T cell-induced inflammation of the small and large intestine in immunodeficient mice

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Ostanin, Dmitry V., Kevin P. Pavlick, Sulaiman Bharwani, Dwain D’Souza, Kathryn L. Furr, Carla M. Brown, and Matthew B. Grisham. T cell-induced inflammation of the small and large intestine in immunodeficient mice. Am J Physiol Gastrointest Liver Physiol 289: G109–G119, 2006. First published August 11, 2005; doi:10.1152/ajpgi.00214.2005.—It is well known that transfer of CD4⁺CD45RB<sup>hi</sup> (naïve) T cells into syngeneic lymphocyte-deficient mice induces chronic colitis. However, no studies have reported the presence of small bowel inflammation in this T cell-dependent model. Therefore, the objective of this study was to evaluate and compare small and large bowel inflammation induced by transfer of naïve T cells into two different immunodeficient recipient mice. T and B cell-deficient recombinase activating gene 1-deficient [RAG knockout (KO)] and T cell-deficient T cell receptor-β × T cell receptor-δ double-deficient (TCR KO) mice were reconstituted with wild-type naïve T cells and observed for signs of disease. We found that reconstituted RAG KO mice developed moderate to severe colitis and inflammation of the entire small intestine at 6–8 wk after T cell transfer. Adoptive transfer of naïve T cells into TCR KO mice induced a milder form of chronic colitis and small bowel inflammation that was confined primarily to the duodenum at 10–12 wk after T cell transfer. T helper cell 1 and macrophage-derived proinflammatory cytokine mRNA levels correlated well with the localization and severity of the chronic large and small bowel inflammation. In addition, we observed comparable homing and expansion of donor lymphocytes in the gut and secondary lymphoid tissues of both recipients. Taken together, our data demonstrate that transfer of naïve T cells into immunodeficient recipient mice induces both chronic small and large bowel inflammation and that the presence of B cells in the TCR KO recipients may play a role in regulating chronic intestinal inflammation.

INFLAMMATORY BOWEL DISEASES (IBD; Crohn’s disease and ulcerative colitis) are idiopathic recurrent inflammatory diseases of the intestinal tract that affect over 1 million individuals in North America and several million worldwide. Patients with active disease suffer from rectal bleeding, severe diarrhea, abdominal pain, fever, and weight loss. Histological examination of biopsies obtained from patients with active disease reveal the infiltration of large numbers of leukocytes such as polymorphonuclear leukocytes (PMNs), monocytes, and lymphocytes into the intestinal and/or colonic interstitium as well as edema, loss of goblet cells, decreased mucus production, crypt cell hyperplasia, erosions, and ulcerations (31). Despite many years of intense investigation, the etiology and specific pathogenic mechanisms responsible for IBD remain poorly defined. Recent experimental and clinical studies suggest that the initiation and pathogenesis of these diseases are multifactorial, involving interactions among genetic, environmental, and immune factors (8). Regardless of exactly how these interactions ultimately promote chronic gut inflammation, it is becoming increasingly apparent that the acquired immune system plays a crucial role in disease pathogenesis. Indeed, data obtained from a variety of experimental studies suggest that the chronic gut inflammation may result from a dysregulated immune response to enteric bacterial antigens (28). This concept of a dysregulated immune response is best exemplified experimentally by the adoptive transfer model of naïve (CD4⁺CD45RB<sup>hi</sup>) T cells into immunodeficient recipient severe combined immunodeficient (SCID) or recombinase activating gene 1 (RAG)-deficient mice, which induces moderate to severe colitis (16, 29). It has been well described that the chronic colitis induced by adoptive transfer develops as a result of enteric antigen-driven activation and polarization of naïve T cells to disease-producing T helper 1 cells (Th1 cells) in the absence of appropriate regulatory T cells such as CD4⁺CD25⁺ Treg, Tr1, and/or Th3 T cells (10, 23, 30, 34).

Although the adoptive transfer model has been used by several different laboratories for more than 10 yr, few studies have reported macroscopic or histological evidence of chronic small bowel inflammation. This is somewhat surprising given the fact that enteric bacteria and their associated antigens are not only found in the colon but are also present in substantial numbers in the lumen of the small bowel and cecum. Indeed, it has been proposed that enteric antigens are taken up by dendritic cells within the small bowel and transported to the draining mesenteric lymph nodes, where naïve T cells interact with these antigen-presenting cells to become activated to a Th1 disease-producing phenotype (for a review, see Ref. 24). If correct, one would predict that these antigen-activated Th1 cells would be recruited back to the small intestine (as well as the colon), where they would reencounter the original antigens, thereby producing small bowel inflammation in addition to colitis. Recent preliminary data from our laboratory (26) as well as the results of Dohi et al. (5, 6) demonstrated that transfer of wild-type (WT) or interferon (IFN)-γ-deficient naïve T cells, respectively, induces chronic small bowel inflammation in T cell receptor (TCR)-deficient mice. To better understand the immunological mechanisms responsible for...
promoting small and large bowel inflammation, we systematically evaluated the small and large bowel for signs of inflammation and injury induced in two different immunodeficient recipient mice. We report, for the first time, that adoptive transfer of syngeneic WT CD4\(^+\)CD45RB\(^{\text{high}}\) T cells into RAG-deficient [RAG knockout (KO)] mice induces chronic inflammation of the entire small and large intestine, whereas transfer of naïve T cells into TCR-\(\beta\) × TCR-\(\delta\) double-deficient (TCR KO) mice induces only duodenal inflammation and colitis. The immunological and pathophysiological significances of these novel findings are discussed.

