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Oral cadmium intake and immune responses in the gut: intestinal inflammation and immune priming of mesenteric lymph nodes

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Introduction: Toxic metal cadmium (Cd) is important food and drinking water contaminant. The majority of ingested cadmium retained in the gastrointestinal tract (GIT) mucosa which allocates GIT as its main target. Mechanisms of induction of intestinal inflammatory response are largely unknown.
Materials and Methods: Effect of subchronic (30 days) oral (in water) cadmium administration (5 ppm and 50 ppm) was examined in Dark Agouti (DA) and Albino Oxford (AO) rats. Beside intestinal immune response, activity of draining mesenteric lymph node (MLN) cells, central place for induction of intestinal immune tolerance and local protective responses, was evaluated.
Results: In both rat strains cadmium consumption resulted in reduction of bacteria (Lactobacillus strain), intestinal tissue damage, modulated antioxidant enzymes activity and inflammation [increased proinflammatory cytokine (TNF, IL-1β, IFN-γ, IL-17) content in DA rats; increased TNF and IL-10 content in AO rats] in duodenal homogenates. Accumulation of cadmium in MLN was followed by stress response [elevation of MLN glutathione and metallothionein mRNA levels] only in DA rats. Stimulation of both adaptive (proliferation, Th1 and Th17 cytokine response) and innate immune activities (NKG2D+, CD68+ cells, selected oxidative activities, IL-1β) in MLN was observed, more pronounced in DA compared to AO rats.
Conclusions: Oral intake of cadmium resulted in intestinal damage, inflammation, and induction of proinflammatory milleu and innate effector cell activities in MLN. Cadmium-induced proinflammatory responses in DA rats but discrete immune responses of AO rats imply strain-dependent effects of oral cadmium administration.
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Zonulin level correlates with density of enteroviruses and tolerogenic dendritic cells in small bowel mucosa in celiac disease patients

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Aim: to investigate the level of Zonulin (ZO), as tight junction regulator, in sera of celiac disease (CD) patients with and without type 1 diabetes (T1D), to elucidate the relationship between ZO level, density of enteroviruses (EV), tolerogenic indoleamine 2, 3-dioxygenase (IDO) + dendritic cells (DCs) in small bowel mucosa.
Materials and Methods: Seventy patients (43 female, median age 10.7 years) who underwent small bowel biopsy were studied: CD without T1D was diagnosed in 18, CD with T1D in 13 patients, normal small bowel mucosa was found in two T1D patients and 37 patients with functional abdominal pain. All CD patients had partial or subtotal villous atrophy (Marsh 3a or 3c). ZO was evaluated using Zonulin ELISA (Hölzel Diagnostika, Germany). Staining for EV and IDO+ DCs was performed by immunohistochemistry on paraffin-embedded specimens.
Results: ZO level was higher in CD patients, particularly in patients with Marsh 3c atrophy, comparing with persons with normal small bowel mucosa (p=0.02). CD patients, especially with coexisting T1D have a positive correlation between ZO level and densities of EV (p=0.0004) and IDO+ DCs (p=0.01).
Conclusions: CD patients, particularly with severe small bowel mucosa atrophy had significantly elevated level of ZO comparing with controls. This finding was strongly correlated with density of EV in small bowel mucosa in CD patients with severe atrophic changes, particularly in cases of concomitant T1D. Increased density of IDO+ DCs in CD indicates possible activation of immunoregulation processes in small bowel mucosa depending on increased intestinal permeability possibly connected to EV.

