T cell-associated CD18 but not CD62L, ICAM-1, or PSGL-1 is required for the induction of chronic colitis

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Ostanin DV, Furr KL, Pavlick KP, Gray L, Kevil CG, Shukla D, D’Souza D, Hoffman JM, Grisham MB. T cell-associated CD18 but not CD62L, ICAM-1, or PSGL-1 is required for the induction of chronic colitis. Am J Physiol Gastrointest Liver Physiol 292: G1706–G1714, 2007; doi:10.1152/ajpgi.00573.2006.—The induction and perpetuation of chronic colitis are thought to involve a complex set of adhesion interactions between T cells and endothelial cells located on the vasculature within secondary lymphoid tissue and the intestine. The objective of this study was to assess the roles of T cell-associated CD18, CD62L (L-selectin), ICAM-1, and P-selectin glycoprotein ligand-1 (PSGL-1) in the induction of chronic colitis in mice. CD4+CD25− T cells derived from either wild-type (WT), CD18-deficient [CD18 knockout (KO)], CD62L KO, ICAM-1 KO, or PSGL-1 KO mice were adoptively transferred into recombinase activating gene-1 (RAG-1)-deficient mice (RAG KO mice) to assess the potential of these T cells to induce chronic colitis. At 8–10 wk following T cell transfer, we observed moderate to severe colitis as assessed by increases in colon weight-to-length ratios and by blinded histopathological analysis. In contrast, we found that transfer of CD18 KO T cells into RAG KO recipients resulted in the significant attenuation of colonic inflammation in these mice. Furthermore, we observed fewer infiltrating CD4+ T cells in the colon lamina propria in the CD18 KO→RAG KO group compared with the WT→RAG KO group. Finally, message levels of colonic TNF-α, IL-1β, and IFN-γ were significantly reduced in CD18 KO→RAG KO mice compared with colitic control animals. We conclude that T cell-associated CD18, but not CD62L, ICAM-1, or PSGL-1, is required for the development of chronic colitis.

T cell trafficking; inflammation; inflammatory bowel disease; cytokines; intracellular adhesion molecule-1; P-selectin glycoprotein ligand-1

The inflammatory bowel diseases (IBD; Crohn’s disease and ulcerative colitis) are idiopathic chronic inflammatory disorders of the intestine and/or colon that are characterized by rectal bleeding, severe diarrhea, abdominal pain, fever, and weight loss. Histological examination of biopsies obtained from patients with active disease reveals the presence of large numbers of leukocytes such as lymphocytes, polymorphonuclear leukocytes (PMNs), macrophages, and monocytes in the intestinal and/or colonic interstitium. A variety of different studies using genetically engineered or immune-manipulated mice have suggested that chronic colitis results from a dysregulated immune response to commensal bacteria. This concept is best illustrated experimentally using the adoptive transfer of naïve T cells into immunodeficient recipient severe combined immunodeficient (SCID) or recombinase-activating gene (RAG)-deficient [RAG knockout (KO)] mice, which induces chronic colitis and small bowel inflammation 6–8 wk following transfer (11, 25, 33, 35, 37, 38, 40, 45). Active disease in these mice resembles Crohn’s disease in that the inflammation is transmural in nature and exhibits erosions, epithelial cell hyperplasia adjacent to areas of epithelial cell injury, goblet cell depletion, and infiltration of large numbers of lymphocytes, PMNs, and monocytes (18, 24, 43).

There is an emerging body of experimental data suggesting that the induction of chronic gut inflammation begins with the migration of naïve T cells from the blood to gut-associated lymphoid tissues (GALTs) such as Peyer’s patches (PPs) and mesenteric lymph nodes (MLNs). In the absence of appropriate regulatory mechanisms, naïve T cells interact with enteric-loaded dendritic cells resulting in the activation, polarization, and clonal expansion of these lymphocytes to produce colitogenic effector cells such as T helper (Th1) and/or Th17 cells (15, 27, 30, 31, 46). These effector cells then exit the intestinal tissue via the efferent lymphatics, enter the systemic circulation, and home to the gut interleukin. Upon secondary activation by their cognate antigen within the gut interstitium, these effector T cells initiate intestinal inflammation (39, 40).

