ANIMAL MODELS OF IBD 1
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OR.41. Intestinal Microsomal Triglyceride Transfer Protein (MTP) Regulates CD1d Function on the Intestinal Epithelium and Protects from Mortality in the Oxazolone Model

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Background and Aim: Natural Killer T (NKT) cells recognize lipid antigens presented by CD1d. MTP is an endoplasmic reticulum-resident protein which can transfer phospholipids onto CD1d and regulate CD1d antigen presentation. We aimed to characterize the role of CD1d and MTP in intestinal epithelial cells (IECs) in oxazolone colitis. Results: VcreERT2MTTPfl/fl mice were generated to allow for tamoxifen-induced deletion of MTP in IECs (MTP-KO). Oxazolone colitis led to rapid mortality of MTP-KO mice, while no mortality was observed in VcreERT-2MTTPfl/fl mice without tamoxifen treatment or in MTP-KO mice in the absence of oxazolone skin sensitization. Inhibition of CD1d-restricted antigen presentation by i.v. injection with a monoclonal anti-CD1d antibody prevented mortality in MTP-KO mice. Microarray analysis of intestinal tissues 6 hours after rectal oxazolone challenge revealed strong induction of gene expression related to innate immunity (IL-1α, IL-1β, cyclooxygenase 2, S100A9) with significantly higher expression in tissues from MTP-KO mice. Baseline expression of several heat-shock proteins (HSPs) involved in barrier protection and immunity was decreased in MTP-KO mice with further down-regulation upon oxazolone administration. CD1d crosslinking and coculture with NKT cells increased HSP expression in wildtype IECs. Conclusion: IECs have an MTP- and CD1d-dependent protective role in oxazolone colitis while hematopoetic cells drive colitis in a CD1d-restricted manner.

OR.42. An Anti-flagellin Antibody Ameliorates Symptoms of Inflammatory Bowel Disease in Experimental Colitis Models

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Disruption of epithelial barrier function, intolerance to gut flora and immune dysregulation, plays dominant role in pathogenesis of IBD. Data from both humans and animal models underscore the critical role of flagellated bacteria in the establishment and maintenance of IBD. Here, we evaluated the efficacy of anti-flagellin monoclonal antibodies (INO-741 and INO-763) in DNBS induced and IL-10 knockout colitis models. In the DNBS induced murine model of colitis, INO-741 and INO-763 significantly attenuated colon macroscopic injury, DNBS induced body weight loss, and histological damage to colon in comparison to (vehicle or non-relevant isotype mAb treated) controls in a dose-dependent manner (5–20 mg/kg). Additionally, INO-763 treatment reduced colonic levels of select chemokines and cytokines. In the murine IL-10 knockout model of spontaneous autoimmune colitis, INO-741 and INO-763 provided protection against histological colon damage and significantly attenuated the induction of select proinflammatory chemokines and cytokines (MIP-1, TNF, IL-1β). Furthermore, the beneficial effects observed following INO-763 treatment were at least as significant as for treatment with Infliximab, a chimeric anti-TNF monoclonal antibody developed to treat IBD, Psoriasis and Rheumatoid arthritis. Both INO-741 and INO-763, at all doses (5–20 mg/kg), significantly attenuated DNBS-induced mortality: Vehicle or control animals (mAb 18.8) developed severe hemorrhagic diarrhoea and died (7/15 and 6/15 respectively at 4 days). Mortality was significantly less in mice pre-treated with INO-763 (0/15) or INO-741 (1/15). These data support the notion that anti-flagellin antibodies may prove useful in the clinical management of IBD.

OR.43. A Polymorphism in the Coding Region of IL12b Promotes IL-12p70 and IL-23 Heterodimer Formation in Colitis Sensitive SJL/J Mice

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IL-12 and IL-23 are key cytokines involved in the induction of Th1 and Th17 immune responses. Both cytokines are heterodimers, sharing a common subunit (IL-12p40) coded for by the IL12b gene. Genome wide association studies have implicated IL12b as a susceptibility gene for Crohns disease and ulcerative colitis. Previously we identified the locus harboring this gene is a susceptibility region for trinitrobenzene sulfonic acid (TNBS)-induced colitis. To investigate whether polymorphisms in the IL12b gene influence the synthesis of IL-12 in the highly susceptible SJL/J mouse strain we first generated two sets of constructs in which IL12p35 was linked to either the polymorphic SJL/J IL-12p40 or the wild type IL-12p40 from C57Bl/6. We then conducted transfection studies using these constructs and determined that constructs containing the SJL/J-derived variant synthesized significantly more IL-12p70 relative to IL-12p40 compared to the C57BL/6-derived variant. Further studies showed that this difference was not due to increasesd synthesis or secretion of the IL-12p40 or IL-12p35 subunits and was thus attributable to a higher affinity of SJL/J-derived subunits for each other. This is supported by studies showing IL-12p40 from the two strains exhibit different glycosylation patterns which could lead to conformational changes in the p40 molecule that influence subunit affinities and thus the efficiency of heterodimer assembly. In parallel studies we showed that a similar situation obtains for IL-23p40 and IL-23p19. We conclude that the higher susceptibility for TNBS-induced colitis of SJL/J mice is due to their greater capacity to link IL-12p40 with its respective IL-12 and IL-23 subunit; this results in a rapid pro-inflammatory skewing of the immune response and distortion of the homeostatic balance.

OR.44. Long-lived Colitogenic CD4+ Memory T Cells can be Maintained Outside the Intestine in the Absence of Commesal Bacteria

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Introduction: Inflammatory bowel disease is the persistent disease with lifelong recurrence. According to present understanding, this disease is caused by colitogenic memory CD4+ T cells, which memorize the intestinal bacterial antigens for a long time. Recently, we have reported that IL-7 is essential for the maintenance of colitogenic memory CD4+ T cells, and that bone marrow (BM), the main source of IL-7 is the reservoir for these cells. On the other hand, it is thought that TCR signaling is also important for the maintenance of memory CD4+ T cells in normal condition. Thus, we have attempted to clarify the role of commensal bacteria for the maintenance of colitogenic memory CD4+ T cells. Methods: We isolated splenic (SP) CD4+ T cells from colitic CD4+CD45RBhigh T cell-transferred SCID mice under specific pathogen-free (SPF) condition, and re-transferred them into SPF or Germ Free (GF) SCID mice. Results: Six weeks after re-transfer into GF mice, one group was continuously maintained in GF (GF→GF), and the other was transferred into SPF (GF→SPF). Donor colitic SP CD4+ T cells have a characteristic of CD44highCD62L-IL-7Rαhigh memory phenotype. SPF, but not GF→GF, mice re-transferred with these cells developed colitis at 4 weeks after GF→SPF movement. Surprisingly, however, a large number of memory CD4+ T cells remained resident in spleen and BM, but not in lamina propria in the GF→GF recipients. In addition, GF→SPF mice rapidly developed colitis. Immunohistochemistry showed that substantial numbers of CD4+ T cells were resident in BM of the three groups consistent with the constant expression of IL-7. Conclusions: These findings suggest that long-lived colitogenic memory CD4+ T cells can be maintained outside the intestine in the absence of commesal bacteria, and participate in the perpetuation of colitis.