12th European Mucosal Immunology Group Meeting

Abstract Booklet



EMIG 2023

July 12-14, Bern, Switzerland

Scientific Program

VonRoll Campus, Room 001,
University of Bern, Fabrikstrasse 6, 3012 Bern

Wednesday July 12, 2023

7:45 – 8:30 Registration

8:30 – 8:40 Welcome and Introduction

8:40 -9:30 **Keynote lecture by Yasmine Belkaid** (NIH, USA)
"Microbiome Control of Host Immunity"
Chairs: Christoph Schneider, Stephanie Ganal-Vonarburg

9:30- 10:00 Coffee break

10:00 – 12:00 **Session I: Innate Immunity in Mucosal Tissues**
Chairs: Andy Wullaert, Jakob Zimmermann
Maria Rescigno (Humanitas University, IT)
"Microbiota in gut-liver-brain axis"
Short talks:
Isabelle Coales (King's College London, UK): *Mucosal epithelial organoids promote the development of tissue-specific innate lymphoid cells (T1)*
Martin Schwarzer (Institute of Microbiology of the Czech Academy of Sciences, CZ): *Nodding to growth: Lactiplantibacillus plantarum WJL improves linear growth of undernourished infant mice (T2)*
Signe Birkeland (Norwegian University of Life Sciences, NO): *Exploring effects of naturalized housing and dietary fiber on DSS-induced low-grade colonic inflammation in mice (T3)*
Andreas Diefenbach (DRFZ Berlin & Charité, DE)
„Innate lymphoid cells and tissue homeostasis"
5' company pitch: Agilent

12:00 – 12:20 **Eulogy for Nils Lycke by Allan Mowat**

12:20 – 13:30 Lunch

13:30 – 15:10 **Session II: Mechanisms of Mucosal Inflammation**
Funded by the EoE Foundation
Chairs: Christoph Schneider, Melanie Conrad
Gitta Stockinger (The Francis Crick Institute, UK)
"*The effect of environmental stimuli on barrier integrity in the gut"*
Short talks:
Francesco Strati (University of Milano Bicocca, IT): *iNKT cells immunomodulation and mucosal healing by microbiota-derived lactate (T4)*
Jakob Zimmermann (University of Bern, CH): *Transient intestinal colonisation of germ-free mice demonstrates long-lived microbiota-specific Th cell memory (T5)*

Hamida Hammad (VIB-UGent, Belgium)
"Neonatal exposure to environmental triggers influences type 2 immunity to allergens later in life"

15:10 – 15:40 Coffee break

15:40 – 17:35 **Session III: Intertissue crosstalk**
Chairs: Francesca Ronchi, Kathalina Lahl
Eugene C. Butcher (Stanford University and PAVIR, USA)
"Cruising the bioinformation highway for new insights into the mucosal immune system"
Short talks:
Soraya Rabahi (Institut Curie, FR): *Interleukin-22 modulates enteric nervous system development and function in zebrafish (T6)*
Marika Falcone (San Raffaele Institute, IT): *The beneficial role of microbial bile acid metabolites in brain autoimmunity (T7)*
Mikael Niku (Veterinary Biosciences, University of Helsinki, FI): *Impacts of maternal microbial metabolites and extracellular vesicles on the fetal development of the immune system (T8)*
Henrique Veiga-Fernandes (Champalimaud Centre for the Unknown, PT)
"Neuroimmune Ecosystems"

17:35-18:35 **Swiss Young Immunologists Society (SYIS) and young European Federation of Immunological Societies (yEFIS) session**
Funded by EFIS and SYIS
Chairs: Annika Hausmann, Hind Hussein
Short talks:
Robin Avanthay (IVI, CH): *NS1 and PA-X act in a concerted manner to manipulate the innate immune response of pandemic H1N1/09 influenza virus (T9)*
Eva-Lena Stange (Uniklinik Aachen, DE): *Microbial influence on the postnatal establishment of hepatic immune homeostasis (T10)*
Anna Pascual Reguant (Deutsches Rheuma-Forschungszentrum & Charité Universitätsmedizin Berlin, DE): *Cellular and molecular characterization of the local immune response in severe COVID-19 by integrated spatial analysis of lungs and draining lymph nodes (T11)*
Maximilian R. A. Koch (Technical University Munich, DE): *CagA-specific Gastric CD8+ Tissue-Resident T Cells Control Helicobacter pylori During the Early Infection Phase (T12)*

18:45-21:00 Dinner and **Poster session I**
Posters 001-094

Thursday July 13, 2023

8:30 – 10:15 **Session IV: Systems Immunology in Host-Microbe Interactions**
Chairs: Cecilia Johansson, Bahtiyar Yilmaz
Nicola Segata (University of Trento & IEO Milan, IT)
"High-resolution metagenomics to mine the hidden diversity of the human microbiome"
Nassos Typas (EMBL Heidelberg, DE)
„Probing the interface of medication and the human gut microbiome"
Short talks:

Vanni Bucci (UMass Chan Medical School, USA): *Microbiota encoded fatty-acid metabolism expands tuft cells to protect tissues homeostasis during Clostridioides difficile infection in the large intestine* (T13)
Victoria von Goetze (Deutsches Rheuma-Forschungszentrum Berlin, DE): *Direct induction of TGF-β signaling by the intestinal gut microbiota bacterium Anaeroplasma reveals new potential anti-inflammatory probiotic (T14)*

10:15- 10:45 Coffee break

10:45 – 12:05 **Session V: Microbe-Immune Interactions at Body Surfaces**
Chairs: Christoph Müller, Andrew Macpherson
Joanne Konkel (University of Manchester, UK)
"Delineating mechanisms of immune tailoring at the oral barrier"
Short talks:
Stefan Fattinger (ETH Zurich, CH): *Gasdermin D is the only Gasdermin that provides non-redundant protection against acute Salmonella gut infection (T15)*
Valérie Gaboriau-Routhiau (INRAE, FR): *MyD88-dependent control of host-SFB symbiosis in SFB-monocolonized mice (T16)*
Lara Grassi (University of Basel, CH): *An innovative human bladder microtissue infection model (T17)*
5' company pitch: Olink

12:05 – 13:30 Lunch

12:20 – 13:20 **Company-sponsored lunch symposium "Advances in spatial biology technologies"**
Mila Basic (Lunaphore): *Sequential immunofluorescence: the novel fully automated method to map the cellular architecture and immune microenvironment.*
Carmela Foti (Merck): *Illuminating multiple dimensions: entirely new generation of antibody technologies to empower complex analysis with superior specificity, high reproducibility and attention to sustainability.*
Placido Pereira (Bio-Techne): *Bio-Techne - Where Science Intersects* Innovation

13:30 – 16:00 **Session VI: Mucosal Infections**
Chairs: Bill Agace, Allan Mowat
Emma Slack (ETH Zurich, CH)
"Growing, flowing and sugar-coating"
Short talks:
Marco Rodari (Institut Imagine, FR): *Aberrant TGF-beta signaling downstream importin 8 deficiency triggers intestinal inflammation (T18)*
Maria Pasztoi (Technical University of Munich and Helmholtz Centre Munich, DE): *Inflammation-driven polarization and tissue-niche formation of intestinal lamina propria stromal cells (T19)*
De Martin Angelina Marisa (Kantonsspital St. Gallen, CH): *Fibroblastic stromal cell-derived IL-33 controls intestinal helminth infestation (T20)*
Konjit Getachew Muleta (Lund University, SW): *cDC1 deficiency affects neonatal humoral immunity to Rotavirus without impacting on protection from reinfection later in life (T21)*
5' company pitch: Transnetyx

16:00 – 16:05 Travel awards

16:05 – 17:40 Coffee and **Poster session II**
Posters 095-187

18:45 – 00:00 Gala Dinner and Party at Kornhauskeller Restaurant, Kornhausplatz 8, Bern

Friday July 14, 2023

9:00 – 9:50 **Keynote lecture by Eran Segal (Weizmann Institute of Science, Israel)**
"*Personalized medicine based on deep human phenotyping"*
Chairs: Christoph Müller, Francesca Ronchi

9:50- 10:20 Coffee break

10:20 – 12:00 **Session VII: The neonatal window of opportunities**
Chairs: Stephanie Ganal-Vonarburg, Ziad Al Nabhani
Gérard Eberl (Institut Pasteur, FR)
"Modulation of immune response to vaccination and autoimmunity by the weaning reaction"
Mathias Hornef (RWTH University Hospital Aachen, DE)
"Macromolecule translocation and mucosal immune maturation in the neonatal host"
Short talks:
Anna Silvia Wenning (University of Würzburg, DE): *Maternal microbiota-derived metabolites drive embryonic epidermal formation and set neonatal skin barrier (T22)*
Gina Ann Lindberg (Lund University, SW): *A method to distinguish neonatal and maternal IgA in young mice (T23)*

12:00 – 13:15 Lunch

13:15 – 14:25 **Session VIII: Mucosal B Cells**
Chairs: Hyun-Dong Chang, Emma Slack
Andrew Macpherson (University of Bern, Switzerland)
"Specific and generic mucosal immune responses to the intestinal microbiota"
Short talks:
Mauricio Guzman (Hospital del Mar d'Investigacions Mèdiques (IMIM), ES): *Secreted IgD Targets Monocytes to Deliver a Complex Signaling Program Characterized by a Prominent Tolerogenic Component (T24)*
Simona Ceglia (University of Massachusetts Chan Medical School, US): *Integrated nutrient sensing controls intestinal IgA plasma cell response against commensals and pathogens (T25)*
5' company pitch: Cancer Tools

14:25 – 14:45 Poster prizes and Closing words

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Abstracts

Short talks
Talks T1-T25 *Pages 8-20*

Poster session I: Wed Jul 12th, 18:45-21:00
Posters 001-094 *Pages 21-67*

Poster session II: Thu Jul 13th, 16:05-17:40
Posters 095-187 *Pages 67-114*

ID: T1
Abstract
Topics: Innate Immunity
Keywords: Innate Lymphoid Cells, organoids, intestine, tissue

Mucosal epithelial organoids promote the development of tissue-specific innate lymphoid cells

Geraldine M Jowett (King's College London, United Kingdom), **Emily Read** (King's College London, United Kingdom), **Luke B Roberts** (King's College London, United Kingdom), **Isabelle Coales** (King's College London, United Kingdom), **Diana Coman** (King's College London, United Kingdom), **Ludovic Vallier** (Cambridge University, United Kingdom), **Graham M Lord** (King's College London, United Kingdom), **Joana F Neves** (King's College London, United Kingdom)

Innate Lymphoid Cells (ILC)-restricted precursors (ILCP) arise from multipotent common lymphoid precursors in the bone marrow, however the final stages of ILC-subset maturation likely occur upon exiting this niche. Deciphering what local stimuli drive the differentiation and function of ILC in the tissues remains a pressing question, as ILC frequencies can become dysregulated in disease.

Here, we introduce murine & human co-culture systems of ILCP with organoids that faithfully capture the maturation of ILC within mucosal tissues.

This approach promotes significant expansion and functional maturation of all ILC subsets in parallel. Notably, gut and lung organoids cells are sufficient to induce tissue-specific patterns of ILC subset frequencies and phenotypes, even in the absence of organ-specific microbial tropism or additional cells. For example, like their ex vivo counterparts, ILC2 are KLRG1high when developed on gut organoids and ST2high when generated on lung organoids. This lung phenotype, is dependent on IL-33, demonstrating the importance of epithelial derived factors on ILC modulation. We will be presenting further transcriptomic and functional characterization of these organoid-generated ILC.

Importantly, using hIPCS-derived organoids we show that human gut epithelium, not stroma, supports proliferation and maturation of human ILCP.

Taken together, our work provides unprecedented insight into in situ ILC maturation and function. Moreover, it introduces an organoid platform that provides exquisite control over both environmental stimuli and host genetics, making it powerful tool for dissecting immune-epithelial interactions in health and disease. Finally, this system has the potential to be used for the generation of ILC for human treatment opening the door to autologous ILC therapies.

ID: T2
Abstract
Topics: Innate Immunity
Keywords: Lactiplantibacillus, chronic undernutrition, growth, IGF-1, NOD2

Nodding to growth: Lactiplantibacillus plantarum WJL improves linear growth of undernourished infant mice

Martin Schwarzer (Institute of Microbiology of the Czech Academy of Sciences, Czech Republic), **Umesh Kumar Gautam** (Institute of Microbiology of the Czech Academy of Sciences, Czech Republic), **Tomáš Brabec** (Institute of Molecular Genetics of the Czech Academy of Sciences, 14220 Prague, Czech Republic), **Dagmar Srutkova** (Institute of Microbiology of the Czech Academy of Sciences, Czech Republic), **Amélie Joly** (Institut de Génomique Fonctionnelle de Lyon, Ecole Normale Supérieure de Lyon, CNRS UMR5242, UCBL Lyon-1, F-69007 Lyon, France), **Filipe De Vadder** (Institut de Génomique Fonctionnelle de Lyon, Ecole Normale Supérieure de Lyon, CNRS UMR5242, UCBL Lyon-1, F-69007 Lyon, France), **François Leulier** (Institut de Génomique Fonctionnelle de Lyon, Ecole Normale Supérieure de Lyon, CNRS UMR5242, UCBL Lyon-1, F-69007 Lyon, France)

The intestinal microbiota is known to influence postnatal growth. We previously found that a strain of Lactiplantibacillus plantarum (strain LpWJL) buffers the adverse effects of chronic undernutrition on the growth of juvenile germ-free mice. Here, we report that LpWJL sustains the postnatal growth of malnourished conventional animals and supports both insulin-like growth factor-1 (IGF-1) and insulin production and activity. We have identified cell walls isolated from LpWJL as sufficient cues to stimulate animal growth despite undernutrition. Further, we found that NOD2 is necessary in intestinal epithelial cells for LpWJL-mediated improvement of intestinal crypt cell proliferation, type I interferon–regulated gene induction, IGF-1 production, and postnatal growth promotion in malnourished conventional animals. Our results demonstrate that bacterial cell walls or purified NOD2 ligands are sensed by the pattern recognition receptor NOD2 in the intestinal epithelial cells and sustain postnatal juvenile growth despite chronic undernutrition. We posit that one of the mechanisms by which LpWJL and its cell wall exert its postnatal growth–promoting properties is the buffering of the deleterious effect of undernutrition on small intestinal crypt cell proliferation through NOD2-dependent bacterial cell walls sensing. Our results suggest that, coupled with renutrition strategies, supplementation of evidence-based probiotics such as LpWJL or defined bacteria-derived postbiotics such as LpWJL cell walls and/or NOD2 agonists have the potential to alleviate persistent stunting, one of the long-term sequelae of undernutrition that still affects >149 million children under 5 years of age in low- and middle-income countries.

Supported by grant 21-19640M from the Czech Science Foundation.

ID: T3
Abstract
Topics: Innate Immunity, Microbe-immune interactions at body surfaces
Keywords: Feralization, dietary fibers, microbiota, NF-kB, Dextran Sulfate Sodium

Exploring effects of naturalized housing and dietary fiber on DSS-induced low-grade colonic inflammation in mice

Signe Birkeland (Norwegian University of Life Sciences (NMBU)), **Marte Senneset** (Norwegian University of Life Sciences (NMBU)), **Ingerid Rohde Mæhlum** (Norwegian University of Life Sciences (NMBU)), **Veronika Motuzova** (Norwegian University of Life SciencesNorwegian University of Life Sciences (NMBU)), **Henriette Arnesen** (Norwegian University of Life Sciences (NMBU)), **Preben Boysen** (Norwegian University of Life Sciences (NMBU)), **Harald Carlsen** (Norwegian University of Life Sciences (NMBU))

"Feralized" mice, i.e., mice living in a farmyard-type habitat, enriched with domestic animal droppings and soil, show a more matured immunological and gut-microbial profile than mice raised under conventional, clean conditions. Feralization has also been shown to reduce colonic tumor burden. We hypothesize that feralization can lead to strengthening of the intestinal barrier and thereby reduce encroachment of bacteria leading to inflammation, which often precedes carcinogenesis as well as systemic inflammation. Unpublished findings by our group indeed support that feralization strengthens the barrier through upregulation of critical barrier-related genes. To extend these findings we here explored if mice subjected to a DSS-induced low-grade colonic inflammation were protected by feralization both locally (colon) and systemically (liver). Furthermore, dietary fibers are known to improve gut health and contribute to barrier integrity, possibly through enhancing gut-microbiota diversity and acting as prebiotics. Hence, we included diets enriched or deprived of fibers to assess their influence on gut health. The two feralized groups were compared to two groups of mice housed under clean conditions with the same two diets. After five weeks on either of the four regimens, mice were treated with DSS and scored for disease symptoms before termination, where fecal material and tissues were collected for further analyses. The use of NF-κB reporter mice further allowed us to assess inflammation by in vivo and ex vivo imaging. Preliminary results demonstrate that feralized mice display slower progression of disease symptoms compared to conventionally raised mice. The role of dietary fibers is currently being analyzed. Results from all the other analyses including microbiota composition, inflammation, mucosal gene expression, immune cell flow cytometry, and more will be presented.

ID: T4
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: iNKT cells; lactate; Crohn's disease; IBD

iNKT cells immunomodulation and mucosal healing by microbiota-derived lactate

Francesco Strati (University of Milano Bicocca, Italy), **Chiara Amoroso** (Policlinico Hospital, Milan), **Federica Facciotti** (University of Milano Bicocca, Italy)

Invariant natural killer T (iNKT) cells are unconventional, CD1d-restricted, T lymphocytes playing a critical role in mucosal immune homeostasis. iNKT cells plastically adapt their functional phenotypes to the surrounding environment and are involved in IBD pathogenesis. Although iNKT cells release pro-inflammatory cytokines in response to the altered gut microbiota of IBD patients, exposure to microbiota-derived metabolites can promote homeostatic IL10-mediated iNKT responses resulting in better clinical outcomes in Crohn's disease (CD) patients. Thus, understanding the mechanisms leading to iNKT cells' functional shaping by microbiota-derived metabolites is important to design novel therapeutic approaches for IBD patients. We hypothesised that iNKT cells, serving as sentinels of tissue integrity, are the primary immune cells sensing microbiota-derived metabolic signals promoting the resolution of inflammation in IBD. In particular, here we show that microbiota-derived lactate can tightly control iNKT cells function promoting iNKT-mediated mucosal tolerance while preventing T-cell-mediated overt inflammation and tissue injury. We performed immunophenotyping of iNKT cells by multiparametric flow-cytometry from surgical specimens of CD patients and correlated NKT10 responses with intra-colonic levels of lactate, gut microbiome functions and tissue injury/healing. We studied the molecular pathway determining the NKT10 phenotype in a lactate-enriched microenvironment by RNA-seq and CHIP-seq. We validated the role of NKT10 responses by microbiota-derived lactate in-vivo by using experimental colitis models. On overall the present work shows i) how microbiota-derived lactate modulates the immunophenotype of iNKT cells, ii) the mechanisms underlying the sensing of microbiota-derived lactate by iNKT cells, iii) iNKT cells role in inflammation resolution.

ID: T5
Abstract
Topics: Mucosal T cells, Microbe-immune interactions at body surfaces
Keywords: Microbiota, CD4 T cells, Intestine, Th17, Immunological Memory

Transient intestinal colonisation of germ-free mice demonstrates long-lived microbiota-specific Th cell memory

Jakob Zimmermann (University of Bern, Switzerland), **Andrew Macpherson** (University of Bern, Switzerland)

The gut microbiota induces specific Th cell responses such as regulatory T cells, Th17, and Tfh cells. Due to the inability of uncoupling T cell induction from persistence of the microbe, the dynamics of such responses – such as dose requirements and longevity – have remained elusive.

We have genetically engineered a mutant strain of Limosilactobacillus reuteri that through cell wall auxotrophies reversibly colonises germ-free mice. This transient intestinal exposure induces L. reuteri-specific Th17 cells that can be followed beyond bacterial clearance. To enable tracing of antigen-specific Th cells, we have identified and validated specific T cell receptors and devised retrogenic mice for adoptive transfer experiments.

This highly defined and controllable system has revealed new insights into the physiology of microbiota-specific Th cells: (1) The T cell response is regulated by antigen dose at various levels with low doses sufficient for T cell expansion in mLN but higher doses required for homing to the small intestine and optimal Th17 differentiation. (2) Microbiota-specific Th cells form long-lived antigen-independent memory. After bacterial clearance following two weeks of transient colonisation, numbers of antigen-specific Th cells in the small intestine are maintained until at least day 84 but are reduced in blood and mLN compared to early after activation. These cells have a CD69+CD103- resident-memory phenotype. While they remain largely RORgt+, their ability to express IL-17A is reduced over time. (3) During stable colonisation with replication-competent L. reuteri, antigen-specific Th17 cells are found in the small intestine but also in mLN and blood suggesting their continuous activation and recirculation. Overall, transient colonization of germ-free mice combined with antigen-specific adoptive T cell transfers demonstrates long-lived Th cell memory against the gut microbiota. These findings may help to explain aberrant responses such as in inflammatory bowel disease but also be leveraged for mucosal vaccine design.

ID: T6
Abstract
Topics: Gut-Microbiota-Brain axis
Keywords: Cytokines, enteric nervous system, intestine, epithelial cells, zebrafish

Interleukin-22 modulates enteric nervous system development and function in zebrafish

Soraya Rabahi (Institut Curie, France)

Cytokines are small secreted proteins that promote tissue defense and homeostasis. Among key cytokines in barrier organs like the gut, interleukin-22 (IL-22) primarily targets epithelial cells, protecting this organ from pathogens and promoting tissue repair. Although it is well established that IL-22 dysregulation can lead to inflammatory bowel disease (IBD) and cancer, whether this cytokine plays a role in the developing gut remains unclear. We have used the zebrafish model to determine the function of il22 in gut development maturation. We generated IL-22-deficient zebrafish and found a novel role of this cytokine in modulating the development and function of the enteric nervous system (ENS). il22 KO fish showed dysregulated expression of several neuronal markers in the gut, as well as impaired peristalsis and food transit.

In addition, using an il22-reporter line that we generated, we identified enteroendocrine cells as the main source of il22 in the larval gut. Furthermore, il22 was expressed by a enteroendocrine subtype that also expressed trpa1 (transient receptor potential cation channel, subfamily A, member 1). Trpa1 is best known as a sensor for pain, cold and itch and it has been recently shown to sense tryptophan-derived metabolites produced by bacteria. Accordingly, we found that treatment of fish with chemical activators of this channel increased il22 expression in enteroendocrine cells.

We are currently deciphering the mechanisms by which microbial-induced enteroendocrine cell production of IL-22 modulates ENS development and physiology. Altogether, this project will provide a better understanding of how disorders such as IBD and those linked to ENS dysfunction such as irritable bowel syndrome are linked via the function of IL-22 in gut development and maturation.

ID: T7
Abstract
Topics: Gut-Microbiota-Brain axis, Microbe-immune interactions at body surfaces
Keywords: Microbiota, Bile Acid Metabolites, Autoimmunity, Multiple Sclerosis

The beneficial role of microbial bile acid metabolites in brain autoimmunity

Marika Falcone (San Raffaele Institute, IT)

The microbiota-induced metabolic profile is crucial to modulate immunity and autoimmunity in the gut but also in extra-intestinal tissues including the brain in Multiple Sclerosis (MS). Secondary bile acid metabolites (BAM) released by gut microbial species such as Deoxycholic acid (DCA) and Lithocholic acid (LCA) play important immune regulatory functions being capable to drive intestinal differentiation of FoxP3+ regulatory T cells at the expenses of effector Th17 and Th1 cells. We analyzed the bile acid metabolism in relapsing-remitting (RR)MS patients and test the beneficial effect of BAM in modulating brain autoimmunity in animal models of MS (EAE).

Our WGS metagenomic analysis revealed a reduced abundance of BAM-producing bacteria in the gut microbiota of RRMS patients compared to healthy controls. Our untargeted metabolomic analysis (LS-MS spectrometry) showed a highly significant reduction of secondary BAM, i.e., deoxycholic acid (DCA), in the intestine of RRMS patients. The alteration of the microbiota-regulated biliary network in RRMS patients was associated with immune dysregulation and increased percentages of Th17 cells in the peripheral blood. We validated the key role of microbial BAM in brain autoimmunity by showing that oral administration of two secondary BAM (DCA and LCA) ameliorated clinical and histopathological signs of EAE. BAM-treated EAE mice displayed decreased Th17 cell frequency and increased percentages of regulatory T cells (FOXP3+ Treg and IL-10+Tr1 cells) in the gut but also in the draining lymph nodes and brain.

Our data suggest that alterations of the bile acid metabolism linked to modification of the gut microbiota are present in RRMS patients and lead to immune dysregulation. The importance of microbial BAM in controlling brain autoimmunity was reinforced by our in vivo experiments showing that BAM administration prevents EAE by promoting immune tolerance. Bile acid metabolism could represent a new therapeutic target to promote immune tolerance.

ID: T8
Abstract
Topics: Gut-Microbiota-Brain axis, Neonatal window of opportunities
Keywords: maternal microbiota, fetal development, microbial metabolites, bacterial extracellular vesicles

Impacts of maternal microbial metabolites and extracellular vesicles on the fetal development of the immune system

Hyuk Nam Kwon (Veterinary Biosciences, University of Helsinki, Finland), **Roselydiah Makunja** (Veterinary Biosciences, University of Helsinki, Finland), **Tiina Pessa-Morikawa** (Veterinary Biosciences, University of Helsinki, Finland), **Aleksi Husso** (Veterinary Biosciences, University of Helsinki, Finland), **Ville Mikael Koistinen** (Food Sciences Unit, Department of Life Technologies, University of Turku; Institute of Public Health and Clinical Nutrition, School of Medicine, University of Eastern Finland; Afekta Technologies Ltd, Kuopio, Finland), **Olli Kärkkäinen** (: School of Pharmacy, University of Eastern Finland, Kuopio, Finland; Afekta Technologies Ltd., Kuopio, Finland), **Kati Hanhineva** (Food Sciences Unit, Department of Life Technologies, University of Turku; Institute of Public Health and Clinical Nutrition, School of Medicine, University of Eastern Finland; Afekta Technologies Ltd, Kuopio, Finland), **Mikael Niku** (Veterinary Biosciences, University of Helsinki, Finland)

The maternal microbiota modulates the fetal development, but the mechanisms of these earliest host-microbe interactions are unclear.

To investigate the impacts of maternal microbial metabolites, we compared full-term fetuses from germ-free (GF) and specific pathogen free mouse dams by non-targeted metabolomics and gene expression profiling. We also analyzed metabolites in human newborns and pig and calf fetuses using the same LC-MS pipeline.

In mice, the developing immune system was strongly dependent on the maternal microbial status. In the fetal intestine of GF mice, critical components mediating host-microbe interactions, innate immunity and epithelial barrier were differentially expressed. Interferon and inflammatory signaling genes were downregulated in the intestine and brain of the GF fetuses. Neural system development and function, translation and RNA metabolism, and regulation of energy metabolism were also affected by the maternal microbial status. In the placenta of GF fetuses, essential regulators of pregnancy were downregulated.

These impacts were strongly associated with microbially modulated metabolite concentrations in the fetal tissues, suggesting that they are largely mediated by maternal microbial metabolites absorbed through the placenta. These included AhR ligands, trimethylated compounds, fatty acid derivatives, dipeptides and multiple metabolites which have not yet been annotated. Many of these, or related compounds, were also observed in the large mammals.

We are now identifying the microbial metabolites with most interesting signatures and probing their physiological functions in vitro. We also investigate bacterial extracellular vesicles as a potential transplacental transport mechanism of microbial macromolecular antigens and develop methods for their specific studies in animal tissues.

ID: T9
Abstract
Topics: Mucosal diseases and infections, Swiss Young Immunologists Society (SYIS) and young European Federation of Immunological Societies (yEFIS)
Keywords: Influenza, Epithelium, pig, infection

NS1 and PA-X act in a concerted manner to manipulate the innate immune response of pandemic H1N1/09 influenza virus

Robin Avanthay[1], **Obdulio Garcias-Nicolas**[2], **Gert Zimmer**[2], **Artur Summerfield**[2]
[1]University Bern, IVI, [2]IVI

Live-attenuated influenza A viruses (LAIV) may be superior over inactivated or subunit vaccines since they can be administered mucosally to induce local immunity in the respiratory tract. In addition, LAIV can trigger cellular immune responses that may protect against a broader range of antigen-drifted viruses. However, the development of LAIV is challenging as attenuation of the vaccine candidates might result in significant loss of immunogenicity while others might be still too virulent. In this study, we took advantage of reverse genetics to generate three LAIV based on the pandemic H1N1 2009 (pH1N1/09) virus strain: NS1(1-126) lacking 93 amino acids at the C-terminus of the NS1 protein, ΔPA-X, which is defective in the synthesis of the accessory PA-X protein, and a combination of both. Characterization of these recombinant viruses using a novel porcine alveolar epithelial cell line revealed that that NS1(1-126) triggered the release of several chemokines and cytokines including type I and III IFN. Nevertheless, replication of NS1(1-126) on these epithelial cells was only marginally attenuated. The ΔPA-X mutant did not modulate the immune response but had a modulating effect on genes involved in cell cycle progression. Interestingly, infection of porcine alveolar cells with the NS1(1-126)/ΔPA-X mutant resulted in a very early and strong innate immune response which led to a significant attenuation of virus replication, indicating that NS1 and PA-X act in a concerted manner to manipulate the innate immune response. These findings may help to develop LAIV with a more favorable balance of virulence and immunogenicity.

ID: T10
Abstract
Topics: Neonatal window of opportunities, Swiss Young Immunologists Society (SYIS) and young European Federation of Immunological Societies (yEFIS)
Keywords: neonate, liver, microbiota, CD4 T cells, immune maturation

Microbial influence on the postnatal establishment of hepatic immune homeostasis

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Although not a classical mucosal organ, the liver is exposed to a substantial amount of microbial product from the gut via the portal vein and is positioned within circulation to link mucosal and systemic immunity. In contrast to the adult liver, the neonatal liver has not fully adopted its metabolic function, but instead harbours a high number of immune cells. Yet, while the impact of microbiota-dependent immune priming in early life is well established in the gut and other barrier tissues, little is known about the establishment of hepatic immune homeostasis and how this process is influenced by the microbiota. To broaden the understanding of neonatal liver immune cell dynamics, this study profiles the hepatic immune environment and the phenotype of liver T cells and investigates how they are shaped by the gut microbiota. RNA sequencing of neonatal liver tissue detected a strong enrichment of GO terms associated with immune cell activation, while cytokine multiplex ELISA and gene expression analysis via qRT-PCR revealed a transient, liver-specific peak of several inflammatory cytokines during the first week of life. Further, high-dimensional flow cytometry and scRNA-Seq revealed a transient wave of CD4 T cells in the neonatal liver, which were enriched in regulatory T cells (Treg) and activated conventional T cells (Tconv) that acquired an anergic phenotype. Microbial colonization contributed to cytokine induction and T cell activation: in germ-free neonates, levels of IFNγ, IFNα and IL-10 and frequencies of activated and anergic Tconv were decreased. Our results suggest that the liver provides a niche for adaptive immune priming early after birth that might be instrumental to the establishment of self and microbial tolerance of T cells upon their initial arrival in the periphery after birth, thus setting the stage for immune responses throughout the individual's course of life.

ID: T11
Abstract
Topics: Mucosal diseases and infections, Swiss Young Immunologists Society (SYIS) and young European Federation of Immunological Societies (yEFIS)
Keywords: COVID-19, adventitial niche, CCL21, exhausted T cells, spatial TCR profiling

Cellular and molecular characterization of the local immune response in severe COVID-19 by integrated spatial analysis of lungs and draining lymph nodes

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Tissues are composed of different cell types that group together into niches. Advances in multiplexed imaging approaches have revealed that the spatial organization of cells is intimately linked to tissue pathology. Lung pathology has been associated with COVID-19, but the underlying cellular participants, molecular mechanisms, and niche-specific functions are still poorly understood.

We have established a multiplexed imaging technique called multi-epitope ligand cartography (MELC), which allows analyzing more than 50 proteins on the same tissue section. We have developed a computational analysis pipeline to analyze the imaging data that allows for accurate cell segmentation, single-cell feature extraction, unsupervised clustering and spatial analysis. We combined MELC with spatial transcriptomics (ST) in the same COVID-19 lungs to complement the protein data, increase throughput and detect cytokines, key mediators of tissue organization. Thereby, we identified activated adventitial niches as crucial lung microenvironments orchestrating prolonged immunopathology, where CCL21 and macrophage-derived CCL18 promote endothelial-to-mesenchymal transition contributing to tissue fibrosis. The local immune response chronifies by the recruitment of CCR7+ T cells into these niches, where they are imprinted with an exhausted, innate-like phenotype with proliferative capacities and eventually form ectopic lymphoid structures.

We have benchmarked a strategy for clonal tracking of T cell populations in their spatial tissue context. The reported accumulation of CCR7+ exhausted T cells and their proliferation within activated niches suggests that clonal expansion occurs within the inflammatory sites. Thereby, SARS-CoV-2-specific T-cell functions would be constrained in the target organ, while bystander activation and immunopathology could take place. The ongoing analysis of spatially resolved TCR profiles of severe COVID-19 paired lungs and draining lymph nodes, and advanced integrated analysis with global transcriptional and protein profiling from the same tissues will shed light into this question and into the development and compartmentalization of T cell responses in COVID-19.

ID: T12
Abstract
Topics: Mucosal T cells
Keywords: CD8+ T cells, Tissue-resident memory T cells, Helicobacter pylori, antigen specificity, CagA

CagA-specific Gastric CD8+ Tissue-Resident T Cells Control Helicobacter pylori During the Early Infection Phase

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Infection with Helicobacter pylori strongly affects global health by causing chronic gastritis, ulcer disease, and gastric cancer. The bacteria's carcinogenic potential is largely determined by the oncogene CagA, which is injected by H. pylori into host cells.

Although extensive research into the strong immune response against this persistently colonizing bacterium exists, the specific role of CD8+ T cells remains elusive.

We comprehensively characterize gastric H pylori-specific CD8+ T-cell responses in mice by flow cytometry, (single-cell) RNA-sequencing, immunohistochemistry, and ChipCytometry, applying functional analyses including T-cell depletion, H pylori eradication, and ex vivo restimulation.

We corroborate our findings in human by analysis CD8+ T cells derived from gastric biopsies and blood samples with the methods described above.

We define CD8+ T-cell populations bearing a tissue-resident memory (TRM) phenotype, which infiltrate the gastric mucosa shortly after infection and mediate pathogen control by executing antigen-specific effector properties. These induced CD8+ TRM cells show monoclonal T-cell receptor usage and are mostly specific for CagA. We confirmed the CagA-targeted CD8+ TRM cell response in human and identify multiple CagA derived peptide:MHC-I epitopes.

Our results point toward a hitherto unknown role of CD8+ T-cell response in this bacterial infection, which may have important clinical implications for treatment and vaccination strategies against H pylori.

ID: T13
Abstract
Topics: Microbe-immune interactions at body surfaces, Systems immunology in host-microbe interactions

Microbiota encoded fatty-acid metabolism expands tuft cells to protect tissues homeostasis during Clostridioides difficile infection in the large intestine

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Succinate, a driver of tuft cells (TCs) differentiation and consequent type 2 immunity-dependent protection against invading parasites in the small intestine, is also a growth enhancer of the nosocomial pathogen Clostridioides difficile in the large intestine. However, none has shown the role of succinate in modulating TC dynamics in the large intestine and the relevance of this pathway to C. difficile pathophysiology. Here we reveal the existence of a three-way circuit between commensal microbes, C. difficile and host epithelial cells which is centered around succinate. Selective microbiota depletion increases succinate concentration and causes higher levels of type 2 cytokines and expands TCs in the colon. Rational microbiome supplementation experiments with stool fraction transplants and microbial consortia of succinate-producing bacteria establish the causal role of the microbiome in modulating colonic TC abundance and subsequent type 2 cytokines induction., Genetic manipulation of Bacteroides thetaiotaomicron to disrupt its ability to produce succinate completely abolishes enhanced type 2 immunity in a mono-colonized setting. Finally we demonstrate that, mice prophylactically administered with the consortium of succinate-producing bacteria or the succinate-producing B. thetaiotaomicron show reduced C. difficile-induced morbidity and mortality compared to mice administered with the production-deficient strain despite higher C. difficile load in the intestine. This effect is nullified by IL-25 neutralizing antibodies thus finally confirming that the protection occurs via the TC pathway.

ID: T14
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal B cells

Direct induction of TGF-β signaling by the intestinal gut microbiota bacterium Anaeroplasma reveals new potential anti-inflammatory probiotic

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Inflammatory bowel disease (IBD) is characterized by a strong inflammation of the gut driven by a continuous imbalance between pro- and anti-inflammatory cytokines. An important regulatory cytokine is tumor growth factor β (TGF-β) which suppresses unwanted immune responses in the gut and induces the immunoglobulin class switch to IgA in B cells. Both TGF-β and IgA are required to maintain gut homeostasis and are therefore a potential targets for the therapy of intestinal inflammation.

How TGF-β is regulated in the intestine has largely been unclear. We have recently identified the bacterium Anaeroplasma within the gut microbiota, which when newly introduced into the intestinal microbiota enhances mucosal IgA levels. Anaeroplasma does so by inducing the expression of TGF-β in T follicular helper (Tfh) cells of Peyer's Patches (PP). Whole genome sequencing of human Anaeroplasma isolates revealed an open reading frame (ORF) with amino acid homology to TGF-β itself. TGF-β via TGF-β receptor signaling is a strong inducer of its own expression. Initial results with a TGF-β receptor reporter cell line indicate that the product of the ORF of Anaeroplasma can induce TGF-β receptor signaling.

Thus, we have identified a bacterial gene product which promotes TGF-β expression in the intestinal mucosal tissue. These results suggest that Anaeroplasma plays a role in the maintenance of immune homeostasis in the intestine.

ID: T15
Abstract
Topics: Microbe-immune interactions at body surfaces
Keywords: Gasdermins, Salmonella, gut mucosa, host-pathogen interaction

Gasdermin D is the only Gasdermin that provides non-redundant protection against acute Salmonella gut infection

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Gasdermins (GSDMs) share a common functional domain structure and are best known for their capacity to form membrane pores. These pores are hallmarks of a specific form of cell death called pyroptosis and mediate the secretion of pro-inflammatory cytokines such as interleukin 1β (IL1β) and interleukin 18 (IL18). Thereby, Gasdermins have been implicated in various immune responses against cancer and infectious diseases such as acute Salmonella Typhimurium (S.Tm) gut infection. However, to date, we lack a comprehensive functional assessment of the different Gasdermins (GSDMA-E) during S.Tm infection in vivo. Here, we have performed littermate-controlled oral S.Tm infections to investigate the impact of all murine Gasdermins. While GSDMA, -C and -E appear dispensable, we show that GSDMD (i) restricts S.Tm loads in the gut tissue and systemic organs, (ii) controls gut inflammation kinetics, and (iii) prevents epithelium disruption by 72h of the infection. Full protection requires GSDMD expression by both bone-marrow-derived lamina propria cells and intestinal epithelial cells (IECs). In vivo experiments, 3D- and 2D-enteroid infections further show that infected IEC extrusion proceeds also without GSDMD, but that GSDMD controls the permeabilization and morphology of the extruding cells and affects extrusion kinetics. As such, this work identifies a non-redundant multipronged role of GSDMD in mucosal tissue defence against a common enteric pathogen.

ID: T16
Abstract
Topics: Innate Immunity, Microbe-immune interactions at body surfaces
Keywords: gut, SFB, MyD88, symbiosis

MyD88-dependent control of host-SFB symbiosis in SFB-monocolonized mice

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The segmented filamentous bacterium (SFB) is a commensal member of the gut microbiota that is crucial to orchestrate the post-natal maturation of the host immune system and to establish a healthy state of physiological inflammation in the intestine, including notably a strong homeostatic Th17 cell response. Strikingly, this function was shown to largely depend on the intimate attachment of SFB to the ileal mucosa. Although this unusual innocuous partnership remains largely enigmatic, it suggests that hosts have evolved potent mechanisms to control SFB colonization and to maintain a symbiotic relationship with SFB. However, which signaling pathways are used by SFB to launch self-limiting immune responses and how such homeostatic responses eventually control SFB colonization remain elusive. Using gnotobiotic approaches in immuno-deficient and chimeric mice to prevent confounding effects of a complex microbiota on the host responses, we observe that adaptive T or B cell-dependent immune responses are dispensable to control SFB growth in SFB-monocolonized mice while MyD88-dependent innate responses are critical. In addition, we show that MyD88 expression is dispensable in epithelial cells but indispensable in hematopoietic cells, and that MyD88-mediated control of SFB overgrowth involves IL-22 production by type 3 innate lymphoid cells and subsequent induction of an IL-22-dependent program. Given the potential role of myeloid cells as mediators of SFB-dependent triggering pathways, our current objective is to determine how MyD88 deficiency affects their distribution and function in the context of SFB monocolonization.

ID: T17
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections
Keywords: urothelium, microtissue, urinary tract infection (UTI), 3D human model

An innovative human bladder microtissue infection model

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Urinary tract infections (UTIs) are a major cause of morbidity, hospitalization and prescription of antibiotics worldwide. The high recurrence rates show that even the gold-standard treatments are suboptimal. Research in the UTI field has been dominated by studies using conventional cancer cell lines or animal models that do not fully recapitulate the ultrastructure, physiology and immunity found in humans.

More complex 3D bladder and kidney models have been advancing our knowledge in the field. Nonetheless, they usually lack critical features needed to elucidate the patient-like scenario, such as full tissue stratification and differentiation, barrier function, innate immunity and urine tolerance.

To fill this gap, we established a 3D microtissue urothelial model in a Transwell® platform that successfully mimics the key features previously mentioned. Namely, we archived 60-70 µm thickness with ~7 cell layers, embodying the three main urothelial subtypes (basal, intermediate and umbrella). Terminal differentiation was confirmed by UPIII and CK20 markers. Tissue integrity and barrier function was shown by the presence of ZO-1 in tight junctions and using transepithelial electrical resistance (TEER) measurements.

As this model tolerates urine for long time period, including under flow, we were able to use it to assess uropathogenic Escherichia coli (UPEC) infection spatial-temporal dynamics in a human-relevant microenvironment. UTI-associated physiological events from both host (cell exfoliation) and bacterial (morphological alterations) sides were monitored at the single cell level in real time. More studies are ongoing regarding the production of cyto/chemokines and other innate responses.

This model provides useful insights at both cell and tissue levels. As it is established in an inverted setup, it facilitates live high-resolution microscopy while challenging the bacteria to use their motility and virulence arsenal to meet and colonize the host. Overall, this model emerges as a promising platform to study patient-like scenario in and beyond the UTI field.

ID: T18
Abstract
Topics: Mucosal diseases and infections
Keywords: TGF-beta, inflammatory bowel disease, immune dysregulation, Loeys-Dietz syndrome, TGF-beta signalopathies

Aberrant TGF-beta signaling downstream importin 8 deficiency triggers intestinal inflammation

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The TGF-beta/BMP cytokine family orchestrates pleiotropic functions during embryonic development, tissue homeostasis and repair, as well as within the immune system. Accordingly, aberrant TGF-beta signaling underlies the pathogenesis of severe congenital disorders associating developmental defects with or without immune dysregulation. Heterozygous loss-of-function (LOF) variants in TGFBR1/R2, TGFB2 or TGFB3, SMAD2, or SMAD3 are notably the cause of the Loeys–Dietz syndrome (LDS), a rare condition presenting with connective tissue disorders, craniofacial and skeletal abnormalities, joint laxity. Increased frequency of allergic manifestations and inflammatory bowel diseases (IBD) has also been reported in LDS patients. We have recently identified bi-allelic LOF variants in IPO8 as cause of a connective tissue disorder largely overlapping with LDS. IPO8 codes for importin 8, a member of the beta-karyopherin family, which shuttles a wide range of cargos from the cytoplasm to the nucleus. We have shown that importin 8 plays a critical role during early stage of development in zebrafish by controlling phospho-Smad (p-SMAD) nuclear translocation downstream and TGF-beta/BMP-dependent transcription. We report here that IPO8 deficient patients also display increased prevalence of immunedysregulation features, early onset intestinal inflammation, hyperIgE and allergies. We then sought to determine whether Ipo8-/- mice might recapitulate the immune phenotypes observed in IPO8 patients. Pointing to spontaneous intestinal inflammation, we found that Ipo8-/- male mice showed failure to thrive over time and about 80% developed rectal prolapse whereas wild-type littermates did not. Significant decrease in colon length and splenomegaly further confirmed underlying low grade inflammation. Overall, our data suggest an immune relevant role of IPO8, and show that Ipo8-/- mice are a valid model to study how dysregulation of the TGF-β signaling dependent on loss of IPO8 predisposes to intestinal inflammation and immune dysregulation, underscoring the prominent role of these pathways in directing immune responses to mucosal antigens.

ID: T19
Abstract
Topics: Mucosal diseases and infections
Keywords: intestine, type 2 immunity, stromal cells, tissue niche, parasite infection

Inflammation-driven polarization and tissue-niche formation of intestinal lamina propria stromal cells

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Mucosal surfaces in the gastrointestinal tract represent key immunological sites, where decisions between tolerance and immunity are constantly made. We have previously shown that stromal cells of the gut-draining mesenteric lymph nodes possess tolerogenic properties and substantially contribute to regulatory T-cell induction. Mesenchymal stromal cells are highly abundant in the intestinal lamina propria (LP) as well, however, their contribution to tolerance or immunity remains largely obscure. Therefore, we aimed to explore key molecular signatures of intestinal LP stromal cells upon different types of intestinal inflammation and their contribution to intestinal tolerance.

In vitro short-term culture of CD31-Pdpn+ intestinal LP fibroblastic stromal cells (FSCs) revealed their responsiveness to inflammatory cues including the hallmark type-1, -2, and – 17 cytokines IFN-γ, IL-13, and IL-17. This cytokine-specific response pattern also indicated that intestinal LP FSCs form distinct “tissue niches” for immune cells depending on the type of inflammation. To corroborate these findings, we performed RNA-seq analysis with sorted intestinal LP FSCs from mice undergoing Nippostrongylus brasiliensis (N.b) parasite and Lymphocytic choriomeningitis (LCMV) virus infection. Here, we aimed to identify polarization-specific in vivo transcriptional signature genes of intestinal LP FSCs overlapping with the previously identified in vitro signature genes. Further flow cytometric characterization of the intestinal LP stromal compartment indicated their substantial reorganization upon N.b helminth infection and the appearance of a highly proliferating progenitor-like subset, which we are currently investigating by single-cell RNA-seq analysis.

