was associated with regulation of IFNγ, induction of IL4, IL10 and TGFβ production in WT Balb/C mice (p<0.001 for each cytokine btw uninfected control and helminth-infected; N=at least 3 independent experiments), while no regulation of IFNγ and modest induction of IL4 or IL10 was seen in STAT6-/- mice. Helminths did not induce TGFβ production in STAT6-/- animals. In an acute GVHD and colitis model, helminths regulated WT donor T cell Th1 cytokine generation, serum Th1 cytokines, colitis and survival in WT and not STAT6-/- recipients (p<0.05 for each parameter btw. helminth infected WT and helminth-infected STAT6-/- recipients, multiple experiments). Helminth infection was associated with the induction of donor and recipient FoxP3+ regulatory T cells (Tregs) in WT and not STAT6-/- bone marrow transplanted mice (p<0.05 btw. helminth infected WT and helminth-infected STAT6-/- recipients, multiple experiments). Addition of TGFβ to STAT6-/- T cell cultures restored T cell IL10 production and Treg generation that regulate Th1 inflammation. **Conclusions:** STAT6 is a critical transcription factor directing mucosal TGFβ producing Th3 cell generation. Helminths utilize STAT6 pathway to induce mucosal Tregs, trigger mucosal T cell IL10 secretion and regulate alloreactive colitis in a TGFβ dependent manner.

Sa1773

Caspase-11 Enhances Killing of Colitogenic Escherichia coli by Macrophages, but Does Not Affect Development of Experimental Colitis

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BACKGROUND: Inflammatory bowel diseases (IBDs) are thought to be due to dysregulated innate and adaptive immune responses to resident enteric bacteria in genetically susceptible hosts. Specifically, functionally altered Escherichia coli are implicated in the pathogenesis of IBDs and experimental colitis. Moreover, altered intestinal lamina propria macrophage responses to bacterial stimuli contribute to the immune dysfunction associated with IBDs. Recent studies have shown that non-pathogenic E. coli activate a non-cannonical inflammasome in macrophages via caspase-11 (Casp11) resulting in interleukin (IL)-1β production and that Casp11 promotes fusion of pathogenic bacteria-laden phagocytic vacuoles with lysosomes. However, the roles of Casp11 in macrophage processing of colitogenic E. coli and development of colitis are unknown. We hypothesize that Casp11 enhances macrophage-mediated killing of colitogenic E. coli and attenuates experimental colitis. METHODS: Gentamicin protection assays were used to measure intracellular killing of colitogenic E. coli NC101 by bone marrow derived macrophages from wild-type (WT) and Caspase-11 deficient (Casp11-/-) mice on the C57BL/6 background. Effects of Casp11 on acute colitis were determined by treating specific pathogen free WT and Casp11-/- mice with 4% dextran sodium sulfate (DSS) for 8 days. Effects of Casp11 on spontaneous, immune-mediated chronic colitis were assessed by quantifying histological inflammation in colon sections and spontaneous IL-12/23 p40 secretion by colon explants from interleukin-10 deficient (Il10-/-), Casp11-/-, and Il10-/-;Casp11-/- mice at 5 and 12 weeks. RESULTS: We detected increased numbers of intracellular E. coli NC101 in Casp11-/- compared to WT macrophages at 24 hours post infection (317 ± 236 vs. 18 ± 18 colony forming units/well, respectively; p<0.05). Weight loss was no different between DSS-treated Casp11-/- and WT mice on day 8 (82.1 ± 5.3 vs. 81.3 ± 2.0 % of initial weight, respectively; p= 0.745). Casp11-/- mice did not develop spontaneous colitis. Furthermore, composite histological colon inflammation scores (2.08 ±1.46 vs. 1.29 ± 0.49, respectively; p=0.25) and IL-12/23p40 secretion by colon explants (5.39 ± 2.05 vs. 6.85 ± 2.18 pg/mg tissue, respectively; p=0.27) were no different in 12-week-old Il10-/- compared with Il10-/-;Casp11-/- mice. CONCLUSIONS: While Casp11 increases the ability of macrophages to kill colitogenic E. coli NC101, it is not involved in the development of experimental colitis in specific pathogen free mice.