**MATERIALS AND METHODS**

**Animals.** Female WT mice (8–10 wk old) as well as male RAG KO and TCR KO mice (5–6 wk), all on a C57Bl/6 background, were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were maintained on 12:12-h light-dark cycles in standard animal cages with filter tops under specific pathogen-free (SPF) conditions in our animal care facility at the Louisiana State University Health Sciences Center (Shreveport, LA). All animals were given standard laboratory rodent chow and water ad libitum. All experimental procedures involving the use of animals were reviewed and approved by the Institutional Animal Care and Use Committee of Louisiana State University Health Sciences Center and performed according to the criteria outlined by the National Institutes of Health.

**Induction of chronic gut inflammation.** Transfer of naïve CD4\(^+\)CD45RB\(^{\text{high}}\) T cells into RAG KO or TCR KO mice was performed as previously described (11, 12). Briefly, spleens were removed from donor C57Bl/6 female mice and teased into single cell suspensions in PBS containing 4% FCS using the frosted sides of glass microslides. Erythrocytes were removed by hypotonic lysis. For enrichment of CD4\(^+\) T cells, the MACS system from Miltenyi Biotec (Auburn, CA) was employed for negative selection by magnetic cell sorting according to the manufacturer’s instructions. Briefly, cells were incubated with FITC-conjugated anti-B220, anti-CD8-\(\alpha\), and anti-MAC-1 monoclonal antibodies (all from BD Pharmingen; San Jose, CA) and subsequently labeled with anti-FITC microbeads (Miltenyi Biotec). Unlabeled cells were separated from labeled cells on a depletion column (column type CS, Miltenyi Biotec) assembled into the magnetic separator (VarioMACS, Miltenyi Biotec). Enriched CD4\(^+\) T cells were labeled with biotin-conjugated anti-CD4 monoclonal antibody (eBioscience; San Diego, CA), followed by streptavidin 670 (Invitrogen; Carlsbad, CA) and phycoerythrin (PE)-conjugated anti-CD45RB monoclonal antibody (BD Pharmingen), and fractionated into CD4\(^+\)CD45RB\(^{\text{high}}\) and CD4\(^+\)CD45RB\(^{\text{low}}\) fractions by two-color sorting on a FACS Vantage (Becton-Dickinson; San Jose, CA). The CD45RB\(^{\text{high}}\)and CD45RB\(^{\text{low}}\) populations were defined as the brightest staining 40% and dullest 15% of CD4\(^+\) T cells, respectively, and were found to be >98% pure on postsort analysis. Male TCR KO and RAG KO mice were injected (intraperitoneally) with either 5 × 10\(^5\) CD45RB\(^{\text{high}}\) or CD45RB\(^{\text{low}}\) T cells suspended in 500 μl PBS. Clinical evidence of disease (e.g., body weight loss and loose stool/diarrhea) was followed and recorded weekly from the time of the injection.

**Tissue lymphocyte analyses.** Lymphocytes were obtained from the spleen, intestine, and colon and analyzed by flow cytometry as previously described (15). Briefly, spleens were removed from WT, RAG KO, and TCR KO mice reconstituted with either CD45RB\(^{\text{high}}\) T cells, CD45RB\(^{\text{low}}\) T cells, or PBS and teased into a single cell suspension using the frosted ends of two glass slides in 4% FACS buffer on ice. The suspension was then passed through a 26-gauge syringe to obtain a single cell suspension. After the suspension was pelleted, red blood cells were removed by hypotonic lysis, and the resulting leukocytes were resuspended in FACS buffer containing anti-Fc receptor antibody at 5 × 10\(^7\) cells/ml. After an incubation with anti-Fc receptor monoclonal antibody, ~1 × 10\(^6\) cells were placed into individual wells of a 96-well plate, pelleted, and stained. The total number of T cells was determined by three-color analysis using anti-CD3-FITC, CD4-allophycocyanin (APC), and CD8a-PE (BD Pharmingen, eBioscience), and the degree of activation was determined using CD3-FITC, CD4-APC, and CD45RB-PE (BD Pharmingen, eBioscience).

Analysis of intestinal intraepithelial lymphocytes (IELs) was performed using a modification of the method described previously (2). Briefly, the small and large intestines were removed from mice, flushed of luminal contents, and trimmed of excess fat and connective tissue. The small and large intestines were then opened longitudinally and cut into small (0.5–1.0 cm) pieces in PBS on ice. The pieces were then incubated in prewarmed (37°C) PBS-4% FCS-0.2 mM EDTA-10 mM D-glucose solution on a rotating shaker for 20 min at 250 rpm at 37°C. After incubation, the intestinal pieces were vortexed for 3–5 s. Supernatants from individual animals were collected in separate 50-ml conical tubes and kept on ice. Incubations were performed at least three times to insure complete removal of epithelium. Intestinal pieces from individual animals were processed separately and never mixed. The resultant pooled supernatants were pelleted by centrifugation and resuspended in 30 ml of 40% Percoll. IELs were further purified by centrifugation over a 40% Percoll gradient for 25 min at 1,000 g at room temperature. After centrifugation, the pellet of IELs was washed and resuspended in FACS buffer containing anti-Fc receptor monoclonal antibody. Viable cells were counted using 0.4% Trypan blue dye-PBS solution.