Taken together, our observations demonstrate distinct polarization capacities of intestinal LP FSCs upon exposure to different inflammatory stimuli and provide key molecular hints for crucial stromal-immune cell networks during type-2 immunity-driven intestinal inflammation.

ID: T20
Abstract
Topics: Mucosal diseases and infections
Keywords: Fibroblastic stromal cells, Interleukin-33, Heligmosomoides polygyrus bakeri, Eosinophils

Fibroblastic stromal cell-derived IL-33 controls intestinal helminth infestation

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Interleukin-33 (IL-33) is a nuclear cytokine of the interleukin-1 family and is released upon cell damage thereby acting as an alarmin. IL-33 plays an essential role in promoting host-protective immune responses against helminth parasites at different mucosal surfaces including the intestine. However, early events after gastrointestinal nematode infection are poorly understood and the cellular source of IL-33 in the gut remains ill-defined. Here we show that Cxcl13-Cre-positive fibroblastic stromal cells (FSCs) of the lamina propria are the main source of IL-33 in the small intestine. Within the first days of nematode infection, lamina propria FSCs showed strongly increased IL-33 expression. Ablation of IL-33 in Cxcl-13-Cre-positive FSCs resulted in an increased infestation with the gastrointestinal nematode Heligmosomoides polygyrus bakeri (Hp). We found that the recruitment of eosinophils to the site of worm invasion early after infestation was dependent on FSC-derived IL-33. Collectively, these data unveil that FSC-derived IL-33 is critical during the initial immune response against gastrointestinal nematodes.

ID: T21
Abstract
Topics: Mucosal B cells
Keywords: humoral immunity, neonatal immunity

cDC1 deficiency affects neonatal humoral immunity to Rotavirus without impacting on protection from reinfection later in life

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Rotavirus is an intestinal pathogen that primarily affects infants and young children. Available vaccines show variable efficacy. Dendritic cells (DCs) are crucial for the initiation of immune responses and therefore important targets for vaccines. The two major DC subsets, called cDC1 and cDC2, differ in their capacity of inducing specific adaptive immunity in adults, but their distribution and potentially differential roles in neonates are largely unknown. After confirming that steady state neonates indeed harbored considerable numbers of both cDC1 and cDC2 in the intestines, we set out to address whether specific DC subsets were required to induce humoral responses towards neonatal rotavirus infection. As in adult mice, rotavirus induced a considerable T cell-dependent accumulation of total B cells in gut-associated lymphoid tissues, which was unaltered in mice lacking cDC1 or cDC2. In contrast, cDC1-deficient suckling mice specifically lacked the substantial plasmablast induction observed in response to rotavirus infection in wild type mice. This differs from the adult setting, in which lack of cDC1 affects IgA induction, but shows no effect on overall plasmablast numbers. Despite the absence of plasmablasts in the mesenteric LNs of cDC1-deficient suckling mice however, both neonatally and adult infected models were equally protected from reinfection. Overall, our data suggest that mechanisms of primary B cell induction and isotype switching differ in the developing versus the adult immune system, but that the absence of cDC1 does not affect the remarkable immune memory initiated in the context of natural rotavirus infection.

ID: T22
Abstract
Topics: Neonatal window of opportunities
Keywords: Maternal microbiota, microbial metabolites, epidermal development, skin barrier, aryl hydrocarbon receptor

Maternal microbiota-derived metabolites drive embryonic epidermal formation and set neonatal skin barrier

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Neonates need a competent skin barrier by birth to deal with external aggressions such as pathogens, hazardous substances and mechanical stress. At birth, the microbiota commensally colonizes mammalian barriers and supports postnatal barrier maturation. Nevertheless, prenatal training by the maternal microbiota through control at stem cell level might foster efficient and long-lasting immune development. We previously showed that metabolites, derived from the maternal microbiota, reach the embryo. We aimed to investigate the potential effect of maternal microbiota-derived metabolites in shaping embryonic skin ontogeny and in training early-life skin barrier function.

Pregnant germ-free mice were either colonized by intragastric delivery of auxotrophic Escherichia coli HA107 for a reversible microbial exposure, or by a cocktail of multiple microbes. Single cell sequencing, flow cytometry and histological analysis of embryonic skin of offspring of gestationally colonized dams illustrated an epidermis composed of a tightly organized stem cell monolayer, overlain with multiple layers of differentiated keratinocytes. In the absence of maternal microbiota-derived metabolites, we observed an underdeveloped epidermis with several layers of undifferentiated and highly proliferative stem cells. Similarly, hair follicle development was enhanced by gestational colonization. Pre-gestational or postnatal microbiota-derived metabolite exposure failed to compensate for their absence during gestation. Metabolites involved in epidermal differentiation and skin barrier development such as vitamins, nicotinamide, tryptophan and derivatives accumulated in embryonic skin after gestational colonization. By using 13C-labelled bacteria, mass-spectrometry analysis and epidermal 3D-organoid models, we demonstrated that aryl hydrocarbon receptor ligands from the maternal microbiota modulate the differentiation capacity of keratinocyte stem cells. Abnormalities in the permeability barrier, illustrated by enhanced trans-epidermal water loss and dye penetration, were present in neonatal skin developed without maternal microbiota-derived metabolite exposure. In summary, our results show that maternal microbiota-derived metabolites shape embryonic epidermal ontogeny and set skin barrier by birth.

ID: T23
Abstract
Topics: Neonatal window of opportunities, Microbe-immune interactions at body surfaces
Keywords: IgA, microbiota, neonates, intestine

A method to distinguish neonatal and maternal IgA in young mice

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Secretory IgA (SIgA) provides immune exclusion of pathogens at mucosal barrier sites such as the intestine, crucially contributing to the maintenance of gut integrity and homeostasis. This is particularly important during the neonatal phase, where maternally breast-milk-derived SIgA protects the vulnerable offspring from infectious diseases and contributes to the balanced maturation of the gut microbiota. Withdrawal of protective milk-derived factors upon weaning induces immune maturation, resulting in the onset of endogenous SIgA in the offspring. Likewise, the complete absence of maternal humoral immunity has been associated with a premature onset of endogenous SIgA. To in detail understand the kinetics of SIgA onset in an unperturbed setting, we here set out to establish a model that would allow for the faithful tracking of the contribution of endogenous SIgA to bacterial coating in offspring born to immunocompetent dams. To differentiate between maternal and neonatal SIgA sources, we took advantage of existing murine IgHa and b allotypes on the C57BL/6 background, which can be tracked using allotype-specific antibodies. We collected feces as well as luminal content from small and large intestines of IgHa/IgHa, IgHa/IgHb and IgHb/IgHb adult mice and stained bacteria with total IgA and IgAa specific antibodies. As expected, both stains linearly correlated and staining intensities for IgAa were dependent on genotype. To quantify the selective contribution of pup-derived SIgA coating of bacteria, we currently use our linear regression model on IgHa/IgHb pups born to IgHb/IgHb dams across different ages. This will clarify the exact kinetics of endogenous SIgA production onset in offspring and will allow future studying of cellular and molecular requirements for such onset in homeostatic and immune-challenged conditions.

ID: T24
Abstract
Topics: Innate Immunity, Mucosal B cells
Keywords: Monocytes, Secreted IgD, Immune Tolerance, Alergy

Secreted IgD Targets Monocytes to Deliver a Complex Signaling Program Characterized by a Prominent Tolerogenic Component

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Secreted immunoglobulin D (IgD) derives from IgD class-switched plasma cells emerging from the nasopharyngeal mucosa. IgD binds to basophils through a receptor complex that includes the tandem repeat galectin family member galectin-9 and the galectin-9-binding protein CD44. Co-ligation of basophil-bound IgD and IgE by antigen interferes with IgE-induced degranulation. Accordingly, antigen-specific IgD responses correlate with a lower risk of anaphylaxis and increased allergen immunotherapy-induced tolerance in allergic patients. Here, we hypothesized that the enhancement of tolerance by secreted IgD involves monocytes, which serve as precursors of tolerogenic dendritic cells and macrophages in mucosal compartments.

Methods

Circulating monocytes from healthy subjects were purified by negative selection. Flow cytometry and confocal microscopy were performed to evaluate monocyte interaction with secreted IgD. To dissect IgD-induced signaling programs and cytokine production, monocytes were first cultured in the presence of control or anti-IgD Fab(2) antibodies and then analyzed by RNA sequencing (RNA-seq) and ELISA.

Results

Secreted IgD specifically targeted a large fraction of monocytes, which led to the cytoplasmic accumulation of IgD. Although inducing only marginal phenotypic changes, crosslinking of IgD for 6 hours extensively shaped the gene expression profile of monocytes, inducing down-regulation and up-regulation of 10.000 and 2000 gene products, respectively. The latter included transcripts implicated in IL-10 signaling through the JAK-STAT pathway or involved in mucosal IgA production. Accordingly, ELISA showed that IgD crosslinking for 24 or 48 hours elicited monocyte secretion of both the tolerogenic cytokine IL-10 and the IgA-inducing cytokine BAFF, but no secretion of the pro-inflammatory cytokine TNF.

Conclusions

The interaction of secreted IgD with monocytes induces a tolerogenic signaling program that may help respiratory surfaces to interact with common environmental antigens in a non-inflammatory manner. This possibility may explain why antigen-specific IgD responses correlate with increased tolerance to allergens in some individuals.

ID: T25
Abstract
Topics: Mucosal diseases and infections, Mucosal B cells
Keywords: Oxysterols, intestinal epithelial cells, CH25H, plasma cells, GPR183, CD98, amino acids

Integrated nutrient sensing controls intestinal IgA plasma cell response against commensals and pathogens

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Immunoglobulin A (IgA) secretion by plasma cells assures microbiome homeostasis and protects the host against enteric infections. Exposure to diet-derived and commensal-derived signals provides immune cells with organizing cues that instruct their effector function and dynamically shape intestinal immune responses at the mucosal barrier. Although, it is emerging that nutrient uptake might impact plasma cell lifespan and function, mechanistic links between diet-derived bioproducts, plasma cell metabolic signaling and antibody secretion it is still unknown.

We discovered that intestinal epithelial cells (IECs) integrate luminal input to produce 7α,25-dihydroxycholesterol (7α,25-HC), a cholesterol metabolite (oxysterol) with immunomodulatory functions. We used mice lacking the oxysterol enzyme CH25H specifically in IECs to study oxysterol generation and activity in the small intestine. Inability of IECs to generate 7α,25-HC enhanced IgA secretion by plasma cells in the gut, suggesting that oxysterol negatively regulate humoral response at the mucosal barriers. We showed that intestinal plasma cell sensed 7α,25-HC via the chemoattractant receptor GPR183 to position in the lamina propria tissue. This IEC-plasma cell axis was rapidly modulated by cholesterol dietary content and tuned Salmonella-specific IgA response.

Interestingly, we observed that intestinal plasma cell that actively secrete IgA also up-regulate the amino acid transporter CD98. Plasma cells exposed to the non-essential amino L-Alanine, enhanced the expression of transcription factors involved in cellular metabolism and boosted IgA secretion. Consequently, inhibition of metabolic regulatory signaling hubs led to reduced antibody production and reactivity to antigens in the intestine, as well as in spleen and bone marrow.

Together, our results suggested that different branches of intracellular metabolism cooperate to assure efficient humoral response in the intestine. Specific targeting of cholesterol absorption by IEC or plasma cell metabolic pathway downstream amino acids uptake might represent a new therapeutic strategy for boosting efficiency of novel mucosal vaccine and to treat of antibody-dependent auto-inflammatory conditions.

ID: 001
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Myelopoiesis, Chlamydia trachomatis, M2 macrophages, Immune suppression, CD40

Chlamydia trachomatis skews myelopoiesis to M2 and induces immune suppression in macropahges

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Chlamydia trachomatis (C.tr), an obligate intracellular pathogen, causes asymptomatic genital infections in women causing pelvic inflammatory disease and ectopic pregnancy, and is also a leading cause of preventable blindness. During chronic and repeated C.tr infections cervical infiltrated lymphocytes predominantly consisted of Th2 and Treg cells accompanied by M2 macrophages. The infiltrated macrophages show poor CD40 expression. Draining lymph nodes also show low Th1 cells and more Treg cells. Acute infection shows higher Th1 cells along with CD8+T cells secreting IFNγ and TNFα which help in bacterial clearance. An increase in exhaustion and anergic (Klrg3, Tim3) T cell markers is also observed during chronic infection. It was observed that chronic C. trachomatis induces myelopoiesis to generate Ly6Chigh MDSCs and alternatively-activated macrophages as observed with increased M2 generation from the bone marrow of chronic infected mice and circulating MDSCs. Chronic infection-induced cytokines also polarised normal BMDMs to M2 phenotype. The observation was supported by the increase in M2 macrophages in the blood. These macrophages are recruited to the genital tract and express low CD40 promoting Th2 and Treg differentiation leading to sustained C. trachomatis genital infection. T cells are inactivated to such an extent that, restimulation of T cells from spleen and lymph nodes shows poor BrdU incorporation and IL-2 secretion. Even the T cells generation after restimulation are mostly CD4+ CD25+ Foxp3+ IL-10+ TGFβ+ Tregs but not Th1 necessary for bacterial clearance. C. trachomatis infection prevents increase in CD40 expression in macrophages. Low IFNγ, as observed in chronic infection leads to incomplete clearance of bacteria and poor immune activation. Thus, C. trachomatis modulate the innate immune cells attenuating the anti-chlamydial functions of T cells in a manner that involves decreased CD40 expression on macrophages and macrophage polarisation.

ID: 002
Abstract
Topics: Microbe-immune interactions at body surfaces
Keywords: tuft cells, Paneth cells, microbiota, type 2 immun responses

Tuft cells mediate Paneth cell-driven gut dysbiosis and inflammation

Philippe Jay (Inserm, France)

Gut microbiota imbalance (dysbiosis) is increasingly associated with pathological conditions within and outside of the gastrointestinal tract. Intestinal Paneth cells are considered to be guardians of the gut microbiota but the events linking Paneth cell dysfunction with dysbiosis remain unclear. We report a three-step mechanism for dysbiosis initiation. Initial alterations in Paneth cells, as frequently observed in obese and IBD patients, cause a mild remodeling of microbiota, with amplification of succinate-producing species. SucnR1-dependent activation of epithelial tuft cells triggers a type 2 immune response that, in turn, aggravates the Paneth cell defaults, promoting dysbiosis and chronic inflammation. We thus reveal a novel function of tuft cells in promoting dysbiosis following Paneth cell deficiency, and an unappreciated essential role of Paneth cells in maintaining a balanced microbiota to prevent inappropriate activation of tuft cells and deleterious dysbiosis. This succinate-tuft cell-inflammation circuit may also contribute to the observed patients dysbiosis chronicity.

ID: 003
Abstract
Topics: Neonatal window of opportunities, Mucosal diseases and infections
Keywords: MUC17, glycocalyx, small intesine, infection

Membrane mucin MUC17 forms a cellular glycocalyx barrier against bacteria in a model of human Inflammatory bowel disease (IBD)

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The gastrointestinal tract is fortified by a monolayer of intestinal epithelial cells (IECs) that creates a firewall between the microbiota and the immune system. Inappropriate encounter of bacteria by IECs and immune cells triggers Inflammatory bowel disease (IBD), allergies, obesity, and even cancer.

Membrane mucins are large, evolutionarily conserved, and sugar-laden transmembrane proteins that protrude up to 1 micrometer from the epithelium into the intestinal lumen. Despite their discovery over 30 years ago, their fundamental biological function in human health and disease remains unknown. We recently uncovered that the homeostatic cytokine IL-22 induces IECs to express membrane mucin MUC17 during the critical suckling-weaning transition in mice. By employing a new imaging technique in intestinal explants, we showed that MUC17 forms a dense cell-attached glycocalyx that prevents direct bacterial contact with IECs. However, due to the lack of appropriate mouse models, the function of MUC17 and the glycocalyx in vivo has remained elusive.

Here, we generated the first-ever mouse model with an IEC-specific Muc17 deletion. Muc17ΔIEC mice lack a glycocalyx barrier and are more susceptible to atypical small intestinal infection with Citrobacter rodentium, a murine model of human Enteropathogenic E. coli (EPEC). In Muc17ΔIEC mice, both commensal and pathogenic bacteria make inappropriate contacts with IECs, thereby triggering classic manifestations of IBD such as epithelial hyperproliferation, epithelial cell shedding, and mucus barrier defects. We also report that Crohn's disease patients exhibit a striking downregulation of MUC17 that makes the small intestinal epithelium susceptible to bacteria.

In summary, we identified glycocalyx-forming membrane mucin MUC17 as a critical line of defense against bacterial encroachment in the human and mouse small intestine. Our findings present MUC17 as a potentially new target for treatments aiming at enforcing the mucosal barrier in Crohn's disease.

ID: 004
Abstract
Topics: Innate Immunity, Mucosal B cells
Keywords: Tissue Injury, Peyer's patches, B cells, Neutrophils, circulating DNA

Peyer's Patch B cells undergo cell death via neutrophil-released toxic DNA following sterile tissue injury

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The loss of systemic lymphocytes is a major hallmark of post-injury immunosuppression and associated bacterial infections. Recent studies have evidenced the severe dysbalance of the intestinal immune compartment after tissue injury. Intestinal Peyer's patches (PP) contain a large number of B cells, but how sterile tissue injury leads to the contraction of B cells in PP has not been explored. In this study, we observed rapid and macroscopically apparent shrinkage of PP after stroke and myocardial infarction. Light-sheet fluorescence microscopy and flow cytometry discovered a strong reduction in the number of PP resident B cells. Mechanistically, tissue injury triggered the activation of neutrophils that released B cell-toxic neutrophil extracellular traps (NETs) decorated with citrullinated histone-H3. Antibody-mediated or genetically induced neutrophil-loss, NETs-degradation or blockade of their generation significantly reversed B cell loss and preserved the tissue architecture of PP. Our findings that NET-like elements are also present in human post-stroke plasma, we propose that targeting NET-generation or -function counteracts post-injury B cell contraction in PP and thereby maintains immune homeostasis at mucosal barriers.

ID: 005
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal B cells
Keywords: Staphylococcus epidermidis, cutaneous B cells, T follicular helper cells, IgG

Host colonization with cutaneous commensal induces humoral immunity via formation of dermal tertiary lymphoid organs

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The skin is one of the largest barrier sites which harbors a plethora of microorganisms. Whether skin microbiota promotes humoral responses remains unknown. Here we show that colonization with common skin commensal Staphylococcus epidermidis profoundly modulates cutaneous immunity that results in serum responsiveness with antigen-specific IgG antibodies. Such response is uncoupled from inflammatory signals and is associated with the induction of dermal tertiary lymphoid organs that closely resemble classical germinal centers. Of note, such responses occur in a tissue autonomous manner as evidenced by the preserved antibody repertoire in mice deficient in professional lymphoid organs. Our data also uncover a non-redundant role for Langerhan's cells for the generation of commensal specific antibodies. Further, our work proposes a model by which commensal specific antigens are collected in the hair follicle leading to the generation of T follicular helper cells that further expand the local B cell pool in an IL-21-dependent manner. We also found that commensal induced antibodies can protect the host against subsequent infection with the same microbe demonstrating the importance of this line of defense in maintaining barrier protection. Collectively, our observations highlight the autonomous potential of skin organizational flexibility and for the first time demonstrate that B cells represent an indispensable local cutaneous compartment that actively maintains skin immunity.

ID: 006
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: Inflammatory bowel disease, stroma cell, T cell, myeloid cell

Cell-specific modulation of A-type lamins in immune and stroma cells as therapy for Inflammatory Bowel Disease

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Maintenance of intestinal homeostasis is crucial to prevent the development of inflammatory bowel disease (IBD). Gut homeostasis strictly depends on the interplay between several cell types including immune and non-immune cells. However, the mechanism of action of intestinal homeostasis-regulating cells remains unknown. Here, we explore the specific role of some immune cells and stroma cells by regulating the levels of lamin A/C, a structural, epigenetic, and transcriptional regulator, in a mouse model of IBD with dextran sulfate sodium salt (DSS).

DSS-colitis development was measured in mice that lack of lamin A/C (Lmna-/-) in all immune cells (Vav1-Cre), T cells (CD4-Cre), some myeloid cells (LysM-Cre), and stroma cells (Co1a2-Cre) or in mice overexpressing lamin A/C in T cells (Lmna++/++ CD4-Cre). Mice were exposed to one or two cycles of DSS to preferably stimulate innate or adaptive immunity, respectively.

Our experiments show that, in comparison to wild-type mice (Lmna+/+), the absence of lamin A/C in T cells, in myeloid cells, or stroma cells upon one dose of DSS protects from colitis. The protection appears to be more accentuated when mice that lack lamin A/C in T cells are exposed to two doses of DSS, hence, highlighting the key role of lamin A/C in the adaptive immunity in colitis. In accordance, lamin A/C overexpression in T cells provokes much more colitis upon two doses of DSS. The lack of lamin A/C in all immune cells protects from colitis upon one or two doses of DSS.

In conclusion, lamin A/C levels in immune cells and stroma cells seems to be important in colitis evolution: its absence protects against colitis while its overexpression worsen the disease, converting lamin A/C regulation in certain immune and non-immune cells in a potential therapeutic target.

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ID: 007
Abstract
Topics: Mucosal T cells, Microbe-immune interactions at body surfaces
Keywords: iNKT, Porphyromonas, CRC, pro-tumorigenic

Porphyromonas gingivalis supports colon cancer progression by switching iNKT cell functions towards a pro-tumorigenic phenotype

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Invariant Natural Killer T (iNKT) cells are lipid-specific T lymphocytes endowed with antitumor activities and whose infiltration is considered a positive prognostic factor in colorectal cancer (CRC). In this context, we have previously demonstrated that, at steady state, iNKT cells eliminate human CRC cells by releasing perforin and granzyme B. However, very little is known about how the function of these cells is influenced by colon cancer-associated bacteria, such as Porphyromonas gingivalis. Hence, our aim was to assess changes in iNKT cell phenotype provoked by this bacterium, and how these changes might contribute to colon cancer progression. For this, we first addressed human iNKT cell phenotype and performed functional assays upon stimulation with P. gingivalis. Subsequently, as validation, we administered P. gingivalis to AOM/DSS-treated mice and evaluated iNKT cell phenotype and tumor progression. We observed that, compared to cells stimulated with aGalCer (a prototypical iNKT cell antigen), iNKT cells primed with P. gingivalis showed lower levels of interferon gamma, together with reduced cytotoxicity against CRC cell lines. These cells were also characterized by an increased expression of GM-CSF, and interleukins -17 and -10, as well as inducing neutrophil chemotaxis. This pro-tumorigenic phenotype was also found in AOM/DSS-treated mice, as P. gingivalis-treated animals showed tumors of greater size, together with an increase in IL-17+ and GM-CSF+ iNKT cells. These results demonstrate that P. gingivalis can promote colon tumorigenesis by acting on unconventional T cells, both by hampering antitumor properties and promoting the release of pro-tumorigenic cytokines, which further act on other immune populations.

ID: 008
Abstract
Topics: Gut-Microbiota-Brain axis, Mucosal diseases and infections
Keywords: gut-lungs axis, gut-brain axis, microbial metabolites

The influence of microbial metabolites on lung and brain immunity

Edyta Bulanda (Nencki Institute of Experimental Biology, PAS, Poland)

The gut microbiota is a collection of microbes, which inhabit our intestines. It is becoming apparent that the interactions between these microorganisms and human cells are central to maintaining health and become dysregulated in disease. Despite this, one question remains unanswered: how does the gut microbiota influence immune function in distal body organs, such as the brain and lungs?

In recent years, we and others have gained important advances towards unravelling the mechanisms that underline the “gut-lung axis” and "gut-brain axis" as we pointed to the presence of gut-derived metabolites in these organs. Following up on this, we identified one metabolite, which inhibited inflammatory responses in cells from these areas. In lung cells, it inhibited the production of cytokines and chemokines characteristic of acute respiratory distress syndrome (IL-6, CXCL1 or CXCL10). In glia, it ameliorated the production of mediators typically upregulated in multiple sclerosis patients (IL-6, CCL2 or CCL20). Testing the efficacy of metabolite’s isomer pointed to the active site of the molecule and allowed us to construct the library of 20 rationally designed chemical derivatives. Some of these modifications exerted a stronger ani-inflammatory effect than the original compound, and some showed a distinct anti-inflammatory profile (i.e. inhibited different mediators). Collectively, these findings constitute the grounds for exploring the efficacy of identified metabolites in vivo and explaining their mechanisms of action.

Overall, our results may constitute the first step towards the development of these compounds as drugs, e.g. in form of a mixture of identified metabolites, a therapeutic solution that simultaneously targets a broad spectrum of proinflammatory mediators.

ID: 009
Abstract
Topics: Swiss Young Immunologists Society (SYIS) and young European Federation of Immunological Societies (yEFIS)
Keywords: primary immunodeficiency, neurologic involvement

Neurological Involvement in patients with Primary Immunodeficiency

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Introduction: Primary immunodeficiency diseases (PID) are a group of diseases defined by recurrent infections, allergies, autoimmunity, and malignancies. A variety of pathological mechanisms, including DNA repair disorders, metabolic abnormalities, and auto-immune phenomena, have also been associated with neurological conditions.

Material and method: We evaluated the neurological involvement in 108 patients with PID in this study retrospectively.

Results: The female/male ratio of the cases was 49/59, and the median age was 13 years (min=1 max=60). Neurological problems were detected at the median age of seven years (min = 0.5, max = 30).DiGeorge Syndrome (DGS) and CVID (common variable immunodeficiency) were the most common diseases in our cohort (n= 31, 30%, n=30,%27). The most frequent findings were cognitive delay (n=63, 58%), epilepsy (n=25, 23%), and ataxia (n=20, 18%), respectively.

Central nervous system involvement was found in 99 %of patients (n=107), and peripheral nervous system complication was found in only one patient with CVID and chronic inflammatory demyelinating polyneuropathy (CDIP). Cranial MRI was abnormal in 74% (n = 80) of patients. MRI findings were cerebellar atrophy (n=33, 34%), white matter lesion (n=27, 28.4%), cerebral atrophy (n=21, 22.3%), gray matter lesion (n=6, 6.3%), hydrocephalus (n=5, 5,3%), and pituitary gland lesion (n=3, 3.2%), intracranial hemorrhage (n=3, 3%), intracranial vasculitis (n=3, 2,7%), and arterio-venous malformation (n=1, 0,9%).

Primary involvement (a component of the disease) was 60% (n=65), secondary (infection or autoimmunity) and tertiary involvement (structural or incidental lesions) were 20% (n=20) and 20% (n=20) in patients. respectively.

Conclusion: The neurologic presentation may constitute the initial manifestation of

certain types of PID. Early diagnosis and treatment are essential to prevent or reduce further neurologic damage.

ID: 010
Abstract
Topics: Systems immunology in host-microbe interactions
Keywords: primary immunodeficiency, Rag gene

Recombinase activating gene defects, phenotypic diversity

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Recombinase activating gene defects, phenotypic diversity

Background and aims:

Mutations in recombinase activating genes 1 and 2 (RAG1/2) are associated with different clinical manifestations, from asymptomatic to severe combined immunodeficiency (SCID). This study aims to present our series of patients who had immunodeficiency with RAG mutations.

Methods:

Demographic data were obtained from the clinical records retrospectively. The clinical status of the patients, treatment modalities, mutation types, laboratory findings, and survival rates were analyzed

Results:

A total of 27 patients were included in the study with a mean age of 10.46 ± 13.87. There were 12

male and 15 female patients. The mean age at the time of diagnosis was 5.29± 10.74 years. The time

between the onset of symptoms and diagnosis was 3.91 ± 8.39 years. RAG 1 mutation was observed

in 18 (66%) patients, and RAG 2 mutation were observed in 8 (29.7%). One patient whom clinically and immunologically has Omenn syndrome phenotype is waiting for genetic analysis. Twenty-six patients (96.1%) received IVIG treatment. Thirteen patients (48.1%) received bone marrow transplantation. In 18 patients, BMT was indicated; 13 of them received BMT, one of them is in the conditioning for BMT, and 4 of the patients are waiting for an available donor. The BMT decision was due to Omenn syndrome in 9 patients and severe combined immunodeficiency (SCID) for nine patients. During the follow-up period, 7 (25.9%) patients died.

Conclusions:

The clinical course of RAG mutation shows great variation. The same mutation in different patients may cause different clinical pictures. The present study is one of the largest series about RAG mutations in the literature. Multicenter studies are needed to improve the knowledge for diagnosing and treating RAG-related immunodeficiencies.

ID: 011
Abstract
Topics: Mucosal diseases and infections
Keywords: COVID-19, Influenza, respiratory tract infection-induced B cell, antibody clones, immune repertoire

Acute respiratory tract infection (COVID-19 and Influenza)-induced immune repertoire profiling in humans

Kuan-Ying Huang (National Taiwan University, Taiwan)

Emerging and re-emerging infections that target the respiratory tract pose a serious threat to human health. The COVID-19 and seasonal influenza are two common respiratory tract infections and community outbreaks are associated with severe complications and mortality around the world. Host immune responses, especially B cell and antibody-mediated immunity, play a key role in prevention and control of illnesses of COVID-19 and influenza. However, the key features of immune repertoire that are linked to SARS-CoV-2 or influenza antigen-specific immune phenotype remain largely unclear in humans. We have been able to characterize the breadth and magnitude of antigen-specific B cell-derived antibody clonal database from COVID-19 and influenza convalescent patients and vaccinated individuals. For instance, the average frequency of anti-SARS-CoV-2 Spike B cells accounts for 0.5-2% of circulating B cells early upon antigen exposure. Among COVID-19 patients and vaccinated individuals, anti-receptor-binding domain (RBD) antibodies constitute one major part of potently neutralizing anti-SARS-CoV-2 Spike antibody clones and a preference to certain gene usage of immunoglobulin variable region of heavy and light chains is observed in some classes of neutralizing anti-RBD antibodies. We further acquire broad sampling and in-depth assessment of immune repertoires and the germline adaptive immune receptor repertoire is utilized to profile immune repertoires among COVID-19 and influenza convalescent patients and vaccinated individuals. The information of germline V, D and J gene usage, complementarity-determining region diversity, somatic hypermutation levels and clonal relationship provides valuable insights into the nature of immune response to these viral pathogens.

ID: 012
Abstract
Topics: Innate Immunity, Microbe-immune interactions at body surfaces
Keywords: intestine, Glycoprotein 2 (GP2), microbiota, IgA, gut homeostasis

Defining the role of the GP2-IgA-microbiota interactome in intestinal homeostasis and disease

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The host immune system is in constant and mutualistic dialogue with the intestinal microbiota - a dialogue that is essential for gut homeostasis. Major alteration in the normal microbiota composition (dysbiosis) and inappropriate immune responses can be key drivers in the development of inflammatory bowel disease (IBD). A central immune response mediating beneficial host-microbiota interactions and preventing dysbiosis is the secretion of immunoglobulin A (IgA) into the gut lumen, where it binds to both the microbiota and pathogens. In contrast to the importance of intestinal IgA to gut homeostasis, the contribution of innate intestinal host factors remains poorly studied. One such factor is Glycoprotein 2 (GP2), an innate protein produced by pancreas acinar cells and, like IgA, secreted into the gut lumen. However, its in vivo functions in the context of gut immunity remain largely unknown. Notably, GP2 also constitutes a major autoantigen in IBD, although why GP2 becomes an autoantigen is unclear. Here, we characterize the underlying mechanisms regulating the binding of GP2 with the microbiota, its role in IgA-microbiota interactions and the relevance of these interactions in intestinal health and disease. Bacterial flow cytometry combined with fluorescent microscopy showed that GP2 in both mice and humans binds to a large fraction of the microbiota, that GP2-bound bacteria are co-associated with IgA (but not IgG) and that GP2-deficient mice have altered IgA-binding profiles to gut bacteria. Moreover, 16S rRNA analysis revealed overall changes in the microbiota composition, particularly in the small intestine, in GP2-deficient mice. These findings suggest that GP2 represents a key innate modulator of host-microbiota interactions. Prospectively, our findings may increase the understanding of how innate and adaptive immune mechanisms synergize to promote intestinal homeostasis thereby providing a basis for novel clinical approaches in the treatment of dysbiosis related inflammatory disease.

ID: 013
Abstract
Topics: Mucosal T cells, Systems immunology in host-microbe interactions
Keywords: LCMV, virus infection, gnotobiotic mouse models

Selective microbiota influence anti-viral immunity in the chronic clone-13 LCMV infection model

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Recent research has shed led light on the important and versatile roles of the intestinal microbiome not least due to its immunomodulatory role in various human diseases including immunotherapies and viral infections like COVID-19.

However, due to its complexity both in humans as well as animal models raised in different animal houses it has been notoriously difficult to pinpoint the level by which the microbiome can impact chronic viral infections. Here, we addressed whether the microbiome influences anti-viral immunity in the chronic strain (clone-13) of the systemic cytopathic lymphocytic choriomeningitis virus (LCMV). To address this question, we infected germ-free mice as well as mice colonized with a limited microbial flora (OMM12) in gnotobiotic isolators with clone-13 LCMV and investigated the T cell response in comparison to SPF-infected animals. Surprisingly, the microbial status did marginally affect the cytotoxic CD8+ T cell response while CD4+ T cells were highly impacted by the microbiome. While germ-free mice lost moderately more weight than the SPF control group during the clone-13 infection, OMM12 mice showed a drastic increased in weight loss and higher expansion of adoptively transferred virus-specific TCR-transgenic and endogenous Th1 cells as well as increased cytokine secretion (GzmB, TNFα, IFNγ). Interestingly, this altered anti-viral immunity was especially pronounced in the small intestine, suggesting that virus-specific T cells may be directly regulated in the intestine. Transcriptional profiling and flow cytometry analysis of adoptively transferred virus-specific CD4+ T cells from SPF and OMM12-colonized mice revealed a diminished population of TFH-like cells in the spleen and an increased population with high cytotoxic and inflammatory potential in the small intestine of OMM12-colonized animals.

In summary, our study shows that specific microbial communities can have a strong impact on anti-viral immunity and immunopathology and that this response is primarily driven by microbiome effects on virus-specific T helper cells.

ID: 014
Abstract
Topics: Mucosal diseases and infections
Keywords: Inflammatory bowel diseases, Proliferation, DNA repair, Bactericidal proteins.

Analysis of the in vivo function of Interleukin-26 in gut homeostasis

Yazan Salloum (Curie Institute, France)

The gastrointestinal tract represents a complex system housing numerous populations of immune cells and beneficial microorganisms and serves as an entry point for various pathogens. In this environment, a dynamic crosstalk between immune cells, epithelial cells, and the microbiota is essential to ensure proper organ homeostasis. This crosstalk is largely mediated by cytokines and its dysregulation can lead to inflammatory bowel disease (IBD).

Human genome-wide association studies have identified interleukin-26 (IL-26) as a risk locus for IBD. Moreover, IL-26 has been found to be over-expressed in IBD lesions. However, The in vivo functions of this cytokine have not yet been deciphered, largely due to its absence in rodents. Interestingly, the zebrafish possesses a unique homolog of the human IL-26 gene.

In this project, we exploit the zebrafish to test whether and how IL-26 plays a role in maintaining gut homeostasis.

During the early stages of IBD, the primary drivers of inflammation are the bacteria that overcome the mucosal defence mechanisms and inflict damage to the intestinal epithelium. Intriguingly, human IL-26 has intrinsic bactericidal activity through pore formation in vitro. However, the in vivo consequences of this activity remain unclear. We found that the zebrafish IL-26 also kills bacteria in vitro, and we are currently determining how this activity impacts gut homeostasis in vivo.

In the course of IBD, prolonged tissue damage could lead to the accumulation of DNA damage, eventually resulting in the proliferation of abnormal cells and cancer. Therefore, it is essential to identify agents that control DNA repair and proliferation in the gut. Interestingly, we generated il26-/- zebrafish and found that IL-26 modulates pathways related to cell cycle, DNA replication, and DNA repair. We are currently analysing the consequences of IL-26 loss on these aspect in epithelial progenitors at steady state and upon inflammation.

ID: 015
Abstract
Topics: Innate Immunity, Swiss Young Immunologists Society (SYIS) and young European Federation of Immunological Societies (yEFIS)
Keywords: IFN-λ, Interferon, organoids, epithelial cells, pyroptosis

Type III interferons control intestinal repair in by inducing pyroptosis in gut epithelial cells

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Tissue damage and repair are hallmarks of the inflammatory process. Despite a wealth of information on the mechanisms that govern tissue damage, mechanistic insight on how inflammatory mediators affect repair is lacking. Here, we investigated how interferons influence tissue restitution after damage to the intestinal mucosa driven by inflammatory or physical injury. We found that type III, but not type I, interferons delay tissue restitution by inducing the upregulation of ZBP1, Caspase-8 activation, and cleavage of Gasdermin C, which drives epithelial cell death by pyroptosis. We also found that this pathway is activated in IBD patients. Our findings highlight a molecular signaling cascade that affects the outcome of the immune response by delaying tissue repair, and that has important implications for human inflammatory disorders.

ID: 016
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Solid Organ Transplantation, Mycophenolate, P.aeruginosa infection, Exotoxin S, respiratory epithelium

EpITRANS, Impact of immunosuppressive drugs of solid organ transplant recipients on epithelial immunity in acute respiratory infections by Pseudomonas aeruginosa

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INTRODUCTION: Solid organ transplant recipients (SOTr) need a lifelong anti-rejection immunosuppressive therapy (ISt) mainly targeting adaptive immunity. Surprisingly, SOTr exhibit an increased risk of Pseudomonas aeruginosa (Pa) acute airway infection (AAI) with a 35% mortality. However, identified risk factors involve innate immunity and epithelial barrier impairment: this increased susceptibility remains unexplained. We characterised ISt-induced modifications on airway epithelium during Pa-mediated AAI, by screening all combinations. We focused on activities of both mycophenolic acid (MPA) and Pa-exotoxins ExoS/T/Y, which imply guanosine triphosphate (GTP).

METHODS: Human bronchial cells (BEAS-2B) were treated with every real-life ISt-combination for 24h and/or GTP, and then infected with Pa (strain PAO1 wild-type at multiplicity of infection 1 -MOI1- or ExoS/T/Y-deleted -PAO1Δ, ΔSTY- at MOI10). We assessed cytotoxicity by LDH release, cell death by real-time propidium iodide incorporation and cytokine production by ELISA and RT-qPCR. C57BL/6J mice were treated with intraperitoneal MPA for 3 days before infection. After sacrifice, broncho-alvolar lavages (BAL) were collected to perform IL-6/TNFα ELISA and neutrophil count.

RESULTS: Of all screened ISt, MPA alone or in combination enhanced PAO1-induced epithelial cytotoxicity of approximately 80% and accelerated cell death (6h-difference). MPA did not affect PAO1ΔS/ΔSTY-induced cytotoxicity. MPA also decreased pro-inflammatory cytokines IL-6 and IL-8 production of 40-80%, by decreasing transcription activities of corresponding genes. These MPA-induced modifications were reversed by GTP. In vivo, IL-6, TNF-α concentrations and neutrophil recruitment were lower in BAL of MPA-treated mice.

CONCLUSION: MPA alters respiratory epithelial integrity and its innate immune response, two major risk factors of Pa-mediated AAI. These alterations rely on the well-known Pa-exotoxin ExoS, and GTP-depletion. Mechanisms underlying these results are still to be explored. Describing additional effects of ISt on respiratory innate and mucosal immunity may allow to both adapt these drug regimens during AAI in SOTr and develop new therapeutic assets against P.aeruginosa.

ID: 017
Abstract
Topics: Microbe-immune interactions at body surfaces
Keywords: Exopolysaccahride, airway allergy, IgA

Extracellular polysaccharide produced by probiotic gram-positive bacteria modulates immune response in mouse model of airway allergy

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The increasing prevalence of allergic diseases in industrialized countries during recent decades has been correlated with reduced microbial burden in western population. Therefore, the use of probiotic bacteria and their bacterial cell components for modulation of allergic immune response has been introduced as an interesting concept.

Two-month old BALB/c mice were two-times sensitized by intraperitoneal injection of OVA and asthma-like symptoms were induced by intranasal application of four doses of OVA challenge. Exopolysaccharide from Lacticaseibacillus rhamnosus LOCK900 (EPS-L900) was applied intranasally before each ovalbumin challenge. One day after the last challenge, mice were sacrificed and asthma-like symptoms were evaluated by differential cell counting in bronchoalveolar lavage (BAL), histological staining of lung tissue, determination of humoral response in sera and cytokine response in splenocyte cultures and BAL fluid.

Intranasal application of EPS-L900 before OVA-challenge decreased number of eosinophils in BAL and reduced the infiltration of cells in lung tissue assessed by histological staining. OVA-specific

humoral immune response in sera of treated mice were not affected by EPS administration, whereas the production of OVA-specific cytokines IL-4, IL-5 and IL-13 was downregulated in spleen cell cultures and BAL fluid. Moreover, we detected significantly enhanced level of TGF-β1 and total IgA in BAL fluid and IgA-producing cells in lung tissue of EPS-treated mice.

Exopolysaccharide isolated from probiotic gram-positive bacteria L. rhamnosus LOCK900 is able to modulate allergic immune response in mouse models of asthma and stimulate IgA production in lung tissue. Intranasal administration of these bacterial antigens with a defined structure and biological properties helps to elucidate the mechanism of bacterial-host interaction and represents a promising new strategy in the treatment of allergies.

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ID: 018
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: regulatory T cells, dendritic cells, type 2 immunity, helminth parasite

RelB-dependent transcriptional programs in migratory dendritic cells regulate protective type 2 immunity via dominant regulatory T cells

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Migratory dendritic cells (DCs) are instrumental for mounting protective immune responses but have also been implicated in the generation and regulation of Foxp3+ regulatory T cells (Tregs). Specific DC subsets or DC-intrinsic pathways regulate immunity vs. tolerance at barrier sites, but underlying mechanisms in the intestinal tract remain poorly defined. By using bulk and single cell sequencing we provide evidence that the alternative NF-kB family member RelB controls key transcriptional programs in migratory DC/cDC2 subsets within mesenteric lymph nodes that result in increased tisTreg but decreased ROR(gt)+ pTreg cell numbers both at steady state and under inflammatory conditions. Genes under RelB control include chemokines (CCL22, CCL17) and co-stimulatory molecules (CD80, CD40, OX40L, CD200) as well as the transcriptional regulator AIRE recently associated to the induction of ROR(gt)+ Treg cells. In contrast to mesenteric lymph node cells, RelB deficicency did only affect the development of a small population of DCs in the intestinal lamina propria surrounding crypto patches and lymphoid follicles. Functionally, increased tisTreg cells in DC-specific RelB knockout animals exerted a dominant control over protective type 2 immune responses in the small intestine after infection with Heligmosomoides polygyrus despite a previous type 2 immune bias at steady state. Thus, RelB in DCs acts as a rheostat for tisTregs accumulation and control of type 2 immunity while it has opposing effects on microbiota-dependent pTreg differentiation.

ID: 019
Abstract
Topics: Innate Immunity, Mucosal diseases and infections

IFNg holds Shigella at bay

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Shigella is a significant human pathogen and the causative agent of shigellosis, a severe diarrheal disease that annually accounts for 200,000 deaths. Equipped with over 30 secreted effector proteins, Shigella has evolved to exploit the nutrient-rich intracellular environment of colonic epithelial cells, thereby escaping and hiding from extracellular defense mechanisms. We recently identified IFNg responses as a central factor counteracting bacterial proliferation. Interferon-stimulated genes (ISG) efficiently restrict bacterial intracellular replication in a race of arms between the pathogen and host. Remarkably, little is known about the beneficial effects of IFNg on infected gut-epithelial cells in vivo. Current experiments aim to identify the relevant ISGs and characterize how these anti-microbial restriction factors limit disease progression. Understanding Shigella pathogenesis and harnessing host immune responses will provide an essential foundation for developing novel vaccine strategies and therapeutics for this important human pathogen.

ID: 020
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Lung, Respiratory infection, RSV, Tissue-resident memory T cells, Type I IFNs

The role of type I interferons in the generation of tissue-resident memory CD8+ T cell responses during RSV infection

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The respiratory tract is the gateway for pathogens that represent threats to human health. Respiratory viruses, most commonly respiratory syncytial virus (RSV), constitute major burdens on healthcare systems. RSV causes severe lower respiratory tract infections, especially in children and the elderly. The lack of vaccines and recurrence of RSV infections indicate the difficulties in eliciting protective immune responses. Tissue-resident memory T cells (TRMs) are key players in memory responses at mucosal surfaces and play a central role in protecting from reinfection by respiratory viruses in mice and humans. In human experimental RSV infection, the presence of lung CD8+ TRMs correlates with a better outcome, however, the requirements for adequate lung TRMs responses during RSV reinfection are not fully understood. Type I IFNs are one of the early drivers of inflammation during viral infections and are critical anti-viral cytokines with pleiotropic effects. Type I IFNs are also important for T cell effector function, memory differentiation and survival.

We use mouse models to assess the role of type I IFNs in the generation and subsequent expansion of the TRMs pool during RSV infection.

We show that lung resident CD8+ TRM cells expand independently from systemic CD8+ T cells after RSV reinfection. Reinfected MAVS, MyD88/TRIF, and IFNAR1 deficient mice lacking key components involved in innate immune recognition of RSV and induction of type I IFNs, display impaired expansion of CD8+ TRM cells. IFN-α treatment of MAVS deficient mice during primary RSV infection restores the TRM cell expansion. Furthermore, bone-marrow chimeric mice show that the effect of type I IFNs on the generation of CD8+ TRMs is both via stromal and bone marrow-derived cells. Our data reveal how the axis controlling type I IFN induction instructs and regulates CD8+ TRM cell responses to RSV infection.