Naïve and effector T cell trafficking to the secondary lymphoid tissue and the intestinal lamina propria are complex processes that involve a variety of specific lymphocyte-endothelial cell interactions. The major T cell-associated adhesion molecules that have been implicated in these processes include CD62L (L-selectin), CD18, P-selectin glycoprotein ligand-1 (PSGL-1), and α4β7-integrin. A great deal of attention has been focused on CD62L because it is found on the surface of naïve T cells and is thought to be critically important for the migration of these lymphocytes from the blood into secondary lymphoid tissues by way of high endothelial venules (HEVs) (26, 47, 48). HEVs associated with PPs contain mucosal addressin cell adhesion molecule-1 (MadCAM-1), whereas MLN-HEVs express both MadCAM-1 and peripheral node addressin (PNAd). It is thought that T cell-associated CD62L can bind to both MadCAM-1 and PNAd to tether lymphocytes to HEVs and initiate T cell rolling (1). Lack of CD62L on T cells results in delayed entry and activation of T cells within peripheral lymphoid nodes but does not affect the ability of activated T cells to migrate to inflammatory sites (1, 41).

Another potentially important adhesion molecule that has been

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implicated in the pathogenesis of autoimmune and inflammatory diseases is CD18. This is the common β2-integrin chain that can be combined with various α-subunits such as CD11a, CD11b, and CD11c to produce lymphocyte function-associated antigen-1 (LFA-1; CD11a/CD18), macrophage antigen-1 (Mac-1; CD11b/CD18), and p150,95 (CD11c/CD18), respectively. It has been suggested that LFA-1 is the only β2-integrin found on T cells; however, more recent evidence has suggested that T cell-associated Mac-1 may also be important in the pathophysiology of T cell-dependent inflammation (5, 51). T cell-associated LFA-1 has been shown to be involved in multiple T cell functions, including naïve T cell migration to and activation within secondary lymphoid tissue as well as homing of effector T cells to target tissue. We (34) have recently demonstrated that CD11a is a critical molecular determinant for the induction of chronic colitis in the T cell transfer, suggesting that LFA-1 is important in disease pathogenesis. However, in view of the work described above, other T cell-associated CD18 adhesion molecules may play important roles in disease pathogenesis as well. Yet, the effects of adoptive transfer of CD18-deficient T cells into immunodeficient recipients have not been assessed in a model of chronic colitis. The vast majority of previous studies have focused on the interactions of lymphocyte β2-integrins (e.g., LFA-1) with endothelial cell ICAM-1 as a major adhesive determinant for promoting naïve T cell rolling and adhesion in lymphoid tissue and homing of effector T cells to target tissues. Indeed, numerous studies have demonstrated that ICAM-1 is expressed on HEVs of lymphoid tissue and postcapillary venular endothelial cells of all tissue (including the gut) and is well known to bind CD11a/CD18 (LFA-1) and/or CD11b/CD18 (Mac-1) expressed on the surface of T cells and myeloid cells. In addition, we as well as others have shown that ICAM-1 is upregulated on the postcapillary microvasculature in the chronically inflamed bowel (19, 20) and that the administration of monoclonal antibodies (MAbs) to ICAM-1 attenuates intestinal inflammation in different models of acute and chronic colitis (2, 3, 6). Although these data have suggested that endothelial cell-associated ICAM-1 may play an important role in the pathogenesis of gut inflammation, it has also been demonstrated that ICAM-1 is expressed on activated T cells and is thought to be important for cell contact-dependent T cell activation (4, 9). However, no studies have investigated the role that T cell-associated ICAM-1 plays in the induction and/or propagation of chronic intestinal inflammation.

Following the initial activation/polarization of naïve T cells to disease-producing Th1 and/or Th17 cells within the GALT, these effector cells enter the systemic circulation via the lymphatics, where they home to the gut and initiate disease. This new pattern of homing is thought to be mediated by the interaction between T cell-associated PSGL-1, α4β1-integrin, and LFA-1 with venular P/E-selectin, MaDCAM-1, and ICAM-1, respectively (26, 47, 48). As mentioned previously, we (34) have shown that T cell LFA-1 is important for the onset and, possibly, propagation of chronic colitis. Using the same T cell transfer model of chronic colitis, Sydora et al. (44) have found that α4β1-integrin is not required for the development of disease, suggesting that additional adhesion molecules may play a role in disease pathogenesis. It is known, for example, that PSGL-1 is expressed on the surface of activated/effector T cells (as well as myeloid cells) and binds to L- and P-selectin, mediating leukocyte rolling on the endothelial surface. Recent studies (16, 42) have demonstrated that the administration of anti-PSGL-1 MAb results in decreased leukocyte rolling on the ileal microvasculature and suppression of ileitis in SAMP/Yit mice. In another study (14), treatment with anti-P-selectin MAb significantly reduced the recruitment of in vitro polarized Th1 cells following their adoptive transfer into Balb/c mice, implicating the role of T cell-associated PSGL-1.

