

3.0 ROLE OF THE IMMUNE SYSTEM IN INTESTINAL INFLAMMATION: WHO ARE THE PLAYERS AND WHAT DO THEY DO?

3.2

DO B CELLS PLAY A ROLE IN IBD?

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B cells play an important role in immunologically-mediated reactions, including autoimmune diseases, by producing antibodies reactive with self or foreign antigens. Recent studies indicate that, like CD4⁺ T cells, pathogenic and regulatory subsets exist within the B cell population. B cell subsets capable of producing regulatory cytokines such as IL-10 and TGF- β 1 prevent the development of autoimmune diseases. Our studies have identified a regulatory role of B cells in Th2-mediated spontaneous colitis of T cell receptor a knockout (TCR α KO) mice; the colitis shares many features with human ulcerative colitis. A regulatory B cell subset, characterized by up-regulation of surface CD1d expression, can be identified after the development of colitis. These regulatory B cells suppress the progression of disease by the production of IL-10. Our studies also indicate that B cells may play a role in Th-1 mediated colitis. TCR α KO mice deficient in both IL-4 and B cells, but not in IL-4 alone, develop granulomatous colitis with features of Chron's disease (CD). Furthermore, there is exacerbation of Th1-mediated colitis of IL-10 KO mice in the absence of B cells. This suggests that B cells can regulate colitis in the absence of IL-10. Our recent findings indicate that immunoglobulins may regulate granulomatous colitis by suppressing the activation of myeloid dendritic cells through their Fc receptors.

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IL-10-producing regulatory B cells can regulate autoimmune encephalomyelitis in mice.

4.0 POSTER PRESENTATIONS

4.1

REGULATION OF IL-8 AND IL-1BETA EXPRESSION WITH CROHN'S DISEASE ASSOCIATED NOD2/CARD15 MUTATIONS

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Crohn's disease (CD) is a chronic inflammation affecting the gastrointestinal tract. The mutations (Arg702Trp, Gly908Arg, Leu1007fsinsC) within the NOD2/CARD15 gene are associated with increased CD susceptibility. To examine the functional effects of these mutations in primary human mononuclear cells, we measured the cytokine induction with a muramyl dipeptide (MDP) or MDP and TNF α . At low MDP dose (10ng/ml), we observed comparable defects in IL-8 expression in all double-dose carriers. Leu1007fsinsC double dose carriers showed no induction of IL-8 protein in response to higher dose of MDP (1mg/ml), in contrast, the double dose carriers of the other two mutations showed a significant induction of IL-8 protein. This suggested that these three genetic equivalent mutations function differently. In heterozygotes, we observe a range of MDP-mediated IL-8 production. Those heterozygotes demonstrating an MDP response similar to double-dose carriers may carry additional risk alleles on this pathway, and may provide a mean of using this intermediate phenotype to identify additional genetic defects increasing CD susceptibility. In wild-type individuals, there is a marked synergistic induction with combination of MDP and TNF- α treatment. In Leu1007fsinsC homozygotes, there is a profound defect in IL-1 β secretion with MDP and TNF α , despite intact TNF α induction of IL-1 β mRNA, demonstrating post-transcriptional dependency on the CARD15 pathway for IL-1 β secretion.

4.2

GENETIC VARIANTS OF NOD2 & STAT6 IN CROHN'S DISEASE

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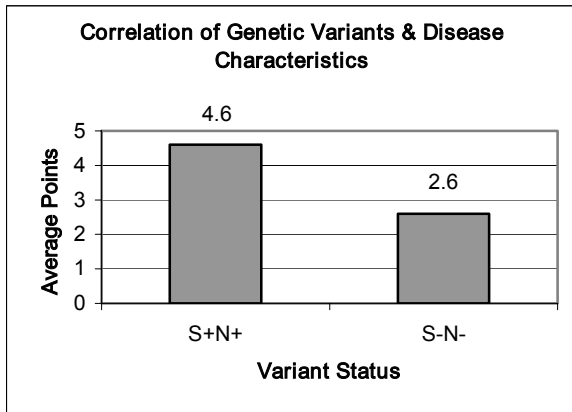
Crohn's disease (CD) is a complex-trait illness in which several genetic loci have been implicated. Allelic variants in the NOD2/CARD 15 gene have been found to correlate with CD in only 30-40% of patients with terminal ileal (TI) disease, suggesting a role for mutations in other immune modifying genes. We have previously identified patients from a familial IBD registry with defects in Stat6 function suggesting it may play a role in CD. Stat6 is a transcription factor for IL-4.

Purpose: To correlate clinical characteristics of CD severity in 14 patients with TI disease with NOD2 allelic variants and Stat6 function (Stat6null, low, or high).

Methods: Detection of NOD2 allelic variants by PCR and Stat6 function by EMSA analysis was performed. A point system was assigned for each clinical disease characteristic (early diagnosis [<40 years), stricturing disease, >2 surgeries, >2 hospitalizations, prednisone dependency, and length of bowel resection (>20 cm)], and the average points derived for patients with both Stat6 defect and NOD2 variant.

Results: 6/14 patients had NOD2 variants and 8/14 had Stat6 defects (Stat6null or low).

Patients who had defects in both Stat6 and NOD2 had higher disease severity scores than those with neither defect.



Conclusion: Severity of disease correlated with the combined sum of defects in NOD2 and Stat6 function.

S+N+ = Stat6 defect+NOD2 variant

S-N- = Neither defect

4.3 ECTOPIC CD40 LIGAND ON B CELLS TRIGGERS INTESTINAL INFLAMMATION

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Several studies indicate that T cells, macrophages and dendritic cells initially mediate intestinal inflammation in murine models of human inflammatory bowel disease (IBD). However, the initial role of B cells in intestinal inflammation remains unclear. Here we show the evidence that B cell can trigger intestinal inflammation using transgenic mice expressing CD40 ligand ectopically on B cells (CD40L/B Tg). We demonstrated that CD40L/B Tg mice spontaneously developed severe transmural intestinal inflammation in both colon and ileum at 8-15 wks of age. The inflammatory infiltrates consisted predominantly of massive aggregated IgM-positive B cells. These mice were also characterized by the presence of anti-colon autoantibodies and elevated IFN-g production.

Furthermore, although mice transferred with CD4+ T cells alone or both CD4+ T and B220+ B cells, but not B220+ cells alone from diseased CD40L/B Tg mice, develop colitis, mice transferred with B220+ B cells from diseased CD40L/B Tg mice and CD4+ T cells from wild type mice did also develop colitis, indicating that the transgenic B cells should be a trigger for this colitis model, whereas T cells are involved as effectors. The present study suggests the possible contribution of B cells in triggering intestinal inflammation in human IBD.

4.4 HEAT SHOCK PROTEINS (HSP)-60 PEPTIDE RECOGNITION IN PEDIATRIC INFLAMMATORY BOWEL DISEASES (IBD)

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Despite rigorous research, etiology of both Crohn's disease (CD) and Ulcerative Colitis (UC) is currently unknown. Activation of the mucosal immune system in response to bacterial antigens with consecutive pathologic cytokine production by T lymphocytes may play a key pathogenic role.

It is our hypothesis that HSP fulfill all requirements as target of the chronic inflammatory process involved in IBD. HSP are proteins with strong antigenic potential, induce production of stimulatory/regulatory cytokines, are present at the site of immune-mediated inflammation and are phylogenetically conserved.

We analyzed the proinflammatory cytokine production profile in colonic biopsies obtained from children diagnosed with CD, UC and a control group. Biopsies were challenged in *ex-vivo* cultures with 6 different HSP60-derived peptides of bacterial and human origin. Quantitative Real-Time PCR was used to evaluate the mRNA cytokine expression level after peptides stimulation. Using such analysis we were able to identify 2 HSP-derived peptides inducing TNF- α production in UC patients and 1 in CD patients. Proinflammatory response measured as TNF- α production was also positively associated with Pediatric CD Activity Index.

Altogether these results support the concept that responses to HSP-derived peptides may play a modulating role in autoimmune inflammation. It is our objective to evaluate the importance of these mechanisms in human IBD and to exploit them for therapeutic purposes.

4.5 CHARACTERIZATION OF POTENTIAL EFFICACY ENDPOINTS IN A MURINE IBD MODEL

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Crohn's disease and ulcerative colitis, collectively known as inflammatory bowel disease, are chronic inflammatory disorders of unknown etiology. A number of murine models which exhibit spontaneous or induced colitis have been developed to examine the pathogenesis of IBD and *in vivo* efficacy testing of therapeutic candidates.

CRF2-4 (IL-10R2) knockout mice spontaneously develop colitis at approximately 12 weeks of age when maintained in conventional housing (1). We have evaluated CRF2-4 colitis as an IBD model for testing biologic and small molecule therapeutic candidates by assessing biologic endpoints which might serve as markers for compound efficacy. A histopathologic scoring system has been established based on one described for the IL-10 knockout mouse colitis. The histologic severity score provides a basis for comparison for other potential efficacy endpoints.