ID: 021
Abstract
Topics: Neonatal window of opportunities, Microbe-immune interactions at body surfaces
Keywords: neonate, DC, Peyer's patch, microbiota

M cell maturation and cDC activation determine the onset of adaptive immune priming in the neonatal Peyer's patch

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Marked differences exist between the mucosal immune system of the neonate and adult host but the signals and mechanisms that determine the pace of postnatal immune maturation have not been fully resolved. Here we analyzed mononuclear phagocytes (MNP) in small intestinal Peyer's patches (PP), the primary inductive site of intestinal immunity. Conventional type 1 and 2 dendritic cells (cDC) and RORgt+ antigen-presenting cells (RORgt+APC) exhibited significant age-dependent changes in subset composition and tissue distribution. Comparative single cell transcriptional profiling and functional assays further revealed an overall retarded cell maturation and decreased antigen processing/presentation capacity during the postnatal period. This resulted in a reduced proportion of CCR7+ migratory/activated cDC2 and RORgt+ APC and a consequent lack in CD4 T cell priming throughout the postnatal development. Although microbial cues contributed to these processes, metagenomic, metaproteomic, and functional immunological analyses revealed a dense and diverse small intestinal core microbiome early after birth making a global lack of innate immune stimuli and immunogenic antigens unlikely. Type I interferon (IFN) induction by oral R848 administration accelerated MNP maturation and enhanced cognate antigen CD4 T cell priming but IFN signaling did not represent the physiological stimulus. Instead, follicle-associated epithelium (FAE) M cell differentiation and uptake of luminal particulate antigen was required and sufficient to drive postweaning PP MNP maturation. Together, our results identify MNPs as rate limiting factor of postnatal immune maturation, highlight the critical role of FAE M cell differentiation in MNP maturation and demonstrate that targeted interventional strategies allow manipulation of mucosal responses in early life.

ID: 022
Abstract
Topics: Neonatal window of opportunities, Microbe-immune interactions at body surfaces
Keywords: Peyer's patches, LysoDC, SFB, Th17, weaning

Peyer's patches LysoDCs as privileged players coordinating the intestinal immune education and gut barrier homeostasis induced by SFB colonization

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Segmented filamentous bacteria (SFB) is a commensal member of the gut microbiota known to strongly induce Th17 responses, thus educating the immune system into a tolerogenic profile that is important to the homeostasis of the host. However, which innate phagocytic population is responsible for bridging SFB to Th17 and the precise site in which SFB is initially sensed by the immune system are both questions yet to be elucidated. LysoDCs are a subset of monocyte-derived cells with the strongest phagocytic activity present in the Peyer's patches (PPs). Given their privileged location and effective functions, they have been highlighted as potential candidates to play such role in mediating SFB-induced immunity; nevertheless, this relationship has never been directly approached. Here we show that freshly isolated subsets of PPs LysoDCs have the ability to prime SFB-specific naïve T cells obtained from SFB-specific TCR transgenic mice. In addition, we show that weaning is an important moment not only for the onset of SFB colonization, but also for driving changes in the profile of LysoDCs in the PPs. In SPF mice, the abundance of the mature/most phagocytic LysoDC subset hereby named TP (Emb+ Jam-A+ CD24+) significantly decreases few days after weaning, remaining at lower levels in adulthood. On the other hand, weaning does not impact LysoDCs profile in germ-free mice, indicating that this effect is dependent on the gut microbiota colonization. Interestingly, SFB itself can recapitulate the phenotype driven by the complex SPF microbiota, as a similar dynamic in the LysoDC profile was observed in SFB monocolonized animals. Within PPs, only LysoDCs seem to be affected by weaning, as no changes were observed in other monocyte-derived cell population. We are currently developing a model for in vivo depletion of LysoDC that will be crucial for elucidating their role in such complex SFB-Th17 scenario.

ID: 023
Abstract
Topics: Innate Immunity
Keywords: Isolated lymphoid follicles, fibroblasts, intestinal immunity, Peyers patches, human

Human gut-associated lymphoid tissues are inhabited by a diverse fibroblast network that steer immune functions

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The intestinal mucosa consists of an epithelial layer and the underlying lamina propria (LP), which harbors a variety of immune effector cells. Additionally to these effector sites, gut-associated lymphoid tissues (GALT), such as the Peyer's patches of the small intestine or isolated lymphoid follicles (ILF) along the entire length of the intestine, represent immune priming sites. These structures form key local hubs where rare antigen-specific lymphocytes can interact with antigen-presenting cells and screen for their cognate antigen.

Studies in mice have shown that lymphoid tissues like Peyer's patches are erected and supported by a dense network of tissue-resident fibroblasts. However, GALT in humans remain poorly understood, due to the difficulty to isolate and study these structures. Using a recently developed technique to separate GALT from the surrounding LP, we aimed to draw a comprehensive MSC roadmap of human intestinal GALT using single cell RNA-sequencing, flow cytometry and immunohistochemistry. Our data demonstrate that human GALT are highly organized and inhabited by a heterogeneous fibroblast network, composed of several discrete subtypes that can regulate lymphocyte attraction, function and survival.

Together, we believe that our experiments expand our current understanding of intestinal immune homeostasis in humans.

ID: 024
Abstract
Topics: Mucosal diseases and infections, Systems immunology in host-microbe interactions
Keywords: Therapeutic antibodies, P. aeruginosa, Long-term protection, Mucosal immunity, Immunomodulation

Mucosal administration of anti-bacterial antibody provides long-term cross-protection against Pseudomonas aeruginosa respiratory infection

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Bacterial respiratory infections are one of the leading causes of mortality worldwide, intensified by the increase of antibiotic-resistant strains. As an alternative treatment, therapeutic antibodies (Ab) have proven to be valuable anti-infective agents. Anti-infective antibodies are usually administered parentally and rely mostly on direct pathogen neutralization. For respiratory pathogens, which are primarily located in the airways, their potency is often suboptimal, as they pass poorly from the systemic compartments into the airways. We previously showed that local delivery of antibacterial Ab directly into the respiratory tract offers a suitable approach to improve their therapeutic index. Using a mouse model of acute pneumonia induced by Pseudomonas aeruginosa, we found that beyond the rapid and efficient containment of the primary infection, anti-infective Ab delivered through the airways harnessed genuine innate and adaptive immune responses to provide long-term immunity, protecting against a secondary bacterial infection.

In vitro antigen-presenting cells stimulation assay, as well as in vivo bacterial challenges and serum transfer experiments indicate an essential contribution of immunes complexes, formed between the Ab and the pathogen, in the induction of a sustained and protective antibacterial humoral response. This effect depends on the amount of immune complexes, as well as the development of a local inflammation. Interestingly, the long-lasting response protected partially against secondary infections with heterologous P. aeruginosa strains. Further studies of the cellular response and molecular partners will be required to provide a complete picture of the immune mechanisms involved in this long-term protection. Overall, our findings suggest that mucosal delivery of Ab promotes rapid pathogen containment and provides long-term protection against secondary infection. This novel modality associated with anti-infective antibody opens new perspectives for the treatment of respiratory infections.

ID: 025
Abstract
Topics: Mucosal diseases and infections
Keywords: Intestinal Epithelial Cells, Organoid Transplantation, Deep Visual Proteomics

Recapitulation of human coloncytes in situ by human colon organoids in vitro and after orthologous transplantation

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The intestinal epithelium plays a central role in human health and disease, and several chronic inflammatory disorders associate with a weakened epithelial barrier. The organoid model allows the cultivation, propagation and analysis of non-immortalized intestinal epithelial cells and has been instrumental in studying epithelial behavior in homeostasis and disease. Recent advances allow the transplantation of human organoids into mice to study epithelial cell behavior within the intestinal tissue context. It remained unclear how realistically human colonic organoids cultured in vitro or transplanted into the murine colon recapitulate protein expression of human colonocytes in vivo, and how the transplantation influences the mucosal microenvironment. To address this, we employ state-of-the-art deep visual proteomics to compare human colonic epithelial stem and differentiated cells in human and mouse and compare them to organoids cultured in vitro. In addition, we sample immune and mesenchymal cells from the lamina propria in the three in vivo conditions to obtain information about changes in the mucosal microenvironment. Taken together, this will provide important insights into cellular functions which are realistically recapitulating human colonocytes in these models.

ID: 026
Abstract
Topics: Systems immunology in host-microbe interactions

Epithelial OPA1 links mitochondrial dynamics to inflammatory bowel disease.

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Intestinal immune homeostasis relies on intestinal epithelial cells (IEC) constituting an efficient barrier but also negotiating signals between the microbiome in the gut lumen and the mucosal immune system in the bowel wall. While dysregulation of the intestinal barrier is considered a key driver of inflammatory bowel disease (IBD), the molecular mechanisms of such dysregulation are not well understood.

Here we demonstrate a critical role of epithelial OPA1, a dynamin-related GTPase required for mitochondrial fusion, for barrier integrity and intestinal immune homeostasis. Mitochondrial fusion-related genes and expression of mitochondrial DNA-encoded genes were negatively regulated during experimental intestinal inflammation in mice. Mice with a conditional knockout of OPA1 specifically in IEC (Opa1ΔIEC), spontaneously developed a chronic intestinal inflammation with ulceration and immune cell infiltration. Opa1ΔIEC mice showed severe epithelial erosions, crypt cell apoptosis and microbial translocation into the bowel wall. Spontaneous inflammation in Opa1ΔIEC mice was dependent on the presence of microbiota. Gut samples of Opa1ΔIEC mice showed diminished transcription of mitochondrial genes and decreased levels of mitochondrial DNA. OPA1 deficient epithelial cells lacked mitochondrial fusion as indicated by disruptions of the mitochondrial network with mitochondrial fragmentation, changes of mitochondrial size and ultrastructure and increased mitophagy. OPA1 expression and expression of mitochondrial DNA-encoded genes were reduced in TNFa-treated organoids and in a TNF-transgenic model of intestinal inflammation. Lastly, we identified reduced levels of mitochondrial fusion-related genes, diminished OPA1 levels and fragmented mitochondrial networks within the crypts of patients with IBD, suggesting a role of mitochondrial fission and fusion in the pathogenesis of these diseases.

Together, our data demonstrate a critical role of OPA1 in regulating intestinal immune homeostasis, maintaining gut barrier function and in protection of IEC from cell death following dysfunctional mitochondrial dynamics.

ID: 027
Abstract
Topics: Systems immunology in host-microbe interactions

Presenilins are key to IEC differentiation and barrier integrity

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The Notch signaling pathway is one of the most important pathways shaping gut development and gastrointestinal epithelial cell homeostasis, since it is required for stem cell maintenance and intestinal epithelial cell (IEC) fate decision into absorptive and secretory cells. Cleavage of the Notch receptor occurs through the γ-secretase complex, leading to the release of the Notch intracellular domain, which translocates to the nucleus driving target gene expression. The catalytic component of the γ-secretase are the presenilins Psen1 and Psen2. Most of the functions of the γ-secretase and the integrated presenilins are known from Alzheimer's disease (AD), since mutations in Psen1 and Psen2 are one of the causes for AD. In these studies, Psen1 was described to be the main presenilin integrated in most of the γ-secretase complexes, whereas Psen2 had a rather unimportant role. Interestingly, through the establishment of IEC-specific Psen1 knockout mice (Psen1ΔIEC) we could show, that Psen2 can fully compensate for the loss of Psen1 within the IECs of the gut. Vice versa, loss of Psen2 in the gut does also not affect gut homeostasis. However, loss of both presenilins in the gut leads to a tremendous increase of all secretory cells, including goblet, Paneth, enteroendocrine and tuft cells, whereas stem cells and absorptive enterocyte numbers are decreased. Surprisingly, Psen double-knockout animals show signs of spontaneous inflammation, along with non-apoptotic cell death, barrier defects and thus bacterial translocation. With this study we are able to shed new light on the role of the presenilins within the γ-secretase complex and the important Notch signaling and its influence on gut homeostasis.

ID: 028
Abstract
Topics: Mucosal B cells
Keywords: plasma cells, microbiota

Shaping of gut B cell repertoire by the microbiota

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The human antibody pool comprises about 1012 unique antibodies produced by the gut plasma cell pool and encoded by the B cell receptor (BCR) repertoire. This diversity derives from processes generating the pre-immune repertoire and subsequent B cell activation, expansion and secondary diversification by somatic hypermutation and affinity-based selection generating the gut immune repertoire. Depending on the particular type of antigen and immunological context different anatomical, cellular and molecular environment can support gut B cell responses and plasma cell generation. The gut microbiota instruct all these steps of the intestinal B cell life cycle and shaping of the intestinal B cell repertoires. We hypothesized that the history of microbiota encounters might reflect in the architecture and cellular phenotypes of the small intestinal plasma cell population.

We performed vertical colonization of mice with defined microbiota and characterized the ensuing plasma cell population by BCR repertoire analysis, single cell sequencing and high dimensional flow cytometry. Additionally, we fate tracked activation induced deaminase-expressing B cells from young or adult age to later follow-up time points. Our first results suggest a rather homogenous small intestinal plasma cell population. Typically, clonally related plasma cells showed a rather broad phenotypic and transcriptional profile that was reminiscent to the overall plasma cell population. Our observations indicate that either alternative pathways of plasma cell generation might play subordinate role in fuelling the gut plasma cell pool or such alternative pathways might not leave phenotypic traces in the mature plasma cell population.

ID: 029
Abstract
Topics: Mucosal diseases and infections, Mucosal B cells

Investigating the immune response using scRNA-Seq in Irritable Bowel Syndrome

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Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder that is estimated to affect 10% of the general population. It is characterized by an altered defecation pattern and abdominal pain in the absence of an identified organic cause. Aberrant pain signalling or visceral hypersensitivity (VHS) is a hallmark symptom of IBS. We have shown that a local IgEresponse to food antigen triggers mast cell degranulation and sensitization of nociceptive neurons in mouse models. Non-allergic patients with IBS develop an edema upon injection of food antigens in the colon mucosa, suggesting that a local immune response also occurs in humans. The immune mechanisms leading to the development of this response are currently unknown.

In this study, we aim to characterize the immune mechanisms and cellular actors involved in the development of pain in IBS patients. To do so, we performed single cell transcriptomics on a cohort of healthy and IBS patients, thereby generating a 280 000-cell dataset. This dataset will allow us to thoroughly investigate the immune mechanisms underlying the development of IBS and abdominal pain. Our preliminary results reveal an increased enrichment of plasma cells within the IBS group, associated with increased IGHE expression. This is in line with our previous findings and supports that a type 2 immune response underlies the development of IBS in humans.

ID: 030
Abstract
Topics: Innate Immunity, Systems immunology in host-microbe interactions
Keywords: ex vivo laboratory platform; host-pathogen interactions; bluetongue virus; Mycoplasma bovis

A bovine blood-based ex vivo platform to dissect early host-pathogen interactions

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The development of rationally designed vaccines benefits from a good understanding of host-pathogen interactions including virulence traits triggering specific host responses. Despite major distinctions between the bovine and human immune systems, such as an overrepresentation of gamma delta T cells in cattle, the bovine immune system remains poorly investigated and still requires an in-depth characterization of both their innate and adaptive immune cell subsets. Herein, considering the importance to adhere to the 3R principles, we developed an ex vivo laboratory platform employing primary blood cells to dissect host-pathogen interactions. The characterization of immunological responses is based on independent readouts such as: i) 12-14 color flow cytometry assay measuring activation (intracellular cytokine detection), maturation (modulation of cell surface marker expression), and proliferation state (reduction in the CellTrace signal from preloaded cells) of monocytes, conventional dendritic cells (cDCs), plasmacytoid DCs (pDCs), NK cells, gamma delta T cells, B and T cells; and ii) Luminex-based multiplex assay to monitor chemokine and cytokine secretion. These assays were tested on primary bovine blood cells from adult cattle that were exposed to viral and/or bacterial pathogens. The later were selected due to their capacity to cause respiratory diseases, either individually or together by cooperating in a co-infection context (viruses: bluetongue virus, bovine respiratory syncytial virus; bacteria: Mycoplasma bovis). Finally, we investigated the impact of incubation temperature (37°C, 39.5°C and 40.5°C), postulating that fever strongly modulates immune cell interaction with those pathogens. In conclusion, our experimental approach combining unprecedented delineation of bovine immune responses with host-pathogen interaction studies has a great prospect to decipher not only concerted actions of immune cells but also to identify virulence factors of bovine respiratory pathogens.

ID: 031
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections
Keywords: aminopeptidase N, gut epithelium, tranpsort, secretory IgA, E. coli

Aminopeptidase N-mediated gut epithelial transcytosis drives gut immunity to oral subunit vaccines

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Most pathogens invade the host at the mucosal surfaces. To protect against these pathogens, the induction of local secretory IgA (SIgA) responses is paramount. While injectable vaccines provide strong systemic immune responses, oral vaccination is the most efficient way to trigger protective gut SIgA responses. Despite this, the development of oral subunit vaccines is challenging due to hurdles inherent to the gut tissues. Oral vaccines need to survive the low pH and degradation by digestive enzymes in the gastrointestinal tract and need to reach the gut-associated lymphoid tissues, which are protected by physical barriers that prevent efficient vaccine uptake. Furthermore, they need to surmount the default tolerogenic responses of the gut immune system. Several strategies have been developed to tackle these hurdles, such as delivery systems that protect vaccine antigens from degradation, mucosal adjuvants that induce robust immune responses and targeting approaches that aim to deliver vaccine antigens to the gut tissues. We have shown that a promising strategy in this context is the targeting of vaccine antigens to the gut epithelial transcytotic receptor aminopeptidase N. I'll present our recent progress on the delivery of oral subunit vaccines to the gut immune system via aminopeptidase N in a large animal model and highlight how this approach triggers secretory IgA responses, which protect against infection with bacterial pathogens, like enterotoxigenic E. coli.

ID: 032
Abstract
Topics: Mucosal T cells
Keywords: Mucosal-associated invariant T cell (MAIT), tissue damage, bleomycin, dendritic cells, DNGR-1

MAIT cells contribute to protection against bleomycin-induced lung tissue damage by promoting monocyte differentiation into type 1 conventional dendritic cells

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Introduction: Mucosal-associated invariant T (MAIT) cells are the most abundant unconventional T cells in the lung and have a recently-described function in maintaining tissue homeostasis, indicating their potential role in promoting pulmonary tissue repair. It is not known what role MAIT cells play during sterile tissue damage, or the functional consequences of their tissue repair capabilities in the lung.

Methods: We assessed MAIT cell activation and function during sterile tissue inflammation using a bleomycin-induced murine lung injury model. We used multi-parameter flow cytometry, spectral analysis (Cytek Aurora), histology, qPCR, immunoassays, bulk and single cell RNA sequencing and adoptive transfer in wild-type and MAIT cell-deficient Mr1-/- mice.

Results: We show in vivo that bleomycin challenge strongly activated murine pulmonary MAIT cells and induced their tissue repair program. Mr1-/- mice exhibited more severe weight loss and a more robust immune response following bleomycin challenge compared with their wild-type counterparts. We further demonstrate that early accumulation of CD103+ type 1 dendritic cell (cDC1) in the lungs of Mr1-/- mice was impaired upon bleomycin challenge, and MAIT cells were required for the early differentiation of CCR2+ Ly6Chi monocytes into cDC1. Finally, the weight loss of Mr1-/- mice was rescued by adoptive transfer of bone marrow-derived dendritic cells (BMDCs) but this effect was lost when DNGR-1 was blocked in the mice.

Conclusion Overall, our data suggested that MAIT cells played a protective role against bleomycin-induced lung tissue damage by promoting monocyte differentiation into cDC1, which limited tissue damage via the DNGR-1 signal. These findings establish a novel mechanism by which MAIT cells function to reduce damage during sterile tissue injury and provide insight into the potential use of MAIT cells in pulmonary tissue repair.

ID: 033
Abstract
Topics: Mucosal T cells

T cells control secretion of chemokines by Keratinocytes

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The massive infiltration of lymphocytes into the skin is a hallmark of numerous human skin disorders. By co-culturing murine keratinocytes with splenic T cells we demonstrate here that T cells affect and control the synthesis and secretion of chemokines by keratinocytes. While pre-activated CD8+T cells induce the synthesis of CXCL9 and CXCL10 in keratinocytes and keep in check the synthesis of CXCL1, CXCL5 and CCL20, keratinocytes dampen the synthesis of CCL3 and CCL4 in pre-activated CD8+T cells. One key molecule is IFN-g that is synthesized by CD8+T cells under the control of NFATc1 and NFATc2. CD8+T cells deficient for both NFAT factors are unable to induce CXCL9 and CXCL10 expression. In addition, CD8+T cells induced numerous type I IFN-inducible 'defense genes' in keratinocytes including the PD1 and CD40 ligands, TNF-a and caspase 1. The enhanced expression of type I IFN-inducible genes resembles the gene expression pattern at the dermal/epidermal interface in lichen planus, an inflammatory T lymphocyte-driven skin disease, in which we detected the expression of CXCL10 in keratinocytes at the infiltration front of T cells. These data reflect the multi-faced interplay of lymphocytes with keratinocytes at the molecular level.

ID: 034
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections
Keywords: Respiratory mucosa, lung inflammation, antigen presentation

The ins and outs of the regulation of MHC I expression in lung epithelial cells.

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Infections of the respiratory tract are a perennial cause of death worldwide. Most of the time, the death is due to an exacerbated inflammation that causes lung dysfunction. These diseases typically affect the lung mucosa, composed of several cell types, including lung epithelial cells (lung ECs). Lung ECs play an essential role in the physiology of the respiratory mucosa (mucus production, immune response, gas exchanges). We recently discovered that those cells express constitutively low amounts of MHC I, which could be detrimental to lung immunosurveillance. We showed that, in the mouse model, lung ECs upregulate their surface expression of MHC I during inflammation. Simultaneously, they decrease genes involved in their specialized functions crucial to preserving lung mucosal integrity. In this context, we raise two questions: i) Are human lung ECs expressing low MHC I amount, and are these levels modulated in a pathological context? ii) Does an MHC I upregulation in lung ECs impact lung mucosal integrity?

By analyzing single-cell RNA-seq data we showed that, in humans, lung ECs are the cell type expressing the lowest amount of MHC I from the lung. We also observed a significant increase of MHC I in lung ECs from cystic fibrosis, chronic obstructive pulmonary disease, and idiopathic pulmonary fibrosis donors. To understand if the increase of MHC I plays a direct role in lung inflammatory disorders, we created a transgenic mice model expressing high MHC I levels in lung ECs. In the short, medium, and long term, we are assessing the effect of this up-regulation on lung physiology.

Our data open new windows in the understanding of chronic lung inflammation. We hope that in the future, those results could help develop more adequate therapeutics for some diseases affecting the lung while respecting the integrity of the respiratory mucosa.

ID: 035
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections

SAMHD1 regulates small Intestinal damage following T cell–induced enteropathy

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Sterile α motif and HD domain-containing protein 1 (SAMHD1) is a mammalian dNTP hydrolase (dNTPase) that regulates intracellular dNTP balance. SAMHD1 has been identified as an important host restriction factor that inhibits the infection of several retroviruses and regulates immune responses. Studies already shown high levels of endogenous SAMHD1 mRNA and protein in resting CD4 T cells. Interestingly, we found SAMHD1 is significantly upregulated in IBD patients, especially in immune cells. In a previously established model of T cell activation-meditated small intestinal inflammation induced by anti-CD3 antibody, SAMHD1 expression was dramatically increased. In SAMHD1 KO mice, acute T cell activation with anti-CD3 mAbinduced milder small intestinal mucosal damage compared with wild-type mice. Reduced mucosal injury was characterized by longer villi, less damage of the crypt architecture, decreased epithelial cell apoptosis, and less inflammatory cytokine and chemokine accumulation in the small intestine. Taken together, we demonstrate an essential role of SAMHD1 in regulating small intestinal mucosal damage following acute T cell activation.

ID: 036
Abstract
Topics: Neonatal window of opportunities, Microbe-immune interactions at body surfaces
Keywords: Early-life microbiota, host-microbiota interaction, window of opportunity, obesity, chronic inflammatory diseases

Food additives induce transgenerational microbiota alterations, inducing intestinal low-grade inflammation and impaired metabolism

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Host-microbiota interaction in early life is a key element in driving proper immunity and related metabolism development. Disturbance of the gut microbiota during this critical time window is associated with increase susceptibility to chronic inflammatory and metabolic diseases. However, maternal diet contribution to offspring early-life microbiota alteration remains poorly understood. Hence, we hypothesized here that microbiota alteration in dams following emulsifiers consumption, dietary additives known to alter intestinal microbiota composition and function, might affect offspring microbiota thus driving adulthood consequences.

We tested this hypothesis using C57/Bl6J breeding pairs treated with select dietary emulsifiers. After birth, half of the obtained offspring were left with their original moms, while the other half were cross-fostered with untreated dams in order to restore proper intestinal microbiota. After weaning, mice were kept under emulsifiers-free regimen and treated with high-fat diet (HFD) or dextran-sulfate-sodium (DSS), to investigate susceptibility to diet-induced obesity and colitis, respectively. Fresh feces were collected for gut microbiota investigation, and tissues were harvested at sacrifice for macroscopical, histological and molecular analysis.

We observed that maternal emulsifiers consumption associates with early-life microbiota alteration in the offspring, while cross fostering of pups born from emulsifiers-treated dams by non-treated dams was enough to restore a normal microbiota. Moreover, offspring carried and fostered by emulsifiers-treated dams presented increased body weight gain and liver steatosis following HFD exposure. When subjected to DSS, such mice demonstrated increased intestinal inflammation. Importantly, all of these phenotypes were eliminated by early-life microbiota normalization through cross-fostering by non-treated dams, demonstrating the central role played by the intestinal microbiota in driving such transgenerational detrimental impacts of emulsifier consumption.

Altogether, these results suggest that maternal emulsifiers consumption is sufficient to drive early-life microbiota alteration in the offspring, which perturbation resulted in increased susceptibility to diet-induced obesity and colitis in adulthood.

ID: 037
Abstract
Topics: Mucosal diseases and infections, Mucosal B cells

Pathogenic transglutaminase 2 in celiac disease: Detection of catalytically active enzyme in the intestinal lumen of mice

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Celiac disease (CeD) is an autoimmune condition affecting the small intestine caused by the ingestion of cereal gluten proteins. The enzyme transglutaminase 2 (TG2) is involved in the pathogenesis of CeD in two ways. First, enzymatically active TG2 modifies gluten peptides to become immunogenic T-cell antigens in subjects expressing certain HLA-DQ allotypes (i.e., HLA-DQ2 and -DQ8), resulting in a highly inflammatory anti-gluten T-cell response. Second, TG2 is the target of the autoantibody response in CeD. The autoantibodies are only formed when patients consume gluten, suggesting that interactions between gluten-specific T cells and TG2-specific B cells are important for driving CeD pathogenesis. A big remaining question is where enzymatically active TG2 encounters gluten. We recently proposed a model in which TG2 released from shed enterocytes in the gut lumen is the source of pathogenically relevant TG2 in CeD. Here, we show that a significant amount of enzymatically active TG2 can be retrieved from mouse small intestinal lavage fluid. No TG2 was detected in the lavage from Tgm2 knockout mice and mice with a specific deletion of Tgm2 in enterocytes (Tgm2 floxed/Villin-Cre mice), supporting our hypothesis that extracellular TG2 in the intestinal lumen is derived from shed enterocytes. We further show that incubation of intestinal lavage with biotinylated-gluten peptides resulted in the incorporation of biotin into a broad range of luminal proteins through TG2-mediated isopeptide-bond formation, confirming that luminal-TG2 is enzymatically active. Our study provides further evidence for a model where TG2 encounters gluten peptides in the gut lumen, forms enzyme-substrate (TG2-gluten) complexes and is taken up from the lumen by the gut-associated lymphoid tissue, leading to the activation of gluten-specific T cells and TG2-specific B cells. Inhibition of TG2 activity is a promising therapeutic target for CeD treatment, and our findings may contribute to the development of more effective treatment approaches.

ID: 038
Abstract
Topics: Mucosal T cells, Microbe-immune interactions at body surfaces

Colon cancer-associated bacteria shape T cell immunity and tumorigenesis

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Colorectal cancer (CRC) develops at the interface between the host and the intestinal microbiota. Insights in the mechanisms of the interaction between three extremely complex, constantly evolving, biological entities - microbiota, tumor cells and immune system – are therefore of high interest in the field of onco-immunology. Regulatory T cells (Tregs) are indispensable for the maintenance of the intestinal homeostasis but can be detrimental during CRC, as they are supportive to a pro-tumorigenic environment. In a mouse model for colitis-associated CRC, we identified that tumor-associated Tregs have highly suppressive features and are equipped with a specific set of molecules strongly associated with enhanced migratory properties, in particular the expression of the chemoattractant molecule GPR15. In a healthy gut, bacteria communicate with the host through the production of immunogenic metabolites, thereby balancing pro- and anti-inflammatory T cell functions, including the expression of GPR15. Thus, we questioned whether the gut microbiota affects T cell functions during CRC. We detected alterations in the gut microbial composition and changes in the expression of bacterial metabolites in the feces of CRC mice. Manipulation of the gut microbiota by antibiotic treatment of CRC-bearing mice inhibited intestinal tumor progression, which was accompanied by reduced Treg frequencies. Interestingly, we could not detect enhanced frequencies of microbiota-induced Tregs within the tumorous tissue nor a direct impact of CRC-associated bacterial metabolites on the expansion, the induction or the suppressive capacity of Tregs in vitro. However, we found significant alterations in pro-inflammatory cytokine secretion after antibiotic treatment of CRC mice. In line, microbial metabolites in the fecal content of CRC mice promoted the induction of pro-tumorigenic Th17 cells and inhibited the killing capacity of CD8+ T cells in vitro. In summary, our data provide evidence that CRC-associated bacteria directly support T cell immunity by inducing a pro-tumorigenic microenvironment, rather than directly affecting Treg functions.

ID: 039
Abstract
Topics: Mucosal T cells, Microbe-immune interactions at body surfaces
Keywords: Inflammatory bowel disease, Mucosal associated T cells, Microbiota, colitis, riboflavin

MAIT cells monitor intestinal dysanaerobiosis enabling host protection during colitis

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Oxygen is almost absent from the colon lumen at steady-state, but becomes available during intestinal inflammation, fueling shifts in microbiota metabolism. How the host monitors and responds to such changes remains unclear. Our findings show a protective mechanism by which the adaptive immune system monitors microbiota metabolites that are produced in response to oxygen. This mechanism relies on Mucosal-associated invariant T (MAIT) cells, an evolutionary conserved T cell population recognizing intermediary adducts from the microbial riboflavin biosynthesis pathway. Experimental colitis is also associated with oxygenation of the colon lumen and with production of MAIT ligands. Metatranscriptomic analyses suggest that the aerotolerant bacterium Mucispirillum schaedleri is the dominant producer of MAIT ligands during colitis. MAIT antigens rapidly cross the intestinal barrier and activate MAIT cells, which produce barrier-promoting and tissue-repair mediators during experimental colitis. As a consequence, mice lacking MAIT cells are more susceptible to colitis and colitis-driven colorectal cancer. Thus, MAIT cells monitor a bacterial metabolic pathway indicative of intestinal inflammation, providing host protection in return.

ID: 040
Abstract
Topics: Mucosal diseases and infections
Keywords: Vedolizumab, etrolizumab, intestinal wound healing, gut homing, monocytes

Anti-β7 integrin treatment impedes the recruitment on non-classical monocytes to the gut and delays macrophage-mediated intestinal wound healing

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Background:

Closing mucosal defects to reach mucosal healing is an important goal of therapy in inflammatory bowel disease (IBD). Monocyte-derived macrophages are centrally involved in such intestinal wound healing. We had previously demonstrated that the anti-α4β7 integrin antibody vedolizumab blocks the recruitment of non-classical monocytes as biased progenitors of wound healing macrophages to the gut and delays wound healing. We hypothesized that the anti-β7 integrin antibody etrolizumab might also play a role for monocyte/macrophage trafficking in the gut and investigated the impact of anti-β7 compared with anti-α4β7 blockade on wound healing.

Methods:

We analyzed the expression of etrolizumab targets on human and mouse monocyte subsets and investigated the impact of etrolizumab on monocyte trafficking in adhesion assays as well as in vivo. We explored wound-associated monocyte recruitment dynamics with multiphoton microscopy and compared the effects of anti-β7 and anti-α4β7 blockade on experimental intestinal wound healing and wound-associated macrophage abundance. Finally, we investigated wound healing macrophage transciptome signatures in patients with Crohn's disease treated with etrolizumab.

Results:

Human and mouse non-classical monocytes expressed more αEβ7 than classical monocytes and were a target of etrolizumab-s, which blocked non-classical monocyte adhesion as well as gut homing in vivo more effectively compared to vedolizumab. Intestinal wound healing was delayed with etrolizumab-s along with a reduction of peri-lesional wound healing macrophages. In patients with Crohn's disease, wound healing macrophage signatures in the colon were substantially downregulated on treatment with etrolizumab, but not with placebo.

Conclusions:

Collectively, our data show that αEβ7 is expressed and functionally relevant on non-classical monocytes, making them a likely target for etrolizumab. The combined blockade of αEβ7 and α4β7 seems to delay macrophage-dependent wound healing even greater than sole anti- α4β7 treatment, which might be a partial explanation for the disappointing observations in the etrolizumab phase III trial.

ID: 041
Abstract
Topics: Gut-Microbiota-Brain axis
Keywords: ketogenic diet, microbiota, epilepsy, drug treatment

Identification of a gut microbiota signature in patients with drug-resistant epilepsy upon ketogenic diet treatment

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Epilepsy is a neurological disease characterized by seizures that affects 50 million people world-wide. Around 30% of the patients do not respond to the current drugs. Ketogenic diet (KD), highly enriched in fats, is an established therapy for drug-resistant epilepsy (DRE) that shows a reduction of >50% of seizures frequency in ~50% of DRE patients. Interestingly, microbiota from DRE patients responsive to KD is different from KD-non-responder DRE patients and it is still unclear why some patients do not respond to KD. Our research project will address microbiota differences in DRE patients, responders and non-responders to KD, and identify strains of bacteria with potential anti-seizure effect. For this purpose, fecal samples will be collected from patients with DRE before and during treatment with KD up to 6 months together with clinical and dietary information. Stool bacterial 16S rRNA will be sequence, stool and blood metabolites (including ketone bodies) will be measured through targeted and untargeted metabolomics, and biochemical and inflammatory parameters (e.g., C-reactive protein, procalcitonin, cytokines) from serum samples will be analyzed. We expect that >30-50% of the patients under KD will have >50% reduction of seizures, ~30% will experience <50% reduction of seizure frequency and ~5-10% will not show any improvement or eventually will get worse upon KD introduction. Healthy relatives and patients with DRE without KD will be analyzed as controls. The ultimate goal of this project is to describe which microbes are associated with KD-dependent epilepsy amelioration and to test their effect in vivo in preclinical animal models of epilepsy. Identification of those microbes could be used as a potential treatment for KD-non-responder DRE patients, as well as alternative to KD, which cannot be kept long-term. We believe that these results will shed light for diet-microbiota based treatments for other neurological diseases and metabolic disorders.

ID: 042
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: Chlamydia, Cytotoxic, T cells, Uterus, Epithelial cells

Cytotoxic CD4+ T cells can provide protection against Chlamydia trachomatis infection of the upper genital tract

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Chlamydia trachomatis (C. trachomatis) is the most common cause of bacterial sexually transmitted infection (STI) in both men and women. Immunity to C. trachomatis involves many cell types, but CD4+ T cells play a key role in protecting the host during natural infection. Specifically, IFNy production by CD4+ T cells is the main effector responsible for bacterial clearance, yet the exact mechanism by which IFNy confers protection is poorly defined. In our efforts to define the specific mechanisms for bacterial clearance, we now show that IFNy upregulates the expression of Major Histocompatibility Complex Class II (MHCII) on non-hematopoietic cells of the upper genital tract of mice during C. trachomatis infection in vivo. We also find that MCHII expression on non-hematopoietic cells contributes to the efficient clearance of bacteria mediated by pathogen-specific CD4+ Th1 cells. As we further cataloged the protective mechanisms of C. trachomatis- specific CD4+ T cells, we found that the T cells also express granzyme B (GzmB) when co-incubated with infected cells. Additionally, during C. trachomatis infection of mice, antigen-naïve effector CD4+ Th1 cells displayed elevated granzyme transcripts (GzmA, GzmB, GzmM, GzmK, GzmC) compared to memory CD4+ T cells in vivo. Finally, using intracellular cytokine staining, we show that that effector C. trachomatis-specific CD4+ Th1 cells express granzyme B upon antigen stimulation in vivo. Together these results have led us to conclude that Chlamydia-specific CD4+ Th1 cells develop cytotoxic capacity that contributes to C. trachomatis clearance

ID: 043
Abstract
Topics: Mucosal diseases and infections
Keywords: Colitis, Inflammatory Bowel Disease, Colon cancer, Obesity, Autophagy

Skeletal muscle DUPD1 links colitis with obesity-associated metabolic diseases.

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Inflammatory bowel disease (IBD), comprising of Crohn's disease and Ulcerative Colitis (UC), is a debilitating inflammatory disease of the gastrointestinal tract with no known cause. Although extensive work has linked numerous genetic loci to IBD, most of these associations remain poorly understood. In addition, some studies have associated IBD to metabolic diseases such as diabetes, although the precise molecular and pathophysiological mechanisms are unknown. Employing multiple murine models of colitis, colitis-associated colon cancer (CAC) and obesity associated metabolic diseases, we have identified DUPD1, a phosphatase of unknown function, links IBD with metabolic diseases. Specifically, Dupd1-/- mice are protected from dextran sodium sulfate (DSS) and Helicobacter hepaticus induced colitis, and DSS/azoxymethane (AOM) induced CAC. Dupd1-/- mice also exhibited protection against high fat diet (HFD) induced obesity and non-alcoholic fatty liver disease (in female mice) and glucose intolerance. DUPD1 is highly expressed in skeletal muscle and exerts its colitogenic effects from the skeletal muscle by autophagy, a pathway whose dysfunction is known to play a role in IBD. Importantly, a dual specificity phosphatase inhibitor (NSC-663284) inhibited DUPD1 enzyme activity and reduced DSS-induced colitis in wildtype mice but not in Dupd1-/- mice. Our study identifies Dupd1 as a central gene that links IBD and obesity-associated metabolic diseases and suggests that it is a new therapeutic target in IBD, and obesity associated metabolic diseases.

ID: 044
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Trained immunity, RSV, alveolar macrophages

Investigating macrophage heterogeneity, dynamics and 'memory' in Respiratory Syncytial Virus Infection

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Alveolar macrophages (AMs) are long-lived cells that form the first line of defence in the airways. However, the composition and behaviour of the AMs changes markedly upon infection with respiratory viruses, including influenza, adenovirus and Sars-CoV2. Moreover, certain viral infections have been shown to induce a form of immunological memory in AMs, in a process known as 'trained immunity', altering their responses to subsequent challenge.

Respiratory Syncytial Virus (RSV) infection is the second largest cause of death in children under 1yr worldwide and RSV-induced bronchiolitis increases the risk of developing chronic wheezing and allergic asthma in later life. If and how infection with RSV alters AM behaviour and whether altered AM function post-RSV contributes to predisposition to respiratory sequelae remains unclear.

In preliminary work, we have used a combination of functional assays, multi-parameter flow cytometry and lineage-tracing to characterise AMs in a model of RSV infection. Importantly, we find that RSV infection alters the responsiveness of AM to TLR stimulation ex vivo, long after viral clearance, supporting the premise that RSV leads to AM 'training'. To understand the cellular and molecular events underlying this heightened responsiveness, we profile the AM compartment longitudinally. We show RSV leads to marked recruitment of monocytes into the airways over the first 2-3 days. These differentiate into CD11b+CD11c–/+ macrophages that are distinct from CD11c+SiglecF+ AM. Lineage-tracing confirms monocytes in the bronchoalveolar space arise from de novo recruitment, but also reveals that despite persisting through viral clearance, in the long-term tissue-resident AM lose the ability to maintain themselves autonomously and are replaced by BM-derived monocytes. Future work seeks to understand the contribution of resident and BM-derived AM to the altered functional profile post-RSV and how AM 'training' impacts responses to secondary insult.

ID: 045
Abstract
Topics: Gut-Microbiota-Brain axis, Swiss Young Immunologists Society (SYIS) and young European Federation of Immunological Societies (yEFIS)
Keywords: molecular mimicry; microbiome; multiple sclerosis; neuroinflammation

Microbial triggering of myelin-specific immune cells in the gut drives central nervous system inflammation

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Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system, in which genetic and environmental factors are thought to lead to dysregulated immune responses targeting myelin antigens. Changes in gut microbiota composition have recently been implicated in MS pathogenesis. However, how alterations in gut microbiota composition influence systemic immune responses in an antigenic specific manner and whether this interaction can trigger myelin-reactive immune responses in the gut through molecular mimicry with commensal bacteria is unknown. Here, we studied antigen-specific triggering of gut immune cells and their encephalitogenic potential in experimental models of autoimmune neuroinflammation. We found that myelin peptide-expressing - but not bacteria expressing ovalbumin - were capable of triggering or exacerbating disease in different experimental murine models of MS. Our results provide novel insights into antigen-specific microbial triggers of MS with implications for the development of novel therapeutic strategies that aim at manipulating the gut microbiome.

ID: 046
Abstract
Topics: Innate Immunity, Neonatal window of opportunities
Keywords: SCFAs, Microbiota, Asthma, dysbiosis, ILCs

Antibiotic use during pregnancy, microbial dysbiosis and early life immune cell populations

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Introduction: Antibiotic use during pregnancy is associated with increased asthma severity in the progeny, however, the underlying mechanisms are not fully understood.

Objective: The goal of this project is to assess how antibiotic use during pregnancy, and the transfer of gut microbial dysbiosis from mother to offspring, alters immune system development along the gut-lung axis in early life.

Methods: Pregnant mice were treated with vancomycin from gestation day 8-17 of pregnancy. Feces samples were collected from mothers and offspring for 16S rRNA sequencing and short-chain fatty acid (SCFA) analysis. On postnatal day 15 (PN) and after asthma induction (PN49), the changes to the developing immune system were studied via flow cytometry of small intestine, blood, and lung samples.

Results: Maternal treatment with vancomycin induced maternal and offspring intestinal dysbiosis, evidenced by increased Escherichia and Parabacteroides, and decreased Ruminococcus and Clostridium. Dysbiosis in the PN15 offspring intestine contributed to decreased SCFA concentrations, and was accompanied by ILC2 activation (IL-17RBhi) and significantly increased pro-inflammatory ILC3s populations (NCR-). LPS was detected in the blood of dysbiotic offspring, with higher Rorgt+ T cell percentages and activated eosinophils (Siglecfhi, CD11bhi). Lung tissue had increased Rorgt+ T cell populations in early development and after allergy induction, ILC2s percentage was higher and displayed higher expression of inflammatory markers (IL-7Rαhi, KLRG1hi). Lung macrophages and eosinophils percentages were also increased after asthma induction.

Conclusion: We show that maternal antibiotic use during pregnancy contributed to offspring gut dysbiosis in early life, accompanied by decreased immunomodulatory SCFAs and increased inflammatory ILC3s in the offspring gut. The increased circulating LPS in these animals could have contributed to the expansion of Rorγt+ T cells in the blood and, subsequently, the lung. We propose that early-life intestinal inflammation may contribute to immune system hyperactivation along the gut-lung axis.

ID: 047
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Intestine, Myeloid, Cytokine, Autoantibody, Crohn's

A personalized approach towards the improvement of Crohn's Disease

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Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract that severely affects quality of life. Despite technological advances, CD diagnoses remain challenging and invasive, resulting in worse prognosis and disease complications if not precise. The multifactorial pathogenesis of CD further complicates the identification of pathways driving the disease and development of targeted treatments. However, the factors all converge in loss of immune homeostasis in CD. Intestinal immune homeostasis is centered around the development and function of mononuclear phagocytes (MNP) which support immune tolerance, antimicrobial activity, and barrier integrity. The development and function of MNP rely on the crucial, locally produced cytokine, Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), in the healthy intestine.

Intriguingly, work by our team demonstrates that GM-CSF autoantibodies (GMAbs) are detected in the serum of every third CD patient and antedate the onset of CD by up to 6 years. Moreover, GMAbs are predictive of disease location and severity and associated with impaired intestinal immune homeostasis. Further characterization revealed GMAbs neutralize the cytokine through binding of glycosylations on GM-CSF.

Here, we have developed a bead-based assay that provides a tool to identify GMAbs in CD patients using minimal serum quantities. Beyond prediction of CD development, our assay allows the stratification of patients based on their GMAb reactivity to glycovariants of GM-CSF and their GMAb isotype profile. This assay will reveal the GMAb reactive epitopes on GM-CSF, enabling strategic use of a GM-CSF glycovariant tailored to the GMAb profile of each patient as a therapeutic. With human GM-CSF already being an FDA approved drug, using our approach to classify CD patients provides an innovative strategy to use engineered GM-CSF glycovariants as an intervention to revert the intestinal immune imbalance driven by deficient MNP function in a subset of CD patients.

ID: 048
Abstract
Topics: Mucosal T cells

The GPR15 - GPR15L axis controls unconventional intraepithelial lymphocyte homing to the large intestines

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Objectives: GPR15 is a well-established colon homing receptor, but its pattern of expression among gut IEL has not been explored. GPR15L is the only ligand for GPR15 described to date, but whether this is the only functional ligand for GPR15 in the colon remains unclear. Here we critically tackle these fundamental gaps in knowledge and significantly extend our knowledge on GPR15 immunobiology.

Design: IEL subsets representation and GPR15 expression were profiled in human healthy donors and mouse intestinal segments by flow cytometry and immunofluorescence using Gpr15-/-, Gpr15L-/-, competitive bone marrow chimeric and humanized mice for GPR15.

Results: GPR15 is widely expressed in normal mouse gut IELs, namely unconventional TCRab CD8aa+/- cells that account for the majority of colon IEL. TCRab CD8aa+/- cells were sharply reduced in Gpr15-/- and Gpr15L-/- mice colon IEL compartment, indicating that GPR15 and its ligand are critical for their recruitment. Competitive bone marrow chimera experiments confirm that GPR15L is the only functional colon-expressed chemokine ligand for GPR15. By profiling the humanized mice for GPR15 we show that human GPR15 is expressed, functional and rescues the Gpr15-/- colon IEL phenotype. Besides, these mice display the typical human GPR15 vs CD103 (αE) expression positive correlation, while in normal mice that is largely mutually exclusive.

Conclusion: GPR15L is the only functional ligand for GPR15 in the colon and both receptor and ligand are critical for TCRab CD8aa +/- cells colon homing in mice. The novel GPR15 humanized mice we here present, unlike normal mice, faithfully recapitulate GPR15 immunobiology within the intestinal IEL.

