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Poster Abstracts

001

***Cryptosporidium parvum* subverts the host innate immune response through manipulation of CRAMP expression during neonatal infection**

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Due to the immaturity of their immune system, neonates are highly sensitive to intestinal infections. During the neonatal period, antimicrobial peptide (AMP) expression differs substantially from that of adults as this is the case for the cathelicidin-related antimicrobial peptide CRAMP expressed preferentially in the neonatal period while conversely other AMPs such as Reg3 γ are expressed later in life.

Among enteric neonatal diseases, Cryptosporidiosis is a zoonotic disease and is highly prevalent in young infant less than 5 years old in underdeveloped country and in neonatal ruminants worldwide. *Cryptosporidium parvum* is the etiological agent of this diarrheal disease and infects exclusively epithelial cells. Innate immunity is important to control the acute phase of infection in neonates with dendritic cells and IFN γ playing a major role. Antimicrobial peptides are important contributors of innate immunity, but the role of CRAMP, which is elevated in the intestine of neonates has never been investigated during Cryptosporidiosis so far.

In this work, we used the neonatal murine model of cryptosporidiosis and unlike all the other antimicrobial peptides analyzed CRAMP expression in the intestinal epithelial cells was significantly reduced during infection. This reduced CRAMP expression is independent of IFN γ , a cytokine strongly produced during infection but also Myd88 and gut flora independent. When *C. parvum* infected neonatal mice orally received exogenous CRAMP to compensate the reduced expression of this AMP, the parasitic load of neonates was significantly decreased. In addition, when free parasites were in direct contact with CRAMP, this AMP affects the viability of sporozoites, the first free infectious form of this parasite. All together, these data suggest that *C. parvum* induces the reduction of CRAMP expression to escape the anti-parasitocidal effect of CRAMP. The molecular mechanism by which the parasite subverts epithelial-derived CRAMP production is currently under investigation.

002

***Faecalibacterium prausnitzii* strain HTF-F and its extracellular polymeric matrix attenuate clinical parameters in DSS-induced colitis**

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Introduction: A decrease in the abundance and biodiversity of intestinal bacteria within the Firmicutes phylum has been associated with inflammatory bowel disease (IBD). In particular, the anti-inflammatory bacterium *Faecalibacterium prausnitzii*, member of the Firmicutes phylum and one of the most abundant species in healthy human colon, is underrepresented in the microbiota of IBD patients.

Method: In this study we investigated the capacity of *F. prausnitzii* strain A2-165, the biofilm forming strain HTF-F and the extracellular polymeric matrix (EPM) isolated from strain HTF-F, to suppress inflammation in the mouse dextran sodium sulphate (DSS) colitis model.

Results: The two *F. prausnitzii* strains have anti-inflammatory effects in the DSS colitis model. *F. prausnitzii* HTF-F is more effective than A2-165 partly because of the immune-regulating properties of the EPM. The immunomodulatory effects of the EPM are mediated through the TLR2-dependent modulation of IL-12 and IL-10 cytokine production in antigen presenting cells. Both *F. prausnitzii* HTF-F and the EPM may have a therapeutic use in IBD.

003

***Roseburia hominis* regulates immunity through TLR-5 dependent responses**

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Commensal bacteria play a fundamental role in maintaining immune homeostasis a complex balance between immune stimulation and appropriate responsiveness and immune suppression and tolerance. Breakdown of these immune regulatory processes lead to chronic inflammation associated with intestinal dysbiosis. The immune modulating properties of *Roseburia hominis*, a flagellate gut anaerobe, were investigated in colonised conventional and colitic mouse models. Oral administration of *Roseburia hominis* to conventional mice with colitis caused by Dextran Sodium Sulphate altered intestinal immune responses, attenuated gut inflammation and reduced the severity of colitis. This protection was associated with a modulation and expansion of the T regulatory cell population triggered by *R. hominis*. The role of flagellin in directing these immune responses was investigated in vitro and in vivo.

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004

A specific tolerance inducing vector for therapeutic treatment of autoimmune diseases**C. Hansson, K. Schön & N. Lycke***Microbiology and Immunology, Gothenburg University, Gothenburg, Sweden*

Introduction: We have developed a novel platform for intranasal treatment of autoimmune diseases in which ADP-ribosylation determines whether immunity or tolerance is induced. Hence, cholera toxin A1-subunit based immunomodulation through the CTA1-peptide-DD fusion protein promotes enhancement, while inactive mutants induce suppression. Whereas the target population after intranasal administration of both constructs was CD103⁺CD11b^{low} DC's, both active and inactive mutants induced strong CD4 T cell priming, but they differentially affected CD4 T cell differentiation. This way inactive mutant constructs generated regulatory CD4 T-cells producing IL-10 and effectively preventing experimental autoimmune encephalitis (EAE) and collagen-induced arthritis (CIA), when carrying relevant peptides. Targeted DCs expressed low levels of CD80, CD86 and CD40, while, by contrast, ADP-ribosylating CTA1-peptide-DD constructs gave significantly enhanced co-stimulation. Suppression was global in that regulatory T-cells following treatment were able to suppress adoptively transferred naïve CD4 T cells and could subsequently maintain tolerance.

Conclusion: The use of specifically tolerance-inducing fusion proteins may be a way forward in the search of effective treatments against autoimmune diseases. The immunomodulating effect on the CD103⁺CD11b^{low} DCs will be described and the requirement for CTA1-binding to Gs alpha evaluated in detail, using Cre-lox Gs alpha-deficient mice.

005

A study on T follicular helper cells (TFH) in human NALT and effect of CpG-DNA on TFH-mediated antibody production**A. Aljurayyan¹, N. Upile², C. Vaughan², C. Xie², R. Sharma², H. Beer³, M.S. McCormick³, S. Gordon⁴ & Q. Zhang¹**

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Background: T Follicular helper cells (TFH) have been identified as a distinct CD4⁺ T cell subset and considered important for germinal centre function and critical for T cell-dependent B cell antibody production. Therefore it may be an effective strategy to enhance vaccine immunogenicity by promoting TFH numbers/function in humans. Adenotonsillar tissues are nasopharynx-associated lymphoid tissue (NALT) and important in response to intranasal vaccination.

Aims: We analysed TFH numbers and function in NALT from children and adults and studied whether and how CpG-DNA promotes TFH function leading to enhancement of mucosal immunity to influenza.

Methods: Mononuclear cells (MNC) were isolated from adenotonsillar tissue of children and adults, and TFH number (CD4⁺/CXCR5⁺/ICOS⁺) and function were analysed by flowcytometry and intracellular cytokine staining. Purified CXCR5⁺ TFH were co-cultured with B cells with/without influenza antigens and CpG-DNA. Haemagglutinin (HA)-specific antibody production was analysed by ELISA and Elispot assay.

Results: A prominent number of TFH were identified in NALT which were considerably higher than PBMC, and the mean NALT TFH number in children was significantly higher than in adults. NALT TFH were shown to express high levels of IL-21 and that were important for B cell antibody production. Co-culture of purified TFH but not non-TFH with B cells promoted antibody production.

Stimulation of adenotonsillar MNC by CpG-DNA significantly increased TFH number and that was correlated with HA-specific antibody production following influenza antigen stimulation. Co-incubation with purified plasmacytoid dendritic cells significantly enhanced the CpG-DNA-mediated antibody production.

Conclusion: A prominent number of TFH were shown in human NALT especially in children and that were highly active in B cell-help for antibody production. CpG-DNA promoted NALT TFH cells which correlated with the enhancement of HA-specific antibody production. To enhance vaccine immunogenicity by promoting TFH function may be an effective vaccination strategy.

006

A20/TNFAIP3 as a brake on intestinal inflammation and tumorigenesis**L. Vereecke¹, S.V. Silva², T. Billiet³, J.H. van Es⁴, C. McGuire¹, K. Slowicka¹, M. Sze¹, M. van den Born⁴, G.D. Hertogh⁵, H. Clevers⁴, J. Raes⁶, P. Rutgeerts³, S. Vermeire³, R. Beyaert¹ & G. van Loo¹**¹*Inflammation Research Centre, Technologiepark 927, Ghent, Belgium,*²*Department of Microbiology and Immunology, Rega In, Belgium,*³*Department of Clinical and Experimental Medicine, Leuven, Belgium,*⁴*Hubrecht Institute for Developmental Biology and S, Utrecht,**Netherlands,* ⁵*Department of Morphology and Molecular Pathology,**Leuven, Belgium,* ⁶*Department of Microbiology and Immunology, Rega In, Leuven, Belgium*

Intestinal immune homeostasis is established through complex interaction between commensal bacteria, the intestinal epithelium and mucosal immune cells. A defective interaction between these compartments can result in intestinal pathology. A crucial mediator in establishing this homeostasis is the transcription factor NF-kappaB, since it regulates multiple protective mechanisms in intestinal epithelial cells (IEC's), and pro-inflammatory responses in mucosal immune cells. NF-kappaB activation is tightly controlled by its negative feedback regulator A20, which in addition has pronounced anti-apoptotic functions. Genetic studies identified polymorphisms in the A20 locus associated with multiple inflammatory and auto-immune pathologies, including coeliac and Crohn's disease. To investigate the physiological role of A20 in intestinal immune homeostasis, we generated tissue specific A20 knockout mice. We found that A20 has predominant anti-apoptotic functions in IEC's and predominant anti-inflammatory functions in myeloid cells. By deleting A20 in both IEC's and myeloid cells, we generated a novel mouse model of intestinal inflammation which is characterized by early Paneth and goblet cell loss. Older mice suffer from ileitis and severe colitis which often progresses to colorectal cancer development. These findings suggest that defects in proper A20 function may contribute to the development and progression of inflammatory bowel disease and cancer in humans.

007

Ablation of macrophages by prolonged blockade of CSF1R signalling depletes M-cell differentiation in the intestinal epithelium**A. Sehgal¹, D. Donaldson², D. Hume² & N. Mabbott²**¹*University of Edinburgh, Easter Bush, Midlothian, Edinburgh, UK,*²*The Roslin Institute and R^DSVS, University of Edinburgh, Easter Bush, Midlothian, Edinburgh, UK*

The gut-associated lymphoid tissue (GALT), including Peyer's patches (PPs) and isolated lymphoid follicles lack afferent lymphatics

and directly sample mucosal antigens by specialized epithelial cells in the follicular associated epithelia (FAE), known as microfold (M) cells. M cell differentiation has been attributed to factors released by cells within the sub-epithelium dome of PP but very little is known about factors within crypts that may affect M cell differentiation. Here we provide evidence of macrophages in close contact with Paneth cells in intestinal crypts may play a role in M cell differentiation. In our study, mice were treated with an anti-CSF1R monoclonal antibody (M279) for 6 weeks. This led to a complete ablation of CD68⁺CD11c⁺Csf1r-EGFP⁻ macrophages in the gut. Our study demonstrates a loss of M cells and a reduction in antigen uptake in the FAE of PP of mice. We also found a loss of Paneth cells in intestinal crypts however we found no expression of CSF1R on Paneth cells indicating this may be due to loss of macrophages in close contact with intestinal crypts. These data suggest that macrophages may have a more intimate relationship with Paneth cells than previously understood and may control M cell differentiation within the FAE by providing a scaffold for intestinal crypt proliferation function.

008

Activation and differentiation of human lamina propria (myeloid) cells during the initiation of intestinal inflammation

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Mucosal lamina propria cells of IBD patients are highly activated in contrast to the hyporesponsive phenotype of these cells under homeostatic conditions. Given, that human studies are based on patient biopsies taken years after disease onset, the molecular events initially causing this inflammation are still largely unknown. In this study, we aimed to characterize early activation mechanisms of lamina propria cells including myeloid cells by utilizing an ex vivo human intestinal organ culture model which was recently described by us. In this model, healthy human colonic mucosa was depleted of epithelial cells by EDTA treatment which resulted in the activation of lamina propria immune cells and their emigration out of the tissue. Global gene expression analysis of lamina propria cells obtained by laser-capture microdissection prior and after loss of the epithelial layer revealed an induction of myeloid (activation) surface receptors such as CD80, CD83, CCR7, as well as inflammatory mediators like IL1B, IL-23p19 in lamina propria cells in situ. Upregulation of these markers/mediators was confirmed in emigrated HLA-DR+CD11c+CD14⁻ and HLA-DR+CD11c+CD14⁺ lamina propria myeloid cells when compared to peripheral blood myeloid cells by qRT-PCR and/ or flow cytometry. In addition, bioinformatic analysis of in situ gene expression profiles revealed the induction of signalling pathways not yet associated with the activation of lamina propria (myeloid) cells under inflammatory conditions, e.g. the response to unfolded protein. The upregulation of known target genes of this pathway such as GADD153, C/EBP α , and HSPA5 in the latter cell population was subsequently confirmed in situ by qRT-PCR and/ or immunofluorescence. These findings provide insight into molecular events underlying the change from a hyporesponsive to a highly activated phenotype in lamina propria (myeloid) cells at the very onset of inflammation.

009

Adjuvants modulate the immune response elicited by oral vaccination with adenovirus based vectors

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Introduction: Oral delivery of vaccines is effective in inducing mucosal immunity and thus represents a highly relevant route for improved vaccines. At present, however, most orally delivered vaccines are live attenuated vaccines, which present a risk of reversion to virulence. Vaccines derived from recombinant adenoviruses (rAd) that have been rendered replication incompetent provide a safer alternative. Such vaccines elicit potent humoral and cellular immune responses (IR) after parenteral delivery, but have proven less effective as oral vaccines, potentially in relation to such factors as insufficient stability of the vector in the gastro-intestinal environment, sub-optimal penetration of the intestinal barrier, insufficient presentation by antigen-presenting cells or a local immunological context biased towards immunological tolerance. It is possible that many of these obstacles could be overcome by appropriate use of adjuvants.

Objective: The objective of this work was to determine the effect of several classes of adjuvant on the amplitude and quality of the immune response after oral administration of rAd.

Method: To this end, a vector derived from a type 5 human adenovirus and encoding the reference antigen ovalbumin (ova) was administered to mice by the intra-gastric route in the presence of various adjuvants. The humoral IR against ova was evaluated in serum, feces and vaginal lavage fluids by ELISA. For two adjuvants, the cellular IR against ova was assessed in spleen, intestine (ileum) and mesenteric lymph nodes by intracellular cytokine staining and flow cytometry.

Results: The initial results indicate that the tested adjuvants can indeed modulate the humoral and cellular IR after oral administration of rAd, but each in a different manner. It should thus be possible to modify, both quantitatively and qualitatively, the IR elicited by orally delivered rAd by using adjuvants. Further work will address the impact of the different adjuvants at sequential steps involved in induction of the IR.

010

CCR2 expression defines a functionally distinct population of conventional DCs in intestinal mucosa

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The origin and functions of mononuclear phagocytes (MPs) in the intestine have been a source of confusion for some time, partly because overlapping markers such as CD11c, CD11b, CX3CR1, MHC class II and CCR2 have been used to distinguish dendritic cells (DCs) from macrophages (MFs). While CD103⁺ intestinal MPs appear to be genuine DCs, the nature of CD103⁻ MPs remains controversial. By combining phenotypic, gene profiling and kinetic analysis, we show that CD103⁻CD11b⁺ MPs in both the small and large intestine comprise distinct populations of DCs and MFs. CD64⁻CD103⁻CD11b⁺ MPs are classical DCs, being derived from Flt3L-dependent DC-committed precursors, migrating in afferent lymph, turning over rapidly in vivo and priming naive CD4⁺ T cells. In contrast, the CD64⁺ subset of CD103⁻CD11b⁺ MPs is sessile and avidly phagocytic, derives from Ly6Chi monocytes in a non-Flt3-dependent manner and turns over slowly in vivo. Surprisingly however, the monocyte/MF lineage marker CCR2 is expressed by a

significant proportion of CD103-CD11b⁺ DCs and there is a selective decrease in CD103-CD11b⁺ DCs in mice lacking this chemokine receptor. CCR2⁺CD103⁻ DCs in the LP express IRF4, their presence is partially dependent on this transcription factor in vivo and they have a selective ability to drive IL-17a production by T cells both in vivo and in vitro. Furthermore, an equivalent population of CCR2⁺CD103⁻ DCs is present in human intestine. Together, these data highlight the heterogeneity of intestinal MPs and reveal a bona fide population of CCR2⁺ conventional DCs, which is involved in priming mucosal Th17 responses.

011

CCR7 expression of intestinal myeloid cells and their migratory potential

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CD103⁺ DCs and CX3CR1⁺ lamina propria (LP) cells represent non-overlapping populations with distinct precursors in the small intestine. Our group suggested that CD103⁺ DCs but not CX3CR1hi cells migrate to the mesenteric lymph nodes (mLN) where they prime naïve T cells. We observed that CX3CR1int cells are present in intestinal lymph but not CX3CR1hi cells (Schulz O et al, 2009). In contrast, recently published data indicated that in microbiota-depleted mice CX3CR1hi cells become migratory and enter into lymphatics and mLN in a CCR7-dependent manner (Diehl GE et al, 2013). We therefore established a novel mouse model to monitor CCR7 expression and its regulation in vivo. In this mouse model insertion of GFP disrupts the function of the CCR7 gene. Upon stimulation, cells from CCR7-GFPki/mice up-regulate CCR7/GFP but are not able to migrate because of the disrupted CCR7. In contrast, cells from CCR7-GFPki/wt mice can up-regulate CCR7 and are capable of migrating to the draining LN. Mice were treated with antibiotic or left untreated, and gavaged with R848 14 hours prior to analysis. Whereas intestinal CD103⁺ and CD103⁻ DCs up-regulated CCR7/GFP after R848 stimulation both in antibiotic treated and untreated mice, CD64⁺ macrophages didn't express CCR7 even after microbiota depletion. CD11c+MHCII+CD64-CD103- LP cells expressed the chemokine receptor. Moreover, in CX3CR1GFP/+ mice, CX3CR1int but not CX3CR1hi cells were found in the mLN after R848 in the antibiotic treated and untreated mice. Thus, CD103⁺ CX3CR1- and CD103-CX3CR1int DCs express CCR7 upon stimulation. In contrast, intestinal macrophages characterized by a CD64⁺CX3CR1hi phenotype did not upregulate CCR7 irrespective of the microbiota status in these mice.

012

CD103+DC play a key role in the innate immune mechanisms protecting the epithelium against neonatal enteric infections

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Neonates are generally more susceptible than adults to infectious diseases. Their intestinal immune system is in development and subject to numerous changes after birth, facing the colonization by the commensal flora, alimentary antigens, and aggression by enteric pathogens. *Cryptosporidium parvum* is a highly prevalent zoonotic protozoan parasite that develops only in the gastrointestinal

epithelium and causes profuse diarrhea that can be life threatening in very young children and immunocompromised individuals. Neonatal mice are highly susceptible to *C. parvum* but the infection is self-limited, whereas adult mice are resistant unless immunocompromised. Accumulating evidences obtained with the neonatal mouse model demonstrate the key role of innate immunity to control the acute phase of the infection. Conditional depletion of CD11c⁺ cells demonstrated their essential role for the control of the infection both in neonates and adults (1). We therefore investigated the contribution of CD103⁺ DC to the age-dependent susceptibility to infection. We found that neonates presented a marked deficit in CD103⁺ DC in the intestinal lamina propria during the first weeks of life and artificially increasing the number of intestinal CD103⁺ DC by administering FLT3-L significantly reduced susceptibility to the infection. We next identified an unexpected mechanism of recruitment of the CD103⁺DC during the infection with several transgenic mouse models. Indeed, rapid recruitment of CD103⁺DC was depending on the production of CXCR3-binding chemokines produced by IEC in response to IFN. In addition to this key role in CD103⁺ DC recruitment, IFN is known to inhibit intracellular parasite development. We demonstrated that during neonatal infection CD103⁺ DC produce IL-12 and IFN in the lamina propria and the draining lymph nodes. Thus, CD103⁺DC are key players in the innate immune control of *C. parvum* infection in the intestinal epithelium. The relative paucity of CD103⁺ DC in the neonatal intestine contributes to the high susceptibility to intestinal infection. Our work makes a substantial increase in the understanding of the role of immune effectors involved in the control of the acute phase of *C. parvum* infection and paves the way to immune modulation strategies (2) targeting intestinal DC for strengthening neonatal immune system against enteric infections.

(1) Lantier et al. PLoS Pathog. 2013

(2) Lantier et al. J Infect Dis. 2014 KW Dendritic Neonate Innate Infection

013

CD11b is required for oral tolerance by regulating Treg frequencies in the small intestine

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Intestinal tolerance requires the activation of FoxP3⁺ regulatory T cells (Tregs) in the mesenteric lymph nodes by CD103⁺ dendritic cells and their subsequent homing to the intestine. Signalling by resident CD11b+F4/80⁺CX3CR1high macrophages present in the intestinal lamina propria leads to further Treg expansion. We have previously shown that the high expression of CX3CR1 by resident macrophages is necessary for their expression of IL-10. In CX3CR1-deficient mice, reduced IL-10 expression leads to a loss in Treg expansion in the small intestinal lamina propria and hence abrogates oral tolerance.

Here, we show that signalling via CD11b, in addition to CX3CR1, is also involved in regulating oral tolerance. Using delayed type hypersensitivity measurements, we observed that CD11b-deficient mice failed to establish oral tolerance. To investigate Treg induction in the mLN and Treg frequencies in the small intestinal lamina propria in vivo, we performed adoptive transfer experiments in CD11b^{-/-} mice. Like in CX3CR1-deficient mice, Treg cells were readily induced in the mesenteric lymph nodes at frequencies comparable to wild type mice. Moreover, expression levels of the gut homing molecules a4b7 and CCR9 were unimpaired. On the contrary, Treg frequencies in the lamina propria were reduced in CD11b^{-/-} mice, possibly explaining the oral tolerance defect.