4.6

T-CELL INDUCTION OF CHRONIC SMALL AND LARGE INTESTINAL INFLAMMATION IN T-CELL RECEPTOR (TCR) BETA X DELTA DOUBLE DEFICIENT MICE

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Crohn's Disease (CD) is a chronic inflammatory bowel disease that affects the small and/or large intestine. Although a number of different rodent models of gut inflammation have been developed to study disease pathogenesis, most, if not all of these models exhibit chronic colitis but no small intestinal inflammation. We have developed a T-cell-dependent mouse model of CD in which transfer of naïve CD4⁺ T-cells into TCR $\beta \times \delta$ double deficient (TCR KO) mice induces chronic inflammation in the proximal small intestine as well as the colon. We found that injection (i.p.) of wild type CD4⁺CD45RB^{high} T-cells into C57Bl/6 TCR KO recipients induced moderate to severe colitis as well as chronic duodenal/jejunal inflammation. Blinded histopathological analyses of the small and large intestine in the reconstituted TCR KO mice revealed transmural inflammation, disruption of crypt architecture and epithelial cell erosions in both tissues as well as villus blunting and muscle layer thickening in the duodenum/jejunum. This novel mouse model of CD may prove useful in studying the immunopathological mechanisms responsible for small bowel vs. large bowel inflammation. (Supported by DK64023)

4.7

LFA-1 DEFICIENT T-CELLS FAIL TO INDUCE CHRONIC COLITIS IN A T-CELL DEPENDENT MODEL OF CROHN'S DISEASE

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The objective of this study was to assess the role of T cell-associated LFA-1 (CD11a/CD18) in initiating and/or

perpetuating chronic colitis in immunodeficient mice *in vivo*. We found that transfer of CD4⁺CD45RB^{high} T-cells obtained from wild type (wt) C57Bl/6 donors into RAG-1^{-/-} recipients induced moderate to severe colitis at 8-10 weeks post transfer whereas injection of CD11a-deficient (LFA-1^{-/-}) CD4⁺CD45RB^{high} T-cells into immunodeficient recipients failed to induce gut inflammation. Blinded histopathological analyses revealed dramatic and significant reductions in colonic inflammation in the LFA-1^{-/-} T-cell injected RAG-1^{-/-} mice compared to wt T-cell injected RAG-1^{-/-}. Flow cytometry demonstrated a reduction in CD3⁺CD4⁺ intraepithelial cells and lamina propria lymphocytes (LPLs) of 11- and 5-fold respectively in LFA-1^{-/-}T-cell injected RAG-1^{-/-} mice compared to wt-injected RAG-1^{-/-} recipients. CD3/CD28 stimulation of colonic LPLs isolated from LFA-1^{-/-} injected mice produced approximately three fold less TNF- α , IFN- γ and IL-2 when compared to LPLs from colitic mice. However, large and equivalent numbers of activated CD4⁺ lymphocytes were observed in spleens of wt and LFA-1^{-/-} injected mice. Taken together, these data suggest that T-cell associated LFA-1 is critical for the recruitment of CD4⁺ T-cells into the colonic interstitium where they induce chronic colitis.

4.8

A ROLE FOR FER PROTEIN TYROSINE KINASE (P94) IN LIPOPOLYSACCHARIDE (LPS)-INDUCED INTESTINAL INFLAMMATION

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Fer kinase (p94) is a ubiquitously expressed protein tyrosine kinase which has recently been shown to play a role in cellular responses to endotoxin. This study examines the role of Fer kinase in LPS-induced leukocyte recruitment and epithelial barrier dysfunction in the gut. Wild type or Fer inactivated mice (6-10 wk, n=5-8) were challenged systemically with LPS (500 μ /kg; i.p.) or saline and examined 4 h later. Epithelial permeability was measured using blood-to-lumen ⁵¹Cr-EDTA leakage *in vivo*. Leukocyte kinetics (rolling flux, rolling velocity and adhesion) in submucosal intestinal venules were studied by intravital microscopy. Leukocyte emigration was measured by FACS analysis of isolated intestinal cell populations. A significant epithelial barrier dysfunction was noted in wild type mice 4 h post LPS treatment (p<0.05). This was accompanied by a decrease in leukocyte rolling velocity and flux (p<0.05) and an increase in adhesion (p<0.05) in intestinal venules. In the absence of Fer kinase, LPS-induced a significant increase in leukocyte adhesion and neutrophil emigration (p<0.05) and epithelial permeability was exacerbated 4 fold (p<0.01). Treatment with anti-neutrophil antibody only partially reduced the exacerbated epithelial dysfunction observed in the absence of Fer. These data suggest a significant role for Fer kinase in regulating LPS-induced epithelial barrier dysfunction *in vivo*. This is mediated in part through emigrated neutrophils. Funded by the Canadian Institutes of Health Research.

4.9

INDUCTION OF TOLL-LIKE RECEPTOR (TLR) GENE EXPRESSION IN ENTEROCYTES BY PLATELET-ACTIVATING FACTOR (PAF) *IN VIVO* AND *IN VITRO*.

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Both PAF and bacteria have been thought to play a role in neonatal necrotizing enterocolitis (NEC) and in adult inflammatory bowel disease (IBD). The purpose of this study was to determine whether PAF plays a regulatory role in TLR expression, thereby sensitizing the intestinal epithelium to bacterial challenge. As measured by quantitative real time RT-PCR (QPCR), in mother-fed (MF) neonatal rats TLR4 gene expression transiently increased on day one, then rapidly declined, unlike in formula-fed stressed neonatal rats where TLR4 expression kept rising, reaching significantly elevated levels by day 3 of life. QPCR demonstrated that perfusion of adult rat ileal loop with PAF resulted in a significant increase of TLR4 expression in mucosal scrapings. Similarly, PAF caused a time and concentration-dependent elevation of TLR2 and TLR4 mRNA in IEC-6 cells, as measured by QPCR and caused increased TLR4 cell surface expression in HT29C119A cells, as visualized using indirect immunofluorescence labeling. Correspondingly, in PAFR-transfected 293 cells there was a concentration-dependent activation of TLR4 promoter-luciferase reporter activity by PAF. In conclusion, TLR4 gene expression is developmentally down-regulated in MF rats, while there is increased TLR4 expression in enterocytes in a neonatal rat model of NEC where elevated PAF is known to play a pathogenic role. Furthermore, PAF is a potent inducer of enterocyte TLR gene expression both *in vivo* and *in vitro*. These data suggest that PAF sensitizes enterocytes to bacterial toxins by increasing TLR gene expression and imply a mechanism by which PAF and bacteria might interact in the pathophysiology of NEC and IBD.

4.10

THE PROTECTIVE EFFECT OF GROUP IIA SECRETORY PHOSPHOLIPASE A2 (sPLA2) IN MURINE DSS-INDUCED COLITIS IS COX-2 DEPENDENT.

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Background & Aims: Group IIA sPLA2 is associated with disease activity in IBD possibly through a pro-inflammatory role via the generation of eicosanoids. We recently showed that Group IIA sPLA2 protects against DSS-induced colitis in C57BL/6 human Group IIA sPLA2 tg mice. **Methods:** Colitis was induced by DSS in 4 groups: i) C57BL/6 mice; ii) Group IIA sPLA2 tg mice fed a control diet; iii) C57BL/6 mice; and iv) Group IIA sPLA2 tg mice (groups iii) and iv) fed a COX-2 inhibitor

(celecoxib) diet). 5-bromo-2-deoxyuridine (BrdU) to assess epithelial proliferation. Colitis was assessed clinically, and by SAA levels and histological scores. IL-6, TNF α and IL-12, as well as PGE2 levels were measured. COX-1 and COX-2 immunoblots were done. **Results:** Group IIA sPLA2 tg mice consistently showed markedly less severe DSS-induced colitis. Colonic epithelial cell proliferation was significantly increased in sPLA2 tg mice compared to wild type mice shown by BrdU labeling and PCNA immunolabeling. In sPLA2 tg mice COX-2 (and PGE2) levels peaked within 3 days and returned to baseline; while in control mice COX-2 continued to increase until the end of DSS treatment. COX-2 inhibition (shown by decreased PGE2 levels) increased colitis severity in both sPLA2 tg and control mice. **Conclusion:** Intestinal Group IIA sPLA2 protects against, rather than promote, the development of colitis. This effect is COX-2 dependent and the underlying mechanisms include increased epithelial cell proliferation.

4.11

PLATELET RECRUITMENT IN INTESTINAL INFLAMMATION IS MODULATED BY ICAM-1, P-SELECTIN AND PSGL-1

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There is growing recognition of cross-talk between platelets and leukocytes during inflammation. The aim of the study was to define the mechanisms responsible for the platelet-WBC and platelet-endothelial cell (EC) interactions that occur in experimental colitis. Colitis was induced in wild type (WT) C57BL/6J and ICAM-1^{-/-} mice with 3% dextran sulfate sodium (DSS) in drinking water for 7 days (d0-6). Control mice received water. At day 6, firm adhesion of fluorescently-labeled platelets (CFSE, from genotype-matched donors) and leukocytes (rhodamine 6G) was monitored in colonic venules at the same time by intravital microscopy. Compared with controls, DSS-treated WT mice showed significantly increased adhesion of platelets, with 2.5% of platelets adhering to ECs directly and 97.5% binding to adherent WBC. ICAM-1 deficiency significantly decreased platelet-WBC adhesion. WT mice rendered neutropenic with anti-neutrophil serum (ANS) exhibited reduced platelet-WBC binding, but significantly increased platelet-EC adhesion. The increase in platelet-EC adhesion in neutropenic animals was prevented by both, a single dose (2mg/kg) of either P-selectin or PSGL-1 blocking monoclonal antibody. In conclusion, in DSS-induced colitis, platelet accumulation in venules is dependent on both, leukocyte adhesion as well as on mechanisms involving the adhesion molecules P-selectin and PSGL-1.

4.12

GROWTH HORMONE REDUCES STAT3 ACTIVATION AND MODULATES CELLULAR APOPTOSIS AND PROLIFERATION IN MURINE COLITIS

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Background: STAT3 regulates disease activity in experimental colitis and human IBD. Suppressor of Cytokine Signaling 3 (SOCS-3) and Src-homology Tyrosine Phosphatase 2 (SHP2) are negative regulators of IL-6 dependent STAT3 activation via the gp130 receptor. Growth hormone (GH) reduces disease activity in experimental colitis; however, the molecular mechanism was not known. We hypothesized that GH would ameliorate colitis by up regulating SOCS-3 or SHP2 and reducing STAT3 activation. Results: GH administration improved weight gain and colon histopathology in IL-10 null mice with colitis. This was not associated with increases in either serum or colon IGF-1. GH increased apoptosis of lamina propria mononuclear cells (LPMC), while reducing apoptosis and increasing proliferation of crypt epithelial cells (CEC). GH reduced STAT3 activation in both CEC and LPMC; the improvement in histopathology was associated with reduced STAT3 activation. GH enhanced SHP2:gp130 binding under these conditions, while SOCS-3 abundance was reduced. Direct effects of GH were investigated in tissue culture. GH treatment decreased constitutive STAT3 activation in IL-10 null mouse colon organ culture. As was observed in vivo, GH increased SHP2:gp130 association and reduced IL-6 dependent STAT3 activation in T84 human colon carcinoma cells. Conclusion: GH administration improved weight gain and reduced disease activity in IL-10 null mice with colitis. This was related to a reduction in STAT3 activation associated with increased SHP2:gp130 binding and alterations in CEC and LPMC apoptosis and proliferation.

