IBD: ANIMAL MODELS
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T.1. Suppression of LPS-induced NFκB and p38 MAPK Activation by FK506 Ameliorates Murine Colitis

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Background: FK506 is a novel immunomodulator that is currently used for the prophylaxis of organ rejections, autoimmune diseases, and inflammatory bowel diseases. However, the mechanism of the immunosuppressive effects of FK506 on macrophage is unclear. We investigated the effect of FK506 on macrophage of IL10 KO mice and the direct effect of FK506 on immune-mediated colitis by rectal administration. Methods: (1) Peritoneal macrophages of IL10 KO mice were stimulated with LPS and FK506 for 24 hours, and the production of IL-12p40, TNFα, and IL-6 in supernatant were measured by ELISA. (2) We investigated whether FK506 affects NFκB and p38 MAPK activation pathways in macrophage of IL10 KO mice. (3) To evaluate the direct effect of FK506 on colonic inflammation of IL10 KO mice, rectal administration of FK506 was performed three times in a week. After 2 weeks, histological evaluation and the gene expression of inflammatory cytokines (IL-12p40 and TNFα) in colonic mucosa were evaluated by RT-PCR. Results: (1) The production of IL-12p40, TNFα, and IL-6 were significantly decreased in supernatant from peritoneal macrophages treated with FK506 compared to non-treated cells. (2) Pretreatment of FK506 suppressed LPS-induced phosphorylation of both NFκB and p38 MAPK in peritoneal macrophage. (3) Histological score was significantly decreased in mice treated with FK506 than in those with PBS. (4) The gene expression of IL-12p40 and TNFα in colonic mucosa were significantly decreased in mice treated with FK506 compared to those with PBS. Conclusion: We firstly demonstrated the immunosuppressive effects of FK506 on macrophage, by which immune-mediated colonic inflammation was attenuated. Blockade of activation of NFκB and p38MAPK by FK506 could be one possible mechanism for reducing intestinal inflammation.

T.2. Differences on the Mechanisms of Inflammation Control During the Development of Colitis in IL-10 Deficient Mice Kept Under SPF or Conventional Environments

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The "hygiene hypothesis" relates the incorrect maturation of the immunological response to a greater susceptibility of developing inflammatory bowel disease. IL10 -/- mice develop colitis of different degree of severity according to the environment. Objective: to assess the immunological changes of the intraepithelial and lamina propria compartments on a spontaneous colitis model. Methods: Twelve wild type (WT) mice and 24 IL10-/- mice (4 weeks old) were maintained under SPF conditions. After four weeks, half of them were transferred to a conventional environment. Mice were sacrificied at 12 weeks of age to assess morphologic changes, hystologic lesion and incidence of colitis. Immunohistochemical analyses for apoptosis, TLR2 and MyD88 were performed. Phenotype, and intraepithelial (IEL) and lamina propria (LPL) lymphocyte apoptosis were analyzed by flux cytometry. TLR2 and TLR9 expression by real-time PCR was also determined. Results: IL10-/- mice kept under conventional environment showed a greater incidence of colitis (66% vs 50%) and their lesions were significantly more severe than colitic animals kept under SPF environment (p=0.009). The IEL activity was significantly higher in both environments (CD3 increase + decreased apoptosis); however, LPL only showed a relevant activity under conventional environment. A significant increase in TLR2 and TLR9 was shown in IL-10-/- when compared to WT mice. Immunohistochemistry demonstrated the loss of TLR2 and MyD88 in the lesioned areas. Conclusions: IL10 deficiency does not prevent the inflammation control of LPL in an environment almost deployed of antigens (in number and variety) such as SPF. The activation of the TLR-MyD88 pathway in SPF is probably one of the involved mechanisms by which LPL control inflammation, maintaining the barrier function.

T.3. A Novel Anti-inflammatory Role of TNFα in Intestinal Inflammation by Inducing Local Glucocorticoid Synthesis

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TNFα is a cytokine with prominent pro-inflammatory activities in a variety of diseases, including inflammatory bowel disease. However, there is increasing evidence for anti-inflammatory actions of TNFα. In contrast, glucocorticoids are steroid hormones with potent anti-inflammatory properties, at least in part also by regulating the expression and action of TNFα. Here we report that TNFα induces the extra-adrenal production of immunoregulatory glucocorticoids in the intestinal mucosa during experimental inflammatory bowel disease. Absence of TNFα results in lack of colonic glucocorticoid synthesis and exacerbation of dextran sodium sulfate- and oxazolone-induced colitis. TNFα seems to promote local steroidogenesis by directly inducing steroidogenic enzymes in intestinal epithelial cells. Critically, therapeutic administration of TNFα restores glucocorticoid synthesis in oxazolone-induced colitis and ameliorates intestinal inflammation. These data describe a novel anti-inflammatory role of TNFα in the pathogenesis of inflammatory bowel disease via the induction of local steroidogenesis.

