.62 [1.94-10.25]; p=0.04) as well as in intestinal mucosa (Pre: 12.83 [4.71-21.06]; Post: 6.19 [2.36-12.69]; p=0.03). This decrease is detected also by IHQ and is independent of steroid response. Treg levels in mucosa did not increase after an effective rescue treatment. Conclusion: A decrease of Treg and restitution after effective rescue therapy was not detected either in blood or in intestinal mucosa of CD. Corticosteroids causes decrease of Treg in PBMC and intestinal mucosa.

P082

Similar Phenotype and Functional Capacities of T Lymphocytes in Different Anatomic Locations of Human Intestine

Anna Carrasco, Fernando Fernandez-Bañares, Merce Rosinach, Carme Loras, Montserrat Aceituno, Jorge Espinos, Montserrat Forne, JosepMaria Viver, Maria Esteve
Hospital Universitari Mútua de Terrassa. Fundació per a la Recerca Mútua de Terrassa. CIBERehd, Terrassa, Spain

INTRODUCTION: There is scarce information concerning T lymphocyte subpopulations in the normal intestine and their phenotypic and functional differences related to anatomic location.

AIM: To asses if there are location related differences in T cell subpopulation and apoptosis in healthy intestine.

METHODS: Ileocolonoscopy for colon cancer prevention was performed to 7 healthy subjects (age 48.6±7) and 10 biopsies were collected from terminal ileum, right and left colon. Flow cytometric analysis was performed to asses cell phenotype (CD3, CD4, CD8), Treg cells (CD25FoxP3), intracellular cytokines (IFNγ, IL-17-A), apoptosis (AnnexinV). Results were compared by using paired Friedman test.

RESULTS: A significant decrease in the total cell count was observed in colon compared to ileum (p=0.03). Intracellular IFNgamma+CD4+ was significantly increased (p=0.04), whereas CD8 apoptosis was significantly decreased in ileum as compared to colon (p=0.03). An increase of Double Positive CD4+CD8+ T Cells (p=0.02), whereas Double negative CD4-CD8- T Cells were reduced in ileum compared to colon (p=0.06). A tendency to a major presence of Treg CD25+FOXP3+ cells in ileum compared to colon was found. These findings were confirmed by immunohistochemistry showing similar results.

CONCLUSION: Phenotype and function of T lymphocytes are similar in different areas of the colon. A decreased apoptosis in ileum compared to colon was found. In addition, an increase of Double Positive Cells and a decrease of Double Negative Cells in ileum compared to colon was detected. Since these subpopulations have important regulatory functions of immune response, these differences should be taken into account in studies of intestinal inflammation.

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Notes