4.13

NOVEL COMPENSATORY VASODILATION MECHANISM IN MICROVESSELS FROM PATIENTS WITH CHRONIC INFLAMMATORY BOWEL DISEASE

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Microvascular endothelial dysfunction with reduced dilation to acetylcholine (Ach) is observed in mucosal arterioles isolated from patients with IBD. Both nitric oxide (NO) & endothelial derived hyperpolarizing (EDHF) mechanisms of dilation are reduced. However, dilation to Ach remains, largely dependent upon an increased production of cyclooxygenase-derived prostanoid metabolites. In the present study, we determined the mechanism of this dilation focusing on the cellular origin of the cyclooxygenase enzyme and prostanoid species generated. HPLC was used to isolate eicosanoid vasodilator products. Ach induced vasoconstriction (-52% +/- 10%; n = 7) in control endothelial denuded microvessels with no additional effect of indomethacin (INDO: a cyclooxygenase inhibitor). In contrast, in IBD denuded arterioles, Ach induced a concentration-dependent but modest vasodilation. INDO reversed the Ach-induced vasodilation, yielding a frank vasoconstriction similar to control denuded arterioles (-54% +/- 9%, n = 6). In denuded arterioles from

IBD patients, but not from controls, Ach stimulated production of an arachidonic acid metabolite that comigrated on HPLC with PGD₂. In separate studies, PGD₂ elicited a concentration-dependent vasodilation (66% +/- 4%, n = 6) of IBD arterioles. In intestinal submucosal arterioles from IBD patients, Ach-mediated dilation shifts from endothelial production of NO and EDHF to non-endothelial generation of a prostaglandin, likely PGD₂. This is the first description of a dilator mechanism arising from non-endothelial vascular tissue that compensates for loss of endothelium-dependent dilation. Prostaglandins such as PGD₂ may be important in regulating mucosal blood flow in patients with IBD.

4.14

LOSS OF CHOLINERGIC MUSCARINIC MEDIATED ION TRANSPORT DURING EXPERIMENTAL COLITIS IS NOT DUE TO REDUCED M₃ EPITHELIAL RECEPTOR EXPRESSION.

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Introduction: Our laboratory has observed that cholinergically-mediated Cl⁻ secretion is perturbed in colonic tissue from mice treated with dextran sodium sulphate (DSS) to induce colitis. Specifically, the muscarinic agonist bethanechol produces no change in short-circuit current (I_{SC}) when applied to tissue from DSS-treated mice, unlike the bethanechol-induced increase in I_{SC} typically observed in control tissue. We hypothesized that this could be due to a decrease in the expression of epithelial M₃ muscarinic receptors. **Methods:** Colitis was induced by 4% (wt/vol) oral DSS for 5 days followed by 3 days of tap water. Colons from both control and DSS-treated mice were excised for analysis of M₃ muscarinic receptor expression. Immunofluorescent staining was performed on fixed cryostat sections using an anti-M₃ antibody. Additionally, either protein or mRNA was extracted from muscle-stripped colonic tissue as well as from freshly isolated epithelial cells for western blotting and RT-PCR, respectively. **Results:** Positive anti-M₃ receptor staining was observed on the epithelium of colonic tissue sections from both control and DSS-treated mice. Levels of staining intensity were not appreciably different between the two groups. This observation was corroborated by semi-quantitative data from both western blotting and RT-PCR, showing similar levels of M₃ protein and mRNA expression levels between the two groups. **Conclusion:** In testing our hypothesis, we have excluded reduced M₃ receptor expression as an explanation for the loss of muscarinic receptor-mediated ion transport during DSS-induced colitis. These data suggest that M₃ receptors are affected at the functional level, possibly through uncoupling of intracellular signals. (CCFC funded)

4.15

ROLE OF TUMOR NECROSIS FACTOR RECEPTORS (TNFRS) IN A TNBS ANIMAL MODEL OF COLITIS

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TNF- α is known to play an important role in IBD; however, the pathophysiological role of its receptors is still unclear.

Aim: To measure mRNA and protein levels of TNF- α and TNFRs in a rat model of colitis. **Methods:** Colitis was induced by intracolonic administration of TNBS. Control rats received the ethanol vehicle. Rats were sacrificed 72 hrs later and samples were collected. TNF- α and TNFR protein levels were measured in plasma and peritoneal fluid (PF) by ELISA. RT-PCR was performed to measure TNF- α and TNFR gene expression. Expression levels were analyzed by densitometry. **Results:** Protein levels of sTNF- α ($p < 0.05$), sTNFR I ($p < 0.01$), and sTNFR II ($p < 0.01$) in PF were significantly increased in experimental rats. Plasma sTNFR II levels correlated with both macroscopic damage and sTNFR II PF levels ($p < 0.05$). TNF- α , TNFR I, and TNFR II mRNA expression in the colon of experimental animals was 4-fold ($p < 0.001$), 3-fold ($p < 0.05$), and 4-fold ($p < 0.001$), higher than in controls, respectively. **Conclusions:** Increased expression of TNFRs and TNF- α in the colon of this rat model of IBD provides further evidence for their involvement in the promotion of inflammation and tissue damage. Increased levels of sTNFRs in the PF of experimental rats, particularly sTNFR II, may be involved in the development of colitis by serving as a reservoir of TNF- α , and thus provide a novel therapeutic target. *Supported in part by NIGMS 1F31 GM68392-01 & NIH-S06GM08239.*

4.16

ALTERATION OF TNF-ALPHA LEVELS BY THE BACTERIAL PEPTIDE FORMYL-METHIONYL-LEUCYL PHENYLALANINE (fMLP) IN ACUTE AND CHRONIC COLITIS.

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The ability of bacterial peptides, such as formyl-methionyl-leucyl-phenylalanine (fMLP), to enhance inflammatory activity via modulation of cytokine expression in intestinal inflammation is unclear. **Aim:** To measure the levels of TNF-alpha after administration of fMLP in acute and chronic animal models of colitis. **Methods:** Rats with acute colitis received fMLP (2.5mM i.c.) 2 hours after administration of TNBS (30mg). Chronic "reactivated" colitis was induced six weeks later by intravenous administration of TNBS (5mg/kg), with or without the bacterial peptide at 24 hour time intervals over three days. Controls received TNBS + vehicle. Colons were scored for damage, and TNF-alpha gene expression and protein levels were measured. **Results:** Administration of fMLP exacerbated both the macroscopic and microscopic damage in the acute model of colitis but not the chronic. TNF-alpha gene expression after administration of fMLP during the

acute or chronic phase was not significantly increased. Levels of TNF-alpha protein were significantly higher after administration of fMLP during the acute phase (14.10 \pm 4.06 pg/ml) compared to controls (3.91 \pm 0.93 pg/ml; n=4-6, $p < 0.05$), but not during the chronic phase (3.30 \pm 3.30 pg/ml) **Conclusion:** Administration of fMLP exacerbated both damage and TNF-alpha levels during the acute phase of colitis, but not during the chronic 'relapsed' phase. These results suggest that the role of bacterial peptides during the acute and chronic phase of colitis is different. *F31DK60245-02(GH) and NIH-NCRR/RCMI 2G12RR03050.*

4.17

INHIBITION OF TNF- [ALPHA] IMPROVES INDOMETHACIN (INDO)-INDUCED JEJUNOILEITIS IN RATS BY MODULATING INOS EXPRESSION

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TNF- α , including other proinflammatory cytokines alone or in combination, induces iNOS expression and upregulates inflammatory responses. To evaluate the relationship between TNF- α and iNOS expression in INDO-induced jejunoileitis. Rats (n =12) were pretreated with 2 phosphodiesterase (PDE) inhibitors; theophylline (TF 50, 100mg/kg) and pentoxifylline (PF 100, 200mg/kg) daily po for 2 days. Jejunoileitis was induced by 2 injections of INDO (7.5mg/kg sc) 24 h apart and TF or PF continued for either 12h or 4 days. Other rats received a single dose of 25, 50 μ g of anti-TNF- α monoclonal antibody (TNF-ab) ip 30 min before INDO. Controls received only INDO. Compared to INDO-induced ulceration and DAI at 4 days, TNF-ab, TF and PF treatment significantly decreased total ulcer length and DAI. Similarly TNF-ab, TF and PF decreased MPO activity significantly. INDO increased serum and tissue TNF- α levels by 10- and 4-fold over basal value at 12h respectively, but at 4 days the changes were only 2-fold. Serum/tissue TNF- α was undetectable in all treatment groups. INDO increased serum NOx level significantly over basal level but iNOS expression detected only after 4 days. Treatment with PF, TF or TNF-ab reduced serum NOx and iNOS expression. INDO-induced increase in serum/tissue TNF- α was inhibited by PDE inhibitors and by TNF-ab, associated with concomitant reduction in INDO-induced jejunoileitis. Downregulation of serum NOx by these inhibitors suggest that TNF- α modulates iNOS expression.

4.18

POLYUNSATURATED FATTY ACIDS (PUFA) ARE POTENT INHIBITORS OF PLATELET-ACTIVATING FACTOR-INDUCED SIGNALING IN INTESTINAL EPITHELIAL CELLS

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Platelet-activating factor receptor (PAFR) is a member of the G protein-coupled receptor (GPCR) family, it is present in the apical plasma membrane of intestinal epithelial cells, it's ligand, PAF, is thought to be a key mediator in the

pathogenesis of neonatal necrotizing enterocolitis (NEC) and PAF is implicated in the pathogenesis of adult inflammatory bowel diseases (IBD). Herein, we investigated PAF-induced signaling mechanisms in enterocytes, we analyzed the role of lipid raft integrity in PAFR signaling and the modulation of this signaling by PUFA. As determined by phospho-specific antibodies, western blotting, and treatments with PAF, the PI3 kinase inhibitor LY294002 or their combination, PAFR signaling elicits apoptosis in enterocytes by causing dephosphorylation in several members of the PI3 kinase/Akt pathway, and PUFA block this effect upstream of PI3 kinase. Furthermore, we localized the PAFR in enterocytes to lipid rafts using density gradient centrifugation, detergent extraction and western blotting. We have found that similar to treatment with PUFA-s, disruption of lipid rafts via cholesterol chelation or inhibition of palmitoylation by 2-bromo palmitate abolishes PAFR signaling. Either arachidonic acid, docosahexaenoic acid, or their combination equally inhibit PAFR signaling. Indomethacin, or the antioxidant vitamin E do not affect PAFR signaling in enterocytes, and do not affect the inhibition of PAFR signaling by any of the PUFA tested. These data, strongly suggest that PUFA do not inhibit PAFR signaling through an effect on prostaglandin synthesis, or as an antioxidant, but interfere with protein palmitoylation and GPCR signaling. Understanding the precise mechanism of PUFA effects might aid designing future preventative strategies for NEC and IBD.

