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Mo1736

Knockdown of STE20-Like Proline/Alanine-Rich Kinase (SPAK) Attenuates Intestinal Inflammation in Mouse

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Background: Inflammatory bowel diseases (IBDs), principally Crohn's disease and ulcerative colitis, are characterized by epithelial barrier disruption and alterations in immune regulation. Ste20-like proline/alanine-rich kinase (SPAK) plays a role in intestinal inflammation, but its underlying mechanisms need to be defined. **Methods:** SPAK knockout mice (KO) colon mucosa was subjected to transepithelial resistance (TER) assay and dilution potential assay with using chamber, and transepithelial permeability assay with fluorescein isothiocyanate-dextran (FITC-dextran). SPAK KO mice were treated with dextran sulfate sodium (DSS) or trinitrobenzene sulfuric acid (TNBS) to evaluate the effect of SPAK on the experimental colitis in terms of clinical and histological characteristics, mouse endoscopic features, enzymatic activities, and production of pro-inflammatory cytokines and translocation of luminal bacteria. The expressions of junction proteins were characterized using real time PCR, western blots and immunohistochemistry. **Results:** SPAK KO mice exhibited significant increases of intestinal barrier function. Knock-down of SPAK significantly decreased paracellular intestinal permeability to FITC-dextran, and also lowered significantly the sodium ion selectivity of tight junctions in intestinal epithelial cells. Further, the expression of junction proteins β -catenin, claudin-2 decreased. In contrast, expression of ZO-1, Occludin, E-cadherin increased significantly, whereas the change of expressions of Claudin-4 and Claudin-1 was not noticeable. **In Vivo** studies using mouse model of colitis induced by DSS and TNBS showed that KO mice were more tolerant to DSS or TNBS treatment than were wild-type animals, as demonstrated by clinical and histological characteristics and MPO enzymatic activities. KO mice also demonstrated ameliorated colon inflammation by mouse endoscopy compared to wild type mice. Consistent with this notion, we found that SPAK knockdown attenuated the increases of inflammatory cytokines production **In Vivo** and also ameliorated bacterial translocation under DSS treatment, which together likely reduce intestinal epithelial permeability. **Conclusion:** Knockdown of SPAK increases intestinal innate immune homeostasis, showing that SPAK is important in control or attenuation of pathological responses in IBD.

Mo1737

Role of Sialylation by St3gal4 Sialyltransferase in Mouse Models of Colitis

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Sialic acid is a common terminal glycan modification that determines ligands for a number of receptors involved in immunity. The carbohydrate sialic acid is also a prominent component of milk oligosaccharides that prevent pathogen colonization and promote development of a specific intestinal flora. Previously we have shown that the absence of α 2,3-sialylation produced by St3gal4 sialyltransferase has a protective action in the model of acute DSS-induced colitis (Fuhrer, A. et al, JEM, 2010). To study the contribution of St3gal4 sialyltransferase in the adaptive immune response we used two mouse models: (1) chronic inflammation induced by DSS treatment; (2) spontaneous inflammation observed in the absence of IL-10 (Il10^{-/-} mice). The effect of α 2,3-linked sialylactose on development of colitis was tested in cross-fostering experiments of WT and St3gal4^{-/-} mice, with a subsequent DSS treatment. We showed that St3gal4^{-/-} (KO) mice were more resistant to chronic colitis induced by DSS treatment than wild type (WT) mice. Reduced inflammation in KO mice was confirmed by colonoscopy and histological analysis. Flow cytometry analysis of lamina propria leukocytes showed significant increase in CD45⁺ infiltrating cells present in WT mice compared to KO mice. Interestingly, WT mice cross-fostered to KO mice were less susceptible to DSS treatment while KO mice cross-fostered to WT mice were more susceptible to colitis when challenged 4-5 weeks after weaning. We analyzed the contribution of α 2,3-linked sialic acid also in a model of spontaneous inflammation (Il10^{-/-} mice). The onset of inflammation was significantly delayed in St3gal4^{-/-}/Il10^{-/-} mice. Supplementation of these mice with 3-sialyl-lactose over the lactation period rescued intestinal inflammation to the comparable level as in Il10^{-/-} mice. We conclude that α 2,3 sialylation by St3gal4 sialyltransferase contributes to mucosal inflammation. The mechanism involved in the altered immune response is under investigation. We are analyzing changes in bacterial flora and examining the immune function changes in the absence of St3gal4^{-/-}.

