Identification of IL-17-producing FoxP3⁺ regulatory T cells in Crohn's disease

Hovhannisyan Z, Treatmen J, Mayer L

Immunology Institute, Mount Sinai School of Medicine, New York, NY

BACKGROUND: IL17-producing CD4⁺ T cells (Th17) constitute a subset of T-helper cells implicated in the pathogenesis of inflammatory bowel diseases (IBD). At mucosal surfaces, Th17 cells are thought to protect the host from infection, whereas regulatory T (Tregs) cells control immune responses and inflammation. The development of both cell types requires TGF-b, but depend on distinct transcription factors, which suggests that Th17 cells and Tregs may be related and may arise from the same precursor in distinct cytokine milieus. Studies on the reciprocal relationship between human Th17 cells and Tregs in intestinal inflammation in IBD will allow for further elucidation of mechanisms regulating the balance between inflammation and tolerance in pathological conditions.

METHODS: Surgical specimens from patients undergoing bowel resection for IBD and cancer (as normal controls) were used as the source of lamina propria mononuclear cells. IL17-production and FoxP3 expression was assessed ex-vivo by intracytoplasmic staining after stimulation with PMA and ionomycin for 4 hours.

RESULTS: Following measurement of IL17-production and FoxP3 expression by CD4⁺ T cells, we detected a distinct population of FoxP3⁺IL17-co-expressing cells in Crohn's disease patients (3.8%±1.1%), but not in healthy controls or in patients with ulcerative colitis. These FoxP3⁺IL17-producing cells were undetectable in the periphery of IBD patients and normal controls. Identified lamina propria FoxP3⁺IL17-producing cells did not show a preference for distinct sites in the bowel but were found in decreased numbers when derived from less inflamed tissues. Furthermore, these cells co-expressed both FoxP3 and RORgt transcription factors, and produced significant amounts of IFNg(38%±6.4%), but not IL10. FoxP3⁺IL17-producing cells shared phenotypic features with conventional Th17 cells, expressing high levels of CCR6, CD161 and co-secreting IL22 and IL21. However, unlike conventional Th17 cells, but similar to Tregs, they expressed high levels of CD101 and low levels of CD127, and were imprinted for gut homing, as indicated by high levels of CCR6, CD103 and integrin a4b7 expression. Furthermore, FoxP3⁺IL17-producing cells strongly suppressed in vitro proliferation of CD4⁺ responder T cells.

CONCLUSION: We identified the existence of a minor but distinct population of FoxP3⁺IL17-producing CD4⁺ T cells in the lamina propria of Crohn’s disease patients. These may represent an intermediary between Tregs and Th17 cells. Ex-vivo secretion of IL17 and constitutive expression of RORgt by FoxP3⁺CD4⁺ T cells suggest that under specific environmental stimuli FoxP3⁺Tregs can possess proinflammatory properties.
Background: The senescence-accelerated (SAMP1) mouse strain develops spontaneous ileitis and recapitulates the pathology of human Crohn’s disease. Further examination of ileal tissue from this strain reveals significant eosinophilic infiltration and remodelling.

Purpose: We hypothesize that eosinophilic infiltration contributes to intestinal tissue inflammation and remodelling. Thus the impact of anti-eosinophil antibody treatment on inflammatory infiltrate and altered tissue architecture in the SAMP1 model of Crohn’s-like ileitis was assessed.

Description: A time-course of histological and molecular features of ileitis in SAMP1 mice were assessed by routine staining and eosinophil specific immunohistochemical staining. Intestinal permeability was measured by the FITC labeled Dextran gavage method. Eosinophil inhibition studies were performed with -CCR3 antibody injections during the chronic stage of disease, from 20 to 30 weeks. Flow cytometry for lamina propria leukocytes including eosinophils was performed and all assessments were compared to age-matched control AKR mice.

