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The Tumor Necrosis Factor Family Member TNFSF14 (LIGHT) is Required for Resolution of Intestinal Inflammation in Mice

Petra Krause, Sonja P. Zahner, Gisen Kim, Raziya B. Shaikh, Marcos W. Steinberg[¶], and Mitchell Kronenberg

Division of Developmental Immunology, La Jolla Institute for Allergy & Immunology, 9420 Athena Circle, La Jolla, CA 92037, USA

Abstract

Background & Aims—The pathogenesis of inflammatory bowel disease (IBD) is associated with a dysregulated mucosal immune response. Expression of the tumor necrosis factor (TNF) superfamily member 14 (TNFSF14, also known as LIGHT) on T cells is involved in their activation; transgenic expression of LIGHT on T cells in mice promotes inflammation in multiple organs, including intestine. We investigated the roles for LIGHT in recovery from intestinal inflammation in mice.

Methods—We studied the role of LIGHT in intestinal inflammation using *Tnfsf14*^{−/−} and wild-type mice. Colitis was induced by transfer of CD4⁺CD45RB^{high} T cells into *Rag1*^{−/−} or *Tnfsf14*^{−/−}*Rag1*^{−/−} mice, or by administration of dextran sulfate sodium salt (DSS) to *Tnfsf14*^{−/−} or wild-type C57BL/6J mice. Mice were weighed; colon tissues were collected and measured, and histology analyses were performed. We measured infiltrating cell populations and expression of cytokines, chemokines, and LIGHT.

Results—Following administration of DSS, *Tnfsf14*^{−/−} mice developed more severe colitis than controls, based on their reduced survival, accelerated loss of body weight, and histologic scores. LIGHT protected mice from colitis via the lymphotoxin β receptor and was expressed mainly by myeloid cells in the colon. Colons of *Tnfsf14*^{−/−} mice also had increased accumulation of innate immune cells and higher levels of cytokines than colons from control mice. LIGHT therefore appears to regulate inflammation in the colon.

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Correspondence Mitchell Kronenberg, La Jolla Institute for Allergy & Immunology, 9420 Athena Circle, La Jolla, CA 92037, USA, mitch@liai.org, Tel: (858) 752-6540, Fax: (858) 752-6990, URL: <http://www.liai.org>.

[¶]M.W.S present address: Tanabe Research Laboratories U.S.A., Inc., 4540 Towne Centre Court, San Diego, CA, 92121.

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Disclosures

The authors have no competing interests regarding this work.

Author contributions

P.K., S.Z., M.W.S. and R.S. conducted experiments and acquired and analyzed data. P.K., S.Z., G.K., M.W.S. and M.K. designed research, P.K. and M.K. wrote the manuscript and P.K. and M.K. obtained funding. All authors approved the manuscript.

Conclusion—*Tnfrsf14*^{-/-} mice develop more severe colitis than control mice. LIGHT signals through the lymphotoxin β receptor in the colon to regulate the innate immune response and mediate recovery from intestinal inflammation.

Keywords

IBD; mouse model; ulcerative colitis; innate immunity

Introduction

The intestinal immune system is tightly regulated, providing tolerance against the commensal microbiota and its products, as well as against dietary antigens, while retaining the ability to launch an effective immune response against invading pathogens. The consequences of dysregulation of this carefully balanced system are obviously manifested in inflammatory bowel diseases (IBD), in which continual activation of the mucosal immune system leads to chronic, unresolved inflammation. The characteristic symptoms of IBD can be attributed to a robust, cytokine-driven, but non-infectious inflammation of the gut, which results from an imbalance of effector and regulatory mechanisms, caused in part by genetically determined defects¹. Understanding the details of the regulatory mechanisms of the intestinal immune system is key to identifying new therapeutic targets for IBD.

The tumor necrosis factor (TNF) superfamily of cytokines plays important roles in fundamental immunological processes and IBD pathogenesis, making them targets for IBD therapy^{2,3}. Interestingly, the human gene for TNFSF14 (LIGHT, “homologous to Lymphotoxins, exhibits I nducible expression, and competes with HSV G lycoprotein D for H VEM, a receptor expressed by T lymphocytes”) maps to a susceptibility locus for Crohn’s disease on chromosome 19p13.3⁴, and data from experimental models of IBD support an involvement of LIGHT in disease pathogenesis. Transgenic mice, in which T cells constitutively expressed LIGHT, developed spontaneous multi-organ inflammation that includes the intestine^{5,6} and mucosal T cells from IBD patients express high levels of LIGHT⁷.