Lamina propria (LP) lymphocytes (LPLs) were prepared by digestion of the finely minced intestinal pieces remaining after IEL isolation with RPMI-1640–4% FBS solution containing collagenase type VIII (200 U/ml) for 40 min at 250 rpm in a 37°C shaker (15). Lymphocytes were further enriched by centrifugation over a 40% Percoll gradient. The LPL pellet was washed and resuspended in FACS buffer containing anti-Fc receptor monoclonal, and the number of LPLs was counted. Approximately 1 × 10\(^6\) cells were placed in individual wells of a 96-well plate and stained using anti-CD3-FITC, CD4-APC, and CD8a-PE (BD Pharmingen, eBioscience) as well as CD3-FITC, CD4-APC, and CD45RB-PE (BD Pharmingen, eBioscience) cocktails. After being stained, the cells were fixed for 15 min on ice in freshly prepared 2% ultrapure formaldehyde (Polysciences; Warrington, PA) and analyzed the next day on the FACSCalibur (BD Biosciences).

**RNA isolation and RT-PCR.** Small pieces of the colon were removed from animals, cleansed of fecal material, and snap frozen in liquid nitrogen. Each small intestine was divided into the proximal and distal halves, gently cleaned with a cotton swab, and snap frozen in liquid nitrogen. Tissue samples were stored at −80°C until further processed. Total RNA was isolated from tissues of three representative samples in each group using TRIzol reagent (GIBCO-BRL; Grand Island, NY) according to the manufacturer’s instructions. RNA was resuspended in RNase-free water and stored at −80°C.

One microgram of DNase-treated RNA was converted to cDNA by RT-PCR using a GeneAmp RNA PCR kit (Applied Biosystems; Foster City, CA) in a 20-μl total reaction volume. Quantitative PCRs for murine interleukin (IL)-1β, IL-10, tumor necrosis factor (TNF)-α, IFN-γ, and transforming growth factor (TGF)-β cytokines were performed using predeveloped primers with 200 ng of input cDNA in a 50-μl total reaction volume using an iQ SYBR green supermix (Bio-Rad Laboratories; Hercules, CA) in the iCycler iQ System (Bio-Rad Laboratories) with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. Each sample was run in duplicate wells. Cycling conditions were as follows: cycle 1, 95°C for 3 min; cycle 2, step 1, 95°C for 10 s and step 2, 55°C for 45 s, repeat 60 cycles; cycles 3–4, 55°C for 2 min; cycle 5, 55°C for 10 s and increase set point temperature after cycle 2 by 0.5°C every cycle, repeat 80 cycles; and cycle 6, hold at 25°C.
Table 1 shows the PCR primer sequences used for the PCR (27). The relative expression for each cytokine was determined by normalizing expression of each representative sample to GAPDH and comparing it with the control, which was also normalized to GAPDH. The resulting value indicates the fold increase in cytokine mRNA expression in the experimental sample (CD45RB<sup>low</sup>) over the control (CD45RB<sup>high</sup>).

**Macroscopic and histopathological analyses.** At 8–12 wk after T cell reconstitution, or when animals lost 15–20% of their original body weight, mice were euthanized, and the colons were removed, cleaned of fecal material, divided into proximal and distal sections, and scored for macroscopic evidence of inflammation using a modification of the method described by Conner et al. (3). Briefly, normal colonic morphology was assessed a score of 0; mild bowel wall thickening in the absence of visible hyperemia was assigned a score of 1; moderate bowel wall thickening and hyperemia was given a score of 2; severe bowel wall thickening with rigidity and marked hyperemia was assigned a score of 3; and severe bowel wall thickening with rigidity, hyperemia, and colonic adhesions was given a score of 4. A small piece of each section was placed in 10% PBS-formalin and fixed overnight at 4°C. The fixed tissue was then rinsed with PBS, partially dehydrated in ethanol, and embedded in JB-4 (Polysciences). These samples were sectioned (5 μm) using glass knives and processed for standard hematoxylin and eosin (H&E) staining for histopathology.

Swiss rolls of the proximal and distal portions of the small intestine were prepared as follows: the entire small intestine was cut into two equal parts, ~10–15 cm, and labeled as proximal and distal. With the use of scissors and a wet cotton swab, the intestines were carefully opened longitudinally and spread onto a piece of cardboard, and the lumen was flushed with saline using a plastic pipette. Excess saline was carefully blotted with a paper towel, and 10% formalin solution was dripped along the entire length of the intestine to cover it completely. Each piece was prefixed in this manner for 5–10 min. Excess fixative was carefully blotted, and both intestinal segments were rolled luminal side up without being stretched onto a saline-dipped wooden stick starting with the most proximal end to the distal end so that the proximal end of each segment was always in the middle of each roll. The ends of each roll were fixed in place with a pin or 26-gauge hypodermic needle. Each roll was placed in a small jar filled with 10% formalin and fixed overnight. After fixation, rolls were placed in cassettes and embedded in paraffin, and 5-μm cross sections were cut and then stained with H&E.

Histopathological scores of colons were assigned in a blinded manner using the method described by Laroux et al. (15). Briefly, eight parameters were used, which included: 1) the inflammatory infiltrate score, given a score ranging from 0 to 3; 2) the extent of inflammation, given a score ranging from 0 to 3; 3) crypt damage, given a score ranging from 0 to 3; 4) the percent involvement, given a score ranging from 0 to 3; and 5) villus atypia, distortion, branching, atrophy, and blunting, given a score ranging from 0 to 3. The severity of the inflammatory changes was based on the sum of the scores reported for each parameter.