T.4. Gene Expression Profiles Differentiate Pathological Outcomes of Johne's Disease: An Infectious Model of Inflammatory Bowel Disease

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The gastrointestinal immune response is unique in its opposing goals of tolerating food antigens and commensal microflora versus the need to activate strong immune responses to control pathogenic infections. When this immunological balance is upset chronic diseases such as inflammatory bowel disease (IBD) can develop. Using an experimental infection model of IBD in red deer (Cervus elaphus), we studied the immune responses during the different pathological states of disease. Johne's disease is a chronic IBD of ruminants caused by *Mycobacterium avium* subspecies paratuberculosis (MAP). A broad spectrum of pathological outcomes are observed following experimental infection with MAP ranging from clearance or containment of bacteria through to excessive inflammation and unrestrained bacterial growth causing death in clinically diseased animals. Gene expression profiling of peripheral blood mononuclear cells and gut-associated lymphatic tissues has shown an association between uncontrolled Th1 and Th17 responses and gut immunopathology. In animals with contained infection an association of Treg and Th2 immune responses in balancing the immune responses has been observed, challenging the dogma that Th2 responses in Johne's disease are characteristic of severe disease. These immune profiles provide novel diagnostic markers for the different pathological states of Johne's disease and other inflammatory bowel diseases.

T.5. Capsaicin-induced Ablation of the Enteric Afferent Nerves Protect SCID Mice Against T Cell-induced Chronic Colitis

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It has become increasingly clear that the enteric nervous system is highly involved in controlling and modulating immune and inflammatory processes in the gut. Previous studies have demonstrated that capsaicin sensitive nerves play a protective role under the conditions of acute experimentally induced colitis, but also, that neurotoxic doses of capsaicin may promote repair under particular experimental conditions or at particular anatomical sites. We have studied the effect of neurotoxic doses of capsaicin on the development of chronic colitis in the SCID mouse transfer model. We find that systemic capsaicin treatment of SCID mice completely abolishes the colitis-inducing effect of transferred CD4+CD25- T cells. Thus intact transient receptor potential vanilloid (TRPV1) receptors expressed by nociceptive, supstance-P containing nerve fibers are mandatory for the development of T cell transfer colitis. T cells obtained from capsaicin-protected mice produced much less cytokine than SCID mice with colitis. We also find evidence for that intrarectal exposure to capsaicin may reduce disease symptoms. Pointing to an important role for the enteric afferent nerve system in controlling chronic inflammation and suggest the possibility that locally applied neurotoxic doses of capsaicin or similar high affinity TRPV1 agonists may show beneficial effects in the human forms of inflammatory bowel disease.

T.6. A Crucial Role for Herpes Virus Entry Mediator-mediated Stimulatory Signals in Murine Colitis

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The recently identified TNF superfamily member TNFSF14 (LIGHT) is over-expressed in inflamed biopsies from inflammatory bowel disease (IBD) patients, however its role in the pathogenesis of colitis is unknown. We have investigated the role of LIGHT and its receptor Herpes virus entry mediator (HVEM) using two different models of experimental colitis in mice. HVEM-/- mice were resistant to DSS-induced colonic inflammation and exhibited severely attenuated pro-inflammatory cytokine and chemokine production by innate cells within the colon. HVEM-/- CD4+ T cells were also unable to mediate colitis following their transfer into immuno-deficient hosts and hosts exhibited strongly reduced pro-inflammatory cytokine production. These data indicate that HVEM co-stimulatory signals, most likely delivered by LIGHT, are essential for the activation of innate immune and CD4+ T cells during experimental colitis. Thus selective blockade of HVEM stimulatory signals may represent an attractive therapeutic approach for the treatment of IBD.

T.7. T Cell-associated L-selectin (CD62L) and β7 are Required for Inducing and Suppressing Chronic Gut Inflammation

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The objective of this study was to assess the importance of both T cell-associated CD62L and β7 for inducing as well as suppressing gut inflammation in the T cell transfer model of chronic colitis in mice. We found that transfer of wild type (WT) CD4+CD45RBhigh (naive) T cells into RAG-1-/- mice induced robust colitis at 8 wks (mean histological score±SEM: 13.2±0.6) whereas transfer of CD62L-/-xβ7-/- double deficient (DKO) T cells induced little or no disease at 8 wks (2.9±0.7) post-transfer. Total numbers of CD4+ T cell numbers were reduced in the colonic lamina propria (LP) of DKO→RAG-/- vs. WT→RAG-1-/- mice at both time points however intracellular staining revealed that both LP CD4+ DKO and WT T cells produce significant amounts of IFN-γ and IL-17 at 8 wks. Analysis of CFSE-labeled WT or DKO T cells transferred into RAG-1-/- mice revealed that although fewer DKO T cells migrated into the MLN and colonic LP, the proliferative profiles of both T-cell populations were similar. In addition, we found that co-transfer DKO regulatory T cells

(Tregs; CD4+CD25+) with naïve WT T cells did not suppress the induction of colitis (histological score±SEM: 10.4±1.6) whereas co-transfer of WT Tregs suppressed the development of disease (histological score±SEM: 4.6±1.6). Taken together, our data suggest that both CD62L and β7 are required for the induction and for suppression of chronic colitis. Furthermore, we demonstrate that the naïve DKO T cells are unable to induce disease because of defects in trafficking rather than defects in T cell activation.

