Mucosal Immunology in Health and Disease.

7th European Mucosal Immunology Group meeting Conference report

Anna Vossenkämper

Barts and The London School of Medicine and Dentistry, Centre for Immunology and Infectious Disease, London E1 2AT, United Kingdom.

The 7th European Mucosal Immunology Group meeting was held in Amsterdam, in the beautiful venue of the Royal Tropical Institute, from the 29th September to the 2nd October 2010 and attracted 300 delegates. This report summarizes some of the highlights of the conference. The meeting started with a fascinating talk by **Reina Mebius** from the VUMC Amsterdam, who presented her work on the development of the lymphoid tissue in the murine gut. Illustrated by confocal microscopy images, she described the important role of lymphotoxin for the development of Peyer's patches (PP) and intestinal lymph nodes (LN). The first immune clusters in the murine gut start to appear by day 13. Lymphoid tissue inducer (LTi) cells express Ltαß which interacts with the LT receptor on stromal cells, which subsequently release chemokines that attract more immune cells into the clusters. Cluster formation is dependent on CXCR5 and CXCL13, since knock-out (KO) mice for these markers show no immune clusters due to a lack of CD4+ CXCR5+ and CD4+CXCL13+ LTi cells. Reina Mebius also focused on retinoic acid (RA) during her talk. The importance of RA in the immune cluster formation is evident since retinaldehyde dehydrogenase type 2 (RALDH2) KO mice (missing the enzyme that converts retinaldehyde into RA) have no intestinal LN formation on embryonic day 13.5. It

was proposed that neurones reaching towards the immune clusters are the source of RA which then stimulates mesenchymal precursor cells to express CXCL13 which is crucial for LN formation. However, CCL21 might be able to compensate for the loss of CXCL13 to some extend, since it can stimulate mesenchymal precursor cells as well. **Ifor Williams** (Emory University, Atlanta) gave an overview of M cell function and explained the important role of RANKL (Receptor Activator for Nuclear Factor κ B Ligand) which facilitates LN development but also determines the differentiation and number of M cells in the gut. RANKL KO mice show a 70%-decrease in M cell numbers and recombinant RANKL (rRANKL) can restore the number of M cells in these mice. rRANKL treatment leads to a diffuse distribution of M cells on villi in the small intestine and to a higher rate of bacterial translocation in these animals. Ifor Williams therefore hypothesized that rRANKL treatment might be a strategy to temporarily increase M cell numbers in the gut which might intensify the immune response to oral vaccinations. An important topic in the following session was RA and its effect on dendritic cells (DCs). **Rosalie Molenaar** (VUMC Amsterdam) talked about RALDH in murine DCs and the role for RA for Foxp3+ regulatory T cells (Treg) function and imprinting of gut homing receptors. RALDH expression in migratory CD103+ DCs and mesenteric lymph node (MLN) stromal cells is controlled by dietary intake of vitamin A; it is, however, not dependent on signaling *via* Toll-like receptors. RALDH activity is markedly decreased in mice that are fed a vitamin A deficient diet. Besides mucosal DCs and MLN stromal cells, RALDH activity is mainly detectable in intestinal epithelial cells and neurons. **Ilaria Spadoni** (European Institute of Oncology, Milan) presented her work on thymic stromal lymphopoetin (TSLP). This cytokine is produced by e.g. epithelial cells and DCs and induces