Despite the data suggesting that one or more of these T cell-associated adhesion molecules may be important in the pathogenesis of experimental IBD, no investigations have directly tested whether T cell-associated CD18, CD62L, ICAM-1, and/or PSGL-1 are required for the induction of chronic intestinal inflammation. Therefore, the objective of this study was to define the importance of each of these adhesion molecules in a murine model of chronic colitis. The implications of our findings are discussed in the context of disease pathogenesis and therapeutic targets.

MATERIALS AND METHODS

Animals. C57Bl/6 wild-type (WT), RAG KO (5–6 wk), and CD62L-deficient (CD62L KO) mice were purchased from the Jackson Laboratory (Bar Harbor, ME), whereas CD18-deficient (CD18 KO), ICAM-1-deficient (ICAM-1 KO), and PSGL-1-deficient (PSGL-1 KO) mice were obtained from the Louisiana State University Health Sciences Center (LSUHSC; Shreveport, LA) animal breeding facility. Animals were maintained on 12:12-h light-dark cycles in standard animal cages with filter tops under specific pathogen-free conditions in our animal care facility at LSUHSC and 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 LSUHSC and performed according to criteria outlined by the National Institutes of Health.

Antibodies. Antibodies were purchased either from BD Pharmingen (San Diego, CA) or eBioscience (San Diego, CA). The following antibodies were used: CD62L-FITC, CD44-PE, CD3-PE-Cy5, and CD4-APC, which were used for quantifying the numbers and activation status of T cells isolated from different tissues. CD11a-FITC, CD49d-PE, ICAM-1-PE, CD18-FITC, and CD62L-FITC were used for quantifying T cell adhesion molecule surface expression.

Induction of chronic gut inflammation. Chronic colitis was induced by the transfer of WT or mutant mouse CD4+CD25− T cells into RAG KO mice using a minor modification of our previously published methods (33, 34). Briefly, spleens were removed from donor C57Bl/6 mice (WT, CD62L KO, or ICAM-1 KO) and teased into single cell suspensions in 1× PBS with 4% FBS (FACS buffer). Erythrocytes were removed by hypotonic lysis. For the enrichment of CD4+ T cells, cells were first incubated with FITC-conjugated anti-B220, anti-CD8a, and anti-MAC-1 MAb and then labeled with anti-FITC microbeads (Miltenyi Biotec, Auburn, CA) followed by negative selection on a depletion CS column. Enriched CD4+ T cells were labeled with anti-CD4 and anti-CD25 MAb and sorted into the CD4+CD25− fraction using FACSAria (Becton-Dickinson, San Jose, CA) and were found to be >98% pure on postsort analysis. Male RAG KO mice were injected (intraperitoneally) with 5–7.5 × 105 CD4+CD25− T cells resuspended 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.

For experiments involving the purification of CD18 KO T cells, isolation and sorting protocols were modified in the following way: erythocyte-free cells were incubated with anti-Fc receptor (FcR;
CD16/32)-FITC, followed by an incubation with FITC-conjugated anti-B220 and anti-CD8a MAbs and then with anti-FITC microbeads (Miltenyi Biotec). FITC-positive cells were separated by negative selection on a depletion CS column. Negatively labeled cells were then labeled with anti-CD4 and anti-CD25 MAbs and sorted into the CD4 + CD25 - fraction using FACS Aria (Becton-Dickinson, San Jose, CA). The control group for these experiments included WT splenocytes prepared in the same manner as the CD18 KO group.