P.B.35.06
Chemerin is an antimicrobial agent in mouse skin

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Introduction: Chemerin is a multifunctional protein implicated in leukocytes migration. It is abundantly expressed at epithelial barriers, including healthy but not diseased epidermis. Chemerin is predicted to share similar tertiary structure with antibacterial cathelicidins and we previously demonstrated that both recombinant chemerin, and chemerin in exudates from primary skin cultures has antimicrobial activity. The role of chemerin in controlling bacterial growth *in vivo* remains to be determined.
Materials and Methods: Chemerin-deficient and WT control mice were ectopically treated with two bacterial strains known to colonize the skin, *S. aureus* and *E. coli*. Skin was retrieved 24h later for histological examinations as well as enumeration of CFU. In some experiments, mice were treated with bacteria in the presence of chemically synthesized chemerin-derived peptides, that differed in antimicrobial activity based on *in vitro* assays or with scramble peptide.
Results: Chemerin-deficient mice harbored significantly higher bacterial loads compared to wild-type mice. In the infected mice, bacteria were seen to have formed a thin layer on the skin surface and invaded from the stratum corneum into the epidermal keratinocytes in KO but not WT mice. Application of an antimicrobial chemerin peptide on the skin significantly diminished the growth of bacteria.
Conclusions: Chemerin-deficient mice are not able to efficiently clear *S. aureus* and *E. coli* when exposed to these pathogens. Chemerin peptide restores the ability of these mice to restrict infection. Manipulation of chemerin levels and bioactivity or the use of chemerin-derived peptides may be a novel therapeutic approach to treat skin infections.

P.B.35.07
Influence of the microbiome in regulating ConA-induced-liver injury

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Introduction: The liver is continuously exposed to gut derived factors and bacteria. It has been shown, that gut microbiota can affect liver diseases like non-alcoholic fatty liver disease, hepatic encephalopathy, as well as viral, and alcoholic hepatitis. However, little is known about the influence of gut microbiota on immune-mediated liver injury. Therefore, we studied the role of gut microbiota in the Concanavalin A (ConA)-induced murine autoimmune hepatitis.
Materials and Methods: The composition of the microbiome in the feces of C57Bl/6, IL-10ko, and IL10fl/fl x Foxp3cre mice were analyzed by 16S rRNA sequencing. We further investigated the influence of antibiotics on ConA-induced liver damage. Immunophenotyping of infiltrating cells was performed via flow cytometry.
Results: We correlated the analysis of different phyla of the microbiome to parameters of liver damage. Preliminary results indicate three different phyla (Verrucomicrobia, Bacteriodetes, Firmicutes) to play either a protective or detrimental role in the progression of autoimmune-liver disease. Moreover, the different knock-outs influence the distribution of the distinct phyla in the gut microbiome. Interestingly, treatment with a broad-spectrum antibiotic cocktail ameliorate ConA-induced liver damage, which correlated with decreased frequency of T regulatory cells in the liver.
Conclusion: We clearly demonstrated a correlation between gut microbiota and ConA-induced liver damage. In order to identify specific species among phyla relevant for ConA-hepatitis, we currently perform further experiments to distinguish between protective or detrimental bacteria. Further immunophenotyping of mice treated with antibiotics may shed light on the involvement of gut derived immune cells on the auto-immune hepatitis.

P.B.35.08
Langerhans cells (CD1a and CD207), dermal dendrocytes (FXIIIa) and plasmacytoid dendritic cells (CD123) in skin lesions of leprosy patients

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Introduction: The clinical course of infection with *Mycobacterium leprae* varies widely and depends on the pattern of the host immune response. Dendritic cells play an important role in the activation of the innate and adaptive immune system and seem to be essential for the development of the disease. To analyze the presence of epidermal dendritic cells (CD1a and CD207), plasmacytoid dendritic cells (CD123) and dermal dendrocytes (factor XIIIa) in skin lesion fragments of leprosy patients, and to associate the presence of these cells with the polar forms of the disease. **Materials and Methods:** Skin samples from 30 patients, 16 with the tuberculoid form and 14 with the lepromatous forms, were used. These samples were submitted to immunohistochemistry using monoclonal antibodies against CD1a, CD207, FXIIIa, and CD123. **Results:** The results showed a larger mean number of Langerhans cells, detected with the CD1a or CD207 marker, dermal dendrocytes and plasmacytoid dendritic cells in patients with the tuberculoid form. A correlation was observed between the Langerhans cell markers CD1a and CD207 in both the tuberculoid and lepromatous forms, and between Langerhans cells and dermal dendrocytes in samples with the tuberculoid form. **Conclusion:** The present results indicate the existence of a larger number of dendritic cells in patients at the resistant pole of the disease (tuberculoid) and suggest that the different dendritic cells studied play an important role, favoring an efficient immune response against infection with *M. leprae*.