Published in final edited form as:
Gastroenterology. 2012 December ; 143(6): 1544–1554.e7. doi:10.1053/j.gastro.2012.08.042.

Signaling Lymphocyte Activation Molecule Regulates Development of Colitis in Mice

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Abstract

BACKGROUND & AIMS—Signaling lymphocyte activation molecule (Slamf)1 is a co-stimulatory receptor on T cells and regulates cytokine production by macrophages and dendritic cells. Slamf1 regulates microbicidal mechanisms in macrophages, therefore we investigated whether the receptor affects development of colitis in mice.

METHODS—We transferred CD45RB^{hi} CD4^{+} T cells into *Rag*$^{-/-}$ or *Slamf1*$^{-/-}$ *Rag*$^{-/-}$ mice to induce colitis. We also induced colitis by injecting mice with an antibody that activates CD40. We determined the severity of enterocolitis based on disease activity index, histology scores, and levels of cytokine production, and assessed the effects of antibodies against Slamf1 on colitis induction. We quantified migration of monocytes and macrophage to inflamed tissues upon induction of colitis or thioglycollate-induced peritonitis and in response to tumor necrosis factor-α in an air-pouch model of leukocyte migration.

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Conflicts of interest The authors disclose no conflicts.

Supplementary Material Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2012.08.042.

RESULTS—Colitis was reduced in *Slamf1*$^{-/-}$ *Rag*$^{-/-}$ mice, compared with *Rag*$^{-/-}$ mice, after transfer of CD45RB^{hi} CD4^{+} T cells or administration of the CD40 agonist. The numbers of monocytes and macrophages were reduced in inflamed tissues of *Slamf1*$^{-/-}$ *Rag*$^{-/-}$ mice, compared with *Rag*$^{-/-}$ mice, after induction of colitis and other inflammatory disorders. An antibody that inhibited Slamf1 reduced the level of enterocolitis in *Rag*$^{-/-}$ mice.

CONCLUSIONS—Slamf1 contributes to the development of colitis in mice. It appears to indirectly regulate the appearance of monocytes and macrophages in inflamed intestinal tissues. Antibodies that inhibit Slamf1 reduce colitis in mice, so human SLAMF1 might be a therapeutic target for inflammatory bowel disease.

Keywords

Immune Regulation; Mouse Model; TNF; Inflammatory Bowel Disease

Signaling lymphocyte activation molecule family (SLAMF) receptors play a role in adaptive as well as in innate immune responses and human SLAMF1 (CD150) and mouse Slamf1 serve several distinct roles in macrophages.[1] A variety of CD150 functions are well characterized in adaptive immune processes, including signaling in the immune synapse of T cells, cytokine production, and natural killer–T-cell development.[2] Although we are starting to comprehend the functions of Slamf1 on innate cells, little is known about the *in vivo* implications of Slamf1 on macrophages, monocytes, and dendritic cells. Because Slamf1 positively regulates microbicidal mechanisms directed at some bacteria in macrophages, we evaluated whether Slamf1 would affect disease in enterocolitis models, which are reminiscent of human inflammatory bowel diseases (ie, ulcerative colitis and Crohn's disease).[3]

Although both innate and adaptive immunity are involved in managing the commensal bacteria in the lumen of the colon, the adaptive immune system responds most aggressively to luminal antigens or bacterially induced host antigens by CD4^{+} T helper (Th)1, Th2, or Th17 cell expansion. For instance, colitis is induced when CD4^{+} T cells are not counterbalanced by immune-suppressive mechanisms.[4–7] However, mice without an adaptive immune system (eg, *Rag*$^{-/-}$ mice), are perfectly capable of coping with the intestinal bacteria through their innate immune defenses. Key players in this defense are macrophages, which show remarkable functional plasticity in response to environmental cues. Under steady-state conditions, in the subendothelial lamina propria, Ly6C^{hi} monocytes can differentiate into tolerogenic F4/80^{hi}CX3CR1^{hi}CD11b^{+} macrophages, which eradicate commensal microbes without eliciting an immune response. By contrast, at the onset of colitis, Ly6C^{hi} monocytes are thought to infiltrate into the colon, where they differentiate into F4/80^{+}CX3CR1^{int}CD11b^{+} inflammatory phagocytes, and produce tumor necrosis factor-α (TNFα) and inducible nitric oxide synthase.[8,9]

Because Slamf1 is expressed on both lymphoid and myeloid cells, we assessed the role of Slamf1 in murine chronic enterocolitis using the transfer of wild-type (*wt*) and *Slamf1*$^{-/-}$ naive and memory CD4^{+} T cells into *Rag*$^{-/-}$ or *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice to induce experimental colitis.[7] To focus on the role of Slamf1 on myeloid cells, we induced disease in *Rag*$^{-/-}$ or *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice with an agonistic αCD40 monoclonal antibody, as described by Uhlig et al.[7] We found that only the absence of Slamf1 in the recipient mice mitigated disease. This appears to be independent of the Slam family–specific adaptors EWS/FLI1 activated transcript 2 (Eat-2)a and Eat-2b. Because the homeostasis of monocytes/monocyte-derived macrophages in the colitic lamina propria is affected by the absence of Slamf1, the receptor may regulate macrophage infiltration into, or retention in, inflamed tissues. Because monoclonal αSlamf1 antibodies also ameliorate colitis in both models, we conclude that Slamf1 partakes in the pathogenesis of experimental enterocolitis.

Materials and Methods

Mice

Slamf1$^{-/-}$ *BALB/c* or *C57BL/6* FITC mice were used to generate *Slamf1*$^{-/-}$*Rag-1*$^{-/-}$ *BALB/c* or *Slamf1*$^{-/-}$ *Rag-2*$^{-/-}$ *C57BL/6* mice. *Rag-1*$^{-/-}$ and *Rag-2*$^{-/-}$ mice were from Jackson Labs (Bar Harbor, ME) and Taconic (Hudson, NY), respectively.[10] Similarly, the previously described *Eat-2a/b*$^{-/-}$ *C57BL/6* mice[11] were crossed with *Rag-2*$^{-/-}$ *C57BL/6*. All mice were kept under specific pathogen–free conditions at the Animal Research Facility with approval by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee.

Antibodies

CD11b–FITC, Ly6C-PerCP-Cy5, F4/80-PE, CD11c–APC, Ly6G-PE, and CD115-biotin were from Biolegend (San Diego, CA). TLR2-PE, F4/80-PacificBlue, CD86-PacificBlue, and I-A^{b} (MHC-II)-APC were from eBioscience (San Jose, CA).