4.19

PRO-INFLAMMATORY CYTOKINES DOWN-REGULATE TRANSLATION OF CYTOPROTECTIVE INTESTINAL EPITHELIAL HEAT SHOCK PROTEINS THROUGH THE 3' UNTRANSLATED REGION

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Heat shock proteins (HSP) confer cytoprotection to cells under stress or injury, which are selectively expressed by colonic epithelial cells in direct contact with luminal fluid and flora. However, in inflammatory bowel diseases (IBD), their expression is paradoxically and selectively decreased, possibly by pro-inflammatory cytokines. We investigated potential mechanisms underlying this effect. Materials and Methods: Intestinal YAMC and IEC-18 cells were treated with different pro-inflammatory cytokines for 8 hours followed by heat shock or butyrate treatment to induce HSPs. The mRNA and protein of HSP 25 and 72 were measured. IEC-18 cells were transfected with luciferase plasmid containing HSP25 3'UTR construct. RNA binding proteins expression was determined after the treatment of cytokines, butyrate and heat shock. Results: We found that pro-inflammatory cytokines, TNF- α and interferon- γ , negatively regulated HSP25 and HSP72 protein expression, while these effects occur in the absence of respective HSP mRNA and protein degradation changing. After heat shock, luciferase constructs in IEC-18 cells exhibited higher activity with 3'UTR presence than the control. RNA binding protein, tristetraprolin (TTP), was induced by TNF-

α , interferon- γ and butyrate both on mRNA and protein, while HuR was diminished. Conclusions: In intestinal cells, the selective inhibition of HSP by TNF- α and interferon- γ is mediated through 3'UTR-dependent block of mRNA translation, possibly through altered expression of the RNA binding proteins, TTP and HuR. The downregulation of cytoprotective HSP could contribute to the extent and severity of mucosal injury associated with IBD.

4.20

SNARING CYTOKINES, TRAFFICKING, SECRETION AND MANIPULATION OF TNF IN INFLAMMATORY MACROPHAGES

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In chronic inflammatory disease the excess secretion of proinflammatory cytokines such as tumor necrosis factor (TNF) by activated macrophages has serious pathophysiological consequences. In studies aimed at characterizing the secretory pathway in macrophages, we used gene and protein screens to identify specific LPS-regulated trafficking machinery proteins that facilitate TNF secretion. Several SNARE complexes, which function in vesicle docking and fusion, were identified at the level of the Golgi and cell surface (syntaxins 4 & 6, Vti1b). Obligate upregulation of these SNAREs upon macrophage activation enables TNF secretion. Activation also involves the movement of specific SNAREs components into lipid rafts to create high affinity exit sites at the macrophage surface for cytokine release. Our findings show that LPS-regulated SNAREs control cytokine secretion; manipulation of these SNAREs in inflammatory macrophages using biological modifiers (antibodies, cDNAs and toxins) blocks TNF secretion, suggesting novel strategies for anti-TNF therapies.

4.21

THE ROLE OF HEME OXYGENASE ON THE BALANCE OF INFLAMMATORY CYTOKINES IN DEXTRAN SODIUM SULFATE-INDUCED COLITIS

Tomohisa Takagi, Yuji Naito, Kazuhiro Katada, Yutaka Isozaki, Masaaki Kuroda, Hisato Tsuboi, Toshimitu Okuda, Satoshi Kokura, Hiroshi Ichikawa, Norimasa Yoshida, Toshikazu Yoshikawa, Kyoto Prefectural University of Medicine, Kawamachi Hirokoji, Kamigyo-ku, Kyoto, 602-8566, Japan

Background: It has been reported Heme oxygenases-1 (HO-1) play a protective role in the process of inflammation. The present study investigated the possible role of HO-1 in the regulation of inflammatory process using a dextran sodium sulfate (DSS) colitis. 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/HO-2 and TNF- α , IFN- γ as pro-inflammatory cytokines and IL-4, IL-10 as anti-inflammatory cytokines was measured. The mRNA expression of CCR5 (Th1 marker) and CCR4 (Th2 marker) was also measured. Moreover we evaluated the

enhancement by treatment of an HO-1 inhibitor, ZnPP (25mg/kg i.p., daily). Results: After DSS administration, DAI score and expression of HO-1 mRNA and protein were increased in a time-dependent manner. Expression of TNF- α , IFN- γ , IL-4, IL-10, CCR4, and CCR5 mRNA were increased after DSS administration. CCR5 expression was more enhanced during DSS administration compared to CCR4 expression. Treatment with ZnPP enhanced the increase in DAI score. The increases in the expression of TNF- α , IFN- γ , CCR4, and CCR5 were enhanced in ZnPP-treated group, and dominance of CCR5 expression was also enhanced. In contrast, the increases in the expression of IL-4 and IL-10 were inhibited in ZnPP-treated group. 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.

4.22

CECAL GENE EXPRESSION IN *HELICOBACTER HEPATICUS*-INFECTED TYPHLITIS-PRONE MICE

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Helicobacter hepaticus infection in A/JCr mice is typically subclinical, and typhlitis develops only after months of infection. The prolonged pre-inflammatory period and the ensuing inflammation in the context of an intact immune system make *H. hepaticus* infection in A/JCr mice an attractive model for studying immune dysregulation during the early and late stages of inflammatory bowel disease.

In this work, we hypothesized that immune dysregulation associated with *H. hepaticus* infection occurs prior to development of any pathologic features of disease, and these alterations are heightened during the inflammatory phase of disease. To address this hypothesis, we focused on three specific aims: 1) we identified the time points that correlate to early and late stages of disease; prior to the onset of pathologic lesions and after typhlitis is fully established; 2) we surveyed the cecal gene expression profile of *H. hepaticus*-infected A/JCr mice in early and late disease; and 3) we selected a subset of genes from the cDNA array data and used real-time RT-PCR to quantify their expression. This study demonstrated that *H. hepaticus* infection in A/JCr mice is clinically and histologically silent during the first month of infection; by month-3, intestinal inflammation is clearly established. Analysis of the gene expression profile in the cecum of infected mice revealed 25 up-regulated and 3 down-regulated genes at month-1 and 31 up-regulated and 2 down-regulated genes at month-3 of infection. From the cecal gene expression profiles, we identified a subset of immune-related genes including CD38, IP-10, MIG, MIP-1 α , and SAA1. Real-time RT-PCR confirmed the increased expression levels of these genes as well as elevated expression of IFN- γ prior to the development of any features of pathology, as well as during the chronic stage of inflammatory bowel disease.

4.23

5-HT₃ RECEPTOR MEDIATED SECRETORY FUNCTION OF THE ILEUM ATTENUATED BY INFLAMMATION AND INHIBITION OF SERT

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5-HT, a key signaling molecule in the intestine, is released from mucosal enterochromaffin cells in response to luminal stimuli, leading to activation of 5-HT₃ receptors on intrinsic neurons. The aim of the study was to determine the effect of elevation of mucosal 5-HT (such as occurs in intestinal inflammation) on cellular distribution and function of 5HT₃Rs. To elevate mucosal 5-HT, rats were treated with the SERT inhibitor, fluoxetine (fluox) or vehicle for 6 days (20 mg/kg daily, po). Colonic inflammation was induced by TNBS (30 mg/0.5 ml 50% ethanol). The terminal ileum was processed for immunohistochemistry to determine the cellular distribution of 5-HT₃R in myenteric neurons. Ileal secretion was assessed by measuring the weight change of a 2.5% agarose cylinder placed in the lumen of anesthetized rats. Fluox treatment increased 5-HT concentration in the luminal fluid and decreased 5-HT₃R immunoreactivity at the membrane of myenteric neurons. In vehicle treated rats, ileal fluid absorption was decreased by 2-Me5-HT (5-HT₃R agonist, 3 mg/Kg sc) and by luminal glucose. Fluox treatment had no significant effect on basal fluid absorption, but significantly attenuated secretory responses to 2-Me5-HT and glucose. Induction of colitis also significantly attenuated the secretory response to glucose. Chronic elevation of mucosal 5-HT increases 5-HT₃R internalization in myenteric neurons and decreased the physiological expression of receptor function. Chronic changes in mucosal 5-HT, induced by inflammation, may alter gastrointestinal secretory function via ongoing loss of receptor from neuronal membrane.

4.24

THE REGULATORY ROLE OF MAST CELLS IN A MURINE MODEL FOR IBD

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The etiology of inflammatory bowel disease (IBD) remains unclear and the immunological mechanisms underlying the pathogenesis are poorly defined. Especially the role of mast cells as a possible source for immunological mediators in the development of IBD is not well described.

In this study a colonic hypersensitivity reaction in mice induced by skin sensitization with dinitrofluorobenzene (DNFB) followed by an intrarectal challenge with the hapten featured as a model to study the role of mast cells in the development of IBD.

The mice were monitored for clinical symptoms and inflammatory features for 72h. DNFB-treated mice stayed behind in bodyweight and developed diarrheic stool compared to vehicle-treated animals. At 72h increased colonic vascular permeability and hypertrophy of colonic lymphoid follicles (colonic patches) were observed in DNFB-treated animals. Increased numbers of mast cells were found in the colon of DNFB-treated mice located in and around colonic patches. This was associated with elevated levels of mouse mast cell protease-1 (mMCP-1) in

serum indicating mast cell activation. TNF α is the major proinflammatory cytokine of mast cell origin and found to be important in the pathogenesis of IBD. Increased levels of TNF α in tissue homogenates of the colon were demonstrated in DNFB-treated mice compared to controls. Finally, neutrophil infiltration was observed in the colon of DNFB-treated mice. Induction of this model in mast cell deficient W/W^v mice shows a profound reduction of characteristics of the colonic hypersensitivity reaction. Reconstitution with bone marrow derived mast cells in W/W^v mice restored the inflammatory response.

This study demonstrates the importance of mast cells in the development of colonic hypersensitivity.