LIGHT signals through two receptors, the Herpes Virus Entry Mediator (HVEM, TNFRSF14) and the lymphotoxin β receptor (LT β R). HVEM has been suggested to contribute to IBD pathogenesis by promoting T cell activation and Th1 responses⁷. LIGHT signaling through HVEM has been shown to provide a co-stimulatory signal for the activation and proliferation of T cells^{7,8}. Thus, blocking LIGHT in IBD to control inappropriate T cell activation and proliferation poses a promising therapeutic concept, which is currently under development.

However, our study provides evidence for an entirely different role for LIGHT in promoting the resolution of intestinal inflammation, which is facilitated through interaction with LT β R and not HVEM. This effect is T cell-independent and involves mainly innate immune cells.

Materials and Methods

Animals

Mice were bred and housed under SPF conditions in the vivarium of the La Jolla Institute for Allergy & Immunology (LJI, La Jolla, CA). For some experiments wild-type C57BL/6J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). *Tnfsf14*^{-/-} mice were generated by Klaus Pfeffer (University of Düsseldorf, Germany). All procedures were approved by the LJI Animal Care and Use Committee.

Reagents and antibodies

Hamster anti-HVEM (LH1) and rat anti-LTβR (LLTB2) specific antibodies were protein G purified from hybridoma cell lines, which were generously provided by Dr. Koji Tamada (University of Maryland, Baltimore). LH1, LLTB2 and appropriate isotype controls were administered at 100μg i.p. twice a week. For flow cytometry, mAb against the following mouse antigens were used: CD45 (30-F11), CD11b (M1/70), Ly6G (1A8), Ly6C (AL-21), CD4 (RM4-5), TCRβ (H57-597), CD19 (1D3), CD45RB (16A), podoplanin (8.1.1), CD31 (MEC13.3) and EpCAM (G8.8). Antibodies were purchased from BD Biosciences (San Diego, CA), eBiosciences (San Diego, CA) or BioLegend (San Diego, CA). Live/dead (Molecular Probes, Eugene, OR) staining was used to exclude dead cells. Cell sorting was performed on an Aria II instrument (BD Biosciences).

Experimental colitis models

T cell transfer model: T cells were isolated from spleens of donor mice using anti-CD4 conjugated microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and sorted for CD4⁺CD45RB^{high}. 4–5×10⁵ cells were injected i.v. into *Rag1*^{-/-} or *Tnfsf14*^{-/-}*Rag1*^{-/-} mice. Disease was monitored by weight loss, diarrhea and appearance.

Chronic DSS-induced colitis: wild-type C57BL/6 and LIGHT-deficient (*Tnfsf14*^{-/-}) or HVEM-deficient (*Tnfrsf14*^{-/-}) mice were given 3% dextran sulfate sodium (DSS; MP Biomedicals, Solon, OH) in the drinking water *ad libidum* for a maximum of four cycles, in which one cycle comprised five days of water plus DSS and two days of regular drinking water without DSS as described previously⁹. Body weight and appearance were monitored daily. Death was defined as physical death or more than 20% weight loss, at which stage animals were euthanized in compliance with our animal protocols.

Histology

Upon termination of the colitis experiment, tissue sections were taken from distal colon and cecum. Tissue was fixed in 10% formalin and embedded in paraffin. 5μm sections were cut and stained with hematoxylin-eosin (H&E) and histological scores were assigned in a blinded fashion and calculated as described in Supplementary Table 1. For collagen staining 7μm sections were stained with sirius red and fast green (Chondrex, Redmond, WA) according to the manufacturer's instructions.

Isolation of lamina propria cells

Large intestines were opened longitudinally, washed to remove fecal content, cut into small pieces and incubated three times with 2.5mM EDTA at 37°C in a horizontal shaker for 20min to remove epithelial cells. Colon pieces were minced and digested for 20min with 1mg/ml Collagenase type VIII (Sigma, St. Louis, MO) at 37°C. Lamina propria cells were filtered and stained for flow cytometry analysis or cell sorting.

Relative mRNA quantification

Total RNA was extracted from 2–3mm long colon sections using the RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genomic DNA was digested with DNase I (Qiagen), cDNA was synthesized using iScript (Biorad, Hercules, CA) and real-time PCR was performed using SYBRgreen (Roche, Indianapolis, IN) on a LightCycler instrument (Roche); primer sequences (Supplementary Table 2). For analysis of mRNA expression at day 12 of DSS-induced colitis, the RT² ProfilerTM PCR array Inflammatory Response and Autoimmunity (Qiagen) was used.

Statistical analysis

The Student's *t* test was used for statistical analysis except for survival and histological scores, for which the Log-Rank test and the Mann Whitney test were used respectively. Differences were considered significant at *P* < .05.