In vivo *Procedures*

Adoptive transfer of CD45RB^{hi}CD4^{+} and CD45RB^{low}CD4^{+} CD25^{+} T cells— Adoptive transfer into *Rag*$^{-/-}$ or *Slamf1*$^{-/-}$*Rag*$^{-/-}$ recipients was described previously.[12] The Disease Activity Index (DAI) and histology scores were determined as previously described.[13]

Induction of colitis by agonistic αCD40—Mice were injected intraperitoneally with 200 μg of rat anti-mouse-CD40 (FGK45, IgG2a, generously donated by Professor Ton Rolink, Basel, Switzerland) or with rat IgG2a (BioXcell, West Lebanon, New Hampshire).[7]

Antibody treatment—A total of 250 μg per mouse of αSlamf1 (9D1 or 12F12 clones) or rat IgG isotypes (IgG1 and IgG2a, respectively) (BioXcell) was injected twice weekly in the CD45RB^{hi}CD4^{+} transfer model. In the αCD40-induced colitis model, 1 mg of αSlamf1 (9D1 or 12F12) and their respective isotype (rat IgG1 or rat IgG2a) were administered on days 0 and 1.

***In vivo* migration experiments—**Peritonitis was induced by one intraperitoneal injection with 2 mL of 4% thioglycollate broth, and 24 or 72 hours later the mice were sacrificed. Cells were harvested with a peritoneal lavage, as described.[14] Recipient mice were anesthetized by isoflurane inhalation and subcutaneously injected into the lower back with an air bubble on days 0 and 3 and with 500 ng TNFα in 200 μL phosphate-buffered saline on day 7. At 4 hours after injection with TNFα, exudate cells were analyzed by fluorescence-activated cell sorter (FACS).[15]

Transwell Migration Assay

In vitro transwell (5-μm^2 pores) migration analysis was performed using a 48-well migration chamber (NeuroProbe, Gaithersburg MD). The lower wells contained monocyte-chemoattractant protein (MCP)-1 (20 ng/mL), TNF-α (50 ng/mL), or macrophage inflammatory molecule (MIP-1α) (50 ng/mL). A 1:1 mixture of Cell Tracker red CMPTX-stained *wt* and carboxy-fluorescein diacetate, succinimidyl ester (CFSE)-stained (Life Technologies, Grand Island, NY) *Slamf1*$^{-/-}$ cells, and vice versa, was added to the upper well to migrate for 70 minutes in a humidified chamber (5% CO_2, 37°C).

Cell Preparation, and Cytokine and Chemokine Analyses

Cells from the lamina propria were obtained as described.[16] Interferon-γ and TNFα were detected in supernatants of 100 mg colonic tissue cultures (36 hours) by enzyme-linked immunosorbent assay (BD PharMingen, San Diego, CA). Chemokine analyses were performed using supernatant or serum by a mouse chemokine Flowcytomix kit (eBioscience, Vienna, Austria).

Flow Cytometry

All samples for flow cytometric analysis were washed with FACS buffer (phosphate-buffered saline, 2% fetal bovine serum), and Fc-receptors were blocked with anti-CD16/32 antibody at 4°C for 20 minutes. Cells were stained using directly conjugated antibodies on ice. Cells were analyzed on a BD LSRII using the FlowJo analysis package (Trees Star, Inc, Ashland, OR).

Immunohistochemistry

Fresh tissue samples from the colon were frozen in optimal cutting temperature compound (Ames Company, Elkhart, IN). Frozen tissue sections (4-μm thick) were stained by the avidinbiotin complex method as previously described.[17] Sixty fields were counted per condition.

Statistical Analysis

Parametric data are shown as the mean ± standard deviation. Nonparametric data were analyzed using the Mann–Whitney test, as described by Liao et al.[16] The median ± standard error of the mean was determined. The statistical analyses were performed with Prism 5 software (GraphPad, San Diego, CA).

Results

The Presence of the Cell Surface Receptor Slamf1 on Nonlymphoid Cells Is Requisite for the Development of Chronic Enterocolitis

To study the role of Slamf1 in chronic enterocolitis, *Slamf1*$^{-/-}$ or *wt* disease-inducing CD45RB^{hi} CD4^{+} T cells were transferred into either *Rag*$^{-/-}$ or *Slamf1*$^{-/-}$ *Rag*$^{-/-}$ mice (Figure 1*A*). Surprisingly, upon transfer of *wt* CD45RB^{hi}CD4^{+} T cells into *Slamf1*$^{-/-}$*Rag*$^{-/-}$ recipients, mice failed to develop colitis as judged by the DAI, histology score, and interferon-γ production by CD4^{+} T cells from the mesenteric lymph nodes, suggesting a role for Slamf1 in the pathogenesis of the disease (Figure 1*B–D*).

Slamf1 is expressed on the surface of activated APCs as well as on the surface of memory and recently activated CD4^{+} T cells.[2,10] We therefore evaluated whether the homophilic interactions between Slamf1 on the surface of the donor CD45RB^{hi}CD4^{+} T cells and the APCs in the *Slamf1*$^{-/-}$*Rag*$^{-/-}$ recipients might play a role in ameliorating colitis. As shown in Figure 1*E*, *Slamf1*$^{-/-}$ and *wt* CD45RB^{hi}CD4^{+} T cells induced disease with the same efficiency in *Rag*$^{-/-}$ recipient mice. Thus, the absence of Slamf1 from the surface of CD45RB^{hi}CD4^{+} T cells did not impact the severity of colitis. Furthermore, when *Rag*$^{-/-}$ mice are reconstituted with both *wt* CD45RB^{hi}CD4^{+} and *Slamf1*$^{-/-}$ CD45RB^{lo}CD25^{+}CD4^{+} regulatory T cells (Tregs), no colitis developed (Figure 1*F*). Thus, the homophilic interaction between Slamf1 on the surface of Treg cells and that on the surface of CD45RB^{hi}CD4^{+} T cells or APCs is not critical for maintaining their suppressive capability.

Consequently, the outcomes of these experiments indicate that the presence of Slamf1 on the surface of nonlymphoid cells, rather than on $CD4^+$ T cells, is required for the onset of the pathogenesis of chronic enterocolitis.

Anti—CD40-Induced Colitis Is Ameliorated in Slamf1$^{-/-}$Rag$^{-/-}$, But Not in Eat-2a/b$^{-/-}$Rag$^{-/-}$ Mice

To directly determine that expression of Slamf1 on the surface of innate immune cells (eg, dendritic cells and monocyte/macrophages), is important in the pathogenesis of experimental colitis, we used an agonistic αCD40 antibody (FGK45) to induce disease in *Rag*$^{-/-}$ mice. In the absence of T and B lymphocytes, agonistic αCD40 primarily activates myeloid CD40-expressing cells by mimicking the CD40 ligand, which is induced on the surface of activated $CD4^+$ T cells. When *Rag*$^{-/-}$ mice are administered αCD40, they develop acute colitis that is driven primarily by macrophages and dendritic cells within 1 week.[7] Reminiscent of the result after $CD45RB^{hi}CD4^+$ T-cell induction, *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice developed considerably milder colitis compared with the *Rag*$^{-/-}$ mice as judged by DAI, histology score, weight loss (Figure 2*A* and *B*, Supplementary Figure 1), and splenomegaly (data not shown). This was not caused by altered CD40 expression on Slamf1-deficient macrophages.[2] Immunohistochemistry staining of the colon tissues with monoclonal antibodies directed against the macrophage markers F4/80 and CD11b indicated a reduced infiltration of $CD11b^+F4/80^+$ macrophages in *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice than in *Rag*$^{-/-}$ mice after αCD40 induction. The numbers of $CD11c^+$ cells were comparable between these mice (Figure 2*C* and *D*).