4.25

THE ROLE OF HEME OXYGENASE ON THE BALANCE OF INFLAMMATORY CYTOKINES IN DEXTRAN SODIUM SULFATE-INDUCED COLITIS

Tomohisa Takagi, Yuji Naito, Kazuhiro Katada, Yutaka Isozaki, Masaaki Kuroda, Hisato Tsuboi, Toshimitu Okuda, Satoshi Kokura, Hiroshi Ichikawa, Norimasa Yoshida, Toshikazu Yoshikawa, Kyoto Prefectural University of Medicine, Kawamachi Hirokoji, Kamigyo-ku, Kyoto, 602-8566, Japan

Background: Heme oxygenases (HOs) are the rate-limiting enzymes in heme degradation. Inducible form of heme oxygenase (HO-1) plays a protective role in the process of inflammation. The present study investigated the possible role of HO-1 in the regulation of inflammatory process using a dextran sodium sulfate (DSS)-induced colitis. Materials and methods: On acute DSS colitis in male BALB/c mice, a disease activity index (DAI) consists of a calculated score based on changes in body weight, stool consistency, and intestinal bleeding. Colonic mRNA expression for HO-1 and TNF- α , IFN- β as pro-inflammatory cytokines, and IL-4, IL-10 as anti-inflammatory cytokines. The mRNA expression of CCR5 (Th1 marker), CCR4 (Th2 marker) was also measured. Moreover we evaluated the enhancement by treatment of HO-1 inhibitor, zinc protoporphyrin IX (ZnPP 25mg/kg i.p., daily). Results: After DSS administration, DAI score, expression of HO-1 mRNA and protein were increased in time-dependent manner. Expression of TNF- α , IFN- β , IL-4, IL-10, CCR4, and CCR5 mRNA were increased after DSS administration. CCR5 expression was more enhanced during DSS administration compared to CCR4 expression. With ZnPP administration, DAI score was increased. The increases in the expression of TNF- α , IFN- β , CCR4, and CCR5 were enhanced in ZnPP-treated group, and dominance of CCR5 expression was also enhanced. In contrast, the increases in the expression of IL-4 and IL-10 were inhibited in ZnPP group. 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.

4.26

GASEOUS MONOXIDES IN DEXTRAN SULFATE SODIUM-INDUCED COLITIS IN MICE

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There is some evidence that carbon monoxide (CO) and nitric oxide (NO) have parallel significant actions via soluble guanylyl cyclase (sGC) in select tissue. We previously demonstrated the marked expression of inducible gene of heme oxygenase and NO synthase (HO-1 and iNOS) in the inflamed intestine. The aim of the present study is to investigate the interaction between CO and NO in the inflamed intestine induced by dextran sulfate sodium (DSS) in mice. HO-1 was induced in epithelial cells as well as interstitial inflammatory cells in DSS-treated intestine. Disease activity index (DAI) was significantly enhanced in the presence of ZnPP, an HO inhibitor. A specific iNOS inhibitor ONO1714 significantly inhibited the increase in DAI after DSS administration with/without ZnPP. Luminal NO_x levels were increased after DSS administration, and these levels were reduced by ONO1714 and not affected by ZnPP. The amounts of mucosal cGMP increased after DSS administration, and the increase was significantly reduced, and the increase was enhanced in the presence of ZnPP. These results suggest that activation of NO-sGC signaling is one of major pathological pathway in DSS-induced intestinal inflammation and CO may act as an anti-inflammatory mediator via inhibiting the NO-induced activation of sGC.

4.27

ALTERED INTESTINAL CYTOKINE AND EICOSA-NOID SYNTHESIS IN THE G α 2-DEFICIENT MOUSE MODEL OF INFLAMMATORY BOWEL DISEASE.

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Mice lacking the G-protein alpha subunit G α 2 spontaneously develop colitis between 6-12 weeks of age and can progress to invasive adenocarcinoma, mimicking human IBD. The colitis phenotype shares some histologic features with ulcerative colitis, but immunophenotypically mimics Crohn's disease: Th-1 associated cytokines are overexpressed by multiple immune cell populations (T-cells, dendritic cells, and macrophages), often before the onset of clinical disease. Healthy G α 2-KO mice had elevated colonic levels of IL-12, IL-18, and the chemokine IP-10. Purified peritoneal and bone marrow-derived macrophages overexpressed these cytokines in response to activation with Zymosan and/or LPS. Because of the protective effects of PGE₂ on epithelial integrity/barrier function, its suppression of IL-12 synthesis, an absence of group II sPLA₂ in colitis-prone G α 2-KO mouse strains, and defects in cPLA₂-IVA activation in G α 2-KO MEF's, we hypothesized that defects in PGE₂ production might contribute to the colitis phenotype. However, G α 2-KO macrophages and intestinal lamina propria mononuclear cells (LPMC) overproduced PGE₂ compared to their wildtype littermates. No defects in Ca²⁺-mediated cPLA₂-IVA mobilization were seen in bone-marrow derived macrophages. LPS-Activated macrophage COX-2 expression was found to be significantly elevated in G α 2-KO mice. These data identify alterations in innate immune responses in G α 2-KO mice and underscore the persistent

development of colitis despite the overproduction of PGE₂ in the lamina propria of the intestine. Elevations in PGE₂ synthesis and COX-2 expression may also contribute to the predisposition towards the development of carcinomas in Gα2-KO mice.

4.28

GM-CSF AND HOST CYTOKINE RESPONSE IN MURINE INFLAMMATORY MODELS.

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Impaired mucosal clearance of microbes may be pivotal in the etiology of Crohn's disease. This idea is consistent with an essential role for gut flora in models of IBD. Sargramostim (rhu GM-CSF) decreased disease activity in a phase II trial in patients with moderate to severe CD. The mechanism of action remains unknown. GM-CSF increases effector function of innate immune populations, thereby promoting microbial clearance. The effect of murine pegylated GM-CSF was examined in DSS-induced colitis by gene expression analysis. DSS reduced epithelial proliferation, leading to impaired epithelial barrier function and increased association between immune cells and resident microbial flora. GM-CSF treatment resulted in significant reductions in histologic disease activity score. We interrogated high density oligonucleotide arrays and analyzed gene expression patterns by clustering methods. Marked reductions in a number of cytokines were observed; notably TNF- α , IFN- γ and IL-1 β . To determine if these effects were due to reduced cytokine expression as a consequence of mucosal healing (indirect) or due to GM-CSF induced modulation of cytokine expression, mice were challenged with 100 μ g LPS administered IP in the presence or absence of GM-CSF. Gene expression changes were assayed in splenocytes isolated 2 hours later. LPS challenge led to significant increases in multiple cytokines, including a major overlap with mediators induced in DSS colitis. In contrast, GM-CSF treatment ameliorated LPS-induced induction of IFN- γ and IL-1 β . Arrays confirmed alterations in downstream transcriptional targets of these genes, including a large IFN-regulated gene cluster. These results support the efficacy of GM-CSF in inflammatory models and further suggests multiple complementary mechanisms of action.

4.29

SPATIAL ORGANIZATION OF BACTERIAL FLORA IN NORMAL AND INFLAMED INTESTINE - A FLUORESCENCE IN SITU HYBRIDIZATION STUDY IN MICE.

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Background: The role of intestinal flora in IBD is poorly specified. Materials and Methods: The spatial organization of intestinal flora was investigated in normal mice and in two models of murine colitis using fluorescence in situ hybridization. Results: The murine small intestine was

nearly bacteria-free. The normal colonic flora was organized in three distinct compartments (crypt, interlaced, and fecal), each with different bacterial compositions. Crypt bacteria were present in the cecum and proximal colon. The fecal compartment was composed of homogeneously mixed bacterial groups that directly contacted the colonic wall in the cecum but were separated from the proximal colonic wall by a dense interlaced layer. Beginning in the middle colon, a mucus gap of growing thickness physically separated all intestinal bacteria from contact with the epithelium. Colonic inflammation was accompanied by a depletion of bacteria within the fecal compartment, a reduced surface area in which feces had direct contact with the colonic wall, increased thickness and spread of the mucus gap, and massive increases of bacterial concentrations in the crypt and interlaced compartments. Adhesive and infiltrative bacteria were observed in inflamed colon only, with *Bacteroides* species dominating. Conclusions: The proximal and distal colon are functionally different organs with respect to the intestinal flora, representing a bioreactor and a segregation device. The highly organized structure of the colonic flora, its different arrangement in different colonic segments, and its differentiated response to inflammatory stimuli indicate that the intestinal flora is an innate part of host immunity that is under complex control.

4.30

E. COLI K1 RS218 INDUCES INTESTINAL EPITHELIAL CELL APOPTOSIS

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E. coli K1 RS218 is able to translocate the intestinal barrier, causing sepsis and meningitis in humans and rats. RS218 induces small intestinal villous damage. The aim of these studies was to determine if this damage was via apoptosis. Sprague-Dawley rat pups were orally inoculated with RS218 or nonpathogenic *E. coli* HB101 and for 72h. TUNEL (TDT-mediated dUTP-biotin nick-end labeling) was performed on intestines and IEC-6 cells. Apoptosis in IEC-6 was quantified by Annexin V-FITC (AV) and propidium iodide (PI) after incubation with RS218 for 20h, tracking cells from AV- PI- (viable) to AV+ PI- (early apoptosis) to AV+ PI+ (end stage apoptosis and death). Caspases 1-9 were measured with VAD Multi-Caspase Substrate. Cells underwent Becton-Dickinson FACSCalibur flow cytometry analyzed with CellQuest Software. Markedly TUNEL+ rat enterocytes were seen at villous tips in contact with RS218. Rats fed HB101 showed rare, diffuse TUNEL+ at the base of the crypts. After incubation, TUNEL+ IEC-6 cells increased from 0.16% to 87.03 +/- 15.5%. Uninfected IEC-6 cells demonstrated 10% AV+, 23% PI+ and 11% AV+PI+ at baseline. After 2, 4, 6, 8 and 12 hours of incubation, AV+ increased to 22%, 39%, 22%, 26% and 50%, PI+ increased to 26%, 71%, 81%, 98%, and 70%, while AV+PI+ was seen in 17%, 52%, 18%, 42%, and 38% of cells at those time points, respectively (all p<0.01 compared to baseline). Caspases

were upregulated from 6% to 87%. Thus, RS218 can induce damage in human and rat intestinal epithelial cells via apoptosis. Induction of apoptosis by *E. coli* K1 may explain its ability to successfully translocate and disseminate. Understanding the mechanisms by which these host microbial interactions take place may lead to novel preventative or therapeutic treatments for IBD.