T.8. IL-7Rα Expression on CD4+ T Cells is Essential for the Development of Colitis

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Introduction: IL-7 has recently emerged as a key cytokine involved in controlling the homeostatic survival of memory CD4+ T cells. Also, we previously demonstrated that IL-7 is essential for the development and persistence of chronic colitis as a survival factor of colitogenic memory CD4+ T cells. However, IL-7Rα is expressed not only on CD4+ T cells, but also on various other cells, such as other hematopoietic cells, fibroblasts, and epithelial cells. Thus, we have attempted to reveal whether the crucial influence of IL-7/IL-7R signal for the development of colitis is mainly led by CD4+ T cells, or by other IL-7Rα expressing cells. Methods: We isolated splenic (SP) CD4+CD25- T cells from IL-7Rα-/- mice or wild type (WT) SP CD4+CD25- T cells as positive control, and transferred them into RAG2-/- mice. As negative control, we transferred (WT) SP CD4+CD25+ T cells in addition to (WT) SP CD4+CD25- T cells. Each group was assessed 10 weeks after transfer. Results: The mice transferred with IL-7Rα-/-CD4+CD25- T cells did not show the sign of colitis both in clinical and histological aspects. The number of memory CD4+ T cells in colonic lamina propria (LP) and SP of IL-7Rα-/-CD4+CD25- transferred mice were less than those of positive control. LP CD4+ T cells of IL-7Rα-/-CD4+CD25- transferred mice produced almost no cytokines, while those of WT CD4+CD25- transferred mice produced Th1 or Th17 cytokines. Conclusions: We have shown the fact that IL-7Rα expression on CD4+ T cell is essential for the development of colitis in this model. This finding suggests that IL-7Rα on CD4+ T cells is the most important target in IL-7/IL-7R signal blocking therapy.

T.9. T Cell Regulation of Neutrophil Infiltrate at the Early Stages of a Murine Colitis Model

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Background and Aims: T cells are a main target in inflammatory bowel disease. T cell suppressors may inhibit T cell-dependent production of pro-inflammatory mediators as well as innate immune cell function. E.g., these drugs may impair innate cell recruitment and activation through inhibition of T cells or act independent of T cell modulation. We explored the extent of immune modulation by the T cell inhibitor tacrolimus in murine colitis. Methods: We induced TNBS colitis in WT and RAG2-deficient mice and determined the effects of Tacrolimus by histological scores and weight loss. We further characterized the inflammation using immunohistochemistry and by analysis of isolated intestinal leukocytes at various stages of disease. Results: Tacrolimus treated WT mice were less sensitive to colitis and had fewer activated intestinal T cells. T cell inhibition was associated with diminished neutrophil recruitment at the early stages of disease. In agreement, immunohistochemistry demonstrated that tacrolimus inhibited production of neutrophil chemoattractants CXCL1 and CXCL2. In T cell deficient mice, tacrolimus did not affect the severity of TNBS colitis or numbers of intestinal neutrophils. Conclusion: Both the innate and the adaptive mucosal immune system contribute to TNBS colitis. Tacrolimus suppresses colitis through inhibition of T cell activation and by suppression of T cell-mediated recruitment of neutrophils.

T.10. IL-13 Orchestrates Resolution of Inflammation in Chronic Experimental Colitis

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To investigate the value of TLR signaling during chronic intestinal inflammation we analyzed chronic TNBS-colitis with intestinal fibrosis in BALB/c mice. The characteristic Th17 response plateaued at 7–9 weeks and then declined in concert with the subsidence of the inflammation and the appearance of IL-13. We therefore addressed the mechanism by which IL-13 production leads to control of Th17 inflammation in this model. We could show that TLR signaling is regulated by glycogen synthase kinase 3 beta (GSK3beta). This kinase, when present in an active state, facilitates TLR-mediated NF-kappaB activation and inflammatory cytokine production. On the other hand, this pattern is reversed when GSK3beta is present in an inactive state. In terms of mechanistic studies, we showed that IL-13 induces GSK3beta inactivation through the IL-13Rα1 and activation of STAT6. To prove that GSK3beta is involved in the regulation of TLR signaling in chronic TNBS colitis, we showed that inhibition of GSK3beta activation prevented subsidence of inflammation in the terminal phase of chronic TNBS-colitis. In summary, these findings suggest that chronic intestinal inflammation is orchestrated by a succession of cytokines that ultimately results in IL-13 production and subsequent resolution of the inflammation through modulation of TLR signaling.