In order to gain a comprehensive picture of the pathways that might be changed in resident macrophages due to the deficiency of CD11b and CX3CR1 we carried out complex transcriptome and cytokine analysis. Based on these studies, we intend to define a profile of intestinal macrophages that is important for their tolerogenic function. These results in turn will open insights into pathways that can be targeted for regulating Treg frequencies and oral tolerance establishment.

014

Changes in the pattern of expression of Fatty Acid Binding Proteins in small intestine in untreated Coeliac Disease

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Coeliac disease (CD) is an immune-mediated enteropathy that develops in genetically susceptible individuals following exposure to dietary gluten. Fully differentiated epithelial cells express two isoforms of fatty acid binding proteins (FABPs): intestinal and liver, I- and LFABP, respectively. I- and LFABPs belong to a family of small cytosolic proteins which bind and transport long chain fatty acids, but also have other important biological roles in signalling pathways, particularly those related to PPARs which link lipid metabolism and inflammatory process.

The aim of this work was to analyse the pattern of expression of I- and LFABP in small intestine in normal tissues and in those from untreated CD patients as well as the evaluation of IFABP serum levels in CD patients and non-CD controls. Expression of I- and LFABP in duodenal tissues was assessed by confocal fluorescence microscopy using specific polyclonal antibodies and by quantitative PCR. Immunofluorescence analysis showed a differential pattern of expression for both FABPs when normal tissue and severe enteropathy were compared. I- and LFABP were expressed in the epithelium in healthy mucosa and in the remaining epithelium in partial or total villus atrophy. Slight labelling for IFABP was also observed in the crypts in healthy mucosa. Remarkably, FABPs expression was clearly increased in the crypts of intestinal mucosa in untreated CD. Quantitative PCR analysis showed that mRNA levels for LFABP were higher than those for IFABP in normal tissue. Employing *hck* as housekeeping gene, I- and LFABP mRNA levels were lower in duodenal samples from adult untreated CD patients than in healthy controls ($P < 0.01$). In paediatric population this difference was not observed. On the other hand, when villin was used as reference, the levels of both FABPs were increased in enteropathy. Using a commercial quantitative ELISA, we observed that IFABP serum levels were higher in adult untreated CD patients compared with healthy controls ($P < 0.0001$), patients on Gluten free diet ($P = 0.023$) and inflammatory bowel disease patients ($P < 0.001$).

In conclusion, IFABP, which is likely released from the damaged enterocyte, can be used as a biomarker providing additional information in diagnosis and follow up of CD. The marked increase in I- and LFABP expression in the crypts may reflect an accelerated rate of enterocyte differentiation as compensatory mechanism due to the increase in epithelial loss.

015

Characterisation of antigen-specific CD4⁺ T cells by using MHC class II tetramers

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In the context of vaccination, CD4⁺ T-cell primary activation can be considered as an early biomarker of vaccine immunogenicity since it is required for both the induction of high-affinity antibodies and immune memory. To this aim, we have here characterized the Ag-specific CD4⁺ T cell response following immunization with a Mycobacterium tuberculosis vaccine antigen plus a liposome-based adjuvant administered by two different routes (parenteral versus mucosal). Ag85b-specific MHC class II tetramers were employed for detecting Ag-specific CD4⁺ T cells. C57/BL6 mice were primed by the subcutaneous or nasal route, and the clonal expansion of Ag-specific CD4⁺ T-cells was analyzed into respective draining lymph nodes (LNs) and spleens by using Ag85b280-294 MHC class II tetramers. Cells were also characterized for the expression of surface markers and the production of cytokines. Prime-boost experiments, that combined the nasal and parenteral routes, were also performed and the cellular, as well as the humoral response were analysed. Tetramer-positive CD4⁺ T cells were detected into respective draining LNs with a peak 7-9 days after priming (about 0.12 and 0.3% of total CD4⁺ T cells, following nasal and parenteral priming respectively), and declined at day 14. Priming by the SC route induced a stronger CD4⁺ T cell clonal expansion respect to the nasal one, and a higher dissemination towards spleens and lungs. Upon parenteral immunization about 20% of Ag85b-specific primed CD4⁺ T cells were T follicular helper (Tfh) cells (CXCR5⁺PD1⁺), and about a 30% Th1 (CXCR3⁺). CD4⁺ T cells primed by the parenteral route were efficiently boosted by nasal as well as parenteral immunization. Ag85b280-294-MHC class II complexes can be efficiently used for studying the immunogenicity of a tuberculosis vaccine in the mouse model, and characterizing the cellular response after both priming and boosting. Here, we showed that a single parenteral immunization with a subunit tuberculosis vaccine induced the expansion of Ag-specific CD4⁺ T cells that differentiated mainly into Tfh and Th1, and that this vaccine formulation primes the immune system better by the parenteral than the nasal route.

016

Characterization of gastric tissue-resident memory CD8⁺ T cells from children, adults and the elderly

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The main orchestrators of protective immunity in the stomach are T cells. However, limited information is available on the presence and function of the gastric T subsets mostly due to the difficulty in isolating high numbers of viable cells from human gastric biopsies. To overcome this shortcoming, we optimized a cell isolation method that yielded high numbers of viable lamina propria mononuclear cells (LPMC) from gastric biopsies obtained during upper GI endoscopy. Classic memory T (TM) subsets were identified in gastric LPMC obtained from children, adults and the elderly using flow cytometry. A dominant effector memory (TEM) phenotype was observed in gastric LPMC CD8⁺ T cells in all age groups. We then evaluated whether these cells represented a population of gastric tissue-resident memory T (TRM) cells by assessing expression of CD103 and CD69. The vast majority of gastric LPMC CD8⁺ T cells

either co-expressed CD103/CD69 (>70%) or expressed CD103 alone (~20%). Thus, gastric LPMC CD8⁺ T cells had the characteristics of TRM cells. In addition, gastric CD8⁺ TRM cells produced multiple cytokines (IFN- γ , IL-2, TNF- α , IL-17A, MIP-1 β) and up-regulated CD107a upon stimulation with mitogens. Furthermore, gastric CD8⁺ TRM demonstrated differences in the frequency, susceptibility to activation and cytokine/multi-cytokine production profiles among the age groups. Most notably, children's gastric CD8⁺ TRM cells responded differently to stimuli than gastric CD8⁺ TRM cells from adults or the elderly. In conclusion, we demonstrate the presence of gastric CD8⁺ TRM which exhibit diverse functional characteristics in children, adults and the elderly.

017

Constitutive Type I Interferon, via STAT1 activation, selectively promotes regulatory T cell function in the healthy human intestine, but not in IBD

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Background: Control of T-cell reactivity with the human intestinal mucosa is poorly understood. Type I Interferon (T1IFN) signals via JAK/STAT, particularly STAT1, and supports Treg function in mouse models of colitis. T1IFN has been used as a treatment in IBD. We therefore hypothesised that constitutive T1IFN had a regulatory role in human intestinal T cells.

Methods: Endoscopic biopsies or resection specimens were frozen for immunohistochemistry (IHC) or cultured in the presence of neutralising anti-IFN β or isotope control. Cells were harvested, stimulated with anti-CD3/CD28 antibodies and analysed for cytokine production by intracellular staining and by multiplex ELISA of culture supernatants. Phosphorylated STAT1 was measured by flow cytometry with or without prior T1IFN stimulation. Colonic sections were stained with anti-IFN β and analysed using fluorescent IHC. CD3⁺ T-cells were FACS sorted and expression of Interferon Stimulated Genes (ISGs) and SOCS 1 and 3 determined by q-RT-PCR.

Results: IFN β was detected in the lamina propria of control and IBD tissue and ISGs (MXA and 250AS) were expressed by intestinal T cells. In vitro, IFN β neutralisation reduced the frequency of pSTAT1⁺ intestinal T cells ($n = 6$, $P = 0.05$) and, in healthy controls, decreased the proportion of IL10-producing intestinal T cells ($n = 8$, $P = 0.01$). There was a trend for more IFN γ -producers and IFN γ levels in supernatants were significantly increased ($n = 10$, $P = 0.016$). In IBD, intestinal T cells were more responsive to IFN β , as assessed by ISG induction, ($n = 10$, $P < 0.03$) and pSTAT1 was increased in T-cells isolated from IBD patients ($n = 30$ IBD, 16 control, $P = 0.03$). Concordantly, SOCS1 expression was decreased in IBD samples compared to controls ($n = 8$ IBD, 6 control), ($P = 0.047$). In contrast to controls, neutralisation of IFN β in IBD samples led to a generalised increase in cytokine production, with an increase in T cells producing all cytokines examined (IL-10, IFN γ , IL-17 and TNF α , $n = 10$).

Discussion: T1IFN is present in the human intestinal mucosa and in health may have a regulatory role via T cell production of IL-10. There is increased responsiveness of the T1IFN pathway in T cells from IBD patients, associated with a generalised suppression of cytokine production. Thus, T1IFN effects are context dependant, which may explain differing clinical effects of therapeutic T1IFN and potentially responses to other environmental factors.

018

CTA1-3M2e-DD; a broadly protective vaccine candidate against influenza virus

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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 M2e epitope conferred a better level of protection than specific serum antibody levels alone. Next we analyzed the TCR-repertoire that recognized M2e and investigated the type of T cell response induced subsequent to immunization. The CD4 T cell response was oligoclonal and the recall response to M2e in immunized Balb/C mice was dominated by IL-17. In addition, vaccinated IL-17 deficient mice showed reduced survival following live challenge, indicating involvement of Th17 M2e-specific CD4 T cells in protection against influenza. The M2e specific T cells were present in low frequencies in draining lymph nodes, spleen and lung for more than 12 month after i.n. immunizations and upon re-encounter with antigen they showed exceptional ability to expand and protect mice from a lethal influenza dose. Moreover, a significant increase in anti-influenza hemagglutinin (HA) IgG antibody levels was found in serum subsequent to live challenge of nude mice that have been reconstituted with memory influenza-specific CD4⁺ T cells, suggesting that those M2e memory T cells could provide B cell help to unrelated naïve B cells.

The CTA1-3M2e-DD fusion protein is, thus, a highly promising mucosal vaccine candidate that promotes both antibody and CD4 T cell M2e-specific immunity, both contributing to protection against live influenza virus infection. Our study highlights the importance of CD4 T cells for protection against influenza infections.

019

Cyclosporine A treatment of T cells induces apoptosis and decreases the production of IL-13 in ulcerative colitis

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Introduction: Inflammatory bowel disease (IBD) is a chronic inflammation of the digestive tract caused by a dysregulated immune response. The two major forms of IBD are ulcerative colitis (UC) and Crohn's disease (CD). The immunosuppressive drug cyclosporine A (CsA) is a potential rescue treatment to avoid colectomy in severe steroid-refractory UC patients, whereas CsA treatment has no beneficial effect in CD patients. The molecular mechanism of action of CsA in UC is nevertheless incompletely understood. The aim of this study was to investigate the effect of CsA on a possible modulation of cytokine production and apoptosis induction by peripheral blood mononuclear cells (PBMCs) of controls and patients with UC or CD.

Methods: Human PBMCs were isolated from whole blood samples and cultured with anti-CD3/CD28 antibodies in the presence or absence of CsA. Supernatants were taken for analysis of cytokine production (TNF α , IFN γ , TGF β IL-4, IL-5, IL-10, IL-13, IL-17A,

IL-17F) by ELISA assays. To determine apoptosis induction FACS analysis for AnnexinV staining and fluorescent stainings for CD4 and TUNEL were performed. Additionally, expression of caspase-8, Bcl-xL, Bcl-2 and Bax were analysed by qPCR.

Results: It was found that levels of IFN γ remained unaffected after the addition of CsA. In UC patients, levels of IL-13 were significantly reduced after CsA treatment in vitro, whereas in PBMCs of controls and CD patients IL-13 production was unaffected by CsA treatment. Levels of TNF α , IL-10 and IL-17A were significantly reduced after CsA treatment in PBMCs of all patients groups. TGF β and IL-4 production was significantly decreased with addition of CsA in controls, but was unaltered in UC and CD patients. Controls showed a significant reduction of IL-5 and IL-17F production after CsA treatment. Levels of IL-5 were also significantly decreased in UC patients in the presence of CsA, whereas levels of IL-17A were significantly reduced in CD patients. Additionally, cultured CD4 $^{+}$ PBMCs of controls and UC patients underwent apoptosis which was induced independent of the mitochondrial apoptosis pathway, but dependent on activated caspase-8 signalling.

Conclusion: IBD patients, suffering from Th2 associated UC, can be treated successfully with CsA. This study demonstrates for the first time that CsA selectively induces apoptosis in CD4 $^{+}$ blood cells of UC patients in a mitochondrial independent way associated with a diminished production of the Th2-like cytokine IL-13.

020

Deletion of IL-4R α on Macrophages renders mice resistant to intestinal inflammation

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Background and Aims: The inflammatory bowel diseases (Crohn's disease (CD) and ulcerative colitis (UC)) are illness characterized by a chronic clinical course of relapse and remission associated with self-destructive inflammation of the gastrointestinal tract. In both UC and CD, leukocyte infiltration into the intestine is fundamental event in disease development and progression. Th2 cytokines IL-4 and IL-13 are known susceptibility factors for IBD and induce their biological functions through a common receptor, the IL-4 receptor alpha chain (IL-4R α). We investigated whether activation of interleukin IL-4R α systemic and on macrophages determines their effector functions and mediates intestinal inflammation in experimental mice model.

Methods and Results: We studied the role of IL-4R α by using the systemic IL-4R α KO and macrophage-specific IL-4R α KO (IL-4R α LysM) mice in a mouse model of dextran sodium sulphate induced colitis. Mice were challenged with 2.5% DSS for 14 days to induce colitis. DSS-treated systemic and macrophage specific IL-4R α KO mice were resistant and showed protection to DSS-induced intestinal inflammation compared to WT mice. IL-4R α KO and macrophage-specific IL-4R α KO (IL-4R α LysM) mice have no/less inflammation, indicating no/less weight lost and no epithelial erosions compared to WT mice. Resistance to DSS-induced inflammation in IL-4R α KO/IL-4R α LysM mice correlated with reduced numbers of inflammatory cells like F4/80 and Neutrophils in the colonic mucosa and showing well-structured epithelial cell layer without any damage.

Conclusions: IL-4R α plays an important role in inflammatory bowel disease. Deficiency of IL-4R α systemic and macrophage-specific IL-4R α LysM mice reduces intestinal inflammation in a mouse model of DSS induced colitis. Decreased inflammation is accompanied by lack of proinflammatory immune response.

022

Depletion of regulatory T cells in the APCmin/+ mouse model of colon cancer enhances T cell recruitment and Th1 associated responses

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Regulatory T cells (Treg) are important to prevent autoimmunity, microbial driven inflammation and allergy. In colorectal cancer, Treg infiltration is associated with a better patient outcome, in contrast to most other tumors. We have previously demonstrated that Treg from cancer patients profoundly reduce transendothelial migration of conventional T cells. To evaluate if Treg also affect lymphocyte infiltration into intestinal tumors, we have used APCmin/+ mice that spontaneously develop intestinal tumors due to a mutation in the APC tumor suppressor gene. The frequencies of FoxP3 $^{+}$ putative Treg, CD4 $^{+}$, and CD8 $^{+}$ T cells were determined by flow cytometry and immunofluorescence and these analyses revealed an accumulation of Treg and a decrease of conventional T cells in the tumors. To evaluate if Treg actively inhibit lymphocyte migration into tumors, APCmin/+ mice were crossed with DEREK mice, in which Treg can be selectively depleted with diphtheria toxin. Short term Treg depletion in tumor-bearing mice resulted in increased frequencies of conventional T cells in the tumors, and an increased frequency of proliferating (Ki67 $^{+}$) T cells, suggesting that the increased T cell frequencies may at least partly result from increased local proliferation in the tumors. Treg depletion also resulted in a strong up-regulation of mRNA for the Th1 associated chemokines CXCL9 ($P < 0.01$) and CXCL10 ($P < 0.001$) specifically in the tumors. In parallel, expression of the corresponding chemokine receptor CXCR3 was increased on conventional T cells migrating into the tumors after Treg depletion. Depletion of Treg also resulted in increased T cell production of IL-17 and IFN-gamma in the tumors. In conclusion, Treg depletion results in increased accumulation of conventional T cells, especially Th1 associated CXCR3 $^{+}$ T cells, in intestinal tumors and targeting of Treg as an anti-tumor immunotherapy may thus improve not only effector functions of activated T cells, but also their recruitment to tumors.

023

Dissecting the role of intestinal epithelial barrier in the pathogenesis of ulcerative colitis

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Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) of unknown aetiology. Alterations in the colonic epithelial barrier contribute to the onset of UC. However, little data is available about the primary role of epithelial cell (EC) dysfunction in this disease. Recently, a novel cell-culture system has been established to study gastrointestinal EC pathophysiology.

Using this approach we aim to investigate whether a primary defect in intestinal EC function is present in UC patients that could drive the development of an inflammatory response.

To this end, we collected biopsies from the sigmoid colons of 6 UC patients and 4 non-IBD controls. Isolated crypts were cultured and stem EC were expanded in Matrigel as "organoids". Total RNA from stem and differentiated organoids was extracted for transcriptional analysis.

Our results show that control and UC organoids follow common differentiation programs with comparable downregulation of stem and proliferation markers (i.e., Lgr5, Ki67) and upregulation of EC differentiation markers (i.e., MUC2, ANPEP, ChrA). However, statistical analysis of microarrays revealed more than 800 genes differentially expressed between control and UC organoids, with cellular movement and development, lipid metabolism, and molecule transport the most significantly altered cellular functions.

In the present study the organoid culture model has been successfully used to detect intrinsic differences in the epithelium of UC patients. Validation experiments are ongoing to confirm our results in an independent cohort of patients and to dissect the functional meaning of such alterations.

024

Early maternal separation induced commensal *e. coli* overgrowth triggering visceral sensitivity and specific humoral response in adult mice

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Small Intestinal Bacteria Overgrowth (SIBO) in favor of Enterobacteriaceae is highly associated to Irritable Bowel Syndrome (IBS) a functional gastrointestinal pathology where a specific humoral response toward bacterial antigens has been observed. Symptoms of IBS and SIBO are similar consisting in abdominal pain, bloating and altered stool forms. Further, IBS symptoms can be generated and/or exacerbated by early life stressful events. In this study, using the well described rodent model of IBS: maternal separation, we developed a mouse model of SIBO and studied its consequences on IBS symptom and on humoral response toward microbiota.

MS increased visceral sensitivity and induced ileal and fecal overgrowth of *E. coli* in adult female mice. This overgrowth was associated with a decrease of fecal lysozyme antimicrobial activity and increase of anti-*E. coli* IgG and IgA. In order to decipher whether or not those alterations were a consequence of *E. coli* overgrowth, adult mice were force fed daily with 109 commensal *E. coli* for 15 days. *E. coli* gavage reproduced ileal and fecal overgrowth as well as visceral hypersensitivity and increased anti-*E. coli* IgG and IgA. However, *E. coli* gavage didn't decrease lysozyme antimicrobial activity in feces suggesting that lysozyme defect is a consequence of MS that can participate to the *E. coli* overgrowth.

This study shows for the first time that (i) maternal separation in mice weakened intestinal antimicrobial defense that might result to intestinal *E. coli* overgrowth and (ii) intestinal *E. coli* overgrowth was responsible for visceral hypersensitivity and systemic humoral response toward microbiota.

025

Early maternal separation leads to abnormal immune response against commensal microbita in adult mice

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Neonatal period is characterized by immature intestinal epithelium and immune system. Occurrence of adverse events during this period induces long lasting alterations of intestinal homeostasis associated with susceptibility to develop gastrointestinal disorders at

adulthood. To characterize the adverse effect of early stress on immune system development, we analyzed the consequences of maternal separation (MS) in mice on intestinal barrier functions and systemic immune response toward microbiota.

In 50-days old male mice, MS increased intestinal permeability to FITC-Dextran-4 kDa and visceral sensitivity. MS decreased fecal lysozyme activity, IgA concentration and ILC3 population. This breach in mucosal barrier function was associated with a systemic IgG response against *E. coli*. Furthermore, MS increased anti CD3/CD28-induced IFN γ and IL10 secretion in splenocytes. These results are in accordance with an increase of CD4⁺CD44^{high}CD62L^{low} active T cells and decreased CD4⁺CD25⁺Foxp3⁺ regulatory T cells. Furthermore, MS decreased CD40 expression in CD11c⁺ and CD11b⁺ leading to decrease of IL10 and TGF β secretion without any modification of IFN γ on *E. coli*-stimulated splenocytes suggesting a defect of antigenic presentation. No modification (quantitative nor qualitative) of fecal *E. coli* population was observed at 50 days in MS mice.

For the first time, we demonstrated that early stressful events impaired intestinal barrier functions leading to an exacerbated humoral response toward microbiota. Inflammatory T cell response was increased in MS mice but dampened by an antigenic presentation defect. Adult mice experiencing early stressful events mimic common symptoms of gastrointestinal disorders and might be an important factor in those pathologies.

026

Early steps to explore mucosal immune components in a marine vertebrate

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Maturation of the mucosal immune system is influenced by the environment, behavior and feeding, and, in carnivores and primates, the first 12 months of life are critical for its proper development. Regrettably, despite being the frontline for defense, little is known about mucosal immunity of free-living mammals, and even less is known about its ontogeny and ecological constraints. In this context, we have used the California sea lion, a long-lived, sexually-dimorphic, amphibious top predator with well-characterized life stages, in an attempt to understand the early development of mucosal immunity and to investigate the contribution of ontogenic and energetic traits on expression of mucosal immune components. At Granito Island (Gulf of California), we collected rectal, genital, nasal and oral swabs from 2-, 5- and 12-month old pups. Body condition and various health parameters were determined for all. SDS-PAGE gels were run on extracted proteins to characterize the profile of each mucosa. Distinct band patterns were evident between mucosal types and between age classes. Genital mucosa proteins differed between sexes ($F = 46.32$, $gdl = 41$, $P < 0.001$), specially at 5-months of age. For all stages of development, the anal mucosa showed the great diversity of proteins (~100) and widest molecular range. Some bands were only found at 2- and 12-month old pups (84 and 26 kDa, respectively). Protein sequencing and immunodetection techniques will now allow us to characterize the proteins, identify those with immune function, and explore their ontogenic and energetic constraints.