Results: Eosinophilic inflammation correlated with establishment of ileitis and remodelling events in the SAMP1 mouse (p<0.05). Intestinal permeability also increased with disease progression. Blockade of lamina propria eosinophil infiltration was observed following 10-weeks of -CCR3 injections (p<0.001) and resulted in attenuation of histological measures and ileal inflammation (p<0.05). Molecular measures of remodelling were also reduced. Cellularity of the reactive draining mesenteric lymph nodes, but not the spleen, were significantly reduced (p<0.05). Furthermore, flow cytometric analysis revealed a reduction in lamina propria lymphocyte populations (p<0.05-0.001). Reduction in tissue remodelling was also observed, as demonstrated by reduced muscle hypertrophy (p<0.01) and reduced goblet cell hyperplasia (p<0.01). The fibroblast chemo-attractant and eosinophil binding protein fibronectin, was the most increased gene during the progression of remodelling in these mice (p<0.001) and was significantly decreased following antibody treatment (p<0.01).

Conclusions: SAMP1 mice demonstrate Crohn’s-like ileitis with histological and molecular features of eosinophilic infiltration and tissue remodelling. Antibody treatment targeting eosinophils are effective in reducing eosinophil infiltration, overall inflammation and tissue remodelling. We anticipate that this model will provide a valuable tool for further elucidating the eosinophil’s role in the pathogenesis of inflammatory bowel diseases.

This project was funded by grants from NIH RO1 DK 62245, NIH R01 DK 080212-01A2 and the NIH CCTSI CMH pilot award.
Long-Term Endoprosthetic Management of Anastomotic Strictures in Crohn’s Disease: Report of Nine Year Follow-Up & Review of the Literature

Levine R, Kadro O

Department of Colon & Rectal Surgery, William Beaumont Hospital, Royal Oak, MI

Background: The role of endoluminal stenting in benign obstruction, especially for Crohn’s disease, is controversial with limited data and widely disparate outcomes. The purpose of this study was to determine the long-term efficacy and safety of this technology in the treatment of fibrostenotic Crohn’s disease and to review the existing literature on this topic.

Methods: We undertook a retrospective review of all patients undergoing endoluminal stenting for Crohn’s disease strictures at our institution from August 2001 to 2010. Outcome measures included technical success, clinical improvement, duration of stent and luminal patency, and need for re-intervention.

Results: The findings of this study are summarized in Table 1. Five patients underwent this procedure with a 100% rate of technical and clinical success. All patients had a history of ileocolonic resection for Crohn’s disease with symptomatic structuring of the anastomosis. Mean follow-up and mean duration of stent patency were 28 months (range 0.75-109 months). There was one delayed complication involving re-obstruction which required surgical intervention and there were no mortalities.

Table 1. Patient characteristics and outcomes after stent placement.

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age/Gender</th>
<th>Site of Stricture (length)</th>
<th>Prior Interventions</th>
<th>Stent Size (type)</th>
<th>Technical/Clinical Success</th>
<th>Complications (time after stent)</th>
<th>Repeat Intervention (time after stent)</th>
<th>Duration of Stent &amp; Luminal Patency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52/M</td>
<td>ICA (5cm)</td>
<td>ICR x2, strictureplasty, balloon dilatation</td>
<td>22 x 60mm (Wallstent UC)</td>
<td>yes/yes</td>
<td>none</td>
<td>none</td>
<td>9 yrs, 1 mo.</td>
</tr>
<tr>
<td>2</td>
<td>67/M</td>
<td>ISA (3cm)</td>
<td>subtotal colectomy, SBR x2</td>
<td>25 x 90mm (Wallflex UC)</td>
<td>yes/yes</td>
<td>none</td>
<td>none</td>
<td>1 yr, 27 wks</td>
</tr>
<tr>
<td>3</td>
<td>52/F</td>
<td>ICA (3cm)</td>
<td>ICR</td>
<td>22 x 90mm (Wallflex UC)</td>
<td>yes/yes</td>
<td>none</td>
<td>none</td>
<td>7 mos.</td>
</tr>
<tr>
<td>4</td>
<td>49/M</td>
<td>ICA (2cm)</td>
<td>multiple bowel resections, strictureplasty</td>
<td>25 x 60mm (Wallflex UC)</td>
<td>yes/yes</td>
<td>re-obstruction (3wks)</td>
<td>ICR, stent removal (3 wks)</td>
<td>3 wks</td>
</tr>
<tr>
<td>5</td>
<td>29/M</td>
<td>ICA (1cm)</td>
<td>ICR, strictureplasty, balloon dilatation</td>
<td>25 x 90mm (Wallflex UC)</td>
<td>yes/yes</td>
<td>none</td>
<td>none</td>
<td>18 wks</td>
</tr>
</tbody>
</table>