ID: 049
Abstract
Topics: Innate Immunity, Microbe-immune interactions at body surfaces

Epithelially expressed Interferon epsilon protects against colitis

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Type I interferon (IFN) signalling is crucial for maintaining intestinal homeostasis and disrupting this immune balance can lead to inflammatory bowel diseases (IBD). The type I IFN family includes multiple IFNα, IFNβ and IFNε; the exact role of the individual type I IFN is unclear. We previously found IFNε is highly expressed by epithelial cells of the female reproductive tract, where it is involved in protection against bacterial and viral pathogens. Its function has not previously been studied in the gastrointestinal tract.

We show here that IFNε is expressed in human and mouse intestinal epithelium and expression is lost in inflamed conditions. Furthermore, our results show IFNε limits intestinal inflammation in the DSS colitis model. Regulatory T cell (Treg) frequencies were paradoxically decreased in DSS-treated IFNε-/- mice suggesting a role for IFNε in maintaining the intestinal Treg compartment. Moreover, colitis symptoms were ameliorated by transfer of wild-type Tregs into IFNε-/- mice. This demonstrates that the presence of IFNε supports intestinal Treg function in the gut, and the loss of Tregs drives disease in our model. Overall, we have demonstrated that IFNε is present in human intestinal epithelium and show a critical role for IFNε in gut homeostasis. It's constitutive and mucosal expression makes it a promising new therapeutic agent for the treatment of IBD.

ID: 050
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: Dendritic cells, transcriptional regulation, Bcl6, cDC2s, Th17

Bcl6 deficiency in dendritic cells affects intestinal cDC2 subset abundancy and Th17 priming in response to Citrobacter rodentium infection

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Conventional dendritic cells (cDCs) comprise two main subsets (type 1 and type 2 cDCs, called cDC1 and cDC2) with distinctive transcriptional dependencies. Subset identity and their transcriptional fine-tuning dictate the outcome of the functional differentiation of primed effector T cell subsets.

We found that while cDC1 and cDC2 deficient in the transcriptional factor Bcl6 (CD11c.Bcl6KO) maintain their core lineage-specific identity, both DC subsets exhibit substantial transcriptional and phenotypical changes across tissues. Within the cDC2 subset, we found a significant and selective loss of ESAMhi CD11b+ cDC2s in the spleen and their CD103+CD11b+ counterpart in the intestine, a phenotype reminiscent of DC-specific Notch2-deficiency. Intestinal Notch2-dependent cDC2s were previously shown to drive Th17 responses towards extracellular bacteria.

To characterize the functional consequences of Bcl6-deficiency in DCs specifically in the intestinal compartment, we infected CD11c.Bcl6KO mice with Citrobacter rodentium. We found that DC-targeted Bcl6-deficient mice display reduced levels of CD4+ T cells producing IL-17A and IL-22 in the colon and draining lymph nodes 9 days after infection. These findings mimic the well-established phenotype observed upon Notch2 deficiency in cDC2s, and suggest a non-redundant role of Bcl6 in DCs with consequences for the response to bacterial pathogens in vivo. Together, this introduces Bcl6 as a new player to the list of transcription factors governing cDC2 subset heterogeneity.

ID: 051
Abstract
Topics: Mucosal T cells
Keywords: MAIT cells, Colon cancer, In vitro expansion, Cytotoxicity, Granzyme B

Tumour-infiltrating MAIT cells kill target cells by a GrB-dependent mechanism

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Mucosal-associated invariant T (MAIT) cells are a class of unconventional T cells, which are terminally differentiated effector cells capable of producing cytotoxic and pro-inflammatory mediators Their presence in multiple tumour types suggests that they may contribute to tumour immunity. However, the capacity of tumour-infiltrating MAIT cells to actually kill tumour cells

is yet to be fully understood. Here we investigated the cytotoxic capacity of MAIT cells isolated from unaffected colon tissue, tumours, and blood from patients undergoing curative surgery for colon cancer. MAIT cells were expanded by adding artificial antigen-presenting cells, activating the cells through the antigen 5-OP-RU, in the presence of IL-2 and IL-7. Circulating

MAIT cells expanded more than MAIT cells from tissues, and MAIT cells from unaffected tissue expanded slightly more than tumour-infiltrating MAIT cells. The cytotoxicity of expanded MAIT cells towards tumour cell lines (HeLa, HT29, and HCT116) and primary patient-derived tumour cells from colon cancer patients was evaluated by flow cytometry. Tumour-infiltrating expanded MAIT cells readily killed target cells of all the cell lines tested in a dose-dependent manner and donor-derived expanded MAIT cells exhibited a robust

cytotoxic activity towards primary patient-derived tumour cells. Inhibition of Granzyme B activity by the serine protease inhibitor 3,4-Dichloroisocoumarine substantially reduced the MAIT cell cytotoxicity towards the target cells. Furthermore, blocking the activity of CD161, TRAIL, and FasL on expanded MAIT cells also resulted in a slight decrease in cytotoxic activity towards some of the target cells tested. In summary, our studies demonstrate that tumour-infiltrating MAIT cells have cytotoxic capacity which is similar to that of MAIT cells from unaffected tissues. These results, together with the possibility of expanding MAIT cells and their lack of MHC-restriction, indicate that MAIT cells may represent a promising new candidate for cell-based cancer therapies.

ID: 052
Abstract
Topics: Microbe-immune interactions at body surfaces, Systems immunology in host-microbe interactions
Keywords: IL-33, gut microbiota, enteropathogen infection, SCFA

Interleukin-33-mediated gut dysbiosis and its consequences for a bacterial enteropathogenic infection

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The interplay between the commensal gut microbiota and the immune system includes several interactions in homeostasis and disease. The microbiota play critical roles in the training and development of the host's innate and adaptive immune system, while at the same time the immune system is involved in the orchestration of the maintenance and the composition of the microbiota. Cytokines and chemokines support intestinal homeostasis but can also be key drivers of intestinal inflammation and inflammation-associated damage. Recently, we identified interleukin (IL)-33, which is highly abundant in the intestine, as a critical regulator of bacterial enteropathogenic infection. We observed that deficiency of the IL-33 signalling pathway attenuates bacteria-induced colitis and conversely, boosting this pathway strongly aggravates the pathogen-driven pathology and makes the mice prone to systemic infection. Interestingly, mice deficient in IL-33 but also with IL-33 supplementation both develop a microbial dysbiosis with alterations in the Firmicutes to Bacteroidetes ratio, which is a relevant marker for gut dysbiosis. Concomitantly, exogenous treatment of mice with IL-33 modified the bacterial metabolite composition in the faeces with reduced levels of short-chain-fatty-acids (SCFAs). SCFAs act on many cell types to regulate a number of important biological processes, including host metabolism, intestinal epithelial cell functions and immune system. Well in line, an essential intestinal response against enteropathogenic infection is diminished in IL-33 treated animals and opens the door for an uncontrolled distribution of a pathogen. Thus, a deeper understanding of cytokine-mediated dysbiosis and the resulting consequences for the hosts' gut immunity are indispensable for the accurate treatment of intestinal infectious diseases.

ID: 053
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: Dendritic cell, T cell, T cell stimulation, Crohn disease

Addressing the function of human colonic and ileal dendritic cell subsets in health and Crohn´s disease

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Human intestinal conventional dendritic cells (cDC) determine the outcome of T-cell mediated immune responses. Indeed, cDC can be divided into several subsets like CD103- cDC2 (CD103-SIRPα+), CD103+ cDC2 (CD103+SIRPα+) and cDC1 (CD103+SIRPα-). Nevertheless, the specific function of each subset, together with the presence of regional differences (colon Vs. ileum) in health and in Crohn´s disease (CD) remains elusive. Hence, human intestinal cDC subsets were enriched from tissue resections from controls and CD patients. As opposed to sorted intestinal macrophages, cDC stimulated naïve T-cells confirming their function. Indeed, all intestinal cDC subsets primed the generation of Tregs as stimulated T-cells produced IL-10 and co-expressed FoxP3, being this capacity increased in both CD103+ subsets (CD103+ cDC2 and cDC1). Moreover, all three ileal cDC subsets stimulated T-cells more efficiently than their paired colonic counterparts in controls, although the acquired T-cell profile did not differ among tissues. None of the cDC subets, on the contrary, induced the expression of IFNγ or IL-17 on stimulated T-cells. Referred to CD patients, all three colonic cDC subsets were more stimulatory over T-cells than their counterparts from the control tissue (although that was not the case in the human ileum). Moreover, CD103+ cDC2 from the inflamed ileum from CD patients primed the generation of IL-17+ responding T-cells. In summary, we hereby have demonstrated not just functional differences between human colonic and ileal cDC, but also that CD103+ cDC have an increased capacity to generate Treg cells which, nevertheless, is compromised in CD patients.

ID: 054
Abstract
Topics: Mucosal diseases and infections

Investigating the role for parasitic annexins in the membrane traversal of non-secretory virulence factors in Giardia lamblia

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Giardia lamblia is a protist parasite colonizing the small intestine of its mammalian hosts. Despite being non-invasive, Giardiasis, leads to multiple symptoms like diarrhea, abdominal pain and malnutrition. Giardia infections result in disruption of the microbiome and were correlated to irritable bowel syndrome. Although Giardiasis presents worldwide distribution and is among the most common parasitic diarrheal diseases, little is known about Giardia's virulence factors and the molecular mechanisms underlying their extracellular deposition.

Several reports on the release of putative non-secretory virulence factors point towards unconventional protein secretion (UPS). UPS at the host-pathogen interface of mammalian systems is involved in crucial processes for survival of both organisms with implications in inflammation, cytokine secretion and invasion.

To dissect the molecular machinery involved in UPS in Giardia, we performed affinity purification and mass spectrometry as well as microscopy-based subcellular localization using a selection of epitope tagged UPS substrates. These investigations led to two main discoveries. First, a subset of the investigated UPS substrates localises to Giardia's peripheral endocytic compartments (PECs), highlighting the possible dual exo/endo- cytic nature of these organelles.

Second, the investigated PEC-associated UPS substrates form a tight complex interacting with human annexin homologs called alpha giardins. Annexins bind negatively charged phospholipids and destabilise membranes. Therefore, alpha giardins, themselves putative virulence factors, may play a role in membrane traversal for UPS of virulence factors. Preliminary results of liposome-based experiments confirm that alpha giardins are capable of binding lipids and suggest that some can destabilize phospholipid membranes, thereby mediating their own traversal.

Taken together, this work shows how parasitic protists have evolved novel trafficking routes and organelle types to establish the molecular communication required for effective pathogen-host interaction to take place, mostly at the host's expense. Excitingly, the exact role for the microbiome in mediating and perhaps promoting this interaction, awaits deeper investigation.

ID: 055
Abstract
Topics: Microbe-immune interactions at body surfaces, Systems immunology in host-microbe interactions
Keywords: Gut immunity, Microbiome, Glycosylation, IBD

Host glycosylation shapes intestinal microbiome and immune response in Inflammatory Bowel Disease

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Inflammatory Bowel Disease (IBD) is a multifactorial immune disorder of the gastrointestinal tract comprising Crohn's disease and Ulcerative colitis. Although the precise aetiology of IBD is still elusive, it seems to result from an abnormal immune response to an altered gut microbiota in a genetically susceptible host. The currently available therapies for IBD are ineffective, being associated with several side effects. Thus, an improved understanding of the causes associated with loss of gut microbial equilibrium (dysbiosis) and the mucosal inflammatory response is crucial for a better clinical management of IBD. Cellular glycosylation displays a key role in IBD immunopathogenesis. The intestinal epithelial glycocalyx, a complex layer of glycans/sugar-chains at the surface of mucosa, is a major interface between host and gut microbiota. Hence, in this study we aim to unveil the impact of an altered host glycocalyx in the microbiota profile and in the initiation of intestinal inflammation. We have resorted to a glycoengineered mouse model with alterations in N-glycosylation to characterize the impact of changes in host glycome in the modulation of microbiota and immune response. Colitis was induced by dextran sulfate sodium (DSS). The intestinal glyco-immune profile was characterized, and faecal microbiota was analysed by 16S rRNA sequencing. We observed that glycoengineered mice displayed an increased susceptibility to severe forms of colitis. These mice also exhibited a dysbiotic gut microbiota. Interestingly, we also observed that KO mice for selected glycogene exhibited an impaired protective immune response, characterized by the decrease frequency of immune populations such as regulatory T cells, as well as a significant reduction in homeostatic cytokines such as IL-10 and IL-22. Moreover, the deficiency in N-glycans was also associated with a pro-inflammatory environment at baseline. Hence, our preliminary results highlight glycans as essential elements that shape gut immunity by modulating intestinal microbiota composition.

ID: 056
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: memory CD38+ T cells, TIGIT, Th1*, IL12p70, Crohn's disease

Mucosal memory TIGITneg T cells proliferate and acquire a Th1* phenotype in an in vitro inflammatory environment

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TIGIT (T-cell immunoreceptor with immunoglobulin and ITIM domains), a coinhibitory receptor expressed by T cells and NK cells, is selectively induced on CD4+ T cells with a memory phenotype. Approximately 20-40% of intestinal memory CD4+ T cells and circulating CD38+ memory (CD62LnegCD4+) T cells, enriched for mucosal antigen specificity, carry TIGIT. TIGIT+CD38+ memory T cells have regulatory function and express co-inhibitory receptors CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) and PD-1 (programmed cell death protein 1). In pediatric Crohn's disease (CD), low frequencies of TIGIT+CD38+ memory T cells identify a subgroup of severe patients whose TIGITnegCD38+ memory T cells are enriched in inflammatory IFNγ+-IL17+ Th1* cells. We hypothesize that CD-related inflammatory cytokines increase TIGITneg memory Th1* frequencies within the CD38+ memory T cell-pool, thereby driving intestinal inflammation in CD. We cultured peripheral blood memory CD4+ T cells from healthy individuals with TCR stimulation, co-stimulation and CD-related inflammatory cytokines, and assessed development of TIGITneg memory Th1* cells. While TCR ligation alone induced TIGIT expression on CD38+ memory T cells, anti-CD28 co-stimulation enhanced TIGITneg cell frequencies. Only cocktails containing IL-12p70 further increased TIGITneg cell frequencies. Interestingly, TIGITneg cells were still expressing PD-1 and CTLA-4. In agreement, IL-12 receptor β2 mRNA expression was higher in TIGITneg compared to TIGIT+CD38+ memory T cells. This suggests that TIGITneg cells preferentially respond to IL-12p70 stimulation. Co-stimulation with IL12p70, IL-1β and IL-6 yielded the highest frequencies of TIGITnegCD38+ memory T cells. Both IFNγ+-IL17+ and Tbet+-RORγt+ cell frequencies increased in TIGITnegcompared to TIGIT+CD38+ memory T cells when cultured with this cocktail. Altogether, these results demonstrate that culture with TCR ligation, co-stimulation and the IL-12p70/IL-1β/IL-6 cocktail drives proliferation of TIGITnegCD38+ memory T cells and initiates a switch to a functional Th1* program. These cytokines may drive selective expansion of pathogenic TIGITnegCD38+ memory Th1* cells in a subgroup of CD patients.

ID: 057
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: COVID-19, mRNA vaccines, lymphoid malignancies, biomarkers, spectral cytometry .

Immune response of COVID-19 mRNA vaccines in older adults and patients with lymphoid malignancies and potential biomarkers.

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COVID-19 affects the population unequally with a higher impact on aged and immunosuppressed individuals. Hence, we aimed to characterize the immune response triggered by mRNA vaccines as well as to identify biomarkers of prognosis in three different cohorts: 25 healthy adults (under 70); 28 institutionalized adults (over 70 years); and 48 oncohematologic patients untreated or treated with B-cells antiproliferative drugs (lenalidomide, ibrutinib and rituximab). Plasma and peripheral blood mononuclear cells (PBMCs) were analyzed before and 3 months following immunization to determine humoral and cellular memory towards SARS-CoV-2 together with the phenotype of the PBMC by spectral cytometry (40-plex panel). While healthy and institutionalized adults developed a serologic response, it was much lower in the oncohematologic cohorts (being absent in rituximab-treated patients). Nevertheless, all cohorts (excluding patients treated with ibrutinib) developed a cellular response. Unsupervised clustering algorithms (UMAP and flowSOM) along with classical hierarchical analyses showed great heterogeneity in the basal condition of the cohorts, with significant differences in multiple subsets related to their vulnerability. There were also significant differences within each cohort following vaccination, responsible for the conferred protection. Finally, an analysis comparing SARS-CoV-2 infected vs. uninfected individuals after vaccination was performed. The acquired humoral and cellular memory did not predict infection. Nevertheless, an in-depth analysis of the cellular immunome by spectral cytometry, both at basal and post-vaccination, revealed several metaclusters within the CD8+ T-cells, plasmablasts, ILCs and Tγδ differentially expressed in infected and non-infected individuals, particularly within the oncohematologic patients. In summary, we hereby have identified cellular subsets strongly related to COVID-19 vulnerability, as opposed to the acquisition of cellular and/or humoral memory and the time that we have also described the immune response to SARS-CoV-2 mRNA vaccines.

ID: 058
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections
Keywords: gut microbiome, Heligmosomoides polygyrus, colorectal cancer, fecal microbiota transplantation

Influence of helminth-induced alterations of the microbiome on the development and progression of colorectal cancer

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Intestinal infections with parasitic worms, so-called helminths, are among the most common infections worldwide. Heligmosomoides polygyrus is a naturally occurring intestinal parasite of mice that is closely related to highly-prevalent human nematode parasites and commonly used as an infection model. To attenuate the T helper 2 (TH2)-type immune response against themselves, helminths release a variety of immunosuppressive molecules that modulate the hosts' immunity to ensure their survival. This immunosuppressive status can make the host more susceptible to other infections or increase the risk of developing cancer, as some helminth species are already described to be associated with certain cancers. Indeed, previous own results demonstrated that infection with Heligmosomoides polygyrus in the early inflammatory phase of colitis-associated colon cancer aggravated intestinal inflammation, altered the immune cell composition in the colon, and promoted tumor development. Since gut homeostasis is maintained by the balanced interaction between host immunity and intestinal microbiota, microbial dysbiosis may affect tumor development. Therefore, we collected feces from helminth-infected mice with and without colorectal cancer and performed 16S rRNA sequencing. First results revealed that the infection with Heligmosomoides polygyrus in the small intestine caused significant differences of the microbial composition in the colon between helminth-infected and untreated mice. Additionally, helminth infection also led to a significant shift of the gut microbiota of mice with colorectal cancer. In ongoing experiments, we aim to clarify whether helminth-induced alterations of the gut microbiome modulate the immune system and foster the hosts' susceptibility to colorectal cancer. This study will contribute to a better understanding of the complex interplay of infection, microbiome, immune system, and cancer and help to develop preventive or therapeutic treatments of intestinal diseases.

ID: 059
Abstract
Topics: Mucosal T cells
Keywords: Microbiota, T lymphocytes, Inflammation

TGF-beta sustains intestinal epithelial integrity through its action on T cells

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In the intestine, microbiota encodes millions of proteins; each of them possessing several potential T lymphocyte antigens to which the host must be tolerant. Thus, the maintenance of the anatomical separation between the microbiota and the immune system is crucial to avoid chronic and deleterious inflammations. Here, we reveal that Transforming Growth Factor Beta (TGF-beta), a cytokine highly enriched in mammal intestine, is responsible for the maintenance of barrier integrity of the intestine. Using genetic approaches and T cell adoptive transfers, we found that intestinal TGF-beta controls the second wave of development of Rorgtpos regulatory T cells (Rorgt Tregs), a T cell population we described as endowed with a unique ability to sustain the barrier integrity by producing IL-22. In addition, intestinal TGF-beta represses the ability of effector T cells to react against microbiota antigens that could reach lamina propria. Subsequently, in the absence of TGF-beta in alpha, beta T cells, bacteria contained within the lumen pass through the epithelium and effector T cells create a chronic inflammation in the intestine. Hence, this study reveals unsuspected functions for TGF-beta on the intestinal barrier through its action on both Tregs and effector T cells, with direct consequences on intestinal chronic inflammations.

ID: 060
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: Colitis-associated colon cancer, helminths, CD8+ T cells, ulcerative colitis

Helminth infection interferes with the specificity and function of CD8+ T cells in colitis-associated colon cancer

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A close relationship between inflammation and tumor development has been described for numerous human cancers over the last years. In terms of ulcerative colitis (UC), it has been clearly demonstrated that these patients are predisposed to develop colorectal cancer. Current treatments of UC aim to alleviate the acute inflammation and maintain patients in remission, but fail to modify or reverse the underlying pathogenic mechanisms. As helminth infections have been shown to provoke an immunosuppressive state in their host, helminth parasites were tested for treating IBD patients. However, some infection–related cancers can be attributed to helminth infection, probably due to the generation of a microenvironment that might be conductive to the initiation and development of cancer. To analyze whether a helminth infection interferes with tumor development, the intestinal helminth Heligmosomoides (H.) polygyrus was applied before tumor induction in a mouse model of colitis-associated colon cancer (CAC). Interestingly, we observed that H. polygyrus infection strengthens the inflammatory response in the colon and significantly boosts the initiation and growth of tumors. H. polygyrus infection was accompanied by long-lasting alterations in the colonic immune cell compartment, with reduced frequencies of CD8+ effector T cells. As cytotoxic CD8+ T cells are crucial for anti-tumor immune responses in CAC, we further analyzed the impact of a helminth infection on the function of colonic anti-tumor CD8+ T cells by means of functional assays and T cell receptor profiling. Thereby, we identified that H. polygyrus infection during tumor initiation significantly altered the activation and the proliferation of CD8+ T cells in the colon. Furthermore, H. polygyrus infected CAC mice also displayed alterations in their specific CD8+ T cell receptor repertoire.

Thus, our study underlines that parasitic helminth infections have implications for cancer development where a protective immune response depends on antitumor CD8+ T cells.

ID: 061
Abstract
Topics: Innate Immunity
Keywords: ILC2, Fibrosis, Hypoxia

The Role of Hypoxia-Inducible Factors in Type 2 Innate Lymphoid Cells

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Innate Lymphoid cells type 2 (ILC2s) play a crucial role in effector function of innate immunity against several organisms including Helminths as well as tissue homeostasis and remodeling. Upon activation with Interleukin(IL)-33, ILC2s release the cytokines IL-13 and IL-5. Although ILC2s only represent 0.5-5% of CD45 positive cells in the lung, they seem to play an important pathological role in the development of allergic airway diseases. Lymphoid cells need to work in highly inflammated tissue with an alternating oxygen supply.

Adaption to low oxygen is mediated by the hypoxia-inducible transcription factors (HIF1α). In hypoxic environments HIFs are stabilized and lead to an adaption of the cell by initiating the transcription of several genes e.g. Glucosetransporter-1 (Glut-1). In several studies it is shown that innate lymphoid cells lacking HIF1α show impaired cell effector function. Although, the lung is the most well-oxygenated organ, we observe GLUT-1 expression in murine lungs treated with IL-33 despite the presence of ample oxygen, suggesting HIF signaling during IL-33-dependent immune responses. Here, we investigate the function of HIF1α in IL-5 producing cells including ILC2s.Therefore we generated a mouseline containing the RED5 reporter and Smart13 for investigating IL-13 production. We treated mice with recombinant IL-33 by oropharyngeal aspiration. We observe a significant increase in IL-13 producing ILC2s in the lung in mice lacking the transcription factor subunit HIF1α after acute and chronic activation with IL-33. Likewise, infection with the helminth Nippostrongylus brasiliensis results in increased IL-13 expression in pulmonary ILC2s. Furthermore, after chronic IL-33 challenge, we observe more goblet cell hyperplasia as well as more peribronchial and perivascular fibrosis in mice lacking HIF1α in ILC2s, hence, morphological findings that are reminiscent to the tissue pathology of patients with severe Asthma. In summary, this suggests, that loss of HIF1α leads to an hyperreactive ILC2 phenotype with increased IL-13 production.

ID: 062
Abstract
Topics: Innate Immunity, Mucosal T cells
Keywords: Human gastric immunome, spectral cytometry, individual variability, phenotipic and functional characterization

High resolution immunome characterization reveals a complex leukocyte network with inter-individual variability and lack of regional variation within the human gastric mucosa.

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The immune cellular landscape from the gastric mucosa remains largely scarce in the human setting, despite its relevance in several inflammatory conditions. Indeed, and although the properties of the intestinal immune system systematically change through its length, it is currently unknown whether the same applies in the human stomach. Therefore, we hereby aimed to perform both an unbiased characterization of the human gastric immune system, but also to determine the presence of regional differences within it. To that end, human gastric biopsies were obtained from the antrum, body and incisura from 10 controls to obtain lamina propria mononuclear cells that were further characterized by spectral cytometry with a panel of 40 markers (OMIP 069).

Phenotypic hierarchical analyses identified a total of 54 different immune cell subsets within the human gastric mucosa revealing that T-cells (>60%) and NK cells (>20%) where the main populations. Within T-cells, CD4+ and CD8+ were equally represented with CD4+ T-cells displaying mainly a central memory phenotype while CD8+ T-cells were much more variable. NK cells, on the contrary, were mainly of the early phenotype. No regional differences were observed for any subsets among the 3 different locations. Following unsupervised analysis by dimensionality reduction algorithms (UMAP and FlowSOM) -including both phenotypic and functional markers- a total of 82 metaclusters were identified. Again, no differences were observed between locations although a great degree of inter-individual variability was found.

In summary, we hereby have unraveled the human gastric cellular subset composition at the time that we have proved a unique interindividual immune fingerprint with no inter-regional variations.

ID: 063
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: bacteria, virus, interaction, asthma, innate

Bacteria-virus interactions in the airway mucosa mediated via the antiviral signalling molecule MAVS and can be modulated directly via macrolide antibiotics

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Introduction: Chronic bacterial infections and viruses have complex interactions in the airway mucosa. The macrolide antibiotic azithromycin (AZM) reduces asthma exacerbations through incompletely described mechanisms.

Methods: We studied bacteria-virus interactions in vitro in human airway epithelial cells and in vivo in mice, and studied the effects of bacteria and AZM in human cohorts using single cell sequencing and Nanopore metagenomics on bronchoscopy samples.

Results: NTHi was the dominant pathogen in severe asthma, and associated with induction of type 1 cytokines and IL-10 in the airways. In vitro NTHi infection reduced IFN-β secretion by airway epithelial cells and promoted RV infection. AZM inhibited RV replication. AZM restored IFN-β levels in cells co-infected with NTHi group in a dose dependent manner, independent of antibiotic effect. Transcriptomic analysis during RV co-infection showed NTHi upregulated IL-1β, and granulocyte chemokines whilst AZM downregulated proinflammatory cytokines, granulocyte migration, and upregulated innate antiviral defense pathways including IFIT3, OAS1, ISIG, IFNL. Proteomic analysis of epithelial cells found AZM upregulated the mitochondrial antiviral signalling protein (MAVS). In a CLICK assay AZM bound to MAVS. RV infection caused degradation of MAVS, but this was restored by AZM. In vivo MAVS mRNA and protein were downregulated by NTHi infection. During NTHi infection MAVS-/- mice experienced less weight loss but had higher bacterial burden, and greater neutrophilia than wild-type mice, due to a deficiency in early production of cytokines including IL-1β, -2, -17 and TNF. In human scSeq analysis 3 months of AZM led to reduction in T cell activation, cytotoxicity, inflammatory infiltrates, chemokines (CCL4, CXCR4, CCL5) and amphiregulin.

Conclusion: NTHi is dominant in the airway mucosa in asthma, where it promotes RV infection and type 1 inflammation, but is sensed by MAVS. Beyond its antibiotic properties AZM has direct antiviral effects on RV and can restore MAVS mediated responses to NTHi.

ID: 064
Abstract
Topics: Gut-Microbiota-Brain axis
Keywords: Gut-Microbiota-Brain Axis, microbiota, gnotobiotic, spatial transcriptomics, ketogenic diet

Ketogenic Diet affects molecular pathways in specific brain areas in a microbiota-dependent manner

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The central nervous system (CNS) and the intestine communicate via peripheral nervous structures, the immune system and via intestinal microbial products and metabolites, building a bidirectional network called gut-microbiota-brain (GMB) axis. The microbiota is highly influenced by the dietary habits of the host. Ketogenic diet (KD) not only changes the microbiota composition but ameliorates conditions in several metabolic and neurologic diseases. However, the mechanisms behind the effect of this dietary intervention are not well understood. We aim to unravel the effects of KD on the function of gut microbiota and the host metabolism, the immune system and the CNS under healthy conditions. To study the microbiota, we performed 16S rRNA sequencing and metatranscriptomics on intestinal bacteria of mice colonized with a diverse and undefined microbiota (specific-pathogen free, SPF) or with a gnotobiotic moderately diverse microbiota and fed purified KD or control diet. KD changed the Firmicutes/Bacteroidetes ratio and the metabolism of Clostridia families. To investigate the effect of KD on the CNS in a microbiota-dependent and -independent way, we analyzed the brain performing spatial transcriptomics in germ-free and SPF mice fed KD and control diet. In the brain, KD induced, in a microbiota-dependent manner, the upregulation of genes involved in neuronal cell communication and development, myelinization, and metabolic processes within specific areas, such as the hypothalamus, thalamus, amygdala and the hippocampus. We are currently investigating the effect of KD and microbiota on the host at the single cell level, via single nuclei RNA seq, in the affected brain areas and via flow-cytometry to identify the role of the immune system in our model. Behavioral tests will be performed to address the CNS functions in the different experimental conditions.

Our experiments provide important findings to unravel the mechanisms behind the effects of KD on the host via the microbiota.

ID: 065
Abstract
Topics: Innate Immunity

High-dimensional analysis indicates the existence of lineage committed and uncommitted cDC precursors in the human intestinal lamina propria

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Unbiased single-cell approaches have increased our understanding of human conventional dendritic cell (cDC) heterogeneity and have provided evidence of the existence of cDC subsets additional to the well-defined cDC1 and cDC2 populations, as well as lineage-specific cDC1 and cDC2 precursors. In the current study, we combined our recently described intestinal isolation protocol (1) with single-cell transcriptomics and CITE-seq to investigate cDC heterogeneity in the human small intestinal and colonic lamina propria (LP). In addition to cDC1 and cDC2, we identified a major subset of cDC that shared a transcriptional profile with blood and bone marrow (BM) cDC3. We also identified several ITGAXlow HLAlow proliferative clusters in both the small intestinal and colonic LP which, based on gene expression profiling, trajectory analysis and RNA splicing patterns, we putatively designate as lineage-committed pre-cDC1, pre-cDC2 and pre-cDC3 precursors. Finally, we identified a rare ITGAXlow HLAlow proliferative cluster that did not transcriptionally align with lineage-committed cDC precursors, but instead transcriptionally resembled BM myeloid precursors. Collectively, our results suggest that the healthy human ileum and colon contains mature cDC1, cDC2, cDC3 as well as uncommitted and lineage-committed cDC precursors.

(1) Jørgensen, P.B., Fenton, T.M., Mörbe, U.M. et al. Identification, isolation and analysis of human gut-associated lymphoid tissues. Nat Protoc 16, 2051–2067 (2021). https://doi.org/10.1038/s41596-020-00482-1

ID: 066
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Emulsifiers, ferroptosis, Inflammatory Bowel Disease, polysorbate 80, epithelial cells, organoids

The dietary emulsifier polysorbate-80 induces cell death in intestinal epithelial cells via ferroptosis

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The microbiota and westernised diet are suggested to contribute to the development of several chronic conditions including obesity, inflammatory bowel disease (IBD), metabolic syndrome etc. In recent years, certain dietary additives e.g., emulsifiers and sweeteners have been implicated in the deterioration of these conditions in experimental models by negatively impacting both the intestinal microbiota and the subsequent host response. However, mechanistic understanding of these dietary additives on the host response including epithelial cells are largely unknown. Preliminary studies revealed that the emulsifier polysorbate 80 (p80) was toxic to human intestinal epithelial cells, while carboxymethyl cellulose (CMC) was not. Thus, the aim of this project was to unravel which cell death mechanism(s) regulates p80-induced death in epithelial cells. By using flow cytometry, ELISA, Seahorse XFe24 Analyzer, light-, fluorescence- and electron microscopy on p80-treated human intestinal epithelial cell lines and murine organoids we identified alterations in mitochondria metabolism, reduced proliferation, and increased cell death, without an increase in cytokine secretion. In combination with small molecule inhibitors targeting the cell death pathways of apoptosis, necrosis, necroptosis, autophagy and ferroptosis, we showed that p80 causes an early structural damage on the mitochondria, an increase in reactive oxygen species (ROS), iron accumulation and lipid peroxidation resulting in an increased ferroptotic cell death over time. These structural findings were validated by alterations in ferroptosis markers such as FTH1, NRF2 and NCO2 by western blotting in p80-treated cells, changes that were reversed by the ferroptosis inhibitors. In conclusion, our data suggest that dietary emulsifying agents such as p80 can contribute to the increased incidence of chronic intestinal conditions and metabolic syndrome by altering the host response such as induction of cell death via ferroptosis in conjunction with bacterial alterations.

ID: 067
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections
Keywords: T cells, flagellins, microbiota-host, IBD

Elevated adaptive immune responses to multiple Lachnospiraceae bacterial flagellins in therapy-naive pediatric CD patients are associated with distinct immune pathology

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In inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), CD4+ T-cell responses to unknown microbial antigens drive intestinal inflammation. Both hypo- or hyperactive anti-microbial innate immunity could underlie these pathogenic T-cell responses. Previously, IgG and T-cell responses to Lachnospiraceae-derived flagellins were detected in spontaneously colitic mice and a subgroup of adult CD patients with complicated disease. Here we aim to elucidate whether anti-Lachnospiraceae IgG and inflammatory flagellin-specific T-cell responses occur in therapy-naïve pediatric IBD patients and assess whether they associate with innate hypo- or hyperresponsiveness. We measured plasma IgG reactivity to 20 flagellins from Lachnospiraceae that colonize mouse and human gut in therapy-naïve pediatric IBD patients (CD n=49; UC n=16), non-IBD (n=12) and age-matched healthy controls (HC n=17). Immunological and clinical profiles were related to the number of flagellins to which patients responded (denoted as "flagellin-reactivity"). A higher proportion of CD patients (41%) had IgG responses to more than 10 flagellins compared to UC (6%), non-IBD (8%) and HC (6%). In CD patients, flagellin-reactivity did not relate to clinical scores. As expected, in blood, both flagellin-specific- and inflammatory gut-homing- memory CD4+ T-cell frequencies increased with flagellin-reactivity. We then assessed whether this increased adaptive immune response relates to innate hypo- or hyperresponsiveness. Colonic lesional tissue of patients with high flagellin-reactivity showed strikingly little immune cell infiltration, epithelial damage and phosphorylated NFκB compared to patients with low flagellin-reactivity. Moreover, flagellin-reactivity was associated with decreased plasma concentrations of neutrophil-derived EN-RAGE. In conclusion, a subgroup of therapy-naïve CD patients has elevated IgG and T-cell responses to multiple Lachnospiraceae-derived flagellins. The colonic lesions of these patients have very limited innate immune cell activation and/or infiltration. These data raise the question whether the enhanced anti-Lachnospiraceae adaptive immune response in a subgroup of CD patients may be driven by insufficient local innate immunity.

ID: 068
Abstract
Topics: Mucosal diseases and infections
Keywords: Obesity, Tolerance, lymph node

Dietary lipids accumulate in mesenteric lymph node cells and influence oral tolerance induction

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Obesity increases the risk of various diseases, such as diabetes mellitus type 2 and cardiovascular diseases, but also affects the immune system. During obesity, more dietary fats are absorbed and transported into the bloodstream via the lymphatic systems. Increased fat accumulation leads to impaired lymphatic fluid and atrophy of the mesenteric lymph nodes (mLN). In these secondary lymphoid organs, the lymph fluid is filtered, immune reactions against pathogens are generated or tolerance to harmless antigens is induced. However, the alteration of these functions in the mLN in obesity is still poorly understood. In this study, the changes in the microarchitecture of the mLN during high fat transport were investigated and the induction of oral tolerance was assessed.

To this end, the effects of diet-induced obesity (DIO) on the mLN were investigated in C57BL/6NCrl mice. Mice were fed a high-fat (HFD) or low-fat (LFD) diet for a maximum period of 14 weeks. After 10 weeks of feeding, the lymph node architecture was analysed and oral tolerance was induced.

Lipid droplets were observed in lymphatic endothelial cells, various reticulum cells and macrophages by transmission electron microscopy, and the size of lipid droplets as well as their number and intercellular spacing increased after 10 weeks of high-fat diet.

After oral tolerance induction, we observed an impaired delayed hypersensitivity response (DTH) during DIO, but this was reversible after switching to standard chow. Furthermore, an influence of the lymph node microenvironment on the development of oral tolerance during DIO was detected, with oral tolerance induced in transplanted peripheral lymph nodes.

The results suggest that changes in microarchitecture and increased accumulation of lipid droplets in stromal cells and macrophages influence the immunological function of mLNs.

ID: 069
Abstract
Topics: Innate Immunity, Neonatal window of opportunities
Keywords: postnatal immunty, neonatal window, gut closure, endotoxin translocation, gut-liver axis

Early life hepatic immune activation by CD36-mediated lipopolysaccharide translocation prior to gut closure

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Macromolecule translocation across the intestinal mucosal barrier is enhanced in the first week of early life before it ceases at a time at what has been termed gut closure. This might expose the neonatal liver, which at this stage in mice shifts from a hematopoietic to a metabolic organ, to gut-derived microbial constituents. Using neonatal wildtype and conditional knock-out mice as well as intestinal epithelial stem cell organoid cultures in combination with molecular tracer and microbial stimulus administration as well as microbiota analysis, we identified biologically relevant intestinal macromolecules, the molecular mechanism of translocation and characterized the hepatic response. Microbiota profiling revealed a transiently enhanced abundance of intestinal lipopolysaccharide (LPS)-producing γ-Proteobacteria early after birth. Orally administered LPS was rapidly translocated across the mucosal barrier during the first week of life causing transiently elevated hepatic Il6 expression most likely by neonatal liver monocytes. LPS translocation was independent of disabled homolog 2 (Dab2)-mediated endocytosis by fetal-like enterocytes. Instead, it was mediated in a caveolin-dependent manner via the fatty acid translocase CD36 that exhibited a strongly enhanced epithelial expression early after birth. Intestinal translocation of LPS was reduced in vivo by irreversibly inhibiting CD36-mediated fatty acid uptake by Sulfo-N-succinimidyl oleate (SSO). Together, our results identify and characterize a transient, postnatal time window of hematopoietic tissue stimulation by gut-derived LPS as a novel immune priming event in early life. Our findings may explain epidemiological studies in humans that associate early life exposure to a farm environment with reduced risk for developing allergic diseases later in life

ID: 070
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: TIGIT, T cells, immunoregulation, IBD

Expression of the coinhibitory receptor TIGIT on memory CD4+ T cells is induced by TCR ligation and retinoic acid co-stimulation and is elicited during microbial colonization of the intestine

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The coinhibitory receptor T-cell immunoreceptor with Ig and ITIM domains (TIGIT) is widely expressed by immune cells, including CD4+ T cells. Previously, we demonstrated that low frequencies of immunoregulatory TIGIT+ cells in circulating gut-homing CD38+ (CD62LnegCD4+) memory T cells associate with more severe inflammatory bowel disease. Therefore we hypothesize that TIGIT+CD4+ T cells regulate immune responses to intestinal bacteria, thereby maintaining intestinal homeostasis. Thereto, we investigated TIGIT expression in subpopulations of murine and human CD4+ T cells. In addition, we assessed the signals required for the induction of TIGIT expression in vitro using human peripheral blood and mouse spleen CD4+ T cells. TIGIT was only expressed on memory but not naive CD4+ T cells in both species. In human blood, 60% of TIGIT+ cells was central memory. In mouse and human intestinal lamina propria, TIGIT was expressed by more than 50% of CD4+ memory T cells. Both Foxp3+ and Foxp3neg intestinal CD4+ T cells expressed TIGIT. Intestinal TIGIT expression depended on the presence of microbial antigens, as conventionalization of germ-free mice with specific-pathogen free microbiota dramatically increased TIGIT expression in both small intestine and colon. Next, we identified the signals required for TIGIT induction on TIGITnegCD4+ T cells from human peripheral blood and mouse spleen. T cell receptor (TCR) ligation and downstream calcium-dependent signaling were essential to induce TIGIT expression on CD4+ T cells. TIGIT expression could only be induced on antigen-experienced CD4+ memory T cells, but not naïve CD4+ T cells. Co-stimulation with retinoic acid, produced by intestinal antigen-presenting cells (APCs), but not TGF-B, significantly enhanced TCR-induced TIGIT expression in CD4+ memory T cells. These data argue that TCR stimulation and retinoic acid production by resident intestinal APCs induces TIGIT expression on CD4+ memory T cells, which in turn may contribute to regulation of immune responses against microbial antigens.

ID: 071
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: Immune profiling, T cells, Inflammation, neutrophils, SLPI

HIGH INTESTINAL SECRETORY LEUKOCYTE PROTEASE INHIBITOR EXPRESSION CORRELATES WITH ABUNDANT NEUTROPHILIC INFLAMMATION AND IDENTIFIES INFLAMMATORY BOWEL DISEASE PATIENTS WITH A STRONG IL-17 RESPONSE

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Inflammatory bowel disease (IBD) comprising Crohn's disease (CD) and ulcerative colitis (UC) is driven by aberrant host-microbial interactions. Heterogeneity in disease severity requires new strategies to target the patients' individual immune disease. As histology of intestinal biopsies is imperative for IBD diagnosis, we investigated histological features of intestinal immune processes that differentiate IBD patients. In mice, upon epithelial barrier breach and microbiota-driven inflammation, colonic epithelial cells increase Secretory Leukocyte Protease Inhibitor (SLPI) expression. SLPI can exert anti-microbial activity and inhibit NF-kB activation. We hypothesized that SLPI expression is increased in the intestine of IBD patients and differentiates patients with a distinctive underlying immune disease.

We collected intestinal biopsies and plasma from therapy-naive pediatric patients with CD (n=41) and UC (n=22) or IBD-negative controls (n=15). Intestinal SLPI protein was detected by immunohistochemistry, scored semi-quantitatively and biopsies were dichotomized into 'SLPI-high' and 'SLPI-low'.

SLPI mRNA and protein expression was increased in intestinal tissue of CD and UC patients, compared to IBD-negative controls. SLPI expression was strongest in macroscopically inflamed tissue, predominantly in colon compared to ileum, and related to histological and clinical disease severity. High colonic epithelial SLPI associated with abundant neutrophil infiltration and calprotectin expression. To assess the epithelial SLPI-neutrophil interaction, we performed RNAseq on SLPI-high versus -low biopsies. Differentially expressed genes identified upregulated pathways, including TNF-α signaling via NF-kB and epithelial-mesenchymal transition. SLPI-high biopsies had a stronger signature of neutrophilic inflammation, including higher expression of OSM, IL-1B, and CXCL8, genes associated with resistance to anti-TNF treatment. Additionally, SLPI-high biopsies had an mRNA signature of increased IL-17A signaling. This agreed with immunohistochemical detection of high frequencies of IL-17A-producing cells co-localizing with epithelial SLPI and higher plasma concentrations of CCL20, MMP10 and IL-17A.

In conclusion, epithelial SLPI expression is a histological parameter identifying therapy-naïve pediatric IBD patients with a Th17/neutrophil-related immune profile.

ID: 072
Abstract
Topics: Mucosal diseases and infections
Keywords: commensals, intestinal inflammation, enteric infections, gnotobiotics

The microbial composition determines the severity of norovirus-induced intestinal inflammation

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Noroviruses are the main cause of non-bacterial gastroenteritis, but are also implicated in disease severity of patients and animal models with chronic gut inflammation. The norovirus-triggered intestinal pathology is microbiota-dependent, as germ-free or antibiotic treated mice do not develop gut inflammation after infection. The intestinal microbiota is a complex ecosystem with a prominent role in host health and disease. However, the exact mechanisms of how commensals modulate the infection outcome and contribute to the intestinal inflammation is still unclear. Gnotobiotic animal models have become valuable tools to assess the causal role of microbiota. Thus, to address the impact of commensals on norovirus-triggered inflammation, we employ gnotobiotic animal models colonized with defined microbial communities. Using this approach, we recently demonstrated that distinct microbial communities differently modulate the outcome of murine norovirus (MNV)-induced pathology. MNV infection exacerbated intestinal inflammation in mice associated with Altered Schaedler Flora (ASF), but not those with Oligo Mouse Microbiota 12 (OMM12). Co-colonization with segmented filamentous bacteria protected the mice from inflammation, whereas the removal of Lachnospiraceae members did not increase the susceptibility to inflammation showing that MNV-triggered pathology depends on the microbial context. The microbiota-mediated protective effects involved enhanced activation of intestinal barrier defense mechanisms such as upregulated expression of tight junctions, mucus and antimicrobial peptides as well as regulatory mucosal immune responses in the chronic and acute phase of MNV infection. The understanding of interactions between microbial communities, pathogens and the host are fundamental for the development of novel strategies that will allow non-invasive modulation of the intestinal microbiota to the host benefit.

ID: 073
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Respiratory, Chronic, Muco-obstructive, Mucoactive, Therapeutic

Carbocisteine promotes mucin regulation and mucus clearance in a murine model of chronic lung disease.

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Mucus secretion is a common feature of both acute and chronic respiratory diseases and promotes defence of the airway. However, hypersecretion of mucus may result in complications including sputum retention and obstruction, mucus dysregulation, and airway inflammation. Mucoactive therapeutics such as carbocisteine interact and regulate mucins, the primary organic component of mucus, and may be employed in severely ill patients to manage airway secretions and promote sputum clearance. However, the entire mechanism of action and effect on mucin regulation of many mucoactive agents, including carbocisteine, is still unclear. Here we aim to evaluate the effect of carbocisteine treatment on mucus expression, composition and obstruction in βENaC-transgenic mice, a murine model expressing a muco-obstructive phenotype.

Carbocisteine was administered intraperitoneally twice daily, and mice were then sacrificed after 5 days. Bronchoalveolar lavage (BAL) fluid was collected as well as lung lobe excision to generate lung homogenates and lung tissue sections. Western blots indicated significantly decreased mucin Muc5ac and Muc5b concentrations in BAL obtained from βENaC-Tg mice treated with carbocisteine. Similarly, reduced airway mucus volume and plugging was observed in βENaC-Tg mice receiving carbocisteine. Furthermore, carbocisteine treatment was shown to elevate gene expression of Muc5ac and Muc5b, while additionally increasing the expression of mucin promoters IL-13 and GOB5. However, contradictory to mRNA expression, IL-13 protein concentrations were shown to decrease in BAL from βENaC-Tg mice treated with carbocisteine.