027

Effect of "acid protect" supplementation on the volatile faecal metabolome of thoroughbred racehorses**N. Larkins¹, C.J. Proudman², J. Travers³, C.J. Probert³ & K. Koare¹**¹Nutritional Laboratories, Monmouth, UK, ²School of Veterinary Medicine, University of Surrey, Guildford, Surrey, UK, ³Department of Gastroenterology

The importance of the intestinal microbiome to mammalian health is now widely recognised. Intestinal bacterial communities not only contribute to digestion of food material but are important partners in host-microbial co-metabolism. Studies in laboratory animals and in humans have demonstrated profound effects of altered gut microbial communities on many facets of mammalian health including immunity, behaviour, heart disease and obesity. As a hindgut fermenter the horse is likely to be even more reliant upon its intestinal microbiome than many other species. Faeces are easily sampled from horses and offer a diagnostic window into the structure and function of gut microbial communities. The volatile faecal metabolome, not previously investigated in the horse, may offer opportunities for monitoring equine intestinal health.

The aim of this study was to identify changes in faecal volatile metabolome in Thoroughbred racehorses after supplementation with Acid Protect

The study population of 8 Tb racehorse was selected using the following criteria:

- * Male and castrated
- * Over 2 years of age
- * In race training
- * Healthy at the beginning of sampling
- * No medication administered in previous 3 months

Fresh faecal samples were collected prior to exercise and frozen at -80C prior to analysis. After the first two faecal samples were collected horses received dietary supplementation with 75 g (tid) for 14 days.

Samples were thawed, warmed in a water bath to 38 C for 20 minutes, and volatile organic compounds (VOCs) adsorbed onto a solid phase adsorption fibre for 30 minutes. VOC chromatograms for each faecal sample were obtained by thermal desorption from the fibre followed by separation by gas chromatography and identification by mass spectrometry (GC-MS). Chromatograms were interpreted by both manual and automated readings to identify and quantify VOCs present.

Conclusions:

- * We have demonstrated the potential utility of faecal VOC analysis by GC-MS for characterisation of the intestinal metabolic environment.
- * We demonstrate repeatable VOC profiles from similarly managed horses.
- * Our analysis can detect small shifts in VOC profile possibly associated with dietary supplementation.
- * Increase in butanoic acid in post-supplementation samples is of particular interest as this compound is associated with enterocyte health.

028

Effect of six strains of probiotics on the integrity of a model of intestinal epithelial monolayer**B. Martínez-Abad¹, E. Montalvillo¹, C. Escudero-Hernández¹, J.A. Garrote² & E. Arranz¹**¹Pediatría & Inmunología, C/Ramón y Cajal 7, Valladolid, Spain, ²Genetics Lab. Hospital U. Rio-Hortega, Dulzaina 2, Valladolid, Spain

Enterocytes represent 80% of cells constituting the intestinal epithelium. These cells act like a barrier and contribute to the local immune responses by secreting several soluble factors. Under inflammatory conditions, the number of non-polarized enterocyte stem cells increases due to the stimulation of the cell renewal process.

In this context, we proposed to analyse the effects of certain probiotic bacteria using an intestinal epithelial culture model, Caco-2 cells. These cells were culture for 7 days (non-polarized and undifferentiated cells) and for 21 days (polarized and differentiated cells) and stimulated with probiotics at different concentrations. We analysed the changes on chemokine expression, trans-epithelial electrical resistance (TEER), distribution of zonula occludens (ZO)-1 by immunofluorescence, and the bacterial capacity of adherence. We found that non-polarized Caco-2 cells were more sensitive to bacterial stimuli than fully polarized Caco-2 cells in chemokine expression. Moreover, the probiotic with the higher effect on chemokine expression on non-polarized Caco-2 cells triggered an increase in the monolayer's TEER, in agreement with the fact that a slight increase in pro-inflammatory response could reinforce the epithelial monolayer. We cannot confirm a relationship between the distribution of ZO-1 and TEER's increase, however we observed some membrane changes in bacteria-stimulated Caco-2 cells. At the highest concentration, some probiotics induced damage to the epithelial monolayer.

In summary, there are probiotics with no effect on the expression of pro-inflammatory chemokines and these could be useful in the maintenance of intestinal homeostasis, whereas others may have a role to prevent damage to the epithelial monolayer.

029

Effects of selective PI3Kδ inhibitors on activation and downstream signalling in T cells**K. Björhall, S.R. Blomqvist, L. Yrlid, M. Gustavsson, M. Uddin, N. Holden & K. Karabelas***AstraZeneca RIA iMED, Pepparedsleden 1, Mölndal, Sweden*

The Class I phosphoinositide-3-kinase delta (PI3Kδ) isoform regulates essential immune functions in a wide variety of cells, including leukocytes. Its importance in inflammation signalling, particularly in B and T cells, has stimulated the clinical development of selective PI3Kδ inhibitors. As Th2-driven allergic airway inflammation plays an important role in the pathogenesis of asthma, our aims was to evaluate the effects of a selective PI3Kδ inhibitor on T cell activation in an ovalbumin (OVA)/ anti-CD3-induced airway inflammation model in transgenic OT.2 mice *in vivo*.

We have developed highly selective PI3Kδ inhibitors that block the release of IL-5 in human PBMCs and IL-17 by human Th17 cells, in a concentration-dependent manner *in vitro*. To elucidate the pharmacodynamic effects of our PI3Kδ inhibitors on effector/memory T helper cells in the lung, we used OVA specific, MHC class II restricted αβ T-cell receptor (TCR) OT.2 transgenic mice. Upon local OVA provocation challenge, T-cells were activated and recruited into the lungs. Five days later, the OT.2 mice were given an intranasal (i.n.) anti-CD3 challenge and the downstream effects on PI3K signalling were measured through phosphorylation of S6 ribosomal protein (pS6RPSer235/236), cytokine release and T cell activation markers (CD62L, CD69 and CD25) in the absence and presence of a selective PI3Kδ inhibitor.

We found that pS6RPSer235/236 were significantly downregulated in CD4⁺T cells by our PI3Kδ inhibitors 6 h after anti-CD3 challenge ($P < 0.002$). However, no significant effects on activation markers (CD62L, CD69 and CD25) on CD4⁺ T cells or on cytokine levels in bronchoalveolar lavage fluid were observed at the same time-point. The mechanism underlying this dissociation is unclear.

Our results show that S6RP is phosphorylated at Ser235/236 in CD4⁺ T cells after anti-CD3 challenge and that this phosphorylation is inhibited by selective PI3K δ inhibitors. We therefore conclude that pS6RPSer235/236 can be used as a target engagement marker reflecting PI3K δ activity in this in vivo model.

030

Epithelial IL-1R2 acts as a homeostatic regulator of inflammatory signals during remission of ulcerative colitis

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Ulcerative colitis (UC) is a chronic colonic disease that presents periods of active inflammation followed by periods of remission. We focused on identifying regulatory mechanisms that favor intestinal homeostasis during disease remission. A total of 180 UC patients (UC active or remission) and 55 healthy non-IBD controls participated in the study. Using real-time PCR and ELISA of culture supernatants from colon biopsies, we found that the interleukin-1 (IL-1) decoy receptor gene (IL1R2) and secreted protein (IL-1sR2) were significantly ($P < 0.05$) up-regulated during UC remission compared to UC active and control mucosa. In contrast, the IL-1 receptor antagonist (IL-1Ra), together with IL-1 β , IL-1 receptor type 1 (IL-1R1) and IL-1 receptor accessory protein (IL-1RAcP), were overexpressed in UC active mucosa. Immunostaining identified both lamina propria IgA⁺ cells and adjacent mucosal epithelium as IL-1R2⁺. Nevertheless, we found by intracellular flow cytometry staining of digested biopsies that epithelial cells were the cellular source responsible for the upregulation of IL-1R2 during UC remission. Culturing whole colonic crypts or epithelial stem cells, we demonstrated that IL-1R2 is negatively regulated by wnt/beta-catenin signaling, and is therefore up-regulated during epithelial cell differentiation. Importantly, IL-1R2 produced by isolated colonic crypts of UC patients in remission, but not controls, reduced IL-8 induced by exogenous IL-1 β . Furthermore, expression of the IL1R2 in the remitting mucosa could be a predictive molecule of UC relapse during a 1-year follow-up. In summary, we hypothesize that IL-1R2 represents a homeostatic molecule, whose increased expression during remission may represent an endogenous mechanism that could dampen local inflammation and prevent disease relapse.

031

Essential role of Dectin-1 in intestinal M cell-mediated reverse transcytosis of SIgA-antigen complexes

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Intestinal microfold (M) cells possess a high transcytosis capacity and are able to transport a broad range of materials including particulate antigens, soluble macromolecules and pathogens from the intestinal lumen to inductive sites of the mucosal immune system. M cells are also the primary pathway for delivery of secretory IgA (SIgA) to the gut-associated lymphoid tissue. However, although the consequences

of SIgA uptake by M cells are now well known and described, the mechanisms whereby SIgA is selectively bound and taken up remain poorly understood. Here we first demonstrate that both the C α 1 region and glycosylation, more particularly sialic acid residues, are involved in M cell-mediated reverse transcytosis. Second, we found that SIgA is taken up by M cells via the Dectin-1 receptor, with the possible involvement of Siglec-5 acting as a co-receptor. Third, we establish that transcytosed SIgA is taken up by mucosal CX3CR1⁺ dendritic cells (DCs) via the DC-SIGN receptor. Fourth, we show that mucosal and systemic antibody responses against the HIV p24-SIgA complexes administered orally is strictly dependent on the expression of Dectin-1. Having deciphered the mechanisms leading to specific targeting of SIgA-based Ag complexes paves the way to the use of such a vehicle for mucosal vaccination against various infectious diseases.

032

Evaluation of immunogenicity and protective efficacy of Type III Secretion System proteins of enteropathogenic bacteria

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Diarrheal diseases caused by bacterial pathogens e.g. Escherichia coli, Salmonella enterica, Shigella flexneri, etc. remain a major public health problem in developing countries, that contribute to the high mortality rate, especially for children younger than 5 years [1]. These bacteria have evolved an of molecules that are mostly secreted by dedicated Type III Secretion System (T3SS). Part of the T3SS forms an extracellular needle and syringe necessary to inject effector proteins in the host cell, subverting normal cellular functions and causing enteric infections [2, 3]. In contrast to the large diversity observed among the effectors proteins [4], the structural proteins that compose the injectisome (OM ring, needle and needle tip) are relatively conserved among the various pathogenic bacteria. Because these proteins are required for pathogenesis and share similarities in all virulent enteropathogenic bacteria, they are ideal candidate antigens for a subunit-based, broad-spectrum vaccine. We will examine the immunogenicity and protective efficacy of the different structural proteins: needle proteins PrgI/MxiH/EscF, needle tip proteins SipD/IpaD/EspA and OM ring proteins InvG/MxiD/EscC of S. enterica, S. flexneri and E. coli respectively, alone or combined, in a mouse model of infection. Keywords: Enteropathogenic bacteria, Type III Secretion System, immunogenicity, vaccine.

033

Follicle homing antigen presenting cells modulate T_H2 bias

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The expression of the chemokine receptor CXCR5 by dendritic cells and their homing to B-cell follicles are suggested requirements for the generation of T-helper type 2 (T_H2) cells in response to infection. Previous studies revealed that bone marrow chimeric mice deficient in CXCR5 in dendritic cells or CD4⁺ T-cells impaired the development of both T-follicular helper (T_{FH}) or T_H2 cells after infection with *Heligmosomoides polygyrus* (Leon, Ballesteros-Tato et al. 2012). Using a refined Cre/LoxP conditional gene expression model we have generated a specific CD11c-mediated CXCR5 knockout transgenic mouse on a C57Bl/6 genetic background. Characterisation of this model has revealed that CD11c⁺ cells are capable of trafficking to and are restricted within T-cell regions of lymph nodes & spleen, and are unable to traffic into the B-cell follicle. Infection with the gastrointestinal nematode *Trichuris*

muris stimulates a T_H2 dominated response in resistant mouse strains such as C57Bl/6 with worm clearance occurring within 21 days. Mouse strains susceptible to *T. muris* infection display a T_H1 dominated response and remain persistently infected. We investigated the ability of CD11c-CXCR5^{-/-} mice to mount an appropriate T_H2 response to *T. muris* infection to facilitate clearance. Unlike CXCR5^{fl} control mice, CD11c-CXCR5^{-/-} mice were unable to clear *T. muris* infection after 30 days. Gene expression analysis of cytokine responses in the mesenteric lymph nodes of *T. muris*-infected mice revealed increased IFNG, IL1B, IL2, IL6, IL10 and reduced IL4, IL9 and IL25 mRNA expression in CD11c-CXCR5^{-/-} mice compared to CXCR5^{fl} control mice. These alterations in cytokine expression were associated with increased expression of both IL-12 receptor beta subunits, IL12RB1 and IL12RB2, and the co-stimulatory molecules CD80 and CD86. We have demonstrated that CXCR5 deficiency in CD11c⁺ cells alters the ability to form a coherent T_H2 type response to *T. muris* infection, preventing worm clearance. These data confirm that for the efficient formation of a T_H2 response to infection with intestinal nematodes, CD11c⁺ cells are required to localise to the B-cell follicle via expression of the chemokine receptor CXCR5. Leon, B., A. Ballesteros-Tato, et al. (2012). "Regulation of TH2 development by CXCR5⁺ dendritic cells and lymphotoxin-expressing B cells." *Nat Immunol* 13(7): 681-690.

034

Fossil fuel derived ambient particulate matter (pm10) induces multiple pathways of activation in human dendritic cells (dc)

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Fossil fuel derived particulate matter (PM) is present within airway myeloid cells of individuals exposed to pollution and is linked with respiratory and systemic inflammatory disease. We hypothesize that exposure of airway DC to fossil fuel PM enhances their ability to induce inflammatory responses. Therefore, we examined the effects of urban ambient PM of <10 µm (PM10) on human monocyte derived DC (MoDC) and on *ex vivo* respiratory tract DC (RT-DC) FACS sorted from induced sputum. DC phenotype was assessed by flow cytometry and qRT-PCR; stimulatory capacity determined in a mixed leukocyte reaction.

Like the TLR4 agonist LPS, PM10 induced 'classical' DC activation by dose-dependent up-regulation of MHC class II, CD40, CD86 and CCR7 as well as the production of IL-6 and IL-12p70. In accordance with their mature phenotype, PM10 treated DC were more stimulatory for naive CD4⁺ T cells. In contrast to LPS, PM10 also induced the release of cytokines associated with inflammasome activation (IL-1β and IL-18) and IL-23. PM10 stimulation, but not LPS, additionally induced aryl hydrocarbon receptor (AhR) signalling in MoDC as indicated by AhR-dependent induction of the target gene *CYP1A1*. PM10 also induced *CYP1A1* in *ex vivo* RT-DC. Carbon black particles, representing the particulate core of PM, did not activate DC indicating a critical role for other components.

Thus, fossil fuel derived PM10 induce a complex programme of DC activation that includes classical maturation, inflammasome-dependent cytokines and AhR signalling. How these pathways interact to influence DC function and immune regulation in the human airway is under investigation.

035

Gut microbial antigen specific CD4⁺T cells from Crohn's Disease patients exhibit a pro-inflammatory Th17 phenotype

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Experimental models have led to the theory that chronic inflammation, as seen in Crohn's disease (CD), results from a loss of tolerance towards commensal microbiota. In fact, antibodies specific for microbial components are found in 50% of CD patients, indicating that a memory T-cell response might be generated as well. There is yet little evidence, however, in human disease proving the later. In testing reactivity to several gut microbial antigens in peripheral blood, we have detected T-cell responses towards antigens including FlaX, Fla2 and YidX in both healthy individuals and CD patients. Interestingly, the proliferative response was significantly higher among CD patients compared to controls, which also correlated with an increased production of IFN-gamma and IL-17 as assessed by ELISA. Intracellular cytokine staining showed the presence of Th17, Th1 and Th17/Th1 cells among the FlaX, Fla2 and YidX-specific T-cell populations, whose frequency was higher in CD. Furthermore, real time-PCR analysis of RORc, IL-17A and IL-17F expression on sorted FlaX, Fla2 and YidX-specific CD4⁺T cells revealed a clear bias towards a pathogenic Th17 phenotype only in CD patients' T cells, but not in T cells from healthy controls. Thus, our data indicate that T cells that react to the same gut microbial antigen have been differently imprinted with a pro-inflammatory phenotype during CD. We hypothesize that these circulating memory T cells may contribute to sustain gut inflammation in CD upon antigen re-encounter; their identification opens new avenues for antigen-directed therapies in CD.

036

High fat feeding alters gut microbiota and protects mice from colitis and colitis-associated colorectal cancer

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Inflammatory Bowel Disease (IBD) appears to be triggered by environmental factors (e.g. diet, microbiota composition) leading to dysregulated immune responses in genetically susceptible individuals. To date, studies exploring the cross-interaction between diet, immune responses and microbiota in the pathology of IBD are limited. A recent study reported that a diet based on milk derived fat led to the expansion of a specific pathobiont resulting in worsened colitis in IL-10^{-/-} mice. In this study, we examined the temporal relationship between diet, the gut microbiota and immune responses in experimental models of Colitis and Colitis-associated Cancer (CAC). Mice were fed lard-based high fat diet (HFD-45%Kcal) or low fat diet (LFD-10%Kcal), followed by AOM injection and 3x DSS-cycles [(1.5%DSS-5 days & water-14 days), CAC-model] or 3xDSS-cycles (Colitis-model). HFD-feeding protected mice from developing colitis and CAC as demonstrated by reduced tumour incidence and numbers, improved colon length, body weight, and

histological scores and reduced plasma and colonic/tumor inflammatory mediators. Mice with Colitis and CAC fed with HFD presented an improved microbial diversity, associated with a reduction in Proteobacteria and an increase in Firmicutes and Verrucomicrobia compared to their LFD-fed counterparts. Collectively and contrary to the previous report using a milk fat based diet, we show that lard-based HFD alters the gut microbial populations protecting against colitis and reducing the risk for CAC. The data highlights a far more complex regulation of lard-based HFD on microbiota composition and host immune responses warranting further investigations on lard-components and other diets as triggers IBD and CAC pathology. Coliti-associated cancer; high fat diet; microbiota; colitis

037

Histone acetylase inhibitors induce caspase-1 independent IL-1 β secretion

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In the course of the last years evidence has accumulated showing that histone deacetylase inhibitors (HDACi) have important immune modulatory activity. In the present work it is shown that in human and murine dendritic cells and murine macrophages HDACi are strong activators of LPS induced IL-1 β processing and secretion. Strikingly, this IL-1 β secretion was independent of the inflammasome components NLRP3, ASC and even caspase-1 and activation kinetics differed completely from that observed after inflammasome activation. Inhibition studies showed that the histone deacetylase HDAC10 is responsible for this HDACi/LPS induced IL-1 β secretion. Mechanistically, HDACi/LPS induced IL-1 β secretion was strictly dependent on Trif and was associated with a functional impairment of autophagic processes. Importantly, these data demonstrate that besides the conventional inflammasome dependent IL-1 β cleavage, dendritic cells and macrophages are capable of activating IL-1 β by a novel, alternative mechanism. Treatment of mice with HDACi during the induction of a dextran sulfate sodium-induced colitis resulted in a strong increase of intestinal IL-1 β . As naturally occurring HDACi like butyric acid are physiologic components of the intestinal milieu, HDACi induced IL-1 β may have a physiological function in intestinal homeostasis.

Altogether the data demonstrate that in addition to the conventional inflammasome dependent IL-1 β activation dendritic cells and macrophages are capable to activate IL-1 β by a novel until now unknown additional mechanism.

038

Homeostatic mechanisms prevent ileitis in mice with deficient MUC2 production

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Introduction and Objectives: Muc2 is the major component of the intestinal mucus layer, and Muc2^{-/-} mice spontaneously develop

colitis. Our aim was to investigate the role of the mucus barrier in small intestine by comparing age-dependent biological responses in mice with deficient Muc2 production.

Materials and Methods: Muc2^{+/+}, Muc2^{+/-} and Muc2^{-/-} mice were reared under SPF conditions and sacrificed at 2, 4, and 8 weeks of age. Total RNA from ileum and colon was purified, and analysed for full genome transcriptome analysis. Microbiota composition of faecal samples was determined using a mouse intestinal chip (MITChip). Morphological and immunohistological studies were performed on segments of ileum and proximal colon.

Results and Discussion: Muc2^{-/-} mice develop colitis in proximal colon after 4 weeks, which worsens by week 8, as evidenced by progressive mucosal thickening, epithelial morphological changes and bacterial invasion. No overt morphological changes were observed in ileum, apart from longer villi due to hyperproliferation in the Muc2^{-/-} mice at weeks 4 and 8. Muc2 expression increased with age in mice^{+/+}; in Muc2^{+/-} mice expression was half that of Muc2^{+/+} mice. Innate immune responses were elevated in ileum in Muc2^{-/-} and Muc2^{+/-} at weeks 4 and 8. By week 4 and 8, adaptive immune responses and inflammatory signalling pathways, including NF- κ B activation, were down-regulated in Muc2^{-/-} and Muc2^{+/-}. Lipid metabolism pathways were down-regulated in ileum at weeks 4 and 8 suggesting altered metabolic functions. Colonic microbial composition is different in Muc2^{-/-} compared to Muc2^{+/+}, whereas the differences are less obvious in ileum. In colon, Muc2 deficiency leads to inflammation and tissue damage. In contrast, there are less histological changes in ileum despite increased cytokine and chemokine expression, and decreased expression of inflammatory pathway genes. In ileum, microbiota composition, lower total numbers of bacteria, and/or intrinsic homeostatic mechanisms appear to prevent tissue damage in Muc2^{-/-}. Muc2^{+/-} mice are a good model to study the role of mucus in intestinal health and have implications for IBD where mucus levels are reduced during active disease and remission.