**Tissue lymphocyte analyses.** Lymphocytes were obtained from the spleen, MLNs, and colonic lamina propria and analyzed by flow cytometry as previously described (24, 33, 34). Briefly, spleens were removed from reconstituted RAG KO mice, teased into a single cell suspension using frosted ends of two glassslides, and passed through a 26-gauge syringe to obtain a single cell suspension. Red blood cells were removed by hypotonic lysis, and the resulting leukocytes were washed and then resuspended in FACS buffer. Cells from MLNs were obtained by grinding the tissue using a glass rod inside a 70-μm nylon tube filter (Falcon, San Jose, CA) in FACS buffer. Lamina propria lymphocytes were prepared by the digestion of the finely minced intestinal pieces remaining after IEL isolation with RPMI-1640, 4% FBS, and collagenase type VIII (200 U/ml, Sigma) for 40 min at 250 rpm in a 37°C shaker. Lymphocytes were further enriched by centrifugation over a 40% Percoll gradient. The lamina propria lymphocyte pellet was washed and resuspended in FACS buffer containing anti-FcR MAb, and viable cells were counted using a solution of 0.4% Trypan blue in 1× PBS. For analysis, ~1 × 10^6 cells were placed in individual wells of a 96-well plate, incubated first with FcR block (CD16/CD32), and then stained with the appropriate antibody cocktails. After being stained, cells were fixed for 20 min on ice in freshly prepared 2% ultrapure formaldehyde (Polysciences, Warrington, PA) and analyzed the next day on the Calibur or LSR II (BD Biosciences).

Absolute numbers of CD3 + CD4 + T cells in the spleens, MLNs, and colonic lamina propria of reconstituted animals were calculated by multiplying the total number of viable cells isolated from each tissue by the percentage of total cells positive for CD3 and CD4 as determined by two-color flow cytometric analysis using the FACS calibur instrument (BD Biosciences).

**In vitro proliferation and cytokine determination.** Splenic CD4 + T cells (10^6) cells were enriched to >90% by negative selection and were plated in triplicate on CD3 MAb-coated 96-well plates. Soluble CD28 MAb (1 μg/ml final concentration) was added to all wells to maximally activate T cells. Activated or unactivated T cells (no CD3 or CD28 MAb added) were incubated for 72 h at 37°C in a 5% CO2 incubator. T cell proliferation was quantified using [3H]thymidine (5μCi/ml) incorporation for the last 18 h. Cell-free supernatants were collected following 72 h of incubation, and cytokine levels were measured using the Bio-Plex Cytokine Assay for mouse cytokines according to the manufacturer’s instructions.

**Histopathology.** Eight weeks posttransfer or when the animals had lost 15–20% of their original weights, mice were killed by cervical dislocation, and their colons were excised, measured, and weighed. A piece of the proximal and distal colon was fixed in 10% formaldehyde and processed for hematoxylin and eosin staining and blinded histopathological scoring using our previously published criteria (33, 34). The remaining colonic tissue was snap frozen and stored at −70°C until further processed for cytokine mRNA determination using real-time PCR (33).

**Statistics.** Data are presented as means ± SE. The statistical significance between experimental and control groups was evaluated using an unpaired t-test with the Welch correction. Statistical significance between more than two groups was evaluated using one-way ANOVA. Statistical significance between selected groups was evaluated using Dunnett’s post hoc test. A probability value (P value) of P < 0.05 was considered significant. All statistical analyses were done using GraphPad InStat software (version 3.06 for Windows).

**RESULTS**

Transfer of CD4 + CD25 - cells from CD62L KO, ICAM-1 KO, and PSGL-1 KO mice but not from CD18 KO mice induces chronic colitis in RAG KO recipients. To determine whether a deficiency in CD62L, PSGL-1, ICAM-1, or CD18 can impact the onset and/or severity of chronic gut inflammation, we adoptively transferred CD4 + CD25 - T cells obtained from WT or mutant mice into immunodeficient recipients and observed these animals for signs of intestinal inflammation, such as weight loss and diarrhea, over a period of 8 wk. We found that animals reconstituted with PSGL-1 KO CD4 + CD25 - T cells developed wasting disease, as characterized by a progressive loss of body weight and the appearance of diarrhea similar to that observed with WT cells (Fig. 1). In addition, the average weight loss in PSGL-1 KO→RAG KO mice at 6 and 7 wk was significantly lower than that in WT→RAG KO mice, and all mice from the former group had to be killed at 7 wk posttransfer due to high disease-associated mortality. Mice reconstituted with T cells from CD62L KO, CD18 KO, or ICAM-1 KO mice did not develop obvious wasting, with only few animals developing loose stools (Fig. 1). We found that colonic weight-to-length ratios for CD18 KO→RAG KO mice, but not CD62L KO→RAG KO, ICAM-1 KO→RAG KO, or PSGL-1 ko→RAG KO mice, were significantly lower than for control WT→RAG KO mice, suggesting an attenuation in colonic inflammation in the CD18 KO group (Fig. 2).