Signal transduction by engagement of 6 of the 9 SLAM family receptors in a variety of hematopoietic cells is modulated by the specific adaptors SAP (SH2D1A) and/or EAT-2 (SH2D1B).[1] Because both Eat-2a and Eat-2b regu-late Slamf1-initiated signal transduction and they are found in myeloid cells, we reasoned that their absence might affect signaling of Slamf1 in colitis-driving phagocytes and hence the development of colitis. To test this, we generated triple-knockout *Eat-2a/b*$^{-/-}$*Rag*$^{-/-}$mice, which responded similarly to the αCD40 antibody as judged by DAI, histology score, and end weight (Figure 2*E* and *F*, Supplementary Figure 2). This result indicates that the absence of Slamf1 from the surface of myeloid cells ameliorates colitis, but that this process does not involve signaling through the adaptor proteins Eat-2a/b.

Reduced Numbers of Infiltrating $CD11b^+F4/80^+$ Macrophages in ColiticSlamf1$^{-/-}$Rag$^{-/-}$ Mice

To correlate the reduced number of $CD11b^+F4/80^+$ cells with the ameliorated colitis in *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice in more detail, we isolated lamina propria cells from *Rag*$^{-/-}$ mice in which colitis had been induced. Cytofluorometric analyses using multiple macrophage markers confirmed that after the transfer of $CD45RB^{hi}CD4^+$ T cells (Figure 3*A*) or after administering agonistic αCD40 (Figure 3*B*), the percentage of $CD11b^+F4/80^+$ macrophages in the lamina propria of the *Slamf1*$^{-/-}$*Rag*$^{-/-}$ recipients was reduced significantly as compared with *Rag*$^{-/-}$ recipients. Most $CD11b^+$ cells isolated from the inflamed colonic lamina propria express Ly6C on their surface, suggesting they are of monocytic origin. The expression of F4/80, TLR2, MHCII, and CD86 on the surface of these cells indicates a proinflammatory M1 macrophage phenotype[18,19] (Figure 3*C*). The expression levels of these functionally relevant molecules are comparable between *wt* and *Slamf1*$^{-/-}$ cells (Figure 3*C*), indicating that *Slamf1* deficiency does not affect the development of this cell population, which confirmed our previous report.[2] However, the absolute number of the $CD11b^+F4/80^+$ macrophages isolated from the lamina propria of *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice was reduced significantly as compared with that from the *Rag*$^{-/-}$ mice after αCD40 induction (Figure 3*D*–*F*). These $CD11b^+F4/80^+$ macrophages that infiltrated the colonic lamina

propria after αCD40 induction express *Slamf1* as judged by reverse-transcription polymerase chain reaction as well as flow cytometry (Supplementary Figure 3). To exclude the possibility that a Slamf1 polymorphism might affect APC-mediated colitis,[1] we also induced αCD40 colitis in *BALB/c Slamf1*$^{-/-}$*Rag-1*$^{-/-}$ mice. This is possibly ruled out because αCD40 antibody also induced milder colitis in *Slamf1*$^{-/-}$*Rag-1*$^{-/-}$ *BALB/c* mice as compared with the *Rag-1*$^{-/-}$ *BALB/c* mice (Figure 3*B*, Supplementary Figure 4).

It was possible that the reduced macrophage levels in the colon of *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice might be caused by an increased apoptosis as a result of the absence of Slamf1. To test this, the colon tissues of αCD40-treated *Rag*$^{-/-}$ and *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice were co-stained with αCD11b-APC and terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling (TUNEL). As shown in Supplementary Figure 5, the percentage of TUNEL-positive, CD11b^{+} macrophages is comparable between *Rag*$^{-/-}$ and *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice after αCD40 induction. Therefore, a reduced number of macrophages in the colon of αCD40-induced *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice is not likely the result of increased apoptosis of macrophages lacking Slamf1.

These outcomes show that Slamf1 governs functions of macrophages that are responsible for the development of enterocolitis. We find fewer M1 macrophages in the inflamed colon of *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice. Because it is well accepted that inflammatory M1 macrophages play a role in colitis,[8,9,20,21] it is likely that the reduced numbers of these macrophages in the *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice during inflammation is causative of ameliorated colitis.

MCP-1 and MCP-3 Serum Levels Are Reduced Upon Induction of Colitis by αCD40 in Slamf1$^{-/-}$ Rag$^{-/-}$ Mice as Compared With Rag$^{-/-}$ Mice

It is well known that chemoattractant molecules play an important role in regulating migration of monocytes to the sites inflammation. To further assess whether the reduced macrophage infiltration in the absence of Slamf1 is caused by an impaired migration of monocytes, we evaluated MCP-1 (Chemokine (C-C motif) ligand 2 (CCL2)) and MCP-3 (CCL-7), which both bind to Chemokine (C-C motif) receptor (CCR)2, a receptor that is expressed on Ly6C^{hi} monocytes,[22] RANTES (CCL-5), MIP-1α, and MIP-1β in the serum of *Rag*$^{-/-}$ and *Slamf1*$^{-/-}$ *Rag*$^{-/-}$ mice in which colitis had been induced by agonistic αCD40.

We find that the serum levels of MCP-1 and MCP-3, but not the levels of RANTES, MIP-1α, and MIP-1β, are significantly lower in *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice compared with *Rag*$^{-/-}$ mice at day 7 after induction of colitis by αCD40 (Figure 4*A*), whereas no difference was detected after isotype treatment (Figure 4*B*). Because these chemokines are markedly up-regulated in inflammation, this finding confirms the notion that inflammation is more severe in *Rag*$^{-/-}$ mice than in *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice. Whether this lower production of chemokines is caused by the Slamf1 mutation is unknown because these chemokines are secreted by a variety of cell types.[22] Furthermore, MCP-1 or MCP-3 were secreted equally well by colon cultures of *Rag*$^{-/-}$ and *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice after treatment with αCD40 (Figure 4*C* and *D*). Thus, the increased levels of chemokines in the serum of colitic *Rag*$^{-/-}$ mice compared with *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice might be caused indirectly by the Slamf1 mutation.

