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T.86. Relationship Between Cells from Intestinal Epithelium and Bronchoalveolar Fluid through the Analysis of IL-7 Modulation

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The compartmentalization between intestinal villi and Bronchus-Associated Lymphoid Tissue shown in our model of secondary immunodeficiency is the result of preferential migration of γδ T cells due to an inflammatory process to both sites. Even if IL-7 influences γδ T cells development, the mechanisms of this modulation has not been clearly understood. The aim of the present report was to analyze IL-7 protein expression and its mRNA in intestinal epithelial cells (IECs) compared with cells from bronchoalveolar fluid (BAL). Rats deprived of protein at weaning were fed with a 20% casein diet for 21 days (renourished group: R21). Well-nourished group were studied simultaneously (C60). Western Blot and semiquantitative RT-PCR were performed on IECs and on cells from BAL of R21 and C60. IL-7 protein expression was detected in cytosolic and microsomal fractions of both groups. An increase of 1.5 fold of IL-7 mRNA was detected in IECs and in BAL of R21 as compared with C60($P<0.05$). We may assume that the increase of γδ T cells observed in the renourished group is due to dietary antigen uptake as well as an improvement of intestinal microbiota. This increase is modulated through IL-7 as intestinal bacterial commensals and the immune system communicate through an innate detection system to generate adaptive lymphoid tissues.

T.87. The Expansion of Gr-1+ Myeloid Cells Derive Hypergammaglobulinemia with Autoantibody through the Generation of T Follicular Helper Cells During Persistent Salmonella Exposure in MyD88-deficient Condition

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Oral administration of recombinant attenuated *Salmonella enterica* serovar *Typhimurium* vaccine (RASV) strain in MyD88$^{-/-}$ mice resulted in systemic sepsis accompanying splenomegaly and lymphadenopathy. Especially, B cell compartment was significantly increased and levels of total serum IgG were dramatically increased, while IgM levels were not altered compared to that of wild-type mice. Further, anti-dsDNA Ab in sera and the deposition of immune-complex in the kidney were detected in RASV-infected MyD88$^{-/-}$ mice. The depletion of CD4^{+} T cells completely blocked the hyper-gammaglobulinemia in RASV infected-MyD88$^{-/-}$ mice. The ICOS and PD-1 on CD4^{+} T cells of RASV-infected MyD88$^{-/-}$ mice were dramatically increased, suggesting similar features of T follicular helper (Tfh) cells. Of note, CD11b^{+}Gr-1^{+} myeloid cells expressing MHC Class II, CD40, CD80, CD86, PD-L1 and ICOS-L were also drastically expanded in MyD88$^{-/-}$ mice after RASV infection. They produced high levels of IL-1β and IL-6 against RASV restimulation, and existed adjacent to CD4^{+} T cells in the extrafollicular region of the spleen. Overall, these results suggesting that Tfh cells which may be activated by expanded CD11b^{+}Gr-1^{+} cells mediated B cell hyperactivation and hypergammaglobulinemia in MyD88-independent manner.

T.88. The Protective Effect of Chymase Inhibitor on NSAID-induced Small Intestinal Enteritis in Rats

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Background and Aims: Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin produce damage not only in the stomach but also in the small intestine, as their side effects. Chymase is a chymotrypsin-like serine protease located in the secretory granules of mast cells and is known to be convert pro-matrix metalloproteinase (proMMP)-9 to matrix metalloproteinase (MMP)-9. In the present study, we evaluated the effect of chymase inhibitor, TY-51469, on indomethacin-induced small intestinal enteritis in rats. Methods: Male SD rats were orally given indomethacin (10 mg/kg) and were sacrificed 3, 6, 12 and 24 hr after the administration. The small intestines were removed for measuring MMP-9 and for evaluating the degree of damage. Each rat was intraperitoneally administered TY-51469 or placebo 3 hr before the administration of indomethacin and was evaluated the enzymatic activities such as MMP-9 and the ulcer index 24 hr after the administration of indomethacin. Results: Indomethacin gradually caused severe lesions in the small intestine time-dependently, and the ulcer index showed a significant increase at the time of 12 and 24 hr after the administration of indomethacin. MMP-9 level was significantly increased to 5.6 and 9.2 times of control intestinal value 12 and 24 hr after the administration, respectively. Twenty four hr after the administration of indomethacin, TY-51469 significantly decreased to 13.9% the ulcer index compared with placebo. MMP-9 activities in TY-51469-treated rats were also decreased to the same level of control rats. Conclusion: We demonstrated the importance of chymase-dependent MMP-9 activation in NSAIDs-induced small intestinal enteritis and the usefulness of chymase inhibitor for preventing the development of enteritis via inhibition of MMP-9 activation *in vivo*.