STAT5 expression. It favors a Th2 differentiation and down-regulates interleukin-12 production by T cells. Ilaria Spadoni found TSLP to be down-regulated in patients with Crohn's disease. Mice deficient in TSLP are prone to helminth infections and develop very severe colitis after DSS challenge which is mediated by increased numbers of CD4+IFNγ+ and CD4+IL17+ cells. Therefore, TSLP seems to have anti-inflammatory effects in mouse models of colitis that are Th1 and Th17 driven. **William Agace** (University of Lund) spoke about RA and gut DCs. CD103+ DCs are the cells that efficiently imprint gut homing receptors and are the main migratory lamina propria DC population. Murine CD103+ DCs metabolise RA; mice fed a vitamin A deficient diet have no decrease of this cell population. However, RALDH activity is drastically reduced in the gut and CD8+ lymphocytes lack expression of the homing receptors CCR9 and $\alpha 4\beta 7$ integrin. William Agace found the RA concentration to be high in bile and speculated that it might be an important source for DCs that imprint gut homing receptors. **Thaddeus Stappenbeck** (Washington University) presented results on inflammatory bowel disease and autophagy. Mutations in the autophagy-related gene ATG16L is one of the genetic risk factors for developing Crohn's disease. He showed that mice and humans with variants of the ATG16L gene have abnormal and dismorphic Paneth cells, loss of the secretory cellular machinery and a diffuse distribution of lysozyme. Since the infection with a murine Norwalk-like virus (a pathogen present in most animal facilities) is required for the observed defect in autophagy in mice, he proposed a virus might also contribute to the pathology in IBD patients. **Emanuel Berger** (Technical University Munich) gave a very interesting talk on mitochondrial stress mechanisms in human colitis and murine colitis models. In T cell transfer colitis in mice, epithelial cells have an increased level of

proteins associated with stress in the endoplasmatic reticulum (ER) and the mitochondria. He pointed out that in colitis models and IBD, the ER chaperone GRP78 as well as the mitochondrial stress-associated chaperonin, Cpn60, a member of the heat shock protein family, are highly expressed under inflammatory conditions. In addition, the protein kinase R (PKR) signaling pathway leading to the expression of Chop60, seems to play a role in intestinal inflammation, since pkr KO mice do not develop DSS colitis. **Manon Wildenberg** (Leiden University Medical Centre) brought the attention back to autophagy. She presented her findings of increased T cell proliferation in mixed lymphocyte reactions (MLR) when the autophagy-related genes ATG16L1 and IRGM in the stimulating DCs were knocked down by using siRNA. These DCs showed no change in the expression of accessory markers CD80, CD83 and CD86; neither was the cytokine secretion altered after the knock-down. However, imaging of these $ATG16L1^{low}$ and $IRGM^{low}$ DCs revealed a change in cell shape and an increased polarization of the DCs towards T cells in MLRs resulting in a more intimate and longer interaction with the T cells. Manon Wildenberg further demonstrated that DCs defective in autophagy stimulated T cells to a higher production of IL-17 compared to controls. These results might help us to understand how defects in autophagy in IBD patients are related to a pronounced Th17 response. The session on regulation in the gut by T cells started with a talk from **Oliver Pabst** (Medizinische Hochschule Hannover) who focused on intestinal tolerance and how the migration of DCs and T cells is crucial for this process. CCR7 KO mice show no oral tolerance. In these mice, DCs in the villi cannot migrate towards the MLN. By using Two-photon microscopy, Oliver Papst's team investigated which DC populations move in the intestinal lymph and found that mainly CD11c+CD103+

DCs but not CXCR1+ DCs migrate *via* the lymph. Since CD103+ DCs also imprint gut homing receptors on T cells, he concluded that this DC population is the main migratory DC population which stimulates T cells in the MLN. Gut homing of T cells is a prerequisite for oral tolerance as $\alpha4\beta7$ integrin KO mice and MAdCAM KO mice do not develop tolerance to ovalbumin challenge. The transfer of $\alpha4\beta7$ integrin + T cells into $\alpha4\beta7$ integrin KO mice restored the ability to develop oral tolerance. During the talk, the role of Foxp3+ Tregs for oral tolerance was emphasized as well. Tregs are found to appear in increased numbers after antigen challenge in the lamina propria but only to a small extend in the MLN. Expression of CX3CR1 on DCs in the lamina propria is essential for expansion of Tregs and transfer of CX3CR1+ DCs into CX3CR1 KO mice restores the expansion of Tregs in the lamina propria and also oral tolerance. The following talk was given by **Mark Travis** (University of Manchester). His work concentrates on intestinal DCs and transforming growth factor-β (TGFβ). This molecule is secreted as an inactive complex and needs cleavage before to gains its immunoregulatory properties. The molecule that cleaves TGFβ is yet unknown but Mark Travis showed results that suggest the integrin $\alpha v\beta8$ on CD103+ DCs might be important. As already published by Travis, mice lacking $\alpha v\beta8$ suffer from autoimmunity; CD103+ DCs are potent inducers of Tregs and able to activate TGFβ. Travis showed data that treatment with antibody against TGFβ in mice abrogates the ability of CD103+ DCs to induce Treg development and CD103+ DCs in $\alpha v\beta8$ KO mice were unable to activate TGFβ. Therefore, he proposed that $\alpha v\beta8$ integrin on CD103+ DCs is able to activate TGFβ which subsequently differentiates naïve T cells into Foxp3-expressing Tregs. **Pim Koelink** (Utrecht University) focused entirely on collagen breakdown products and how they