039

How can differentially acting ADP-ribosylating toxins effectively prime CD4 T cells and affect germinal center size and function?

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The ultimate aim of vaccination is to induce long term protective immunity. Accumulating evidence support a critical role of the choice of adjuvant for an effective stimulation of immunological memory. While cholera toxin (CT) is a potent adjuvant also pertussis toxin (PT) host adjuvant functions, albeit it is better known to inhibit leukocyte migration. Both toxins are ADP-ribosylating enzymes, but act on two different G-proteins, Gsa and Gi, in targeted dendritic cells (DC). While CT acts on Gsa and stimulates adenylate cyclase, leading to increased intracellular cAMP-levels, PT also promotes intracellular cAMP-levels, but only indirectly. We have undertaken extensive studies of the adjuvant effect of CTA1-DD, a gene fusion protein that exploits the enzymatic activity of CT, combined with a DC-targeting D-dimer from Staphylococcus aureus protein A. Unexpectedly, targeted DC failed to show increased cAMP--levels, while the adjuvant effect clearly required Gsa in DC. However, the corresponding S1-DD fusion protein from PT was found to host potent adjuvant effects and similar to CTA1-DD intranasal immunizations stimulated strong specific mucosal IgA and systemic IgG responses. Both CTA1-DD and S1-DD induced enhanced germinal center (GC) reactions and primed CD4 T cells to produce stronger IL-10, IL-17 and INF- γ levels. Here we have asked to what extent cAMP is involved and which signaling pathways mediate the adjuvant functions of CTA1-DD and S1-DD by exploring a mouse model with a distinct defect of Gsa in the DC cell subset.

040

Human buccal epithelium acquires microbial hyporesponsiveness at birth, a role for secretory leukocyte protease inhibitor

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Repetitive interaction with microbial stimuli renders epithelial cells (EC) hyporesponsive to microbial stimulation. Previously, we have reported that buccal EC from a subset of pediatric Crohn's disease patients are not hyporesponsive and spontaneously released chemokines. We now aimed to identify kinetics and mechanisms of acquisition of hyporesponsiveness to microbial stimulation using primary human buccal epithelium. Buccal EC collected directly after birth and in later stages of life were investigated. Chemokine release and regulatory signaling pathways were studied using primary buccal EC and the buccal EC line TR146. Findings were extended to the intestinal mucosa using murine model systems. Directly after birth primary human buccal EC spontaneously produced the chemokine CXCL-8 and were responsive to microbial stimuli. Within the first weeks of life these EC attained hyporesponsiveness, associated with inactivation of the NF- κ B pathway and upregulation of the novel NF- κ B inhibitor SLPI but no other known NF- κ B inhibitors. SLPI protein was abundant in the cytoplasm and the nucleus of hyporesponsive buccal EC. Knock-down of SLPI in TR146-buccal EC induced loss of hyporesponsiveness with increased NF- κ B activation and subsequent chemokine release. This regulatory mechanism extended to the intestine, as colonization of germfree mice elicited SLPI expression in small intestine and colon. Moreover, SLPI deficient mice had increased chemokine expression in small intestinal and colonic EC.

We identify SLPI as a new player in acquisition of microbial hyporesponsiveness by buccal- and intestinal- epithelium in the first weeks after microbial colonization.

041

Human mucosa-associated invariant T (MAIT) cells are present in colon adenocarcinomas, but secrete reduced amounts of IFN-gamma

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Mucosa-associated invariant T (MAIT) cells are innate-like T cells with a conserved T cell receptor alpha-chain which recognize bacterial metabolites of B vitamins presented on the invariant MR-1 molecule. They are present in intestinal tissues and liver, and they are capable of rapid production of IFN-gamma and IL-17 in response to bacterial insult. In colon cancer, IL-17-driven intestinal inflammation promotes tumor progression, while IFN-gamma-production is associated with improved patient prognosis. However, virtually nothing is known about MAIT cells in human tumors. Here we determined the presence and functional capacity of human colonic MAIT cells, both in colon tumors and tumor-free tissue. Flow cytometric analyses of

CD45⁺CD3⁺CD4-Valpha7.2+CD161high MAIT cells showed somewhat higher frequencies in tumor than unaffected colon tissues ($0.72 \pm 0.17\%$, versus $0.43 \pm .11\%$ of CD45⁺ leukocytes, $P < 0.05$), but there was no correlation between MAIT cell frequencies and tumor stage. The large majority of colonic MAIT cells are recently activated memory/effector cells based on their expression of CD69 and CD45RO. The majority of MAIT cells in unaffected colon tissues ($77 \pm 15\%$, mean \pm SEM) produced IFN-gamma, while only $3 \pm 2\%$ produced IL-17. Colonic MAIT cells also produced TNFalpha ($29 \pm 9\%$) and IL-2 ($27 \pm 7\%$), as well as Granzyme B (GrB; $49 \pm 6\%$). In the tumors, significantly lower frequencies ($P < 0.01$) of IFN-gamma-producing MAIT cells were seen ($49 \pm 9\%$), while there were no significant differences in the frequencies of IL-17-, TNFalpha-, IL-2-, and GrB-producing cells. In conclusion, we show that MAIT cells are able to infiltrate colon tumors but that their ability to produce IFN-gamma is substantially reduced. We suggest that MAIT cells have the capacity to promote the local immune response to tumors, but that factors in the tumor microenvironment act to reduce MAIT cell IFN-gamma production.

042

Identification of signaling pathways involved in Rankl-Induced M cell differentiation using 3-D enteroid cultures

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Microfold (M) cells are specialized antigen-sampling epithelial cells restricted to the Peyer's patches and isolated lymphoid follicles of the small intestine. M cells take up particulate antigens from the intestinal lumen and deliver these antigens to the lymphoid follicles beneath the epithelium where adaptive immune responses are initiated. Previous studies identified RANKL-RANK signaling and the Ets transcription factor Spi-B as essential mediators for M cell differentiation. In the past, it has been challenging to study M cell differentiation in vivo due to their low numbers. We have established RANKL-supplemented 3-D enteroid cultures in Matrigel as an in vitro model system for studying the process of M cell differentiation. Primary enteroid cultures derived from wild type mice were stimulated with RANKL at the time of the first subculture. Two days of RANKL treatment is sufficient to strongly induce multiple M cell-associated genes including *Anxa5*, *Spib* and *Gp2* compared to unstimulated control cultures. These genes are not induced in enteroids derived from conditional knockout mice lacking RANK on intestinal epithelial cells. In other RANKL-responsive cells, RANKL can activate multiple signaling pathways including NF- κ B, MAP kinases, PI-3 kinase and Src kinase. We used the enteroid model system to test whether specific RANKL-activated signaling pathways play a critical role in M cell differentiation. Inhibitors of MAP kinases, PI-3 kinase and Src kinase did not interfere with RANKL-induced M cell differentiation in C57BL/6 enteroid cultures. No induction of M cell-associated genes was observed in enteroids from aly/aly mice with a nonfunctional mutant allele of NF- κ B-inducing kinase (NIK). These results pinpoint the noncanonical NF- κ B signaling pathway that requires NIK as a key proximal event downstream of RANK engagement for intestinal epithelial stem cells to commit to the M cell lineage.

043

IL-17 is required for vaccine induced secretory IgA transport and protection from Oral Cholera Toxin Challenge**C. McEntee, C. Davitt, A. Abautret-Daly, R.M. McLoughlin & E.C. Lavelle***School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Dublin 2, Ireland*

A role for interleukin (IL)-17 in the regulation of polymeric immunoglobulin receptor (pIgR) expression at mucosal surfaces has recently been described (Jaffar et al, Cao et al). In this study we investigated whether a deficiency in the IL-17 pathway impacted on oral vaccine induced IgA responses and susceptibility to oral cholera toxin challenge (OCTC). In our experience, three rounds of vaccination with CT and CTB can induce sufficiently high CTB-specific IgA titres to confer protection against OCTC. Thus, wild-type (WT) C57BL/6 mice and IL-17R^{-/-} mice were vaccinated three times, two weeks apart with CT + CTB and challenged with CT two weeks later. Vaccinated WT mice were fully protected from CT-induced fluid accumulation following challenge whereas IL-17R^{-/-} mice exhibited substantial fluid accumulation in the caecum, indicative of CT enterotoxicity. This susceptibility to OCTC correlated with lower levels of faecal CTB-specific IgA in IL-17R^{-/-} mice when compared to WTs. However, this was not due to a general defect in specific IgA production as CTB-specific antibodies in intestinal tissue were comparable between WT and IL-17R^{-/-} mice. Furthermore, intestinal exposure to CT triggered a very rapid transport of IgA into the lumen which was dependent on IL-17, IL-1 and the NLRP3 inflammasome. These findings suggest a key role for the inflammasome-IL-1-IL-17 axis in CT-induced IgA secretion and protective immunity and that IL-17 may be a valuable biomarker of oral vaccine efficacy.

044

Immunization via conjunctiva and CALT as a route for chlamydia vaccine delivery: Immune responses to PmpC, an outer membrane protein of *C. trachomatis***A. Inic-Kanada¹, S. Belij¹, M. Stojanovic², E. Marinkovic², I. Stojicevic², J. Montanaro¹, E. Stein¹, N. Bintner¹, N. Schürer¹, A. Ladurner¹ & T. Barisani-Asenbauer¹**¹*LBCE OCUVAC, Medical University of Vienna, Vienna, Austria,*²*Institute of Virology, Vaccines and Sera - TORLAK, Belgrade, Serbia*

Trachoma is the world's leading cause of preventable infectious ocular disease. Although antibiotics are effective in treating active cases of the illness, they do not prevent re-infection, which occurs with high frequency in susceptible populations. Nevertheless the immunological mechanisms responsible for protective immunity versus immunopathology are still not well understood, although it is widely accepted that T cell driven IFN γ Th17 responses are critical for clearing the infection.

C57BL/6 mouse strain was immunized via the conjunctiva and subcutaneously with polymorphic membrane protein C (PmpC), an outer membrane protein of *Chlamydia trachomatis*. The amount of PmpC administered per dose was 40 μ g/mice. Three immunizations were performed at 2 weeks interval and the evaluation of local and systemic immune response was assessed 2 weeks after the last immunization. Conjunctival immunization with PmpC elicited higher concentrations of SIgA in tears in comparison to subcutaneous immunization. C57BL/6 mice showed the highest proliferation values when heat-inactivated *Chlamydia trachomatis* was used as a stimulator. The conjunctival application of PmpC induced skewing of PmpC-specific immune responses toward a Th1/Th17 profile, as determined by the stimulation of IFN γ and IL-17A secretion and/or the concurrent pronounced reduction of IL-4 secretion.

Immunization via conjunctiva is a new approach to vaccine delivery against ocular surface infections. Our results revealed that PmpC has a potential activate type 1 T-cellular immune response, which holds essential for resolving chlamydial infection to higher extent when applied via conjunctiva vs. subcutaneous immunization.

045

Immunogenic effects of a new alpha-gliadin peptide on a dendritic cell culture model**E. Montalvillo¹, C. Escudero-Hernández¹, J.A. Garrote² & E. Arranz¹**¹*Pediatrics & Immunology, C/Ramón y Cajal 7, Valladolid, Spain,*²*Genetics Lab. Hospital U. Rio-Hortega, C/Dulzaina 2, Valladolid, Spain*

We have previously described a new gliadin peptide released by the action of gliadin-degrading proteases from the duodenum of celiac disease (CD) patients. The ability of this peptide to develop a humoral immune response in vivo in CD patients was confirmed by the detection of IgA anti-deamidated 8-mer (d8mer) peptide in plasma samples from these patients. Our aim here was to study the immunogenic effects of this peptide in an in vitro model of dendritic cells. Peripheral blood-derived dendritic cells were obtained from CD patients (both active CD, and on GFD) and healthy volunteers. Cell culture for 24 hours was stimulated with 100 μ g/ml of each peptide (native 8mer and d8mer, d33mer, 19mer, and control peptide). Dendritic cell maturation and activation markers were analyzed, as well as the ability of these cells to induce T cell proliferation in an autologous co-culture.

Dendritic cells from CD patients stimulated with d8mer show an increased expression of CD80 (aCD, $P < 0.001$, GFD, $P < 0.01$) and CD86 (aCD, $P < 0.01$, DSG, $P < 0.05$); whereas 33mer increased CD83 (aCD, $P < 0.001$) and CD86 (aCD, $P < 0.01$, GFD, $P < 0.05$) expression, compared to the corresponding basal culture. Dendritic cells stimulated with both d8mer and d33mer induced proliferation of IFN γ -producing T cells in samples from all CD patients. Both, 19mer and native 8mer have no effects.

In summary, as with 33mer peptide, the d8mer peptide is involved in the stimulation of dendritic cells and proliferation of IFN γ -producing T cells, probably independent of whether antigen presentation is mediated HLA-DQ2 + or DQ2 dendritic cells.

046

Immunological differences between left and right-sided colonic tumours of patients with colorectal cancer**H.O. Al-Hassi¹, G.H. Lee¹, G. Malietzis¹, D. Bernardo¹, E.R. Mann², D. Reddi¹, N.R. English¹, R. Man³, R. K.S. Phillips⁴ & S.K. Clark⁴**¹*Immunology, Antigen Presentation Research Group, Watford Road, London, UK,* ²*Department of Medicine, Johns Hopkins University, School of Medicine, Baltimore, USA,* ³*Wolfson Unit for Endoscopy, St Mark's Hospital, London, UK,* ⁴*Department of Surgery, St Mark's Hospital, Watford Road, London, UK*

Introduction: Colorectal cancer (CRC) is one of the major causes of cancer related mortality. Dendritic cells (DC) promote either tumour immunity or tolerance but their effectiveness is dependent on tissue-microenvironment. Growing evidence suggests that different sites within the colon may have different tumour incidence, histopathological and prognostic outcomes. There are embryological, histological and functional differences between the proximal and the distal sides of the colon. However, the immunological differences between the compartments in CRC remain unknown; we aim to explore

differences and provide a mechanistic rationale for them according to the activity of DC in these compartments.

Methods: Phenotype, activation and migration markers of DC in mucosal and tumour biopsies from proximal versus distal colon of CRC patients were investigated using flow cytometry and immunohistology. Functional studies using migration assays were performed to assess the effect of tumour microenvironment on the migratory capacity of DC.

Results: In the tumour tissues, expression of the activation markers CD40, CD86 was significantly higher on DC from the proximal colon. Expression of $\beta 7$ (a gut homing marker) and ILT3 (immature DC marker) did not differ between compartments. Expression of CCR7 (a lymph node migration marker) was significantly higher on DC from the distal colon. In the mucosae, expression of CD40, CD86 and CCR7 on DC was similar to that in tumour tissues. Significantly greater migration towards CCL19 in vitro by DC from the distal colon showed that CCR7 expression was functional. Expression of $\beta 7$ and ILT3 was significantly higher on DC from the distal colon.

Conclusion: These immunological differences suggest that tumours in the proximal colon have more activated DC and should be treated as different entities from their distal counterparts. Right side tumours are less frequent, more aggressive and the survival rate is lower compared with left side CRC patients. This difference could be due to evolution of these tumours so that some mutations in adenomas in the right side of the colon enable survival of the higher immune activity/surveillance at that site. Immunological differences between left and right-sided CRC and its potential impact on disease outcome may inform classification of patients with the same risk stratification for clinical trials and for the development of personalised treatment strategies. (Dendritic cell - Colorectal cancer)

047

Immuo-modulatory properties of probiotics in DSS-colitis: Impact on micro-RNA expression and bacterial diversity

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Introduction: Probiotics have been reported to be useful in IBD treatment through different mechanisms, including their immunomodulatory properties and the impact in the microbiota composition. These effects can promote the down-regulation of inflammation mediators including micro-RNAs. The aim was to evaluate the immunomodulatory properties of different probiotics in DSS model of mice colitis, emphasizing the relation between micro-RNA expression and bacterial diversity.

Methods: Male C57BL/6 mice were pretreated with E. coli Nissle 1917 and L. fermentum CECT5716 at 5x10⁸ UFC/mice/day for two weeks before DSS-induced colitis (3% solution for 6 days in the drinking water). Non-colitic and non-treated colitic groups were included as reference. Six days after colitis induction, mice were sacrificed. The inflammatory status was evaluated by a disease activity index (DAI), qPCR of inflammatory markers and micro-RNAs, and changes induced in microbiota populations characterized by pyrosequencing. Statistical significance is set at $P < 0.05$.

Results: Probiotics administration resulted in an intestinal anti-inflammatory effects evidenced macroscopically by lower DAI values. Biochemically, it was observed a decreased expression of pro-inflammatory cytokines (IL-1 β) and an increased expression in mucins (MUC-2 and MUC-3) and tight junction proteins. Also, it was established a micro-RNA expression profile which showed a reduced

expression of miR-143 and miR-375 while miR-155, miR-223 and miR-150 were increased in this model. Probiotics were able to restore the expression of these markers. Finally, dysbiosis and lowering bacterial diversity was characterized in colitic mice by 454-pyrosequencing, being restored by the treatment with each probiotic.

Discussion and Conclusion: Probiotics are able to increase the diversity of microbiota as well as to alter the expression of different inflammatory markers and micro-RNAs. These results support the immuno-modulatory effects of probiotics and its use in IBD.

048

Immunoproteasome subunit LMP7 drives the development of colitis-associated carcinogenesis

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Colorectal cancer (CRC) is the second leading cause of cancer-related death worldwide. Chronic colonic inflammation often progresses to CRC, whereby NF- κ B-controlled expression of proinflammatory cytokines such as TNF- α , IL-6 and IL-17A provides a crucial functional link between augmented immune responses and development of colitis-associated carcinogenesis (CAC). We have recently demonstrated the involvement of immunoproteasomes in the regulation of NF- κ B activity. Here, we show that the immunoproteasome subunit LMP7 is essential for development of CAC. We found a striking difference between wild-type (WT) and LMP7 deficient mice with respect to number of polyps and development of dysplasia in the AOM/DSS murine model of colon carcinogenesis. On day 76 after induction of CAC, all WT mice developed macroscopically visible adenocarcinomatous lesions, but no such lesions were detected in LMP7 deficient mice. During the course of treatment with AOM and DSS, a massive infiltration of immune cells into the lamina propria of WT mice was accompanied with elevated expression of TNF- α , IL-6, IL-17A and INF- γ . On the other hand, a decreased influx of neutrophils and low production of proinflammatory cytokines were observed in LMP7 KO animals. Importantly, the treatment with the selective LMP7 inhibitor (ONX-0914) blocked the production of proinflammatory cytokines and attenuated progression of CAC in WT mice. Thus, the immunoproteasome might be a specific target for development of anti-inflammatory drugs to dampen the ongoing chronic inflammation and development of CAC.

049

In vitro modelling of the effects of gluten and poly:IC on mucus-secreting epithelium

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Celiac disease is a chronic autoimmune enteropathy of the small intestine triggered by ingestion of gluten and related prolamins in individuals with tissue type HLA DQ2 or 8. In the lamina propria of the intestinal mucosa, gluten peptides are taken up by antigen-presenting cells which induce inflammation by activation of gluten-specific CD4 T cells. The integrity of the intestinal epithelial

barrier depends on a mucus layer and intercellular tight junction formation. Coeliac disease is characterized by enhanced intestinal permeability, but the reason for this is largely unknown.

In the present work, we have grown a monolayer of gut epithelial cells expressing tight junctions (caco-2) and gut epithelial cells producing mucus (HT-29). We investigated if gluten had immunotoxic effect on the epithelium and if these effects were changed by the presence of a mucus layer.

We added chymotrypsin-treated gluten peptides to the apical side of the membrane of caco-2- and HT-29 cells alone, and to a co-culture. Trans-epithelial electric resistance (TEER) was used as a measure of confluency and tight junction formation of the cells. In addition, we added poly:IC simultaneously with gluten in order to mimic a viral infection to test if this had any toxic effect on the epithelium or a synergistic effect with gluten.

Our preliminary results show no significant changes between the different treatments of the epithelium compared to bovine serum albumin-treated controls. In future experiments we will use pepsin/trypsin-treated gluten peptides, and preincubate the epithelium with poly:IC before the addition of gluten.

050

Interactions between intestinal epithelial cells and Coxsackievirus B and the role of the Type III Interferons

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The Coxsackievirus B (CVB) family of enteroviruses (EVs) are single-stranded RNA viruses that have been implicated in a number of diseases including Type 1 diabetes (T1D), myocarditis, hepatitis and meningitis. CVBs infect via the intestinal tract and studies suggest that T1D is associated with CVB infection in the gut mucosa, although little is currently known about these interactions. Type III interferons (IFN λ s) are important regulators in the permissiveness of both primary human pancreatic islets and hepatocytes to CVB infection, and they have also been implicated in the control of rotavirus infection in intestinal epithelial cells (IECs). However, to date, their role in limiting EV infection, and more specifically CVB infection in the gut is not known. Therefore, the focus of this study was to examine the interactions between CVBs and IECs and to determine whether the IFN λ s can regulate the permissiveness of IECs to infection. To examine this, we have used the two human colonic cell lines HT-29 and CaCo2 and the common EV, CVB3.