**RESULTS**

**Transfer of naïve T cells into TCR KO mice induces inflammation of the proximal small intestine and colon.** TCR KO mice are devoid of all peripheral T cells and IELs but retain functionally active B cells (21). In contrast to the original report (20) describing the generation of TCR KO mice, we found that these mice did not develop spontaneous colitis when maintained in SPF conditions (data not shown). In addition, we observed no colitis when these mice were reconstituted with CD4<sup>+</sup>CD45RB<sup>low</sup> cells. However, upon reconstitution with WT CD4<sup>+</sup>CD45RB<sup>high</sup> T cells, TCR KO mice developed a wasting syndrome over a period of 10–12 wk characterized by diarrhea, piloerection, and loss of activity as well as loss of body weight (Fig. 1A). Increased colonic weight-to-length ratios were consistent with the developed chronic colitis in these animals (Fig. 1B). Blinded histopathological evaluation of the colon revealed moderate to severe disease characterized by extensive transmural infiltration of PMNs and mononuclear leukocytes, epithelial hyperplasia, Goblet cell loss, abnormal crypt architecture, and crypt abscesses compared with the CD4<sup>+</sup>CD45RB<sup>low</sup>- or PBS-injected control groups (Figs. 2A and 3A).

In addition to colitis, we also observed bowel wall thickening and hyperemia of the proximal-most portion of the small intestine (duodenum) in TCR KO mice injected with CD4<sup>+</sup>CD45RB<sup>high</sup> T cells. To ascertain whether these mice developed chronic small bowel inflammation in other regions, we prepared Swiss rolls of the entire small intestine from each animal, fixed the tissue in paraffin, sectioned it, and then stained it with H&E. Histopathological inspection revealed significant inflammation in the duodenum compared with TCR KO control mice (Fig. 4A). Inflammation was characterized by the infiltration of PMNs and mononuclear leukocytes into the...
LP, villus blunting/atrophy, substantial crypt hyperplasia, and thickening of the muscle layer. Inflammation in the distal portion of the small bowel of these animals was minimal or absent (Fig. 4B). Blinded histopathological evaluation of the small bowel revealed moderate duodenitis with little or no inflammation in the distal small intestine of TCR KO mice injected with CD45RBhigh. No inflammation was observed in TCR KO mice injected with CD45RBlow (Fig. 5).

Morphometric analyses were performed on the small bowel, and the results are presented in Table 2. We observed that the villus height-to-crypt depth ratio was reduced by over twofold in the proximal but not distal portion of the small bowel (Table 2). In addition, we noticed a more than a twofold increase in muscle layer thickness in both the proximal as well as distal small intestine. Interestingly, in some cases, we observed modest increases in Goblet cell numbers in TCR KO mice injected with CD45RBhigh along the entire length of the small intestine as well as increased numbers of Paneth cells in cell crypts compared with CD4+CD45RBlow-injected TCR KO mice (data not shown).

Transfer of naïve T cells into RAG KO mice induces moderate to severe inflammation of the entire small intestine and colon. A recent report by Dohi et al. (6) suggested that B cells may play an important role in promoting distal small bowel inflammation in TCR KO mice injected with IFN-γ-deficient naïve T cells. Because TCR KO mice have fully functional B cells but lack T cells and IELs, we wanted to ascertain whether the absence of B cells would eliminate the T cell-mediated proximal small intestinal inflammation we observed in TCR KO mice. To address this possibility, we utilized RAG KO mice because they are devoid of all T cells, IELs, and B cells. We found that in contrast to TCR KO mice, where pathological changes were most prominent in the colon and duodenum, transfer of naïve T cells into RAG KO mice induced moderate to severe colitis as well as a more severe form of small bowel inflammation that affected the entire length of the small intestine. Whereas reconstituted TCR KO mice required 10–12 wk to lose more than 10% of their body weight, RAG KO mice injected with naïve T cells began to lose weight as early as

**Fig. 1.** A: adoptive transfer of CD4+CD45RBhigh (RBhigh) but not CD4+CD45RBlow (RBlow) T cells into recombinase activating gene 1 (RAG) knockout (KO) or T cell receptor-β × T cell receptor-δ (TCR) KO mice induces wasting disease. **Significant difference (P < 0.05) compared with initial weight. Data represent means ± SE for n = 29 TCR KO + CD45RBhigh, n = 7 for TCR KO + CD45RBlow, n = 10 for RAG KO + CD45RBhigh, and n = 7 for RAG KO + CD45RBlow. B: transfer of CD4+CD45RBhigh but not CD4+CD45RBlow T cells into RAG KO or TCR KO mice induces macroscopic evidence of colitis. Weight-to-length ratios were determined at 6–8 wk after T cell transfer into RAG KO mice and 10–12 wk after transfer into TCR KO recipients. Data represent means ± SE for n = 28 TCR KO + CD45RBhigh, n = 9 for TCR KO + CD45RBlow, n = 9 for RAG KO + CD45RBhigh, and n = 7 for RAG KO + CD45RBlow. **Significant difference (P < 0.05) between TCR KO mice that received CD45RBhigh and CD45RBlow T cells; #significant difference (P < 0.05) between RAG KO groups that received CD45RBhigh and CD45RBlow T cells.