T.11. The Preventive Role of Pulverized Konjac Glucomannan (PKGM) on Oxazolone-induced Colitis in Mice

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Background and Aim: Konjac is a glucomannan-rich Japanese traditional food. Pulverized Konjac glucomannan (PKGM) has recently been demonstrated to prevent both plasma IgE elevation and developing dermatitis in a model of atopic dermatitis. In this study, we investigated the role of PKGM in oxazolone (OXA)-induced colitis in mice. Methods: C57BL/6 mice were fed by control food, PKGM and re-granulated PKGM (g-PKGM) 2 weeks before inducing colitis by OXA. Body weight, colon length, and histological change of the colon were examined. Mononuclear cells were purified from spleen and mesenteric lymph nodes (MLN), and stimulated with LPS or PMA + ionomycin. TNF-α, IFN-γ, and IL-4 levels in the supernatant were measured by ELISA. Results: PKGM-fed mice showed the improvement of body weight loss, shortening of colon length, and histological inflammation. TNF-α production in PKGM-fed mice was significantly lower than that in control mice in MLN and spleen. Likewise, IL-4 level was significantly lower in spleen of PKGM-fed mice than in control and g-PKGM mice. PKGM mice demonstrated significantly lower IL-4/IFN-γ ratio in spleen compared with control mice. Conclusion: These results suggest that PKGM may prevent mice from developing OXA-induced colitis by modulating Th1/Th2 ratio.

T.12. IEX-1 Deficiency Protects from Colonic Inflammation and Cancer in Mice

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IEX-1 (Immediate Early response gene X-1) is a stress-inducible gene involved in growth control, regulation of apoptosis, and intracellular ROS homeostasis. To address its physiological role in colitis and colorectal cancer, newly developed IEX-1-deficient (IEX-1 KO) mice were subjected to dextran sulfate sodium (DSS)-induced colitis and azoxymethane (AZO)/repetitive DSS colorectal cancer model. Surprisingly, IEX-1 KO animals were protected from colitis induced by 5% DSS ingestion and developed significantly fewer colorectal tumors. Analysis of colon homogenates and intestinal intraepithelial lymphocytes (IELs) from IEX-1 KO and WT animals treated with DSS for 3 days revealed a 3-fold increase in IL-17 levels in the absence compared to the presence of IEX-1, concomitant with increased levels of CD3-positive IL-17-producing cells in the mice. In parallel, apoptotic rates of CD3+ T cells were also elevated 2.5 times. We suspect that an increase in apoptosis of Th1 cells as well as in production of IL-17 by Th17 and gammadelta T cells both contribute to the protection. Therefore, inhibition of IEX-1 expression may represent a novel strategy for colitis treatment and colon cancer prevention.

T.13. Heme Oxygenase Regulates the Intestinal Inflammation via the Regulation of Th1/Th2 Cytokines

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Aims: Heme oxygenase-1 (HO-1) has been shown to mediate antioxidant, anti-inflammatory, and anti-apoptotic functions as well as the regulation of vascular tone in several experimental models. The present study investigated the possible role of HO-1 in dextran sodium sulfate (DSS)-induced intestinal mucosal injury. Materials and Methods: Acute colitis was induced with DSS in male BALB/c mice. A disease activity index (DAI) was determined on a daily basis for each animal, and consists of a calculated score based on changes in body weight, stool consistency, and intestinal bleeding. Colonic mRNA expression for HO-1 and IFN-gamma as Th1 cytokine and IL-4, IL-10 as Th2 cytokines, were measured by RealTime-PCR at different points after DSS induction. The expression of t-bet and GATA3 was also evaluated by RealTime-PCR and western blotting. Moreover, we evaluated the enhancement by treatment of an HO-1 inhibitor, ZnPP (25 mg/kg i.p., daily). Results: After DSS administration, DAI score and expression of HO-1 mRNA were increased in time dependent manner. Expression of IFN-gamma and IL-4, L-10 mRNA also was increased after DSS administration. Administration with ZnPP enhanced the increase in DAI score. The increases in the expression of IFN-gamma were enhanced in ZnPP-treated group. The increases in the expression of IL-10 were inhibited in ZnPP-treated group. Furthermore, ZnPP treatment attenuated the expression of GATA3, but not the expression of t-bet. Conclusion: These results indicate that HO-1 plays a protective role in the intestinal mucosal injury induced by DSS. These effects probably result from the regulation of Th1/Th2 cytokine balance in intestinal tissues.