Results

LIGHT-deficiency aggravates disease in experimental models of colitis

Previous studies by our laboratory and others have shown that constitutive expression of LIGHT by T cells in transgenic mice caused a variety of autoimmune syndromes, including intestinal inflammation^{5,6}. Because over-expression of LIGHT caused inflammatory disease, and LIGHT plays a role in T cell co-stimulation, we considered it possible that LIGHT-deficiency might lead to reduced inflammation. To test this possibility, we used the T cell transfer model of colitis, in which disease is initiated by the transfer of naïve CD4⁺CD45RB^{high} T lymphocytes, devoid of Foxp3⁺ regulatory T cells, into immune-deficient recipients. Transfer of wild-type naïve T cells into *Rag1*^{-/-} recipients with genetic ablation of LIGHT (*Tnfrsf14*^{-/-}*Rag1*^{-/-}) led to greatly accelerated weight loss, which was surprisingly not caused by an increased frequency of T cells in colonic lamina propria (Figure 1). Moreover, TNF, IL-17 and IFN- γ levels were similar in colon tissue of *Tnfrsf14*^{-/-}*Rag1*^{-/-} and *Rag1*^{-/-} mice, suggesting that LIGHT-deficiency in the host did not alter the differentiation of the transferred T cells. In contrast, LIGHT-deficiency correlated with elevated levels of IL-6 mRNA expression in colon tissue (Figure 1). Because these data suggested that the accelerated disease observed in LIGHT-deficient recipients was not driven primarily by T cells, we employed a second model of experimental colitis, which is initiated by dextran sulfate sodium salt (DSS)-induced damage to the colon epithelium, and is predominantly driven by innate immune cells. Chronic DSS-induced colitis was established by administration of four cycles of 3% DSS as described previously⁹. Wild-type mice started to lose weight after 5–6 days of DSS treatment, but recovered from weight loss between days 10 and day 12. LIGHT-deficient mice, however, lost weight similarly but

could not recover from the initial weight loss (Figure 2A), which correlated with a strong decrease in their survival (Figure 2A, B). LIGHT-deficient mice showed extreme shortening of the colon and cecum and increased histological scores (Figure 2C, D, E), with greater histological differences in cecum than in distal colon (Supplementary Figure 1). LIGHT-deficient mice had massive inflammatory infiltrates, epithelial disruption and substantial widening of the submucosal layer in the distal colon as well as in the cecum compared to wild-type mice (Figure 2F). Importantly, LIGHT-deficient animals showed no signs of inflammation in the large intestine under steady state conditions before treatment with DSS (Figure 2F). Therefore, in two different mouse models of colitis, the absence of LIGHT expression led to exacerbated disease. In a previous study, the absence of LIGHT was shown to ameliorate acute DSS-induced colitis¹⁰. To elucidate whether this discrepancy was due to acute versus chronic DSS administration, we performed acute DSS-induced colitis experiments. When we followed the protocol as described by Jungbeck et al. (1.5% DSS), we could not induce weight loss in our wild-type mice, while *Tnfsf14*^{-/-} mice showed decreased body weight, increased histological scores and shortened colons at day 8 (Supplementary Figure 2A). With 3% DSS we could induce weight loss in wildtype mice, but again LIGHT-deficient mice exhibited more severe disease (Supplementary Figure 2B).

Severe colitis in LIGHT-deficient mice is due to innate immune cells

In mice chronically exposed to DSS, analysis of the cellular infiltrate in colon lamina propria revealed that the T cell frequencies in the colon and blood of wild-type and LIGHT-deficient mice were equivalent (Figure 3A), suggesting that the aggravated disease in *Tnfsf14*^{-/-} animals was not due to an enhanced T cell expansion and T cell mediated immune response. To confirm that T lymphocytes did not contribute to the exacerbated disease in the absence of LIGHT, we induced chronic DSS colitis in LIGHT-deficient *Rag1*^{-/-} (*Tnfsf14*^{-/-}*Rag1*^{-/-}) mice. Compared to *Rag1*^{-/-} mice, *Tnfsf14*^{-/-}*Rag1*^{-/-} mice showed decreased survival (data not shown) and unresolved inflammation in distal colon and cecum, even seven weeks after DSS removal (Supplementary Figure 3).

In contrast, the frequency of neutrophils was significantly increased in the colon of LIGHT-deficient mice during chronic DSS-induced colitis after two cycles and remained elevated through four cycles (Figure 3B, Supplementary Figure 4). The increase in neutrophils infiltrating the large intestine of *Tnfsf14*^{-/-} animals correlated with higher frequencies of these cells in the blood suggesting an increased recruitment of neutrophils from the bone marrow during chronic colitis (Figure 3B). We also found elevated frequencies of monocytes in colon lamina propria and blood of LIGHT-deficient mice after two cycles of DSS (Figure 3C). Importantly, unchallenged LIGHT-deficient mice had similar frequencies of neutrophils, monocytes and T cells compared to healthy wild-type mice in large intestine lamina propria and blood (Figure 3). Sirius Red staining of colon sections revealed massive deposition of collagen (Figure 3D) suggesting increased numbers and activation of fibroblasts in the submucosal colonic layer of mice lacking LIGHT.