Mo1738

Psgl1 Expression in Non-Lymphocyte Populations is Required to Prevent Colitis in Adoptive Transfer Mice

Jeffrey Brown, Elizabeth Managlia, Terrence A. Barrett

Background/Aims: P-selectin glycoprotein ligand-1 (PSGL1)'s role in leukocyte recruitment has been well characterized. Non-trafficking roles have been described but remain poorly defined, including the regulation of immune responses in the gut. The current study examined the role of PSGL1 within the colonic innate immune system to regulate establishment of homeostasis or induction of T helper-mediated colitis after CD4⁺ transfer to Rag^{-/-} mice. **Methods/Results:** Wild type (WT) splenic CD4⁺ T cells were transferred i.p. to Rag^{-/-} mice who were moved to conventional housing on day 7, and tissues were examined on day 42. As expected, recipients gained weight in parallel with Rag^{-/-} controls and developed minimal clinical evidence (diarrhea, occult stool blood) of colitis. Rag^{-/-} mice developed limited histologic changes (colitis score 0.5±0.2 out of 5) and produced low levels of colonic effector cytokine, measured by ELISA on colon explant cultures. To examine the role of PSGL1 in innate signaling to adaptive reconstitution, PSGL1^{-/-} mice were crossed to Rag^{-/-} mice. Rag/PSGL1^{-/-} mice underwent an identical transfer protocol. Compared to Rag^{-/-} recipients, Rag/PSGL1^{-/-} mice developed clear clinical colitis with significantly worse diarrhea and occult blood after week 1 and began losing weight after day 28. PSGL1 deletion increased colitis scores (4.3±0.8) and effector cytokine production 5-10-fold (IFN γ , IL17, IL1 β , IL6, TNF). FACS analysis demonstrated a dramatic expansion in lamina propria CD4⁺ T cells (50-fold) in Rag/PSGL1^{-/-} vs. Rag^{-/-} recipients. However, the proportion of FoxP3/CD25⁺ CD4⁺ cells (Tregs) was reduced 60% in colitic Rag/PSGL1^{-/-} mice (3.9±0.9% vs. 9.9±1.2%). Urzainqui

et al. J Imm 2007 suggested PSGL1 plays a tolerogenic role in dendritic cells related to IL10 production influencing the development of Tregs. To specifically examine innate IL10 production, CD4⁺ transfer was repeated using cells from Il10^{-/-} mice, where the addition of piroxicam (a NSAID) after T cell transfer results in colitis. Both Rag^{-/-} and Rag/PSGL1^{-/-} mice developed colitis, but colitis scores and effector cytokine were increased more in Rag/PSGL1^{-/-} mice. However, IL10 levels were 2.4-fold higher in Rag^{-/-} mice. **Conclusion:** PSGL1 deletion in Rag^{-/-} mice results in severe colitis after adoptive transfer of CD4⁺ cells with enhanced proliferation of effector CD4⁺ cells, impaired IL10 production and reduced proportion of Tregs. The transfer of splenic WT CD4⁺ cells in Rag^{-/-} and Rag/PSGL1^{-/-} mice permitted us to examine the role of PSGL1 in the non-lymphocyte/innate population. This novel detection of severe colitis in Rag/PSGL1^{-/-} recipients suggests PSGL1 expression in non-lymphocytes regulates mucosal inflammatory responses independent of lymphocyte recruitment.

Mo1739

Amelioration of 5-Fluorouracil-Induced Oral Mucositis in Hamsters by TJ-14 (Hangeshashinto), Inhibitor of Inducible Prostaglandin E2 and Proinflammatory Cytokines