T.14. Neonatal Model of Bone Marrow Reconstitution-Identifying the Critical Cell Type for Colitis Development in FVB.mdr1a-/- Mice

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P-glycoprotein (P-gp) is the product of the multi-drug resistance gene (MDR). P-gp is an ATP-dependent transmembrane pump which extrudes substrates from the intracellular environment. It is expressed on the apical surface of epithelial cells and cells of a hematopoetic lineage. Studies of IBD patients indicate a correlation between MDR polymorphisms and diminished P-gp function. Conventionally housed FVB.mdr1a-/- mice develop spontaneous colitis at an early age. Previous studies utilizing bone marrow chimeras indicated colitis was contingent upon P-gp deficiencies in innate cell populations. However, as adult FVB.mdr1a-/- demonstrate altered expression of inflammatory genes prior to colitis development; we hypothesized that these adult FVB.mdr1a-/- would not be appropriate models to study

the cell type critical for colitis development. Therefore, we developed a neonatal model of reconstitution. FVB.mdr1a-/- pups were lethally irradiated, and reconstituted with stem cells from FVB.mdr1a-/- or FVB/N mice. Mice were followed for colitis development and real-time PCR was used to calculate colonic gene expression. FVB.mdr1a-/- reconstituted with stem cells from FVB.mdr1a-/- mice demonstrated 100% mortality, stunted growth curves and up-regulation of colonic IL17, IFNγ and IL1β. Animals reconstituted with cells from FVB/N animals demonstrated 20% mortality. Surviving animals demonstrated normal growth curves and were histologically free of colitis. Supported-NIH Grants T32A107051, P01 DK071176 and the UAB Digestive Diseases Research Development Center Grant #P30 DK064400.

T.15. Distinct Roles for CXCR6$^+$ and CXCR6$^-$ CD4$^+$ T Cells in Pathogenesis of Chronic Colitis

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Colitogenic CD4$^+$ T cells play a central role in the development of inflammatory bowel diseases (IBDs) via vigorous production of IFN-γ and IL-17A. To better characterize the colitogenic CD4$^+$ T cells, we examined their expression of CXCR6, a chemokine receptor expressed upon activation in T and NKT cells. We found that approximately 90% of colonic lamina propria CD4$^+$ T cells expressed CXCR6 in CD45Rb^{hi}-tansferred mouse colitis model. Concomitantly, its ligand CXCL16 was upregulated in the inflamed intestinal mucosa of mouse colitis model and human IBD patients. Although both CXCR6$^+$ and CXCR6$^-$ CD4$^+$ T cells in the lamina propria showed the CD62L^{lo}CD44^{hi}IL7Rα$^+$ effector memory phenotype, only CXCR6$^+$ population abundantly produced IFN-γ and IL-17A. Nevertheless, retransfer of lamina propria CXCR6$^+$CD4$^+$ T cells into RAG-1$^{-/-}$ mice failed to induce colitis with no expansion of transferred T cells. In contrast, retransfer of CXCR6$^-$CD4$^+$ T cells gave rise to colitis similar to the CD45Rb^{hi}-tansferred model, with their phenotypic change into CXCR6$^+$ Th1 and Th17 cells in colonic lamina propria. These data suggest that CXCR6$^+$CD4$^+$ T cells function as terminally differentiated effector T cells, whereas CXCR6$^-$CD4$^+$ T cells serve as colitogenic memory T cells that could be responsible for persistent and/or recurrent inflammatory response in the colon.

T.16. Interleukin-19 Protects Mice from Innate-mediated Colonic Inflammation

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Inflammatory bowel disease results from chronic dysregulation of the mucosal immune system involving aberrant activation of innate and adaptive immune responses. Here, using gene-targeting, we have identified a novel role for interleukin (IL)-19 as a regulator in colonic inflammation. We initially investigated the susceptibility of IL-19 deficient mice to the development of DSS-induced acute colitis by analyzing mortality, the disease activity index (DAI), and histology of the distal colon. IL-19 deficient mice showed severe mortality on administration of 3% DSS in drinking water for 7 days. In accordance with the observed difference in survival, IL-19 deficient mice showed much more severe weight loss compared with WT control mice. IL-19 deficient mice had a significantly higher DAI score compared with WT control mice on day 4 to day 8 after DSS administration. We next show that distal colon of IL-19 deficient mice produced extremely high levels of IFN-gamma, IL-1beta, IL-6, IL-12, TNF-alpha and KC on day 5 after DSS administration, indicating inflammatory cytokines and chemokine production are controlled by IL-19 *in vivo*. Our findings indicate that IL-19 has previously undocumented roles in the protection of mucosal epithelial cells and the elimination of acute inflammation in the colon.

T.18. EBI3 Regulates Protective Intestinal Immune Responses in Experimental and Spontaneous Colitis

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EBI3 is as part of IL-27 and IL-35 involved in the regulation of immune responses. Since EBI3 and the IL-27/IL-35 partner subunits p28 and p35 have been shown to be upregulated in IBD, we evaluated here the contribution of EBI3 expression to disease in experimental and spontaneous colitis. First, we analyzed EBI3$^{-/-}$ mice in the DSS colitis model. Interestingly, wasting disease, colonoscopic and histological inflammation scores and as a result mortality was significantly higher in the EBI3$^{-/-}$ group. A multicytokine analysis with organ cultures and isolated LP and MLN cells showed a strongly increased expression of T_H17 related cytokines including IL-17A, IL-17F and GMCSF in the gut of EBI3$^{-/-}$ with colitis. Next we crossed EBI3$^{-/-}$ mice to a strain with a conditional ablation of STAT3 in myeloid cells, a well established model for spontaneous colitis due to deregulated activation of innate immune cells. These double deficient mice developed early very severe signs of intestinal inflammation, produced high levels of proinflammatory cytokines in the gut and all died before reaching an age of 10 weeks, whereas EBI3 expressing control mice survived several month longer. These results from both colitis models suggest that EBI3 cytokines are crucial regulators of immune cell activation in the gut.