T.89. Starvation Induces Expression of IL-13 Receptor a2 in the Mouse Intestine in a Commensal Flora-dependent Manner

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Intestinal mucosa undergoes atrophic changes during starvation, but refeeding induces rapid recovery of its morphology

and function. The final goal of this study is to understand the gut immunity associated with this dramatic response to starvation and refeeding. Four-week-old BALB/c mice were starved for 36 hours and then refed. Samples from the jejunum and the colon was collected at the various time point after refeeding and subjected to histological analysis and quantitative RT-PCR. In some experiments, antibiotics were orally administrated to eliminate commensal flora. Transcripts of interleukin-13 receptor α2 (IL-13Ra2) was upregulated about 10-fold and 5-fold after starvation in the small and large intestine, respectively. Twenty-four hours after refeeding, expression of IL-13Ra2 returned to the normal level or lower in both small and large intestine. This up-regulation of IL-13Rα2 was mostly eliminated when mice were treated with antibiotics. These results suggest that expression of IL-13Rα2 is induced by starvation, directly or indirectly mediated by the factor derived from bacterial flora. Since our previous studies have shown that neutralization of IL-13 by IL-13 receptor α2 (IL-13Rα2) promoted epithelial cell regeneration, its involovement in the intestinal morphological changes during starvation-refed is now under investigation.

T.90. Immunomodulatory Effects of Colostrum and Amniotic Fluid

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Infants born prematurely are susceptible to gastrointestinal infections such as necrotizing enterocolitis (NEC). In a premature piglet model of NEC we have previously shown that oral feeding with porcine and bovine colostrum and porcine amniotic fluid protects the immature intestine from inflammation and decreases the incidence of NEC relative to formula feeding. We hypothesized that colostrum and amniotic fluid contain similar immunomodulatory compounds able to down-regulate inflammatory mechanisms in the intestine, and these may be conserved in the two fluids and across different species. We used bone marrow-derived murine dendritic cells (DC) to study the effects of porcine, bovine and human amniotic fluid and whey fractions of porcine and bovine colostrum and human milk on the response to bacterial stimulation. Down-regulation of IL-6, TNF-α and IL-12 by porcine and human whey and amniotic fluid was observed, and the modulations seemed to be strongest by the porcine fluids. The modulation by bovine fluids was bacteria dependent. Overall, effects of amniotic fluid and whey from same species were similar. Immunomodulatory compounds affecting the cytokine response from stimulated DC are present in both colostrum whey and amniotic fluid, and we are currently working to identify some of these more specifically.

T.91. A Novel Role for the Rho Associated Kinase, ROCK, in IL-1 Stimulated Intestinal Epithelial Cell Responses

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Activation of the IL-1 signaling pathway in intestinal epithelial cells results in the production of the proinflammatory cytokines IL-8 and MCP-1. Recent reports have suggested that the Rho activated kinase, ROCK, may be involved in regulating IL-1 signaling in some cell types. Using Caco-2 and IEC-6 cells, we have determined that inhibition of ROCK activity with the inhibitor Y-27632 resulted in a significant reduction in IL-1 induced IL-8 and MCP-1, respectively, protein secretion and mRNA levels. Yet, unlike other cell types, Y-27632 inhibition of ROCK activity had no effect on IL-1 induced phosphorylation of IκBα. Furthermore, ROCK inhibition yielded a significant suppression of JNK phosphorylation suggesting that ROCK activity may be important for IL-1 induced activation of JNK/AP-1, but not IκBα/NF-κB. IL-1 stimulation also significantly increased ROCKI activity, but had little effect on the activity of ROCKII, suggesting that ROCKI may be involved in the effect. Finally, IL-1 stimulation did not affect the activity of the common upstream activator of ROCK, RhoA, which may indicate a role for other Rho proteins in the ROCK/IL-1 interaction. Taken together, these experiments suggest a novel role for ROCK in the regulation of IL-1 responses in intestinal epithelial cells.

T.92. Mucosal Injury and Activation of NFkB in the Initiation of Intestinal Inflammation

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Intestinal macrophages lack expression of pattern recognition receptors and costimulatory molecules. Given that these receptors are indispensable for induction of innate /adaptive immune responses, initiation of inflammation in the intestinal mucosa remains obscure. We applied an intestinal organ culture model to study early activation of the local lamina propria lymphocyte population in response to an inflammatory stimulus. Briefly, mucosal damage is induced by detachment of epithelial cells from normal non-inflamed mucosa pieces by EDTA treatment. Loss of epithelial cells causes emigration of LPMC ("walk-out" WO-LPMC) through pores onto the denuded basement membrane. Flowcytometric and gene expression analysis reveals that WO-LPMC are activated: WO-LPMO express surface antigens like CD14, CD16, CD54, CD86, CD98, HLA-DR and TLRs, while expression of CD25, CD69, CD98 is up-regulated on WO-LPT. Furthermore, cytokines and chemokines (e.g. IL-1b, IL-6, MCP-1) known to be up-regulated during intestinal inflammation can be detected in the organ culture supernatant by cytokine array analysis. Culturing mucosal samples in the presence of the NFkB-inhibitor APDC at least partially inhibits activation of WO-LPMC as well as inflammatory cytokine /chemokine

production. In conclusion, mucosal injury induces a local immune response characterized by the up-regulation of pattern recognition receptors and costimulatory molecules on LPMC as well as release of pro-inflammatory cytokine /chemokines. Mucosal injury therefore represents one possible requirement for the initiation of an innate and antigen-specific intestinal immune response.Activation of NFkappaB seems to be an essential signalling event during the initiation of intestinal inflammation.