In this study we demonstrate that both HT-29 and CaCo2 cells express the two receptors used by CVBs to infect cells, namely CAR and DAF. Moreover, we also show that both cell lines are susceptible to infection with CVB3 as confirmed by plaque assay and the monitoring of cytopathic effects. 3 h and 6 h post infection, both cell lines also appear to up-regulate the expression of IFN λ 1 (interleukin-29, IL-29) and IFN λ 2 (IL-28a). RT-PCR analysis of the two IFN λ receptor subunits, IFN λ -R1 and IL-10R2, confirmed that the IEC lines express both subunits at the mRNA level. Furthermore, treatment of the IECs with IFN λ 1 and IFN λ 2 for 6 h and 24 h up-regulated the expression of a number of IFN stimulated genes involved in antiviral defence mechanisms at both the mRNA and protein level, as confirmed by RT-PCR and Western blotting.

From this study we can conclude that the two human IEC lines, HT29 and CaCo2 are susceptible to infection with CVB3 and that upon infection they up-regulate the expression of the IFN λ s. Moreover, the IECs also enter an antiviral state after treatment with the IFN λ s. However, whether the IFN λ s protect against CVB infection remains to be established. Further studies using a novel method for

culturing human intestinal tissue ex vivo will confirm whether these findings are applicable in a more relevant model.

051

Interleukin-10 inhibits human IFN γ -secreting effector T cells indirectly by controlling antigen-presenting cell function

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Chronic inflammation of the gastrointestinal tract, as seen in inflammatory bowel disease, arises from abnormal reactivity of T cells to commensal microbiota. Interleukin-10 (IL-10) plays a crucial role in suppressing microbiota-specific T cell responses. However, it is unknown how IL-10 prevents reactivation of effector T cells in the human intestine. We have recently identified a homozygous loss-of-function mutation in the IL-10RA gene of a pediatric patient with early-onset colitis. In contrast to recently reported cases, disease remission could be achieved without stem-cell transplantation, allowing in-depth analysis of the mechanism by which IL-10 controls human effector T cells. Lesional intestinal tissue taken at onset of disease contained high numbers of IL-17+ and T-bet+ cells. In agreement, IL-10 failed to control IFN γ and IL-17 production by activated T cells derived from the IL-10RA-deficient patient in vitro. By coculturing CD4⁺ T cells and monocyte-derived dendritic cells (DC) from the IL-10RA-deficient patient and a healthy control, we revealed that IL-10R expression on DC, and not on T cells, is important for controlling IFN γ production, and to a lesser extent IL-17 production, by effector T cells. Importantly, we demonstrated that IL-10 plays a pivotal role in controlling the differentiation of immature monocyte-derived DC into inflammatory DC with a disease promoting phenotype. Taken together, our study demonstrates that IL-10 is essential to limit IFN γ and IL-17 secretion by human effector T cells and reveals that IL-10 exerts its suppressive function on IFN γ -secreting effector T cells mainly indirectly by controlling antigen-presenting cell function.

052

Intestinal inflammation induced by dietary cholesterol in Zebrafish

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Inflammatory bowel disease and other chronic, often systemic, auto-inflammatory human disorders have considerably increased over the past decade, coincidentally with a rise in the consumption of diets enriched in cholesterol or saturated fatty acids. The intestinal mucosal barrier first encounters dietary components, however, in vivo information regarding their local and direct inflammatory effect on the intestinal epithelium is lacking. We found that a single exposure to a high cholesterol diet (HCD) results in localized accumulation of myeloid cells in the intestine in both mice and zebrafish. Pharmacological interventions demonstrated that this acute myeloid cell accumulation is dependent on cholesterol uptake via NPC1L1, NFkB, NADPH oxidase and Cathepsin B, leading to caspase-1 activity in

intestinal epithelial cells. Through a novel morpholino oligonucleotides delivery approach in zebrafish we found that inflammasome activation in epithelial cells is dependent on ASC. Extending the HCD exposure to 10 days results in localised functional dysregulation, dependent on cholesterol binding/uptake and inflammasome activation, and systemic pathologies. Our model reveals a novel route by which dietary cholesterol can initiate intestinal inflammation and suggest its involvement in long-term pathologies of the intestine. Further, our model illustrates the power of the zebrafish system to study pathophysiological responses induced in the intestinal epithelium in an intact organism and aspects of mucosal immunology research that have been hampered in the past.

053

Intestinal mucin-dendritic cell crosstalk in gut homeostasis

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The gastrointestinal tract is lined with mucus, mainly composed of the goblet cell secreted mucin MUC2. Despite this mucus layer, the intestine still comes into contact with the commensal microbiota and potential enteric pathogens. Thus, a fine regulation of the immune system in the intestine is required to maintain gut homeostasis and protect from infection. Intestinal dendritic cells (DCs) are essential for sampling luminal antigens and promoting the appropriate immune responses to the intestinal environment, promoting tolerogenic responses to commensals while orchestrating effector responses against pathogens. Recent evidence has suggested that interactions between intestinal DCs and mucins may modulate DC function and subsequent immune responses. To determine the potential for mucins to regulate DC function, we treated human monocyte-derived DC with purified MUC2/Muc2 obtained from intestinal cell lines and mouse large intestine. We found that expression of the pro-inflammatory chemokine interleukin 8 (IL-8) is significantly upregulated by human DCs in the presence of MUC2/Muc2. Additionally, mucin-treated DCs are able to enhance recruitment of neutrophils in transmigration assays. Thus, in contrast to recent published results suggesting potential anti-inflammatory properties of MUC2, we find that this mucin may induce important pro-inflammatory functions in DC. Further investigation is therefore required to explore mucin-DC interactions during health and infection.

054

Intestinal myeloid DCs display an activated phenotype and are less susceptible to HIV-1 infection compared to blood DCs

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We recently showed that human colonic lamina propria (LP) CD11c⁺ DC actively shuttle R5 HIV-1 across an intact epithelial barrier and transfer infection to CD4⁺ T cells (Cavarelli, EMBO Mol-Med 2013). However the susceptibility of intestinal DC to HIV infection has been poorly investigated, due to difficulties in isolating mucosal DC.

CD11c⁺ myeloid DC obtained from the colonic LP were further characterized as CD103⁺ and CX3CR1⁺ and the expression of HIV-1 receptors analyzed in comparison to blood DCs. Supernatant

obtained from an ex vivo culture of healthy human colonic mucosa was used to condition monocyte-derived DC in an in vitro model as to mimic the exposure of DC to intestinal microenvironment. Conditioned-DC (C-DC) were analyzed by flow cytometry for the expression of HIV-1 receptors and activation markers, and incubated in vitro with either R5 or X4 HIV-1 to study their susceptibility to infection.

C-DC displayed an activated phenotype, a significant down-regulation of CCR5, CD4 and CX3CR1, an up-regulation of CXCR4 and a moderate modulation of DC-SIGN expression compared to unconditioned DC. No change in the CD103 expression was observed. Interestingly, both R5 and X4 HIV-1 integrated their genome and replicated less efficiently in C-DC compared to unconditioned DC. Colonic supernatants contained the CCR5-binding chemokines Mip1 β and MCP-1 and the CX3CR1 ligand fractalkine, whereas the CXCR4 ligand SDF-1 α was absent. IL-10 and IL-2, described to induce CXCR4 up-regulation on DC, were also detected. Thus, this specific intestinal milieu may determine the observed phenotype. Both CX3CR1⁺ and CD103⁺ CD11c⁺ DCs were detected in human colonic LP. Interestingly CD11c⁺ DC showed lower CCR5 and higher CXCR4 expression compared to blood DC, and a similar activation profile, which confirmed the results obtained after intestinal conditioning.

Thus, the intestinal microenvironment modulate the expression of HIV receptors on DCs and their capability to replicate the virus. Keywords: HIV-1, mucosal transmission, dendritic cells.

055

Isolation, culture and monitoring of Chlamydia trachomatis and suis using the pig model

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Chlamydia trachomatis infections are the most common sexually transmitted bacterial disease worldwide and infection rates are especially high in aboriginal communities. C. trachomatis infects genital tract mucosa and can cause severe health problems including infertility and an increased risk for infections like HIV/AIDS. A vaccine against C. trachomatis is currently not available but urgently needed. In order to develop such a vaccine it is important to study the immune response in a relevant animal model to understand the host pathogen interactions in human chlamydial infections. Pigs are the natural host of C. suis and infection with C. trachomatis closely resembles the infection in humans. Therefore, the pig is a relevant large animal model to study the host pathogen interactions of C. trachomatis. Additionally, the methodology for a detailed analysis of the relevant T-helper and regulatory T-cell immune responses is available. The first steps in this establishment are the propagation, purification and titration of chlamydia on one side and the isolation of the host cell target of chlamydia, genital tract epithelial cells, on the other side. Most of the available protocols were established in the 1980s. This time gap creates two main problems: First, some of the incorporated chemicals are no longer easily available and second, most of the incorporated methods are obsolete. Therefore, we updated existing protocols with available chemicals and included state of the art techniques like Taqman probe RT-qPCR, multi-colour confocal microscopy and flow cytometry analysis. These protocols enable the production of a highly purified and precisely titrated stock of chlamydia as well as the isolation and detailed characterization of genital tract epithelial cells. These protocols will provide the basis for a comprehensive analysis of the host immune response on chlamydial infection.

056

Local responses to virulent and avirulent Clostridium perfringens in broilers

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The main causative agent of necrotic enteritis is *C. perfringens* (Cp) type A, which produces several toxins, including netB. Early host responses therefore an in situ duodenal loop broiler model was developed to dissect complex host-pathogen interactions at the early stages of disease pathogenesis. Here, we investigate host responses to virulent, netB+, and avirulent, netB-, strains of Cp and their culture supernatants.

Six isolated duodenal loops were prepared surgically in anaesthetised broilers. Loops within birds were infused with: control (growth medium), Cp culture supernatant alone or bacteria and culture supernatant from either the virulent or avirulent strain. Intestinal segments were removed for histology and gene expression analysis at 0.5 or 4 h post-infusion. Expression of genes related to disease pathogenesis (FAS, GIMAP8), immune cell activity (BLA, NBL1) and inflammation (IL-6, IL-1 α and IFN- α) were measured.

The main effects detected were that IL-6 expression was significantly down-regulated in virulent treated birds while FAS was significantly increased in loops containing bacteria and culture supernatant at 4 h in comparison to the control and culture supernatant alone. There were no other significant changes in pathology and heterophil infiltration between the treatments at these early time points. The implication of the results will be discussed.

057

Macrophage subsets exhibit selective endotoxin tolerance induced by Escherichia coli LPSK. Alshaghдали¹, C. Haywood², J. Beal³ & A. Foey³*¹School of Biomedical and Healthcare Sciences, University of Plymouth, Plymouth, UK, ²Derriford Hospital, Plymouth, Plymouth, UK, ³School of Biomedical and Healthcare Sciences, Plymouth, UK*

Macrophages (M Φ s) control gut mucosal responses; facilitating tolerance to commensal bacteria and food components, while keeping the ability to trigger immune defences to pathogens. M Φ reactions are determined by both differentiation and activation stimuli, giving rise to two distinct subsets; pro-inflammatory M1- and anti-inflammatory/regulatory M2- M Φ s. M2-like M Φ s take control in homeostatic environments whereas M1-like M Φ s predominate in inflammatory pathologies. Suppression of M Φ responses can be beneficial to either the host or pathogen. Chronic stimulation by bacterial pathogen associated molecular patterns (PAMPs), such as LPS, is well established to induce tolerance. The aim of this study was to investigate the susceptibility of M Φ subsets to *Escherichia coli* K12 LPS-induced suppression. M1- and M2-like M Φ s were generated in vitro from the THP-1 monocyte cell line by differentiation with PMA and vitamin D3, respectively. M Φ subsets were pre-treated K12-LPS prior to stimulation by bacterial PAMPs. Modulation of inflammation was measured by TNF α , IL-1 β , IL-6, IL-10 ELISA. K12-LPS suppressed PAMP induced TNF α expression in M1 and M2 but selectively suppressed IL-6 and IL-10 expression in M1 and M2 M Φ s. In conclusion, *E. coli* LPS selectively tolerises cytokine production between proinflammatory M1 and regulatory M2 M Φ s; differential suppression exerting knock-on effects on both immunopathology and homeostasis.

058

Microbial PAMP co-stimulation initiates neutrophilic steroid resistant asthmaS. Hadebe¹, G. Brown¹, P. Redelinguys¹, R. Drummond¹, K. Fierens², B. Lambrecht², F. Brombacher³, F. Kirstein³ & S. Vautier¹*¹Immunity, Infection and Inflammation Programme, University of Aberdeen, Aberdeen, UK, ²Respiratory Diseases, University of Ghent, Laboratory of Immunoregulation and Mucosal Immunity, Ghent, Belgium, ³Immunology, University of Cape Town, Institute of Infectious Disease and Molecular Medicine, Cape Town, South Africa*

Introduction: Asthma is a complex obstructive airway disease characterised by airway hyper-reactivity to innocuous allergens. It may be categorised as either classical eosinophilic, T helper 2 type of disease or as one driven by neutrophils that may be associated with T helper 17 cells and that is corticosteroid resistant. While the pathogenesis of the disease is not fully understood, there is increasing evidence for the role of environmentally-derived pathogen-associated molecular patterns (PAMPs) including fungal β -(1,3)-glucans and bacterial lipopolysaccharide (LPS) in inducing and exacerbating airway inflammation.

Results: We investigated the effects of these components, either alone or in combination, in several models of pulmonary inflammation and discovered that they modified airway responses in vivo. Notably, a combination of PAMPs drove a profound neutrophilia that was associated with synergistic IL-17A and RANTES production. Moreover, in allergic models using house dust mite, sensitisation with these agonists resulted in corticosteroid resistant airway hyper-responsiveness.

Conclusion: Sensitisation with multiple microbial PAMPs may therefore play an important role in the pathogenesis of steroid resistant asthma.

059

Microbial signals control inflammation and autoimmunity induced by hypomorphic RAG defectsR. Rigoni¹, V. Maina¹, S. Mantero¹, V. Marrella², F. Grassi³, G. Pesole⁴, J.R. Mora⁵, A. Villa² & B. Cassani²*¹Humanitas Research Hospital, Via Manzoni 113, Rozzano MI, Italy, ²IRGB-CNR, Via Manzoni 113, Rozzano MI, Italy, ³Institute for Research in Biomedicine, via Vela, Bellinzona, Switzerland, ⁴Universita' di Bari, Bari, Italy, ⁵Massachusetts General Hospital, Harvard Medical School, Boston, USA*

Hypomorphic mutations in the RAG genes result in profound lymphopenia associated with multisystem autoimmune manifestations in humans and mice. The role of gut homeostasis and microbial signals in the immune dysfunctions and disease pathogenesis is still debated. The Rag2R229Q/R229Q mutant mice developed an inflammatory bowel disease involving the small intestines, characterized by marked infiltration of CD4⁺ T cells and, intriguingly, also of Treg cells, in the lamina propria compartment. Increased expression of the gut homing receptors CCR9 and $\alpha 4\beta 7$ on peripheral CD4⁺ T cells confirmed the abnormal lymphocyte trafficking to this environmental interface. A pro-inflammatory profile, characterized by a Th1/Th17 skewing, distinguished the intestinal immune responses in the Rag2R229Q/R229Q mice. Remarkably, similar pattern was also evident in the periphery. On the contrary, B cells were poorly present in the gut of mutant mice and the fecal level of IgA was obviously reduced. Interestingly, these findings correlated with augmented intestinal permeability and enhanced epithelial innate responses. Metagenomic analysis revealed substantial changes in the composition of the gut microbial communities in the mutant mice, with an

overall reduced bacterial biodiversity. Importantly, decreasing microbial load with antibiotic treatment significantly limited the lymphocytic infiltration, as demonstrated by the reduction in the frequency of peripheral CCR9⁺ T cells, and ameliorated both the intestinal and systemic inflammation by dampening pro-inflammatory Th1/Th17 immune responses. Overall, these results suggest that microbial factors may play a substantial role in the pathogenesis of human autoimmune disease associated with hypomorphic RAG defects.

060

Milieu-adjusted (physiological) oxygen concentration reduces the production of IL-8 and CCL-20 upon stimulation with Flagellin in CaCo-2 cells

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Background: 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, the partial pressure of oxygen in tissues is far below this. As many cellular and immune functions are influenced by oxygen level, it may also be of importance in IECs.

Methods: The colon epithelial cell line CaCo-2 was investigated under a physiological oxygen concentration of 1%. Proliferation was measured by the incorporation of 3H-Thymidin and viability by MTT-assay. Chemokine production was measured by ELISA or intracellularly by FACS, gene expression by RT-PCR and proteins were detected by Western Blot.

Results: Proliferation and viability of the cells were not significantly altered compared to conventionally cultured cells. While transepithelial electrical resistance (TEER) was initially elevated under low oxygen, it reached comparable levels over time. When stimulated with the TLR 5-ligand Flagellin, IL-8 and CCL-20 production was diminished at 1% O₂. Interestingly, mRNA levels of IL-8 and CCL-20 were not significantly reduced under physiological oxygen concentration. Analysis of MAPK by Western Blot showed a generally higher phosphorylation of p38, JNK and ERK1/2 under low oxygen, which was further enhanced by stimulation with Flagellin. Inhibition of MAPK did not alter the chemokine secretion. Intracellular IL-8 measurements pointed to an alteration in chemokine transport.

Conclusions: 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.

061

Modeling T-cell dissemination upon nasal immunisation using a systems biology approach

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The application of systems biology in vaccinology has recently been proposed as powerful tool to characterize immune responses to vaccination and to predict vaccine immunogenicity and efficacy. Our group has recently employed a branching process to model in vivo CD4⁺ T-cell priming and estimate the probabilities of a cell to enter

in division, rest in quiescence or migrate/dye. This model has been successfully applied to analyse CD4⁺ T-cell priming following different mucosal routes of immunization such as vaginal or nasal immunization and has allowed to estimate the probability of CD4⁺ T cells in the draining lymph nodes to enter in division [Pettini E. et al., PlosOne 2013 8:e80545]. Here, a systems biology approach was employed to get quantitative information on the dissemination of primed CD4⁺ T cells from draining lymph nodes (LNs) to distal lymph nodes and spleen following nasal immunization. The adoptive transfer model of transgenic OT-II ovalbumin(OVA)-specific CD4⁺ T cells was employed to study the antigen-specific T-cell primary activation following nasal immunization. OT-II CD4⁺ T cells were labeled with CFSE and adoptively transferred into recipient mice, that were immunized with OVA and CpG ODN by the nasal route. Groups of mice were sacrificed 0, 48, 60, 72, 84, 96 and 120 hours following immunization, and cervical, mediastinal, iliac, and mesenteric lymph nodes and spleen were collected. Antigen-specific CD4⁺ T-cell clonal expansion was observed in draining LNs already 2.5 days following mucosal immunization, while OVA-specific proliferated T cells appeared in distal lymph nodes and spleen since 3.5 days following immunization. A Galton Watson multitype branching process with emigration was successfully employed to model in vivo dissemination of proliferated T cells. The model has allowed to estimate the probability of CD4⁺ T cells to migrate from draining lymph nodes to distal lymph nodes and spleen. In conclusion, the modeling analysis shows that the developed model is reliable and can be fruitfully exploited for predictive simulations related to the study of primary T-cell activation and dissemination. **Keywords:** Systems biology, T-cell dissemination, nasal immunization, mathematical modeling.

062

Neonatal dendritic cell development

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The intestinal immune system has to discriminate between inducing tolerance and active immunity to food proteins, commensal bacteria and pathogens. Dendritic cells (DCs) in the gut wall and draining lymphoid tissues are central to these processes. We and others have identified four functionally distinct populations of intestinal DCs based on CD11b and CD103 expression. These appear to develop from a common DC precursor, however little is known about how the local intestinal microenvironment might determine their differentiation and subsequent fate. We have begun to explore these issues, focusing on the neonatal period when the intestinal immune system has to adapt to rapid changes in diet, anatomical structure and microbiota.

All four DC subsets are present in the intestinal lamina propria (LP) from birth, although there are fewer CD103⁺CD11b⁺ DCs compared with the adult. However during the first three weeks of life, there is a progressive increase in the proportion of CD103⁺CD11b⁺ DCs, and these become the majority population around weaning, resembling the composition of the DC compartment in the adult.

Few migratory or resident DCs are found within the draining mesenteric lymph node (MLN) at birth, but both populations increase gradually in number thereafter. Consistent with their migratory origin from the LP, the numbers of CD103⁺CD11b⁺ and CD103⁺CD11b⁻ DCs show delayed appearance compared with the mucosa. However by three weeks of age, the proportions of DC subsets in the MLN are comparable to those observed in adults.

Together these data support the idea that intestinal environmental factors drive differentiation of DC subsets in distinct, age-dependent

ways. Specifically CD103⁺CD11b⁺ DCs, unique to the intestine, are highly dependent on changes within the local environment. Identification of these factors will aid understanding of how DCs induce tolerance against commensal bacteria and food proteins.

063

Novel markers to evaluate immunomodulatory effects of antibiotics in DSS-colitis: micro-RNA expression and microbiota populations

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Introduction: Minocycline and doxycycline exert immunomodulatory effects that could be beneficial in IBD. Micro-RNAs have been recently reported to play a key role in intestinal homeostasis that can be influenced by microbiota populations. The aim of the study was to evaluate the effect of these antibiotics in the DSS-colitis model in mice, characterizing the modifications induced in the micro-RNA expression and bacterial diversity.

Methods: Male C57BL/6J mice were assigned into non-colitic and DSS-colitic groups. Colitis was induced by dextran sodium sulfate (DSS) in the drinking water (3%) for 6 days. Once the colitis process was established, colitic mice were divided in three groups: DSS-control (without treatment), MNC (receiving minocycline 50 mg/kg/day) and DXC (receiving doxycycline 10 mg/kg/day). After six days of treatment, all mice were sacrificed. The inflammatory status was evaluated by a disease activity index (DAI), qPCR of inflammatory markers, including micro-RNAs. Also, changes in microbiota populations were characterized by pyrosequencing.