T.19. Role of Secretory Antibodies and Commensal Microbiota in DSS-induced Colitis

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Inflammatory bowel disease (IBD) is a complex disorder where epithelial integrity, intestinal microbiota, and host immune system contribute to disease development. To investigate the role of secretory antibodies in IBD, colitis was induced in polymeric Ig receptor (pIgR)-/- and wt mice by dextran sodium sulfate

(DSS). Animals were monitored daily for body weight, rectal bleeding, diarrhea, and general signs of morbidity. We found that DSS-treated pIgR-/- mice displayed greater loss of bodyweight and had severe clinical illness compared to similarly treated wt animals. To determine the role of intestinal microbiota, mice were depleted for commensals with broad-spectrum antibiotics for 10 days prior to colitis induction and throughout the experiment. Depletion was verified by bacteriologic analysis of colonic feces. The effect of depleting the intestinal microbiota depended on the DSS-dose: at low concentration (1.5% DSS) all morbidity ceased, whereas at moderate concentration (2.0% DSS) a different phenotype of morbidity, which was attenuated by oral LPS treatment, appeared in commensal-depleted animals. Thus, the pIgR or secretory antibodies contribute to protection of the colonic mucosa against DSS-induced epithelial injury. Intestinal bacteria and /or their products play a pathogenic role at low DSS concentration, but a protective role at higher concentrations of DSS.

T.20. Increased Susceptibility of Germ-free Mice to Dextran Sodium Sulfate (DSS)-induced Colitis

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Colitis is the inflammation of the colon, caused by an abnormal immune response to gut constituents, including the indigenous microbiota. Here, we investigated how germ-free (GF) mice respond to Colitis. GF or Conventional mice (CV) were given DSS 4% for 7 days for colitis induction. The severity of clinical score and the weight loss was greater in GF mice than in CV mice after DSS administration. Furthermore, DSS-treated GF mice presented reduced leukocyte recruitment and altered cytokine production when compared to the CV mice submitted to colitis. At the end of DSS treatments, all GF mice were dead while CV presented no lethality. GF mice that received CV feces 14 days before DSS treatment (for microbiota reposition), presented reduced weight loss, minor clinical score and decreased lethality rates after colitis induction. Then we assessed if LPS injection, one of the major microbial components, could revert the susceptible phenotype of GF to DSS administration. The results showed that LPS administration induced partial protection of GF to colitis induction characterized by weight loss and death despite of reduced clinical score. Then, these data suggest that continuous stimuli of the commensal microbiota are of major importance to host protection to DSS-induced colitis.

T.21. Tumor Necrosis Factor Receptor Signaling in Intestinal Epithelial Cells may be Directly Involved in Colitis-associated Carcinogenesis

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Background and Aim: It is unclear whether the TNF signaling in intestinal epithelial cells is involved in the development of colitis-associated cancer (CAC) in the context of inflammatory bowel disease (IBD). To investigate this, we studied the effects of anti-TNF mAb in animal models of CAC. Methods and Results: Increased TNF expression was observed in the colonic tissues from dextran sodium sulfate (DSS) receiving mice. NF-κB pathway was activated in the epithelia from colitis in association with increased expression of TNF receptor (TNFR)2. The silencing of TNFR2 in a mouse intestinal cell line resulted in the down-regulation of myosin light chain kinase, which is responsible to the permeability of epithelial barrier. CAC model was induced by pretreatment with azoxymethane in DSS-receiving mice. The NF-κB pathway was further activated in the tumors in association with more upregulated TNFR2 compared to non-tumor area. Sequential treatment with an anti-TNF mAb reduced the numbers and size of tumors in association with the NF-κB inactivation and restored epithelial tight junction. Conclusions: Our studies suggest that the TNFR2 signaling in intestinal epithelial cells may be directly involved in the development of CAC, and imply that the anti-TNF mAb therapy may prevent from CAC in the patients with IBD.