**Fig. 2.** Development of chronic colitis after transfer of CD4+CD45RBhigh T cells into TCR KO (A) or RAG KO mice (B). Colons were fixed, embedded in JB-4 plastic, sectioned at 5 μm, and stained with hematoxylin and eosin. Representative histological images show extensive transmural inflammation in both the proximal and distal parts of the colon with infiltrating poly- and mononucleated cells and loss of Goblet cells. Top and bottom left: normal architecture of the proximal and distal colon 8–12 wk after the transfer of CD4+CD45RBlow T cells. Magnification: ×100 in both A and B.
4 wk, and all mice had to be killed by 7–8 wk, when they lost, on average, >15% of their initial body weight (Fig. 1A). The loss of body weight corresponded to enhanced macroscopic and histological evidence of chronic inflammation in the colons of RAG KO mice injected with naïve T cells (Figs. 1B, 2B, and 3A). Interestingly, histopathological scores of the proximal and distal colon of RAG KO mice injected with CD45RBhigh were significantly greater than those of TCR KO mice injected with CD45RBhigh (Fig. 3A). In addition, the incidence of moderate and severe disease was substantially greater in the former than in the latter group (Fig. 3B).

Histopathological evaluation of the small bowel revealed an extensive inflammatory cell infiltrate in the mucosa and submucosa with a dramatic loss of villus architecture and crypts, increased mitotic activity in the remaining crypts, crypt abscesses, and thickening of the muscle layer (Figs. 4 and 5). In contrast to the TCR KO mice receiving naïve T cells, reconstituted RAG KO mice exhibited a loss of Goblet cells along the entire length of the small intestine, which was most noticeable in the distal portion. In addition, we observed a loss of Paneth cell staining in the crypts of the distal small bowel of the RAG KO mice reconstituted with naïve T cells (data not shown). Furthermore, small bowel inflammation was associated with decreased villus height-to-crypt depth ratios in both the proximal and distal small intestine, whereas muscle layer thickness was significantly increased in the proximal small bowel and showed tendency for increase, although not statis-
message levels were upregulated 76- and 60-fold in RAG KO versus 42- and 66-fold in TCR KO proximal and distal colons, respectively (Fig. 6). In addition, TNF-α message levels were also higher in the RAG KO recipients compared with TCR KO mice (16- and 11-fold vs. 4- and 5-fold, respectively, for the proximal and distal segments). Expression of IL-10 and TGF-β mRNA was elevated about two- to fivefold and was similar in both groups.

The cytokine profiles in the proximal versus distal regions of the small intestine of RAG KO mice revealed a 66- and 12-fold increase in IFN-γ, 133- and 4-fold increase in IL-1β, and 13- and 3-fold increase in TNF-α message levels, respectively (Fig. 6). The differences in mRNA levels of these proinflammatory cytokines were dramatically higher in the proximal versus distal parts of the small intestine; however, the histological scores were very similar between the two parts (Fig. 5). This discrepancy could be explained by our inability to differentiate, histologically, between maximal and nearly maximal inflammation. It is also possible that there is a regional susceptibility between the proximal and distal small bowel such that a relatively small increase in proinflammatory cytokine production in the distal small bowel can result in dramatic histopathological consequences. Alternatively, proximal small bowel inflammation may be mediated by Th1 cytokines, whereas distal bowel inflammation is not.

Message levels of the different cytokines in the small intestine of reconstituted TCR KO mice displayed only modest elevations in IFN-γ, IL-10, and TGF-β in the proximal and distal small bowel and were not statistically significant (Fig. 6).

Intestinal and splenic lymphocyte analyses in immunodeficient mice reconstituted with CD4<sup>+</sup>CD45RB<sup>high</sup> T cells. To ascertain whether differences in homing and/or activation and proliferation of the injected naïve T cells could account for the differences in anatomic localization, severity, and incidence of the disease we observed in reconstituted TCR KO versus RAG KO recipients, lymphocytes were isolated from the spleen and small and large intestine of representative animals from each group and analyzed for the surface expression of CD3, CD4, CD8, and CD45RB. As expected, injected CD4<sup>+</sup> T cells underwent extensive expansion/proliferation in both groups of immunodeficient mice (Fig. 7). The total numbers of CD4<sup>+</sup> cells in all tissues analyzed were comparable between TCR KO and RAG KO animals (on average, 16–19 × 10<sup>6</sup> T cells could be recovered from the spleen and small and large bowel, which indicates a 30- to 40-fold expansion of naïve T cells in recipients).

Table 2. Morphometric analyses of the proximal and distal portions of the small intestine

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Results are means ± SE; n = no. of mice. Muscularis propria thickness (muscle thickness) and villus height-to-crypt depth ratios were quantified using Metamorph software (Universal Imaging; Downing town, PA). <sup>*</sup>Significant difference between T cell receptor-β x T cell receptor-δ (TCR) knockout mice that received CD45RB<sup>high</sup> (RB<sup>high</sup>) and CD45RB<sub>low</sub> (RB<sub>low</sub>) T cells; <sup>†</sup>significant difference between recombinase activating gene 1 (RAG) knockout mice that received RB<sub>high</sub> and RB<sub>low</sub> T cells.