Results: According to the DAI values, minocycline and doxycycline treatment improved the recovery of colitic mice, ameliorating some of the inflammatory markers, including IL-1 β , IL-17, TGF β , MMP-2, MUC-2, MUC-3, ZO-1 and occludin. A micro-RNA expression profile was established for this model of colitis showing an increased expression of miR-155, miR-223 and miR-150 while miR-143 and miR-375 were decreased. Both antibiotics partially restored the expression of some of these markers. Pyrosequence characterization of microbiota showed that minocycline and doxycycline treatments increased the bacterial diversity, reverting the dysbiosis produced by DSS-colitis.

Discussion and Conclusion: Minocycline and doxycycline are able to modify the expression of different inflammatory markers and microRNAs, as well as to increase the intestinal bacterial diversity. These observations confirm the combined contribution of antibiotic and immunomodulatory properties ascribed to these compounds that could be of great interest to control the complex pathogenesis of IBD.

064

Optimization of a flow cytometric peripheral eosinophil assay aiding in diagnosis and monitoring of allergic disorders

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Introduction: Eosinophilic inflammation is an important component of the pathology seen in allergic disorders such as eosinophilic gastrointestinal disorders and asthma. Expression of an integrin, CD11b

on eosinophils is one sensitive surrogate biomarker for activation, accumulation and recruitment to inflammatory sites.

Method: We sought to optimize the assay in terms of sample post-collection stability, staining duration and gating strategy for clinical implementation purposes. The criteria for this evaluation are based on the percent and CD11b expression in median fluorescent intensity of eosinophil. The gating strategy in identifying eosinophils are augmented by their auto-fluorescent properties and CD11b expression instead of the classical definition characterized by CD9^{hi} and CD16^{dim} phenotype which shows overlap with our new method. Basically, the eosinophil events, derived from total granulocytes, are identified on the bi-variate plot with FITC, an empty channel versus side scatter parameters, as they are most distinctive on the lower spectrum of wavelength. We chose an acceptance range for the criteria to be < 20%CV comparing to those parameters at 0, 4, 6, 24 and 48-hour.

Results: Among five different anti-coagulant collection tubes, K2EDTA and 3.2% Sodium Citrate anti-coagulants shown to have at least 24-hour stability window, which is important to further this assay for clinical diagnostics. As a result, the optimized flow panel design in conjunction with this new gating strategy and the extended sample stability give the assay more flexibility, efficiency and an additional clinical tool aiding in allergic disorders evaluation as well as its utilization in clinical trials.

065

Oral antigen modulates primary systemic KLH-specific T- and B-cell responses in humans

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Oral tolerance is an antigen-specific process and refers to specific non-reactivity of the immune system against an antigen that has been fed to the organism and thus comes into contact with the immune system via the mucosa of the gastrointestinal tract. Especially in humans, the underlying mechanisms are poorly understood.

In our clinical study we administered 50 mg KLH/d for 10 days to healthy volunteers of the oral ($n = 8$) but not the control group ($n = 8$). Afterwards both groups were subcutaneously immunised with KLH. A DTH skin reaction was then provoked by an intradermal KLH injection. Follow-up investigations were done after 6 months. During the study PBMC were isolated at defined time points and KLH-specific T and B cells were analysed. The proportions of cytokine-producing KLH-specific CD4 T cells and KLH-induced proliferation of CD4 T cells were analysed after in-vitro restimulation. KLH-specific B cells were identified by fluorescent-labeled KLH. KLH-specific serum IgG, IgA and IgM as well as IgG subclasses were measured by ELISA.

Only 2/8 volunteers of the oral group but 7/8 volunteers of the control group developed a subsequent skin reaction. KLH-specific proliferation and frequencies of KLH-specific CD154⁺ CD4⁺ T cells producing IFN-gamma, IL-2 and TNF-alpha were higher in volunteers with DTH compared to those without a DTH. Oral KLH primed rather than suppressed KLH-specific B cells. Primary KLH-specific plasma cells were not predominantly IgM⁺ but commonly expressed IgA.

Tolerance induction in humans by oral antigen was confirmed only in part. In particular, KLH-specific proliferation was not abolished after oral KLH.

066

Oral tolerance failure upon neonatal gut colonization with *Escherichia coli* producing the genotoxin colibactin

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Introduction: The commensal bacterium *E. coli* belongs to the pioneer microflora colonizing the mammalian gut immediately after birth. *E. coli* can be differentiated in four major phylogenetic groups (A, B1, B2 and D). Recent studies showed that B2 group, including strains producing the genotoxin colibactin, is over-represented while its prevalence is increasing among *E. coli* strains persisting in the microbiota of humans from developed countries, including infants. In parallel, recent evidences demonstrate that inflammatory-related diseases are on the increase in developed countries. Neonatal intestinal microbiota, through the modulation of metabolism and immunity may have long-term consequences on host susceptibility to such diseases.

Aims and Methods: Using an original rodent model of neonatal gut colonization allowing the natural vertical transmission and persistence of *E. coli* strains from mother to the offspring we analyzed the effects of genotoxic commensal B2 *E. coli* on the host oral tolerance to intestinal antigen. Pregnant rats were fed with a human commensal B2 *E. coli*, an isogenic non-genotoxic mutant, or an isogenic genotoxic complemented mutant. After weaning, rats were submitted to an OVA oral tolerance and delayed-type hypersensitivity (DTH) protocol.

Results: We report here that colibactin expression increased intestinal permeability and enhanced intestinal translocation of B2 *E. coli* in neonates. At adulthood, we observed impaired intestinal barrier together with an increase of the permeability through Peyer's patches correlated with enhanced IFN α levels. This alteration of the gut barrier homeostasis exacerbated the mucosal and systemic immune responses against a luminal antigen through an experimental model of OVA-driven oral tolerance. Animals early colonized by colibactin-producing *E. coli* exhibited enhanced OVA-specific response, which was correlated with a defect in Tregs population. Submitted to a mucosal DTH reaction, colibactin-producing *E. coli* colonized animals displayed exacerbated signs of inflammation which was also correlated to a decreased in Tregs population.

Conclusion: The neonatal gut colonization by *E. coli* producing the genotoxin colibactin enhances intestinal translocation and subsequently alters oral tolerance. Thus, through the production of colibactin, early gut colonization by commensal *E. coli* may represent a risk factor for the development of immune-mediated diseases.

067

Oral tolerance susceptibility is related to exacerbated granuloma development in schistosomiasis.

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The *Schistosoma mansoni* infection was studied in two strains of mice genetically selected for extreme phenotypes of susceptibility (TS) and resistance (TR) to oral tolerance. TR strain present good inflammatory responses and a non-tolerogenic profile while TS are non-inflammatory but high-tolerogenic, with high percentages of CD4⁺CD25⁺Foxp3⁺ T regulatory cells, and able to produce high lev-

els of inhibitory cytokines such as IL-10. The aim of this study is to correlate the cytokines production to the pathology caused by infection. TS strain have higher weakness, apathy, prostration and mortality due to hepatosplenomegaly more intense due to the larger size of hepatic granulomas and extensive fibrosis of these granulomas. Both strains produced IFN- α , but TS produced IL4 and IL-10 in a larger quantity, however IL-10 was not able to regulate the growth of hepatic granulomas exacerbated this lineage. High levels of IL-4 in TS strain are consistent with the exacerbation of granulomas, since IL-4, as well as IL-13, induces collagen synthesis and is related to the development of fibrosis in schistosomal granuloma. Additional studies are needed to confirm our proposals and to understand the mechanisms underlying the difference in immune response of these strains in the schistosoma-host relationship.

068

Oral tolerance susceptibility is related to higher reduction of B lymphocytes subpopulations in schistosomiasis.

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The *Schistosoma mansoni* infection was studied in two strains of mice genetically selected for extreme phenotypes of susceptibility (TS) and resistance (TR) to oral tolerance. TR strain present good inflammatory responses and a non-tolerogenic profile while TS are non-inflammatory but high-tolerogenic, with high percentages of CD4⁺CD25⁺Foxp3⁺ T regulatory cells, and able to produce high levels of inhibitory cytokines such as IL-10. The aim of this study is to correlate the cytokines production to the pathology caused by infection. TS strain have higher weakness, apathy, prostration and mortality due to hepatosplenomegaly more intense due to the larger size of hepatic granulomas and extensive fibrosis of these granulomas. Both strains produced IFN- α , but TS produced IL4 and IL-10 in a larger quantity, however IL-10 was not able to regulate the growth of hepatic granulomas exacerbated this lineage. High levels of IL-4 in TS strain are consistent with the exacerbation of granulomas, since IL-4, as well as IL-13, induces collagen synthesis and is related to the development of fibrosis in schistosomal granuloma. Additional studies are needed to confirm our proposals and to understand the mechanisms underlying the difference in immune response of these strains in the schistosoma-host relationship.

070

Oral tolerance susceptibility is related to ultrastructural alterations in adult female and male *Schistosoma mansoni*

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The *Schistosoma mansoni* infection was studied in two strains of mice genetically selected for extreme phenotypes of susceptibility (TS) and resistance (TR) to oral tolerance. The objective was to analyze by Transmission Electron Microscopy the influence of the host immune regulatory profile on the worm morphology. Parasites recovered from TR mice showed no morphological changes. However, specimens collected from TS mice, exhibited tubercle swelling with blunted and shortened spines in lower density. These tegument alterations were similar to those described with artemether or praziquantel treatment, supporting observations that the host immune

system influences the tegument development and function of worms harbored in non anti-helminthic treated TS mice. The ileum oogram from TS mice showed a higher percentage of dead eggs and a lower percentage of immature eggs than TR mice, but had similar quantities of collected eggs. This suggests that in TS mice the alterations in adult worm tegument prevented egg development, as a consequence of decreased glycogen granules and extensive lysis of internal structures, but not egg production reduction. These results corroborate our previous Scanning Electron Microscopy study indicating the influence of the host immune regulatory profile on the development and function of the worm reproductive system and tegument.

071

Pathogenesis of pouchitis--analysis of adherent junction proteins

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Introduction: Pouchitis, which is defined as the inflammation of the ileum mucosa in the pouch, is one of the major complications after restorative proctocolectomy and Ileoanal Pouchimplantation (IAP) in ulcerative colitis (UC) patients. The severity of symptoms varies and quality of patient's life are impaired. There are indications that the pathogenic mechanisms of the underlying disease, i.e. ulcerative colitis are associated with a disturbed response to bacterial antigens in the pathogenesis of pouchitis. An important mechanism in this case represents a failure in the intestinal barrier, which is formed by antimicrobial peptides, the mucus itself, tight and adherent junctions between the intestinal epithelial cells and the mucosal immune system.

Methods: Screening of adherent junction proteins by RTqPCR profiler arrays in a defined collective of pouchitis samples. Validation of target genes by qPCR in an independent collective. Analysis of target antigens by immunohistochemistry. Results: Severe pouchitis in patients with ulcerative colitis shows a significant dysregulation of various adherent junctionproteins compared to the pouch samples of FAP patients.

Conclusion: Pouchitis in ulcerative colitis patients is characterized by a dysregulation of adherent junction proteins compared to FAP patients and may contribute to a fundamental difference between the two disease entities.

072

Perinatal exposure to bisphenol A (BPA) leads to inappropriate innate and adaptive immune responses in intestinal and systemic compartments at adulthood

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Oral route is the major route of exposure to the food contaminant BPA, and intestine the first organ exposed. In rodent models, BPA has demonstrated its ability to interfere with the gut immune system, particularly when perinatal exposure occurred, and predisposing to food intolerance at adulthood. In this study, we report that adult mice perinatally exposed to a low dose of BPA [5 µg/kg BW/day] showed reduced retinoic acid (RA) production by intestinal epithelial cells (IEC). A decrease in lysozyme secretion, and total IgA and IgG production in feces was also observed. These alterations were associated with a defect in dendritic cells maturation (DC) from

lamina propria (LP) and spleen. Frequency of these cell subsets increased in gut mucosa while it decreased in spleen, suggesting a domiciliation defect after perinatal BPA treatment. Concomitantly, a decrease of regulatory and activated T cells in LP and mesenteric lymph node was observed while these sub-populations were increased at splenic level. Interestingly, an alteration in the frequency of innate lymphoid cells ILC3 producing IL-17 and IL-22 occurred in LP, associated with a decrease of aryl hydrocarbon receptor (AhR) expression, known to drive the development of gut ILC22. Our results demonstrated that perinatal exposure to BPA weakens protective and regulatory immune functions of IEC associated with an alteration of DC and T cells populations and ILC3 development. These disturbances in the progeny might impair mucosal tolerogenic responses, and favored inflammatory systemic immune responses, thus increasing susceptibility to food adverse reactions.

073

Post-natal mucus barrier formation in the small intestine and distal colon

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Intestinal mucus is secreted by epithelial goblet cells (GC) and is composed of a limited number of proteins including the gel-forming mucin Muc2. The mucus forms a barrier that restricts microbiota access to the epithelium by region-specific mechanisms. In the colon this is dependent on stacked, tissue adherent multimeric sheets of Muc2 which form an impenetrable filter between bacteria and the colonic tissue. In the small intestine the Muc2 network is non-adherent and penetrable, but forms a matrix containing antimicrobial peptides. Although developmental aspects of GC biology and mucin composition/expression have been partially explored, surprisingly little is known regarding the timing of functional mucus barrier formation and the factors which influence this process. This is of interest considering the susceptibility of neonates to certain intestinal pathogens and necrotizing enterocolitis.

Initial mucus barrier formation was characterised in the small intestine and distal colon by *ex-vivo* analysis of rat tissue using a horizontal Ussing chamber-like system that allows quantification of mucus thickness, penetrability and growth rate. Immunohistochemical staining of methanol-Carnoy fixed tissue sections with antibodies against both the apoprotein and mature forms of Muc2 allowed *in-vivo* assessment of mucus barrier formation, Muc2 synthesis/secretion and GC distribution. Tissue obtained from both conventionally-raised (ConvR) and germ-free (GF) mice was used to assess the potential role of bacterial colonisation in barrier formation. Analysis of the microbiota was undertaken using qPCR targeting the bacterial 16S gene with universal and taxon-specific primers.

Although GCs expressed and secreted Muc2 *in-utero*, generation of a functional mucus layer in both the small intestine and the distal colon occurred post-natally. Colonic barrier formation was characterised by rapid alterations in mucus properties and GC distribution preceding a more steady increase in the rate of mucus processing. Slower barrier formation was observed in the small intestine, characterised by the gradual release of stored Muc2 from villus GCs. ConvR barrier development was strongly correlated with increased bacterial numbers and absent in GF animals providing a strong causative link between colonization and initial barrier formation.

074

Protease-activated receptor 2 is required for inflammation of the small intestine triggered by *T. gondii* in C57BL/6 mice**C. Bonnard¹, G. Feuillet¹, V. Vasseur¹, N. Cenac¹, B. Gilmore² & N. Blanchard¹**¹*Inserm U1043 CPTP, place Baylac, Toulouse, France,* ²*School of Pharmacy, 97 Lisburn Road, Belfast, UK*

Toxoplasma gondii (T.g.) is a widespread parasite present on all continents. Infection by T.g. persists in one third of the human population and can cause severe neurological and ocular pathologies called toxoplasmosis. T.g. naturally enters the organism via the oral route and crosses the small intestinal barrier to reach internal organs of the body. Interestingly, in several mammals, small intestine inflammation is a pathological feature associated with T.g. infection, which is highly reminiscent of the Crohn's disease (CD) pathology. Previous studies have highlighted the contribution of proteases, protease-activated receptors (PAR) and their signaling pathways, as inflammatory modulators in the gut. Using the gut inflammation model triggered by T.g. infection in C57BL/6 mice, we show that the development of intestinal inflammation requires active PAR2 signaling. Following gavage with T.g. cysts, PAR2-deficient animals exhibited milder inflammation, characterized by lower microscopic and macroscopic inflammation scores and lower up-regulation of pro-inflammatory mediators IL-6 and KC. In addition, a number of pro-inflammatory polyunsaturated fatty acid metabolites were increased in infected wild-type animals but not in PAR2-deficient animals. Interestingly, production of IFN γ and TNF α , two key effector cytokines in this pathology, was not affected by PAR2 deletion. Finally, using innovative protease activity-based probes, our data revealed the existence of a clear disequilibrium in active serine proteases between inflamed and normal gut tissues. Identification of these dysregulated proteases is underway to investigate their role in the PAR2 signaling cascade leading to gut inflammation in the small intestine. This study may pave the way towards the discovery and modulation of new key proteases, potentially relevant for the treatment of intestinal inflammation disorders.

075

Querying the intestinal host-pathogen response to *salmonella* infection using high-throughput kinomics**R. Arsenault, C. Swaggerty, K. Genovese, H. He & M. Kogut***United States Department of Agriculture, College Station, USA*

Enteric pathogens have a profound impact of the functioning of the gastrointestinal tract. Serovars of *Salmonella* elicit a strong pro-inflammatory response in species such as humans and bovine. However, in species such as mice and chickens a significant immune response is not observed and the bacterium appears to be well tolerated. *Salmonella* only elicit disease in mice through the use of antibiotic pre-treatment and in chickens by infecting the animals very early post-hatch. This differential response is of significant interest as it may elucidate mechanisms of *Salmonella* pathogenesis, host response and microbiota tolerance. Our group has considered two serovars of *Salmonella*, Typhimurium and Enteritidis, and the host-pathogen interactions in the chicken gut. We have performed this analysis at the kinome level; the differentially active kinases within the gut tissue. We compared infected and non-infected animals for host response. Our observations point to the induction of a response at the interface of immunity and metabolism. The key cellular signaling intermediates that have appeared in our analyses include mTOR, GSK3B, MAPKs, AKT, and AMPK. We have observed that approximately 4 days post-infection the response switches from one that is pro-inflammatory to one that suppresses immune response generating a tolerance of infection. In addition, in a study involving the *Salmonella* Typhimurium mutant

lacking the *AvrA* virulence gene we observed distinct differences in the pattern of phosphorylation within the ErbB signaling pathway. This included not only the ceca, the natural site of infection, but also the adjoining jejunum, indicating that the *AvrA* mutant *Salmonella* is able to alter signaling at alternative sites along the gastro-intestinal tract.

076

Regulation of Intestinal Immune Responses by the Atypical Chemokine Receptor ACKR4**C. Thomson, A. Mowat & R. Nibbs***Institute of Infection, Immunity and Inflammation, University of Glasgow, UK*

Whether they are aimed at conferring protective immunity or tolerance, immune responses in the small intestine are fundamentally dependent on the action of chemokines and their cognate receptors. For example, CCL19 and CCL21 facilitate the migration of CCR7⁺ mucosal dendritic cells from the lamina propria to the mesenteric lymph nodes (MLN), whilst CCL25 recruits CCR9⁺ gut-homing leukocytes into the lamina propria from the circulation. Interestingly, CCL19, CCL21 and CCL25 also bind to ACKR4 (CCRL1), an atypical chemokine receptor that is expressed in both the intestine and the MLN. In vitro studies show that, rather than inducing cell migration, ACKR4 binds and internalises its ligands, targeting them for intracellular degradation. ACKR4 is therefore proposed to regulate chemokine receptor dependent migration by scavenging extracellular chemokines. However, the expression and function of ACKR4 in vivo, particularly in the intestinal immune system, has not been investigated in any great depth. Here, using ACKR4-eGFP reporter mice and ACKR4-deficient mice, we have identified which cell types express ACKR4 in the lamina propria and gut-associated lymphoid tissues. Furthermore, we have examined the impact of ACKR4 deficiency on immune responses in the small intestine. These studies aim to establish the role of ACKR4 in intestinal immunity, and dissect the complexity of chemokine networks in the small intestine.

077

Relationship between Th17 and Treg in nasopharynx-associated lymphoid tissue and their association with age and pneumococcal carriage in humans**A. Mubarak¹, V. Casey², N. Upile², C. Xie², R. Sharma², H. Beer³, M. McCormick³, M. Ahmed¹, P. McNamara⁴ & Q. Zhang¹**¹*Clinical Infection and Immunology, Liverpool, UK,* ²*ENT Department, Alder Hey Children's Hospital, Liverpool, UK,* ³*ENT Department, Royal Liverpool University Hospital, Liverpool, UK,* ⁴*Department of Women's and Children's Health, University of Liverpool, Liverpool, UK*

Streptococcus pneumoniae is a leading cause of respiratory tract infection in humans. *S. pneumoniae* colonizes human nasopharyngeal mucosa, and carriage is common in young children that may account for the high incidence of pneumococcal disease in this age group. Pneumococcal carriage rate in humans decreases with age due to natural immunity. However, the immunological factors responsible for the clearance of pneumococcal carriage remain undefined. Recent studies in mice suggest Th17 is important in host clearance of *S. pneumoniae*. We have studied the relationship between numbers of mucosal Th17 and T regulatory cells (Foxp3⁺ Treg) in human nasopharynx-associated lymphoid tissue (NALT) and age and their association with pneumococcal carriage in children and adults. Numbers of Th17 and Treg in adenotonsillar tissue were significantly higher than in peripheral blood ($P < 0.01$) in both children and adults. There was a negative correlation between numbers of Th17 and Treg in adeno-

tonsillar tissue ($r = -0.52$, $P < 0.01$). Numbers of adenotonsillar Th17 were shown to correlate with patients age ($r = 0.62$, $P < 0.01$), whereas numbers of Treg were inversely correlated with patients age ($r = -0.50$, $P < 0.01$). Furthermore, there was a positive correlation between the Th17/Treg ratio in NALT and age of patients. Also, the Th17/Treg ratio was significantly higher in pneumococcal carriage negative than positive children ($P < 0.05$). Our findings suggest that the balance/ratio of mucosal Th17 and Treg in nasopharynx is a critical determinant of pneumococcal clearance/carriage, and support efforts to promote mucosal Th17 in vaccination strategy against pneumococcal infection in humans.