T.22. 15-deoxy-delta^{12,14}-prostaglandin J_2 Induces Heme Oxygenase-1 and Protects Colonic Mucosa from DSS-induced Inflammation

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Background: 15-deoxy-delta^{12,14}-prostaglandin J_2 (PGJ_2) induces heme oxygenase-1 (HO-1) through the Nrf2-Keap1 pathway. Carbon monoxide, biliverdin and bilirubin are degradation products of heme, which exert antiinflammation and antioxidation. In this study, we investigated the effect of PGJ_2 and the involvement of HO-1 in acute murine colitis. Methods: Acute colitis was induced with 8% DSS in mice. PGJ_2 was administrated via rectal enema everyday. We determined the disease activity index (DAI) score based on the stool consistency and the levels of intestinal bleeding for each mouse. The expression of HO-1 in colonic mucosa was evaluated by RT-PCR, western blotting and immunohistochemistry. Thiobarbituric acid-reactive substances (TBA) and myeloperoxidase activity (MPO) were measured as indices of lipid peroxidation and neutrophil infiltration, respectively. Pro-inflammatory chemokine, KC, was measured by ELISA. We studied the

influence of co-treatment with HO-1 inhibitor (zinc protoporphyrin IX) or PPAR-gamma antagonist (GW9662). Furthermore, we evaluated the HO-1 inducibility of PGJ_2 in RIE cells by realtime RT-PCR. Results: PGJ_2 enema enhanced the expression of HO-1 in colonic mucosa. DAI score, TBA, MPO and KC levels were increased by DSS administration for seven days, and however, all were suppressed by treating with PGJ_2. Co-treatment with zinc protoporphyrin IX abrogated the effect of PGJ_2, but not with GW9662. Furthermore, HO-1 expression was enhanced by PGJ_2 in cultured cells, PPAR-gamma independently. Conclusion: PGJ_2 ameliorates DSS-induced colitis through its HO-1 inducibility. Inducing HO-1 will be a promising approach for treating colitis.

T.23. Therapy of Murine DSS Colitis by Mesenchymal Stem Cells

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Introduction: Mesenchymal stem cells (MSC) are already studied in clinical trials for inflammatory bowel disease (IBD), but there is limited data on their mode of immunosuppression. Aim: Exploring MSC function for IBD therapy in a murine model. Material and Methods: Acute DSS colitis was induced in BALB/c mice (n=6 to 9 per group). Treatment consisted of 3×10^6 cultured bone-marrow-derived MSC administered intraperitoneally at day +4. Therapeutic outcome was assessed by daily monitoring of clinical parameters with an objective grading system (murine IBD score, range 0–4) and by macroscopic/microscopic pathology at day+8. Results: Administration of MSC prevented weight loss (7% vs. 16% w/o MSC; P = 0.018) and resulted in a lower IBD score (median 1.0 (range 0–3.3) vs. 3.3 (2–4); P=0.026). Reduction of colon length was less pronounced in MSC-treated animals (7.7 cm (6.7 cm–8.5 cm) vs. 5.9 cm (5.0 cm–6.8 cm); P < 0.05). However, pathologic signs of colitis were less impressive but still present in MSC-receiving animals. Conclusion: DSS colitis is a feasible murine model for exploring MSC function in IBD therapy. Currently, a detailed analysis of their mode of action *in vivo* is conducted.

T.24. Therapeutic Effects of Exogenous Lysozyme in a Porcine Model of Experimental Colitis

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Inflammatory bowel disease (IBD) is a chronic and recurring inflammation of the gastrointestinal tract. Recent evidence suggests that altered innate immunity, including defective mucosal antimicrobial protein/peptide expression, may contribute to IBD pathophysiology. We examined the therapeutic effect of supplementation with lysozyme, a well known antimicrobial protein, on intestinal inflammation using a porcine model of colitis. Acute colitis was induced using dextran sodium sulfate (DSS), followed by intra-gastric administration of hen egg lysozyme at a dose of 150 mg/kg body weight. Lysozyme administration attenuated DSS-induced weight loss and intestinal permeability, reduced neutrophil influx, and markedly improved colon histology. Moreover, treatment with lysozyme resulted in a significant reduction in local expression of inflammatory mediators including TNF-α, IL-6, IFN-γ, IL-8, and IL-17, while increasing the expression of IL-4, TGF-β, and Foxp3. In order to elucidate the mechanisms by which lysozyme reduced colitis symptoms, the effects of lysozyme administration on both gut epithelial cells and activated immune cells in intestinal inflammation were examined. These results suggest that exogenous lysozyme may function as a potent anti-inflammatory and immune regulator, and may be a novel therapeutic for the treatment of intestinal inflammation.

T.25. Possible Role for CCR6 in Regulatory T Cell Function in the T Cell-transfer Model of Colitis

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CC chemokine receptor 6 (CCR6) is expressed on most Th17 cells and subpopulations of γ/δ, NK, Th1, Treg, B, and immature dendritic cells. CCR6 expression is increased in colonic tissue from humans or mice with intestinal inflammation, however its role in disease pathogenesis remains obscure. To address this issue, we analyzed the disease course and mucosal T cell phenotypes in Rag2-KO mice given CD4+CD45RBhiCD25- T cells from wild-type (WT) or CCR6-KO (KO) mice. Mice given KO cells had a higher incidence and severity of colitis with increased IFN-γ producing cells, compared to the mice given WT cells. While equivalent percentages of IL-17-producing cells were found in the colitic tissues and MLNs from both groups, the frequency of induced CD4+Foxp3+ regulatory T cells (iTregs) was decreased in the colon and MLNs in KO group. When naturally occurring CD4+CD45RBloCD25+ Tregs (nTregs) from WT or KO mice were co-transferred with WT CD4+CD45RBhi T cells, KO nTregs were less capable of suppressing colitis induction compared to WT nTregs. These data suggest a role for CCR6 in the trafficking and/or suppressive function of regulatory T cells in this colitis model.