Fig. 5. Small intestine histopathological scores. Duodenal and ileal sections were scored in a blinded fashion, and data are expressed as means ± SE for n = 6 TCR KO + CD45RB<sub>low</sub>, n = 4 for TCR KO + CD45RB<sub>high</sub>, n = 6 for RAG KO + CD45RB<sub>high</sub> and n = 4 for RAG KO + CD45RB<sub>low</sub>. $Significant difference (P < 0.05) between TCR KO mice that received CD45RB<sub>high</sub> and CD45RB<sub>low</sub> T cells; #significant difference (P < 0.05) between RAG KO groups that received CD45RB<sub>high</sub> and CD45RB<sub>low</sub> T cells; $significant difference (P < 0.05) between scores of ileal segments of TCR KO and RAG KO groups that received CD45RB<sub>low</sub> T cells.

Cytokine mRNA determinations in the small and large intestine. Quantitative determinations of cytokine message levels isolated from colonic tissue of TCR KO and RAG KO recipients injected with CD45RB<sub>high</sub> T cells revealed significant increases in mRNA expression of Th1 and macrophage-derived cytokines such as IFN-γ, TNF-α, and IL-1β over those mice that received CD45RB<sub>low</sub> T cells (Fig. 6). Interestingly, mRNA levels of regulatory cytokines such as TGF-β and IL-10 were also slightly elevated, but the overall balance of pro- and regulatory cytokines was heavily skewed toward a proinflammatory profile (Fig. 6). Data obtained using quantitative determinations of colonic mRNA demonstrated that tissue obtained from reconstituted RAG KO mice exhibited a larger increase in the expression of certain proinflammatory genes than did colons obtained from reconstituted TCR KO recipients. For example, colonic IFN-γ message levels were increased dramatically (81- and 88-fold in RAG KO vs. 27- and 70-fold in TCR KO proximal and distal colons, respectively). IL-1β message levels were upregulated 76- and 60-fold in RAG KO versus 42- and 66-fold in TCR KO proximal and distal colons, respectively (Fig. 6). In addition, TNF-α message levels were also higher in the RAG KO recipients compared with TCR KO mice (16- and 11-fold vs. 4- and 5-fold, respectively, for the proximal and distal segments). Expression of IL-10 and TGF-β mRNA was elevated about two- to fivefold and was similar in both groups.

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Intestinal and splenic lymphocyte analyses in immunodeficient mice reconstituted with CD4<sup>+</sup>CD45RB<sub>high</sub> T cells. To ascertain whether differences in homing and/or activation and proliferation of the injected naïve T cells could account for the differences in anatomic localization, severity, and incidence of the disease we observed in reconstituted TCR KO versus RAG KO recipients, lymphocytes were isolated from the spleen and small and large intestine of representative animals from each group and analyzed for the surface expression of CD3, CD4, CD8, and CD45RB. As expected, injected CD4<sup>+</sup> T cells underwent extensive expansion/proliferation in both groups of immunodeficient mice (Fig. 7). The total numbers of CD4<sup>+</sup> cells in all tissues analyzed were comparable between TCR KO and RAG KO animals (on average, 16–19 × 10<sup>6</sup> T cells could be recovered from the spleen and small and large bowel, which indicates a 30- to 40-fold expansion of naïve T cells in recipients).
The absolute numbers of splenic CD4+ T cells in TCR KO and RAG KO animals were \(7 \times 10^6\) and \(2 \times 10^6\) cells, respectively, compared with \(12 \times 10^6\) CD4+ cells from the healthy WT spleen (Fig. 7). In addition, we found that CD4+ cells in the spleens of TCR KO and RAG KO animals accounted for 14% and 13%, respectively, compared with 16% for WT animals (Fig. 7). Not surprisingly, the absolute numbers of CD8+ splenocytes in both groups of reconstituted animals were almost 30-fold lower compared with WT mice and accounted for \(\leq 1\%\) of the gated cells (Fig. 7).

Analysis of IELs in the small intestine showed a comparable reconstitution with CD4+ cells in both lymphocyte-deficient groups. These cells accounted for \(\approx 40\% - 50\%\) of gated cells based on the forward and side scatter of analyzed cells (Fig. 7). In contrast, the WT small intestine was populated primarily with CD8+ T cells, which represented \(\approx 75\%\) of gated cells with a very small population of CD4+ cells accounting for only \(6\%\) (Fig. 7). Colonic IELs obtained from reconstituted mice also revealed comparable reconstitution with CD4+ cells in both groups of recipients. Similar to the small intestine, colonic CD4+ IELs represent \(\approx 40\% - 50\%\) of the total lymphocyte population in both groups, with CD8+ T cells comprising only \(2\% - 3\%\) (Fig. 7). Interestingly, in both the small intestine and colon, there was a significant population of CD4+CD8+ double-positive cells, a phenomenon originally reported by Reimann and Rudolphi but also observed by others (14, 32).

Therefore, when we calculated the absolute numbers as well as percentages, all values had to be adjusted to subtract this double-positive population, which accounted for about 20–40% of gated cells isolated from small intestinal IELs and 10–20% of gated cells for colonic IELs (data not shown). This CD4+CD8+ double-positive population of cells was not seen to such a dramatic extent in the spleen or LP, where these cells accounted for \(\leq 1\%\) (spleen) and 2–3% (LP) and may reflect local influences in the IEL compartment on naïve T cells to acquire this double-positive phenotype. Overall, we found that IELs obtained from reconstituted animals express in vast majority CD4 and not the CD8 coreceptor. As a comparison, in healthy WT animals, CD8+ T cells are mostly predominant in the IEL compartment of the small and large intestines (75% and 28% of the gated cells, respectively), whereas CD4+ cells comprise only 6% and 3% of the gated cells in the small and large bowel, respectively.