To characterize the inflammatory response of LIGHT-deficient and wild-type mice exposed chronically to DSS, we assessed cytokine and chemokine expression in colon tissue (Figure 4). As observed in the transfer model, we found elevated levels of IL-6 mRNA, but not TNF

or IL-17 mRNA in LIGHT-deficient animals. Moreover, expression levels of IL-1 β and oncostatin M (Osm) were significantly increased in the absence of LIGHT (Figure 4A). The analysis of chemokine mRNA revealed elevated expression of mediators driving recruitment of innate immune cells in LIGHT-deficient mice (Figure 4B). CXCL1 and CXCL2 are essential for the recruitment of neutrophils to damaged sites, and their enhanced expression in the colon of LIGHT-deficient animals correlated with increased neutrophil accumulation in the large intestine (Figure 3B). Furthermore, CCL3, CCL7 and CXCL10, which are associated with the recruitment of other myeloid lineage cells, such as monocytes, macrophages and eosinophils, were also elevated in the colon of DSS-treated LIGHT-deficient mice (Figure 4B). Cytokine and chemokine levels were comparable in colon tissue or serum of unchallenged LIGHT-deficient and healthy wild-type controls (data not shown). Moreover, to rule out an intestinal barrier defect as the primary cause of the exacerbated colitis phenotype in LIGHT-deficient mice, we assessed intestinal permeability in untreated LIGHT-deficient compared to wild-type mice by means of FITC-dextran uptake. Intestinal barrier function was uncompromised in healthy *Tnfrsf14*^{-/-} mice (Supplementary Figure 5). However, LIGHT-deficient mice showed increased intestinal barrier permeability during the course of chronic DSS-induced colitis, in accordance with the observed disruption of the epithelial layer in sections from distal colon and cecum in LIGHT-deficient mice (Figure 2).

The protective effect of LIGHT is mediated through LT β R

LIGHT interacts with two receptors, HVEM and LT β R. To elucidate which receptor mediates the signal provided by LIGHT to prevent exacerbated colitis, we subjected HVEM-deficient mice to chronic DSS-induced colitis. HVEM-deficiency did not lead to more severe disease (Figure 5A). Additionally, blocking HVEM:LIGHT binding using a specific anti-HVEM antibody (LH1), which only blocks binding of HVEM to LIGHT but not to BTLA¹⁰, did not induce accelerated weight loss in the transfer model of colitis (Figure 5B). In contrast, administration of a LT β R antibody (LLTB2), which specifically blocks LT β R:LIGHT binding, but not LT β R binding to lymphotoxin (LT $\alpha_1\beta_2$)¹¹, recapitulated the LIGHT-deficient phenotype of severe body weight loss in both the chronic DSS-induced and the T cell transfer model (Figure 5C, D). Importantly, LLTB2 did not have celldepleting properties, as we found comparable frequencies of LT β R-expressing cells in the gut before and after treatment (Supplementary Figure 6). Taken together, these data provide evidence that the protective effect of LIGHT during chronic colitis is mediated through the LT β R rather than HVEM. Furthermore, inhibition of LIGHT:LT β R interactions after the first cycle of DSS administration could prevent the recovery from the initial weight loss in wild-type mice, mimicking the phenotype we observed in LIGHT-deficient animals and thus supporting a role for LIGHT during the recovery from intestinal damage (Figure 5E, F).

LIGHT and LT β R expression in colon cell populations

We addressed which cell population(s) in large intestine express LIGHT under steady state conditions and during chronic colitis. The lack of an antibody to detect mouse LIGHT by flow cytometry, prompted us to sort different cell populations from colon lamina propria and to analyze LIGHT mRNA expression by real time PCR. We separated CD45⁺ and CD45⁻ cells, and found LIGHT expression primarily in CD45⁺ cells in unchallenged mice and this pattern and the expression level did not change after chronic DSS administration (Figure

6A). To further define the expression of LIGHT by CD45⁺ cell populations, we sorted neutrophils (CD11b⁺Ly6G^{high}), CD11b⁺ (Ly6G⁻) and CD11b⁻ cells, and identified neutrophils as the major source of LIGHT mRNA (Figure 6B).