ICA = ileocolic anastomosis; ISA = ileosigmoid anastomosis; ICR = ileocolic resection; SBR = small bowel resection; UC = uncovered
**Conclusions:** Endoluminal stenting of anastomotic strictures in Crohn’s disease is a safe and effective alternative to surgery which can provide lasting benefit in select patients. Further studies are necessary to clarify the full impact of this technology on long-term management of this complex disease.

[Keywords: Crohn’s disease, stents, anastomosis, stricture, obstruction, endoscopy]
**Background:** This study examines the correlates of death in Inflammatory Bowel Disease (IBD) by following a cohort of patients from an ongoing population-based registry in Monroe County, NY.

**Methods:** During the years 1995-2000, community-derived Monroe county patients with a diagnosis of IBD were sent a questionnaire and consent form. After exclusions, 727 subjects who had signed consent forms were used to form a cohort. Cause of death information was obtained from our Monroe County IBD Registry and the NY State Department of Health using data from 1995-2006. Correlates of survival were evaluated.

**Results:** Survival time from onset of disease to death for CD vs. CUC patients revealed no significant differences (p=0.13). Overall survival was no different than that expected for Monroe County, NY. The SMR was 1.11 (95% CI: 0.86-1.36). Among the cohort, there were 74 deaths (10.2%). Of all deaths, 28.4% were due to IBD, 28.4% to non-related malignancy and 31.1% to cardiovascular disease. Seven of the deaths related to IBD were from colorectal carcinoma (CRC). Four of these cancers occurred in post-surgical retained rectum or colon. These cancers occurred in spite of close follow-up.

**Conclusions:** A population-based cohort of 727 patients from Monroe County in western NY, initially diagnosed as IBD, was followed for a median of 9 years. Mortality of the cohort was not significantly different from the general population. Four CRC deaths in patients with post-surgical retained colon reflect a unique cancer risk in both chronic ulcerative colitis and Crohn's disease colitis.

**Key Words:** Colorectal Carcinoma (CRC), Mortality, Inflammatory Bowel Disease (IBD), Crohn’s Disease (CD) Chronic Ulcerative Colitis (CUC)
P-182. CD8+ CD28- regulatory T cells mediating contact dependent inhibition are absent in the lamina propria but present in the peripheral blood of patients with Crohn’s disease

Rabinowitz K, Treatman J, Berin C, Mayer L.

Immunology Institute, Mount Sinai School of Medicine, New York, NY

**INTRODUCTION:** In the normal state, the interaction between IEC and LP lymphocytes gives rise to a population of CD8+ CD28- T cells with regulatory function that can be identified phenotypically and functionally in normal LPL. CD8+ T cells isolated from the LP of CD patients show a defect in their suppressive activity.

**AIM:** Characterize the requirement for expansion of these regulatory CD8+ T cells, define the location of precursors and the mechanism by which the cells mediate suppression.