These data indicate that carbocisteine exhibits a potent therapeutic effect in this model although increased gene expression of mucins and their promoters are observed secondary to the effect of the mucoactive agent, as a potentially homeostatic response.

ID: 074
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: Tissue-resident memory T cells, mucosal immunology, Helicobacter pylori infection

The Protective Function of Tissue Resident Memory T cells during Helicobacter pylori Infection

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It was recently reported that tissue-resident memory cells (TRM) show protective function against different bacterial and viral infections by producing protective cytokines. T cells seem to actively participate in protection against Helicobacter pylori, but the specific role of TRMs in this infection is unclear.

We characterized TRM cells at different time points after H. pylori infection in mice, and defined specific subgroups by CD69 and CD103 and Hobit expression, which were dependent on the presence of the virulence factor CagA. These TRMs, which secret pro-inflammatory and cytotoxic cytokines, such as IL-17, IFN-γ and Granzyme B, are recruited from the periphery during H. pylori infection. After H. pylori eradication, Hobit+ TRMs reside in the stomach, and play a protective function against secondary H. pylori infection by rapid expansion with the help of neutrophil infiltration, and show a different transcriptome profile compared to TRMs during initial infection.

These results demonstrate that Hobit+ TRMs induced by H. pylori infection reside in the stomach also without ongoing antigen stimulation, and protect the host from re-infection, which may have potential implications for future prophylactic and therapeutic approaches.

ID: 075
Abstract
Topics: Innate Immunity, Microbe-immune interactions at body surfaces
Keywords: Colon, intestinal barrier, wild mice, co-housing, naturalization

Different sources of microbial exposure convey distinct effects on the mouse intestinal mucosa

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Concerns have been raised that laboratory rodents fail to develop a diverse microbiome and representative immune responses, potentially leading to poor translatability in preclinical models. Various ways to “naturalize” mice have recently showed intriguing effects, but comparative studies between such approaches are lacking. We have established a system where laboratory mice are raised under a full set of environmental conditions present in a farmyard-type habitat, including soil and fresh fecal material from domestic animals. These so-called feralized mice shift their gut microbiota, display signs of a matured immunophenotype, and were shown to develop reduced severity of colorectal cancer. Ours and others’ earlier studies have sometimes included co-housing with wild or pet store raised mice as microbial donors, but how essential these are for the resulting mouse phenotype is unknown. We here present the first comparative study of different approaches to naturalize laboratory mice, showing distinct effects on the intestinal barrier, microbiota and immune cell composition.

Farmyard housing as well as co-housing with wild mice recreated a significant change in the composition of fecal and mucosal-associated microbiota. Increased presence of resident memory T cells was seen in the colonic lamina propria, while in mesenteric lymph nodes NK cells had upregulated KLRG1, and T-cells were more easily triggered to produce IFN-gamma. Furthermore, significant upregulation of genes related to barrier integrity and antimicrobial functions was seen in intestinal epithelial cells, with the greatest effect in the colon. Differential data from the groups will be presented, indicating an overall stronger shift conferred by wild mouse co-housing, while farmyard housing alone produced milder effects. Clarifying the significance of the source of microbial exposure may elucidate differences in environmental cues on the immune system and may aid decisions in future efforts to design more translatable environmental animal models.

ID: 076
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: IBD, fibroblast, myeloid cells

Single-cell analysis uncovers fibroblast heterogeneity in Crohn’s disease and identifies pathological fibroblasts as a new therapeutic target

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Recurrent episodes of intestinal inflammation and tissue remodeling progressively result in fibro-stenosis and bowel obstruction in Crohn’s disease (CD). This irreversible end-stage complication is the main indication for surgical intervention but is often associated with postoperative recurrence of inflammation and repeated tissue resection.

To understand transmural inflammation-induced fibro-stenosis, full-thickness ileum from CD patients (n=10) undergoing ileocecal resection were profiled using single-cell RNA sequencing (scRNA seq) on 10x Genomics platform. Our tissue sampling strategy aimed at recapitulating different disease stages from the same patient including normal ileum, chronic active inflammation and stenotic ileum, allowing a better definition of cell heterogeneity, inter-cellular communication, and tissue organization in CD. Healthy ileum from CRC patients (n=5) undergoing right hemicolectomy were included as external control.

Using scRNA seq, we identified a specific subset of activated fibroblasts during chronic inflammation and fibrosis. FACS showed increasing FAP+ fibroblasts in both inflamed and stenotic ileum, compared to control ileum and proximal ileum ($p<0.01$). Computational methods predicting ligand-receptor signaling suggest that fibroblast activation is mostly mediated by myeloid cell-derived inflammatory signals resulting in collagen deposition and tissue fibrosis. Intestinal fibroblasts co-cultured with inflammatory monocyte supernatant and predicted ligands expressed higher protein levels of FAP, COL3A1 and showed signs of EMT transformation. Furthermore, stimulated intestinal fibroblasts secreted higher levels of ECM proteins, including COL1 ($p<0.001$), and COL3A1 ($p<0.005$). After inhibiting an EMT-related transcription factor identified in the activated fibroblasts, collagen expression and extracellular matrix accumulation were decreased in fibroblasts. Finally, inhibition of EMT transformation in chronic DSS colitis resulted in reduced ECM deposition, compared to vehicle mouse ($p<0.05$).

Our findings suggest that inflammatory monocytes mediate local activation of fibroblasts promoting excessive collagen deposition. We furthermore show that targeting activated fibroblasts was able to reduce tissue remodeling and may therefore prevent fibrostenosis in CD.

ID: 077
Abstract
Topics: Mucosal T cells
Keywords: Mucosal vaccine, respiratory tract, airborne pathogens, T cells, tissue resident T cells

Mucosal vaccination to improve immunity to SARS-CoV-2

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Impressive progress has been made during the COVID-19 pandemic to deliver effective and safe vaccines for SARS-CoV2. However, while current vaccines can reduce disease severity, they are still not entirely able to prevent infection and transmission. In this context, immunity at barrier sites may play an important role. We are investigating mucosal vaccination approaches to improve the generation of tissue resident memory T cells (TRM) at the respiratory mucosa. Mice were immunized intramuscularly (i.m.) with Spike protein and poly I:C followed by an intranasal (i.n.) boost with the same formulation 3 weeks later and viral spike epitope -specific T cells analyzed using fluorescently labeled MHC class I and II tetramers for CD8+ and CD4+ T cells, respectively. After i.m. priming, we observed modest expansion of spike-specific CD4+ and CD8+ T cells in the draining iliac lymph nodes (iILN) as well as lower levels of expansion in the lungs, nasal mucosa (NM), and mediastinal and mandibular lymph nodes (medLN/mandLNs), but these cells waned after 21 days in all organs analyzed. After i.n. boosting, spike-specific T cells increased in iILNs to levels similar to that during priming, while in the lungs, NM, medLNs and mandLNs, their expansion was much more robust, with the cells persisting in these tissues for at least 65 days. Moreover, upper airways immunization induced spike-specific cells in the NM and lungs, while total airways immunization increased spike-specific CD4+ and CD8+ T cells in the lungs. Most of the spike-specific CD4+ T cells expressed CD69 while the CD8+ T cells expressed both CD69 and CD103, which was consistent with a TRM phenotype. Our data suggest that antigen exposure in the respiratory tract is necessary for the generation of local viral-specific T cells that will persist long term in the respiratory mucosa.

ID: 078
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Tofacitinib, dendritic cell, macrophage, ulcerative colitis, IBD

Immunomodulatory effect of tofacinitib on human intestinal antigen presenting cells from patients with ulcerative colitis.

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Tofacitinib is used to treat patients with ulcerative colitis (UC). Human intestinal monocytes (Mo), macrophages (MΦ) and conventional dendritic cells (cDC) are the main antigen presenting cells of the immune system. Indeed, they are essential to maintain the mechanisms of immune homeostasis in health. However, their phenotype and function are altered in patients with inflammatory bowel disease (IBD) including UC. Hence, we aimed to characterize the effect of tofacitinib on mucosal antigen presenting cells from controls and patients with UC (both active -aUC- and quiescent -qUC) to determine whether tofacinitib can restore their profile. In health, MΦ produced more IL-10 and TNFa than cDC, while the latter produced more IL-15. Also, MΦ expressed higher levels of TLR4 and displayed lower expression of JAK1 and JAK3 referred to Mo and cDC, while the latter expressed the highest levels of JAK3. In resting conditions, the aUC mucosa produced lower amounts of soluble IL-1b, CCL2, IL-6 and IL-10. This was in agreement with the lower production of TNFa and IL-6 displayed by MΦ, together with the lower production of IL-6 by Mo and TNFa by mucosal cDC in these patients. Following LPS stimulation in controls, tofacitinib restored the increased expression of IL-10, JAK1, TLR2 and TLR4 displayed by Mo and restored the diminished CXCR3 expression of MΦ and IL-15 production by cDC. Specifically focused in UC, tofacitinib restored MΦ expression of CXCR3, IL-10 and TNFα in aUC and IL-15 in qUC. Last, but not least, following mucosal cDC sorting, Tofacitinib did not modulate their capacity to generate Th1, Th17 and Treg cells. Hence, our results suggest that tofacitinib main mechanisms of action refers to the modulation of the phenotype and function of tissue resident MΦ and newly arrived pro-inflammatory Mo rather than over cDC.

ID: 079
Abstract
Topics: Innate Immunity
Keywords: Tuft cell, ILC2, IL-25

Tuft cell expression of IL-17RB controls IL-25 availability in the small intestine and prevents chronic activation of ILC2s

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Group 2 innate lymphoid cells (ILC2s) are crucial for the mounting of a rapid type 2 response following intestinal helminth infection. Luminal cues engage chemosensory pathways in epithelial tuft cells that, as a result, secrete e.g. IL-25. ILC2s perceive this activation signal via expression of IL-17RB, the more specific component of the heterodimeric IL-25 receptor complex. Ensuing release of ILC2 derived IL-13 then drives epithelial progenitors towards a secretory cell fate, triggering tuft cell hyperplasia and further stimulation of this tuft cell – ILC2 circuit. Using ILC2-specific IL-17RB ablation (Il5Cre; Il17rbflox), we confirmed reduced circuit activation. Intriguingly, tuft cells also displayed substantial IL-17RB expression, indicating presence of both IL-25 and its' receptor. Working under the hypothesis that dual expression could denote autocrine signalling, we generated Vil1Cre; Il17rbflox mice which lacked IL-17RB expression in all epithelial cells, including tuft cells. Instead of displaying evidence of decreased circuit activation, these animals showed evidence of the reverse. The homeostatic ILC2 activation level, assessed by IL-13 and KLRG1, proved significantly elevated, suggesting increased IL-25 signalling and a regulatory role of tuft cell IL-17RB. Indeed, additional deletion of IL-25, in a Vil1Cre; Il17rbflox; Il25flox model, abrogated the chronic activation. Notably, the tuft cell – ILC2 circuit of Vil1Cre; Il17rbflox mice was hyporesponsive to succinate stimulation. In sum, our results identify a regulatory role of tuft cell IL-17RB which functions to limit steady state availability of IL-25 in the small intestine, thereby contributing to the maintenance of a dynamic circuit responsiveness.

ID: 080
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections
Keywords: Lung-Gut Axis; Infection: Inflammation; Intestinal Microbiota; HMPV

Human metapneumovirus infection affects intestinal immunity and microbiota composition in a murine model

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Respiratory infections are one of the main causes of morbidity and mortality worldwide, mainly in children, immunocompromised people, and the elderly. Several respiratory viruses can induce intestinal inflammation and alterations in the composition of the intestinal microbiota. Human metapneumovirus (HMPV) is one of the major respiratory viruses contributing to infant mortality in children under 5 years of age worldwide and the effect of this infection at the gut level has not been studied. Here, we analyzed the distal effects of HMPV infection on intestinal microbiota and inflammation in a murine model, analyzing several post-infection times (days 1, 3 and 5). 6–8-week-old C57BL/6 mice were infected intranasally with HMPV and mice inoculated with a non-infectious supernatant (Mock) were used as a control group. We confirm that hMPV does not have the ability to infect the intestine, but we observed significant changes in the expression of proinflammatory cytokines in intestinal tissue analyzed by qPCR at days 1 and 3 post-infection compared to the control group. Furthermore, we analyzed the frequencies of different innate and adaptive immune cells in the colonic lamina propria using flow cytometry. Frequency of monocyte populations was altered in the colon of HMPV-infected mice at days 1 and 3 but were not significantly different to control mice at day 5 post-infection. Moreover, colonic CD8+ T cells were significantly increased in HMPV-infected mice at day 5, suggesting that HMPV may also alter intestinal adaptive immunity. Additionally, significant changes were observed in the abundance of the genus Bacteroides in the intestinal microbiota of HMPV-infected mice, using 16S qPCR and 16S sequencing. Therefore, these results indicate that HMPV can affect intestinal immunity and the microbiota and further research is required to understand the mechanisms inducing these distal effects in the intestine.

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ID: 081
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections
Keywords: Goblet cell dynamics, Flow cytometry, Citrobacter rodentium infection, mucus response

Flow cytometry analysis of colonic goblet cells under healthy, inflammation and infection conditions

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The gastrointestinal tract is continuously working to respond efficiently to the different stimuli to which it is exposed. In this task, the single layer of epithelial cells along the intestine play an important role in host defense by regulating mucus secretion processes. Epithelial goblet cells are a heterogeneous population specialized in the synthesis and secretion of the mucus that covers the epithelium and imbalances in this protective system trigger dysbiosis and are linked to diseases such as ulcerative colitis.

In this study, we have sought to develop new flow cytometry-based methods for isolating, identifying and quantifying mucosal epithelial cell subsets by utilising transgenic mice expressing fluorescently tagged Muc2 in combination with fluorophore-conjugated lectin staining to identify different goblet cell populations.

Using this approach we have tested different staining protocols to improve the specificity and sensitivity to different goblet cell subsets detected using the lectins UEA1 and WGA. Furthermore, certain goblet cell markers have been screened to allow the identification of distinct goblet cell populations. We have subsequently applied these methods to study inflammation induced by interleukin-13 (IL- 13) and pathogenic response triggered by Citrobacter rodentium infection in order to determine how goblet cell dynamics are influenced by these challenging conditions.

ID: 082
Abstract
Topics: Systems immunology in host-microbe interactions
Keywords: RelA, Stat3, transcription, hepatocyte, bile acid, colitis, inflammation

A hepatocyte-specific transcriptional program driven by RelA and Stat3 exacerbates experimental colitis in mice by modulating bile synthesis

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RelA/NF-κB and Stat3 collaborate to induce genes involved in acute phase response, inflammation, cell proliferation, and survival. Indeed, crosstalk between these factors in hepatocytes orchestrates a protective acute phase response against lung infections. Importantly, RelA/NF-κB and Stat3 functions in immune cells and intestinal epithelial cells have been implicated in experimental colitis. However, if their hepatic activity also impacts intestinal inflammation remains unclear. Here, we reveal that the combined deficiency of RelA and Stat3 in hepatocytes ameliorates DSS-induced acute colitis in mice. Our transcriptomics and metabolomics analyses identified that these factors activate a gene expression program, which directs the alternate bile synthesis pathway in the colitogenic gut. Our immunophenotyping studies suggested that reduced accumulation of specific bile acids in the gut protected knockout mice from experimental colitis. In sum, we suggest that inflammatory transcription factors engage also in distant tissues promoting intestinal pathologies and such multiorgan crosstalk provide therapeutic opportunities.

ID: 084
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections

The DPP-IV inhibition ameliorates intestinal inflammation and colorectal cancer associated with colitis.

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Colorectal cancer (CRC) associated with colitis (CAC) is a subtype of CRC related to intestinal inflammation. The incretins (GLP-1 and GIP) are peptide hormones secreted by the enteroendocrine cells (EECs). DPP-IV, expressed in Th17 and Tregs, controls the activity of incretins hormones. Here, we aimed to evaluate the role of incretins, and the enzyme DPP-IV in Treg/Th17 cell differentiation and the CAC progression. To explore the potential of incretins and DPP-IV in the CAC, we performed in silico analysis from CRC samples from mice and humans. Interestingly, differential expression genes and GO analysis showed downregulation of the hormones-secreted pathways, along with a diminished expression of EECs-related genes in CRC tissue compared with healthy tissue. Notably, lower mRNA levels of GCG (gene that encodes GLP-1) were correlated with poor colorectal cancer patient survival. In an experimental model of CAC, C57BL/6 mice under DSS administration in the drinking water treated with DDP-IV inhibitor (DDP-IVi) exhibited a reduction of colitis score, followed by less shortening of colon length when compared with non-treated animals. Analyzing the CAC mouse model, the treatment with DPP-IVi (50mg/kg/day) only during the time of DSS cycles (3 cycles of 7 days of DSS) delayed the tumor appearance, along with a lower disease score and tumor number, while increased the frequency of CD45+ CD64+ MHCII+ in colon lamina propria compared with non-treated. In addition, the GLP-1 receptor was high in the colonic epithelial cells of DPP-IVi treated mice. In vitro, naïve CD4 T cells polarized to Th17 and treated with DDP-IVi inhibited IL-17 production. Interestingly, DPP-IVi treatment in Th17 cells increased the expression of FoxP3. Overall, DPP-IV treatment modulates immune cells profile and increase GLP1R epithelial cells, indicating a positive action of the EECs and incretins in inhibiting CAC formation and progression.

ID: 085
Abstract
Topics: Microbe-immune interactions at body surfaces
Keywords: intestinal microbiota, autoimmune disease, rheumatoid arthritis, Prevotella copri, outer membrane vesicles

Characteristic lymphocyte responses to subcellular fractions of the human gut bacterium Prevotella copri in patients with rheumatoid arthritis

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Rheumatoid Arthritis (RA) is an autoimmune disease caused by an interaction between genetic and environmental factors, including microbial dysbiosis. Recent findings evidence that Prevotella (P.) copri, member of the gut microbiota, might be implicated in the immunopathogenesis of RA. P. copri is overrepresented in patients with pre-clinical/new-onset RA, promotes arthritis in susceptible mice and induces specific lymphocyte responses in RA patients.

To gain further insight into the role of P. copri in RA and the origin of immunodominant P. copri antigens, we analyzed lymphocyte responses towards distinct subcellular fractions and outer membrane vesicles (OMVs) of P. copri in relation to genetic predisposition and abundance of P. copri in the gut.

Peripheral blood mononuclear cells of RA, osteoarthritis and healthy subjects were stimulated with P. copri fractions of membrane, periplasm, cytoplasm and OMVs and the percentage of activated memory CD4+ T cells producing IFN-g or TNF-α was determined by flow cytometry. Specific antibodies to P. copri fractions were analyzed in serum by ELISA. The abundance of P. copri in stool samples was determined by qPCR. The presence of HLA-DR risk alleles was confirmed by NGS high throughput HLA genotyping (DKMS Life Science Lab).

While control subjects showed T helper cell responses to cytosolic P. copri antigens, P. copri membrane fraction stimulated Th1 cell responses particularly in RA patients. 39% and 25% of RA patients presented IgA antibodies to P. copri membrane fraction and OMVs, respectively, which were specific to RA. The lymphocyte response to P. copri was elevated in subjects with P. copri overabundance but not associated with RA risk alleles.

The results suggest that a differential lymphocyte response to P. copri antigens might, independently of the genetic predisposition, contribute to autoimmune inflammation in RA patients, e.g., through molecular mimicry.

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ID: 086
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections
Keywords: IBD, IL-10-/- mice, AIEC, inflammasome, piroxicam

An IBD-associated pathobiont synergises with NSAID to promote colitis which is blocked by NLRP3 inflammasome and Caspase-8 inhibitors

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Conflicting evidence exists on the association between consumption of non-steroidal anti-inflammatory drugs (NSAIDs) and symptomatic worsening of inflammatory bowel disease (IBD). The mechanisms regulating NSAID-induced symptoms is unknown. Pathobionts such as adherent-invasive Escherichia coli (AIEC) are widely prevalent in Crohn's disease (CD)-mucosa, activate the inflammasome and are considered relevant to the pathogenesis of Crohn's disease. Caspase-8 is a master regulator protein regulating both programmed cell death and intestinal and inflammation. In this project, we hypothesise that the heterogeneous prevalence of pathobionts such as AIEC might explain the inconsistent correlation between NSAIDs and IBD. Using IL-10-/- mice, we show an aggravation of colitis in AIEC-colonised mice subsequently fed with the NSAID piroxicam due to a reduction in barrier function, and activation of pro-inflammatory cytokines, the NLRP3 inflammasome, caspase-8 and the apoptosis/pyroptosis executors caspase-3, PARP and Gasdermin-D, respectively. In addition, multiplex staining revealed colocalization of NLPR3, Caspase-8 and ASC mostly in epithelial cells and in few lamina propria immune cells in the AIEC-colonised mice exposed to piroxicam. These features were not seen in mice exposed to AIEC or NSAID alone, revealing an AIEC/NSAID synergistic effect. Using small molecule inhibitors targeting NLRP3 (MCC950) and caspase-8 (Z-IETD-FMK), we show an amelioration in colitis due to a reduction in NLRP3 inflammasome activation, apoptosis/pyroptosis markers, activated T-cells and macrophages, improved histology and expression of tight junction proteins, increased numbers of mucus producing cells as well as an increased abundance of Clostridium cluster XIVa species. Collectively, our findings provide new insights into how NSAIDs and an opportunistic gut-pathobiont can synergize to worsen IBD symptoms. Targeting the NLRP3 inflammasome or Caspase-8 could be a potential therapeutic strategy in IBD patients with gut inflammation, which is worsened by NSAIDs.

ID: 087
Abstract

ILC2 require cell-intrinsic ST2 signals to promote type 2 immune responses

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Type 2 immunity is a type of immune response that is a type of immune response that is characterized by the production of specific cytokines, including IL-4, IL-5, and IL-13, and the activation of cells such as eosinophils, mast cells, and group 2 innate lymphoid cells (ILC2s). This type of immune response is critical for host defense against parasitic infections, but it can also contribute to the pathogenesis of chronic inflammatory diseases such as allergic asthma. The initiation of type 2 immune responses at mucosal barriers is regulated by rapidly secreted cytokines called alarmins, including IL-33, IL-25, and TSLP. These alarmins are mainly released by stromal and epithelial cells following infection or tissue damage and can activate various immune cells, including ILC2s, being an early source of type 2 cytokines. Recent research has focused on the role of the IL-33 receptor in ILC2 activation, which is a critical step in the initiation of type 2 immune responses. However, there have been limitations in targeting genes specifically in ILC2s, which has made it challenging to fully understand the precise contribution of the IL-33 receptor in this process. Using the newly established Nmur1iCre-eGFP mouse model with conditional genetic ablation of the IL-33 receptor subunit ST2 in ILC2s, we were able to show that ST2-deficient ILC2s are unresponsive to IL-33 and produce limited amounts of type 2 cytokines IL-5 and IL-13. While ST2-deficient ILC2s were still able to respond to other alarmins, such as IL-25, the absence of ST2 resulted in impaired eosinophil homeostasis in steady state conditions. During allergic lung inflammation, ST2-deficient ILC2s were unable to expand and promote eosinophil recruitment, further highlighting the importance of ST2 signaling in ILC2 activation. Additionally, the susceptibility to the worm Nippostrongylus brasiliensis was higher in conditional knockout mice, indicating that ILC2-intrinsic ST2 signals are necessary for mounting an effective type 2 immune response against this pathogen. These findings highlight the importance of cell-intrinsic ST2 signals for the proper activation of ILC2s and the initiation of type 2 immunity, and suggest that targeting the IL-33 receptor could be a potential therapeutic strategy for allergic diseases and parasitic infections.

ID: 088
Abstract
Topics: Microbe-immune interactions at body surfaces, Swiss Young Immunologists Society (SYIS) and young European Federation of Immunological Societies (yEFIS)
Keywords: Th17, ILC3, Candida albicans, mucosal infection

Extrathymic Aire-expressing cells control mucosal antifungal immunity by direct antigen presentation

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The autoimmune regulator (AIRE) is fundamental for the enforcement of central tolerance within the thymus. AIRE deficiency leads to Autoimmune Polyendocrine Syndrome type-1 (APS-1), a severe autoimmune disorder which is frequently accompanied by chronic mucocutaneous Candida albicans infections. While the mechanism behind autoimmunity stemming from AIRE deficiency is well established, the susceptibility of APS-1 patients to fungal infections is incompletely understood. In recent years a rare population of lymph node-resident extrathymic Aire-expressing cells (eTACs) has been identified. eTACs bare major characteristics of LTi-like ILC3 cells including the expression of Rorγt, while also expressing high levels of MHCII and costimulatory or fungal sensing molecules. Focusing on Candida infections associated with AIRE deficiency, we previously demonstrated that eTAC-intrinsic expression of AIRE is critical for the induction of effective Candida-specific Th17 response during gastrointestinal colonization. Here we show that direct antigen presentation by this population is required for this process. Intrudingly, mechanism behind eTACs ability to mount anti-fungal Th17 responses remains incompletely understood. Our preliminary data shows that upon Candida stimulation eTACs secrete several chemokines with the capacity to activate or attract mononuclear phagocytes (MNPs). We hypothesize that eTACs could cooperate with MNPs within lymph nodes, forming signalling hubs that support anti-fungal Th17 cell polarization. Here we present our single cell RNA sequencing analysis aiming on delineating this interaction. Understanding the molecular mechanisms underlying susceptibility to mucosal fungal infections will contribute to a more complete understanding of the regulation of Th17 responses, which represents the first necessary step in designing more effective therapeutic approaches.

ID: 089
Abstract
Topics: Mucosal diseases and infections
Keywords: dendritic cells, type 2 immunity, tolerance, immunomodulation, intestine

Helminth infection model elucidate how the intestinal dendritic cells maintain the equilibrium between pro- and anti-inflammatory responses

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Dendritic cells (DCs) in the intestine orchestrate the decision-making processes that control the homeostatically critical balance between active immunity and tolerance. DCs do this by interacting with T cells to instruct them to mount an immune response. Thus, understanding factors affecting DC:T cell interactions is critical. In the intestine, DCs are highly heterogeneous and it remains unclear if and how individual DC subsets may contribute to the immune outcome.

This issue has been particularly difficult to address in type 2 immune responses, as helminths often regulate host immune responses to try and maintain its persistence in tissues. Here we have used Heligmosomoides polygyrus bakeri (Hp) infection in mice to study intestinal DC function. The role of DCs in Hp infection has been unknown, largely because the standard intestinal cell isolation methods have been ineffective. By applying our recently published protocol which allows DC to be isolated from the Hp-infected intestinal mucosa, we have identified CD103- DC2 that drive Th2 effector differentiation, and a substantial increase in CD103+ DC2s with unknown function. Here we show that the CD103+ DC2s from Hp-infected mice have an altered phenotype and superior ability to induce Foxp3+ regulatory T cells. To understand how Hp might drive these changes in DCs, we generated DC in vitro in the presence of molecules isolated from Hp. TGFβ mimic (TGM), which contributes the immunomodulatory effects of Hp in vivo also altered DC development and function in vitro to mimic CD103+ DC2 found in the intestine during the infection.

Together, these results delineate a mechanism by which Hp can modulate host immune responses by taking advantage of TGFβ signalling pathway and altering the differentiation and function of local DCs. This knowledge provides novel insights into strategies to modulate or enhance intestinal immune responses.

ID: 090
Abstract
Topics: Innate Immunity, Microbe-immune interactions at body surfaces
Keywords: NADPH Oxidase 1 (NOX1), Reactive oxygen species (ROS), gut inflammation, Dextran Sulfate Sodium (DSS), mucosa

The ROS-generating enzyme NADPH oxidase 1 modulates the colonic microbiota but offers minor protection against dextran sulfate sodium-induced low-grade colon inflammation in mice

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The enzyme NADPH oxidase 1 (NOX1) is a major producer of superoxide which together with other reactive oxygen and nitrogen species (ROS/RNS) are implicated in maintaining a healthy epithelial barrier in the gut. While previous studies have indicated NOX1's involvement in microbial modulation in the small intestine, less is known about the effects of NOX1-dependent ROS/RNS formation in the colon. We investigated the role of NOX1 in the colon of NOX1 knockout (KO) and wild type (WT) mice, under mild and subclinical low-grade colon inflammation induced by 1% dextran sulfate sodium (DSS). Ex vivo imaging of ROS/RNS in the colon revealed that absence of NOX1 strongly decreased ROS/RNS production, particularly during DSS treatment. Furthermore, while absence of NOX1 did not affect disease activity, some markers of inflammation (mRNA: Tnfa, Il6, Ptgs2; protein: lipocalin 2) in the colonic mucosa tended to be higher in NOX1 KO than in WT mice following DSS treatment. Lack of NOX1 also extensively modulated the bacterial community in the colon (16S rRNA gene sequencing), where NOX1 KO mice were characterized mainly by lower α-diversity (richness and evenness), higher abundance of Firmicutes, Akkermansia, and Oscillibacter, and lower abundance of Bacteroidetes and Alistipes. Together, our data suggest that NOX1 is pivotal for colonic ROS/RNS production in mice both during steady-state (i.e., no DSS treatment) and during 1% DSS-induced low-grade inflammation and for modulation of the colonic microbiota, with potential beneficial consequences for intestinal health.

ID: 091
Abstract
Topics: Microbe-immune interactions at body surfaces
Keywords: IBD, gut, microbiota, evolution, oxidative stress

The effect of oxidative stress on evolutionary dynamics within intestinal microbiota of inflammatory bowel disease patients

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An initially mutualistic relationship between the host and its microbiota can lead to alterations in microbial consortia and their metabolic functions, accompanied by a loss of fitness of the host — this may manifest a disease such as inflammatory bowel disease (IBD). The gut microbiota in IBD is relatively different from non-IBD, mainly with an increase of enteropathogenic strains due to the generated reactive oxygen species in an altered metabolic niche. However, the effect of oxidative stress on gut residents in a long-term adaptive manner is unknown. Knowing these led us to ask how IBD microbiota under oxidative stress genetically adapts over time within individuals and how this relates to the trajectory of activity and treatment of IBD.

Using mucosa-associated samples from IBD patients with the active and non-active disease, we found numerous altered taxa in IBD, including Bacteroides, Faecalibacterium, Lachnospiraceae, Muribaculaceae, and Akkermansia, are altered and is directly associated with lack of oxidative stress responsiveness in the host. We are currently processing the biopsies longitudinally collected from the Swiss IBD cohort to characterize the variants in gut microbiota and to understand how disease activity contributes to this.

Lastly, we aim to characterize gut microbial strains for their contribution to oxidative stress and gut homeostasis using mouse-model. We have been isolating over 150 strains from subjects with a stoma using different selected media and pre-treatments. We aim to address whether these strains are intrinsically capable of exacerbating or resisting oxidative stress and potentially make the host more susceptible to further inflammation with ongoing oxidative stress.

Overall, understanding how oxidative stress affects genomic variation in IBD gut microbiota and whether this is beneficial to the aetiopathogenesis of pathobionts in an altered metabolic niche will uncover the molecular biomarkers, including particularly relevant variants to human health and well-being.

ID: 092
Abstract
Topics: Neonatal window of opportunities

Deciphering microbiota and diet-driven immune system programming of the offspring in utero and early life

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Early-life perturbations can influence short- and long-term health of an individual, ranging from increased susceptibility to early-life infections to predisposition of developing non-communicable diseases in adulthood. Gestational malnutrition is one such early-life perturbation and a major global health concern. Endemic in developing countries, gestational malnutrition is increasing at an alarming speed in developed countries. Attempts to prevent gestational malnutrition and attenuate its consequences in the offspring are of limited success due to the lack of understanding the complex interactions between gestational malnutrition, the maternal gut microbiota and the offspring's developing immune system during this critical window of opportunity.

We perform dietary interventions in gnotobiotic pregnant mice using defined and purified experimental diets, mirroring gestational malnutrition. Flow cytometry analysis of liver and small intestinal lymphocyte populations revealed profound changes in several immune cell populations. Most striking differences were observed in liver natural killer T cell numbers in mice born to dams fed purified diets. In addition to flow cytometric analyses, we test if perinatal nutrition affects incretin-dependent glucose homeostasis later in life. For that, we colonize germ-free young male mice which were in utero exposed to purified or normal chow diet with the stable defined microbiota SDMDMm2 and simultaneously start high-fat diet (HFD)-feeding with the aim to incite diet-induced-obesity (DIO). After 8 weeks of HFD feeding, we assess incretin-dependent glucose homeostasis by oral glucose tolerance testing (OGTT). First experiments indicate that mice in utero exposed to purified diets had a worse response to oral glucose challenge than mice that where in utero exposed to normal chow. We will continue to investigate the effect of perinatal nutrition on immune cell populations in the liver and small intestine and potential interactions of these cells on glucose homeostasis and postnatal growth.

ID: 093
Abstract
Topics: Neonatal window of opportunities, Systems immunology in host-microbe interactions
Keywords: Bcells, antibody, repertoire, weaning

Distinct reshaping of the systemic B cell repertoire by the gut microbiota in early-life

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Commensal microbiota promotes immune maturation and homeostasis. We have previously demonstrated idiosyncratic B cell repertoires depending on the priming route of intestinal microbes explaining why preferential mucosal IgA responses are seen to pathogens or pathobionts that are more capable of penetrating mucosal barriers. The immune system undergoes profound changes in early-life, especially around the weaning period, and it remains unclear how the plasticity of the immune system especially the systemic B cell repertoire accommodates microbial exposure along this crucial developmental window. Using a reversible model of colonization of germ-free mice with an E. coli auxotrophic strain at specific physiological stages over the host early development, we detected a temporal signature of microbial exposure on host B cell repertoires which appears to be long lasting at least until the midlife of the host in an unperturbed context. Preliminary results from the characterization of the antigen-specific patterns within the B cell repertoire using high-throughput mapping of B cell receptor sequences to antigen specificity, suggests that microbial-specific responses are preferentially selected after the weaning period while constraining the natural polyreactive repertoire seen after birth.

ID: 094
Abstract
Topics: Mucosal B cells

Generation of cross-specific mIgA antibodies against Klebsiella pneumoniae glycans

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Klebsiella pneumoniae (Kp) is a gram-negative, encapsulated, opportunistic pathogen and a leading cause of nosocomial bacterial infection with frequent antibiotic resistance. Monoclonal antibody (mAb) therapy is one promising approach to circumvent antibiotic resistance. Kp has four lipopolysaccharide O-serotypes (O1, O2, O3 and O5), the structural variants of which account for ~75% of clinically relevant isolates. While protective cross-reactive mAbs have been identified between the O3 and O5 mannan-based serotypes they have yet to be described for the common backbone of the O1 and O2 galactan-based serotypes. Here we aim to generate O1/O2 cross-reactive mAbs by bi-colonization of germ-free mice with O1 and O2 Kp strains paired with single B cell Ig gene sequencing and monoclonal antibody generation. Cross-reactive mAbs will be identified by flow cytometric- and ELISA-based methods and their protective capacities will be measured in Kp mouse models. The resulting data may define antibody cross-reactivity as a driving force of intestinal B cell clonal selection and identify mAbs to treat antibiotic-resistant Kp infections.

ID: 095
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections

Atg16l1 preserves intestinal homeostasis by differentially regulating CD4+ peripheral T cells.

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A single-nucleotide polymorphism in the essential autophagy gene Atg16l1 is associated with an increased susceptibility to inflammatory bowel disease, specifically Crohn's disease. While the role of autophagy in intestinal immune homeostasis remains unclear it has been demonstrated that conditional deletion of Atg16l1 in murine CD4+ T cells leads to spontaneous intestinal inflammation which is characterized by an aberrant Th2 response and a loss of Foxp3+ regulatory T cells. However, the mechanisms underlying these T cell changes in the presence of defective autophagy are unknown. To investigate how autophagy impacts different peripheral CD4+ T cells subsets on a molecular level, we performed single-cell RNA sequencing of Atg16l1-deficient and wildtype CD4+ T cells isolated from murine colonic lamina propria. In Atg16l1-deficient CD4+ T cells, bioinformatic analysis confirmed the previously reported increase in Th2 cell proportions and reduction in regulatory T cell proportions. Additionally, an increase in naïve and differentiating T cell proportions was found, indicating impaired generation of T effector cells. Focussing on regulatory T cells, trajectory analysis indicates the presence of intermediate developmental stages of regulatory T cells in the intestine and suggests that these are absent in Atg16l1-deficient mice. Our results suggest that Atg16l1-deficient cells have enhanced activation of the proteasome degradative pathway and impaired IL-10 expression. Based on these results we hypothesise that autophagy may play a unique role in regulatory T cells and could be targeted to modify therapeutic outcomes in chronic inflammatory disorders.

ID: 096
Abstract
Topics: Mucosal diseases and infections
Keywords: antibody responses, mucosal immunity, SARS-CoV-2, mRNA vaccination, ACE2 inhibition

COVID-19 mRNA vaccination drives differential mucosal neutralising antibody profiles in naïve and SARS-CoV-2 previously infected individuals

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Background: SARS-CoV-2 licensed vaccines are efficacious for preventing severe disease. However, the current approved COVID-19 vaccines are administered intramuscularly which may not reflect the most efficient route to induce mucosal immunity protecting against transmission.

Methods: we performed a comparative analysis of SARS-CoV-2 IgG, IgA and neutralizing responses before and after 1st, 2nd and 3rd mRNA vaccine dose in oral fluid and plasma from 200 healthcare workers who were naïve or SARS-CoV-2 previously-infected using MSD® pan-coronavirus multiplex binding and ACE2 inhibition assays.

Results: Prior to vaccination, we found that spike-specific IgG levels in oral fluid positively correlated with IgG levels in plasma from previously-infected individuals (Spearman r=0.6858, p<0.0001) demonstrating that oral fluid could be used as a proxy for the presence of plasma SARS-CoV-2 IgG. However, the sensitivity was lower in oral fluid (0.85, 95% CI 0.77-0.91) than in plasma (0.94, 95% CI 0.88-0.97). Similar kinetics of mucosal and systemic spike-specific IgG levels were observed following vaccination in naïve and previously-infected individuals, respectively. In addition, a significant enhancement of OC43 and HKU1 spike-specific IgG levels was observed in previously-infected individuals following one vaccine dose in oral fluid (OC43 S: p<0.0001; HKU1 S: p=0.0423) suggesting cross-reactive IgG responses to seasonal beta coronaviruses. Mucosal spike-specific IgA responses were induced by mRNA vaccination particularly in previously-infected individuals (71%) but less frequently in naïve participants (23%). Neutralizing responses to SARS-CoV-2 ancestral and variants of concerns were detected following vaccination in naïve and previously-infected participants, with likely contribution from both IgG and IgA in previously-infected individuals (correlations between neutralizing responses and IgG: Spearman r=0.5642, p<0.0001; IgA: Spearman r=0.4545, p=0.0001). We also observed that breakthrough infections or a third vaccine dose enhanced mucosal antibody levels and neutralizing responses.

Conclusions: These data contribute to show that a previous SARS-CoV-2 infection tailors the mucosal antibody profile induced by vaccination.

ID: 098
Abstract
Topics: Microbe-immune interactions at body surfaces
Keywords: Intestinal epithelial cells, LC3-associated phagocytosis, Dectin-1, commensal microorganisms

Human intestinal epithelial cells internalize luminal fungi and bacteria via LC3-associated phagocytosis

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Background: Intestinal epithelial cells (IECs) are the first to encounter luminal microorganisms and actively participate in intestinal immunity. We reported that IECs express the β-glucan receptor Dectin-1, and respond to commensal fungi and β-glucans by Syk and ERK signaling in a Dectin-1- dependent manner. However, only β-glucans directed cytokine secretion suggesting epithelial tolerance towards commensal fungi. In phagocytes, Dectin-1 mediates LC3-associated phagocytosis (LAP) utilizing autophagy components to process extracellular cargo. Dectin-1 can mediate phagocytosis of β-glucan-containing particles by non-phagocytic cells. We aimed to determine whether human IECs phagocytose luminal fungal and bacterial particles via LAP.

Methods: Colonic and ileal organoids from individuals undergoing bowel resection were grown as monolayers. Fluorescent dye conjugated zymosan and heat-killed (HK) C. albicans and E. coli particles were applied to differentiated organoids and to human IEC lines. Confocal microscopy was used for live imaging and immuno-fluorescence. A fluorescence plate-reader was used for phagocytosis quantification.

Results: Zymosan and HK- C. albicans were phagocytosed by IEC lines and by every tested colonic (n=18) and ileal (n=4) organoid. Phagocytosis was significantly diminished upon blockade of Dectin-1 by laminarin, actin polymerization by Cytochalasin-D and NAPDH oxidases by DPI. Moreover, E. coli particles were similarly phagocytosed by all tested organoids and cell lines. LAP was identified by recruitment of LC3, Rubicon and ATG16L1 to phagosomes and lysosomal processing of internalized particles was demonstrated by co-localization with lysosomal dyes and LAMP2

Conclusions: Our results show that human IECs sense luminal fungi and bacteria and internalize them via LAP. This novel mechanism of luminal sampling suggests that IECs may contribute to the maintenance of mucosal tolerance towards commensal microorganisms.

ID: 099
Abstract
Topics: Innate Immunity
Keywords: pneumonia, pneumococcus, antibiotic, innate immunity, immunotherapy

Airway neutrophils contribute to the therapeutic efficacy of flagellin in pneumococcal pneumonia

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Host-directed therapy using the respiratory administration of the Toll-like receptor 5 agonist flagellin combined to antibiotic is effective against pneumococcal pneumonia. Here, we investigated the immune mechanisms involved in the synergistic antibacterial effects between antibiotic and flagellin. Transcriptomic analysis of the lung during the treatment of infection showed that, on top of infection, flagellin further stimulates pathways associated with immune cell infiltration into airways and antimicrobial functions. Indeed, flagellin promoted the early and transient mobilization of neutrophils. Depletion of neutrophils as well as CXCR2 deficiency in hematopoietic cells abrogated the flagellin-mediated protection. The data also suggested that flagellin activity is related to the number of neutrophils rather than to change in phagocytic activity or capacity to generate reactive oxygen species. Moreover, the treatment imprinted the location of neutrophils at the periphery of alveoli, bronchi and bronchioles. In conclusion, mobilizing neutrophils into airways during antibiotic treatment can improve the outcome of pneumococcal pneumonia.

ID: 100
Abstract
Topics: Microbe-immune interactions at body surfaces, Systems immunology in host-microbe interactions

MUCUS COMPOSITION ALTERATIONS IN INFLAMMATORY BOWEL DISEASES

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Introduction: Intact mucus layer is crucial for intestinal mucosal barrier function. Mucins, mucus layer building blocks, are secreted by goblet cells and composed of glycans that are essential for resisting bacterial interactions. Mucins composition alterations may contribute to intestinal inflammation such as that in inflammatory bowel diseases (IBD).

Aim: To assess intestinal mucus composition and mucins expression in IBD, and inflammation-induced alterations.

Methods: MUCs gene expression was assessed in intestinal biopsies from 52 subjects; 32 patients with IBD (Crohn's disease, Ulcerative Colitis and pouchitis) and 20 controls, were clustered to generate a heatmap, using microarray data. Alterations in mucins expression and composition were assessed in mucosal biopsies of patients with IBD, by immunostaining MUC2\MUC4, and Fucose (glycan-binding lectin), respectively. Intestinal organoids generated from healthy controls were differentiated to enrich goblet cells and increase mucus secretion. Following treatment with pro-inflammatory cytokines MUCs mRNA levels were assessed by RT-PCR.

Results Hierarchical clustering analysis revealed significant increase in MUC1\MUC4\MUC6 expression and slight increase in MUC2 expression in patients with IBD with active inflammation compared to non-IBD controls. Immunostaining revealed: higher MUC2\MUC4 expression, while discordant Fucose expression in inflamed compared to non-inflamed ileal and colonic explants from patients with Crohn's Disease (n=4). Moreover, mucins expression was altered in organoids treated with cytokines; MUC1 was upregulated in response to IL-22 or IL-6 ($p<0.001$, $p<0.05$, respectively, n=3); MUC4 was upregulated in response to IFN-γ, IL-22, or IL-6 ($p<0.0001$, $p<0.0001$, $p<0.05$, respectively, n=3); and MUC6 was upregulated in response to IL-13 or IL-4 ($p<0.001$, n=3).

Conclusion: Mucin expression levels are upregulated under inflammatory conditions while, glycan-Immune-mediated changes in mucus layer vary between colon and small bowel. Therefore, quantitative and qualitative mucus layer alterations may be part of the intestinal inflammatory cascade, potentially having a role in IBD pathophysiology.

ID: 101
Abstract
Topics: Mucosal T cells, Mucosal B cells
Keywords: Fibroblastic reticular cells, mesenteric lymph node, lymph node development, single-cell transcriptomics

Defining the origin and differentiation trajectory of perivascular reticular cells in the murine lymph node

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Fibroblastic reticular cells (FRCs) underpin microenvironmental niches of secondary lymphoid organs and regulate immune responses through the interaction with immune cells. The delineation of FRC subset identity unveiled the presence of heterogeneous FRC subsets, which govern attraction of immune cells to their respective niche and provide niche-specific cytokines. The entry and guidance of immune cells in lymph nodes is regulated in the perivascular niche and underlies dynamic expansion and remodeling to support developing immune responses. Perivascular reticular cells (PRC) furnish the perivascular niche of lymph nodes and closely interact with immune cells. Importantly, PRCs are still ill-defined and a profound characterization of their phenotype, origin and function is missing. Recently, early mesenchymal lymphoid tissue organizer (mLTo) cells have been described as FRC progenitors, but a complete delineation of FRC differentiation dynamics is still lacking. Here, we combine specialized fate-mapping mouse models and time-course transcriptomics to dissect the FRC progenitor-progeny relationship and differentiation trajectories at single cell resolution. Fate analysis of embryonic FRC progenitors in peripheral and mesenteric lymph nodes revealed the differentiation of specialized FRC subsets from two populations of perivascular Ccl19+ mLTo cells in embryonic lymph node anlagen, that populate distinct functional niches in the adult lymph node. Subsequent molecular characterization using single cell RNA-sequencing confirms a lymphotoxin beta receptor (LTβR)-independent differentiation of PRCs and Pi16+ RCs from early embryonic precursors.