We next used an *in vitro* approach to assess whether migration of monocytes in response to relevant chemokines is impaired as a result of Slamf1 deficiency. To this end we used CD115^{+}Ly6C^{+} monocytes isolated from bone marrow and allowed them to migrate in response to MCP-1, MIP-1α, or TNF-α across the membrane of a transwell chamber.[23] Both *wt* and *Slamf1*$^{-/-}$ monocytes migrated equally well in response to the two chemokines and to TNF-α (Figure 4*E*). These data indicate that our *in vivo* observations most likely

arose through a Slamf1-dependent mechanism that is not dictated directly by CCR1, CCR2, or CCR5 signaling.

Altered Homeostasis of Slamf1$^{-/-}$ Macrophages During Peritonitis and Subcutaneous Inflammation

The altered homeostasis of intestinal macrophages suggested the possibility of an impaired migration of monocytes to the site of inflammation. Because the impaired migration of *Slamf1*$^{-/-}$ monocytes is not mimicked by our *in vitro* transwell migration assay, we then tested this possibility in two in vivo models. First, we induced peritonitis in *wt* and *Slamf1*-deficient mice by administering thioglycollate[14] and characterized the infiltrating cells (Supplementary Figure 6*A*). Seventy-two hours after injection of thioglycollate, we found that fewer CD11b^{+}F4/80^{+} macrophages were present in the peritoneal cavity of *Slamf1*$^{-/-}$ mice as compared with *wt* mice (Figure 5*A*). In contrast, the number of CD11b^{+}Ly6G^{+} neutrophils, which peaked at 24 hours after injection as expected,[24] did not differ between *wt* and mutant mice at either time point, as judged by flow cytometry (Figure 5*B*) and myeloperoxidase (MPO) production (Supplementary Figure 6*B*). The latter also indicated that no major phenotypic differences existed between *wt* and *Slamf1*$^{-/-}$ neutrophils.

Although many factors determine recruitment of macrophages to sites of inflammation, TNFα is a major component, which is produced by lymphoid and nonlymphoid cells in both murine enterocolitis and Crohn's disease.[4] To test the hypothesis that monocytes might migrate to a TNFα—primed environment, we used a second *in vivo* assay using cytofluorometric analysis. Recruitment of leukocytes to a subcutaneous air pouch in which TNFα has been injected was assessed.[15] The number of macrophages recruited by TNFα was reduced in *Slamf1*$^{-/-}$ mice compared with *wt* animals, yet the number of neutrophils remained constant (Figure 5*C* and *D*). Together with the colitis experiments, the peritonitis and *in vivo* migration assays indicated that *Slamf1*$^{-/-}$ monocytes may have a general diminished ability to migrate to sites of inflammation.

Administration of Anti-Slamf1 Monoclonal Antibody Ameliorates Colitis

To investigate whether blocking the Slamf1/ Slamf1 homophilic interaction would ameliorate the development of colitis, we used the transfer of *wt* CD45RB^{hi}CD4^{+} T cells into *Rag*$^{-/-}$ mice in the presence of an IgG1 preparation of αSlamf1 (9D1) (Figure 6*A*). As judged by DAI (Figure 6*B*), histology scores (Figure 6*C* and *D*), αSlamf1-antibody treatment ameliorated CD45RB^{hi}CD4^{+} T-cell–induced colitis as compared with an isotype control (rat IgG1).

Next, 2 monoclonal antibodies directed against Slamf1 (ie, 9D1 and 12F12) were tested in αCD40-induced colitis in *Rag*$^{-/-}$ mice (Figure 7*A*). Injection of each of the αSlamf1 antibodies mitigated disease as evidenced by histology (Figure 7*B* and *C*), DAI (Figure 7*D*), and TNF-α serum levels (Figure 7*F*). Flow cytometry analyses of the lamina propria cells isolated from the *Rag*$^{-/-}$ mice treated with αSlamf1 or its isotype showed that αSlamf1 treatment reduces the number of CD11b^{+}Ly6C^{+} inflammatory macrophages in the lamina propria (Figure 7*E*). Similar to the experiments with *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice (Figure 5), MCP-1 levels were reduced significantly in the serum of αSlamf1-treated mice (Figure 7*E*). The studies with the monoclonal antibodies directed against mouse Slamf1 show not only that treatment mitigates colitis, but they also support the notion that Slamf1 influences the homeostasis of macrophages in the lamina propria of the colon.

Discussion

The co-stimulatory molecule Slamf1a homophilic adhesion molecule, is known to regulate several functions in T cells, macrophages, and dendritic cells.[1] To assess the role of Slamf1 in regulating immune responses of T-cell subsets *in vivo*, we adopted a well-known colitis model, namely the transfer of *wt* or *Slamf1*$^{-/-}$ disease inducing CD45RB^{hi}CD4^{+} T cells or memory CD4^{+} T cells into *Rag*$^{-/-}$ mice. Upon an evaluation of the ensuing chronic enterocolitis, we found that the homophilic interaction between Slamf1 on CD45RB^{hi}CD4^{+} T cells with Slamf1 on APCs is not required for inducing colitis. In addition, the interactions between Slamf1 adhesion molecule on the surface of Tregs and effector T-cells or Tregs and APCs are not required for maintaining the suppressive function of the CD25^{+}CD4^{+} Tregs.

Surprisingly, when *wt* CD45RB^{hi}CD4^{+} T cells were transferred into *Slamf1*$^{-/-}$*Rag*$^{-/-}$ recipients, colitis was ameliorated. Consistent with the milder disease, the number of monocyte-derived macrophages in the colon of *Slamf1*$^{-/-}$*Rag*$^{-/-}$ recipients was reduced as compared with *Rag*$^{-/-}$ recipients. This was not caused by a lack of the ability of naive CD4^{+} T cells to skew toward a pathogenic Th1 response by the *Slamf1*-deficient APCs[10] because this phenotype was recapitulated by the induction with an agonistic anti-CD40 antibody. Furthermore, because the αCD40-induced colitis was not dependent on the presence of the Slam family–specific adaptors Eat-2a and Eat-2b and as neutralizing antibodies to Slamf1-mitigated disease, the Slamf1/Slamf1 interactions on the cell surface of macrophages/monocytes are likely to play a role in the development of colitis.