To further evaluate the development of disease in these groups, we performed blinded histopathological analysis of tissue obtained from the five different groups. We found that transfer of T cells obtained from CD18 KO donors induced a much milder disease than did adoptive transfer of T cells derived from WT, CD62L KO, ICAM-1 KO, or PSGL-1 KO mice (Fig. 3, A–C). Although transfer of PSGL-1 KO CD4 + CD25 - cells into RAG KO mice appeared to increase colitis compared with WT→RAG KO mice, the increase was not statistically significant (Fig. 3B). When we compared the overall incidence and severity of colitis in all five groups, the majority of animals in the WT→RAG KO, CD62L KO→RAG KO, ICAM-1 KO→RAG KO, and PSGL-1 KO→RAG KO groups ex-
pressed moderate to severe disease, whereas only 25% of the mice in the CD18 KO→RAG KO group exhibited this degree of colitis (Fig. 3C).

T cell-associated adhesion molecule expression. One explanation as to why the CD18 KO→RAG KO mice expressed attenuated disease is that CD18 deficiency may alter the expression of other T cell-associated adhesion molecules that are important for T cell trafficking and/or activation. Thus, we assessed the surface expression of CD62L, LFA-1, ICAM-1, α2-integrin (CD49d), and CD16 on CD4+ T cells obtained from CD18 KO mice and compared their expression to that obtained from WT mice using flow cytometric analysis. As expected, we found that T cells isolated from CD18 KO mice showed no expression of CD16 and LFA-1 but expressed similar levels of α2-integrin compared with WT T cells (Fig. 4A). Interestingly, we observed very little CD62L and substantially more ICAM-1 expression on the surface of CD18 KO T cells compared with WT lymphocytes (Fig. 4A). In addition, we observed a trend for increased proliferation of CD18 KO versus WT T cells when cells were stimulated with plate-bound CD3 and soluble CD28 mAbs; however, this increase did not reach statistical significance (Fig. 4B). Interestingly, we found that activated CD18 KO T cells produced significantly more Th2 and regulatory cytokines (e.g., IL-4, -5, and -10) than did WT CD4+ T cells, whereas the levels of IL-17, IFN-γ, and TNF-α were not significantly different between the two groups (Fig. 4C). It should be noted that the concentrations of IFN-γ produced by WT and CD18 KO T cells were ~30- to 40-fold higher than either of Th2 or regulatory cytokines, indicating that both cell types were skewed predominately to a Th1/Th17 phenotype (Fig. 4C).

RAG KO mice reconstituted with CD18-deficient T cells have lower numbers of CD4+ cells in the colonic lamina propria, but they express an activated/memory phenotype. A deficiency in T cell-associated CD18 may alter the trafficking behavior of these lymphocytes following adoptive transfer into immunodeficient mice. Therefore, we compared the ability of these CD18 KO T cells to traffic to and expand within peripheral tissues and GALTs with that of WT T cells. We found that although transfer of CD18 KO cells induced only mild colitis, T cells did repopulate the spleen and MLNs to the same extent as did adoptively transferred WT T cells (Fig. 5A). Interestingly, absolute numbers of CD4+ T cells obtained from CD18 KO→RAG KO mice were significantly lower in the colonic lamina propria compared with WT→RAG KO mice (Fig. 5A), which correlated well with the blinded histological scores for leukocyte infiltration in this group (Fig. 3B). It should be noted that the numbers of CD3+CD4+ T cells obtained from the spleens of CD62L KO→RAG KO mice (means ± SE: 1.85 ± 0.29 × 10^6 cells) and ICAM-1 KO→RAG KO mice (0.92 ± 0.18 × 10^6 cells) were not statistically different from those obtained from WT→RAG KO mice (1.46 ± 0.19 × 10^6 cells), suggesting that the transferred cells repopulated all recipient mice with similar efficiency.