ID: 102
Abstract
Topics: Innate Immunity, Systems immunology in host-microbe interactions
Keywords: Engineered Mycoplasma mycoides subsp. capri, primary blood cells, antigen-presenting cells (APCs), immunosuppressive effects, cytokine/chemokine induction

Deciphering Mycoplasma mycoides subsp. capri-interactions with ruminant immune cells

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Mycoplasmas are minute cell wall less bacteria that cause a variety of infectious diseases in humans and livestock. Mycoplasma vaccines if available at all have low efficacy and induce often only short-term protection. The interplay between livestock mycoplasmas and host cells is not fully understood but the development of rationale vaccines would benefit from a better understanding. Mycoplasma mycoides subsp. capri GM12 (Mmc-GM12) is a highly virulent strain, causing severe septicemia in goats. GM12 can be genetically modified using synthetic genomics techniques. We engineered a spectrum of Mmc-GM12 mutant strains lacking one or several genes encoding candidate virulence traits and tested the virulence of a number of mutant strains in vivo. Considering the 3R principles, in this study we developed an ex vivo platform employing ruminant primary blood cells, multi-parameter flow cytometry and Luminex-based multiplex assay to characterize host-pathogen interactions. Fresh bovine or caprine blood cells were stimulated with capsule deficient Mmc-GM12 (Δglf) and a fully attenuated mutant strain lacking 68 genes (Δ68). Incubation with the Δglf mutant exposing surface lipoproteins affected the viability of blood cells, while stimulation with the Δ68 strain did not show significant differences when compared to Mmc-GM12. Moreover, the Δglf mutant strongly suppressed the expression of MHC-II on antigen-presenting cells, indicating a potential immunosuppressive effect. However, at the same time, blood cells stimulated with this strain secreted a bulk of pro-inflammatory cytokines and chemokines that could promote a robust T-cell mediated inflammatory response. In conclusion, the capacity of engineered Mmc strains to differentially modulate the host immune system is a useful tool to systematically characterize candidate virulence traits and the immune responses they affect, which is likely to promote to develop better vaccines in the future.

ID: 104
Abstract
Topics: Neonatal window of opportunities, Mucosal diseases and infections
Keywords: Neonatal period, Cryptosporidiosis, Immunostimulation

Targeting innate immunity during the neonatal period, with NOD2 ligand, to protect against Cryptosporidium parvum

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During the neonatal period, the immune system is still immature, giving at newborns susceptibility to infections such as Cryptosporidiosis. C. parvum is the causative agent for the zoonosis Cryptosporidiosis. This parasitic disease is due to an orally transmitted protozoan that develops in intestinal epithelial cells and causes a moderate-to-severe diarrhea in children under 2 years of age, responsible for 18% of the deaths in children under 5 years [1]. In addition to children, Cryptosporidiosis also causes acute diarrhea in very young ruminants and immune-compromised hosts. Today, there is no vaccine to prevent infection nor efficient treatment, and there is therefore an urgent need for the development of new methods to control this zoonosis directly involved in a One Health process. In recent years, several studies have improved our knowledge on the immune mechanisms responsible for the control of the acute phase of the infection and have highlighted the importance of innate immunity [2].

We hypothesize that modulation of the innate immune system during the neonatal period could enhance the immune defense at young ages and favor the control of cryptosporidiosis. Different assays were performed to study the impact of in vivo stimulation of the innate immune system of neonatal mice with NOD2-based agonists to improve their immediate defense against C. parvum infection.

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ID: 105
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Type 2 Refractory Coeliac Disease, iCD3+ Innate cells, Lymphomagenesis, Jak1/Stat3 GoF mutation

ESTABLISHING A MOUSE MODEL OF COELIAC DISEASE-ASSOCIATED LYMPHOMAGENESIS

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The frequent first step in coeliac-disease (CeD)-associated lymphomagenesis is an intraepithelial clonal lymphoproliferation called type 2 refractory CeD (RCD2). RCD2 arises from a small subset of iCD3+ innate-like intraepithelial lymphocytes (IELs) carrying somatic gain-of-function (GoF) mutations in JAK1 and/or STAT3 that license their clonal expansion in the interleukine-15 (IL-15)-rich coeliac intestine. Our current objective is to establish a preclinical mouse model to evaluate the driver role of JAK1 p.G1096D or STAT3 p.D661V mutations and the impact of other recurrent mutations in RCD2 lymphomagenesis.

Preliminary experiments have shown that in vitro generated iCD3+ innate-like cells are predominantly found in the gut epithelium after adoptive transfer in immunodeficient IL-15 transgenic mice. In order to recapitulate the mutations observed in RCD2 cells and to define the combination of oncogenic mutations required to induce transformation, we tested a CRISPR/Cas9-based editing approach. After 11 days of in vitro differentiation, cells were edited for Jak1 p.G1096D or Stat3 p.D661V and the selective advantage of each mutation was investigated by following the number of cells generated in vitro and by sequencing.

Interestingly, sequencing data suggest that edited cells are homozygous for Jak1 mutation and displayed a clear proliferative advantage in vitro. On the other hand, Stat3 GoF mutation did not increase cell proliferation, but the percentage of Homologous Directed Recombination (HDR) increased to reach 50% of Indels 24 days after editing suggesting that all cells are heterozygous. The selective growth advantage of Jak1 and Stat3 GoF mutations will be confirmed in vitro.

Finally, we will assess whether both mutations, alone or in combination, are sufficient to drive lymphomagenesis in injected immunodeficient IL-15-transgenic mice, thereby establishing a preclinical mouse model allowing to test therapeutic strategies.

ID: 106
Abstract
Topics: Mucosal diseases and infections

Epithelial Presenilin-1 is a driver of colonic tumor development in vivo

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Presenilin 1 (PSEN1) is the catalytic subunit of the γ-secretase, a complex involved in the processing of the Amyloid Precursor Protein (APP), and hence, in Alzheimer´s disease. After a first cleavage by the β-secretases, APP is further processed by the γ-secretases, giving rise to Aβ, the component of the neuritic plaques and a hallmark in Alzheimer´s disease. Surprisingly, our own research revealed that Psen1 expression in the gut is second only to that in the brain. Hence, we aimed to explore the role of PSEN1 in intestinal diseases. Interestingly, the expression of all the components of the γ-secretase complex is significantly upregulated in human colorectal cancer (CRC). To understand the role of PSEN1 in intestinal tumorigenesis, we established a novel mouse line lacking the expression of Psen1 in intestinal epithelial cells (Psen1ΔIEC). In two different experimental models of CRC (AOM/DSS, inflammation associated-CRC and Apcmin/+ mice, sporadic cancer model), we could describe how the absence of Psen1 in intestinal epithelial cells lead to a marked reduction in tumor size in vivo. Accordingly, tumor organoids derived from Psen1ΔIEC Apcmin/+ exhibited a stunted growth and an augmented cell death. In depth analysis of Psen1-deficient and proficient tumoroids revealed that Psen1 regulates the EGFR-mediated proliferative responses. Altogether, these data suggest PSEN1 as a driver of colonic tumor development in vivo and in vitro. To translate our results from mice to human CRC, we knocked out PSEN1 in two different human CRC cell lines. The genetic ablation of PSEN1 was associated with an increased cell death in vitro in response to chemotherapeutic treatment and a reduced tumor growth in vivo. Our data uncover an unexpected involvement of PSEN in CRC. Collectively, these results suggest that Psen1 could be a potential therapeutic target in CRC.

ID: 107
Abstract
Topics: Microbe-immune interactions at body surfaces, Swiss Young Immunologists Society (SYIS) and young European Federation of Immunological Societies (yEFIS)
Keywords: Microbiota, Host-Microbiota-Crosstalk, Multi-parameter Microbiota Flow Cytometry, IBD

Single-cell bacterial phenotyping as diagnostic tool for microbiota-based classification of inflammatory bowel disease and identification of disease-associated bacteria

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Inflammatory bowel disease (IBD) with its two major forms Crohn's Disease (CD) and Ulcerative colitis (UC) shows alterations in mucosal immune response and composition of intestinal microbiota, referred to as dysbiosis. Alterations of the intestinal microbial community are primarily determined by 16S rRNA gene amplicon sequencing of the entire bacterial community present in a stool sample. We developed an approach to analyze cellular properties of human gut microbiota from stool samples on single cell-level by multi-parameter microbiota flow cytometry (mMFC). We acquire information about coating with endogenous host immunoglobulins by isotype-specific stainings to capture the mucosal immune response of the host and the expression of specific sugar moieties on the bacterial surface by staining with plant-derived lectins, potentially reflecting adaption of the bacteria to altered micro-environmental conditions in the intestine. Our results demonstrates that the phenotypic signature of the intestinal microbiota is able to classify IBD and distinguish CD and UC. The direct isolation of immunoglobulin-coated and lectin-stained bacterial populations by fluorescence-activated cell sorting (FACS) followed by whole 16S rRNA gene sequencing (PacBio) revealed that the phenotypic signature as represented by immunoglobulin coating not only correlates with but also enhances the observed taxonomic dysbiosis. Thus, mMFC can not only be applied as a diagnostic tool for disease classification but can also help to understand host-microbiota crosstalk in disease by enabling the identification and isolation of disease-associated bacteria.

ID: 108
Abstract
Topics: Microbe-immune interactions at body surfaces
Keywords: Gut microbiota, diet, inflammation, tumorigenesis

High-protein diet consumption promotes colorectal cancer development in mouse model

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Diet is undoubtedly strong modifier of mucosal microenvironment in the gut. Recently, we described proinflammatory monocytes as main drivers of experimental colitis severity in mice fed with high-protein diet (HPD). Here, we studied the hypothesis whether HPD consumption can also influence colorectal cancer development in mouse model.

Mice were fed control diet (CD) or HPD three weeks before induction of the disease. Then, we subcutaneously injected the mice with azoxymethane and one week later, let them drink 3% dextran sodium sulfate (DSS) solution in drinking water for 5 days. HPD mice showed significantly more severe weight loss after DSS treatment when compared to CD mice. After 45 days, when the experiment was terminated, the HPD versus CD mice had significantly higher calculated clinical score (3.11 vs. 1.68, p=0.015) and developed more tumors in the colon (30 vs. 15.33, p=0.033). More severe course of the disease was also associated with the fact that only 60% of animals survived in HPD group compared to 85% in CD group. Subsequent histological analysis revealed that high percentage of tumors in HPD mice showed tendency to invade into submucosa (20% vs. 0% in CD). By ELISA, we measured cytokine production and we found non-significantly increased production of TNFalpha, IL-1beta and IL-33 in the site of tumorigenesis in HPD mice. Flow cytometric analysis showed that HPD mice had lower infiltration of activated monocytes and neutrophils into the colon.

Our results suggest that HPD consumption changes immune response of gut mucosa making it more sensitive to tumorigenesis.

The study was supported by the Czech Science Foundation (grant nr. 20-03997S) and by the Czech Academy of Sciences (grant nr. LQ200202105).

ID: 109
Abstract
Topics: Mucosal diseases and infections, Swiss Young Immunologists Society (SYIS) and young European Federation of Immunological Societies (yEFIS)
Keywords: Intestinal tumorigenesis, intestinal inflammation, IL-33, T cells

Investigation of the role of IL-33 for intestinal tumorigenesis

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Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths, with chronic inflammation in the gut microenvironment being a prominent catalyst. Interleukin-33 (IL-33) signaling plays a controversial role in intestinal inflammation and CRC, and its exact mode of action in the tumor environment is unclear. We have established that ST2, the specific moiety of the IL-33 receptor, promotes intestinal tumorigenesis both in mice and humans. Specifically, activation of IL-33/ST2 signaling compromises the integrity of the intestinal barrier and triggers the production of pro-tumorigenic IL-6 by immune cells. Moreover, ST2 expression promotes in mice with chemically-induced CRC the accumulation of FOXP3+ regulatory T cells (Treg) specifically in intestinal tumors. This is associated with reduced frequencies of cytotoxic lymphocytes and increased tumor size. Genetic ablation of St2 reduces Treg infiltrations and concomitantly enhances the frequencies of effector CD8+ T cells, thereby restraining intestinal tumorigenesis. Mechanistically, IL-33 curtails IL-17 production by FOXP3+ Tregs and inhibits Th17 differentiation. Numbers of activated ST2-expressing Tregs are also increased in blood and tumor lesions of human CRC patients, suggesting a similar mode of regulation. Together, these data indicate a central role of IL-33/ST2 signaling in shaping an immunosuppressive environment during intestinal tumorigenesis. Yet, our recent findings have also unveiled another, protective role of IL-33 during the establishment of tumors in the colon, and ongoing research aims at addressing the underlying mechanisms.

ID: 110
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: IL10-/- mice, piroxicam, apoptosis, caspase-8, inflammasome

Caspase-8 blockade attenuates NSAID-induced colitis and cell death via NLRP3 inflammasome and NF-κB activation

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Background: Non-steroidal anti-inflammatory drugs (NSAIDs) are believed to exacerbate symptoms and inflammation in patients with inflammatory bowel disease (IBD), potentially due to high NSAID consumption, although the literature is inconsistent. In this study, using the piroxicam-accelerated model of colitis (PAC) in IL-10-/- mice, we examined the effect of short vs long term piroxicam-treatment in inducing colitis and examine mechanism(s) regulated by piroxicam.

Methods: IL-10-/- mice were fed 100ppm piroxicam in their food for 5-9 days followed by water until day 14. In the inhibitor study, mice were injected intra-peritoneally with the caspase-8 inhibitor (Z-IETD-FMK, 10mg/kg) every other day starting day -1 before piroxicam-feeding. Colon tissue was collected for analysis on inflammation and cell death markers by qRT-PCR, MesoScale Discovery (MSD)-assay, western blot, and histology.

Results: A significantly higher expression of colonic inflammatory markers and a reduced expression of epithelial barrier proteins and elongated crypts were found in the IL-10-/- mice fed piroxicam for 9 days but not in animals fed 5 days of piroxicam. An activation of members of the NLRP3 inflammasome family (e.g., caspase-1 and IL-1β), NFκB markers (phosphorylated IκBα and p65), Caspase-8, and apoptotic markers (cleaved caspase 3 and PARP) were associated with piroxicam-induced colitis. No activation of necroptosis or pyroptosis markers were observed. Inhibition of caspase-8 ameliorated NSAID-provoked colitis and apoptosis by preventing mitochondrial damage, and downregulating NF-κB and NLRP3 activation, as well as by improving colonic epithelial morphology and by reducing immune cell infiltration to the lamina propria.

Conclusions: Our data provides evidence on how a longer-term of NSAID exposure promotes intestinal inflammation by activating caspase-8, NLRP3 inflammasome and NF-κB, resulting in the induction of inflammation and apoptosis. Targeting Caspase-8 could be a potential treatment strategy for patients with NSAID-worsened symptoms and inflammation.

ID: 111
Abstract
Topics: Innate Immunity
Keywords: Goblet cell: Distal colon: Antigen-presenting cell: Goblet cell associated antigen passage: MHC class II.

Goblet cell mediated regulation of colonic immunity by interactions with antigen-presenting cells.

Victor Pettersson (The University of Gothenburg/Sahlgrenska Academy., SE)

Colonic goblet cells (GCs) are specialized cells devoted to the production and secretion of the mucus layer which plays a pivotal role in the intestinal defense by limiting microbial contact with the epithelium. In addition, GCs have been shown to sample and deliver luminal antigen to lamina propria antigen-presenting cells (LP-APCs), by the formation of goblet cell associated antigen passages (GAPs). The process of GC mediated sampling of the luminal content has been shown to play an important role in induction and maintenance of tolerance to the luminal content. We have previously shown that in the distal colon at steady state, approximately 10% of GCs form GAPs, and that GAP formation occurs primarily in the lower half of the colonic crypts. Whether GC mediated regulation of colonic immunity is restricted to GCs forming GAPs is currently unknown. To address this question, using immunohistochemistry and confocal microscopy, we quantified GC – LP-APC interactions along the crypt – surface axis, and started phenotyping the subtypes of LP-APCs that interact with GCs. Our results show that at steady state, 20% of GC interact with an LP-APC, and that GC – LP-APC interactions occur at a similar frequency along the crypt – surface axis. Initial phenotyping of LP-APC subtypes suggests that GCs interact with both MHC class II+ dendritic cells and macrophages. Taken together, these results show that GC – LP-APC interactions are not restricted to GCs forming GAPs and occur at a similar frequency along the distal colon crypt – surface axis. The physiological role of interactions between GC not forming GAPs and LP-APCs remains to be determined.

ID: 112
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections

Mechanisms behind antigen focusing and selection in bacterial infections of mucosal surfaces

Tom Kloter (ETH Zurich, Switzerland), **Emma Slack** (ETZ Zurich, Switzerland)

Infections with gram-negative bacteria get continuously harder to treat because of their large potential to acquire antibiotic resistances. Mucosal surfaces present main infection points for these bacteria. Vaccination offers a strategy for protection against such bacterial infections and thereby also prevention of their associated disease phenotypes; however, effective vaccine design remains a non-trivial challenge. This work will contribute to this challenge by investigating the mechanism of antigen selection by antigen presenting cells.

Even though remaining unknown, the presence of an antigen selection mechanism is illustrated by the phenomenon of surface antigen focusing: In bacterial infections of the gut, it was observed that there is a strong bias towards immune responses being raised against surface antigens rather than cytosolic antigens.

Bacterial lysis kinetics within the antigen presenting cell can provide an explanation to this phenomenon. We suggest that bacterial lysis within the endosome pathway is delayed to an endo- lysosomal compartment of a late maturation state, where MHC II loading is abolished or inefficient. This temporal separation of antigen sampling and availability of cytosolic antigens would allow to focus immunity onto surface antigens.

To test this hypothesis, we are using bone marrow-derived dendritic cells, infected with E.coli that produce a cytosolic, fluorescent pH-sensor. Upon lysis of the bacteria, the pH of the surrounding compartment will suddenly modulate the fluorescence spectrum of the sensor. This switch in fluorescence determines timepoint of bacterial lysis and simultaneously indicates the pH of the compartment, which corresponds to its maturation state. In a second step, antigen sampling will be followed by staining of MHC II together with protein structures of the antigen loading machinery.

Our findings will provide novel insights into the role of detachability and digestibility of bacterial antigens in the process of antigen selection, which has strong implications on how to design efficacious vaccines.

ID: 113
Abstract
Topics: Gut-Microbiota-Brain axis
Keywords: multiple sclerosis, ocrelizumab, serum biomarkers, barrier function

Dynamics of serum biomarkers of gut barrier function and immune system regulation during treatment with ocrelizumab in patients with multiple sclerosis

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The pathophysiology of multiple sclerosis (MS) is driven by chronic inflammation of the central nervous system, and previous studies suggest that intestinal barrier disruption may play a role in the pathogenesis of MS. Because biologics are an important tool in the treatment of MS, we analyzed how ocrelizumab, a monoclonal antibody to CD20, affects biomarkers of intestinal barrier function and immune response regulation in MS patients.

We collected serum from nine MS patients treated with ocrelizumab before therapy and three and six months later, from 11 MS patients treated with ocrelizumab for more than three years, and healthy controls. These samples were analyzed by ELISA for 15 biomarkers related to gut barrier function (I-FABP, L-FABP) and immune system regulation (CD14, LBP, MBL, IL-18, osteoprotegerin, osteopontin, calprotectin, IGF-II, TIMP-1, CXCL13, CCL3, CCL17).

Patients with multiple sclerosis have significantly higher levels of LBP, TIMP-1, OPG, and significantly lower levels of IGF-II regardless of the duration of treatment with ocrelizumab than healthy controls. MBL and CCL3 decrease within the first months of ocrelizumab therapy, and CXCL13, CD14, and L-FABP decrease only after several years.

Here, we described the dynamics of specific biomarkers of gut barrier function and immune response regulation during ocrelizumab therapy in MS patients. These data will be used for correlation in microbiota changes during ocrelizumab therapy.

This work was supported by the Ministry of Health of the Czech Republic (NU20-04-00077).

ID: 114
Abstract
Topics: Mucosal B cells
Keywords: B cell, IgA, Microbiota, Intestinal barrier integrity, Immune topology.

PhD thesis: The Immune Topology of the Gut Barrier: a Focus on Immunoglobulin A.

Asmae Laouina (Uniklinik RWTH Aachen, Germany), **Oliver Pabst** (Uniklinik RWTH Aachen, Germany)

Immunoglobulin A (IgA) is a key component of the gut barrier. IgA binds to gut microbiota, affecting microbial metabolism, growth, motility, bile acid sensitivity, host-microbe interaction, and effectively tuning microbiota diversity and niches. In addition, IgA has also been suggested to modulate sampling of luminal antigen into Peyer´s patches and to enable retrograde transport of antigen from lamina propria into the gut lumen. A lack of secretory antibodies has been reported to be associated with transcriptome changes of the gut epithelium and with microbiota composition. However, other barrier-related functions of IgA and the outcome on the intestinal functionality and integrity are less well understood.

We speculate that Ig exerts multiple direct and indirect effects on the gut barrier that include but are not limited to the modulation of the microbiota. We developed an AI-assisted histology-immunohistochemistry analysis that can provide large scale quantitative morphological and immune features of the gut tissue, showing for instance, that recently proliferating plasma cells are having distinct localization inside the lamina propria. A multitude of IgA settings are investigated in vivo and in vitro. The latter is making use of intestinal organoids that transport exogenous polymeric IgA coming from polymeric Ig receptor (pIgR) deficient mice. In vivo models consist of intra venous transfer of polymeric IgA that, via bile, is secreted into the gut lumen. These tools are adopted to compare IgA-deficient and sufficient conditions, in microbiota-related and unrelated context. First data suggest that IgA has limited impact on epithelial transcriptome in vitro in an microbiota-independent manner.

We expect that this project will contribute to define an integrated view of how IgA influence the intestinal barrier and to identify potential therapeutic opportunities aiming at reinforcing the gut barrier integrity.

ID: 115
Abstract
Topics: Gut-Microbiota-Brain axis
Keywords: microbiota, bacterial lysate, Parabacteroides distasonis, T-cell response, experimental autoimmune encephalomyelitis

Lysate of Parabacteroides distasonis reduces the severity of experimental autoimmune encephalomyelitis

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The gut microbiota influences the immune system and contributes to differences in host susceptibility to inflammatory diseases. Patients with multiple sclerosis have low abundance of Parabacteroides distasonis (Pd) in their gut microbiota. and Llive Pd suppress the T-cell responses and prevent severe forms of experimental autoimmune encephalomyelitis (EAE) in mice. The aim of this study is to determine whether a protective effect can also be achieved by Pd lysate and whether this administration affects the gut microbiota composition.

Lysate of anaerobically cultured Pd was administered orally to C57BL/6 mice in four weekly doses. Subsequently, EAE was induced by subcutaneous administration of myelin oligodendrocyte glycoprotein emulsified in complete Freund's adjuvant and intraperitoneal injection of Bordetella pertussis toxin. After 3 weeks, we compared the neurological impairments with EAE controls and measured the immune profile by flow cytometry and ELISA. Microbiota composition was analyzed by next-generation sequencing.

Pd lysate significantly delayed and decreased the severity of EAE. It significantly increased the frequency of regulatory T cells in mesenteric lymph nodes, but not in axial lymph nodes. The increase in regulatory T cells was accompanied by a significant increase in IL-10. In addition, Pd-lysate significantly increased gut abundance of the genera Ruminococcus and Parabacteroides in mice with EAE, but not in EAE control group. Administration of Pd lysate without disease induction resulted in significantly higher abundance of the genus Lactobacillus. Induction of EAE resulted in higher abundance of Prevotellaceae in both study groups.

Oral administration of Pd lysate delays clinical symptoms and prevents severe forms of EAE by inducing a T regulatory response. Even killed Pd can protect against severe forms of central nervous system inflammation.

This work was supported by Ministry of Health of the Czech Republic (NU20-04-00077) and the Academy of Sciences of the Czech Republic (LQ200202105).

ID: 116
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections
Keywords: Secretory IgA, mucosal immunology, microbiota, bacterial glycans, protein engineering

Exploring the biophysical principles underlying sIgA function in the intestine

Cheickna Diawara (ETH Zurich, Switzerland)

Secretory IgA is our immune system's primary tool to maintain microbial homeostasis in the intestine. Yet our mechanistic understanding of IgA function remains incomplete. Despite binding pathogens and commensals, it only appears to mediate elimination of undesirable species. How does IgA distinguish the two? We propose that this sophisticated function arises from simple biophysical properties – affinity, avidity and binding geometry – in the context of a flowing ecosystem and modulated by the growth rate of targeted bacteria. I will test this hypothesis by developing IgA mimetic structures that replicate exclusively the multivalent binding properties of IgA. These will consist of proteinaceous nanoparticle scaffolds displaying polysaccharide binding proteins – targeting bacterial surface polysaccharides – with defined affinity, valency, and geometry. After establishing that these structures bind to the target bacteria, I will explore their impact on bacterial growth and physiology in controlled microfluidic systems. These results will then be translated to the complex in vivo situation by studying the impact of IgA mimetics on bacterial population dynamics in the murine gut and making careful comparisons with infection- and vaccine-induced IgA. We expect this project to elucidate the simple biophysical properties that underlie IgA-mediated control of the gut microbiota.

ID: 117
Abstract
Topics: Microbe-immune interactions at body surfaces
Keywords: gut microbiota; mucosal immunity; inflammation; carcinogenesis; diet

Pro-tumogenic potential of HPD diet is decreased by depletion of macrophages

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Diet is the main environmental factor influencing gut microbiota, which has a critical role in carcinogenesis. The key host-microbe interactions underlying this process are dependent on immune cells. Here, we analyze the role of macrophages as drivers of carcinogenesis in animals fed with high-protein diet. Colon cancer was induced by azoxymethane and sodium dextran sulfate in BALBc mice fed with high-protein (HPD) or control synthetic diet (CD). To study their role, macrophages were depleted with regular intraperitoneal injections of clodronate liposomes after the colon cancer induction. Cell populations were determined using a flow cytometry. HPD increases intestinal inflammation and it induces higher number of colonic tumors and depletion of macrophages significantly decreases this effect. This depletion also led to decrease in typical M1 macrophages in the spleen regardless of diet and to significant increase in INFγ+ producing CD8+ T lymphocytes in HPD-fed mice. This suggests that HPD promote carcinogenesis by increasing inflammatory response but the proper cytotoxic T cell response is partially blocked by macrophages.

ID: 118
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: IL-22, IL-26, zebrafish, enteric nervous system, epithelial proliferation

Zebrafish as a model to study how cytokine-microbiota crosstalk modulates gut organ maturation and physiology

Pedro Hernandez (Institut Curie, France)

While the role of vertebrate immune cytokines in infection control has been widely studied, their impact on tissue development and the establishment of organ physiology remains less understood. We are addressing these questions exploiting the advantages of the zebrafish model such as rapid ex utero development, transparency and the early onset of physiological gut function. We are currently focusing on cytokines we found are active in the developing gut, and whose dysregulation is linked to inflammatory gut diseases in humans. These include two related cytokines that have unique ortholog genes in zebrafish: IL-22 and IL-26.

We recently found that guts of fish deficient for IL-22, in addition to showing impaired gut immunity, have dysregulated expression of neuronal markers as well as impaired function of the enteric nervous system. By generating an il22-reporter line, we identified enteroendocrine cells as the main source of il22 in the zebrafish larval gut and that its expression is induced by metabolites from bacteria. We are currently deciphering the mechanisms by which IL-22 modulates gut neuronal development and gut physiology. Regarding IL-26, little is known about its in vivo function as it is absent in rodents, main models for studying vertebrate gene function. The zebrafish has a single il26 gene and we found that the IL-26 cytokine can directly kill bacteria, like the human IL-26. In addition, using gain- and loss-of-function approaches, we revealed that IL-26 suppresses gut epithelial proliferation. We are currently studying whether these functions of IL-26 mediate gut homeostasis upon tissue damage. We propose the zebrafish as a powerful model to study the role of cytokine signaling in the development, homeostasis, and the physiological function of the intestine.

ID: 119
Abstract
Topics: Microbe-immune interactions at body surfaces, Systems immunology in host-microbe interactions
Keywords: atopic dermatitis, gut barrier function, microbiome, serum biomarkers

Levels of ASCA IgA, ASCA IgG, CCL17, L-FABP and IL-18 in serum are associated with disease severity in children with atopic dermatitis

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Introduction: Atopic dermatitis (AD) is common chronic inflammatory skin disorder that affects up to 20% of children in industrialized countries during their first years of life. Complex etiology of AD involves abnormal immunological and inflammatory pathways including defective skin barrier or exposure to inflicting environmental agents. However, no standard predictive biomarkers reflecting objective and subjective signs and symptoms of AD and offering reliable and objective outcome measures have been identified so far. In this study, we aimed to identify potential biomarkers that could correlate with AD severity and progression in infants and toddlers.

Methods: We collected serum from 98 infants or toddlers with AD at the first patient visit (age 3-12 months) and one year later. The severity of the disease was evaluated using SCORAD index. Serum samples were analysed by ELISA for 22 biomarkers related to the gut barrier function, microbial translocation and immune system regulation.

Results: We found a statistically significant correlation between Anti–Saccharomyces cerevisiae antibody IgA (ASCA IgA), Anti–Saccharomyces cerevisiae antibody IgG (ASCA IgG), CCL17, L-FABP and IL-18 with the severity of AD. The strongest correlation was observed for CCL17 ($p=0.0044$; $r=0.4634$) and L-FABP ($p=<0.0001$; $r=0.4808$). This was followed by IL-18 ($p=0.0175$; $r=0.2854$), ASCA IgA ($p=0.0192$; $r=0.2877$) or ASCA IgG ($p=0.0263$; $r=-0.2674$). There was no significant correlation between the severity of AD and serum levels of calprotectin, CD14, defensin, E-FABP, I-FABP, IgE, IL-6, IL-10, IL-31, LBP, MBL, osteoprotegerin, TSLP, CCL3, CCL11, CXCL13 and osteopontin.

Conclusions: Our findings suggest that ASCA IgA and ASCA IgG antibodies, L-FABP, CCL17, and IL-18, could be potential biomarkers associated with AD pathogenesis and severity in infants and toddlers. These findings could lead to better diagnosis, prognosis, and treatment of AD in the future.

This study was supported by the Ministry of Health of the Czech Republic grant no. NU20-05-00038.

ID: 120
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: T cells, Crohns, Intestine

Deciphering CD4+ T cells responses in inductive sites of the human intestine in health and inflammatory bowel disease.

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CD4+ T cells play an essential role in intestinal immune homeostasis but in the setting of chronic intestinal inflammation, such as inflammatory bowel disease (IBD), are thought to contribute to immune mediated intestinal pathology. While intestinal CD4+ T cell responses are initiated in gut-associated lymphoid tissues (GALT) and intestinal draining mesenteric lymph nodes (MLN), our understanding of the types of CD4+ T cell responses that are generated in human GALT and MLN, and how these change in the setting of IBD remains limited. In the current study we are combining our recent techniques to isolate immune compartments along the human intestine (1, 2) with scRNA-seq, CITE-seq, and TCR-seq to (1) assess CD4+ T priming and differentiation in human GALT (Peyer's patch and isolated lymphoid follicles) and MLN and (2) to track the migratory properties of these cells to the ileum and colon lamina propria. We anticipate that these studies will enhance our understanding of CD4+ T cell responses in human intestinal inductive site in health and IBD and potentially lead to the identification of novel avenues for modulating these responses for the benefit of human health.

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ID: 121
Abstract
Topics: Mucosal T cells, Neonatal window of opportunities

Investigating how maternal anti-microbiota IgA regulates neonatal T cell immunity

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The postnatal intestinal immune system undergoes significant development during initial bacterial colonization postpartum. The developing T cell population is tightly regulated, as aberrant T cell responses during development can predispose individuals to auto-inflammation. Breast feeding is negatively associated with onset of inflammatory bowel disease (IBD), which is characterized by aberrant T cell responses to the microbiome. However, the mechanism of protection conferred by breastfeeding, and the connection to the induction of microbiota specific T cells, are incompletely understood. Breast milk supplies antimicrobial peptides and antibodies from the maternal mucosal immune system, the most abundant of which is Immunoglobulin A (IgA). To understand the isolated role of mIgA on the developing microbiome and intestinal immune system, we utilized a heterozygous breeding scheme which enabled characterization of IgA -/+ mice breastfed with either a B6 or IgA -/-. Analysis of the small intestine lamina propria immediately following weaning revealed increased RORγt+ T cells when breastfed with an IgA deficient dam. These changes persisted into adulthood and could be reversed with limited antibiotic treatment in the preweaning period. Analysis of the microbiome by 16S sequencing revealed increased Enterobacteriaceae and Pseudomonadaceae in the absence of mIgA prior to weaning, however the microbiome of each group had no significant differences after the cessation of breastfeeding. This suggests a role of mIgA in preventing the induction of microbiota specific T cells prior to weaning through control of Pseudomonadota, which results in long term alterations to the small intestinal T cell compartment. We hypothesize that microbiota specific Th17 responses generated in the absence of mIgA will increase susceptibility to inflammation in an IL-10 knockout model of IBD. Our results and future studies will provide a potential mechanism for how breastfeeding confers long term protection from aberrant T cell driven intestinal auto-inflammation.

ID: 122
Abstract
Topics: Gut-Microbiota-Brain axis, Microbe-immune interactions at body surfaces
Keywords: ketogenic diet, experimental autoimmune encephalomyeltis, autoimmunity, neuroinflammation, gut-brain axis

Ketogenic diet decreases severity of experimental autoimmune encephalomyelitis

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Ketone bodies produced in the gut from dietary fiber by gut bacteria have potent anti-inflammatory properties. Ketogenic diet leads to production of ketone bodies, especially β-hydroxybutyrate, which interfere with inflammatory processes in a similar way. Here, we studied the effect of the ketogenic diet in mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE).

C57BL/6 mice were fed with ketogenic or standard diet starting 14 days before EAE induction with myelin oligodendrocyte glycoprotein peptide (MOG35-55) mixed with complete Freund's adjuvant, together with intraperitoneal injection of Bordetella pertussis toxin. The disease development was monitored for three weeks, and the state of T cell activation was analyzed in mesenteric or lumbar lymph nodes by flow cytometry.

Ketogenic diet delays EAE onset and significantly decreases its severity. Nevertheless, this mitigation is not accompanied with significant increase in regulatory T cells or decrease in major pro-inflammatory cytokines employed in EAE (IFN-γ, IL-17A or TNF-α) produced by T cells. Polarization of CD4+ cells into Th1 and Th17 phenotypes, and populations of CD4+ and CD8+ T cells within mesenteric and lumbar lymph nodes also remain unaffected by the diet.

We conclude that ketogenic diet decreases severity of EAE by yet unrecognized immunomodulatory mechanism. Supported by Lumina Quaeruntur (Czech Academy of Sciences, project number LQ200202105) and by programme “Foods for the Future” (Strategy of Czech Academy of Sciences AV21).

ID: 124
Abstract
Topics: Mucosal T cells
Keywords: microbiota, IBD, T cell repertoire, Treg

Shaping lymphocyte repertoires in the gut

Oliver Pabst (RWTH Aachen University, Germany)

Key aspects of intestinal T cells including their antigen specificity, their selection by the microbiota and other intestinal antigens and the contribution of individual T cell clones to regulatory and effector functions are unresolved. We have modified the adoptive transfer colitis model based on the transfer of clonally defined effector (Teff) and T regulatory (Treg) populations, i.e. different recipients were transferred with collections of T cells comprising identical T cell clones. T cell populations accumulating in the recipient colon were analyzed by flow cytometry, TCR repertoire analysis and single cell sequencing. Accumulation of in vitro expanded Teff and Treg clones was harmonized in independent recipients, i.e. expanded clones detected in one recipient were also expanded in other recipients resulting in highly similar T cell repertoires shared between individual mice. Similarity recipients was reduced when recipients harbored major differences in their microbiota and no clonal sharing was observed when mice were transferred with non-harmonized T cell samples. These observations suggest that cognate antigen interaction was critical to shape the colonic Teff and Treg population. This observation extended to stability of TGFβ-induced Treg (iTreg) and the repertoire of stable iTreg was distinct from the repertoire of T cells that lost Foxp3 expression in vivo. However, Treg clonally matched to Teff did not show any superior suppressive capacity as compared to non-matched Treg/Teff transfers. Finally, Treg clonotypes acquired similar expression characteristics in vivo. We conclude that the TCR-context in response to the microbiota generates similar functional outcomes in the adoptive transfer colitis model. We speculate that dominant T cell clones might provide a therapeutic target in human inflammatory bowel disease.

ID: 125
Abstract
Topics: Mucosal T cells, Microbe-immune interactions at body surfaces
Keywords: IL-6, microbiota, colorectal cancer

IL-6 promotes colorectal cancer by modulating gut microbiota

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Gut microbiota interaction with the host organism is critical for the maintenance of epithelium integrity and for control of tumor-inducing inflammation. IL-6 is known for its ability to drive tumor progression in the gut. However, IL-6-mediated changes in the composition of intestinal microbiota were not previously addressed. To test whether IL-6 may enhance tumor growth via modulation of gut microbiota we compared susceptibility of IL-6 knockout (IL-6 KO) and wild type (WT) mice to colorectal cancer in AOM/DSS model under separate housing or cohousing conditions.

We found that the induction of AOM/DSS-colorectal cancer in separately housed IL-6 KO resulted in a reduced number of tumors as compared to WT controls. Strikingly, when cohoused with IL-6 KO mice, WT mice also developed fewer tumors in the colon. Sequencing of 16S RNA revealed differences in gut microbiota composition of naive WT and IL-6 KO separately housed mice: cohoused and separately housed mice mostly differed in Firmicutes and Bacteroidetes content. Moreover, cohousing with IL-6-deficient mice decreased the proportion of pathogenic IL-17A+ Th17 cells in the lamina propria of WT mice, which was paralleled by altered systemic level of IL-23 and the reduction in blood neutrophil counts. These results indicate that IL-6 plays an essential role in maintaining gut microbiota composition as well as microbiota-responsive subsets of immune cells. Thus, IL-6 abundance may directly impact susceptibility to intestinal carcinogenesis.

Taken together, our data suggest that IL-6 promotes colorectal tumor growth via modulating gut microbiota, and that manipulation with the composition of the microbiota may reduce tumor burden in the AOM/DSS model.

This work was supported by RSF grant № 22-25-00534.

ID: 127
Abstract
Topics: Innate Immunity
Keywords: Myeloid cells, Multiplexed tissue imaging, tonic interferon, large intestine, spatial proteomics

Regulation of tonic interferon signaling by spatial regulation of intestinal mononuclear phagocytes

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Barrier mucosae are continuously exposed to sterile and non-sterile immune cues. Such exposure leads to the constitutive expression of interferons (IFN), which under tight immune regulation confer a protective state. The mononuclear phagocyte (MP) compartment, including monocytes, macrophages, and dendritic cells (DC), has been implicated in orchestrating tonic IFN signaling. However, the exact mechanisms and spatial regulation of these processes remains elusive.

To investigate the spatial distribution, diversity, and involvement of MPs in the regulation of constitutive IFN responses, we employed a high dimensional 50-plex Co-Detection by Indexing (CODEX) imaging approach onto the Mx1-gfp reporter model. Unsupervised cell classification identified various clusters of MPs in large and small intestine, differentiated them from cells of the lymphoid compartment, and revealed their microanatomical location along the intestinal tube and within the intestinal wall. GFP expression (surrogate marker for IFN signaling) indicated a major contribution of the MP compartment, specifically multiple macrophage populations, to the tonic IFN production in the gut.

To further characterize the niche of these IFN-producing MPs, cellular neighborhood analyses were performed and revealed longitudinal as well as layer-dependent cellular associations. The latter were altered early during acute dextran sulfate sodium (DSS)-induced colitis, but not the source of IFN. Taken together, the workflow presented here allows for the identification of diverse MP cellular neighborhoods and their involvement in constitutive IFN production, shedding light on the regulation of these responses and their biological function.

ID: 128
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Macrophage, intestine, colitis, monocyte

Investigating the effects of inflammation on the heterogeneity, longevity and imprinting of colonic macrophages

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Macrophages are vital for intestinal homeostasis, yet dysregulated macrophage behaviour is considered a key feature of gastrointestinal diseases such as inflammatory bowel disease (IBD). Why these peacekeepers become powerful aggressors in IBD is unclear. Here, we have used a combination of scRNA-seq, lineage tracing and confocal microscopy to characterise the monocyte/macrophage compartment in health, inflammation and inflammation resolution using a resolving model of DSS-induced colitis. We define at least two populations of macrophages in the healthy colon defined by expression of CD11c and CD163, which occupy discrete anatomical niches and display distinct replenishment kinetics from monocytes. During acute colitis, classical monocytes and their progeny accumulate in vast numbers. Importantly, despite sharing the same surface identity, in the context of acute colitis monocytes acquire a unique transcriptional and functional profile, and their depletion ameliorates colitis. Complementary analysis of mucosal biopsy tissue from newly diagnosed, treatment naïve Crohn's disease patients identified an evolutionarily conserved molecular signature of inflammation-associated monocytes. During inflammation resolution and tissue repair, monocyte numbers subside and CD11c/CD163 mature macrophage dominate again. Cx3cr1¬-based fate mapping shows that inflammation has differential effects on the replenishment of CD11c- and CD163-defined macrophage populations. Moreover, marked transcriptional differences exist between these cells and their counterparts in the healthy colon demonstrating that inflammation leads to transcriptional rewiring of these cells in a subset-specific manner. Finally, by combining scRNAseq with genetic fate mapping, we were able to demonstrate that residency in the colon limits the plasticity of colonic macrophages in the context of inflammation.

ID: 129
Abstract
Topics: Innate Immunity
Keywords: Innate lymphoid cells, colorectal cancer, ILC2

Role of type 2 Innate Lymphoid Cells upon Colorectal Cancer Stem Cells

Inês de Paula Costa Monteiro (Unibe, Switzerland)

As the knowledge about the role of type 2 innate lymphoid cells (ILC2s) in physiological conditions evolves, so grows the interest regarding the function of these cells in the cancer context. Colorectal cancer (CRC) is one of the most common malignancies for which there are still limited treatment options in the metastatic setting. Cancer stem cells have a pivotal role in CRC, often being responsible for therapy resistance and disease relapse.

We have established a 3D co-culture of mouse colonic ILC2s with CRC organoids isolated from AOM/DSS-treated (azoxymethane/dextran sodium sulfate) mice or ApcMin/+, Vil-Cre, Krasf/f mice. In the setting of AOM/DSS CRC organoids, the presence of ILC2s or their supernatant significantly decreases organoid formation. Accordingly, the lack of ILC2s in the AOM/DSS CRC mouse model leads to a significantly higher tumor load. Using a multiplexed immunofluorescence technique, ILC2s were identified in the CRC samples from 35 patients and associate with a Crohn's-like lymphoid reaction. This type of inflammation has been consistently associated with a favorable prognosis for CRC patients.

Overall, these results suggest that ILC2s are present in the CRC microenvironment and might have a direct antitumorigenic effect. It is the aim of this project to further describe the role of ILC2s directly upon the cancer stem cells of CRC in mouse and human. Ultimately, understanding the interaction between ILC2s and colorectal cancer cells has the potential to shed new light on potential therapeutic strategie

ID: **130**

Abstract

Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections
Keywords: Oral vaccine, niche competition, microbiota, IgA, Salmonella

Oral vaccination combined with niche competition can generate sterilizing immunity against entero-pathogenic bacteria

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Infections with antimicrobial resistant bacterial were responsible for almost 5 million deaths worldwide, in 2019. Therefore, alternative treatment strategies are urgently needed. Many of these pathogens – such as E. coli and non-Typhoidal Salmonella – colonize mucosal surfaces prior to driving disease. However, this stage is often overlooked by current vaccines.

While live-attenuated vaccines against non-Typhoidal Salmonellosis (NTS) exist for the use in livestock, direct translation to humans is challenging as these vaccines pose a major safety concern in immunocompromised individuals. Moreover, these vaccines provide protection from invasive disease but are known to have limited effects on colonization. Instead, we propose a two-pronged strategy that combines a negative selective pressure induced by inactivated oral vaccines, with benign bacterial niche competitors.

We have validated this approach in a murine NTS model and compared it to the classical live-attenuated vaccines and could achieve complete elimination of pathogenic Salmonella Typhimurium (S.Tm) from the gut lumen. This is based on the proven ability of specific secretory IgA to generate a fitness disadvantage for a targeted bacterium, allowing a non-targeted competitor to rapidly overtake its niche. Comparing different modes of oral vaccination further reveals separation in the mechanisms protecting systemic sites from S.Tm (spleen and liver), which are largely T-cell driven, from those protecting the gut lumen and tissues. This highlights the importance of microbial ecology for designing effective mucosal vaccines, with broad implications for the elimination of pathogenic bacterial reservoirs and microbiota engineering.

Preliminary data indicates that this technique will be broadly applicable to other Enterobacteriaceae.

Taken together, vaccination/competitive exclusion approaches hold great promise in the prevention, elimination and/or treatment of antimicrobial resistant infections. Moreover, the generation of sterilizing immunity against pathogenic bacteria raises the possibility to drive pathogen extinction.

ID: 131

Abstract

Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Cry1Ac Macrophages, IBDs, DSS cytokines

CHARACTERIZATION OF MACROPHAGE POPULATIONS IN THE LARGE INTESTINE INDUCED BY CRY1Ac TOXIN IN MODELS OF INTESTINAL INFLAMMATION.

Ximena Ilbet Betanzos Laguna (UNAM, Mexico), **Damaris Ilhuicatzi Alvarado** (UNAM, México), **Leticia Moreno Fierros** (UNAM, México)

Cry1Ac toxin, form Bacillus thuringensis is widely used as biopesticide and expressed in genetically modified (GM) plants used for human and animal consumption. Since Cry1Ac is also immunogenic and able to activate macrophages, it is crucial to thoroughly evaluate the immunological effects elicited after intra-gastric administration. The intestine contains the largest pool of macrophages in the body which are essential for maintaining mucosal homeostasis in the face of the microbiota and the constant need for epithelial renewal but are also important components of protective immunity and are involved in the pathology of inflammatory bowel disease (IBD). We evaluated the potential of Cry1Ac for induce IBD and compared the effects with a model of ulcerative colitis DSS-3% induced. Mice were daily intragastrically administered for 7 days with: a) vehicle phosphate-buffered saline (PBS), b) TCry1Ac 2µg, c) TCry1Ac 20µg and d) TCry1Ac50µg. During the administration we recorded the IBD-associated physical parameters. We assessed the cytokines in gut culture explants and the results shows the intragastric administration of TCry1Ac with the three doses tested can provoke changes in some cytokines crucial for intestinal homeostasis like IL-6, IL-10 IL-1β and TNF-α. Interestingly we observed reduction in tolerogenic IL-10 levels. Furthermore, we evaluated the lamina propria cells with flow cytometry to identify the intestine Mo phenotype, based on the expression of CD11b+, CCR2+, F4/80+ and CX3CR1+. We observed in the group TCry1Ac 2µg an increase pro-inflammatory monocyte cells. As well we observed a raise in the expression of CD86 in all the groups receiving TCry1Ac in any of the doses tested, and the expression of CD80 was raised in TCry1Ac 2µg group. This work demonstrated the potential of Cry1Ac to induce alterations in the intestinal homeostasis, this could provide important insights to identify the dysregulated immune factor in IBDs.