Likewise, we analyzed LTβR mRNA expression and, as expected, CD45⁻ cells expressed high levels of LTβR. In addition, neutrophils and CD11b⁺ cells in large intestine proved to express LTβR to an at least equal extent, which was unaltered during chronic DSS challenge. This pattern of expression in different cell types and the expression levels also were not changed after chronic DSS challenge (Figure 6D). Within the CD45⁻ population, podoplanin⁺ CD31⁻ fibroblasts expressed high levels of LTβR, and this expression was also unchanged after DSS challenge (Figure 6C).

Mechanism of protective effect of LIGHT

It is striking that we found increased IL-6 in LIGHT-deficient mice in both chronic DSS-induced colitis and in the T cell transfer model, although the causes of inflammation are very different in these contexts. This suggests that LIGHT may control a common mechanism in the resolution of inflammation in the intestine. To understand the mechanism that leads to increased IL-6 production in the absence of LIGHT, we identified the IL-6 producing cell populations in the large intestine during chronic DSS-induced colitis. Surprisingly, IL-6 mRNA was exclusively produced by CD45⁻ cells, and within the CD45⁻ population, podoplanin⁺ CD31⁻ fibroblasts were the main producer of IL-6 (Figure 7A). Fibroblasts from unchallenged mice did not produce IL-6 (data not shown). Known physiological stimuli for IL-6 production in fibroblasts include IL-1β and Osm, both of which were increased in colon tissue of *Tnfrsf14*^{-/-} mice (Figure 4A). IL-1β and Osm were mainly produced by CD45⁺ cells, and predominantly by neutrophils, although other CD11b⁺ cells contributed (Figure 7B, C). Using a fibroblast cell line (NIH3T3), we assessed the effect of IL-1β and Osm on IL-6 production and found that, while IL-1β or Osm alone could induce IL-6 mRNA expression, the combination of both stimuli had a strongly synergistic effect (Figure 7D). Therefore, we suggest that the increased levels of IL-1β and Osm in LIGHT-deficient mice during chronic DSS-induced colitis lead to increased IL-6 production by fibroblasts in the colon.

Discussion

We examined the role of LIGHT in colitis pathogenesis in models that differ greatly with regard to disease induction. The chronic DSS model is initiated by epithelial damage, and pathogenesis is not highly dependent on adaptive immunity. Intestinal inflammation in the T cell transfer model, by contrast, is initiated by the stimulation of activated CD4 T lymphocytes to produce IFNγ and IL-17. Despite these different causes, we found that the effects of LIGHT deficiency or blockade were surprisingly similar. Three novel findings emerge from the experiments we have carried out. Firstly, as noted, we observed a more severe disease phenotype in both colitis models in the absence of LIGHT expression, or when LIGHT interaction with one of its receptors was blocked. Secondly, we found that LIGHT-deficiency primarily affects innate immunity, and finally, the ability of LIGHT to

limit the innate immune response in the intestine is due primarily to interactions with the LT β R, rather than with HVEM.

The augmented disease severity in the absence of LIGHT expression was unexpected, because constitutive over-expression of LIGHT on T lymphocytes has been shown to induce multi-organ inflammation^{5,6}. Furthermore, LIGHT had been reported to costimulate T lymphocytes by engaging HVEM, and LIGHT expression on T cell is increased in Crohn's disease patients⁷. A previous study had demonstrated that LIGHT-deficiency ameliorates acute DSS-induced colitis¹⁰. However, in our animal colony, LIGHT-deficient mice developed exacerbated colitis in both the chronic and acute DSS-induced model. Differences in the microflora in the different colonies could explain the discrepancy between our findings and the published results. Even within a vivarium, such differences can be substantial, with an important maternal influence on the microbiota¹². Therefore, we have addressed the possibility of microflora differences as a reason for the exacerbated colitis phenotype in our LIGHT-deficient colony by comparing co-housed littermates. Consistent with the other results presented, LIGHT-deficient littermates did not recover from the initial weight loss in chronic DSS-induced colitis and showed decreased survival (Supplementary Figure 7), arguing that environmentally determined differences in the microbiota were not a factor.

Our data suggest a role for LIGHT in the resolution of inflammation caused by innate immune cells, as opposed to the lack of LIGHT causing an enhanced initiation of the innate response. In the chronic DSS-induced model, LIGHT-deficiency did not lead to exacerbated weight loss and inflammation up to day 12, but rather led to a persistent inflammation that could not be resolved. Furthermore, blocking the relevant LIGHT receptor, LT β R, beginning at day seven also led to unresolved disease.

The results of our study indicating a role for LIGHT in limiting chronic inflammation are consistent with recent results obtained using a cutaneous wound-healing model, in which the absence of LIGHT led to chronic wounds with excessive inflammation¹³. Similar to the chronic DSS colitis model, the inflammatory lesions were characterized by increased myeloid cell infiltration and elevated levels of pro-inflammatory cytokines and chemokines, such as CXCL1 and CXCL10¹³. Furthermore, collagen deposition and matrix composition were abnormal in the absence of LIGHT¹³. However, the effects of LIGHT and LIGHT deficiency on fibroblasts and other innate immune cells likely are tissue and/or context-specific. In fact, in an asthma model, LIGHT-deficiency reduced rather than augmented lung fibrosis through suppression of TGF- β and IL-13 expression¹⁴.