Sa1774

Regulation of Intestinal Mucosal Barrier Function by Specific Microbial Metabolites

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Background: Intestinal mucosal epithelium acts as a first line of barrier against environmental factors. Indeed, any alteration in the integrity of intestinal epithelium is linked to intestinal inflammation (e.g., IBD). Intestinal microbiota is known to influence host immune homeostasis. This mutually beneficial relationship is crucial for healthy living and any perturbation of this association has been strongly associated with human disorders. However, the complexity of the interacting network between intestinal microbiota and host made it extremely difficult to identify specific molecular mediators that are responsible for mutually beneficial host-microbe interaction. Recent literature suggests that orphan nuclear receptors may serve as a mechanistic link between environment and host immunity but the detailed mechanism has remained elusive. One such receptor, pregnane X receptor (PXR) is most compelling in this function because of its large ligand binding pocket (LBD), that can allow binding to diverse molecules. The goal of the study was to determine beneficial effects of indole metabolites (exclusively produced by intestinal microbiota) on intestinal mucosal homeostasis through the actions of PXR. Methods: Mice experiments were performed according to the institutional guidelines. Intestinal enterocyte isolation, qPCR analysis, luciferase assay, myeloperoxidase (MPO) assay, molecular docking studies, FITC-dextran assay were performed using standard protocols. Results: In our study, we found that indole and its metabolites (e.g., indole propionic acid, IPA) are potent endogenous activators of PXR in the intestine and induce PXR target gene transcription. We have shown that while IPA alone is a weak human PXR (hPXR) agonist, IPA in combination with indole significantly activates hPXR. By contrast, IPA is a potent activator of mouse PXR. In silico docking studies suggest that both indole and IPA can be accommodated simultaneously in PXR LBD. We also observed that IPA decreases Tnf-α gene expression (3.73 fold over control, p≤0.01). Further, we have shown that blocking indole and IPA production resulted in enhanced Tnf-α gene expression and reduced junctional regulator expressions. Ex vivo rescue of indole and IPA depletion resulted in significant reduction in Tnf-α expression and increased junctional regulator expressions only in Pxr+/+ mice but not in Pxr-/- mice, thus establishing PXR specific effect of IPA (p≤0.01). Conclusion: Our results demonstrate that presence of PXR is necessary and important for maintaining intestinal epithelial integrity by acting as a key mechanistic link between intestinal microbiota and host immune system. Based on these observations, endogenous microbiota derived novel PXR activators (e.g., IPA) can be designed for effective non-toxic therapeutic strategies to combat IBD-like intestinal pathophysiologies.

Sa1775

TCRγδ+ Intraepithelial Lymphocytes Protect Intestinal Mucosa Injuryby IL-22 Production Through Aryl Hydrocarbon Receptor Signaling in a Ischemia/Reperfusion Mouse Model

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BACKGROUND:The pathogenesis of intestinal ischemia/reperfusion (I/R) injury is believed to be involved an altered intraepithelial lymphocytes(IELs) function. Aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor that mediates the T-cell responses.We investigated the role of AhR in inflammation and pathogenesis of intestine in anI/R mouse model.METHODS: Small bowel I/R was establishedinmale C57BL/6J mice.Perioperative6-formylindolo(3,2-b)carbazole (FICZ, AhR agonist) treatmentgroups were compared to vehicle-treatedanimals and non-operated controls (n = 6/group).The small bowel was harvested for histological examinationand intestinal barrier function (IBF) was detected by the transmembrane resistance (TER).AhR, cytokine expressions and IELs were detected through immunofluorescence and flow cytometry.The protective effects of IL-22 on epithelial barrier functions on polarized T84 cell monolayers were evaluated by TER and Western blot. RESULTS: Pretreatment with FICZsignificantly inhibited I/Rinduced decrease of IBFand increased IL-22 expression by about 5 folds when compared to vehiclegroup. Confocal microscopy showed a high level of co-localization between theIELs and IL-22. Interestingly,flow cytometryresults indicated that TCRγδ+ IELs are main producers ofIELs-derived IL-22. Neutralization of endogenous IL-22 disrupted the protective effect of Ficz on I/R-induced intestinal injury.Furthermore, pretreatment with rIL-22 could attenuate the decreased expression of ZO-1 (0.34±0.13 vs. 0.78±0.22) and occludin (0.51±0.16 vs. 0.88±0.19) and the loss of TER (86±10.8 vs. 113±8.9 Ω.cm2) under hypoxia in a cell cuture model. CONCLUSION:These findings indicate that activation of AhRattenuates intestinal mucosal injury induced by I/R through a pathway involving the TCRγδ+ IELs-derived IL-22. These resultssuggest that AhR activation has therapeutic value for the treatment of intestinal I/R.