activate neutrophils in the course of IBD. One of these molecules is proline-glycine-proline (PGP), a peptide which leads to chemotaxis of neutrophils in the inflamed gut. PGP is formed during collagen breakdown by matrix-metalloproteinases 8 and 9 and prolyl endopeptidase which are abundant in inflamed tissue. Mice treated with antibodies against PGP suffered from less weight loss and disease activity in the DSS colitis model. PGP might therefore be a target in the treatment of IBD.

In the following session on Celiac disease, **Ludvig Sollid** (University of Oslo) gave an overview on the genetic risk factors of the condition and explained that the enzyme transglutaminase cleaves α2-gliadin into a 33-aminoacid long peptide which has six T cell epitopes. These epitopes are recognised by reactive T cells that mediate the strong Th1 response in Celiac disease. Sollid's group also studies the anti-transglutaminase (TG) antibodies, an important diagnostic marker for the disease, and showed that these Abs are dependent on gluten in the diet. They were further able to demonstrate that approximately 10% of CD138+ plasma cells in the gut were TG-specific CD138+ in Celiac patients. However, upon the omission of gluten from the diet, these specific plasma cells disappear. **Anne Kozijn** (Erasmus Medical Centre, Rotterdam) presented recent work on a mouse model of Celiac disease. She used transgenic mice which are deficient for MHC-II and transgenic for human HLA-DQ2 and the gliadin-γ1 specific T cell receptor (TCR). She showed that all the T cells in these mice express the TCR specific for deamidated gliadin-γ1. The CD4+ T cells of the DQ2.gliadin-TCR mice were isolated and injected to single DQ2 mice. However, upon gliadin challenge, only T cell proliferation in the spleen could be detected, none in the MLN or the gut. Why these mice do not have gliadin-reactive T cells in the gut needs further investigation. One

of the presentations focusing on probiotics was given by **Laurent Favre** (Nestec Research Centre, Lausanne) who used complexes of secretory IgA (sIgA) and *Bifidobacterium lactis* to study the adhesion to intestinal epithelial cells *in vitro* and TSLP production in mice. He found that sIgA+ *B. lactis* bacteria together induced more IgA production than the bacteria alone. Mice treated with the complex also had higher anti-LPS IgG titers after immunization with *Salmonella typhimurium* compared to controls. How the sIgA-bacteria-complex mediates immune stimulation and how specific the process is remains unclear. Some insight into how the probiotic strain *Lactobacillus paracasei* acts on the immune system was provided by **Marie-Anne von Schillde** (Technical University Munich). She convincingly demonstrated that this strain expresses the protein lactocepin which degrades the proinflammatory molecule interferin-inducible protein (IP)-10 *in vitro*. Lactocepin is cell-wall associated and also secreted protease. It was found to have no effect on tight junctions and did not affect the viability of intestinal epithelial cells. Therefore, Lactocepin was identified as an important probiotic structure of *L. paracasei*. The meeting was closed by **Severine Vermeire** (Catholic University of Leuven) with a summary of current and future clinical approaches to IBD. She pointed out that although more and more research is taken from bench to bedside, the clinical situation is often more complex than *in vitro* data suggest. She highlighted the importance of research into genetic factors in IBD and how these factors might help to predict which therapies are most appropriate for individual patients. The 7^{th} EMIG meeting was certainly a very successful and interesting meeting which showed a broad spectrum of human and murine research into mucosal immunology. The next EMIG meeting will take place from the 10^{th}-12^{th} of October 2012 in Dublin.