Our data demonstrate that LIGHT-deficiency selectively affected the innate immune system. In particular, T cell frequencies in the colon were not increased in either model in the absence of LIGHT, and this finding was supported by lack of an increase in mRNA for T cell-derived cytokines involved in driving colitis pathogenesis in other contexts, including IL-17 and IFN γ . Moreover, LIGHT-deficient *Rag1*^{-/-} mice could not resolve intestinal inflammation. By contrast, we detected increased colonic IL-6 mRNA expression in the absence of LIGHT in the T cell transfer model, and in a more extensive cytokine survey carried out in the DSS model, we found increased amounts of mRNA for IL-6, IL-1 β and

Osm. Consistent with alterations in innate immunity, in the DSS model we also found increased frequencies of neutrophils and monocytes in the colon in LIGHT-deficient mice, which correlated with elevated levels of mRNA for chemokines that attract these and other innate leukocytes.

LIGHT binds to two receptors, HVEM and LT β R, both of which have been implicated in models of IBD. We show here that it is exclusively the LT β R that is engaged by LIGHT to limit innate immunity during intestinal inflammation. We eliminated a role for HVEM by analyzing HVEM-deficient mice in DSS-induced colitis. Furthermore, an antibody that blocks the interaction of HVEM with LIGHT, but not HVEM with BTLA¹⁰, did not affect disease in either model. In contrast, treatment of wild-type mice with an LT β R antibody, selectively blocking binding of LIGHT but not lymphotoxin¹⁰, recapitulated the aggravated colitis phenotype we observed in LIGHT-deficient mice in both the chronic DSS-induced and the T cell transfer models. Taken together, these findings indicate that the preventive signal in colitis provided by LIGHT is mediated through the LT β R.

LIGHT has been reported to enhance inflammation-induced cell death by signaling through LT β R^{15,16}, and therefore the increased numbers of myeloid cells accumulated in the colon of LIGHT deficient mice might reflect the absence of this mechanism. It has been demonstrated in a cutaneous wound-healing model, that LIGHT promotes apoptosis of macrophages through LT β R, which is critical for the resolution of inflammation¹⁷. A recent study showed increased accumulation of inflammatory macrophages/microglia in LIGHT-deficient mice in an experimental autoimmune encephalomyelitis model¹⁸. It is possible that the absence of LIGHT also could directly affect the survival of fibroblasts, thereby contributing to fibrosis during colonic inflammation.

We ruled out an intestinal barrier defect in unchallenged LIGHT-deficient mice as the cause for the observed exacerbated disease phenotype. However, it is not surprising that LIGHT-deficient mice displayed normal intestinal barrier function under steady state conditions, as Tnfsf14^{-/-} animals do not develop spontaneous colitis. Furthermore, signaling induced by T cell-derived LIGHT has been shown to impair intestinal integrity¹⁹, a signal absent in LIGHT-deficient mice.

We have assessed in detail for the first time the expression of LIGHT mRNA by intestinal cell populations and found LIGHT to be produced mainly by CD45⁺ hematopoietic cells. Although we could detect some LIGHT mRNA expression in CD45⁻ cells, and this expression increased after chronic DSS exposure, the expression level was 100 to 1000 fold lower compared to CD45⁺ cells. Therefore it is unlikely that this low amount of LIGHT mRNA makes a substantial contribution to pathogenesis. We identified neutrophils as the major source of LIGHT mRNA in healthy mice as well as after DSS challenge. Of course the number of neutrophils in the gut increases dramatically after DSS challenge and with that the availability of neutrophil-derived LIGHT. These findings are consistent with data from microarray analyses, in which LIGHT mRNA was detected in neutrophils (Immgen.org).

We found increased expression levels of IL-6 and IL-1 β mRNA in LIGHT-deficient mice with chronic colitis. The importance of these cytokines in several IBD models has been previously demonstrated. Genetic deletion of IL-6 ameliorates DSS-induced colitis with reduced inflammation, infiltration of inflammatory cells and mucosal cell disruption²⁰, and blockade of IL-6 signaling prevents colitis in the T cell transfer model²¹. As a key cytokine in the onset of IBD, IL-1 β has been shown to control its own expression in a positive feedback loop as well as inducing IL-6 expression, and blockade of IL-1 β can attenuate DSS-induced colitis²². Moreover, limiting IL-1 β levels in the colon has been correlated with amelioration of DSS-induced colitis²³.