078

Retinoic acid modulates the early expansion and differentiation of CD4⁺ T cells during the development of intestinal inflammation

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Epidemiological studies of vitamin A-deficient populations have illustrated the importance of vitamin A and its metabolite all-trans retinoic acid (RA) in mucosal immune responses. In support of that finding, we and others have shown that RA produced by intestinal dendritic cells is required for the generation of gut-tropic T cells and IgA-secreting plasma cells. Evidences from the literature suggest antagonistic roles for RA in intestinal T cell biology. In the homeostatic intestine RA plays anti-inflammatory functions, sustaining the development of foxp3⁺ regulatory T cells, while restraining Th17 effector T cell differentiation. In contrast, the role of RA in the control of intestinal CD4 T cell responses under infectious or inflammatory conditions remains to be more clearly defined.

RA has been shown to control the CD4⁺ T cell compartment by signaling through the RA receptor RAR α . We therefore took advantage of mice expressing a dominant negative form of the RAR α , specifically in the CD4 T cell compartment (RARdn CD4Cre⁺ mice), in order to conditionally ablate RA signaling in CD4⁺ T cells during the development of intestinal inflammation in the T cell transfer colitis model.

Histological, flow cytometry and real time PCR analyses indicate that RA signaling negatively modulates the early expansion of CD4⁺ T cells following their transfer into RAG^{-/-} mice, but also their differentiation into IL-17-producing cells in a ROR γ t-dependent manner. Indeed RA signaling-deficient CD4⁺ T cells selectively accumulate and showed increased capacities to differentiate into IL-17⁺, IL-17⁺IFN γ ⁺ and foxp3⁺ cells, but reduced capacities to differentiate into IFN γ ⁺ cells and were less potent at inducing intestinal inflammation. RA signaling-deficient CD4⁺ T cells were also unable to differentiate into cytotoxic T lymphocyte (CTL)-like cells within the intraepithelial lymphocyte compartment of the colon. We are currently deciphering the mechanisms of these effects of RA on CD4⁺ T cells.

079

T cell receptor repertoire in refractory celiac disease as revealed by next generation sequencing

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Introduction: Refractory celiac disease (RCD) is a Marsh III enteropathy with accompanying malabsorption in spite of stringent gluten-free diet (GFD) for >1 year. Contrary to type-I RCD, a clonal T cell population builds up in type-II RCD and develops further to overt T cell lymphoma in a high percentage of cases. In most institutions, diagnostics to differentiate the two subtypes is based on GeneScan technology determining fragment sizes of PCR-amplified T cell receptor (TCR) DNA sequences and immunohistology. However, phenomena as “oligoclonality” and “pseudoclonality” are frequently found in GeneScans and add to a specificity problem. Moreover, the sequences of the variable regions of the involved TCRs are unknown. **Aims:** To shed further light on TCR biology in RCD and to do a proof-of-concept study for diagnostic testing.

Methods: Determination of the T cell repertoire of duodenal mucosa by multiplex PCR of TCR β -CDR3 regions. Next generation sequencing (NGS, Illumina HiSeq 2000) of amplicons in controls, active celiacs (ACD), celiacs with GFD, unclassified Marsh I cases, RCD type-I and RCD type-II.

Results: On average, 10⁶ sequences per sample were analyzed, yielding ~1000 TCR rearrangements. Clonotypes were defined as identical sequences of CDR3 amplicons. Averaging all RCD type-II, the most frequent clonotype was 52.5% of all clonotypes per sample, which was significantly higher than in controls (16.7%; $P < 0.01$) or RCD type-I (17.3%; $P < 0.001$, cut-off>0.5%). ACD revealed a non-significant tendency towards a higher frequency. Interestingly, patients with high clonotype frequencies developed significantly more often a T cell-lymphoma. The technique was validated by identifying the same top clonotypes in various individuals receiving two examinations months apart. When searches for alignments with previously published CDR3 sequences were done, a significant portion of these sequences was found in our celiac samples, however not within the high frequency clonotypes (HFC). Moreover, HFC's of individual RCD II patients did not reveal cross-homology, arguing against specific TCR motifs in RCD II pathophysiology.

Conclusion: TCR β -NGS-analysis reproducibly detects monoclonal T cell populations in RCD-II. The TCR-CDR3-HFCs in RCD-II are not previously described, gliadin-associated TCR sequences, which might explain their potential to expand gliadin-independently.

080

T cell specificity towards native versus deamidated gluten epitopes in celiac disease

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Background: Celiac disease (CD) is a chronic, inflammatory disease of the small intestine, which occurs in genetically predisposed individuals after ingestion of gluten from wheat, barley and rye. The gluten-specific CD4⁺ T cells play a crucial role in the pathogenesis of CD, where T cell receptors (TCR) recognize gluten epitopes displayed by HLA-DQ2/8 molecules. Deamidation of gluten epitopes by

tissue transglutaminase 2 is known to enhance the binding capacity to HLA-DQ2/8. TCRs contain highly variable regions called complementarity-determining regions (CDR). In this study, we investigated whether different mucosal gluten-specific CD4⁺ T cells from CD patients recognize native and/or deamidated peptides.

Methods: We established five gluten-reactive T cell lines (TCLs) from duodenal biopsies obtained from four children and one adult with CD. The reactivity of the TCLs was tested in proliferation assays against a panel of four deamidated and three native gluten epitopes. A pair of one deamidated and one native epitope that elicited the strongest T cell proliferation in all TCLs was used in nested-PCR-based CDR3 diversity determination of one TCL.

Results: We found that all TCLs proliferated after stimulation with both native and deamidated peptide. The response to deamidated peptide was significantly stronger. The CDR3 length distributions of responding cells in one TCL displayed overall three different patterns according to the CDR3 lengths.

Conclusion: We confirmed that the deamidation of gluten epitopes enhance the T cell proliferation, but that native peptides also induce a response, albeit lower. We cannot yet make conclusions based on our preliminary results from CDR3 diversity determination of one TCL.

081

Th9 cells drive IL-9 mediated colitis-associated colorectal cancer (CAC)

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Introduction: Interleukin 9 is a pleiotropic pro-inflammatory cytokine mainly produced by T cells, beside B cells and mast cells. Recently, a specialized subset of T cells has been identified, dedicated to produce IL-9 and therefore named Th9 cells. In these cells the IL-9 gene is regulated by transcription factors like PU.1 and IRF4. IL-9 has been found to exert many biological functions, but the role of Th9 cells in colitis and colitis-associated cancer is still unclear. High concentrations of IL-9 in the colon tissue of colitis patients point out the important role for this cytokine in the development of colitis, but the involvement of Th9 cells in antitumor immunity is still not known. For this reason we started to investigate the role of IL-9 and Th9 cells in the development of colitis-associated colorectal cancer (CAC).

Methods: IL9KO mice were subjected to the experimental oxazolone model to induce colitis and treated with AOM/DSS for the development of colon cancer. Miniendoscopic analysis has been done to monitor the inflammation and the number and size of tumors. For restoration of the IL-9 deficient phenotype an IL-9 expressing mini-circle DNA vector was injected into IL9KO mice by hydrodynamic injection and the model of AOM/DSS has been investigated. Tumors of the colon have been isolated for histological sections and stained with T cell specific antibodies and PU.1. For analysis of cytokines and transcription factor expression RT-PCR was performed.

Results: As IL9KO mice were protected in the experimental oxazolone-colitis model, we analysed those mice in the tumor model. We observed a significant reduction of tumor size and numbers in IL9KO mice. Furthermore, we explored the effect of IL-9 on tumor growth by overexpression of IL-9 via mini-circle DNA and AOM/DSS treatment, which led to the development of tumors when IL-9 is absent. Additionally, in cryosections of tumorigenic colon tissue more PU.1⁺ T cells were found, pointing out the important relevance for Th9 cells in colon cancer.

Conclusion: Our findings uncover a crucial role of IL-9 producing effector T cells in murine ulcerative colitis and colitis-associated neoplasias. The presence of PU.1⁺ CD4⁺ -expressing cells in inflamed and tumorigenic tissue suggests that these cells belong to the recently described Th9 T cell subset.

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The anatomical origins of migratory dendritic cells in the intestine

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Dendritic cells (DCs) are fundamental in controlling immune responses in the small intestine (SI) and colon; they migrate to the mesenteric lymph node (MLN) and prime gut-tropic effector or regulatory T cells. The aim of this work was to determine whether the DCs that migrate to the MLN from the intestine originate in the lamina propria (LP), or from the Peyer's patches (PP). First, the anatomical organisation of the lymphatic vessels draining to the MLN from the SI and colon was investigated. Once this had been established, the migration of DCs from the LP and PPs to the MLN was examined.

Here, I demonstrate the anatomical segregation of DCs that migrate to the MLN from the SI and colon. After SI photoconversion in fluorescent transgenic Kaede mice, converted DCs were found only in the SI-draining MLN. Likewise following photoconversion of the colon in Kaede mice, converted DCs were detected only in the colonic-draining MLN. To examine the functional consequence of this anatomical compartmentalisation of lymphatic drainage, mice that express ovalbumin only in the SI epithelium were used. Only DCs isolated from SI-draining MLN, but not the colon-draining MLN, were able to present SI derived antigen and cause proliferation of naïve T cells.

To investigate DC migration from PPs to the MLN, PPs in Kaede mice were photoconverted. DCs were then detected in the SI-draining MLN, indicating that some DCs migrate from the PP to the MLN. Migration of PP DCs to the MLN was reduced in CCR7^{-/-} mice and in mice pre-treated with FTY720. Thus, we have identified that a proportion of DCs migrate from the PP to the MLN by a CCR7 and S1P dependent mechanism. These findings open new avenues for controlling the functions of intestinal DCs, and therefore manipulating the intestinal immune response, with exquisite precision.

083

The effect of oral *Salmonella Typhimurium* infection on intestinal mononuclear phagocytes

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Recent literature contains contradictory data regarding the functions, and even the identification of the antigen presenting cells (APCs) in the intestine that induce responses after infection with *Salmonella typhimurium* (STM). We have developed tools to unambiguously identify intestinal CD11c⁺ APC populations, and have begun to examine the changes in dendritic cells (DCs) and macrophages that occur after oral infection of antibiotic-treated mice with STM.

To investigate intestinal phagocytes, WT C57BL/6 or CX3CR1^{+/GFP} mice were treated with an antibiotic cocktail for 14 days, rested for 48 hours and infected orally with Δ invG strain of STM. Mononuclear phagocyte populations in the small intestinal lamina propria (LP) and mesenteric lymph nodes (MLNs) were analysed by flow cytometry 48 h after infection with STM. For lymph analysis, the thoracic duct of mesenteric lymphadenectomised mice was cannulated 24 h after STM infection. Lymph was collected for 16 h, and analysed by flow cytometry.

No differences were observed in the frequency or number of total migrating CD11c⁺ MHCII^{hi} DCs in the MLNs after STM infection, nor were there changes in the frequencies of DC subsets. However, analysis of small intestinal LP phagocyte populations revealed small, but significant, differences in both CD64⁺ macrophages as well as in DC subsets between infected and non-infected mice. Furthermore, significantly higher proportions of CD11c⁺ MHCII⁺ DCs were present in lymph collected from infected animals, and within these there was a significant increase of the CD103⁺ CD11b⁺ DC subset. Furthermore, no CX3CR1^{hi} expressing cells were detected in lymph of STM infected animals.

In conclusion, we provide here a detailed analysis of the intestinal phagocyte populations after STM infection. Oral infection with STM is reported to cause specific changes in the myeloid cells that migrate from the intestine. We have now been able to conclusively show that oral STM infection does indeed lead to an increase in the proportion of migrating DCs from the intestine, and to changes in the frequencies of migrating DC subsets. In addition, we demonstrate that CX3CR1^{hi} macrophages do not migrate into lymph after STM infection.

084

The effect of probiotic consumption on routine haematological and immune markers in a cohort of healthy active adults

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In recent meta-analyses, a positive effect of probiotics against upper respiratory tract illnesses has been noted, but the underlying immune system changes are still mostly obscure. We have reported in a three-arm clinical trial ((i) Bifidobacterium lactis BI-04 (BI-04), (ii) Lactobacillus acidophilus NCFM and Bifidobacterium lactis Bi-07 (NCFM+Bi-07), or (iii) placebo) that daily consumption of BI-04 by healthy active adults reduces the risk of experiencing upper respiratory illness (URI) episodes during 5-month winter season. To study immune system changes, pre and post supplementation blood samples were drawn from a cohort ($n = 144$) of the study subjects. The samples were analyzed for electrolytes, liver and thyroid function, leukocyte counts, plasma immune biomarkers ($n = 34$), tumoricidal NK-cell activity, and phagocytic activity of granulocytes and monocytes. Differences in plasma calcium ($P = 0.03$) and urea ($P = 0.015$) were observed between the probiotic and placebo groups, however these were within assay-specific established laboratory reference ranges. The results showed that in BI-04 group the level of plasma chemokine MIP-1 δ (CCL15) increased by 29 % compared to placebo. In NCFM+Bi-07 group there was a 7.7 % increase in total lymphocytes and 11 % increase in plasma matrix metalloproteinase 1 level. There were no changes in granulocyte, monocyte or NK-cell activities between the study groups. In conclusion, probiotic supplementation with BI-04 or NCFM+Bi-07 is considered safe and did

not have a major effect on the biomarker profile or innate cell functions in healthy active adults.

085

The gut-associated lymphoid tissues in small intestine, not the large intestine, play a critical role in oral prion disease pathogenesis

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Many prion diseases, characterised by the accumulation of PrP^{Sc}, an abnormally folded isoform of the cellular prion protein (PrP^C), are acquired orally. Following oral exposure, high levels of PrP^{Sc} accumulate upon PrP^C-expressing follicular dendritic cells (FDC) in gut-associated lymphoid tissues (GALT) prior to neuroinvasion. Accumulation of PrP^{Sc} in large intestinal GALT has been used diagnostically in humans and other animal species; however, the role of GALT in large intestines in oral prion disease pathogenesis was unknown. In order to precisely distinguish between the influence of GALT in the small and large intestines in oral prion pathogenesis, we created mice that were specifically deficient in FDC-containing GALT only in the small intestine. Oral prion disease susceptibility was blocked in the specific absence of the GALT in small intestine. Although these mice had FDC-containing GALT throughout their large intestines, these tissues did not accumulate prions and the mice were refractory to infection after oral exposure. We also studied whether pathology specifically within the large intestine might influence prion pathogenesis. Congruent infection with the nematode parasite *Trichuris muris* at the time of prion exposure did not significantly affect disease pathogenesis. Together, these data definitively demonstrate that FDC-containing GALT in the small intestine are the critical early sites of prion accumulation and neuroinvasion after oral exposure. In contrast, large intestinal GALT does not influence prion pathogenesis during steady-state or inflammatory conditions. The demonstration that GALT in the small intestine, not the large intestine, are the important sites of prion accumulation and neuroinvasion has important implications for our understanding of the factors influencing the risk to infection and the pre-clinical diagnosis of disease.

086

The inhibition of IKB kinase ameliorates experimental colitis in rats

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Introduction: The transcription factor NF κ B seems to play a crucial role in the pathogenesis of IBD conditions. In this study we have investigated the impact of IKK inhibition on TNBS experimental colitis in rats.

Methods: Wistar rats (200 g) were assigned to five groups ($n = 10$): Non colitic, control colitic and colitic groups treated intraperitoneally with IKK 16 (inhibitor of IKK) (0.3 and 1 mg/kg/day), and dexamethasone (1.2 mg/kg/day), starting the same day of TNBS colitis induction (10 mg). Rats were sacrificed one week after, and colonic damage was assessed histologically and biochemically, by myeloperoxidase activity (MPO). In addition, colonic qPCR analysis was performed to evaluate the expression of pro-inflammatory cytokines (TNF α , IL-1 β , IL-6, IL-12, IL-17), the enzyme iNOS, chemo-

kines (MCP-1 and CINC-1), the adhesion molecule ICAM-1, as well as proteins involved in the preservation of intestine epithelia (mucins MUC-2 and MUC-3, trefoil factor TFF-3 and villin).

Results: IKK 16 administration resulted in an intestinal anti-inflammatory effect as evidenced macroscopically by a reduced extension of colonic damage in comparison with control colitic rats. Biochemically, a decrease in colonic MPO activity was observed, indicating a lower neutrophil infiltration in the inflamed tissue. qPCR evaluation of cytokines expression showed that IKK 16 treatment decreased colonic expression of the proinflammatory cytokines TNF α , IL-1 β , IL-6, IL-12 and IL-17. In addition, the IKK inhibitor reduced the expression of the chemokines CINC-1 and MCP-1, the inducible enzyme iNOS, and the adhesion molecule ICAM-1. In addition, this compound significantly up-regulated the expression of markers of intestine epithelial integrity. These effects were similar to those obtained with dexamethasone.

Discussion and Conclusion: The inhibition of NF κ B signalling pathway by the IKK inhibitor IKK 16 results in beneficial effects in TNBS rat colitis through an improvement of the exacerbated immune response that characterizes the colonic inflammatory process.

087

The intestinal mucus phenotype in Clca3 deficient mice

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The intestinal mucus has been shown to be important for innate immunity by keeping bacteria at a distance. The MUC2 protein is the main component in the mucus, giving the mucus its features and network-like structure. Proteomics has also revealed Clca3 (chloride channel, calcium activated 3) as one of the most abundant non-mucin proteins found in the intestinal mucus. Clca3 was first proposed to be a calcium regulated chloride channel but is today considered to be fully secreted. Despite extensive research the function of Clca3 in the mucus is still obscure.

The domain architecture which includes a metallohydrolase domain followed by a von Willebrand factor type A domain suggests both protease activity and potential binding to other proteins. The abundance of Clca3 in mucus together with the domain architecture has led to the hypothesis that Clca3 has an important and structural role in the intestinal mucus.

We have examined the mucus phenotype in Clca3 deficient mice to test our hypothesis. MUC2 expression and distribution has been investigated by immunohistochemistry which has also allowed examination of the mucus structure. Further, the mucus layer thickness, growth, tissue attachment and responsiveness to stimulation were examined in an Ussing chamber-like ex vivo explant system. The barrier function of the mucus against bacteria was assessed by an ex vivo penetrability assay in parallel with fluorescent in situ hybridization of Carnoy-fixed tissue sections. In addition, a proteomic comparison between wild-type and Clca3^{-/-} mucus was performed to look at differences in other major mucus components.

088

The liver: An underestimated effector site of the intestinal IgA response?

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IgA, the most abundant immunoglobulin of the body, maintains host-microbiota homeostasis and protection against infectious microbes, without inducing overt tissue inflammation. IgA isotype switching and B cell differentiation into plasma cells [PC] are mainly initiated in mucosal-associated lymphoid tissues [MALT] and involve a range of cells and factors involved in oral tolerance. Capitalizing on our recent demonstration that the liver is a critical inductive site of T cell tolerance to intestinal antigens [Ag] (drained to the liver via the portal vein), and is enriched in tolerogenic dendritic cells which favor IgA responses, we explored the role of the liver in the IgA response at steady state and after oral immunization.

Mouse liver at steady state contains around 20% of B cells, including a majority of IgD⁺ follicular B2 cells, few B1 cells, and is enriched in IgA PC (75% of liver PC), compared to the bone marrow, mesenteric LN, spleen and blood. In addition, we found that human liver contains around 5% of CD19⁺ cells, mostly represented by PC, half of which producing IgA. These IgA PC are localized in the hepatic parenchyma close to portal tracts.

Oral immunization with the T-cell-independent Ag NP-Ficoll and cholera toxin as adjuvant generated specific IgA producing cells in the liver and intestinal lamina propria, suggesting that the liver might constitute an alternative site of the IgA response. Using a transfer model of NP-specific transgenic B cells from Quasi Monoclonal mice, we found that oral immunization with NP-Ficoll induced IgA-expressing B cells initially in Peyer's patches, and later IgA⁺CD19^{low}CD138⁺ cells in the liver, indicating that IgA isotype switching in MALT was rapidly followed by homing of IgA plasmablasts/PC to the liver. In this respect, a unique phenotype in term of i) proliferative state, ii) expression of B cell and PC markers, and iii) homing molecules (chemokine receptors CXCR-4, CCR-9, CCR-10, integrin α 4 β 7) was observed on liver IgA PC at homeostasis. Taken together, these data indicate that the liver constitutes an early site of homing of IgA PC primed in the intestine.

We propose that both in mice and human, the liver may be an underestimated effector site for the mucosal IgA response, which may maintain local homeostasis and fuel the duodenum with IgA translocating via the bile.

089

The mucosal environment influences the systemic inflammation observed in Spondyloarthritis patients

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Ankylosing Spondylitis (AS) is a chronic systemic inflammatory disease belonging to the spondyloarthritis (SpA) group of genetically and pathophysiologically related diseases. Alongside inflammation within the vertebral column and sacroiliac joints, AS patients often develop extra-articular disease manifestations, includ-

ing inflammatory bowel disease (IBD). Specifically, approximately 10% of AS patients develop overt IBD whilst 60–70% of AS patients show evidence of subclinical intestinal inflammation. Dendritic cells (DCs) activate naïve T cells and maintain the balance between immunity and tolerance, and may therefore contribute to controlling inflammation in AS. To understand the contribution of DCs to AS, it is important to establish their role in driving systemic and tissue-localised inflammatory responses in patients. We therefore characterised circulating and intestinal DC populations, to elucidate the unique and shared immunological pathways driving these interconnected diseases.

Compared to age-matched HCs, blood from AS patients contained a significantly higher ratio of pro-inflammatory (CD16⁺ CD14⁻ CD11c⁺) mononuclear cells compared to CD1c⁺ DCs. This CD16⁺ population induced CCR6 expression on responding naïve T cells and promoted secretion of the Th17-associated cytokines IL-1 β and IL-6. Interestingly, the frequency of circulating CCR9⁺ CD4⁺ T cells in AS patients increased with inflammation, indicating potential mucosal involvement. We have now developed robust methods for purification of live cells from fresh intestinal tissue, allowing enumeration and functional analyses of intestinal myeloid populations. Initial analyses of intestinal DC subsets (CD45⁺ CD14⁻ CD64⁻ CD11c⁺ MHC II⁺ CD103^{+/}-) in health and disease have been performed.