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Background and Aims: Chemotherapy-induced oral mucositis (COM) is a common toxicity, whereas the optimal treatment is not well established. COM is characterized by a painful inflammation, which involves pathogenically in pain-evoking prostaglandin E2 (PGE2) and proinflammatory cytokines. TJ-14 (Hangeshashinto), a traditional Japanese herbal medicine, is prescribed to treat oral mucositis, but the mechanism is still unknown. A recent clinical study revealed that topical application of TJ-14 as a gargle was effective in the treatment of COM. The aim of this study is to clarify the topical effect of TJ-14 on the PGE2 production and proinflammatory cytokines. **Methods:** COM was induced in hamsters by a combination of 5-fluorouracil administration and mild abrasion of the cheek pouch, and the healing process was examined by measuring the lesion size. The PGE2 contents in the inflamed sites were measured by EIA assay after purification of PGE2. Hamsters were given a diet containing 1% TJ-14 or a control diet throughout the experiment. In analysis with cultured cells, human oral keratinocytes (HOK) and acute monocytic leukemia cells (THP-1) were used. PGE2 in HOK and phorbol ester-treated THP-1 cells was induced by stimulation with 10 ng/mL of interleukin-1 beta (IL-1 β) or 200 ng/mL of lipopolysaccharide (LPS), respectively. Enzymes related to PGE2 synthesis, and proinflammatory cytokines were measured by real-time PCR and ELISA. **Results:** TJ-14 ameliorated COM accompanied by a prominent increase of PGE2 at the inflamed sites. Inducible PGE2 production in HOK and THP-1 cells was significantly decreased by addition of TJ-14, while constitutive PGE2 was not changed at all. The gene expressions of cyclooxygenase-2 (COX-2), cytosolic phospholipase A2, and prostaglandin E synthase were down-regulated by exposure to TJ-14, whereas cyclooxygenase-1 was not affected. A screening test for investigation of the active ingredients that inhibit PGE2 production showed that several main ingredients including 6-geringerol, 6-shogaol, and wogonin decreased PGE2 strongly at 1 μ mol/L or less. IL-1 β production was inhibited in levels of mRNA and protein by TJ-14 and berberine, another main ingredient of TJ-14. **Conclusions:** TJ-14 evidently inhibited PGE2 production with COX-2 selectivity and proinflammatory cytokine production, resulting in the improvement of COM in the animal model. Our study clarified the mechanism of the topical anti-COM effect of TJ-14.

Mo1740

Defects in Autophagy Favour Adherent-Invasive E. coli Persistence Within Macrophages Leading to Increased PRO-Inflammatory Response

Pierre Lapaquette, Marie-Agnès Bringer, Arlette Darfeuille-Michaud

Ileal lesions in Crohn's disease (CD) patients are abnormally colonized by pathogenic adherent-invasive *Escherichia coli* (AIEC). AIEC bacteria are able to replicate within epithelial cells after lysis of the endocytic vacuole and within macrophages in a large vacuole. CD-associated polymorphisms in NOD2, ATG16L1 and IRGM affect bacterial autophagy, a crucial innate immunity mechanism. We previously determined that defects in autophagy impaired the ability of epithelial cells to control AIEC replication. AIEC behave differently within epithelial cells and macrophages and so we investigated the impact of defects in autophagy on AIEC intramacrophagic replication and pro-inflammatory cytokine response. AIEC bacteria induced the recruitment of the autophagy machinery at the site of phagocytosis, and functional autophagy limited AIEC intramacrophagic replication. Impaired ATG16L1, IRGM or NOD2 expression induced increased intramacrophagic AIEC and increased secretion of IL-6 and TNF-alpha in response to AIEC infection. In contrast, forced induction of autophagy decreased the numbers of intramacrophagic AIEC and pro-inflammatory cytokine release, even in a NOD2 deficient context. On the basis of our findings, we speculate that stimulating autophagy in CD patients would be a powerful therapeutic strategy to concomitantly restrain intracellular AIEC replication and slow down the inflammatory response.

Mo1741

Bile Salts Induce Long Polar Fimbriae Expression and Favour Crohn's Disease-Associated Adherent-Invasive *Escherichia coli* Interaction With Peyer's Patches

Benoit Chassaing, Arlette Darfeuille-Michaud

Ileal lesions of patients with Crohn's disease (CD) are colonized by adherent-invasive *Escherichia coli* (AIEC). The earliest lesions of recurrent CD are erosions of Peyer's patches (PP), and we recently reported the presence of a functional lpf operon in AIEC, encoding long polar fimbriae (LPF), that allows AIEC bacteria to interact with murine and human PP and to translocate across M cells. The aim of the present study was to analyze the effect of gastrointestinal conditions on the expression of LPF in AIEC strains. We report that LF82 bacterial growth in an acid pH medium or at high osmolarity has no effect on lpf transcription level, in contrast to bacterial growth in the presence of bile salts, which promotes high lpf