ID: 132
Abstract
Topics: Innate Immunity, Mucosal diseases and infections

Inmunological Evaluation of Multiepitopic Vaccine Prototype againsts SARS-CoV2, using VP2-VLP´s

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The acute respiratory syndrome caused by the SARS-CoV-2 virus, is a delicate heath issue problem, because since its first appearance in 2019 to these days, is a illness that maintain in constant warning all the population. Actually the number of cases and deceases have diminish remarkably, due to the vaccination campaign, nevertheless, the potential develop of more aggressive virus mutations is a latent risk.

The present study develops a multiepitope vaccine prototype against SARS-CoV-2 using VLP platform, that are used as antigens carriers. Lineal epitopes from SARS-CoV-2-SPIKE protein that are reported as crucial sites to induce neutralizing antibodies were selected and put them in tandem sequence and Spy-tag protein was added at the end of the sequence, that construction is named meSpiketag and was designed to be expressed in E.coli. VP-2-Spycatcher VLPs were ensembled and then were associated with meSpikeTag protein. Viral particles stability was measured by DLS and then used to inmunize intramuscular C57BL6 mice and igG antobody production was measured.

ID: 133
Abstract
Topics: Mucosal diseases and infections
Keywords: IBD, Microbiota, Intestinal epithelial cells, immune system, Inflammasome

Dissecting the role of epithelial inflammasome activation in regulating immune responses in colitis

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During inflammatory bowel disease (IBD) the homeostatic relationship with the intestinal microbiota is disrupted and bacteria become key drivers of inflammation. In particular, specific commensals, such as Prevotella, can exacerbate intestinal inflammation activating several pro-inflammatory host pathways and perturbing the microbiota composition and metabolic functions. One of the host mechanisms involved in this is the inflammasome. Inflammasome activation in intestinal epithelial (IEC) and myeloid cells controls the activity of the inflammatory mediators caspase-1, IL-1b and IL-18, which in turn are key regulators of adaptive immunity which has a crucial role in intestinal inflammation. Inflammasome can be activated by microbial signals, however, how exactly some bacteria, such as Prevotella, can activate the intestinal inflammasome and adaptive immune response in colitis induction remains enigmatic.

To explore this crosstalk, we aim to elucidate the regulation of inflammasome activation in intestinal epithelial cells (IECs) by specific bacteria, as well as determine how IEC-microbiota interaction contributes to pro- and anti-inflammatory adaptive immune responses in colitis. For this, we use murine genetic knock-out models targeting various inflammasome components, such as Pycard-/- mice that lack the expression of the inflammasome adaptor protein Apoptosis-associated speck-like protein (ASC) or Il18-/- mice. These mouse models are housed under different hygiene conditions either with known defined or undefined microbiotas (e.g. specific pathogen free, SPF). Through a challenge with the pathobiont Prevotella copri we induce alteration of microbiota composition and we study its effects on the host immune system and IECs before and after DSS-colitis induction. We analyse the effect of P.copri colonisation under our different hygiene conditions on the integrity of the epithelial barrier through expression of tight junction proteins and via RNA sequencing of IECs. Changes in microbial compositions and adaptive immune cells are analysed via 16S rRNA sequencing and flow cytometry respectively.

ID: 134
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections

mRNA alternative splicing in intestinal epithelial cells is essential for microflora colonization and gut immune function

Robert Anthony Gaultney (University of Bern, Switzerland)

Inflammatory bowel disease is an increasing problem in industrialized countries and is responsible for huge costs in patient quality of life and productivity loss. Surprisingly, although several risk factors have been identified, the primary drivers causing patients to become prone to develop IBD remain largely unknown. The microenvironment of the intestine relies on a delicate balance of the interactions between the microflora, host immune system, and cells comprising the epithelial barrier. While first two components are well studied, less attention has been afforded to the critical role played by the intestinal epithelial cells. To achieve and maintain the proper epithelial phenotype, these cells employ varied strategies including epigenetics, transcriptional control, and post-transcriptional regulation such as alternative splicing. We have developed a model of mouse epithelial alternative splicing defect by mutating the epithelial splicing regulatory protein (ESRP1). We found that mice with this mutation are more susceptible to experimental colitis and are less able to heal wounds but show no overt signs of disease before challenge. To investigate the causes of these phenotypes, we colonized germ-free mice with microflora of different complexities and tracked the microbial colonization and immune cell populations of the intestine over time. Our data show a time and competition-dependent alternation in the microflora of epithelial AS-dysregulated mice. We also show that the response of the intestinal immune system depends on the microflora introduced to these alternative splicing-deficient mice, with the most complex flora increasing the number of pro-inflammatory T cells in the intraepithelial space. Overall, our data indicate that mice lacking epithelial alternative splicing display a phenotype like that of individuals primed to develop intestinal disease. Further studies are ongoing to determine the exact factors controlling bacterial colonization and immune maturation.

ID: 135
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: Tissue resident T cells, Interferon gamma, Helminth, Tissue Repair, Immune-Stroma Interactions

Ifng Production by Tissue Resident CD8 T Cells Promotes Tolerance to Intestinal Helminth Infection

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Combatting infection requires a balance between mounting an effective anti-pathogen immune response and limiting collateral tissue damage. During infection with large, multicellular intestinal helminths this balance is tipped such that tissue damage control is prioritized over parasite killing. Our previous studies demonstrated that infection of mice with the roundworm, Heligmosomoides polygyrus bakeri (Hpb), induces an early IFNg-dominant type 1 immune signature during the tissue-invasive stage of infection that limits tissue damage without reducing parasite burden: a defense strategy referred to as disease tolerance. However, the cellular source of IFNg and its downstream targets that mediate host tolerance remained unclear. Using a combination of cell transfer, bone marrow chimeric and gnotobiotic approaches, we demonstrate that tissue-resident CD8 T (Trm) cells are the critical source of IFNg during early Hpb infection. Importantly, CD8+ Trms are activated in an antigen-independent yet microbiota-dependent manner. Eliminating IFNg receptor signaling during Hpb infection resulted in enhanced myofibroblast remodeling of the submucosa and compromised recruitment of neutrophils to the site of infection. These structural and immune cell alterations were associated with accelerated helminth growth, enhanced intestinal damage and chronic defects in tissue repair. Overall, our studies describe an unexpected role for CD8+ Trm cells in mediating disease tolerance during intestinal helminth infection and reveal an immune-stromal cell network that could be targeted for protection against tissue injury.

ID: 136
Abstract
Topics: Innate Immunity
Keywords: ILC2s, SHIP1, COPD, Asthma, Pulmonary Inflammation

Role of type 2 innate lymphoid cells in PI3K signaling-driven chronic lung inflammation

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Airway inflammatory diseases like asthma and chronic obstructive pulmonary disease (COPD) are common disorders of increasing prevalence, for which no curative therapies currently exist. The phosphoinositide-3 kinase (PI3K) is often hyperactivated in these chronic lung inflammatory disorders. Styx mice express hyperactivated PI3K signaling, leading to spontaneous development of a chronic airway inflammatory disorder. Here we found that genetic ablation of the signal induced by IL-33, a key mediator of type 2 immune responses, rescues the lethal lung disease in styx mice. Type 2 innate lymphoid cells (ILC2s) were the main cells expressing of IL-33 receptor in lung infiltrates of styx mice, suggesting these cells as disease initiators. Indeed, styx mice crossed onto a Rag2-/-;Il2rg-/- background, and thus lacking ILCs, did not manifest airway inflammation. However, lung infiltrations were re-established in these mice upon intranasal transfer of styx ILC2s. In addition, styx mice-derived ILC2s showed a hyperactivated phenotype. In humans, GATA3, a transcription factor essential for ILC2 development and function, was increased in lung tissue from severe versus mild COPD patients. Moreover, there was a negative correlation between the expression of a PI3K negative regulator and GATA3 during COPD progression. Together, these results suggest that the PI3K pathway is involved in ILC2 activity during airway inflammation, and that patients suffering from asthma or COPD may benefit from modulation of the PI3K pathway.

ID: 137
Abstract
Topics: Mucosal T cells, Microbe-immune interactions at body surfaces
Keywords: iNKT cells; CRC; neutrophils; Fusobacterium nucleatum

iNKT cell-neutrophil crosstalk promotes colorectal cancer pathogenesis

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iNKT cells account for a relevant fraction of effector T-cells in the intestine and are considered an attractive platform for cancer immunotherapy. Although iNKT cells are cytotoxic lymphocytes, their functional role in colorectal cancer (CRC) is still controversial, limiting their therapeutic use. To gain insights into iNKT cells role in CRC we examined the immune cell composition and iNKT cell phenotype of CRC lesions in patients (n=118) and different murine models. High-dimensional single-cell flow cytometry, metagenomics and RNAseq experiments revealed that iNKT cells are enriched in tumor lesions; the tumor-associated pathobiont Fusobacterium nucleatum inducesIL17 and GM-CSF expression in iNKT cells blunting their cytotoxic capability and promoting iNKT-mediated recruitment of neutrophils with PMN-MDSCs-like phenotype and functions. Lack of iNKT cells reduced tumor burden and recruitment of immune suppressive neutrophils. iNKT cell in-vivo activation with αGalCer restored their anti-tumor function suggesting that iNKT cells can be modulated to overcome CRC-associated immune evasion. Tumor co-infiltration by iNKT cells and neutrophils correlates with negative clinical outcomes highlighting the importance of iNKT cells in the pathophysiology of CRC. Our results reveal a functional plasticity of iNKT cells in CRC suggesting a pivotal role of iNKT cells in shaping the TME with relevant implications for treatment

ID: 138
Abstract
Topics: Neonatal window of opportunities, Microbe-immune interactions at body surfaces
Keywords: microbiota, neonatal window of opportunity, immune imprinting, autoimmunity

Consequences of diet-microbiota interplay at weaning on lifelong immunity

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The intestinal microbiota plays a key role in the development and functional maturation of the immune system. Weaning is a critical time window early in life during which the vast expansion of bacteria colonizing the intestine, caused by the introduction of solid food, induces a strong immune response termed "weaning reaction". This reaction is necessary to prevent increased susceptibility to gut inflammation in adult mice, a phenomenon called "pathological imprinting". However, the cellular and molecular mechanisms behind the weaning reaction leading to a pathological imprinting remain unknown.

This project aims to define how dietary and microbial components early in life affect the generation of the weaning reaction and the susceptibility to develop autoimmune diseases later in life.

We characterized the gut microbiota composition and immune responses in mice exposed to diets with different fat/fiber ratios early in life and assessed the long-term consequences on the development of autoimmunity using a model of streptozotocin-induced diabetes. We found that the diet strongly affected fecal phyla composition of the weaning mouse, leading to increased levels of Firmicutes in response to a high fiber diet, while a low-fiber diet caused expansion of Verrucomicrobiota. Our results also show that intestinal CD4+ T cell responses are highly dependent on dietary and microbial components. Mice fed a diet rich in fat early in life develop more severe type 1 diabetes compared to mice fed a high fiber diet or normal chow. Transfer of the microbial dysbiosis to neonatal germ-free mice, but not to adult, was sufficient to recapitulate the phenotype observed in conventional mice.

Our findings demonstrates that the gut microbiota composition early in life imprints the immune system with long-term consequences on the development of autoimmune diseases and provides fundamental evidence that early life exposure to commensal bacteria can prevent pathological imprinting.

ID: 139
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections

Indole-3-aldehyde reduces inflammatory responses and restores intestinal epithelial barrier function partially via aryl hydrocarbon receptor (AhR) in experimental colitis models

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Background and Aims: Indole-3-aldehyde (IAld), a bacterial metabolite derived from tryptophan, has been shown to improve intestinal epithelial barrier (IEB) function through the aryl hydrocarbon receptor (AhR) in murine colitis models. However, the impact of IAld on intestinal tissue inflammation remains unexplored. This study aimed to investigate the effects of IAld on the inflammatory responses of the gut both in vivo and in vitro and probe the mechanisms by which IAld attenuates colitis.

Methods: Male C57BL/6 mice (n=8 per group) were orally gavaged with IAld, IAld and an AhR inhibitor (CH-223191 (CH-22)) or olive oil as a vehicle for 8 days, followed by supplementation of 2.5% dextran sulfate sodium salt (DSS) in drinking water for 7 days to induce colitis. The basic indicators related to inflammatory response and IEB functions were assessed. To further probe the function of IAld in vitro, macrophage cells (RAW264.7 and THP-1) and intestinal epithelial cells (Caco-2) were stimulated with lipopolysaccharide (LPS) with IAld, IAld and CH-22, or DMSO as a control. The inflammatory responses and epithelial barrier functions were measured.

Results: IAld reduced IL-6, IL-1β and TNF-α protein levels in both colonic tissues from mice with colitis and LPS-stimulated macrophages. The IAld-mediated reduction of IL-6, but not IL-1β and TNF-α was through AhR activation. Furthermore, Nuclear Factor-κB (NF-κB) pathway was found to be inhibited by IAld treatment via AhR activation both in animals and cells. Gut permeability was significantly improved by IAld in both DSS-treated mice and LPS-stimulated Caco-2 cells. These effects were through downregulation of phosphorylated myosin light chain (pMLC), which is mediated by AhR activation.

Conclusions: IAld improved DSS-induced colitis by inhibiting the inflammatory responses and restoring intestinal epithelial barrier function, partially via AhR activation. This work provided insight into the function of IAld in modulating gut inflammation.

ID: 140
Abstract
Topics: Systems immunology in host-microbe interactions
Keywords: nose microbiome, bronchectasis, serum inflammatory biomarkers

Correlations of upper airway microbiome and serum inflammatory biomarkers in bronchiectasis patients

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Background. Human body hosts many distinct microbial ecosystems, both inside and outside. Increasing amount of research reports a microbiota inhabiting the airways and lungs and maintaining healthy state. In states like bronchiectasis, disease course and severity is associated with a significantly divergent bacterial communities. Sampling human lung microbiota is an invasive procedure, thus we assessed microbiomes of upper respiratory tract (nose) in bronchiectasis patients and donors, compared those to the bronchial lavage fluid microbiomes and serum biomarkers.

Objectives. Our aim was to compare the composition of nose microbiota in patients with bronchiectasis and in healthy individuals, and select biomarkers, which could reveal information about the progress and prognosis of the disease.

Methods. Samples were collected by anterior nasal swabbing and sequenced using Oxford Nanopore Technologies 16S Barcoding Kit (SQK-RAB204) and sequenced using Flongle Flow Cell (R9.4.1). Taxonomic classification was performed by using Emu and NanoCLUST pipelines. Serum and saliva biomarkers were assessed in parallel.

Results. Over 40 nose swab samples were collected (from healthy individuals and patients with bronchiectasis). No significant differences were observed between the microbiome richness and diversity in healthy individuals vs. bronchiectasis patients. However, we detected differences in microorganism composition, and several possible biomarkers could be suggested to be associated with bronchiectasis. Some of the microorganisms selected were also associated with systemnic inflammation biomarkers.

ID: 141
Abstract
Topics: Neonatal window of opportunities

The impact of intestinal colonization on T lymphocytes in breast milk

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Early life represents the most decisive period when it comes to the development of host-microbiota mutualism and immune homeostasis. Hence, this period is often referred to as the window of opportunity. During this time window, environmental exposures have the most notable impact on the microbiota and immune system. Breast milk, as the predominant diet during the first months of life, is one exposure, which shapes both the developing microbiota and the immune maturation of the host. Apart from providing essential nutrients for neonatal growth, it is a rich source of immunological constituents, such as antibodies, that protect the offspring from infections and imprint its immune system. The composition of breast milk itself is very dynamic and influenced by factors, including the lactation stage. Recently, metabolites derived from the microbiota during pregnancy have been found in breast milk, suggesting that intestinal colonization affects the composition of breast milk and may consequently have functional effects on the neonatal immune system. We thus aim to understand to which extent maternal microbiota shapes the phenotypic composition of breast milk. Our focus lies on its immunological constituents, including immune cells and cytokines. We compare the breast milk of germ-free and specific pathogen-free mice.

Interestingly, we found differences in the frequency of CD4+ T cells in the milk of mice with distinct microbiota composition, which were mirrored in the mammary glands. These differences resulted in an altered CD4:CD8 T cell ratio and we further detected altered cytokine profiles between the groups. Whether these differences arise from altered T cell proliferation or effector function is currently investigated. Our research will reveal how the microbiota influences T cells in the mammary gland and breast milk to uncover understudied factors that may improve neonatal health.

ID: 142
Abstract
Topics: Mucosal T cells, Neonatal window of opportunities
Keywords: Early life intestine, γδ T cells, Lactobacillus reuteri

Early-life L. reuteri colonization facilitates a defense-to-maintenance transition of the intestinal γδ T cell compartment

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At birth, bacteria rapidly colonize the newborn intestine and due to milk-dominated nutrition, highly adapted microbial communities assemble. They lastingly imprint the host's immune system and disease susceptibility, a phenomenon coined 'window-of-opportunity'. As the adaptive immune system is still immature, the newborn intestine is protected by innate-like γδ T cell populations. These γδ T cells then transition from a defense to a maintenance phenotype but whether this trajectory is genetically hard-wired or dependent on microbial cues has remained unknown. To address this question in a gnotobiotic, reductionist system, we have focused on Lactobacilli as they are among the most abundant bacterial pioneers in both mice and men.

In line with earlier reports, the expansion of 'maintenance' Vγ7 γδ T cell populations in the intestinal epithelium was microbiota-independent. However, we also identified a simultaneous contraction of RORγt+ γδ T cells associated with an innate-like immune defense. To assess if components of the early life microbiota could regulate these cells, we mono-colonized newbornmice with Lactobacillus reuteri. Strikingly, we discovered that L. reuteri alone accelerated the pre-weaning reduction of RORγt+ γδ T cells in both small intestinal epithelium and lamina propria. We characterized this effect to be independent of butyrophilin-like molecules, aryl hydrocarbon receptor signaling and neither found L. reuteri to influence lineage plasticity of RORγt+ γδT cells. Based on these findings we propose that L. reuteri facilitates a defense-to-maintenance maturation of γδ T cells pre-weaning to prepare the host for a more level-headed encounter with the myriad of foreign antigens and xenobiotics during the first ingestion of solid food.

ID: 143
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections

Gasdermin D independent canonical inflammasome responses cooperate with caspase-8 to establish host defense against gastrointestinal Citrobacter rodentium infection

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Citrobacter rodentium is an enteropathogen that causes intestinal inflammatory responses in mice reminiscent of the pathology provoked by enteropathogenic and enterohemorrhagic Escherichia coli infections in humans. C. rodentium expresses various virulence factors that target specific signaling proteins involved in executing apoptotic, necroptotic and pyroptotic cell death, suggesting that each of these distinct cell death modes performs essential host defense functions that the pathogen aims to disturb. However, the relative contributions of apoptosis, necroptosis and pyroptosis in protecting the host against C. rodentium have not been elucidated. Here we used mice with single or combined deficiencies in essential signaling proteins controlling apoptotic, necroptotic or pyroptotic cell death to reveal the roles of these cell death modes in host defense against C. rodentium. These mouse genetic experiments revealed an essential cooperation between caspase-8 signaling and GSDMD-independent canonical inflammasome signaling to establish intestinal and systemic host defense against gastrointestinal C. rodentiuminfection.

ID: 144
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Inflammatory bowel disease, glucocorticoids, intestinal barrier function, intestinal mucosa

Glucocorticoids exert administration pathway dependent intestinal barrier weakening effects in experimental colitis

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Glucocorticoids (GC) are important antiinflammatory and immunosuppresive agents in the management of inflammatory bowel disease and many other conditions. However, GC also display deleterious effects on the intestinal barrier which may limit their clinical benefit. These effects may be ascribed partly to actions on the epithelium. In this regard, mice carrying a deletion of the GC receptor in intestinal epithelial cells are protected against experimental colitis. It may be therefore beneficial to limit GC epithelial actions in the inflamed intestine. In this study, experimental colitis was induced by administration of 2.5% dextran sulfate sodium for 7 days to C57BL/6J female mice and treated with prednisolone 200 µg/day by either the oral or parenteral route (as prednisolone phosphate). Prednisolone ameliorated colitis, based on downregulated expression of inflammatory markers and lower colon thickening. However, some GC treated mice exhibited bacterial translocation to the liver (4 of 10). Nevertheless, oral but not intraperitoneal prednisolone augmented 4 kD FITC-dextran permability. This correlated with plasma lipopolysaccharide (LPS) levels, which was higher with oral prednisolone. In addition, hemorrhage occurred earlier in oral prednisolone treated mice, and blood loss was enhanced by the GC. Besides, mice treated with oral prednisolone show an increased disease activity index (DAI, based on body weight loss, feces consistency and rectal bleeding). On the other hand, GC administration suppressed Cyp11a1 expression in the colonic mucosa and in jejunal organoids, suggesting inhibition of colonic steroidogenesis by exogenous treatment. These findings were largely reproduced using 6 µg/day of budesonide but, in this case, body weight loss was also augmented by glucocorticoid treatment. Our data are consistent with GC weakening of the mucosal barrier coexisting with antiinflammatory response, which may be enhanced by oral administration.

ID: 145
Abstract
Topics: Innate Immunity, Systems immunology in host-microbe interactions
Keywords: Sepsis, glucocorticoids, intestinal barrier function

Glucocorticoid receptor intestinal epithelial knockout mice show attenuated colonic inflammatory response but unaffected permeability in early experimental sepsis

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Sepsis is defined as an organic dysfunction due to an abnormally regulated response to infection. One of the main sources of infection in sepsis is believed to be the intestinal microbiota via traslocation through the mucosa to the bloodstream, which is associated to a weakened intestinal barrier function (IBF). Even if the management of sepsis has advanced in the last decades, mortality is still high and there are blanks in terms of pathological systems and long-term consequences. Glucocorticoids (GC) are part of the drugs used in sepsis, but they have only shown a moderate therapeutic effect. This fact may be caused by harmful effects of GCs on IBF. Previous results of our research group have shown that mice with induced deletion of the GC receptor (GR) in intestinal epithelial cells (i.e. NR3C1ΔIEC mice) are protected against dextran sulphate sodium (DSS)-induced colitis. In this study, we aim to understand the role of the intestinal epithelial GR and its involvement in IBF regulation in experimental sepsis, with the ultimate goal of improving the management of sepsis with GCs. The cecal ligation and puncture (CLP) model of sepsis was applied to WT C57BL/6J and NR3C1ΔIEC mice. After 24 h, CLP mice exhibited hypoglycemia and splenomegaly. IBF was weakened, as indicated by increased FITC-dextran plasma levels and downregulation of Tjp1 expression in colon. A modest increase in inflammatory markers (S100a8, Cxcl1) was noted in the colon and jejunum but also in lung and kidney. Similarly, the colonic expression of Cyp11a1 and Lrh1, involved in local steroidogenesis, was lower in CLP mice. Besides, CLP mice exhibited hypercorticosteronemia, which was associated to increased Cyp11a1 in the adrenes. Of note, both parameters were less pronounced in KO mice. Further studies will characterize the microbiota composition and phenotype in later stages and in the response to glucocorticoid treatment.

ID: 146
Abstract
Topics: Mucosal diseases and infections

A nervous gut: Effects of beta-adrenergic hyperstimulation, liver cirrhosis and ACLF on the small intestinal gut-vascular barrier (GVB)

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Acute on chronic liver failure (ACLF) is often characterized by precipitating events of which pathological bacterial translocation (BT) from the gut and/or bacterial infections have been proposed to be of particular pathophysiological relevance. In ACLF 1) excessive adrenergic drive has been shown by markedly increased serum levels of norepinephrine (Jalan et al. (2015) Liver international) and 2) non-selective beta-blocker (NSBB) therapy appears to improve short-term mortality (Mookerjee et al. (2016) Journal of Hepatology). The gut-vascular-barrier (GVB) is fundamental for limiting access of pathological BT to the gut-liver-axis but has not been addressed as for changes induced by adrenergic drive and/or ACLF.

We have demonstrated adverse effects of experimental liver cirrhosis, ACLF and beta-adrenergic hyperstimulation on the GVB using confocal laser endomicroscopy to visualize the extravasation (=pathological loss) of FITC-albumin into the duodenal lamina propria in mice. Further we have shown, that chronic peritoneal hyperstimulation by beta-adrenoceptor agonist isoproterenol reduces intercellular junctions in the GVB, such as claudin-5 and VE-cadherin, suggesting a paracellular loss of albumin in pathophysiological conditions and GVB impairment. To test the potential protective effect of NSBBs, isoproterenol treated mice with propranolol (NSBB) showed a delayed pathologcial extravasation of FITC-albumin.

ID: 147
Abstract
Topics: Mucosal diseases and infections
Keywords: human oral mucosa, chronic inflammation, fibroblast

Structural Remodelling of the Human Oral Mucosa in Chronic Inflammatory disease

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The relationship between tissue specificity and pathological signals in defining cell phenotype and heterogeneity in oral chronic inflammatory disease is not well understood. Here we asked whether diseased tissue structure drive changes in oral mucosa cell decisions, and whether there is a connection between immune composition and tissue architecture. We deconvolved expression for resolution enhancement of spatial transcriptomic data of human oral mucosa and defined highly specialised epithelial and stromal compartments describing location-specific immune programs. We found that the lamina propria can be divided in highly specialised spatial compartments distinctively involved in epithelial maintenance, ECM remodelling and immune regulation. Furthermore, we spatially mapped a rare pathogenic fibroblast population localised in a highly immunogenic region, responsible for lymphocyte recruitment through CXCL8 and CXCL10 and with a possible role in pathological angiogenesis through ALOX5AP. We hypothesise that this fibroblast may help in the transition from innate to adaptive immunity and in the development of persistent inflammation characteristic of oral chronic disease. Collectively, our findings suggest that tissue-specific transcriptional programmes shape the identity of local functional cellular subsets. This influences the composition of populations in pathology and their associated functions, which may explain the predisposition of anatomical sites to certain types of disease, such as the junctional epithelium in oral chronic inflammatory disease.

ID: 148
Abstract
Topics: Innate Immunity, Systems immunology in host-microbe interactions
Keywords: sepsis, alkaline phosphatase, TNAP

Tissue-nonspecific alkaline phosphatase (TNAP) enzyme alters systemic inflammation in cecal slurry-induced sepsis

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Sepsis is defined as an organic dysfunction due to an abnormally regulated response to infection. Alkaline phosphatases (APs) catalyze the hydrolysis of phosphate groups from different substrates, such as bacterial lipopolysaccharide (LPS), an important mediator in the pathophysiology of sepsis. Different studies have demonstrated that intestinal AP (IAP) reduces intestinal permeability, intestinal inflammation, bacterial translocation and, as a consequence, systemic inflammation. However, a number of studies have demonstrated the importance of the tissue-nonspecific alkaline phosphatase (TNAP) isoenzyme in inflammatory processes. We investigated the effect of TNAP on polymicrobial sepsis induced by cecal slurry (CS) injection. WT and heterozygous TNAP mice (TNAP+/-) were administered with 1 mg CS/kg intraperitoneally and sacrificed after 24 h. CS-induced sepsis resulted in significantly higher weight loss in TNAP+/- mice but with no differences in behavioural outcomes. Oral fluorescein isothiocyanate-dextran 4 kD administration showed intestinal barrier deficiency in CS administered mice, with no differences between genotypes. Interestingly, heterozygous septic mice exhibited attenuated inflammatory markers in colonic tissue (Cxcl1 and S100a8). Moreover, in the liver and lung, TNAP+/- mice displayed a reduced inflammatory response compared to WT. These preliminary results show that TNAP plays an important role in CS-induced systemic inflammation producing a lower activation state.

ID: 149
Abstract
Topics: Innate Immunity, Systems immunology in host-microbe interactions
Keywords: alkaline phosphatase, experimental colitis, TNAP

Mice carrying a conditional intestinal epithelial deletion of tissue-nonspecific alkaline phosphatase exhibit increased infiltration but reduced expression of inflammatory markers during experimental colitis

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Alkaline phosphatases (APs) catalyze the hydrolysis of phosphate groups from different substrates. Recently, APs have received attention due to their protective role in inflammation and their implication in intestinal barrier function. It has been described that there is an increased expression of the tissue-nonspecific AP (TNAP) isozyme during intestinal inflammation, not only due to the infiltration of immune cells, but also to the increased expression of TNAP in intestinal epithelial cells (IECs). However, the specific physiological role of TNAP in the intestinal epithelium cells during inflammation is not well known. We studied the role of TNAP in experimental colitis using epithelial-specific Alpl deficient mice (Alplflox/flox-Villin-CreERT2, referred to as AlpIIEC-/-) employing dextran sodium sulfate (DSS) at 2.5 % for 7 days. Mice receiving DSS exhibited substantial loss of body weight between days 4 and 7, which was enhanced in AlpIIEC-/- mice. The disease activity index (DAI) score was increased early after DSS exposure in AlpIIEC-/- mice owing to early bleeding and sticky stools, although it was later normalized. No differences were observed between genotypes in colon shortening. Histopathological assessment of the colon revealed a higher leukocyte infiltration in DSS-treated AlpIIEC-/- compared to WT mice. However, AlpIIEC-/- mice exhibited an attenuated expression of various inflammatory response markers measured by qRT-PCR, including S100a8, Il6 and Tnf. Colonic AP activity was greater in DSS-treated mice, with no differences between genotypes. Interestingly, AlpIIEC-/- mice displayed higher colonic expression of genes involved in mucosal barrier maintenance, Muc4 and Tff3. This increased expression was observed both in baseline conditions and with DSS administration. Lack of Alpl in the intestinal epithelium also caused an increased expression genes related to the signaling pathway of LPS (Cd14 and Lbp) in the liver. Therefore, AlpIIEC-/- mice present greater colonic infiltration and histological damage but lower expression of proinflammatory markers in the intestine.

ID: 150
Abstract
Topics: Mucosal diseases and infections

Role of innate immunity in modulating infection and drug treatment in a human lung organoid model

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While commensal bacteria generally respect natural barriers of the human body, pathogens are able to breach epithelia, invade deeper tissue layers and cause life-threatening infections. Pseudomonas aeruginosa, an opportunistic human pathogen, is a leading cause of severe hospital-acquired pneumonia, with mortality rates as high as 50% in mechanically ventilated patients. Effective colonization and breaching of lung mucosa are hallmarks of P. aeruginosa pathogenesis. However, infection studies are hampered by the lack of experimental models that faithfully recapitulate the physiology of human tissue while offering the experimental power to investigate the infection process with high temporal and spatial resolution.

Here, we establish an in vitro 3D lung infection model from human stem cells, with air-liquid interface in a Transwell. Immunocytochemistry- and histology staining confirmed that the architecture and cellular composition of the tissue closely resembled the human bronchial epithelium. Using live cell microscopy, we demonstrate that the tissue recapitulates lung functions such as production of mucus and cilia beating. Next, we established standard infection conditions for P. aeruginosa. The lung tissue protects itself by secreting mucus, shedding infected cells, repairing tissue damage and accelerating cilia beating. However, P. aeruginosa still efficiently colonizes the tissue. Together, our preliminary results establish lung tissue Transwells as a versatile in vitro tool to study bacterial infections and host response in a human-like environment.

Next, we aim to integrate appropriate immune cells into the 3D model to study innate immune response upon P. aeruginosa infection in a complex and physiologically relevant setting.

ID: 151
Abstract
Topics: Mucosal diseases and infections, Swiss Young Immunologists Society (SYIS) and young European Federation of Immunological Societies (yEFIS)
Keywords: mucosal immunity, SARS-CoV2 vaccine, IgA, smell, taste

Long-term systemic and mucosal SARS-CoV-2 IgA response and its association with persistent smell and taste disorders

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Current approved COVID-19 vaccines, notably mRNA and adenoviral vectored technologies, still fail to fully protect against infection and transmission of various SARS-CoV-2 variants. The mucosal immunity at the upper respiratory tract represents the first line of defense against respiratory viruses and is thus critical to develop vaccine blocking human-to-human transmission.

After a cluster of COVID-19 at the Percy teaching military hospital in March 2020, we have followed a cohort of hospital workers and a sample of subjects has been selected to measure systemic and mucosal Immunoglobulin A (IgA) response. We have analyzed serum and saliva from 133 healthcare workers following a mild infection (Wuhan strain, n=58) or not infected (n=75), and after SARS-CoV-2 vaccination (Vaxzevria®/Astrazeneca and/or Comirnaty®/Pfizer). IgA were quantified using an electrochemiluminescent multiplex immunoassay. Seroneutralization assay were performed for all serum samples and for saliva, an ACE2 inhibition assay have been conducted.

While serum anti-SARS-CoV-2 Spike IgA response lasted up to 16 months post-infection, IgA response in saliva had mostly fallen to baseline level at 6 months post-infection. Vaccination could reactivate the mucosal response generated by prior infection, including neutralizing antibodies since the inhibition of ACE2 binding to SARS-CoV-2 has increased with the number of doses received. But in naive individuals, vaccination failed to induce a significant mucosal IgA response by itself. Anti-Spike N terminal domain (NTD) IgA displayed interesting results as early post-COVID-19 serum anti-NTD IgA titer correlated with seroneutralization titers while its saliva counterpart positively correlated with persistent smell and taste disorders more than one year after mild COVID-19.

As breakthrough infections could be correlated with IgA levels, other vaccine platforms inducing a better mucosal immunity are needed to control COVID-19 infection in the future. Our results encourage further studies to explore the prognosis potential of anti-Spike-NTD IgA in saliva at predicting persistent smell and taste disorders.

ID: 152
Abstract
Topics: Innate Immunity, Mucosal diseases and infections

Protective effects of pterostilbene on experimental colitis via activation of the Nrf2 pathway

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Background & Aim: Pterostilbene has been suggested to be a promising dietary supplement to regulate the immune response in multiple disease models due to its agonistic effect on nuclear factor erythroid 2-related factor 2 (Nrf2). However, the role of pterostilbene-mediated Nrf2 activation in experimental colitis has not yet been investigated. Therefore, the aim of this study was to investigate the role of pterostilbene-mediated Nrf2 activation in regulating mucosal inflammation during colitis.

Methods: A total of 24 male wild-type C57BL/6 mice were used (n=6/per group). Mice were administered pterostilbene by oral gavage. A total of 2.5% dextran sodium sulfate (DSS) in drinking water was given to mice to induce colitis. To further investigate the underlying mechanism of pterostilbene in regulating mucosal inflammation, macrophage cell lines (RAW264.7 cells and THP-1 cells) were treated with lipopolysaccharide (LPS) with or without pterostilbene. The indicators related to inflammatory and oxidative responses in colonic tissue or cells were measured correspondingly. In addition, faecal samples from mice were collected and analysed by 16S rRNA sequencing.

Results: In mice with colitis, pterostilbene remarkably improved the disease activity index of the mice and reduced the expression of the proinflammatory cytokines TNF-α and IL-6 and ROS levels in the colonic tissue. Pterostilbene also upregulated the expression of Nrf2 pathway-related proteins. 16S rRNA analysis revealed that pterostilbene improved the diversity of the gut microbiota by increasing the abundance of probiotic bacteria and decreasing the abundance of harmful bacteria in mice with colitis. In vitro, pterostilbene-mediated Nrf2 activation markedly reduced proinflammatory cytokine TNF-α and IL-6 production and oxidative stress induced by LPS.

Conclusion: Pterostilbene attenuated mucosal inflammation in mice with colitis by suppressing the oxidative stress and inflammatory response of macrophages. These beneficial effects of pterostilbene on colitis may occur through the activation of Nrf2 pathway.

ID: 153
Abstract
Topics: Innate Immunity
Keywords: Amino acids, dendritic cells, immunity, metabolic reprogramming

Glutamine metabolism and transport regulates TLR-induced IL-23 production in dendritic cells

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Dendritic cells (DCs) integrate both immune stimuli and metabolic signals to modulate their function. We previously demonstrated that fatty acids induce a distinct immunometabolic profile in TLR-activated DC, characterized by high IL-23 production and reduced glycolysis. Here, we demonstrate that glutamine and related amino acids affect IL-23 production and its exacerbation by palmitic acid. TLR-mediated activation increases expression of key amino acid-related enzymes such as Asns, while decreasing Gls, Glud1, and Got2. Depletion and siRNA experiments show that glutamine and aspartic acid deficiency, like palmitate supplementation, increases IL23 production in TLR-activated DCs. Palmitate modulates these changes, notably decreasing expression of the important glutamine transporter Slc1a5, suggesting that fatty acids may induce amino acid deprivation. Fatty acid and amino acid signals may converge at the integrated stress response and hexosamine pathway. These results shed new light on the complex role of amino acid metabolism in the tuning of innate immune responses.

ID: 154
Abstract
Topics: Neonatal window of opportunities
Keywords: epigenetics, maternal microbiota, intestinal barrier, pregnancy, neonate

The maternal microbiota during pregnancy modifies the neonatal epigenome to refine epithelial energy homeostasis and improve intestinal barrier integrity

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To anticipate the overwhelming amounts of antigens at birth, the gathering of our immune strategies takes place prenatally. The maternal microbiota plays a vital role in this process by transferring microbial metabolites and antibodies via the placenta. Using the auxotrophic E. coli strain HA107, it is possible to reversibly colonize germ-free dams during gestation and distinguish between the contribution of the maternal microbiota from the influence of postnatal colonization. We hypothesize that microbe-host interactions in the maternal gut shape the offspring cell physiology and maturation of intestinal epithelial cells by conditioning its epigenome prenatally.

Transient gestational colonization triggered a DNA hypomethylation in genes relevant to the intestinal barrier function and energy homeostasis in the 14-day old progeny. While the accessible chromatin in gene networks mediating barrier integrity allowed an immediate increase in transcription, genes for energy homeostasis were only epigenetically modified, suggesting the necessity of an additional stimulus. Postnatal SPF conventionalization at birth could not re-establish a DNA methylation loss neither for barrier function genes nor in energy homeostasis, indicating an exclusive and long-lasting role of in utero priming. Only during the metabolic adaptation at weaning the integrated epigenetic marks driven by transient HA107 exposure were able to increase aerobic respiration and further drive gene expression of xenosensors.

The modifications of the epigenetic landscape in the offspring enhanced functional plasticity and translated into accelerated epithelial maturation, which rendered the gestationally exposed offspring more resilient to cellular stress and intestinal damage. Our research will reveal essential insights into epigenetic mechanisms due to interactions between the maternal microbiota, the embryo, and the neonate. It will strengthen the importance of a healthy maternal microbiota during pregnancy for neonatal health and display its durable consequences.

ID: 155
Abstract
Topics: Innate Immunity, Microbe-immune interactions at body surfaces
Keywords: Innate immunity, Bordetella pertussis, nasal mucosa, myeloid cells, mouse model

Deciphering the early innate immune response to Bordetella pertussis

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Bordetella pertussis, a strictly human pathogen, elicits a highly contagious respiratory disease known as pertussis, or whooping cough. Current mouse models enabled identification of many bacterial virulence factors and development of pertussis vaccines, but the mechanisms underlying the process of B. pertussis transmission during the catarrhal phase of pertussis disease remain largely unexplored due to lack of a convenient animal model. Recently, we have introduced immunodeficient MyD88 knock-out mice to achieve a human-like high level of nasal mucosa infection that triggers rhinitis and catarrhal shedding of bacteria from mouse nasal cavity and transmission of the infection onto co-housed adult animals (Holubova et al., 2022, PLoSPathog 18: e1010402). Here, we characterized the early innate immune response of the conventional C57BL/6 mice and the MyD88-deficient mice upon intranasal challenge with B. pertussis. Flow cytometry analysis of cells from nasal tissue shows how multiple immune cell populations infiltrate the nasal mucosa after infection in both strains of mice. Single-cell RNA sequencing revealed that nasal mucosa response of conventional mice infected with B. pertussis is characterized by the expansion of a highly activated neutrophil subset characterized by an interferon-stimulated gene signature, that does not develop in the MyD88KO mice. Moreover, the B. pertussis infection is associated with an upregulation of expression for genes encoding antimicrobial peptides (lipocalin 2) and chemoattractant molecules (Lix and Cxcl10) as detected by qPCR analysis. These results open the way for a detailed understanding of B. pertussis clearance from nasal mucosa of the host and highlight the differences in early immune responses between the conventional and immunocompromised mouse models.

ID: 156
Abstract
Topics: Systems immunology in host-microbe interactions
Keywords: Probiotics, Influenza virus, Gut-lung axis, pigs, Bacillus

Probiotics against Influenza A virus in pigs

Katrine Winther (University of Copenhagen, Denmark)

Swine Influenza Virus (SwIV) is a pathogenic virus that results in swine disease with a huge economic impact worldwide. SwIV can lead to acute respiratory infection in pigs and is generally considered one of the most important viral diseases in commercial pig production.

Clinical studies in pigs and mice indicate that probiotics can reduce the risk or duration of respiratory tract infections and that some probiotics have antiviral effects; however, the exact antiviral mechanisms of

probiotics are unclear.

Studies have suggested that probiotics can enhance the resistance to enteric viral infections, and recently, it is becoming apparent that probiotic-immune cell interactions in the intestine can modulate immune function in other mucosal tissues such as the respiratory tract; the so-called gut-lung axis.

In the study, pigs were either fed a diet with Bacillus subtilis-975 (2.6x10^6 CFU/g feed) or a control diet for 14 days before being challenged intranasally with 1,58x10^5 TCID50/ml Influenza A virus (IAV strain A/sw/Denmark/12687/03 (H1N2). The microbial composition of feces collected prior and during the challenge was profiled via 16S rRNA gene sequencing. Nasal swabs collected during the study and tissue from lung and ileum collected at necropsy was analyzed using microfluidic high throughput qPCR to investigate expression of more than 90 different immune related genes. Lung samples were collected for histology analysis. Preliminary results show that gross lung lesions are reduced in pigs fed the probiotic strain.

ID: 157
Abstract
Topics: Neonatal window of opportunities, Mucosal B cells

Tacking the fates and function of early life origin B cells in the adult mouse.

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The adult immune system consists of cells that emerged at various times during ontogeny. We aimed to deduce the numerical and functional contribution of early life origin B cells to the adult B cell pool in mice housed under specific pathogen-free conditions during unperturbed development. Lineage tracing mediated by the CD79aCreERT2 allele induced at various time points during ontogeny revealed that a substantial portion of adult B and plasma cells originated within a restricted neonatal window. In addition to B-1a cells, early-life time-stamped B cells included clonally interrelated IgA plasma cells in the gut and bone marrow. These were actively maintained by B cell memory within gut chronic germinal centers and contained commensal microbiota reactivity. Neonatal rotavirus infection recruited recurrent IgA clones that were distinct from those arising by infection with the same antigen in adults. Thus, a complex layer of neonatally imprinted B cells confer unique antibody responses later in life.

Single cell RNAseq confirmed that early life time-stamped B cells are unevenly distributed among molecularly defined B cell clusters in the adult mouse and revealed unique gene expression signatures compared to their adult derived counterparts. To deduce their combined role in the adult immune system we performed diphtheria toxin receptor mediated ablation of the entire layer of early life time-stamped B cells in adult mice. This treatment led to exacerbated T dependent B cell responses following prime/boost immunization by NP-KLH. We hypothesize that early life B cells harbor an immune regulatory function and that early life antigenic imprinting on B cell memory may control the set-point for immune responsiveness later in life.

ID: 158
Abstract
Topics: Mucosal T cells

T-box transcription factor Eomes is actively excluded from the epithelium of the small intestine

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T-box transcription factors play an important role in the formation of tissue resident memory CD8 T cells (TRM). Whereas graded expression of T-bet is a positive regulator of TRM formation, current evidence suggest a negative role for Eomes.

Using several complimentary in vivo models, we analysed the organ-specific regulation of Eomes at steady state, during infection with lymphocytic choriomeningitis virus (LCMV) and after adoptive transfer in the small intestine, the spleen and the kidneys. Intriguingly, we observed a specific absence of Eomes expression in T cells that adopt a tissue resident phenotype in the epithelium of the small intestine. This was further corroborated by the inverse upregulation of canonical TRM markers, such as CD69 and CD103. Adoptive transfer experiments of Eomes-expressing conventional CD8 memory T cells from EomesGfp/+ mice demonstrated that the majority of cells migrating into the spleen, liver and kidney maintained high expression of Eomes, while cells migrating into the epithelium of the small intestine showed a striking downregulation. Finally, using a transgenic mouse model, in which Eomes expression is driven by the Tbx21 (encodes for T-bet) promotor further suggested that Eomes, at least in part, cannot only be regulated at the transcriptional but also at the translational level.

In summary, our data underline a unique and organ-specific exclusion of Eomes in the epithelium of the small intestine for TRM formation.

ID: 159
Abstract
Topics: Mucosal T cells

Tumour-infiltrating non-Vδ1Vδ2 γδ T cells have tumour-promoting functions in humans

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γδ T cells are unconventional T cells that produce both anti-tumour and tumour

promoting cytokines and effector proteins. Generally, γδ T cell infiltration in tumor tissues is associated with an improved patient outcome. However, γδ T cells separate into functionally different subsets, based on the composition of the γδ T cell T cell receptor (TCR), and the contribution of these subsets to tumour immunity is not yet fully understood. Here we investigated specific γδ T cell subsets defined by their TCR, in colon tumours, unaffected colon mucosa, and blood from colon cancer patients. These studies showed that γδ T cell numbers were reduced in colon tumours compared to unaffected colon mucosa. Furthermore, the δ-chain composition of γδ T cell TCRs varied from patient to patient but there was a clear difference between different tissues. Circulating γδ T cells were predominantly Vδ2 cells, while both unaffected colon mucosa and tumours contained substantial fractions of non-VδVδ2 and Vδ2 cells but few Vδ1 γδ T cells. Additionally, we used mass cytometry to evaluate the expression of several markers for tumour-immunity and found that Vδ1, Vδ2 and non-Vδ1Vδ2 cells from colon tumours form separate clusters with different expression profiles. mRNA analyses revealed that the non-Vδ1Vδ2 cells expressed several genes with tumour-promoting functions such as IL-8, Galectin 3, and Transforming Growth Factor beta Induced (TGFBI). After polyclonal stimulation, we also found that the expression of IFNγ, TNF, and Granzyme B was lower in tumour-infiltrating non-Vδ1Vδ2 cells compared to both the Vδ1 and Vδ2 subsets. Infiltration of non-Vδ1Vδ2 cells seem to correlate positively with tumour differentiation and microsatellite status but inversely with advancing stage. In summary, our results show that substantial numbers of non-Vδ1Vδ2 □□ T cells infiltrate human colon tumours, and they are characterised by an expression profile indicative of tumour-promoting functions in colon tumours.