T.26. Oral Treatment with Soluble Beta-glucan Protects Against Experimental Ulcerative Colitis

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Background: β-Glucans are glucose polymers with an array of stimulatory effects on the immune system although little is known about the mechanisms behind the beneficial effects. Objective: To

investigate the effect of oral treatment with soluble Saccharomyces cerevisiae-derived β-1,3/1,6-glucan (SBG) on experimental ulcerative colitis (UC). Results: Oral SBG administration reduced colitis-associated mortality and body weight loss. Furthermore, colonic inflammation, tissue damage, colon shortening, thymic involution and systemic inflammation was attenuated by SBG treatment. SBG administration to control mice increased the number of macroscopically visible Peyer's patches (PP) and the cross section area of isolated mesenteric lymph nodes (MLN) compared to mice receiving regular drinking water. Moreover, the number of proliferating epithelial cells and the size of the proliferative zone were increased compared to controls. Conclusions: We demonstrate a beneficial effect of oral SBG administration in experimental UC and propose that SBG has a potential as a therapeutic agent in IBD management. We suggest that SBG protects against DSS-induced colitis, in part, by effects on epithelial proliferation and intestinal restitution. SBG stimulated expansion of PPs and MLNs, key mucosal inductive sites. Our data supports the hypothesis that β-glucans may enhance host protection, in part, by effects on the mucosal immune system.

T.27. Colitogenic CD4+ T Cells Home to the Large Intestine Independent of CCL25/CCR9 Interactions

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Background: The chemokine CCL25 and its receptor CCR9 constitute a unique chemokine/receptor pair, which regulates trafficking of T lymphocytes to the small intestine under physiological conditions. Objective: In this study we aimed to determine whether CCL25 is expressed in murine large intestine and whether its intestinal expression is required in an experimental inflammatory bowel disease (IBD). Method: In order to assess whether colitogenic T cells require CCL25/CCR9 interactions to induce IBD, we investigated a T cell-dependant large intestinal inflammation. We adoptively transferred either WT or CCR9-/- sorted CD45RBhi CD4+ T cells into immunocompromised RAG-/- mice to induce IBD or a mixture of WT CD45RBhi + WT CD45RBlo CD4+ T cells to prevent the disease. Results: RT-PCR analysis showed that CCL25 transcripts are present in WT large intestinal mucosa. We did not observe any modulation of CCL25 transcripts in RAG-deficient animals that received only CD45RBhi or a mixture of CD45RBhi and CD45RBlo CD4+ T cells. Thus, CCL25 expression is not up-regulated upon induction or prevention of experimental IBD. RAG-deficient mice transferred with either WT or CCR9-/- colitogenic T cells develop a severe wasting disease associated with massive inflammation and leukocyte infiltration to the colon. Flow cytometry analysis revealed that both WT and CCR9-/- colitogenic T cells home and accumulate in colonic lamina propria of immuno-compromised recipient mice and that WT colitogenic cells do not up-regulate CCR9 expression. Conclusion: Homing of colitogenic T cells to the colon occurs independent of CCL25/CCR9 interactions. This suggests that different chemokine/receptor pairs might control pathogenic T cell trafficking to the large intestine. Supported in part by Harvard Digestive Diseases Center, NIH Grant P30 DK34845.

T.28. RELM Proteins Promote Th17 Cell Responses and Intestinal Inflammation

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REsistin-Like Molecules RELMα and RELMβ belong to a family of secreted mammalian proteins that have potential immunoregulatory functions. Employing dextran sodium sulfate (DSS) as a murine model of intestinal inflammation and repair, we observed the recruitment of RELMα-expressing macrophages to the inflamed colon and increased expression of goblet cell-derived RELMβ. Strikingly, mice deficient in RELMα (Retnla-/-) or RELMβ (Retnlb-/-) were resistant to DSS-induced inflammation, exhibiting reduced weight loss and less severe intestinal inflammation than wild-type mice. Additionally, in comparison to wild-type mice, Retnla-/- and Retnlb-/- mice showed reduced expression of CD4+ T cell-derived IL-17. Since DSS-induced colitis does not allow tracking of antigen-specific T cells, we are employing Citrobacter rodentium infection as a natural model of bacterial-induced colitis to test how RELMα and RELMβ regulate bacterial clearance, pathogen-specific Th17 cell responses and intestinal inflammation. Together, these results will provide mechanistic insights into the functions of RELMα and RELMβ in intestinal inflammation, and their potential as therapeutic targets in treatment of intestinal inflammatory disorders.