In addition to T cell numbers, we also examined the activation status of CD4+ T cells in the spleen, MLNs, and colonic lamina propria of CD18 KO→RAG KO mice. Figure 5B shows that T cells in both groups displayed high levels of CD44 and loss of CD62L expression, indicating an activated/memory phenotype (Fig. 5B).

Message levels of TNF-α, IFN-γ, and IL-1β in the colons of RAG KO mice reconstituted with CD18 KO or WT T cells. Because colonic T cell numbers correlated well with histological evidence of chronic colitis, we wished to assess the overall cytokine milieu within the colonic lamina propria. Using quantitative real-time RT-PCR, we found increases in message levels of IFN-γ, TNF-α, and IL-1β in the colons of RAG KO mice reconstituted with WT CD4+CD25− T cells compared with the colons obtained from healthy WT mice (Fig. 6). In addition, message levels of these proinflammatory cytokines were significantly reduced in the colons obtained from CD18 KO→RAG KO animals, indicating reduced inflammatory mediator production within the colons of those mice (Fig. 6). Furthermore, we found that the mRNA levels of IL-10 and TGF-β were reduced by approximately twofold in CD18 KO→RAG KO mice compared with WT→RAG KO mice, demonstrating that the attenuated disease in the CD18 KO group was not due to increased expression of regulatory cytokines (data not shown).

DISCUSSION

Data obtained from a variety of different experimental studies have suggested that chronic intestinal inflammation is a T cell-dependent process that results from a dysregulated immune response to normal enteric antigens (17). It is becoming increasingly appreciated that, in the absence of appropriate regulatory mechanisms, naive T cells migrate from the circulation into GALTs (e.g., MLNs and PPs), where enteric antigens (presented by dendritic cells) promote the activation, polarization, and expansion of these antigen-inexperienced T cells to yield disease-producing effector cells (15, 27, 30, 31, 46). These colitogenic T cells then enter the systemic circulation via the lymphatics and home to the gut, where they activate interstitial macrophages and intestinal endothelial cells, thereby initiating the inflammatory response (23, 39, 40). Thus, T cell trafficking to GALTs and the intestine/colon is thought to play an important role in the induction as well as...
perpetuation of chronic intestinal inflammation. Several T cell-associated adhesion molecules (e.g., CD62L, CD18, and PSGL-1) have been proposed to be important for mediating these types of immune responses by virtue of their ability to promote T cell trafficking to secondary lymphoid and target tissues (8). However, the roles that these adhesion molecules play in initiating chronic colonic inflammation have not been defined. The data obtained in the present study demonstrate that T cell-associated CD18 but not CD62L, ICAM-1, or PSGL-1 is required for the induction of chronic colitis in the T cell transfer model. The attenuated disease in CD18 KO→RAG KO mice correlated with reduced numbers of Th1-producing CD4⁺ T cells in the colonic lamina propria but not in MLNs or the spleen, indicating that T cell activation and proliferation in GALTs and peripheral lymphoid tissues were not altered in these mice.

Certain aspects of our data agree well with previous work from our laboratory as well as others. We (34) have recently demonstrated that T cell-associated CD11a is required to induce colitis in this model, suggesting that LFA-1 (CD11a/CD18) plays a major role in mediating lymphocyte trafficking and/or activation in the T cell transfer model of chronic colitis. A previous study (28) has suggested that LFA-1 is the only β₂-integrin found on T cells; however, more recent data have implicated T cell-associated Mac-1 (CD11b/CD18) in other autoimmune/chronic inflammatory disorders (5, 51). In addi-