ID: 160
Abstract
Topics: Microbe-immune interactions at body surfaces, Systems immunology in host-microbe interactions
Keywords: IgA repertoire, dietary influences, gnotobiology

Diet determines IgA induction and repertoire characteristics independently of the microbiota

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A hallmark of IgA is the high mutational load that accumulates throughout life. This has been mainly interpreted in terms of differences in microbial induction, although once established in early life the microbiome of humans and experimental mice is relatively stable and mutational activity has been shown to be independent of B cell receptor signaling. Using different diets in germ-free and colonized mice formulated with proprietary grain-based processing or from purified chemicals with different principal macronutrient calorie sources, we show that diet affects IgA induction, its repertoire and mutational diversification independently of microbial exposure. Despite the vast number of chemical differences revealed by mass spectrometry between the different diets, strain combination experiments showed that differences in lipopolysaccharide (LPS) contamination signaling through TLR-4 and affecting germinal center activity and dendritic cell trafficking, were sufficient to explain differences between mucosal immune effects of the diets. Dietary composition and formulation can therefore be distinguished from the microbiota as a key factor in the extent of IgA induction as well as its characteristic repertoire.

ID: 161
Abstract
Topics: Gut-Microbiota-Brain axis
Keywords: Host-microbe interaction, small intestinal microbiome, metabolism, microbiota

Metabolic dynamics of the small intestinal microbiota upon nutritional challenges

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The small intestine is the main absorptive interface of the human intestinal tract, where most of the nutrients, vitamins and 90% of all calories are resorbed. A complex microbiota interacts intimately with the host and critically shapes intestinal function. However, due to difficult accessibility the human small intestinal microbiota remains barely investigated.

In our project, we utilize ileostomies as an efficient and non-invasive access route to the small intestinal microbiota. In a clinical study with two nutritional challenges (high carb vs. high fat) designed as a randomised cross-over trial, we are recruiting 70 stoma patients (50 with ileostomies, 20 control patients with colostomies). On two separate study days, stomata are sampled hourly (0–6h) after the nutritional challenges. Additionally, we acquire 3 blood and urine samples (0h, 3h, 6h).

We aim to characterise the small intestinal microbiota regarding its taxonomy (16S sequencing), biomass and metabolome. These data will assist us in choosing meaningful timepoints for metagenomic and mRNA sequencing analyses. Our goal is to perform a comprehensive “multi-omics” analysis: i) Co-occurrence analysis and other clustering approaches will identify networks of bacteria-bacteria and bacteria-metabolite interactions. ii) Metagenomic assembly and mRNA sequencing will allow interpretation of networks and their dynamics. Additionally, we are isolating individual bacterial strains from patient samples to assemble a bacteria library of 2’000 strains. With these isolates, we plan to reproduce key bacteria-metabolite interactions in vitro.

This will be the first comprehensive study of small intestinal microbiota dynamics using a network approach. Our project aims improve the currently limited understanding of the physiology of the small intestine in humans. Data and samples derived from our clinical study will enable testing of hypothesis in vitro and lay the ground for future disease-specific projects.

ID: 162
Abstract
Topics: Systems immunology in host-microbe interactions, Mucosal B cells
Keywords: Microbiota, Antibody, Immune response, Plasmodium, Malaria

Interplay between the Malaria Parasite and the Host Microbiota

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Most malaria-related deaths (> 80 %) in developing countries in sub-Saharan Africa affect fetuses of infected pregnant women and children under 5 years of age, who are highly susceptible to a malaria infection. Children with severe malaria are at increased risk for invasive bacterial disease, possibly related to gastrointestinal disorders such as diarrhea (5 – 38 %), suggesting a bidirectional relationship between malaria disease severity and gut dysbiosis. This includes shifts in gut microbiota composition and increased gut permeability. In addition, African children with severe malaria have been shown to frequently develop opportunistic infections caused by invasive enteropathogens, particularly non-typhoidal Salmonella.

In this project, we aim to investigate the effects of the humoral immune response during a Plasmodium blood stage infection in the context of the presence or absence of microbiota. We want to elucidate the influence of the microbiota on the generation of protective functional antibodies against the parasite and vice versa. Therefore, we use an infection model established by us, in which germ-free mice will be transiently colonized with an auxotrophic E. coli HA107 strain before and during malaria infection with attenuated P. berghei knockout parasites. Important readouts will include flow cytometric measurements of immune cell populations in lymphoid organs and antibody repertoire sequencing to study the clonality and maturation of the immune response. Furthermore, we plan to investigate how the exposure history affects the ability to control secondary infections (e.g., co-infection with Salmonella enterica).

Thus, we anticipate that the data of this study will provide insights into how the composition of the gut microbiota can be strategically modulated to reduce the risk of Plasmodium infection in mice.

ID: 163
Abstract
Topics: Microbe-immune interactions at body surfaces
Keywords: Transcriptional recording, Intestinal microbiome, Intestinal function, Noninvasive diagnosis, Engineered Escherichia coli

Assessment of human intestinal composition and function using transcriptional recording sentinel cells

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Malnutrition can cause disordered development, growth, and immunity. Suitable methods to examine intestinal composition and function are central for diagnosing and treating disease. However, diagnostics to assess dietary sufficiency and intestinal functionality are mainly indirect and/or require fasting or purging. The challenge remains to have a non-invasive system sensing nutrient biochemistry in fed and fasted individuals at the intestinal sites of nutrient uptake and host-microbial exchange. We address this challenge with a sentinel-cell-based transcriptional recording system composed of E. coli harbouring a plasmid-bound RNA CRISPR spacer acquisition complex (RT-Cas1-Cas2). Upon plasmid induction, the sentinel cells 'record' their gene expression on DNA-encoded CRISPR arrays and thereby, when passing through the intestine, incorporate characteristic physiological and pathological signatures. By deep sequencing of the recording plasmids from fecal samples termed 'Record-Seq', these transcriptional histories are recovered. Murine experiments have shown the capacity of Record-Seq to capture dynamic conditions present in vivo, including quantitative shifts in molecular and chemical features resulting from alterations in intestinal inflammation, nutrition, and bacterial composition. Hence, we think Record-Seq might be useful as a non-invasive diagnostic tool in human patients. We aim to characterize the functionality of the transcriptional recording sentinel cells ex vivo in human stoma-derived intestinal cultures. Specifically, we will establish a culture system recapitulating the intestinal environment under different nutritional conditions; assess the capacity of Record-Seq to report clinical-relevant scenarios in stomal culture perturbation experiments; and apply and refine the Record-Seq system with patients stratified according to micronutrient deficiencies and chronic malnutrition. Demonstrating the value of Record-Seq in these proof-of-concept experiments could serve as a turning point towards initiating further translational research and eventually open the door to broad application in personalized diagnostics.

ID: 164
Abstract
Topics: Mucosal T cells, Mucosal B cells
Keywords: early life, microbiota, immune cell development, T cells, HSC

Early life microbiota exposure shapes immune cell development

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Immunological dysregulation is the cause of many non-infectious human diseases such as autoimmunity, allergy and cancer. The gastrointestinal tract is the primary site of interaction between the host immune system and microorganisms, both symbiotic and pathogenic. Colonization with microbiota starts at birth when the neonate leaves the sterile environment of the uterus. We wondered whether the maternal microbiome also affects the offspring's immune system during gestation.

By transiently colonizing pregnant female mice, we showed that the maternal microbiota shaped different immune cell development of the offspring. Gestational colonization increased hepatocyte proliferation, accompanied by an altered liver transcriptional pattern in postnatal day 14 (P14) pups. These altered gene patterns at P14 suggested a deconditioning role of maternal microbiota in liver B cell ontogeny and HSC niche function, but higher cholesterol and retinol metabolism, cell adhesion, cell cycle regulation, protein stability and endopeptidase inhibitor activity. B cell repertoire in the offspring's liver was also altered by maternal microbiota, despite an aging effect that dominates the B cell repertoire. However, the diversity of B cell repertoire maturation in the liver was not influenced. Moreover, gestational colonization increased CD4 T cells and CD4 central memory T cells in the spleen from the pups at P14 and was maintained until P56. There was also an increase in CD8 effector memory T cells at P14, while a decreased naive T cells from both CD4 and CD8 single-positive T cells in the spleen of the pups at P14, but restored at P56. Not only the phenotype and function, but the T cell receptor (TCR) can also act as a molecular barcode in mice and humans to track T cells with clonal-relatedness through processes such as migration, differentiation, and proliferation. TCR sequencing will be performed to enrich the T cells of interest due to maternal microbiota exposure.

ID: 165
Abstract
Topics: Mucosal T cells, Mucosal B cells
Keywords: Immuno Oncology Cancer Human

Immuno-Oncology Imaging Mass Cytometry Study of the Structural and Cellular Composition of the Tumor Microenvironment in Human

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High-plex imaging is becoming an increasingly valuable tool to study the tumor microenvironment (TME) in immuno-oncology. Imaging Mass Cytometry™ (IMC™) enables the visualization and analysis of the complex spatial biology of the TME. IMC permits detailed assessment of cell phenotype and function using 40-plus markers simultaneously at subcellular resolution on a single slide without spectral overlap or autofluorescence. The ability to analyze multiple markers can facilitate accurate prediction of disease progression and clinical outcome measures from precious tissue samples and microarrays. Here, we demonstrate how our reagents and technology support these applications.

ID: 166
Abstract
Topics: Innate Immunity, Microbe-immune interactions at body surfaces

Dampening type 2 properties of group 2 innate lymphoid cells by a gammaherpesvirus infection reprograms alveolar macrophages

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Immunological dysregulation in asthma is associated with changes in exposure to microorganisms early in life. Gammaherpesviruses (γHVs), such as Epstein-Barr virus, are widespread human viruses that establish lifelong infection and profoundly shape host immunity. Using murid herpesvirus 4 (MuHV-4), a mouse γHV, we show that after infection, lung-resident and recruited group 2 innate lymphoid cells (ILC2s) exhibit a reduced ability to expand and produce type 2 cytokines in response to house dust mites, thereby contributing to protection against asthma. In contrast, MuHV-4 infection triggers GM-CSF production by those lung ILC2s, which orders the differentiation of monocytes (Mos) into alveolar macrophages (AMs) without promoting their type 2 functions. In the context of γHV infection, ILC2s are therefore essential cells within the pulmonary niche that imprint the tissue-specific identity of Mo-derived AMs and shape their function well beyond the initial acute infection.

ID: 167
Abstract
Topics: Mucosal T cells, Neonatal window of opportunities
Keywords: Intraepithelial lymphocytes, IEL progenitors, extra-thymic lymphopoiesis

An Sstr2-Somatostatin chemotactic axis drives T cell progenitor homing to the intestines

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Progenitors of intraepithelial T cells migrate from the thymus to the intestines after birth where they develop into unconventional TCRgd and TCRab lymphocytes in a process of extra-thymic lymphopoiesis within cryptopatches. Mechanisms of IELp migration have remained unclear. Here we show that thymic IELp lack conventional chemokine receptors for gut homing, but express the somatostatin receptor Sstr2 and migrate to somatostatin and cortistatin. IELp homing to the gut is Sstr2-dependent and correlates with neonatal induction of Sst in neuro-endocrine cells and mesenchymal stromal cells of the lamina propria. Human fetal thymic IELp-like CD34+ cells express SSTR2 and intestinal stromal cells express SST at the time of initial T cell population, suggesting conserved mechanisms of progenitor seeding of the developing intestines. The results reveal an unexpected role for the Sstr2-Sst axis in early immune system development and extend to small peptide receptors the range of GPCRs that control developmental lymphocyte trafficking.

ID: 168
Abstract
Topics: Mucosal T cells, Microbe-immune interactions at body surfaces
Keywords: microbiome, metabolites, T cells, Gut, lymph nodes

Role of the ALPK1-ADP heptose signaling pathway in controlling immune tolerance towards the gut microbiota.

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The symbiotic relationship between the gut-resident microbiota and the host immune system promotes homeostasis ensuring the microbial ecosystem. Disruptions in these interactions can drive inflammation. Alpha protein Kinase 1 (Alpk1) is a newly described Pattern Recognition Receptor (PRR) sensing the Gram-negative bacteria metabolite ADP-heptose (ADPh). Alpk1 transcripts have been shown to be expressed by several different cell types, predominantly intestinal epithelial cells and innate immune cells.

Alpk1-deficient mice develop severe colitis in the Helicobacter hepaticus (Hh)/anti-IL10R model, showing enhanced T helper 1 (Th1) cells compared to control mice. How the Alpk1-ADPh signaling pathway directs T cells to adopt distinct functions remains poorly understood. To address this question, we used adoptive transfer of Hh specific T cells (HH7-2tg) into wild type (WT) or Alpk1-defective mice followed by Hh infection and immune characterization. Seven days post transfer, naïve HH7-2tg cells were primed to become induced T regulatory (iTreg) and T follicular helper (TFH) in the mesenteric lymph node (mLN) of WT mice and this correlated with enhanced iTreg expansion in the colonic lamina propria. By contrast in Alpk1 deficient hosts, there was reduced iTreg development with enhanced accumulation of RORγt-expressing Hh-specific T cells in the cecal patch and LP. Two-photon imaging studies revealed that HH7-2tg cells preferentially populate the gut isolated lymphoid follicles (ILFs) in Alpk1-defective mice. Cytokine composition of specific environmental niches is one of the key factors known to influence T cell differentiation and function. Relevant to that, in-vitro bone marrow dendritic cell co-stimulation with ADPh and LPS showed ALPK1 signaling modulation of proinflammatory cytokine secretion.

Understanding the pathophysiology behind Alpk1 deficiency may shed light on a novel process driving immune tolerance towards the gut microbiota.

ID: 169
Abstract
Topics: Neonatal window of opportunities
Keywords: tolerance, early life, microbiota

Early life imprinting of intestinal tolerance

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The intestinal immune system must establish tolerance to trillions of harmless commensal microbes that colonize the gut shortly after birth. We recently identified a novel lineage of RORgammat+ antigen-presenting cells (Thetis cells; TCs) that instruct tolerance to microbial antigens through generation of peripheral regulatory T (pTreg) cells in early life. Interestingly, TCs comprise four subsets (TC I-IV), distinguished by unique transcriptional and functional programs. TC I and III express tissue-restricted self-antigens and the autoimmune regulator Aire, whereas TC IV are distinguished by critical molecules for pTreg differentiation, suggesting distinct roles in tolerance to self vs foreign antigens. Importantly, TCs are enriched in gut lymph nodes during the first few weeks of life and decline sharply post-weaning, suggesting that TC abundance regulates the early life window for establishing intestinal immune homeostasis. However, the ontogeny of TCs and the cues that drive their differentiation and functional specialization remain unknown. Here, using genetic fate-mapping approaches, bone marrow and fetal liver chimeras, and single-cell multiomic analyses, we define the developmental pathway for TCs during embryogenesis and the neonatal period. My work so far has identified a key role for receptor activator of NF-kappaB (RANK) and its ligand (RANKL) in the transcriptional regulation of TC heterogeneity. Using multiple in vivo approaches to address the role of RANKL/RANK/NF-kappaB signaling in TC differentiation, we found that RANK signaling is essential in shaping TC heterogeneity, most notably TC I differentiation. Furthermore, we found that mesenteric lymphoid tissue organizer (LTo) cells are the critical source of RANKL. These findings support a model whereby cues within the developing lymph node promote TC differentiation. By delineating the development pathway for TCs, these studies will reveal how intestinal immune tolerance is established during early life and identify potential pathways that can be therapeutically targeted in settings of intestinal inflammation.

ID: 170
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal diseases and infections
Keywords: Polyreactive mucosal antibodies, Haemophilus, respiratory tract, infections

Polyreactive mucosal antibodies and Haemophilus abundance of the respiratory tract: predictors of recurrent respiratory tract infection severity in young children

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Background: Recurrent respiratory tract infections (rRTIs) are common in young children, and their clinical assessment is typically done by measuring serum antibody levels. RTIs, however, usually remain confined to the respiratory mucosa and serum antibody measurements may not accurately reflect local immune protection. We here address the role of airway mucosal antibodies and their interplay with the respiratory microbiota in RTI susceptibility.

Methods: We conducted a prospective cohort study in 100 children <10 years with rRTIs and their family members. Saliva and nasopharyngeal samples were collected, and serum immunoglobulin levels recorded. The level and reactivity of antibodies were determined (ELISA), and respiratory microbiota composition (16S-rRNA-sequencing) and respiratory viruses (qPCR) were measured in nasopharyngeal swabs. RTI symptoms were monitored using a daily mobile phone application.

Results: We found that 28% of saliva antibodies is polyreactive, while this is 2% for serum antibodies ($p<0.001$). Serum antibody levels and/or deficiencies were not associated with RTI severity, while mucosal polyreactive sIgA and IgG were negatively associated with recurrent lower RTIs ($p=0.01$ and $p=0.001$, respectively) and the number of respiratory viruses detected ($p=0.03$ and $p=0.06$, respectively). Increased abundance of Haemophilus influenzae/haemolyticus was associated with

lower levels of polyreactive saliva sIgA and IgG ($p=0.023$ and $p=0.049$, respectively), an increasing number of viruses ($p=0.04$) and RTI symptoms in winter ($p=0.04$).

Conclusion: These findings emphasize the importance of mucosal antibodies in preventing RTI infections and suggest that mucosal polyreactive antibody levels and H. influenzae/haemolyticus abundance may predict infection risk and severity in children with rRTIs.

ID: 171
Abstract
Topics: Gut-Microbiota-Brain axis
Keywords: GABA, probiotic, gut-microbiota-brain axis, CNS inflammatory demyelination

Protective GABAergic responses triggered by a genetically engineered Lactococcus lactis probiotic

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The gut-microbiota-brain (GMB) axis has emerged as a critical pathway in the regulation of neuroinflammation. Our past work demonstrates that the microbiota's composition affects the severity of neuroinflammation and that neuroinflammation impacts its composition. Induction of CNS disease resulted in significant reductions in gut lactic acid bacteria members and gamma-aminobutyric acid (GABA)-producing bacteria. We hypothesized that increasing intestinal GABA levels would reduce the severity of neuroinflammation and GABAergic responses in the CNS of mice. We genetically engineered a Lactococcus lactis with increased GABA production and tested it in experimental autoimmune encephalomyelitis (EAE) mice obtained from two commercial vendors with different microbiota compositions. GABA-specific ELISA showed a significant increase in neurotransmitter production when exposed to increasing concentrations of glutamic acid and time. In vivo, five times/week oral gavages with 5 x 108 CFU/mouse of GAD L. lactis but not with the empty-plasmid carrier L. lactis protected against EAE compared with sham-treated mice while preventing weight loss. However, protection depended on the host's gut microbiota makeup. Our results show that the increase of GABA at the intestinal level with the oral treatment with a probiotic strain protects against neuroinflammation.

ID: 172
Abstract
Topics: Innate Immunity

An innate immune process-driven pathway under control of PI3Kδ sustains intestinal immune fitness by integrating microbial surveillance with DC antigen presentation.

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Phosphatidylinositol-3-OH kinases (PI3Ks) participate in microbial surveillance signaling downstream of pattern recognition receptors (PRRs) and coordinate vesicular trafficking events in dendritic cells (DCs). Inborn mutations causing loss-of function or deficiency of PIK3CD gene encoding PI3Kδ results in immunodeficiency and colitis, but the immune mechanisms underlying its safeguarding function in mammalian antimicrobial immunity remain elusive. In this study, we show colitis driven by PI3Kδ inactivation involves enteric bacteria-reactive DCs triggering excessive CD8+ T cell and insufficient Treg responses. DC-based studies reveal that PRR-activated PI3Kδ optimizes innate responses within the immunophagosomes by promoting NOX2-dependent superoxide (NOX2-ROS) generation, dampening excessive inflammasome activation and IL-1β secretion and antigen priming of adaptive T cell responses. We demonstrate that PI3Kδ inactivation suppresses NOX2-ROS, causing abrupt phagosome acidification, and protein degradation overall impeding MHCII-restricted antigen presentation critical for Treg development. Our data show PI3Kδ is a vital component of DC immunophagosomes whereby microbial surveillance signals with antimicrobial processes are spatio-temporally united to fine-tune intestinal immune responses.

ID: 173
Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Plasmacytoid Dendritic Cell, Gut-Associated Lymphoid Tissue, Crohn's Disease

Assessing the distribution and functions of plasmacytoid dendritic cells in the human intestine during homeostasis and inflammation

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Intestinal immune homeostasis relies on the balance between tolerating commensal microbiota and being able to mount protective immune responses to pathogens. This balance is disturbed in inflammatory bowel diseases (IBD), such as Crohn's disease (CD), where disease pathology is associated with overly activated immune cells.

Plasmacytoid dendritic cells (pDC) have a central role in antiviral responses by secreting pro-inflammatory molecules such as type I interferons, tumor necrosis factor (TNF) and interleukin-12. However, other roles of pDC during homeostasis or inflammation are less understood, especially in peripheral tissues such as the intestine. Here, we used our novel human gut-associated lymphoid tissues (GALT) and GALT-free lamina propria (LP) isolation technique (Fenton et al., Immunity, 2020, Jørgensen at al., Nat Protocol, 2021) to assess pDC location and transcriptional profile in healthy and CD intestine. We find that pDC are located within GALT, including Peyer's patches and isolated lymphoid follicles, but not in GALT-free LP. Within GALT, pDC were restricted to the T cell zone in close contact with both CD4+ and CD8+ T cells. Further, scRNA-seq analysis suggested that GALT pDC express several factors relevant for T cell function including GZMB indicating potential roles in the regulation of T cell responses. Surprisingly, GALT pDC in health and CD did not express type 1 inteferons (IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17, IFNA21, IFNB1) and, in contrast to blood derived pDC, did not produce IFNA2 following in vitro stimulation with CpG. Indeed, our preliminary transcriptional analysis suggests that pDC have a more regulatory function in GALT in both health and CD. Further experiments are underway to functionally validate putative pDC interactions with T cells and their role in health and CD.

ID: 174

Abstract

Topics: Mucosal T cells, Systems immunology in host-microbe interactions
Keywords: IBD, T cells, Intestinal epithelial cells, microbiota, colitis

Understanding the Microbiota – Intestinal Epithelial cells - T cells Crosstalk in Inflammatory Bowel Disease

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The intestinal health is reflected by the composition and the diversity of the microbes that reside in it and inflammatory bowel diseases are associated with alterations in microbial composition. However, it is still unclear how the microbial composition influences the initiation and maintenance of inflammation. In previous work, we could demonstrate that RAG-deficient recipient mice housed in different facilities show different susceptibility to T cell transfer-induced colitis when T cells lack the transcription factor T-bet. To understand how the microbiota regulates colitis induction, we analysed the intestinal epithelial cells (IEC) and T cells in detail by transcriptional profiling. We gavaged RAG-deficient mice with the more “colitogenic” microbiota and could observe the induction of a proinflammatory interferon response in the IEC even before T cell transfer. Following transfer of T cells lacking either T-bet, RORgt or both, we observed colitis induction to varying degree in all mice. Single cell and TCR sequencing of reisolated intestinal lamina propria CD4+ T cells revealed certain effector T cell clusters shared across all genotypes and certain clusters unique to each genotype. Additionally, we observed a striking non-overlapping oligoclonal T cell response suggesting that specific but different bacteria drive the colitic response of the T cells of different genotypes. The colonic IECs show a transcriptional profile corresponding to the cytokine response of the T cells in addition to an increased DNA damage response and metabolic reprogramming. We hope that the detailed analysis of the crosstalk between the intestinal microbiota, IEC and T cells contribute to a better understanding of the etiology of inflammatory bowel disease.

ID: 175

Abstract

Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Epithelial Cells, Barrier disruption, MAPK´s, Cry1Ac, Transgenic crops

The stimuli of Cry1Ac toxin and protoxin activates MAPK´s and modify Zo-1 expression in differentiated monolayers of Caco-2 cells

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The Cry proteins, produced by Bacillus turingiensis are widely use as bioinsecticides to control crops plagues, and also had been considered innocuous for vertebrates. Nevertheless, our group has demonstrated that Cry1Ac proteins protoxin and toxin are mucosal and systemic immunogens able to activate macrophages via the activation of MAPK pathways, while the protoxin is also a promising adjuvant able to improve protection in different infection mouse models.

Currently genetically transformed crops, are able for human consumption and express Cry1Ac proteins. Because of this, humans are exposed to Cry1Ac proteins by the ingestion of these crops. It has also been published that the intragastric administration of Cry1Ac toxin provokes moderately allergy and lymphoid hyperplasia in large intestine. Therefore, it is important to characterize its effects in the intestines, particularly in epithelial cells.

We chose an in vitro model with CACO-2C2BBe1 cell line, that mimics a gut monolayer with brush border proteins and well stablished tight junction proteins expression, to determine the responses generated in the colon epithelial cells, after the stimulation with Cry1Ac. So, the aim of this study was to evaluate whether Cry1Ac proteins were able to induce the activation of MAPK signaling pathways in well stablished Caco-2 monolayers and if this activation modifies the tight junction protein expression. The results indicate that Cry1Ac proteins induce the activation of ERK, p38 and JNK. Also, the activation of MAPK´s, is linked to permeability changes and ZO-1 dysregulation. Finally, several binding to Cry1Ac proteins in intestinal epithelial cells CACO-2BB2e1 some of the identified proteins are HSP, cadherin and alkaline-phosphatase. In conclusion, Cry1Ac, toxin and protoxin proteins are not innocuous for vertebrate cells, because in human epithelial cells, CACO-2BBe1 cell line, they are able to activate MAPK´s and there were recognized by specific membrane binding proteins.

ID: 176
Abstract
Topics: Microbe-immune interactions at body surfaces

Sample logistics may impact on structural and functional profiles of faecal microbiota

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The intestinal microbiota is of pivotal importance for human health. To understand how the microbiota contributes to disease primarily faecal samples are being analysed due to their accessibility. However, logistics, e.g. collection and transportation of faecal samples for microbiota analyses are not standardised and methodological differences may introduce bias or impact on the results. We aimed to experimentally determine optimal parameters for sample logistics that preserve the faecal microbiota structurally and functionally.

We investigated the impact of time, temperature, cryoprotectant and oxygen exposure on the composition and metabolome of faecal microbiota. Faecal samples (n=6) were put under anaerobic conditions directly after defecation (baseline) and stored at RT, 4°C, -20 °C or at -20°C with 12.5% glycerol, with and without prior exposure to oxygen. After 4 h, 24 h, 48 h and 168 h, we determined community structure by microbiota flow cytometry (MFC) and functionality by short-chain fatty acid (SCFA) profiling and untargeted metabolomics using LC-MS/MS.

Hierarchical clustering showed a donor-dependent grouping of MFC-, SCFA- and global metabolome profiles. To elude the donor effect, we quantified the degree to which the different storage conditions altered the microbiota compared to baseline using the Bray-Curtis (BC) similarity. With increasing time, the BC similarity decreased, showing a significant negative correlation between BC similarity and time, again donor-dependent. Storage condition and oxygen exposure had little to no effect on microbiota community structure and SCFA abundances. However, the untargeted metabolome fingerprint showed a significant decline in BC similarity with increasing storage temperatures and the addition of glycerol altered the global metabolome profiles across donors.

Based on our results, we propose that transport as naïve sample at 4°C or -20°C without cryoprotectant for up to 24h before processing and subsequent storage at -80°C best conserves microbiota community structure, SCFA and untargeted metabolome profiles.

ID: 177
Abstract
Topics: Mucosal diseases and infections
Keywords: Goblet cell, Citrobacter rodentium, Nlrp6, Spdef, Tgm3

Using a model bacterial pathogen to test different aspects of goblet cell (GC) - intrinsic intestinal defence

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The intestinal epithelial surface is maintained essentially sterile from microbial insults by means of a functional inner mucus layer. Nonetheless, disruption of intestinal homeostasis is associated with the development of inflammation and colitis. In such an eventuality, GC associated responses ensure localised protection of the crypt thereby restoring homeostasis. We infected mice lacking functional sentinel GCs (Nlrp6) and the terminal GC differentiation factor Spdef with C. rodentium and evaluated their defensive capabilities. Mice lacking Nlrp6 (primarily females) displayed decreased protection to early epithelial invasion while mice lacking Spdef (primarily males) showed delayed pathogen clearance. Interestingly, mice without TGM3 (an enzyme that crosslinks Muc2) did not display phenotypic differences to C. rodentium susceptibility. Our results indicate that GC defence mechanisms are functionally compartmentalized and are focused on categorical expulsion of C. rodentium.

ID: 178
Abstract
Topics: Microbe-immune interactions at body surfaces
Keywords: EVs, exosomes, parasites, neutrophils

Neutrophil targeting by H. polygyrus bakeri extracellular vesicles

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The gastrointestinal parasite Heligmosomoides polygyrus bakeri has evolved multiple pathways of immune suppression to promote long-term survival in the mouse gut. Recent work has uncovered a novel pathway in which extracellular vesicles (EVs) secreted by the parasite suppress host immune responses using RNA interference. H. poly EVs contain small RNAs and an argonaute protein called exWAGO, which work together to supress specific host genes. Mice immunized against H. poly EVs show a reduced parasite burden, and the EVs are able to supress activation of leukocytes in vitro. However, it is currently unknown whether the EVs are taken up by all cells in the gut wall indiscriminately, or if they are targeted to specific cells.

Here, we developed antibodies against different parasite components, and used immunofluorescence microscopy to investigate uptake of parasite EVs by host cells during infection. In the granuloma surrounding larval stages of the parasite, H. poly EVs could be found inside certain host cells. Immunophenotyping showed that the EVs were taken up by neutrophils, but not by other phagocytes in the granuloma. This data suggests that parasite EVs have the potential to enter host cells and may exhibit cellular tropism. We are currently investigating how EVs specifically target neutrophils, and what the functional effects of this targeted host-pathogen interaction are.

ID: 179
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: Colitis-associated colorectal carcinogenesis, gut mucosa, N-glycosylation, T cells

Branched N-glycans of colonic stromal cells as key players during colitis-associated colorectal carcinogenesis

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**** Equal contribution***

Inflammatory Bowel Disease (IBD)-associated Colorectal Cancer (CAC) is a major concern in the clinical management of patients with chronic Inflammatory Bowel Disease (IBD). Inflammation is a driving factor both in IBD and cancer, but the mechanism underlying the progression to CAC remains poorly understood. Dysregulation of mucosal glycosylation has been described as a key regulatory mechanism associated both with colon inflammation and colorectal cancer (CRC) development. Our group showed that decreased expression of MGAT5 glycogene on intestinal T lymphocytes from IBD patients was associated with disease severity. On the other hand, we also demonstrated that the aberrant expression of MGAT5 glycogene in CRC was associated with immune escape. However, whether this differential expression of glycans in colitis and cancer contexts mediated by MGAT5 glycogene constitutes a new mechanism in CAC remains completely unknown. A well-characterized cohort of CAC patients (from Porto University Centre Hospital – Portugal and Hospital Clinico San Carlos – Madrid, Spain) at different stages of carcinogenesis (colitis, dysplasia and cancer) was assessed to evaluate the N-glycosylation profile of stroma cells. Furthermore, in vivo studies (using AOM/DSS to induce CAC) were conducted in MGAT5 WT and KO mice. The glycoprofile and immunoprofile of colon T cells was analyzed. Our cross-sectional study showed a distinct glycoprofile in the stroma, characterized by an increase of branched N-glycans along the carcinogenic cascade, with an increased expression of MGAT5 gene in high-risk colitis. Additionally, colonic T cells isolated from AOM/DSS MGAT5 glycoengineered mice presented a switch on N-glycans composition along CAC development, followed by different inflammatory profiles, which showed an effect in tumor development. Our preliminary results demonstrate a distinct glyco-signature of colonic stromal cells along the colitis-associated carcinoma cascade, which appears to impact tumor development.

ID: 180
Abstract
Topics: Innate Immunity
Keywords: Granuloma, small intestine, Heterogeneity of macrophages, TNF-driven inflammation, Crohn's disease

Defining macrophage heterogeneity in TNF- driven ileitis

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Granulomas are structures formed by immune cells in response to persistent antigenic stimulation. They are typically found in chronic inflammatory diseases such as tuberculosis, sarcoidosis, and Crohn's disease. Macrophages are key immune cells that play a critical role in the formation and maintenance of granulomas. The presence of granulomas in the small intestine is an important diagnostic feature of Crohn's disease. However, their role in the pathogenesis of the disease is not fully understood. Some studies suggest that granulomas may play a protective role by containing the inflammation and preventing it from spreading to other parts of the intestine. Other studies suggest that they may contribute to tissue damage and scarring by releasing harmful molecules and promoting fibrosis. Here, we have employed the TNFΔARE/+ mice, a TNF-dependent ileitis model, to study the role of granulomas in Crohn's disease and to explore the heterogeneity and compartmentalization of macrophages in the small intestine during homeostasis and inflammation. Our findings show a significant reduction in F4/80high perivascular macrophages within the villi during TNF - driven inflammation. In contrast, F4/80high and CD206+ macrophages were found in association with granulomas and their presence was limited to the submucosa and muscularis externa of the small intestine. Homeostatic and inflammation-associated macrophages were found engaged in different interactions with vascular structures. Current work addresses the transcriptional heterogeneity underlying distinct subsets of intestinal macrophages. In conclusion, we have identified phenotypically distinct macrophage subpopulations and their tissue niches in a mouse model of TNF-dependent ileitis. This line of work may lead us to identify new approaches to promote tissue healing and allow us to develop new therapeutic targets for the treatment of TNF-driven chronic intestinal inflammation.

ID: 181
Abstract
Topics: Mucosal T cells, Mucosal diseases and infections
Keywords: Regulatory T cells, RORgt, HIF1a, colitis-associated colorectal cancer

The role of HIF1a in the function and metabolism of RORgt Tregs

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Colorectal cancer is responsible for 700,000 deaths annually and is the fourth most deadly cancer. Chronic ulcerative colitis has been associated with an increased risk of CRC, classified as colitis-associated colorectal cancer. In this case, inflammatory factors are related to carcinogenesis, disease progression, and metastasis. Recently, a population of Foxp3 RORgt T cells was identified in the region of the lamina propria of the colon, which is dependent on the interaction with microbiota. However, its metabolic profile and its participation during the progression of CAC have not yet been fully clarified. Thus, we hypothesize that the inflammatory microenvironment observed in the experimental model of CAC is essential for the definition of the metabolic program of Foxp3 RORgt T cells since it is expected that genetic deletion of the HIF1a component, regulated by the mTOR pathway in Foxp3 T cells modulates the susceptibility of animals to the disease. This study demonstrated that genetic-specific deletion of HIF1a in RORgt cells leads to a significant decrease in RORgt Tregs expansion in lamina propria of the colon, mesenteric lymph nodes, and spleen of mice with CAC. This specific HIF1a ablation impaired the inflammation in cLP and CAC progression by upregulating immunosuppressive checkpoints and decreasing the production of pro-inflammatory factors, such as IL-17A. Sorted Treg-like and Th17-like cells from cLP of RORgt Cre HIF flox mice were more OXPHOS-dependent when compared with control mice, suggesting a correlation between the effects of HIF1a deletion in the immunophenotype and the metabolism. Using our protocol developed for in vitro polarization of Foxp3 RORgt T cells, we observed that the chemical inhibition of HIF1a leads to a Foxp3 T cell accumulation and an upregulation of OXPHOS, corroborating the in vivo model observations. We are currently performing experiments for mechanism investigations behind the HIF1a and RORgt interactions in RORgt Tregs.

ID: 182
Abstract
Topics: Microbe-immune interactions at body surfaces
Keywords: antibiotics, microbiota, delayed-type hypersensitivity, Peyer's patches, cytokines

Oral metronidazole reduces delayed-type hypersensitivity by modulating mucosal immunity

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The gut microbiota is a critical stimulus for immune system development and thus shapes an individual's susceptibility to inflammation. Here, we investigated whether these immunomodulatory properties can be altered by oral antibiotics (ATB) and whether they are microbiota-dependent or -independent.

We fed BALB/c mice specific ATB (metronidazole (M), colistin or vankomycin) or placebo for 2 weeks and induced delayed-type hypersensitivity (DTH) either in the second week of treatment or 7 or 14 days after the end of treatment. The involvement of the gut microbiota and immune system was tested by a similar experiment under germ-free conditions, by transferring the gut microbiota altered by M or in immunodeficient mice adoptively transferred with leukocytes from placebo- or M-treated mice. We analyzed the effect of ATB on gut microbiota by 16S rRNA gene sequencing, on local or systemic immune response by gut fragment culture or flow cytometry in vivo and on anti-CD3/CD28 stimulation in vitro.

All tested ATB decreased DTH, with oral M having the greatest effect apparent even 2 weeks after the end of M treatment. M decreases the production of pro-inflammatory cytokines in Peyer's patches and decreases the ability of T cells to respond to TCR triggers, but has no effect on T cells when introduced directly in vitro. M also decreases DTH in the absence of the microbiota and its effect can be transmitted by leukocytes. Interestingly, transfer of the microbiota from M-treated mice has the opposite effect.

Orally administered M has a microbiota-independent immunosuppressive effect that persists for 2 weeks after cessation of ATB treatment and requires tuning in Peyer's patches. M-induced dysbiosis ultimately leads to stimulation of the immune system.

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ID: 183
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal B cells
Keywords: B-cells, glycan, lipopolysaccharide

Understanding the biophysical constraints at play in robust B-cell responses against bacterial glycans

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High-affinity IgA confers protection against Salmonella by enchaining growth and can be achieved by target-specific oral vaccination. Bacterial surface glycans are ubiquitously expressed and the dominant target for protective antibody responses in E. coli and Salmonella infections, two common bacterial enteropathogens. However, while systemic immune responses against proteins and haptens are well studied, little is known about efficient mucosal priming and glycan-antigen processing. Here, we develop two novel B-cell receptor knock-in mouse lines showing 1000-fold differences binding affinities to six Salmonella Typhimurium O-antigens variants and exhibiting different biochemical and biophysical properties. Using antigen-bound artificial planar lipid bilayers and in-vivo oral immunization, we examine the effects of glycan length, flexibility and structure on antigen sampling, B-cell priming, activation and affinity maturation. Combined with live microscopy and adoptive transfer, we hope in the following steps to accurately track the localisation of activated B-cells. following different modes of vaccinations. In addition, investigating the recruitment and limitation of available T-cell help will provide a better understanding of mucosal responses to glycan antigens. This novel knowledge can be applied to improve rational vaccine design, offering a robust alternative to antibiotics.

ID: 184
Abstract
Topics: Mucosal diseases and infections

Mucosal protection against SARS-CoV-2 with topically applied plant-produced secretory IgA

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Passive delivery of antibodies to mucosal sites might be a valuable adjunct to vaccination to prevent infection, treat viral carriage, or block transmission of respiratory pathogens like SARS-CoV-2. Neutralising monoclonal IgG antibodies are already approved for systemic delivery. For topical mucosal delivery, secretory IgA would be preferred, as the polymeric complex is adapted for the harsh, unstable external mucosal environment. However, exploration of secretory IgA as a potential therapeutic have been hampered by low production yields in conventional expression systems. Here, we showed the feasibility of producing neutralising monoclonal secretory IgA antibodies against SARS-CoV-2 in engineered N. benthamiana plants with expanded ER-phenotype. We identified two class-switched mAbs that express well and show high capacity for assembly into full-length dimeric secretory IgA variants. The yields were increased by co-expression of ER-resident molecular chaperones reaching 1 g/kg leaf fresh weight. Recombinant SIgAs showed increased antigen binding and stability in mucosal secretions compared to their IgG counterparts, had stronger virus neutralisation activities than IgG mAbs and were protective in vivo in a SARS-CoV-2 infection murine model. Furthermore, we demonstrated the feasibility of aerosolization for delivery of recombinant SIgAs to mucosal surfaces using a commercial nebuliser. These findings provide a persuasive case for developing recombinant SIgAs for mucosal application as a new tool in the fight against COVID-19 or other emerging respiratory infectious diseases using plant-based manufacturing platforms that addresses economic and scalable production to meet population-wide demand.

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Abstract
Topics: Neonatal window of opportunities, Microbe-immune interactions at body surfaces
Keywords: Pregnancy, hypertension, superimposed preeclampsia, microbiota, short chain fatty acids

Maternal gut microbiome alterations precede the onset of superimposed preeclampsia in a spontaneously hypertensive rat model.

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Background: Superimposed preeclampsia (SPE), which occurs in 25-40% of pregnant individuals with chronic hypertension, is associated with high maternal and infant morbidity. Growing evidence suggests a role for the maternal gut microbiota in the development of this disorder. Our previous studies demonstrated that the pregnant Stroke-prone Spontaneously Hypertensive Rat (SHRSP) displays an SPE phenotype, characterized by hypertension, renal distress and fetal growth restriction at gestation day (G)18. Using this model, we investigated changes in the maternal gut microbiota at G14, a time point that precedes the onset of clinical SPE symptoms.

Methods: SHRSP (hypertensive-SPE-model) and Wistar Kyoto (control) females were mated to congenic males and checked daily for vaginal plugs, denoted as gestation day (G)1. On G14, feces was collected for 16S rRNA sequencing of the gut microbiome and analysis of short chain fatty acid concentrations. Additionally at G14, clinical features of SPE were assessed including: systolic blood pressure, renal parameters (24 hour urine volume, protein content and creatinine clearance) and fecundity (number of implantations, fetal weight).

Results: Though pregnant SHRSPs had significantly increased blood pressure at G14, there were no other clinical manifestations of SPE at this time point: similar renal function, number of implantations and fetal weight compared with controls. Analysis of the G14 maternal gut microbiota however, showed significant differences in the SHRSP group including: decreased alpha diversity, increased relative abundance of Prevotella and Bifidobacterium and decreased relative abundance of Oscillospira, Ruminococcus, Blautia and Faecalibacterium. These changes were correlated with SPE in our model. Pregnant SHRSPs also showed significantly increased concentrations of fecal acetate, propionate and valerate compared with pregnant controls.

Conclusion: The altered maternal gut microbiota observed at G14 in pregnant hypertensive rats precedes the development of SPE. These results indicate potential predictive capabilities of the gut microbiome for determination of SPE susceptibility.

ID: 186
Abstract
Topics: Microbe-immune interactions at body surfaces, Mucosal B cells
Keywords: anti-glycan immunity, glycan length, antibody-glycan affinity, bacterial surface decoration, glycan-antigen sampling

Characterization of carbohydrate-specific antibodies against Salmonella typhimurium O-antigen

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Lipopolysaccharides (LPS) and capsular polysaccharides (CPS) comprise a large number of virulence factors of pathogenic microbes. They are located at the outermost surface of bacteria and represent the first structure to be perceived by the human immune system. However, little is known about what parameters manifest efficient glycan-antigen sampling to elicit a protective antibody response. Here we characterized two human IgG antibodies STA5 and STA121 that were isolated from a patient chronically infected with Salmonella typhimurium and that target the same O12-specific O-antigens. FACS results showed different binding capacity towards serological S.tm variants. The affinity of both STA5 and STA121 was stronger when used against O4 than against O5-positive strains. Furthermore, STA5 binds equally well to single-repeat (delta-wzyB) and to full-length O-antigen, whereas STA121 shows no detectable binding to the short O-antigen suggesting that the two monoclonals use different modes of binding. The binding kinetics of the purified S.tm O-antigen derivatives to immobilized STA5 and STA121 IgG were analyzed by SPR and revealed comparatively fast Kon rates for both antibodies, while the Koff rates were very slow for STA121 compared to STA5 and confirmed that STA5 does not interact with single-repeat O-antigen. Cryo-electron tomography of S.tm cells with either long or single-repeat O-antigen labeled with gold-conjugated STA5 and STA121 was performed to visualize the distribution and relative localization near the outer membrane, showing that STA121 binds in very close proximity to the outer membrane of the delta-wzyB strain, whereas STA5 shows no binding. Together these results suggest that STA121 binds across two repeating units of the O-antigen, whereas STA5 may bind to the monosaccharides of one repeating unit or to terminal elements. By X-ray analysis of STA5 and STA121 Fabs in complex with O-antigen, we plan to decipher the exact binding epitopes that manifest these different binding modes.

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Abstract
Topics: Innate Immunity, Mucosal diseases and infections
Keywords: Cry1Ac toxin intragastrically, inflammation intestinal mucosal

Bacillus thuringiensis Cry1Ac toxin provokes intestinal inflammation via oral administration

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The Bacillus thuringiensis biopesticide Cry1Ac toxin can be expressed by genetically modified plants for human and animal consumption. Cry1Ac is not toxic for vertebrates; however, it produces immunologic effects. We evaluated the inflammatory potential in acute model of intestinal inflammation, performing histopathologic analysis, cytokine production and recruitment of immune cells.

Materials and methods.

For comparison we used a DSS-induced model of acute intestinal inflammation administered for 7 days. In the experimental groups mice were intragastrically administrated with: i) vehicle phosphate-buffered saline, ii) tCry1Ac 2ug/ul iii) tCry1Ac 20 ug/ul iv) tCry1Ac 50 ug/ul) for 7 days. After that we evaluated the characteristics like blood in feces, large intestine size, weight loss, inflammatory production of cytokines in serum and intestinal histopathologic changes.

Results.

The Cry1Ac receiving groups; ii) tCry1Ac 2ug/ul iii) tCry1Ac 20 ug/ul iv) tCry1Ac 50 ug/ul developed moderate inflammation related changes. While some severe inflammatory parameters such as blood in feces, weight loss, large intestine size only were observed in the DSS 3% positive group. In the tCry1Ac 2ug group was observed increased IL6 production and reduction of IL10. Interestingly, this group also showed colonic lymphoid hyperplasia, an effect that has been associated with intestinal inflammation in humans. Other, findings were the presence of edema with recruitment the immune cells suggested by the increased presence the macrophages and neutrophils in the colonic intestinal mucosa.

Conclusion. Cry1Ac is not innocuous, especially the lower toxin dose 2 ug/ul, induced IL 6 production. More important, Cry1Ac provoked intestinal lymphoid hyperplasia, edema and recruitment the immune cells in colon.