4.31

PATIENTS WITH ACTIVE INFLAMMATORY BOWEL DISEASE LACK IMMATURE PERIPHERAL BLOOD DENDRITIC CELLS THAT SHOW AN ABERRANT RESPONSE TO MICROBIAL SURROGATE STIMULI

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Background: Breakdown of tolerance against the commensal microflora is believed to be a major factor in the pathogenesis of inflammatory bowel disease (IBD). Dendritic cells (DC) have been implicated in this process in various animal models, while data on human DC in IBD is very limited.

Patients and Methods: Peripheral blood was obtained from 106 patients (Crohn's disease (CD) n=49, ulcerative colitis (UC) n=57) and healthy controls (n=19). Disease activity was scored using the modified Truelove Witts (MTWSI) for UC and the Harvey Bradshaw severity indices (HBSI) for CD. FACS analysis was used to identify, enumerate and phenotype DC. DC were also cultured and stimulated with CpG ODN 2006 or LPS.

Results: IBD patients in remission (PDC UC: 0.39%, CD: 0.35%, MDC-1 UC: 0.23%, CD: 0.22% of PBMC) have slightly lower numbers of circulating DC compared with healthy controls (PDC 0.41%, MDC-1 0.25% of PBMC). In acute flare-ups IBD patients experience a significant drop of DC (PDC UC: 0.04%, CD: 0.11%, MDC-1 UC: 0.11%, CD: 0.14% of PBMC) that correlates with disease activity (Correlation Coefficients: PDC MTWSI: 0.93, HBSI: 0.79; MDC-1 MTWSI: 0.75, HBSI: 0.81). Moreover, both express $\alpha 4\beta 7$ -integrin and display an immature phenotype, which rapidly changes upon culture and stimulation.

Conclusion: IBD patients lack immature blood DC during flare-ups which possibly migrate to the gut. An aberrant response to microbial surrogate stimuli suggests a disturbed interaction with commensals. Correspondence to daniel.baumgart@charite.de

4.32

BACTERIAL INFECTION IN MULTIPLE DRUG RESISTANCE DEFICIENT (MDR1A-/-) MICE RESULTS IN COLITIS CHARACTERIZED BY DYSPLASIA AND IS ASSOCIATED WITH INCREASED EXPRESSION OF C-MYC AND INTERLEUKIN-1AB

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Mdr1a^{-/-} mice lack P-glycoprotein and spontaneously develop colitis which can be accelerated by *Helicobacter* infection. *H. bilis* infection produces a rapid, severe colitis characterized by hyperplasia, crypt abscesses, and inflammation, while we noted that 50% of *mdr1a*^{-/-} mice infected with both *H. bilis* and *H. hepaticus* develop colitis characterized by low to high grade dysplasia. In order to explore this phenomenon, we followed uninfected and *Helicobacter*-infected *mdr1a*^{-/-} mice for 35 weeks and compared severity of colitis and degree of dysplasia by histopathology. Purified colonic epithelial cells were examined for changes in expression of *c-myc* and cytokines by real-time PCR. To decrease bacterial load and increase survival of mice with chronic colitis, animals were fed medicated chow. None of the uninfected, broth mice (0/10) had any evidence of large bowel neoplasia, while 40% (4/10) of *H. hepaticus*-infected mice showed low & high grade dysplasia and 68% (13/19) of *H. bilis*/*H. hepaticus*-infected mice showed low & high grade dysplasia. Epithelial cells from co-infected *mdr1a*^{-/-} mice showed increased expression of the oncogene *c-myc* (3 fold relative to uninfected *mdr1a*^{-/-}) by realtime PCR. In addition, animals with dysplasia showed increased expression of the proinflammatory cytokine interleukin-1 (15-30X relative to uninfected wildtype and *mdr1a*^{-/-}). The presence of differing species of enteric *Helicobacter* can modulate the colitis phenotype and degree of dysplasia which is associated with increased expression of *c-myc* and interleukin-1.

4.33

EXPRESSION AND FUNCTIONAL RELEVANCE OF INTESTINAL EPITHELIAL BACTERICIDAL PERMEABILITY-INCREASING PROTEIN (BPI)

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Epithelial BPI may constitute an important component of innate defense. The aim of the current study were firstly, to gain insight into the molecular mechanisms of BPI expression in mucosal epithelia and also to further explore its functional relevance.

Luciferase promoter constructs of cloned full length (1100 bp) and truncated (693 and 222 bp) BPI promoters were made and transiently transfected into Caco2 intestinal epithelial cells. Expression of minimal BPI promoter constructs (222bp) were readily detectable. The 222 bp promoter contains binding sites for a number of transcription factors and mutations encoding changes to binding sites for Sp1/3 and C/EBP revealed that Sp1/3 and C/EBP are, at least in part, necessary for basal promoter activity.

We also generated a cell line (Caco2-BPI) which stably overexpresses BPI. Significant increases in killing of a rough strain of *S. typhimurium* were attributable to Caco2-BPI compared to wildtype cells. Moreover, *Salmonella*-induced neutrophil transmigration across Caco2-BPI cells was significantly diminished compared to wild-type Caco2, indicating that BPI enhances bacterial killing and attenuates bacterial-elicited signal transduction in epithelia.

4.34

HELICOBACTER INFECTION TO ELUCIDATE THE ROLE OF MHC CLASS II AND DENDRITIC CELLS IN INFLAMMATORY BOWEL DISEASE IN CD11C TRANSGENIC/MHCII/RAG2 DEFICIENT (CD11CTGRII) MICE

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The CD4+CD45RBhigh adoptive transfer (AT) and Helicobacter models have been used to understand the role of T cells and bacteria in colitis. To understand the role of MHC Class II antigen processing in IBD, we generated a transgenic model in which Class II expression is directed to dendritic cells via the CD11c promoter on a Class II-/- genetic background. CD11cTgClass II-/- mice were backcrossed onto Rag2-/- mice to eliminate regulatory T and B cells; CD11cTgRII mice were used as recipients in an AT assay using *H. bilis* to trigger disease. Two replicate experiments were done using mice. Mice were orally given Hb (2x10e6) or broth 1 week prior to AT of 2x10e5 CD4+CD45RBhigh cells. Experimental groups (n=5-13) comprised CD11cTgRII mice+Hb+AT, CD11cTgRII +Hb, CD11cTgRII +AT and nTg controls. Large bowel was graded histopathologically (maximum path score=80). CD11cTgRII mice infected with Hb+AT had the most severe colitis (mean score=57.7) and were similar to positive control Rag2-/- mice (mean score=55.3). CD11cTgRII mice given AT alone (mean score 8.8) or Hb alone (mean 19.6) had colitis that was significantly less severe (p=0.004, p=0.005, respectively). These studies demonstrate the role of dendritic cells, Class II, T cells, and bacteria in the pathogenesis of IBD. Notably, Hb triggered mild colitis in nTg CD11cTgRII mice in the absence or presence of AT cells (mean scores=10.8 and 11.8), demonstrating that Helicobacters can trigger disease in animals lacking both T and B cells, and Class II expression. Studies were funded by NIH R01 DK056204-07

4.35

INTESTINAL LACTOBACILLUS REUTERI-BASED COMBINATION THERAPY DIRECTLY MODULATES MUCOSAL PRO-INFLAMMATORY CYTOKINE PRODUCTION IN IL-10-DEFICIENT MICE.

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Lactobacillus reuteri is the only known commensal *Lactobacillus* common to mice and humans and represents a probiotic model organism. *L. reuteri* clones with cytokine-inhibitory activity may have anti-inflammatory effects in the intestine. We investigated the probiotic activities of TNF- α inhibitory *Lactobacillus* clones in a pathogenic bacterium-induced murine colitis model.

Methods: Murine-derived *Lactobacillus* clones including *L. reuteri* were selected for their ability to inhibit TNF- α secretion by stimulated RAW 264.7 macrophages in cell culture. Interleukin-10 (IL-10)-deficient mice were pre-colonized with a *Lactobacillus reuteri/paracasei* probiotic regimen and challenged with pathogenic *Helicobacter hepaticus*. Ten weeks post-oral gavage, the severity of typhlocolitis was assessed by histologic examination of the cecocolic region. Intestinal pro-inflammatory cytokine responses were evaluated by real-time quantitative RT-PCR and immunoassays, and levels of intestinal *Lactobacillus* and *H. hepaticus* were evaluated by real-time quantitative PCR.

Results: Intestinal colonization by TNF- α -inhibitory *L. reuteri/paracasei* clones diminished typhlocolitis in *H. hepaticus*-challenged IL-10-deficient mice. Net colonic IL-12 transcript and LPS-induced mucosal TNF- α levels were reduced in *L. reuteri/paracasei* treated animals independent of *H. hepaticus* levels.

Conclusions: In *H. hepaticus*-challenged IL-10-deficient mice, mouse intestine-derived *L. reuteri/paracasei* clones demonstrated direct mucosal anti-inflammatory effects independent of pathogen antagonism.

4.36

BROMELAIN DECREASES SEVERITY OF INFLAMMATORY BOWEL DISEASE IN IL-10 DEFICIENT MICE

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Background: Bromelain is a natural proteinase product that specifically removes certain cell surface molecules and affects leukocyte activation and cytokine production *in vitro*. Oral bromelain has been anecdotally reported to induce remission of ulcerative colitis in two patients whose disease was refractory to multi-agent medical therapy.

Methods: C57BL/6 IL-10 deficient mice were treated orally with bromelain or vehicle (water) once daily from 5 to 29 wks of age. IL-10 deficient mice with established colitis following exposure to piroxicam were also treated once daily with bromelain or vehicle (100 mg/ml NaHCO₃). Inflammation was rated histologically using a scale that incorporates mucosal hyperplasia, leukocyte infiltration, and the % of colon involved by these changes (maximum score = 75).

Results: IL-10 deficient mice treated with 5 mg bromelain/day for 24 wks developed significantly less spontaneous colon inflammation than control mice (mean histologic score \pm SEM = 15 \pm 3 vs. 29 \pm 5 at age 29 weeks; p=0.04). Bromelain treatment of IL-10 deficient mice with established colitis also decreased colonic bleeding and gross and histologic colon inflammation (mean histologic score \pm SEM = 16 \pm 2 on day 16 of treatment vs. 32 \pm 6 for vehicle-treated mice).