In summary, we have identified a shift in circulating myeloid populations towards the CCR6-inducing CD16⁺ population that may contribute to the induction of T cell-mediated AS pathogenesis. Furthermore, our data implicate mucosal DCs in driving systemic inflammation in AS patients. Our analyses of live cells prepared from fresh intestinal tissue enable precise examination of the disease-inducing tissue resident effector populations. We anticipate that comparisons of these immune cell populations in AS and IBD patients with healthy controls will reveal important details about pathogenic mechanisms controlling disease development.

090

The neoantigen KLH induces antigen-specific regulatory T cells in humans

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Oral tolerance can prevent and treat autoimmunity in animal models, but its efficacy in humans is unclear. We use the neoantigen keyhole limpet hemocyanin (KLH) to healthy volunteers to investigate antigen-specific tolerance. Foxp3(+) CD4(+) CD25(+) regulatory T cells (Treg) are known for their suppressive function. Here we report our investigations about the systemic Treg population. Two groups of eight healthy volunteers each were compared. One group received 50 mg KLH per day orally for ten days, the other did not and served as control. Both groups were then subcutaneously immunized with KLH and a DTH skin reaction was provoked by an intradermal KLH injection. Follow-up investigations were done six months later. At selected time points, PBMC were isolated and samples were stimulated for 16 hours by anti-CD28, by anti-CD28 in combination with KLH, and by staphylococcal enterotoxin B (SEB). Afterwards the samples were stained for CD4, Foxp3, CD25, CD127, CD45RA, CD137, CD154, CD14, CD19, and analyzed by flow cytometry.

The study is ongoing and three subjects were analyzed so far. Comparing cells stimulated by anti-CD28 combined with KLH and anti-CD28 alone, KLH-specific Treg were identified as the Foxp3(+) CD4(+) CD25(+) population after the exclusion of CD14(+) CD19(+) L/D aqua(+) cells. Treg were seen only after parenteral immunization but not after oral antigen alone. Treg appeared ten days after

parenteral immunization and were detectable for at least two weeks with at most 1.5 % KLH-specific Treg per CD4(+) T cells. At follow-up after six months no KLH-specific Treg were found but these reappeared quickly three days after reimmunization; similar kinetics were found in the CD4(+) CD127(-), the CD4(+) CD137(+) CD154(-) and the CD4(+) CD45RA(-) Foxp3(+) populations. Ten days after initial parenteral immunization with the neoantigen KLH antigen-specific Treg can be detected in the peripheral blood. As expected for a secondary immune response Treg reappear quickly after reimmunization at follow-up. The effect of oral KLH on antigen-specific Treg induction remains to be analyzed.

091

The novel oral vaccine delivery technology SmPill® significantly increases vaccine-specific intestinal antibody responses

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Enteric infections are a major worldwide health problem causing high levels of mortality and morbidity especially in developing countries. Oral vaccination is regarded as the optimal means to fight intestinal infections as it elicits immune responses at intestinal and systemic levels. However, one of challenges of oral vaccination is the degradation of active components by stomach acids and enzymes. This challenge can be overcome by the use of a novel technology named Single Multiple Pill (SmPill®). SmPill® comprises 1-mm microspheres allowing encapsulation of active components. A unique coating surrounds the core of the microspheres protecting the payload from destruction at low pH and allows its specific release at higher pH in the small intestine. A formalin-killed whole cell enterotoxigenic Escherichia coli (ETEC) candidate vaccine and the effective oral adjuvant α -Galactosylceramide have been enclosed inside the microspheres. Microscopy studies clearly demonstrated aggregates of bacteria inside the microspheres. In addition, simulated intestinal fluid (pH 6.8) was shown to induce the disorganization of the internal structure of microspheres as well as the release of bacteria. Finally, we assessed that SmPill® significantly increased the ability of the ETEC vaccine to rapidly generate high titres of vaccine-specific intestinal IgA and IgG in mice. Intestinal T and dendritic cell responses as well as the underlying mechanisms of intestinal B cell responses leading to enhanced antibody responses are currently being analysed. In summary, these data highlight the potential that SmPill® technology has to enhance the effectiveness of oral vaccine delivery.

092

The phagocyte oxidase in infection: A gut perspective

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Well recognized for their role in phagocyte-mediated pathogen killing, reactive oxygen species (ROS) such as O₂ and H₂O₂ are also an integral part of the host defense system and inflammatory responses at mucosal surfaces. The cellular sources of pathogen-triggered ROS generation are predominantly NADPH oxidases. The best characterized member of the NADPH oxidase family is the phagocyte NADPH oxidase (Nox2 complex). Its critical role in host defense is

highlighted by the life-threatening recurrent fungal and bacterial infections observed in patients with chronic granulomatous disease (CGD), a genetic disorder in which Nox2 function is defective due to mutations in its essential subunits. We used Nox2 knockout (KO) mice for investigating the interplay between inflammation, anti-bacterial immunity and microbiota after *Citrobacter rodentium* infection. Infection with upregulated expression of Nox1 and Duox2, the main oxidases expressed in the intestinal barrier, in a similar manner in both wild type (WT) and KO mice. However, loss of Nox2 activity and consequently of ROS generation increased the overall pathology after oral challenge with *C. rodentium*. Nox2-deficient mice showed increased pathogen colonization followed by pronounced mucosal and submucosal colon damage. Changes in the microbiota composition affect the physiology and homeostasis of the host GI tract. Although *C. rodentium* infection changed the microbiota complexity in WT and KO mice, the shift was more pronounced in Nox2 KO mice with a significant increase of the *Bacteroidetes* phyla. Loss of Nox2-derived ROS caused overgrowth of *Enterobacteriaceae*, a family of bacteria previously linked to host gut inflammation. Long term antibiotic treatment induces dysbiosis and susceptibility to infection and colitis. Our results reveal that dysbiotic Nox2 KO mice infected with *C. rodentium* exhibited poor survival despite enhanced Nox1 and Duox2 levels. Even though pathogen colonization of the GI tract was similar to dysbiotic WT mice, Nox2 KO mice showed bacterial translocation to liver, spleen and cardiac blood. These findings suggest a role of phagocyte-derived ROS in limiting bacterial dissemination and systemic disease in the absence of microbiota-mediated protection.

093

The role of dendritic cells in inducing Th2 responses to schistosoma mansoni eggs

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Schistosoma mansoni eggs (SME), which are the main cause of chronic Schistosomiasis, provoke a strong Th2 immune response that can lead to granulomatous reactions and fibrosis in affected organs. Recently, CD11c depletion has been shown to severely disrupt Th2 immune responses against *Schistosoma mansoni*, suggesting that antigen presenting cells, which express CD11c, mediate this immune response. Dendritic cells (DCs) are the most likely CD11c-positive candidates, as they continuously migrate from epithelia, carrying antigens, to the draining lymph nodes where they activate naive T-cells. However, the precise roles of DCs in inducing immune responses against SME have not been studied.

We have developed an in vivo mouse model to inject SME in the subserosal layer of the small intestine of C57BL/6 mice, the anatomical location where SME naturally become lodged. This provokes a Th2 response in the draining mesenteric lymph nodes (MLNs). To test the role of DCs in this response, we will purify migrating DCs from egg challenged mice and control animals and then transfer these cells into the subcapsular region of the MLN of naive mice. Detection of an antigen-specific Th2 response in those mice will indicate that migratory DCs are sufficient to carry and present egg antigens and drive Th2 responses in our model.

This knowledge will contribute to a better understanding of how the early immune response against SME is mediated. This information can be applied to battle chronic Schistosomiasis and may help understand Th2 driven responses against other parasites or Th2 allergic responses.

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The role of follicular dendritic cells in CTA1-DD mediated germinal centre formation

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In the germinal centre (GC), follicular dendritic cells (FDC) provide a depot of antigen and support the survival of antigen-specific B cell clones, which can then be induced to differentiate into high-affinity B cell clones, long-lived plasma cells or memory B cells by follicular T helper cells (T_h). Specific targeting of adjuvants and vaccines to cell populations critical for the induction and augmentation of GC responses could greatly improve vaccine safety and enhance vaccine efficacy. A derivative of cholera toxin, the CTA1-DD adjuvant was exceptionally effective at promoting the formation of large and numerous GC, as well as enhanced antibody responses and memory B cells development. We previously explored the *in vivo* distribution of CTA1-DD in tissues and observed that CTA1-DD-mediated activation of complement and binding to follicular dendritic cells via complement receptor 2 was crucial for adjuvant-induced GC formation and antibody production. On-going analysis of gene-expression levels in FDCs following CTA1-DD immunisation suggests a direct effect of the adjuvant on FDC functionality and their capacity to promote GC formation. In order to isolate and track FDCs more easily, we have created a mouse model expressing the GFP marker gene under the CD21 promoter. Using this model, we are assessing the activation status and localisation of FDCs and T_h in response to CTA1-DD, which will delineate the key elements required for the adjuvant's effects and potentially identify key regulators of GC B cell activation, memory development and vaccine-induced protection.

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The viability of lactobacillus fermentum CECT5716 is not required to exert its intestinal anti-inflammatory activity

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Introduction: *Lactobacillus fermentum* CECT5716 is a probiotic with immunomodulatory properties that has been reported to show intestine anti-inflammatory activity in experimental colitis. Some studies have proposed that the viability of the probiotics is not essential to exert their beneficial effects. In the present study, we studied if the administration of viable *Lactobacillus fermentum* is necessary to obtain its intestine anti-inflammatory effect. Also, in vitro studies were performed to confirm these observations.

Methods: *Lactobacillus fermentum* CECT5716 was administered orally (5 x 10⁸ CFU/rat/day) to Wistar rats, live or dead (95°C for 30 min) for two weeks before colitis induction, and thereafter until colonic evaluation one week later, both macroscopically and biochemically: myeloperoxidase activity, glutathione content, TNF α and IL1 β levels, as well as iNOS expression. In vitro experiments were performed in Caco-2 (intestine epithelium) and RAW 264.7 (macrophages) cells, which were incubated with the probiotic (live or dead) (10⁸ CFU/ml), and stimulated with LPS (100 ng/ml) or IL-1 β (1 ng/ml) for 30 minutes (western blot) or 24 h (IL-8 or nitrite determination).

Results: The probiotic, live or dead, showed intestine anti-inflammatory effect, by reducing the colonic damage area. Biochemically, reduction in MPO activity, indicating lower neutrophil infiltration, and increase in glutathione content was observed in treated groups. Also, both treatments reduced the levels of the proinflammatory

cytokines TNF α and IL1 β , and inhibited the increased iNOS expression in colitic rats. In vitro studies showed that live or dead probiotic inhibited the stimulated production of IL-8 (Caco-2 cells) and nitric oxide (RAW 264.7) in a similar way. In the epithelial cells, this inhibitory effect was associated with a reduced phosphorylation of p42/44 ERK and SAPK/JNK when compared with stimulated cells without probiotic.

Discussion and Conclusion: The viability of *L. fermentum* CECT5716 to downregulate the stimulated immune response and exert its intestine anti-inflammatory effect is not essential.

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TLO Developmental Program: Divergent paradigm of lymphoid organogenesis

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Tertiary lymphoid organs (TLOs) represent the histological hallmark of many immune-mediated inflammatory diseases. TLOs are characterized by a functional leukocyte aggregation and network of lymphoid-like stromal cells (LLSc). LLSc express lymphoid chemokines (CXCL13, CCL19, and CCL21), survival factors (BAFF and IL-7), lymphoid markers and adhesion molecules (gp38, RANKL, ICAM-1 and VCAM-1) that locally support lymphocytes survival and organization in ectopic sites areas. Using a combination of inducible models of TLO formation we demonstrated that the acquisition of this lymphoid phenotype by the non-activated resident stroma requires a multistep process, fundamentally different from that responsible of secondary lymphoid organ formation. We showed that early, during TLO formation, IL-4R α engagement via IL-13 on quiescent tissue-resident fibroblasts induces LLSc priming and mediates the up-regulation of gp38 and lymphoid-associated adhesion molecules. Expansion of this activated lymphoid stroma requires IL-22/IL-22R mediated signaling. Lack of IL-22 or its receptor induces defective LLSc proliferation, abrogation of CXCL13 expression and TLO involution. Finally we demonstrated that, similarly to secondary lymphoid organ, stabilization of the stroma to a functional lymphoid phenotype requires lymphocyte infiltration and lymphotoxin beta expression. This work highlights critical differences between the embryonic program responsible for SLO formation and the inflammatory development of TLOs and unveils novel potential targets for TLO targeting.

098

TLR2 located in subcellular compartments negatively regulates TLR4-mediated inflammatory response of macrophages to internalized bacteria

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Recognition of microbes by toll-like receptors (TLRs) is critical for initiation of appropriate innate and adaptive immune responses. How cross-talk between TLRs situated in various cellular locations contributes to host-microbe dialogue and immunoregulation is presently unclear. Here we report a dual role for TLR2 in regulating the

response of macrophages to internalized bacteria. Cell surface TLR2 initiated an inflammatory response while subcellular TLR2 negatively regulated a TLR4-mediated hyper-inflammatory response that was phagocytosis-dependent and driven by a type I interferon autocrine loop. TLR2-deficient macrophages were hyper-responsive to bacteria and developed an M1-like phenotype, which was absent in TLR2/TLR4-deficient macrophages. Our findings identify subcellular TLR2 as being important for the negative regulation of inappropriate TLR4-mediated inflammatory responses to bacteria.

099

TRPV1 expression in splenocytes of different mouse strains could be critical in understanding variations in immune responses

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Objectives: This study discusses for the first time TRPV1 expression, functionality and subsequent release of CGRP from immune cells in the spleens of AKR, BALB/c and C57.

Material and Methods: TRPV1 expression on splenocytes: Spleen cells were disassociated and stained with different dilutions of 6 flouorochromes. conjugated antibodies for CD11c, F480, CD4, CD8, CD19, IgM, IgD, Gr1 were then along with TRPV1 biotinylated antibody were administered. Calcium signaling: Indo-1 was administered to splenocytes at 5x10⁵ cells/sample. samples were treated with either capsaicin, anandamide or both at concentrations 6, 12, 25, 50, 100uM. specific cell types' calcium signaling was examined with a single concentration of agonists (100uM). CGRP release from splenocytes: 1x10⁶ cells per sample were treated with 100uM with capsaicin, anadamide or both. The supernatants were collected 5 minutes post treatment. The remaining cells were collected and sonicated. both supernatants and sonicated cells were examined for CGRP via ELISA.

Results:

1-TRPV1 is expressed on immune cells in spleen of all mouse strains in study (AKR, BALB/c and C57)

2-BALB/c have a higher percentage of TRPV1 on CD11c+ cells, F4/80⁺ cells and CD11c+F4/80⁺ cells

3-AKR had higher percentage of TRPV1 on IgM+ cells, IgD+ cells and IgD+IgM+ cells.

4-C57 had significantly higher percentage of TRPV1 in CD8⁺ cells.

5-CGRP release appears to be impaired in AKR compared to BALB/c post capsaicin treatment.

6-CGRP inhibited Th1 cytokines in all three strains while promoting Th2.

Conclusion: This study shows for the first time TRPV1 expression on immune cells in the spleen. Also, it discusses TRPV1 signalling and subsequent release of CGRP. Findings are then studied in the light of the known immune phenotypes that all three mouse strains develop in reponse to the helminth trichuris muris (t.muris). This study suggests that lack CGRP release in AKR is impaired compared to BALB/c and C57 mice.

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Vagus nerve modulates colonic inflammation in an alpha-7 nicotinic receptor ($\alpha 7$ AChR) independent mechanism**M.D. Giovangiulio, N. Stakenborg, G. Bosmans, G. Farro, P.J. Gomez-Pinilla, G. Matteoli & G.E. Boeckstaens***Department Clinical and Experimental Medicine, KU Leuven, Leuven, Belgium*

To preserve intestinal immune homeostasis, our immune system has evolved redundant regulatory strategies to prevent inappropriate immune reactions against innocent luminal antigens. Recently, we have shown that the parasympathetic nervous system through the vagus nerve (VN) dampens intestinal muscular macrophages via alpha-7 nicotinic receptor ($\alpha 7$ AChR) in a model of postoperative ileus. Similarly, we showed that vagotomy induces loss of oral tolerance by reducing the conversion of antigen-specific Tregs in the lamina propria and increases the severity of colitis. However, the role of $\alpha 7$ nAChR and the immune cells involved in the vagal control of colitis is still not clearly defined. To study the influence of $\alpha 7$ nAChR on Th cells, two groups of RAG1-/- mice were injected respectively with naïve Th cells from WT or $\alpha 7$ nAChR-/- mice. To define the role of $\alpha 7$ nAChR on innate immune cells, $\alpha 7$ nAChR-RAG1 double knockout mice were injected with WT naïve Th cells. In both experimental approaches, recipient mice developed similar severity of colitis as shown by histology score, cytokine expression and colonic Th cells polarization. Altogether, our results suggest that $\alpha 7$ nAChR deficiency either on Th or on innate immune cells is not crucial for controlling colonic inflammation. Further, studies will be required to define if other nicotinic receptors or other neurotransmitters are responsible for the vagal anti-inflammatory effect in the colon.

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Visualizing macromolecule passage through epithelia**J.F. Richter¹, R. Schmauder², A. Gebert¹ & M. Schumann³**¹*Department of Anatomy II, University Hospital Jena, Jena, Germany,*²*Department of Physiology II, University Hospital Jena, Kollegiengasse 9, Jena, Germany,* ³*Department of Gastroenterology, Charité University Medi, Hindenburgdamm 30, Berlin, Germany*

Introduction: Epithelial layers are the body's interfaces between internal and external compartments. As physical barriers to potentially antigenic macromolecules epithelia prevent uncontrolled access to immune competent compartments. The current discussion about disease initiation in IBD focusses on mucosal barrier function, since loss of barrier function appears to trigger an untempered immune reaction to the commensal intestinal flora. Progress in understanding such barrier defects is largely hampered by a lack of methods that can locally resolve passage sites of macromolecules. To date, epithelial macromolecule passage is analyzed using global measurements such as tracer flux studies which average contributions from many cells. As these global measurements do not locally resolve barrier defects, leak property distribution cannot be analyzed and rare events may be missed. Therefore, local identification of barrier breaks is interesting and needed.

Aims: To localize and characterize macromolecule passage sites in epithelia.

Methods: Using MDCKII, Caco-2/BBe, HT-29/B6 epithelia and rat colonic mucosae we applied fluorescent dextran tracers of various sizes which bind to cell-adherent scavenger molecules at the basolateral membranes of single-layered epithelial sheets in vitro and ex

vivo. Tracer molecule binding was analyzed using fluorescence microscopy.

Results: When administered from the apical side, only dextrans that passed the epithelial barrier bind to the scavenger and hence label sites of increased permeability to macromolecules at a resolution in the μ m-range. Using standard epithelial two-compartment culture systems spontaneous macromolecule passage was detected in all cell lines analyzed. Passage was generally rare, occurred through big leaks very inhomogeneous in distribution and magnitude and was reduced with increased culture duration. The basolateral label increased when barrier function was disrupted with EGTA or TNF α , and correlated well with a corresponding global flux measurement.

Conclusion: We propose a new imaging approach that allows for local analysis of macromolecule passage which provides new insights in immunologically relevant leaks in epithelial barriers in health and disease.

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Vitamin-D status and its regulation of $\gamma\delta$ T-cells in Behçet's disease**M.D. Samiul Hasan, L.A. Bergmeier & F. Fortune***Institute of Dentistry, Queen Mary University, 4, Newark street, London, UK*

Background: Behçet's disease (BD) is a multisystem inflammatory disorder characterized by oro-genital ulcerations, ocular manifestations, arthritis, vasculitis etc. with episodes of exacerbations and remissions. BD is often regarded as at the crossroad between autoimmunity and auto-inflammation however, the pathogenesis remains inconclusive. Besides genetic predisposition, immune-dysregulation involving cytotoxic lymphocytes such as $\gamma\delta$ T-cells is reported to have a role. V $\gamma 9$ V $\delta 2$, a major subset of these atypical T-cells are present in the inflammatory lesion and lead to rapid cytokine production, particularly of Th1-type when exposed to bacterial phosphoantigens and thus might be responsible for inducing and/or maintaining the pro-inflammatory environment characteristic of the disease. Vitamin-D (Vit-D) has regulatory properties for immune system function and has been implicated as a protective factor in some diseases but to date, no studies have addressed the effect of Vit-D on $\gamma\delta$ T-cells in BD.

Materials and Methods: Serum 25-hydroxyvitamin-D levels of BD patients were measured. V $\gamma 9$ V $\delta 2$ T-cells from BD patients were pretreated with Vit-D, followed by stimulation with the phosphoantigen, HDMAPP. Antigen induction of IFN γ , TNF α and IL10 expression was measured using flow cytometry and ELISA.

Results: 80% of BD patients are deficient in serum 25-hydroxyvitamin-D levels. In-vitro treatment with Vit-D significantly reduced IFN γ ($P = 0.002$) and TNF α ($P = 0.001$) expression by V $\gamma 9$ V $\delta 2$ T-cells. There was no effect on IL10 production.

Discussion and Conclusion: The data indicate a role of Vit-D in V $\gamma 9$ V $\delta 2$ T-cell regulation in BD. BD patients are deficient in Vit-D suggesting that this may contribute to the prolonged inflammation. V $\gamma 9$ V $\delta 2$ T-cell is a poor source of IL10 and there is IL10 polymorphism in some BD patients which might explain the IL10 data. Our data support the notion that Vit-D negatively regulate the proinflammatory function of $\gamma\delta$ T-cells and may be implied as a protection against autoinflammatory and autoimmune disorders including BD.