Perhaps more importantly, the outcomes of these studies support the interpretation that Slamf1 regulates the number of macrophages in the inflamed colon. A characteristic of an early inflammatory landscape in the colon is an increased recruitment of monocytes, which are skewed to become inflammatory mononuclear phagocytes instead of hyporesponsive resident macrophages. This monocyte population is defined as CD11b^{+}Ly6C^{hi}CX3CR1^{+} and differentiates to CD11b^{+}F4/80^{+}CX3CR1^{int} phagocytes *in situ*, perpetuating a preliminary response and driving inflammation through secretion of TNFα and a number of other effector mechanisms.[9,25,26] We find reduced numbers of inflammatory (M1) macrophages in the colon of the *Slamf1*$^{-/-}$ *Rag*$^{-/-}$ mice, which received either CD45RB^{hi}CD4^{+} T cells or αCD40. Furthermore, administering monoclonal antibodies directed against Slamf1 ameliorates colitis in both models. These results were unexpected because Slamf1 plays a role in phagosomes of macrophages in the killing of several gram-negative bacteria (ie, *Escherichia coli* or attenuated *Salmonella typhimurium*).[2] Although it would be expected that an impaired clearance of a subset of commensal colonic gram-negative bacteria by *Slamf1*$^{-/-}$ macrophages exacerbates colitis, the opposite is the case. Furthermore, although in the absence of Slamf1 the phagosomal Nox2 (p40phox) activity is reduced in response to *E. coli*, a recent publication describes increased colitis in the absence of p40phox.[27]

The possible explanations for how Slamf1 might regulate the homeostasis of macrophages during chronic or acute enterocolitis can be grouped as follows: (1) Slamf1 controls production proliferation, and/or survival of CD11b^{+} macrophages, or (2) Slamf1 is requisite for migration to, or retention in, the inflamed tissues.

It is unlikely that the reduced number of macrophages in the lamina propria of the colitic mice would be caused by a defective bone marrow production of the precursors of CD11b^{+} macrophages.[28] Although Slamf1 is one of the markers on the surface of hematopoietic stem cells, the numbers of myeloid cells in Slamf1-deficient mice were not altered.[10,28] The possibility that the Slamf1 deficiency may change the survival of macrophages in the gut is not supported by our TUNEL-staining experiments. In addition, there is no indication that

the proliferation of *Slamf1*$^{-/-}$ macrophages is impaired when *Slamf1*$^{-/-}$ bone marrow was used to generate macrophages and dendritic cells *in vitro* under stimulation with macrophage colonystimulating factor or granulocyte-macrophage colonystimulating factor and interleukin-4 (unpublished observations).

The notion of a reduced migration of Slamf1-deficient monocytes into inflammatory sites is suggested by the outcomes of two *in vivo* migration experiments. The number of CD11b^{+} F4/80^{+} cells that were recovered from both the inflamed peritoneum as well as from the subcutaneous cavity in the air pouch experiments was reduced in the *Slamf1*$^{-/-}$ mice. Furthermore, we do find reduced levels of key factors that are involved in monocyte migration, namely MCP-1 and MCP-3 in the serum of *Slamf1*$^{-/-}$ mice. Although the latter result confirms that the state of inflammation in *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice is less than in *Rag*$^{-/-}$ mice upon induction of colitis, the result only indirectly supports the notion of an impaired migration in the absence of Slamf1.

A number of experiments were conducted to explain how the absence of a homophilic adhesion molecule, Slamf1, could be involved directly in the proper infiltration into an inflamed colon. First, the results of our *in vitro* transwell experiments did not support the concept that in the absence of the self-ligand adhesion molecule Slamf1 monocytes migrate less well. The intrinsic propensity of Slamf1-deficient monocytes to respond to several inflammatory chemokines is unaltered. It is unlikely that a difference in migration could be established in an assay using monocyte migration across the endothelial lining because Slamf1 is not expressed on the surface of endothelial cells.[1] Second, bone marrow–derived CD115^{+} monocytes were transferred into *Rag*$^{-/-}$ mice after induction of colitis by either αCD40 or by the transfer of CD45RB^{hi} CD4^{+} T cells. Although a modest number of bone marrow–derived *Slamf1*$^{-/-}$ monocytes repopulated the splenic Ly6C^{+} monocyte reservoir with the same efficacy as *wt* monocytes, no donor cells were found in the lamina propria (data not shown).

Taken together, the presence of the self-ligand adhesion molecule Slamf1 is instrumental in the development of enterocolitis in the mouse. Interestingly, because αSlamf1 mitigates the pathogenesis of colitis, monoclonal antibodies directed against human SLAMF1 could represent a potential therapeutic target for treating inflammatory bowel diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Boaz van Driel, Gongxian Liao, and Xavier Romero contributed equally to this article.

Funding Supported by grants from the National Institutes of Health (AI-15066 and DK-52510 to C.T.; DK-80070 to E.M.; DO-136028 to F.W.L.; DR-068181, AI-093588, and DR-033506 to H.C.R.) and the Center for the Study of IBD (DK-43351). Xavier Romero and Scott Berger were supported by fellowships from the Crohn's and Colitis Foundation of America, and Veronica Azcutia was supported by an American Heart Association Fellowship.

Abbreviations used in this paper

APC	antigen-presenting cell
CD	Crohn's disease
DAI	Disease Activity Index
EAT-2	EWS/FLI1 acti-vated transcript 2

MCP	monocyte-chemoattractant protein
MIP	macrophage inflammatory molecule
Slamf1	signaling lymphocyte activation molecule family 1 or CD150
Th	T helper
TNF	tumor necrosis factor
Treg	regulatory T cell
TUNEL	terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling
wt	wild type

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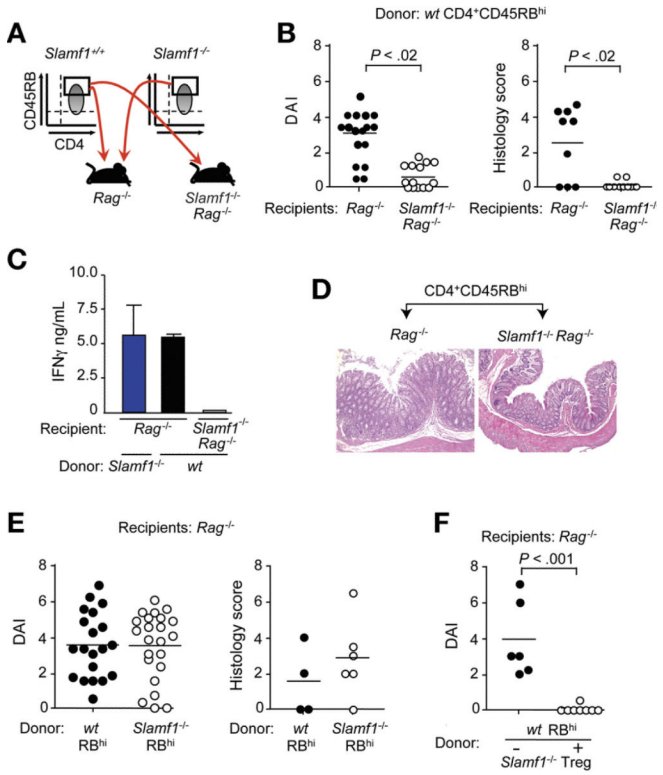

Figure 1.