Analysis of the LP compartment in the reconstituted animals revealed that the CD4+ T cell population in the small intestine of TCR KO mice was increased 17-fold over RAG KO mice (3.5 \(\times\) \(10^6\) vs. 0.2 \(\times\) \(10^6\) cells, respectively; Fig. 7). In addition, in small intestines of reconstituted RAG mice, the absolute number as well as percentage of LP CD4+ cells were comparable to those in WT mice. Interestingly, the CD4+ T cell population in the colonic LP was more than twofold less in TCR KO than RAG KO animals (0.7 \(\times\) \(10^6\) vs. 1.6 \(\times\) \(10^6\) cells,
respectively; Fig. 7). In addition, CD4\(^+\) T cells obtained from healthy WT colons accounted for only 4% of total colonic lymphocytes, whereas in both reconstituted animals they accounted for ~55% of the gated cells. Using CD45RB expression as an indicator of T cell activation, we found that virtually all CD4\(^+\) cells within the spleen and IEL and LPL compartments of reconstituted animals converted from the CD45RB\(^{high}\) (naïve) to CD45RB\(^{low}\) (activated/memory) phenotype (data not shown).

**DISCUSSION**

Much of what we understand regarding the immunological and pathophysiological mechanisms responsible for chronic gut inflammation has come from studies using a variety of different T cell-dependent models of colonic inflammation. One model that has been particularly helpful in delineating mechanisms responsible for the initiation as well as regulation of chronic colitis is the adoptive transfer of naïve (CD4\(^+\)CD45RB\(^{high}\)) T cells into immunodeficient recipient SCID or RAG KO mice (16, 29). Data obtained using this model have shown that in the absence of regulatory cells, chronic colitis develops as a result of enteric antigen-driven activation and polarization of naïve T cells to disease-producing Th1 cells (10, 23, 30, 34). One of the more perplexing questions posed using this model (as well as most of the other mouse models of spontaneous colitis) is why the inflammation...
appears to localize specifically to the colon. Indeed, to our knowledge, there have been no reports demonstrating the presence of small bowel inflammation in SCID or RAG KO recipients. Fundamentally, this is an important consideration given the fact that enteric bacterial antigens from both the small and large intestine will gain access to the secondary lymphoid tissue draining the gut (e.g., mesenteric lymph nodes), where naïve T cells interact with antigen-presenting cells to become activated to a Th1 disease-producing phenotype (24). One would predict that these Th1 cells would be recruited to the small intestine as well as the colon, where they would induce both small bowel inflammation and colitis. Recent studies from our laboratory as well as others (5, 6, 26) have demonstrated that chronic inflammation may be induced in specific regions of the small bowel in TCR KO mice, suggesting that intestinal inflammation may be induced under certain conditions using this adoptive transfer model. In an attempt to better understand the immunological mechanisms responsible for promoting small and large bowel inflammation, we systematically evaluated intestinal inflammation induced in TCR KO mice and compared this inflammatory response with that produced in RAG KO recipients. In addition, we employed a straightforward approach by using WT T cells in our transfer experiments. We provide evidence in the present study demonstrating that transfer of naïve T cells into immunodeficient mice induces both small and large bowel inflammation with varying degrees of involvement and severity depending on the specific recipient. Our data suggest the presence of additional regulatory mechanisms in TCR KO mice.

A major finding of this study was that RAG KO mice reconstituted with naïve T cells developed chronic inflammation of the entire small bowel and colon. To our knowledge, this is the first study demonstrating that chronic small bowel inflammation and colitis may be induced in mice deficient in both T and B cells. Small intestinal pathology was characterized by villus blunting and extensive cell infiltrate into the LP, bowel wall and muscularis thickening, and crypt hyperplasia. In the most severe cases, we saw a complete loss of crypts to inflammatory infiltrate and formation of crypt abscesses. In addition, we observed a loss of Goblet cells and Paneth cells along the entire small bowel and a loss of Goblet cells in the colon. Finally, high levels of Th1 and macrophage-derived cytokines supported the severe inflammation observed histologically.

There have been numerous reports over the past 8 yr describing the immunological and pathophysiological mechanisms involved in the initiation and regulation of the chronic colonic inflammation observed in SCID or RAG KO mice injected with naïve T cells (28–30). In these previous studies, there was either no indication of the presence of small bowel inflammation or the authors stated, with data not shown, that intestinal inflammation was not observed. The reasons for the differences between our results and those from other laboratories are not obvious but may be due to differences in 1) the strain of animal used, 2) housing conditions for the mice, and/or 3) the method of tissue sampling. For example, most of the original studies describing the T cell transfer model used WT donors and SCID recipients on a Balb/c background, whereas all of our studies used C57BL/6 mice. There are well-known strain differences in response to the induction of different autoimmune diseases as well as strain-related variations of host immune response to challenge with pathogens between Balb/c and C57BL/6 mice (7, 9, 13, 22, 33, 35). Alternatively, differences in animal housing conditions may explain some of these differences. All mice used in the present study were raised and maintained under SPF conditions and were given standard rodent chow and tap water. It may be that some of the other investigations used conventional or other types of housing and/or acidified or autoclaved water and food that may have limited or prevented small intestinal inflammation. Another possible explanation for these differences may be related to the methods used to sample tissue for histological evaluation. We prepared Swiss rolls from the entire small bowel so that we could systematically evaluate the presence (or absence) of small intestinal inflammation and its anatomic localization. Because intestinal inflammation is patchy in this model, random selection of tissue biopsies may not consistently demonstrate bowel inflammation.