Sa1777

Novel Regulatory T Cell Population Co-Expressing RorγT+ or GATA3+ Is Increased in Intestinal Lamina Propria of IBD Patients

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BACKGROUND AND AIMS: The polarization of T helper cells contributes to the pathogenesis of IBD. Regulatory T cells could adopt effector functions through expressing key transcription factor of effector Th cells under inflammatory conditions. We recently reported Treg plasticity such as the increased prevalence of double expressing IL-17+FoxP3+ T cells in the peripheral blood of IBD patients. The aim of this study was to clarify whether there is double expressing regulatory T cells in lamina propria of IBD patients. METHODS: Colonoscopic biopsies were obtained from the actively inflamed lesion of 20 IBD patients (9 CD, 11 UC) and from 7 healthy controls (HC). Lamina propria T lymphocytes were isolated and multicolor flow cytometry was used to detect the CD25+FoxP3+CD4+Tcells (Treg) in intestinal lamina propria which co-expressed key transcription factors and cytokines (T-bet and IFN-γ for Th1, Gata3 and IL13 for Th2, RoRγt and IL-17 for Th17). RESULTS: The Treg cell population (%CD3+T cells) significantly elevated in both CD (7.01) and UC (8.24) compared with that in HC (2.49)(P<0.01). The RoRγt+ Treg cell population was increased in both CD (4.57) and UC (5.61)relative to HC (1.06)(P=0.03, P<0.01 respectively) (Figure 1A), as was the IL-17+ Treg cell population (CD 0.56 vs. HC 0.15, P=0.02; UC 0.68 vs. HC 0.15, P<0.01). The Gata3+ Treg cell population was more prevalent in UC (4.77) than that in CD (1.29) and HC(1.38) (P=0.02, P=0.04 respectively) (Figure 1B), as was the IL-13+ Treg cell population (UC 0.49vs.CD 0.14, P<0.01; UC 0.49vs.HC 0.15, P<0.01). There was no significant difference in T-bet+FoxP3+ or IFN-γ+ FoxP3+T cell population among all groups. However, the ratio of T-bet+FoxP3+ / FoxP3+ T cells was significantly lower in UC(0.46) than that in CD (0.65) and in HC(0.74)(P<0.05). CONCLUSIONS: Lamina propria T lymphocytes in patients with both CD and UC show an increased prevalence of co-expressing RoRγt+ or IL17+ Treg cell population compared with HC. Other types of co-expressing Gata3+or IL-13+ Treg cell population also increase in UC. Such plasticity of Treg cells may alter their function, as we have previously demonstrated in the circulating blood compartment. Reference: 1. Ueno A, Jijon H, Chan R, et,al. Inflamm Bowel Dis. 2013 Nov;19(12):2522-34.

Sa1778

Constitutive Type I Interferon, via STAT1 Activation, Selectively Promotes Regulatory T Cell Function in the Healthy Human Intestine, but Not in Inflammatory Bowel Disease

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Background Control of T-cell reactivity with the human intestinal mucosa is poorly understood. Type I Interferon (T1IFN) signals via the JAK/STAT pathway, particularly STAT1, and supports Treg function in mouse models of colitis. T1IFN has been used as a treatment in Inflammatory Bowel Disease (IBD). We therefore hypothesised that constitutive T1IFN had a regulatory role in human intestinal T cells. Methods Endoscopic biopsies or resection specimens were frozen for immunohistochemistry (IHC) or cultured in the presence of neutralising anti-IFNβ or isotype-matched control antibody. Cells were harvested, stimulated with anti-CD3/CD28 antibodies and analysed for cytokine production by intracellular staining and by multiplex ELISA of culture supernatants. Phosphorylated STAT1 was measured by flow cytometry with or without prior T1IFN stimulation. Frozen sections of colonic mucosa were stained with ant-IFNβ and analysed using fluorescent IHC. Finally, CD3+ T-cells were FACS sorted and expression of Interferon Stimulated Genes (ISGs) and Suppressors of