Surprisingly, in chronic DSS-induced colitis IL-6 mRNA was mainly produced by fibroblasts. Physiological stimuli for IL-6 expression in fibroblasts include IL-17 and TNF, which were not increased in LIGHT-deficient mice, as well as Osm and IL-1 β , both of which were increased. Moreover, we demonstrated that Osm and IL-1 β synergistically induced IL-6 mRNA expression in fibroblasts *in vitro*. Therefore, we suggest that the simultaneous increase of IL-1 β and Osm in LIGHT-deficient mice caused the increased IL-6 expression in fibroblasts.

Neutrophils and other CD11b⁺ cells were the major producers of IL-1 β and Osm. Since these populations also express LT β R, we propose two models, which are not mutually exclusive, for how LIGHT modulates intestinal inflammation. First, LIGHT might limit production of inflammatory stimuli like IL-1 β and Osm by LT β R-expressing neutrophils and other CD11b⁺ cells. Second, LIGHT might act directly on LT β R-expressing fibroblasts to counteract inflammatory signals from CD45⁺ cells, namely IL-1 β and Osm. In either case, LIGHT could act by decreasing the survival of the activated target cells.

In conclusion, here we identified a new role for LIGHT, expressed by myeloid cells, in interacting with the LT β R to limit chronic intestinal inflammation. We show that LIGHT-LT β R interactions contribute to limiting both innate cell numbers recruited to the intestine, and as a consequence, inflammatory cytokines produced by innate immune cells, including stromal cells. In the absence of LIGHT, or when LIGHT-LT β R interaction is blocked, inflammation is prolonged and not resolved after mucosal damage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BTLA	B and T lymphocyte attenuator
DSS	dextran sulfate sodium salt
HVEM	herpes virus entry mediator
IBD	inflammatory bowel disease
IL	interleukin
LIGHT	homologous to Lymphotoxins, exhibits Inducible expression, and competes with HSV Glycoprotein D for HVEM, a receptor expressed by T lymphocytes
LP	lamina propria
LTβR	lymphotoxin β receptor
Osm	oncostatin M
Pdpm	podoplanin
Rag1	recombinase-activating gene 1
SPF	specific pathogen free
Th1	T helper type 1
TNF	tumor necrosis factor

References

1. Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest.* 2007; 117:514–521. [PubMed: 17332878]
2. Watts TH. TNF/TNFR family members in costimulation of T cell responses. *Annu Rev Immunol.* 2005; 23:23–68. [PubMed: 15771565]
3. Perrier C, Rutgeerts P. New drug therapies on the horizon for IBD. *Dig Dis.* 2012; 30(Suppl 1):100–105. [PubMed: 23075877]
4. Granger SW, Butrovich KD, Houshmand P, Edwards WR, Ware CF. Genomic characterization of LIGHT reveals linkage to an immune response locus on chromosome 19p13.3 and distinct isoforms generated by alternate splicing or proteolysis. *J Immunol.* 2001; 167:5122–5128. [PubMed: 11673523]
5. Shaikh RB, Santee S, Granger SW, Butrovich K, Cheung T, Kronenberg M, Cheroute H, Ware CF. Constitutive expression of LIGHT on T cells leads to lymphocyte activation, inflammation, and tissue destruction. *J Immunol.* 2001; 167:6330–6337. [PubMed: 11714797]
6. Wang J, Lo JC, Foster A, Yu P, Chen HM, Wang Y, Tamada K, Chen L, Fu YX. The regulation of T cell homeostasis and autoimmunity by T cell-derived LIGHT. *J Clin Invest.* 2001; 108:1771–1780. [PubMed: 11748260]
7. Cohavy O, Zhou J, Granger SW, Ware CF, Targan SR. LIGHT expression by mucosal T cells may regulate IFN- γ expression in the intestine. *J Immunol.* 2004; 173:251–258. [PubMed: 15210782]
8. Tamada K, Shimozaaki K, Chapoval AI, Zhai Y, Su J, Chen SF, Hsieh SL, Nagata S, Ni J, Chen L. LIGHT, a TNF-like molecule, costimulates T cell proliferation and is required for dendritic cell-mediated allogeneic T cell response. *J Immunol.* 2000; 164:4105–4110. [PubMed: 10754304]
9. Takedatsu H, Michelsen KS, Wei B, Landers CJ, Thomas LS, Dhall D, Braun J, Targan SR. TL1A (TNFSF15) regulates the development of chronic colitis by modulating both T-helper 1 and T-helper 17 activation. *Gastroenterology.* 2008; 135:552–567. [PubMed: 18598698]