Conclusions: Oral bromelain markedly decreases colonic inflammation in IL-10-deficient mice. The use of oral proteinases to locally modify inflammation in the gut deserves further study as a potential new therapy for IBD.

4.37

REFERENCE THERAPIES IN MURINE CD4⁺CD45RB INDUCED COLITIS.

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Ulcerative Colitis (UC) and Crohn's Disease (CD) are collectively referred to as inflammatory bowel disease (IBD). These heterogeneous disorders arise from unknown pathogenesis that involves elements of genetics and environment.

Clinical therapies rely on the use of corticosteroids to induce remission. These agents inhibit the production of inflammatory lipids and cytokines, thus decreasing inflammatory infiltrates in the gastrointestinal tract. A common immunosuppressive agent, dexamethasone (DEX), has been shown to inhibit disease in several rodent models of IBD.

T cell costimulatory molecules are known to be upregulated in inflammation. One of the costimulatory molecules, CTLA4, inhibits T cell activation by competing with CD28 for binding to B7 family members on antigen presenting cells. CTLA4.Fc has shown effectiveness in clinical trials of RA and psoriasis, as well as in mouse models of IBD.

Given these preliminary data from colitis, we wished to examine whether dexamethasone or CTLA4.Fc could be of use as a positive control reagent to alter disease in our IBD model. Colitis was induced in *scid* hosts who received sorted CD4⁺CD45RBhi T cells. Adoptive transfer recipients (ATRs) were treated with DEX or various doses of CTLA4.Fc or in a late dosing regimen (from four weeks post-transfer) with a moderate dose of CTLA4.Fc.

Maintenance of DEX or CTLA4.Fc treatment each modified elements of disease for CD4⁺CD45RBhi hosts. Use of DEX was effective at altering but not preventing disease as evidenced by decreased systemic inflammation and increased survival. Hosts treated with CTLA4.Fc experienced greatly improved health in all symptoms and parameters surveyed. It has been recommended that CTLA4.Fc be used as a reference standard for the elimination or prevention of disease in this T cell induced model of IBD.

4.38

APOLIPOPROTEIN A-IV INHIBITS EXPERIMENTAL COLITIS IN MICE

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The anti-atherogenic properties of apolipoprotein A-IV (apo A-IV) suggest that this protein may act as an anti-

inflammatory agent. We examined this possibility in a mouse model of acute colitis. Mice consumed 3% dextran sulfate sodium (DSS) in their drinking water for 7 days, with or without daily intraperitoneal injections of recombinant human apo A-IV. Apo A-IV but not apo A-I significantly and specifically delayed the onset, and reduced the severity and extent of DSS-induced inflammation, as assessed by clinical disease activity score, macroscopic appearance and histology of the colon, and tissue myeloperoxidase activity. Intravital fluorescence microscopy of colonic microvasculature revealed that apo A-IV significantly inhibited DSS-induced leukocyte and platelet adhesive interactions. Furthermore apo A-IV dramatically reduced the upregulation of P-selectin on colonic endothelial cells during DSS-colitis. Apo A-IV knockout mice exhibited a significantly greater inflammatory response to DSS than their wild type littermates; this greater susceptibility to DSS-induced inflammation was reversed upon exogenous administration of apo A-IV to knockout mice. These results provide the first direct support for the hypothesis that apo A-IV is an endogenous anti-inflammatory protein, an effect that likely involves inhibition of P-selectin mediated leukocyte and platelet adhesive interactions.

4.39

AMELIORATION OF DEXTRAN SODIUM SULFATE-INDUCED COLITIS BY AN ORALLY ACTIVE SMALL MOLECULE INHIBITOR OF MACROPHAGE MIGRATION INHIBITOR FACTOR, AVP-13748

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Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine, endocrine factor and enzyme that is crucial in regulating immune-mediated diseases. Neutralizing anti-MIF antibodies prevent the development of colitis and reduce established disease activity in mice, and MIF ^{-/-} mice fail to develop chronic colitis. We have identified small molecule inhibitors of MIF that block the biological activity of MIF in several *in vitro* assays. AVP-13748, the prototype of a series of compounds, inhibits MIF, in an assay using LPS-stimulated THP-1 cells, with an IC₅₀ of 80 nM. The compound interacts specifically with MIF to induce a conformational change that results in loss of biological activity of MIF *in vitro* and inhibit the production of multiple pro-inflammatory cytokines. Oral administration of AVP-13748 prior to LPS challenge shows a dose dependent inhibition of TNF- α serum levels and a concurrent increase of IL-10. Encouraged by these results, we investigated the effect of this molecule in a model of experimental mouse colitis induced by dextran sodium sulfate. AVP-13748 significantly reduced the severity of inflammation, as measured by clinical signs and histological scoring, and also reduced colonic TNF- α levels as well as serum MPO levels in a prophylactic treatment mode. Moreover, in a therapeutic model, AVP-13748 was given 5 days post disease induction, and was also efficacious in reducing inflammation, albeit to a lesser extent. This is the first example of a small molecule MIF inhibitor that is orally active in a model for Inflammatory

Bowl Disease and it can provide a starting point for the development of novel anti-inflammatory agents.

4.40

ACUTE COLITIS INDUCTION BY OIL OF MUSTARD AND CORRECTION BY A CANNABINOID RECEPTOR AGONIST.

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Oil of mustard (OM) is a potent neuronal activator that when given intracolonic rapidly promotes visceral hyperalgesia. The extent to which this agent is also pro-inflammatory in the GI tract is not known, and we investigated colitis induction after intracolonic OM administration to mice. Mice (CD-1) were given a single administration of 0.5% OM in 50 μ L of 30% ethanol. Body weight losses peaked at day 3 (10.6 ± 2.8 %) and returned to normal by day 7. Similarly, OM treated animals developed a severe colitis that was maximum at day 3, and was essentially absent by day 14. At day 3 there was colon shrinkage (2.8 ± 0.5 cm), colon thickening and wet weight increases (160 ± 40 mg), and diarrhea (score = 2.6 ± 0.2 ; maximum = 3). In the distal colon there was evident inflammation, macroscopic damage and histological evidence of epithelial and smooth muscle destruction. Cannabinoid receptor signalling blocks OM-induced neuronal stimulation (Pain, 99: 546, 2002) and its absence is associated with exacerbated experimental colitis development (J. Clin. Invest., 113: 1202, 2004). Consistent with these reports, the mixed CB1/CB2 agonist, WIN 55212-2, (2.5 mg/kg per day, ip), significantly reduced OM-induced colon weight gain (73 ± 14 %), colon shrinkage (61 ± 17 %), colon damage score (55 ± 10 %) and diarrhea scores (76 ± 13 %). These findings indicate that neuronal activation can engender a severe inflammatory response in the large intestine and lends support to the emerging idea that cannabinoid receptors mediate protective mechanisms in experimental colitis.

4.41

DIETARY PHYTOCHEMICALS AS NOVEL THERAPEUTICS IN IBD

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TNF- α is a pro-inflammatory cytokine that plays a major role in the pathophysiology of Crohn's Disease (CD). Targeting TNF- α is a treatment strategy. Here we determined the mechanism by which two dietary phytochemicals, curcumin and galangin regulate TNF- α mediated NF- κ B activation and downstream gene expression. Curcumin and galangin are active ingredients in turmeric (*Curcuma longa*) and siamese ginger (*Alpinia galanga*), respectively.

Methods: Both *in vivo* and *in vitro* experiments were carried out. Mice were fed 1% curcumin in diet for one week before lipopolysaccharide (LPS, 0.5mg/kg) challenge.

HCT116 cells were treated with extracts and phytochemicals before TNF- α stimulation. Western blot and gel shift assays were performed to determine the expression of I κ B, COX-2 and NF- κ B. Real time PCR and promoter driven luciferase assays were performed to determine IL-8, MIP2 and COX-2 expression. PGE₂ and IL-8 levels were determined by ELISA.

Results: Curcumin significantly inhibited *in vivo* intestinal COX-2, PGE₂ and MIP2 expression in LPS treated mice. *In vitro*, both curcumin and crude *Curcuma longa* extracts inhibited TNF- α mediated nuclear translocation of NF- κ B in HCT116 cells resulting in decreased IL-8 and COX-2 expression. Galangin and crude *Alpinia galanga* extracts also inhibited NF- κ B and IL-8 expression. Furthermore, the combination of curcumin and galangin was synergistic in reducing TNF- α activity. Both phytochemicals effectively inhibit I κ B kinase activity.

Conclusion: Taken together, the data suggest that the mechanism of action of the two phytochemicals and the crude extracts involves inhibition of TNF- α activity. Thus, a combination of these phytochemicals may be a potent and effective novel therapeutic strategy for IBD.

4.42

TREATMENT OF INFLAMMATORY BOWEL DISEASE BY MEMBRANE-ANCHORED PHOSPHOLIPASE A2 INHIBITORS LINKED TO GLYCOSAMINOGLYCANS: NOVEL MULTI-FUNCTIONAL ANTI-INFLAMMATORY DRUGS.

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Hydrolysis of cell membrane phospholipids by phospholipase (PLA2) initiates the production of inflammatory lipid mediators, including arachidonic acid (AA)-derived eicosanoids, lysophospholipids and PAF. Due to their role in IBD, eicosanoid suppression has been considered for treatment. However, inhibition of one production pathway (e.g., COX) diverts the AA to the other (e.g., LOX), and often exacerbates the condition. Hence, inclusive control of lipid mediators, by PLA2 inhibition, seems preferable for treating IBD.

In addition, denudation of intestinal epithelia from cell surface glycosaminoglycans (GAG) is believed to trigger IBD, and their enrichment has thus been proposed for treatment.

Multifunctional anti-inflammatory drugs (MFAID), that address both needs, have been designed and synthesized in Yedgar's lab, by linking PLA2inhibiting lipids to GAG. The MFAIDS, given IP, IV or orally, are found effective in treating animal inflammations, including endotoxin-induced sepsis, and IBD induced by TNBS, indomethacin or DSS, as expressed by survival, histology, clinical parameters, and biochemical markers (cytokines, PLA2, eicosanoids).

Current therapies usually address a single, presumably central, inflammatory mediator. However, growing evidence shows that tissue injury involves the synergism of a number of mediators. The MFAID control the production of a host of inflammatory mediators, and thus introduce a

novel therapeutic approach and drug prototype for treatment of IBD.