**METHODS:** Freshly isolated LPLs and PBLs from normal controls, UC and CD patients were stimulated with visilizumab (Nuvion), a humanized anti CD3 mAb (100ng/ml), for 5 days followed by addition of IL7 (10ng/ml) and IL15 (20ng/ml) and irradiated PBLs. After 14 days CD8+ T cells were purified by magnetic bead sorting, and cultured in the presence of IL7 and IL15. Cell lines were analyzed for suppressor activity (inhibition of CD4+ T cell proliferation in response to anti-CD3/CD28 beads), surface molecule expression (flow cytometry) and cytokine secretion profile (CBA). Suppressive cell lines were used as immunogens to generate murine mAbs against surface molecules involved in the suppressor function. RNA from lines generated from the 3 groups was used in a microarray comparison.

**RESULTS:** All cell lines did not express CD28, CD25, ICOS, PDL1, CD16, perforin, granzyme B, CTLA4, FoxP3 and CD103, but were positive for CD3, CD8b, CD2 and CD101. Following stimulation with anti-CD3/CD28, cell lines secreted IFN-g and TNF-a but not IL10, IL2, IL4, IL6 or IL17. Lines generated from the LP of normal and UC patients were able to suppress CD4+ T cell proliferation in a contact dependent fashion. In contrast, lines generated from CD patients either did not or at best had a markedly decreased capacity to mediate suppression. Moreover these lines also produce greater amounts of IFNg, TNF IL10 and IL2 in the cultures. Lines generated from the PBLs of CD patients showed normal suppressor activity as well as a comparable cytokine secretion profile to the lines generated from the LP of controls. Lastly, sera from 4 mice immunized with normal CD8+ LPL lines but not unimmunized control sera demonstrated the ability to block suppression.

**CONCLUSION:** We developed a method for generating regulatory CD8+ T cell lines from the LP and PB and showed that CD8+ T cell lines from the LP of control and UC but not CD patients exhibit contact dependent regulatory function. A cell surface molecule recognized by sera from immunized mice block this suppressive function. Lines generated from the PB of all 3 groups showed normal suppressor activity suggesting that the precursors for regulatory CD8+ T cells are present in the PB which will further our understanding of IBD pathogenesis.
P-183. CX3CR1 regulates colitis through intestinal macrophage homeostasis

Medina O, Denning T

Dept. of Pediatrics, Dept. of Pathology, School of Medicine, Emory University, Atlanta, GA

Dendritic cells (DCs) and macrophages residing in the intestine play a central role in regulating innate and adaptive immune responses and inflammation. Lamina propria (LP) macrophages and DCs are among the first phagocytic cells of the innate immune system to come in contact with luminal bacteria. CX3CR1 expressing LP DCs are thought to be involved in the sampling of luminal antigens and clearance of entero-invasive pathogens, while the phenotype and functions of LP macrophages are less well defined. Since numerous subsets of DCs and macrophages exist within the LP and the expression and function of CX3CR1 on these cells has not been carefully delineated, we initially examined its expression using 10-color flow cytometry and real-time PCR. Our results demonstrate that CX3CR1 is expressed almost exclusively in CD11b^+ F4/80^- macrophages and not in CD11c^-CD103^- DCs. The functional role of CX3CR1 was analyzed in two independent strains of CX3CR1 deficient mice (cx3cr1^GFP/GFP and cx3cr1^-/-). During the steady state, CX3CR1 deficiency led to a decrease in the frequency and absolute cell number of LP macrophages, while LP DCs were not affected, suggesting a role for CX3CR1 in the homeostasis of LP macrophages. Consistent with the ability of intestinal macrophages to appropriately sense and respond to bacteria and bacterial by-products, CX3CR1 deficient mice had an increase in bacterial translocation to secondary lymphoid organs, such as MLN. During inflammatory conditions, such as DSS-induced acute colitis, both cx3cr1^GFP/GFP and cx3cr1^-/- showed a significantly increase in fecal blood, diarrhea, and tissue damage, evidencing more sensitivity to the effects of DSS compared to cx3cr1^+/+ mice. Collectively, our data demonstrate an important role for CX3CR1 in regulating LP macrophage homeostasis and intestinal inflammation. This work was supported by a Career Development Award, from the Crohn’s and Colitis Foundation of America, and NIH awards (AA017870 and AI083554).