INTESTINAL INFLAMMATION IN HUMANS
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OR.65. Human β-defensin 3 Protein Accumulation is Increased and Redirected in Crohn's Ileitis *in vivo* and Modulates T Cell Responses *in vitro*

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Human beta-defensin 3 (hBD-3), an epithelial cell-derived inducible antimicrobial peptide, has multiple immunomodulatory functions linking innate and adaptive immunity. HBD-3, synthesized in various mucosal tissues, including the skin, oral cavity, and lung, is overexpressed during chronic inflammation of these mucosal sites. However, hBD-3 expression and function are poorly characterized within the healthy intestine and during chronic intestinal inflammation such as inflammatory bowel disease (IBD). We hypothesize hBD-3 modulates mucosal immune responses, and its overproduction in Crohn's disease (CD) regulates a chronic adaptive immune response. By ELISA and confocal microscopy, hBD-3 protein is detected in the terminal ileum (TI) and throughout the entire length of the colon in control patients, localized to Paneth cell granules and along the apical epithelial surface. Notably, hBD-3 protein is selectively increased 2-fold only in the TI of CD patients, and its localization switches to the basolateral epithelial surface and becomes interspersed within the lamina propria. *In vitro* hBD-3 enhances IL-2 ($n=5$, $p<0.01$) and IL-10 secretion 2-fold from anti-CD3/anti-CD28 costimulated blood T cells, but does not affect IFN-γ ($n=3$, $p>0.5$) production. These results suggest an important immunomodulatory role for hBD-3 within the healthy intestine, which may function to restore tolerance during periods of chronic inflammation.

OR.66. Adipose Tissue as a Contributor to Colonic Inflammation in Crohn's Disease

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In Crohn's disease the hypertrophy of the mesenteric fat is a characteristic finding. However, the biological significance remains to be elucidated. The production of immune regulating mediators as well as the expression of functional pattern recognition receptors (PRR) by murine adipocytes and preadipocytes indicates a role in the immune system. To estimate the incidence of a direct contact between antigens from the intestinal flora and the mesenteric fat, bacterial translocation was assessed in models of intestinal inflammation. To transfer our initial findings from the murine system, expression and functionality of PRR was tested in human preadipocytes. In chronic DSS-induced colitis, bacterial translocation into various mesenteric tissues including the mesenteric fat was evident. Interestingly, in mice deficient for the TLR-adaptor protein MyD88, bacterial translocation was increased. In parallel to our studies in murine cells, human preadipocytes express TLR and NOD mRNA and respond to PRR-specific stimulation by increased IL-6 production. Our data depict, that bacterial translocation is common during intestinal inflammation. Since functional PRR are expressed on preadipocytes and adipocytes across species, this could subsequently increase the local production of pro-inflammatory adipokines in the adipose tissue. This, in turn might contribute to both, the mesenteric fat hypertrophy and the chronic inflammation in the adjacent colonic mucosa in Crohn's disease.

OR.67. Central Role of IL-6 and MMP-1 for Cross Talk Between Human Intestinal Mast Cells and Human Intestinal Fibroblasts

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Mast cells (MC) are key effector cells in allergic reactions but also involved in host defense and tissue remodeling during wound healing, angiogenesis, and fibrogenesis. We have shown previously that human intestinal fibroblasts (FB) suppress apoptosis in human intestinal MC independent of the known human mast cell growth factors SCF, IL-3, or IL-4, but the implicated factor remained elusive. Here, we identify this factor as IL 6. Intestinal FB produced IL-6 upon direct stimulation by intestinal MC in co-culture or by MC mediators such as TNF, IL-1β, tryptase or histamine. MC incubated with IL-6 survived for up to 3 weeks similar to MC co-cultured with FB. MC survival in the presence of FB could be blocked using a neutralizing anti-IL-6 Ab. Moreover, FB stimulated by MC mediators upregulated their expression of matrix metalloproteinase-1 (MMP-1), a key fibrolytic enzyme. MMP-1 expression in FB triggered by MC was dependent on the MEK/ERK cascade. Noteworthy, FB co-cultured with MC or treated with MMP-1 lost confluence and showed increased numbers of apoptotic cells. Taken together our data indicate an intimate cross talk between MC and FB resulting in MC survival and induction of a fibrolytic rather than a profibrotic state in FB.

OR.68. Oral KLH Modulates Subsequently Induced Systemic Antigen-specific Immune Responses in Humans

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Oral antigen can induce diverse systemic immune responses ranging from tolerance to immunity. Especially in humans, the underlying mechanisms are poorly understood. We investigated the human immune response to oral administration of the neo-antigen keyhole limpet hemocyanin (KLH, 5 mg/d for

10 and, enhanced, for 3x10 days) and its effect on subsequent parenterally induced systemic immune responses. KLH-specific TH cells were identified by the expression of CD154 after short-term in vitro stimulation of PBMC and analyzed by flow cytometry. KLH-specific serum antibodies were assessed by ELISA. Oral KLH alone induced antigen-specific TH cells positive mainly for the gut-homing receptor integrin β7, the cytokines IL-2 and TNF-α, and a subset produced the TH2 cytokine IL-4. In addition, oral KLH accelerated systemic TH and B cell responses to subsequent parenteral challenge. B cell responses were amplified and the cytokine pattern of KLH-specific TH cells was shifted toward more IL-4- and IL-10- as well as less IFN-γ-, IL-2- and TNF-α– producing cells. The enhanced oral protocol did not reduce systemic T cell responses but primed for a cutaneous DTH reaction. Our findings indicate that oral antigen can induce a TH2-biased T cell response and effectively modulate subsequently induced systemic antigen-specific immune responses.