4.43

CARBON MONOXIDE GAS MEASUREMENT; APPLICATION TO HUMAN AND RODENTS

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Aims: There are only a few reports in regard as intestinal luminal gas. Heme oxygenases (HOs) are the rate-limiting enzymes in heme degradation, catalyzing the cleavage of the heme ring to form carbon monoxide (CO), ferrous iron and biliverdin. Inducible form of heme oxygenase (HO-1) is induced and may play a protective role in the process of inflammation. Recently we have developed a new portable gas chromatography, and applied to the measurement of CO gas in intestinal lumen. **Materials and methods:** The simultaneous analysis of intestinal luminal gas (CO, hydrogen (H₂), methane (CH₄)) were performed under Trilyzer mBA-3000 (gas chromatography with semi conductor detector, Taiyo Inc. Osaka). Acute colitis was induced with dextran sulfate sodium (DSS) in male BALB/c mice with/without receiving 25mg/kg i.p. of zinc protoporphyrin IX (ZnPP), an HO inhibitor, daily. For clinical trial, human colonic gas was collected under colonoscopy. **Results:** In intestine of mouse and human, CO, H₂ and CH₄ were detectable at 0.1 ppm order. After DSS administration, intestinal CO was markedly increased. The increases of CO in DSS colitis were significantly inhibited in ZnPP-treated group. The levels of CO gas were 0.6ppm in healthy volunteers, and these levels were not significant differences among several parts of the large intestine. The CO levels in patients with ulcerative colitis were significantly higher than those of healthy volunteers. **Conclusion:** These results indicate that CO derived from heme oxygenase may play a protective role in the intestinal inflammation.

4.44

CTLA-4-IG ABROGATES TNBS COLITIS

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Background: CTLA-4-Ig is a biological agent consisting of the extracellular domain of Cytotoxic T cell Late Antigen - 4 (CTLA-4) fused to the Fc region of IgG. CTLA-4-Ig potentially promotes *tolerance* both via costimulatory blockade as well as through the induction of indoleamine 2,3-dioxygenase (IDO) in professional antigen presenting cells (APCs) like dendritic cells.

Objectives: We sought to determine if CTLA-4-Ig administration could abrogate trinitrobenzene sulfonic acid (TNBS) colitis, and if so, to determine if IDO played a role in this process.

Methods: Intra-rectal TNBS and intra-peritoneal CTLA-4-Ig were administered to SJL/J mice along with a specific

IDO inhibitor. Clinical and histological criteria were assessed to determine colitis severity. IDO protein and mRNA expression were assessed by western blotting and real time PCR respectively.

Results: We found that CTLA-4-Ig induced IDO in cultured LPMNCs as well as in the murine colon after systemic administration. Colonic IDO induction by CTLA-4-Ig was associated with an induction of IFN γ mRNA. Intra-peritoneal CTLA-4-Ig administration prior to TNBS administration significantly abrogated colitis both clinically and by histological criteria. Mice treated with CTLA-4-Ig and TNBS had a 100% survival rate and a significant reduction in colonic TNF α mRNA expression regardless of IDO inhibition. IDO inhibition with 1-methyl-tryptophan (1mT), however, prevented colitis abrogation by CTLA-4-Ig both clinically and by histological criteria, and decreased colonic TGF β mRNA expression.

Conclusion: This study suggests that CTLA-4-Ig promotes *tolerance* in TNBS colitis both via IDO induction as well as through costimulatory blockade, and suggests a potential therapeutic role for CTLA-4-Ig in the treatment of IBD.

5.0 INNATE RESPONSES IN INFLAMMATORY BOWEL DISEASE: ROLE OF THE VASCULATURE

5.1

LEUKOCYTE, PLATELET AND ENDOTHELIAL CELL INTERACTIONS IN INTESTINAL INFLAMMATION

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The microcirculation plays an important role in the recruitment of different circulating blood cells into the inflamed intestine. This role is achieved through phenotypic changes in vascular endothelium that lead to a more pro-adhesive surface and which allows for blood cell attachment and extravasation. A variety of endothelial cell adhesion molecules, including P- and E-selectins, ICAM-1, VCAM-1 and MAdCAM-1 are upregulated on the surface of endothelial cells in the gut microcirculation and these glycoproteins can mediate the recruitment of diverse cell populations, including neutrophils, T-lymphocytes, and platelets. A consequence of these responses is that vascular endothelial cells assume both an inflammatory and thrombogenic phenotype. In human and experimental IBD, platelets circulate in an activated state and more readily form aggregates with leukocytes. Platelets represent an important source of inflammatory mediators and can modulate the activation state of inflammatory cells as well as endothelial cells. Experimental evidence indicates that the increased thrombogenic potential in IBD is related to disease severity and is linked to the adhesion and activation of leukocytes in the gut microcirculation.

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5.2

RECIPROCAL CONTROL OF CD8 T CELL HOMING BY DENDRITIC CELLS.

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In order to accomplish their functions, T cells must leave the blood and reach the different tissues in the body. The integrin $\alpha 4\beta 7$ and the chemokine receptor CCR9 are essential for efficient T cell migration into the small intestine lamina propria. On the other hand, E- and P-selectin ligands and the chemokine receptors CCR4 or CCR10 are required for T cell homing into the skin. We have shown that T cells activated by dendritic cells (DC) from gut-associated lymphoid tissues, like Peyer's patches, express $\alpha 4\beta 7$, CCR9, and present gut-tropism. More recently, we have demonstrated that, reciprocally, CD8 T cells activated by DC from cutaneous-draining lymph nodes express the skin homing receptors E- and P-selectin ligands, CCR4, interact more efficiently with dermal microvessels, and accumulate in inflamed skin. This selective imprinting of T cell homing was independent of Th₁/Th₂ cytokines or of some particular DC subpopulation. Furthermore, re-activation of effector/ memory CD8 T cells with an already committed skin- or gut-homing phenotype, by using either DC from gut- or cutaneous-associated lymphoid tissues respectively, reverted significantly their homing commitment. Thus, tissue-specific T cell homing potential is a dynamic property feasibly modified in a reciprocal fashion by DC from skin- and gut-associated lymphoid tissues.

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8.0 BACTERIAL/HOST INTERACTIONS IN THE PATHOGENESIS OF IBD

8.2

BACTERIAL-HOST INTERACTIONS IN THE MDRIA-/- MODEL OF INTESTINAL INFLAMMATION

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Inflammatory bowel diseases (IBD) are characterized by chronic intestinal inflammation. This persistent inflammation is the result of poorly controlled mucosal immune responses to normal intestinal microbiota. The mechanisms that initiate this aberrant response are not well elucidated, however the disruption of epithelial barrier function appears to play a role in many animal models of disease. We have utilized the p-glycoprotein deficient murine model of IBD to investigate the role of epithelial barrier function in intestinal inflammation. P-glycoprotein is an adenosine triphosphate-binding cassette transporter expressed in the intestine. It functions to pump foreign chemical, as well as naturally occurring microbial products, out of intestinal epithelial cells. The use of this murine model has resulted in the discovery that loss of p-glycoprotein expression leads to both a physical and a functional change in epithelial barrier function. This alteration leads to an enhanced immune response to bacterial antigens and a loss of intestinal homeostasis, which ultimately results in severe intestinal inflammation. Supported by the National Institute of Health (DK059911) and the American Cancer Society (MBC-103126).

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8.3

CROHN'S DISEASE-ASSOCIATED BACTERIA: LESSONS FROM P. FLUORESCENS PFIT (I2 PROTEIN)

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Immune colitis of the mouse, and probably Crohn's disease (CD) in the human, involves aberrant mucosal inflammation directed to members of the commensal enteric microbiota. Model studies in the mouse indicate that there is an exquisite matching of selected bacterial taxa with host genetic susceptibility traits in the formation of colitis. Conversely, analysis of human CD reveals apparent non-selectivity, including mucosal adherence by diverse bacterial taxa, and diverse anti-microbial specificities of serum antibodies. Among several explanations, we considered that the pathobiology of host-microbial interaction in CD would be clarified by identification of discriminating bacterial species pertinent to patient subsets in the CD population. Using representational difference analysis of mucosal CD biopsies, we identified a bacterial sequence (I2) in approximately 50% of active CD lesions. I2 proved to originate from the pfiT gene of *P. fluorescens*, and encodes a CD4+ T cell superantigen that promotes IFN- γ production in colitis-susceptible mouse strains. Whereas *P. fluorescens* is not colitigenic in common mouse IBD models, anti-I2 antibodies identify a subset of human CD patients distinguished by greater disease severity, independence from CARD15 genetic susceptibility, and responsiveness to fecal diversion. The latter is notable because of the minimal capacity of Pseudomonadaceae for enteric colonization, suggesting that host susceptibility traits permitting increased levels of luminal bacterial products (such as the immunoreactive pfiT), or the host response they elicit, may be a non-invasive pathogenetic mechanism in certain CD patients. γ

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9.0 NEW THERAPEUTIC STRATEGIES IN THE TREATMENT OF IBD

9.2

ANTI-ADHESION THERAPY IN THE TREATMENT OF IBD

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The localization of leukocytes to inflammatory areas is a finely regulated event that has key implications in the pathogenesis, diagnosis and treatment of IBD. A major effort has been directed towards identifying and characterizing the adhesion glycoproteins that enable leukocytes to bind to vascular endothelial cells. Expression of endothelial adhesion molecules, including selectins and members of the immunoglobulin superfamily, is increased in intestinal microvessels in active IBD. Detection of upregulated adhesion molecules can be used to define areas of inflammation by imaging techniques. Drugs that specifically target adhesion molecules involved in leukocyte recruitment are effective in the treatment of intestinal inflammation. Experimental studies have shown that blockade of VLA-4, VCAM-1, and P-selectin afford significant amelioration of intestinal inflammation. In experimental models, response to adhesion molecule blockade varies according to the type of inflammatory intestinal condition. In humans VLA-4 immunoneutralization has been shown to be effective in inducing remission in Crohn's disease, but no data is available for ulcerative colitis. Experimental and clinical controlled trials comparing the effectiveness of different strategies of CAM blockade are warranted. This therapeutic approach has to be compared with current therapeutic modalities. *Supported by Grant SAF2002/02211 From Ministerio de Ciencia y Tecnologia. Spain.*

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