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**Fig. 3.** Histopathological images of distal colons obtained from RAG KO mice reconstituted with various types of CD4⁺CD25⁺ T cells. A: representative histopathology of distal colons demonstrating reduced inflammation in CD18 KO→RAG KO mice compared with all other groups. Representative pieces of the distal colon were obtained from reconstituted mice and were processed for hematoxylin-eosin staining as described in MATERIALS AND METHODS. Images were taken from representative samples at ×100 magnification. B: histopathological scores of distal colons were blindly assigned by an experienced pathologist using 7 previously described inflammatory parameters (33). The severity of the inflammatory changes in the distal colon was based on the sum of the scores reported for each parameter, with 17 being the maximal possible score. Data represent means ± SE for n = 9–12 mice in each experimental group and n = 45 mice in the WT control group. *Significant difference (P < 0.05) from the WT→RAG KO group. C: percent incidence and severity of disease in the distal colon in each group. Classification criteria were based on the following histopathological scores: no colitis, histopathological score of 0–1; mild colitis, histopathological score of 2–6; moderate colitis, histopathological scores of 7–11; and severe colitis, histopathological scores of 12–17.
tion, some studies (29, 51) have shown that CD18 KO T cells respond very differently to antigenic stimulation in vitro or to inflammatory stimuli in vivo than do CD11a KO T cells, suggesting that CD18 KO T cells may have different trafficking properties that affect the onset and severity of colitis in the T cell transfer model compared with adoptively transferred CD11a KO T cells. To address this possibility, we reconstituted RAG KO recipients with T cells obtained from CD18 KO donors and quantified colonic inflammation. We found that CD18 KO RAG KO mice expressed significantly less disease than WT RAG KO animals and that this attenuation was similar to what we (34) recently reported with the transfer of CD11a KO T cells into RAG-1 KO recipients. Taken together, these data suggest that both CD11a and CD18 are important for the induction of chronic gut inflammation and are consistent with studies from other investigators who found CD18 to be critical for mediating experimental psoriasis, a T cell-dependent model of chronic inflammation (13, 21). Not surprisingly, the attenuated disease in CD18 KO→RAG KO mice was associated with decreased numbers of CD4+ T cells in the colonic lamina propria and significantly reduced message levels of IFN-γ, TNF-α, and IL-1β (Fig. 6). Although we did observe fewer numbers of T cells within the colonic lamina propria, these cells were found to exhibit an activated phenotype by virtue of their increased surface expression of CD44 and loss of CD62L (Fig. 5). Interestingly, we found that prior to transfer, CD18 KO T cells expressed substantially less CD62L than did WT T cells (Fig. 4), suggesting that the attenuated disease in the CD18 KO→RAG KO mice may be due to defects in CD62L rather than or in addition to a deficiency in CD18. However, this possibility does not appear likely since we demonstrated in this study that adoptive transfer of T cells obtained from CD62L KO donors induced chronic colitis that was not significantly different from the
disease induced by transfer of WT T cells (Fig. 3). To our knowledge, this is the first report showing that deletion of CD18 alters the expression of additional adhesion molecules on CD4⁺ T cells. At first glance, one may anticipate that the loss of L-selectin would indicate an increased state of activation in CD18 KO versus WT T cells prior to transfer. Indeed, we and others (24, 36) have shown that transfer of activated cells from mice with colitis into another immunodeficient recipient induces more rapid and severe disease. However, we found that transfer of CD18 KO T cells into RAG KO recipients produced much less disease compared with RAG KO mice that received WT T cells (Fig. 3).

The fact that adoptive transfer of CD62L KO T cells into lymphopenic mice induced chronic colitis was unexpected given the fact that this T cell-associated adhesion molecule has been demonstrated by several different groups to be critical for trafficking of naïve T cells to different lymphoid tissues such as peripheral lymph nodes, MLNs, and PPs using short-term in vivo migration assays (22, 49, 50). Yet, no studies have examined how CD62L deficiency affects the onset or severity of colonic inflammation. Our data clearly demonstrate that CD62L is not required in this model of chronic intestinal inflammation and agree with the results of a recent investigation (10) showing that it was also not required for the development of acute, T cell-dependent colitis induced in a mouse model of graft-versus-host disease.

Another T cell adhesion molecule that has received a substantial amount of attention over the past few years is PSGL-1. Indeed, T cell-associated PSGL-1 has been found to be involved in the pathogenesis of experimental allergic encephalopathy (12, 32). However, it may be that this adhesion molecule may play a more important role for the recruitment of neutrophils rather than T cells to inflammatory sites (52). Using intravital videomicroscopy to visualize T cell-endothelial cell interactions, some studies have shown that PSGL-1 is required for Th1 (and Th2) cell rolling along the inflamed endothelium, suggesting that this P-selectin ligand may be important for mediating the early events associated with T cell extravasation and disease pathogenesis (53). The data obtained in the present study show that T cell-associated PSGL-1 is not required for the induction of chronic colitis in vivo, suggesting that other T cell adhesion molecules (e.g., LFA-1) or combinations of other integrins and/or selectins play more critical roles for T cell trafficking and disease pathogenesis. Although our data appear a bit surprising given the number of studies implicating CD62L and PSGL-1 in T cell trafficking to lymphoid and peripheral tissue, respectively, they emphasize the