Another important finding of our study was that adoptive transfer of WT naïve T cells into TCR KO mice induced both colitis and duodenal inflammation. Duodenal inflammation was characterized by villus blunting, crypt hyperplasia, inflammatory cell infiltrate, bowel wall and muscularis thickening, and elaboration of Th1 and macrophage-derived cytokines. Interestingly, the remaining 80–90% of the small intestine appeared normal with only a few infiltrating leukocytes but no pathological signs of inflammation. In contrast to the small bowel inflammation induced in RAG KO mice, we observed Goblet cell hyperplasia in several TCR KO mice injected with CD45RBhigh, in agreement with the recent report by Dohi et al. (6). In addition, we observed significant Paneth cell hyperplasia in both the diseased and apparently normal segments of the small bowel. These data and those reported by us in an earlier study (26) differ from what was originally reported by Dohi et al. (6), who found that transfer of WT CD45RBhigh T cells to TCR KO mice induced severe colitis but not small bowel inflammation, whereas transfer of IFN-γ-deficient naïve T cells induced ileitis but not colitis. This group provided evidence to suggest that a dysregulated Th2 response was responsible for the distal small bowel inflammation observed in TCR KO mice (6). However, a recent report (5) by this same group showed that TCR KO recipients will, in fact, develop proximal small bowel inflammation and gastritis when given CD45RBhigh T cells isolated from either WT or IL-4 KO donors. In addition, a recent report by Olson et al. (25) reported the development of ileitis but not colitis when SCID recipients were infected with CD4+CD45RBhigh and, surprisingly, CD4+CD45RBlow T cells isolated from SAMP1/Yit donors, suggesting that the latter population also contains pathogenic cells.

Another aspect of the present study that deserves some discussion is the inability of our TCR KO mice to develop spontaneous colitis when maintained in SPF conditions. We used PBS-injected TCR KO mice that remained disease free, both symptomatically and histologically, for at least 1 yr (data not shown). These data differ from those originally communicated by Mombaerte et al. (20). In their original report, they showed that 50% of their TCR KO offsprings developed mild colitis at 4–9 mo of age, whereas the other 50% developed little or no colonic inflammation. One obvious reason for these differences may be due to differences in animal housing conditions. It was not clear in their report whether mice were raised under conventional or SPF conditions.
The mechanisms responsible for the milder form and regional-specific nature of the small bowel inflammation in TCR KO mice compared with the more severe pan-enteritis observed in RAG KO mice are not entirely clear at the present time. One possibility may be differences in recruitment and/or activation and proliferation of donor T cells to the small bowel. However, we observed similar numbers of activated CD4+ T cells within the IEL compartment of the small and large bowel of both immunodeficient recipients. Interestingly, the LP of the small bowel obtained from TCR KO mice contained 17-fold more CD4+ T cells compared with their RAG KO counterparts. These latter findings are puzzling given the fact that more severe small intestinal inflammation was observed in reconstituted RAG KO mice. Nevertheless, when the total number of CD4+ cells is taken into account, our data show comparable reconstitution in both types of recipients and suggest that alterations in homing and proliferation of adoptively transferred naïve T cells in the recipient TCR KO and RAG KO mice did not correlate with location or severity of disease. We did, however, observe much less Th1 and macrophage-derived cytokine mRNA in both the proximal and distal portions of the small bowel in TCR KO recipients, suggesting that, although CD4+ cell numbers were comparable (or even greater) to those observed in RAG KO mice, proinflammatory cytokine production was blunted.

Another possible explanation for the reduction in cytokine mRNA levels and consequent attenuation in inflammation of the distal 80–90% of the small bowel in TCR KO mice may be due to the presence and/or anatomic distribution of B cells within the small intestine. TCR KO mice lack peripheral T cells and IELs but retain fully functional B cells, whereas RAG KO mice are devoid of all T and B cells (21). There is a continuing controversy over whether B cells are protective or pathogenic in IBD, and this seems to largely depend on the model used in the studies. Mizoguchi et al. (18, 19) reported that B cell administration to TCR-α−/− mice attenuated the chronic colitis observed in these mutant mice. In addition, they demonstrated that B cell-deficient TCR-α−/− × Igμ−/− mice developed more severe colitis than TCR-α−/− mice, suggesting that B cells may suppress colonic inflammation. In contrast to these studies, Dohi et al. (6) provided evidence that B cells are responsible for the induction of ileal inflammation in TCR KO mice reconstituted with CD4+CD45RBhigh T cells isolated from IFN-γ-deficient mice. Taken together, our data appear to support a regulatory/suppressive role for B cells, because we observed only moderate duodenitis with no evidence of inflammation in more than 80% of the small intestine of TCR KO compared with RAG KO mice as well as a less severe form of colitis in the former group compared with the latter. Studies to address the regulatory role of B cells in this model of gut inflammation are currently underway.

In summary, our study demonstrates that the adoptive transfer of WT CD4+CD45RBhigh T cells into RAG KO mice induces chronic inflammation of the entire small and large intestine, whereas transfer of these same T cells into TCR KO mice induces only modest duodenitis and colitis. There are only a few mouse models that express small intestinal inflammation (1, 5, 6, 17) and virtually no studies demonstrating both small and large intestinal inflammation. These novel findings presented in the present study may prove useful in studying the disease pathogenesis as well as additional pathways of immune regulation.

GRANTS

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REFERENCES