T.93. A Transient Breach in the Epithelial Barrier is Associated with Increased IL-25, Reduced IL-23, Increased IL-17 and IL-10 Production

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Background: We recently demonstrated that a transient breach in the epithelial barrier leads to regulatory T cell generation as response to mucosal flora and that this phenomenon involved IL-10 production. We further investigated the local immune-response by characterizing cytokine and cellular response. Methods: We administered 7-8 weeks old SJL male mice with ethanol 50% intrarectally. At different time points (1-2-3 days after treatment), mice were sacrificed, colon collected and analyzed for cytokine content in homogenized tissue by ELISA. Isolated lamina propria mononuclear cells (LPMC) were analyzed for latency associated peptide (LAP) expression. Results: Administration of ethanol is associated with an early significant increase of IL-6, TGF-beta and IL-17 and a significant reduction of IL-23 in colonic tissue of treated mice when compared to untreated mice. IL-17 increase is transient and restricted at day 1. In addition a significant increase of IL-25 and IL-10 was observable. Flow cytometry analysis of LPMC isolated at day 3 after ethanol administration, when compared with LPMC of untreated mice, showed an increase of (LAP) expression in CD4+ gated T cells. Conclusions: Transient increased exposure of the LPMC to the bacterial flora is associated with regulation of IL-25 and IL-23 production and expansion of regulatory T cells.

T.95. Secretory Factors Contribute to Nod2-mediated Tolerance

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In contrast to acute stimulation of Nod2, chronic Nod2 stimulation in primary human monocyte-derived macrophages downregulates the induction of proinflammatory cytokines upon restimulation with TLR2 and TLR4 ligands. We now show that pre-treatment with the Nod2 ligand muramyl dipeptide (MDP) also downregulates proinflammatory cytokine production upon restimulation of the pattern recognition receptors (PRRs) Nod1, TLR3, TLR5 and TLR9, as well as upon restimulation by the proinflammatory cytokine IL-1beta. We previously identified that IL-1beta induced by Nod2 augments Nod2 responses. Now we demonstrate that this autocrine loop contributes to Nod2-mediated tolerance, as blocking IL-1beta receptor signaling reduced Nod2-mediated downregulation of cytokines upon restimulation with PRRs. We next questioned if additional secretory factors contribute to Nod2-mediated tolerance. We find that in macrophages pretreated with MDP, blocking the anti-inflammatory mediators IL-10 and IL-1Ra also reduces the ability of MDP to mediate optimal downregulation of proinflammatory responses during restimulation with PRRs. The degree to which distinct secretory factors contribute to Nod2-mediated tolerance varies among individuals. Taken together, Nod2-mediated tolerance extends to a broad range of PRRs, and secreted mediators contribute to this tolerance. These findings add to our understanding of the role of the Crohn's disease relevant protein Nod2 in intestinal immune homeostasis.

T.96. Differential Effects of TGF-β on Effector/Memory Lamina Propria T Cells: Regulation of Cell Cycle Progression and TCR Signal Transduction

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Crohn's disease (CD), a chronic inflammatory disorder of the GI tract, is characterized by aberrant T cell responses to antigens derived from commensal microflora. TGF-β secreted by regulatory T cells is one of the mediators responsible to maintaining mucosal immune homeostasis. However, the direct effect of TGF-β on effector memory T cell subsets in the normal mucosa and the mechanism by which TGF-β mediated immune homeostasis is dysregulated in CD remain poorly understood. Previously we reported that priming of peripheral blood T cells (PBT) to TGF-β results in enhanced and sustained signaling through the T cell receptor (TCR), while inhibiting proliferation, IL-2 secretion and cell cycle progression. Current data using CD4+ lamina propria T cells (LPT) from CD patients show that in contrast to PBT, TGF-β preconditioning leads to a biphasic signaling response upon TCR engagement that correlates with reduced proliferation and IL-2 secretion. However TGF-β primed CD8 LPT cells are unchanged for these biochemical and biological responses, suggesting that the mechanism by which TGF-β redirects immune effector function varies among T cell subsets. We propose that TGF-β fails to suppress CD4+ LPT in CD thereby influencing the overall outcome of disease.