Reduced chronic enterocolitis in the absence of Slamf1. (*A*) Outline of the CD45RB^{hi}CD4^{+} T-cell transfers into either *Slamf1*$^{-/-}$*Rag*$^{-/-}$ or *Rag*$^{-/-}$ mice.CD45RB^{hi}CD4^{+} T cells obtained from *wt* spleens by fluorescence-activated cell sorting were injected intraperitoneally into *Rag*$^{-/-}$ or *Slamf1*$^{-/-}$*Rag*$^{-/-}$ hosts (5×10^5 cells/mouse).[20] Alternatively, CD45RB^{hi} CD4^{+} T cells from *Slamf1*$^{-/-}$ donors were injected into *Rag*$^{-/-}$ hosts. Each *open circle* and *filled circle* represents 1 mouse. (*B*) DAI of 3 pooled experiments and histology scores. Mean and individual values of each group are indicated. (*C*) Interferon (IFN)γ production in mesenteric lymph node CD4^{+} T-cell culture. IFNγ secretion by CD4^{+} T cells from mesenteric lymph nodes of *wt* → *Rag* (n = 5), *Slamf1*$^{-/-}$→ *Rag*$^{-/-}$ (n = 5), and *wt* → *Slamf1*$^{-/-}$*Rag*$^{-/-}$ (n = 5) mice. Cells were activated with 10 μg/mL of plate-bound αCD3 for 36 hours. Supernatant was analyzed for IFNγ by standard enzyme-linked immunosorbent assay. (*D*) Representative histology of colon tissue from *wt* → *Rag*$^{-/-}$ and *wt* → *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice. Original magnification, 10×. (*E*) DAI and histology scores of *Rag*$^{-/-}$ mice injected with *wt* CD45RB^{hi}CD4^{+} or *Slamf1*$^{-/-}$ CD45RB^{hi}CD4^{+} T cells. Mean and individual values of each group are indicated. (*F*) DAI of *Rag*$^{-/-}$ hosts that received an injection with *wt* CD45RB^{hi}CD4^{+} T cells or a mix of *wt* CD45RB^{hi}CD4^{+} and *Slamf1*$^{-/-}$ CD45RB^{low}CD25^{+}CD4^{+} 'regulatory' T-cells. Means and independent values of each group are indicated. *P* values are shown.

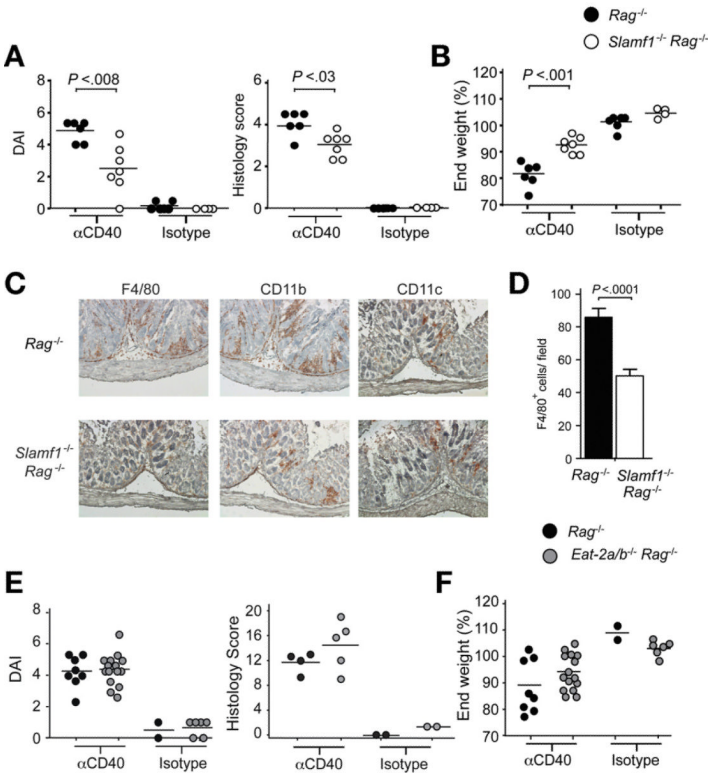

Figure 2.

Anti-CD40 antibody–induced colitis is reduced in *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice but not in *Eat-2a/b*$^{-/-}$*Rag*$^{-/-}$. Anti-CD40 was injected intraperitoneally into *Rag*$^{-/-}$ and *Slamf1*$^{-/-}$ *Rag*$^{-/-}$ mice or into *Rag*$^{-/-}$ and *Eat-2a/b*$^{-/-}$*Rag*$^{-/-}$ mice (200 μg) or with rat IgG2a control. Each *open circle* and *filled circle* represents 1 mouse. The data are representative of 3 separate experiments. (*A*) DAI and histology score. Mean and individual values of each group are indicated. (*B*) Weight loss at day seven as a percentage of the initial weight. (*C*) Representative immunohistochemistry colon sections prepared from *Rag*$^{-/-}$ and *Slamf1*$^{-/-}$*Rag*$^{-/-}$ mice at day seven after injection with αCD40. Colon samples were stained with antibodies directed against F4/80, CD11b, or CD11c, and counterstained with hematoxylin. Original magnification, 40×. (*D*) Graphic representation of the number of F4/80^{+} macrophages per 1-mm^{2} highpower field in sections depicted in panel *C*. Sixty fields were counted for each mouse. Statistical significance was determined by a 2-tailed Student *t* test. (*E*) DAI from 3 separate experiments and histology score. Each *grey circle* and *filled circle* represents 1 mouse. (*F*) Weight loss.

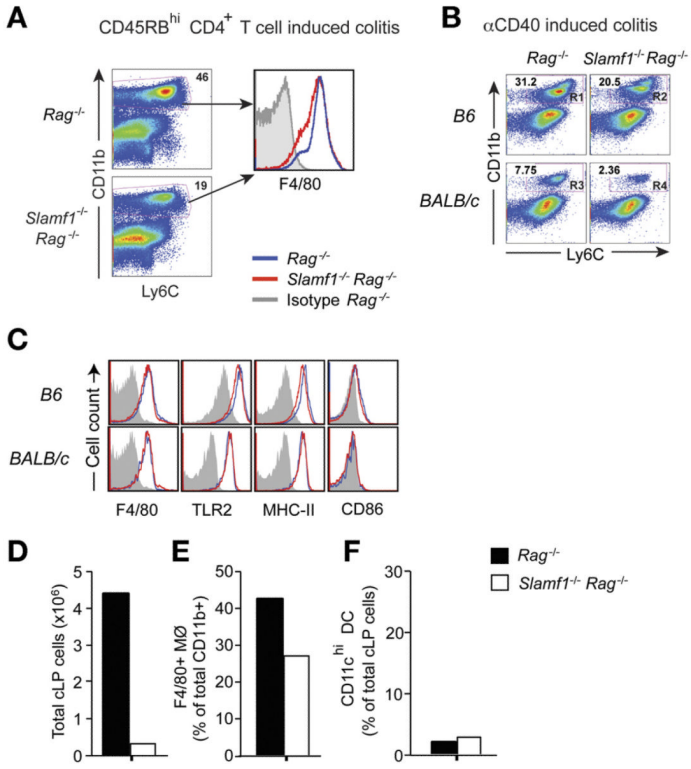

Figure 3.