**Fig. 5.** CD4⁺ T cell numbers obtained from different tissues of WT→RAG KO and CD18 KO→RAG KO mice and their activation status. A: absolute numbers of CD3⁺ C4⁺ T cells in spleens, mesenteric lymph nodes (MLNs), and colonic lamina propria (LP) of WT→RAG KO and CD18 KO→RAG KO mice were obtained by multiplying the total number of viable cells isolated from each tissue by the percentage of total cells positive for CD3 and CD4 as determined by two-color flowcytometric analysis. Data are presented as average numbers of cells per tissue ± SE of at least 2 separate experiments with 2–3 animals/group. *Significant difference (P < 0.05) between the CD18 KO→RAG KO and WT→RAG KO group as determined by an unpaired t-test (Welch corrected). B: the activation status of T cells was determined by surface expression of CD44 and CD62L on CD3⁺CD4⁺ cells. The histograms shown were obtained by first gating on a CD3⁺CD4⁺ double-positive population and are representative for cells obtained from WT→RAG KO and CD18 KO→RAG KO mice. Colonic LPL, colonic LP lymphocytes.

**Fig. 6.** Cytokine message levels in colons obtained from RAG KO mice reconstituted with WT or CD18 KO T- cells. Real-time RT-PCR was used to quantify cytokine message expression in colons of reconstituted mice. Data are expressed as fold increases in cytokine mRNA levels in tissues of experimental animals (those that received CD4⁺CD25⁺ T cells) over those determined in healthy WT colons and were normalized to GAPDH expression as described in MATERIALS AND METHODS (n = 6–14 mice in each group). *Significantly different (P < 0.05) from WT→RAG KO mice.
importance of determining how different adhesion molecules impact disease pathogenesis. Another interesting observation is that the disease severity in PSGL-1 KO→RAG KO mice appeared to be greater than that in the WT→RAG KO group, although this difference did not reach statistical significance. This finding suggests that PSGL-1 may, in addition to T cell trafficking, play an inhibitory role in T cell activation and/or proliferation, such that lack of a PSGL-1-mediated signal may contribute to their increased pathogenic potential in vivo. However, little information regarding this potentially important role of PSGL-1 is available, with only one study (7) showing that ligation of this molecule by a MAb triggers the death of Th1 effector T cells.

Another unexpected observation made in the present study was that CD18 KO T cells express substantially more ICAM-1 than do WT T cells (Fig. 4). Again, we know of no published data demonstrating that ICAM-1 is upregulated on CD18 KO T cells. The role that T cell-associated ICAM-1 plays in T cell trafficking is unknown at the present time. Indeed, a wealth of data demonstrating that ICAM-1 is upregulated on CD18 KO lymphoid tissue and homing of effector T cells to inflamed tissue via its interaction with leukocyte LFA-1 (CD11a/CD18) and/or Mac-1 (CD11b/CD18). However, it has been demonstrated that ICAM-1 is expressed on activated T cells (4, 9). One report (8) did show that ICAM-1 KO virus-specific effector T cells were significantly impaired in their ability to migrate to sites of inflammation. However, no studies have investigated the role that T cell-associated ICAM-1 plays in the induction and/or propagation of acute or chronic intestinal inflammation. In the present study, we found that adoptive transfer of T cells obtained from ICAM-1-deficient mice induced a more variable disease with approximately half of the reconstituted mice developing moderate to severe colitis, whereas the other 50% expressed mild or no disease (Fig. 3). Overall, however, the blinded histopathological scores were not significantly different than those obtained for WT→RAG KO mice.

In summary, our data define, for the first time, the roles that T cell-associated CD18, CD62L, ICAM-1, and PSGL-1 play in initiating and/or perpetuating chronic colitis in mice. We conclude that CD18, but not CD62L, ICAM-1, or PSGL-1, is important in disease pathogenesis and represent potentially important therapeutic targets for treating chronic intestinal inflammation.

GRANTS

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REFERENCES