Slamf1$^{-/-}$*Rag*$^{-/-}$ mice have decreased numbers of infiltrating inflammatory-type phagocytes, in the colonic lamina propria. (*A*) Flow cytometry analysis of isolated lamina propria cells of *Rag*$^{-/-}$ or *Slamf1*$^{-/-}$*Rag*$^{-/-}$ *C57BL/6* mice, in which colitis was induced by CD45RB^{hi}CD4^{+} T-cell transfer. The CD11b^{+} population is gated and the gate numbers represent the percentage of total isolated cells and depicted in a histology graph representing F4/80 expression. Each plot represents a pool of 5 mice. The figure is representative of 2 separate experiments. (*B*) Flow cytometry analysis of isolated lamina propria cells of *Rag*$^{-/-}$ or *Slamf1*$^{-/-}$*Rag*$^{-/-}$ *C57BL/6* or *BALB/c* mice, in which colitis was induced by injection of αCD40. The CD11b^{+} population is gated and the gate numbers represent the percentage of total isolated cells. Each plot represents a pool of 5 mice. The figure is representative of 3 separate experiments. (*C*) Flow cytometry analysis of inflammatory CD11b^{+} phagocytes, as represented in R1 and R2 of *C57BL/6* mice or R3 and R4 of *BALB/c* mice described in panel *B* were stained with monoclonal antibodies directed against F4/80, TLR2, MHC-II (I-A^{b}), and CD86. (*D*) *Bars* represent the total number of infiltrated cells after percoll isolation. The total cells were analyzed further by flow cytometric analysis. (*E*) CD11b^{+}F4/80^{+} phagocyte or (*F*) CD11c^{hi} dendritic cell percentages of the total pool of isolated cells. Each bar represents pooled samples obtained from 5 mice.

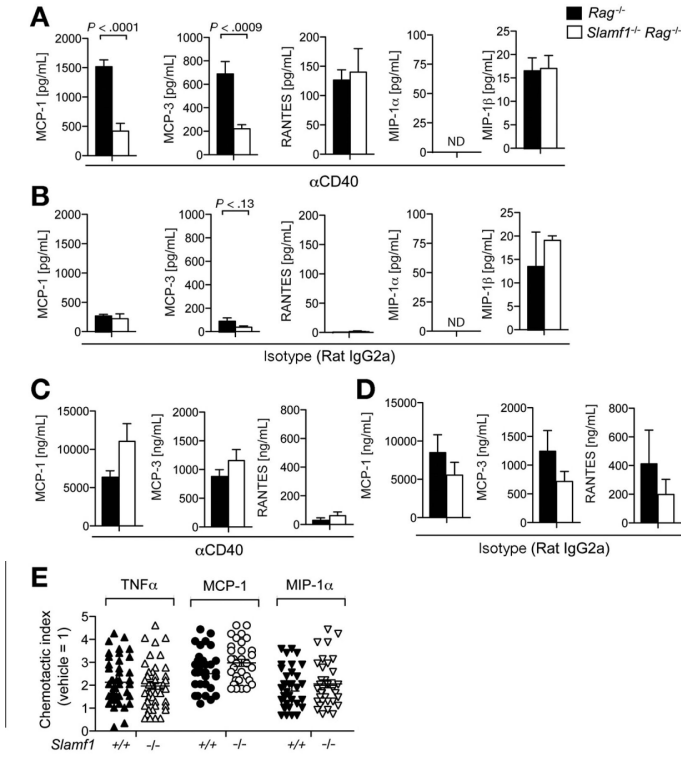

Figure 4.

MCP-1 and MCP-3 levels in the serum of *Slamf1*$^{-/-}$ *Rag*$^{-/-}$ mice are lower than in *Rag*$^{-/-}$ mice in which colitis had been induced by αCD40. (*A* and *B*) Serum and (*C* and *D*) colon culture supernatant were collected from *Slamf1*$^{-/-}$*Rag*$^{-/-}$ and *Rag*$^{-/-}$ mice (n = 8) that were injected either with (*A* and *C*) anti-CD40 or (*B* and *D*) isotype, seven days before harvest. *Bars* represent the amount of chemokines (ie, MCP-1, MCP-3, MIP-1α, MIP-1β, and regulated and normal T cell expressed and secreted (RANTES)) that was measured in the respective samples. Statistical significance was determined by the Student *t* test, mean ± standard error of the mean. Experiments were performed twice. (*E*) *In vitro* transwell migration analysis of bone marrow–derived CD115^{+} monocytes obtained from *wt* and *Slamf1*$^{-/-}$ mice. The lower wells contained MCP-1, TNF-α, or MIP-1α. A 1:1 mix of red (CMPTX) *wt* and green (CFSE) *Slamf1*$^{-/-}$ cells, and vise versa, was added to the upper well and left to migrate. Regulated and normal T cell expressed and secreted (RANTES).

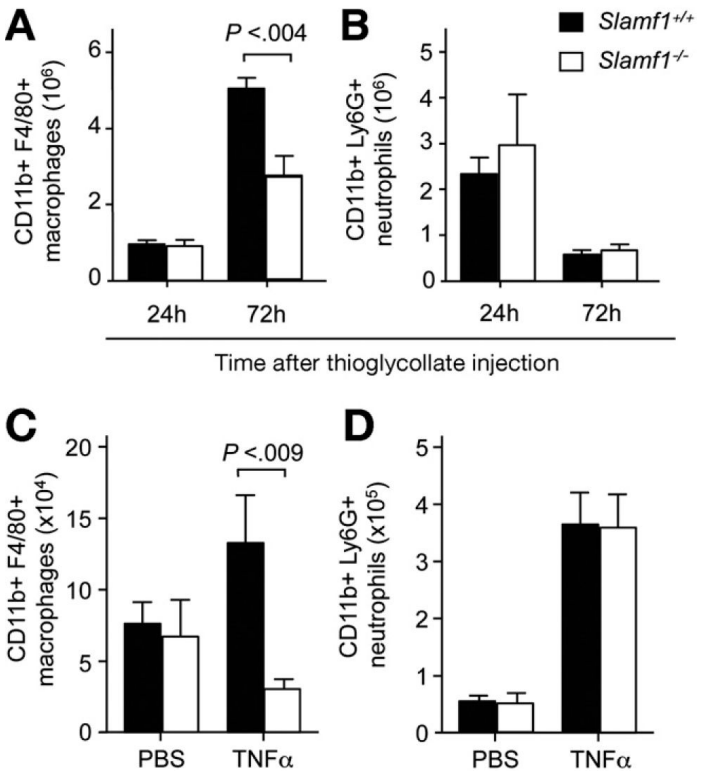

Figure 5.

Fewer *Slamf1*$^{-/-}$ macrophages accumulate at the site of inflammation than *Slamf1*$^{+/+}$ macrophages in two *in vivo* models. *Slamf1*$^{-/-}$ and *wt* mice (n = 5) were injected intraperitoneally with thioglycollate broth (2 mL, 4%). The number of macrophages and neutrophils in the peritoneal lavage at 24 and 72 hours after injection of thioglycollate were analyzed by FACS analysis. *Bars* represent the total number of (*A*) CD11b^{+}F4/80^{+} macrophages or (*B*) CD11b^{+}Ly6G^{+} neutrophils in the peritoneal lavage. *Slamf1*$^{-/-}$ and *wt* mice (n = 5) were injected subcutaneously twice with sterile air. A third injection consisted of TNFα (500 ng/mouse). Macrophages and neutrophils that migrated into the subcutaneous air pouch 4 hours after injection of TNFα were characterized by flow cytometric analysis and quantified. Bars represent the total number of (*C*) CD11b^{+} F4/80^{+} macrophages or (*D*) CD11b^{+}Ly6G^{+} neutrophils that infiltrated the air pouch. PBS, phosphate-buffered saline.

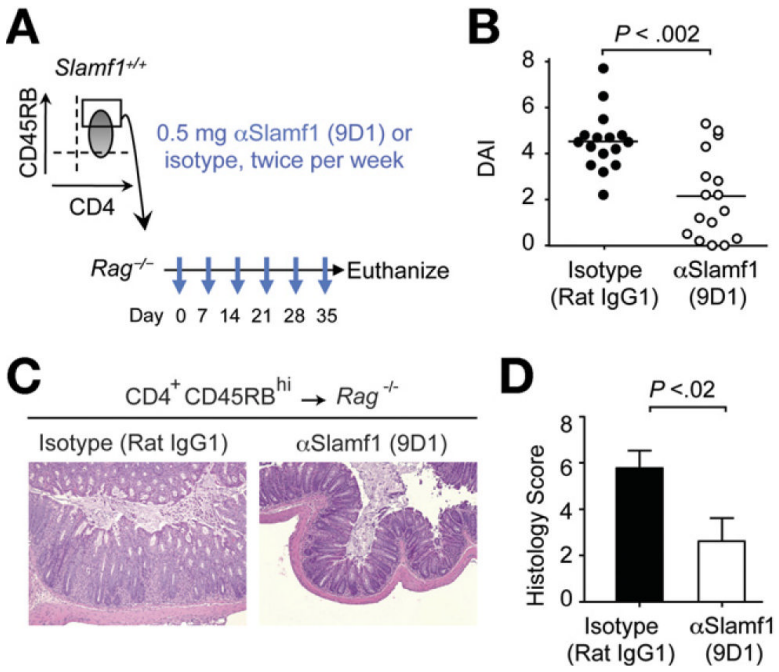

Figure 6.

Anti-Slamf1 antibody ameliorates chronic T-cell transfer colitis. (*A*) Outline of the αSlamf1 injection protocol. The $CD45RB^{hi}CD4^{+}$ T cells were transferred to $Rag^{-/-}$ recipients and intraperitoneally injected with αSlamf1 (9D1) or an immunoglobulin G isotype twice weekly starting at the day of the cell transfer (500 μg/mouse/injection). Mice were euthanized 8 weeks after T-cell transfer. Each *open circle* and *filled circle* represents 1 mouse. (*B*) DAI. (*C*) Representative histology. Colon sections prepared from $Rag^{-/-}$ after treatment and (*D*) histology scores. Statistical significance was determined by the Mann–Whitney test. *P* values are shown.

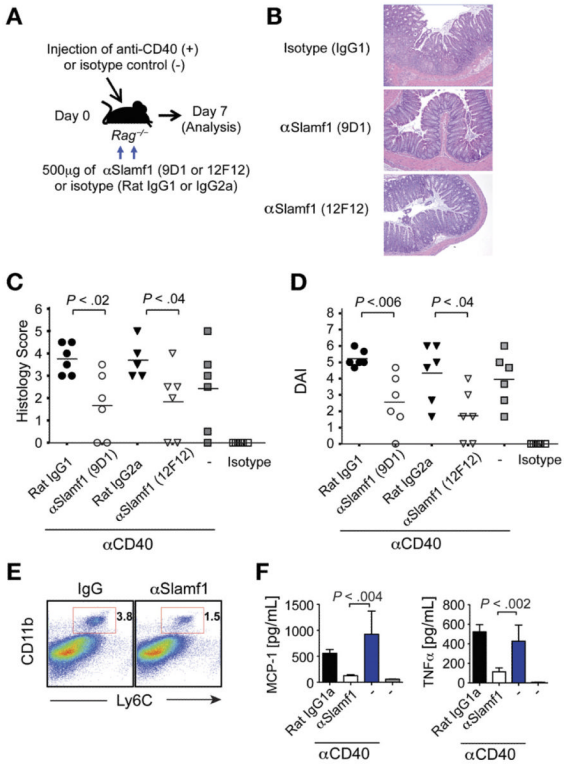

Figure 7.

Anti-Slamf1 antibody ameliorates αCD40-induced colitis. (*A*) Outline of the αSlamf1 injection protocol. Anti-CD40 or rat IgG2a were injected intraperitoneally into $Rag^{-/-}$ mice (200 μg/ mouse). αSlamf1 (9D1 and 12F12) or IgG isotype control were injected intraperitoneally on the same day as αCD40 injection and 1 day later (500 μg/mouse). (*B*) Representative histologic analysis of colon tissues from mice injected with αCD40 and treated with 9D1, 12F12, or immunoglobulin isotypes. Original magnification, 10×. (*C* and *D*) DAI and histology score. Mean and individual values of each group are indicated. *P* values are shown. The data are representative of 3 separate experiments. (*E*) Flow cytometry analysis of lamina propria cells isolated from $Rag^{-/-}$ mice were injected with αCD40 and co-injected on days 0 and 1 with either αSlamf1 (9D1) or an isotype. The $Ly6C^{+}CD11b^{+}$ population is gated, and the gate numbers represent the percentage of total isolated leukocytes. Each plot represents 5 pooled mice. (*F*) MCP-1 and TNFα levels in the serum that was collected at day seven after colitis induction. The experiment was performed twice.