10. Jungbeck M, Daller B, Federhofer J, Wege AK, Wimmer N, Mannel DN, Hehlhans T. Neutralization of LIGHT ameliorates acute dextran sodium sulphate-induced intestinal inflammation. *Immunology*. 2009; 128:451–458. [PubMed: 20067544]
11. Anand S, Wang P, Yoshimura K, Choi IH, Hilliard A, Chen YH, Wang CR, Schulick R, Flies AS, Flies DB, Zhu G, Xu Y, Pardoll DM, Chen L, Tamada K. Essential role of TNF family molecule LIGHT as a cytokine in the pathogenesis of hepatitis. *J Clin Invest*. 2006; 116:1045–1051. [PubMed: 16557300]
12. Ubeda C, Lipuma L, Gobourne A, Viale A, Leiner I, Equinda M, Khanin R, Pamer EG. Familial transmission rather than defective innate immunity shapes the distinct intestinal microbiota of TLR-deficient mice. *J Exp Med*. 2012; 209:1445–1456. [PubMed: 22826298]
13. Petreaca ML, Do D, Dhall S, McLelland D, Serafino A, Lyubovitsky J, Schiller N, Martins-Green MM. Deletion of a tumor necrosis superfamily gene in mice leads to impaired healing that mimics chronic wounds in humans. *Wound Repair Regen*. 2012; 20:353–366. [PubMed: 22564230]
14. Doherty TA, Soroosh P, Khorram N, Fukuyama S, Rosenthal P, Cho JY, Norris PS, Choi H, Scheu S, Pfeffer K, Zuraw BL, Ware CF, Broide DH, Croft M. The tumor necrosis factor family member LIGHT is a target for asthmatic airway remodeling. *Nature Med*. 2011; 17:596–603. [PubMed: 21499267]
15. Rooney IA, Butrovich KD, Glass AA, Borboroglu S, Benedict CA, Whitbeck JC, Cohen GH, Eisenberg RJ, Ware CF. The lymphotoxin-beta receptor is necessary and sufficient for LIGHT-mediated apoptosis of tumor cells. *J Biol Chem*. 2000; 275:14307–14315. [PubMed: 10799510]
16. Chang YH, Chao Y, Hsieh SL, Lin WW. Mechanism of LIGHT/interferon-gamma-induced cell death in HT-29 cells. *J Cell Biochem*. 2004; 93:1188–1202. [PubMed: 15486969]
17. Petreaca ML, Yao M, Ware C, Martins-Green MM. Vascular endothelial growth factor promotes macrophage apoptosis through stimulation of tumor necrosis factor superfamily member 14 (TNFSF14/LIGHT). *Wound Repair Regen*. 2008; 16:602–614. [PubMed: 19128255]
18. Maña P, Liñares D, Silva DG, Fordham S, Scheu S, Pfeffer K, Staykova M, Bertram EM. LIGHT (TNFSF14/CD258) is a decisive factor for recovery from experimental autoimmune encephalomyelitis. *J Immunol*. 2013; 191:154–163. [PubMed: 23720813]
19. Schwarz BT, Wang F, Shen L, Clayburgh DR, Su L, Wang Y, Fu YX, Turner JR. LIGHT signals directly to intestinal epithelia to cause barrier dysfunction via cytoskeletal and endocytic mechanisms. *Gastroenterology*. 2007; 132:2383–2394. [PubMed: 17570213]
20. Naito Y, Takagi T, Uchiyama K, Kuroda M, Kokura S, Ichikawa H, Yanagisawa R, Inoue K, Takano H, Satoh M, Yoshida N, Okanoue T, Yoshikawa T. Reduced intestinal inflammation induced by dextran sodium sulfate in interleukin-6-deficient mice. *Int J Mol Med*. 2004; 14:191–196. [PubMed: 15254764]
21. Yamamoto M, Yoshizaki K, Kishimoto T, Ito H. IL-6 is required for the development of Th1 cell-mediated murine colitis. *J Immunol*. 2000; 164:4878–4882. [PubMed: 10779797]
22. Kwon KH, Murakami A, Hayashi R, Ohigashi H. Interleukin-1beta targets interleukin-6 in progressing dextran sulfate sodium-induced experimental colitis. *Biochem Biophys Res Commun*. 2005; 337:647–654. [PubMed: 16202978]
23. Sävendahl L, Underwood LE, Haldeman KM, Ulshen MH, Lund PK. Fasting prevents experimental murine colitis produced by dextran sulfate sodium and decreases interleukin-1 beta and insulin-like growth factor I messenger ribonucleic acid. *Endocrinology*. 1997; 138:734–740. [PubMed: 9003009]

Figure 1. LIGHT-deficiency accelerates T cell transfer induced colitis
 Wild-type CD4⁺CD45RB^{high} T cells were transferred into *Tnfsf14*^{-/-}*Rag1*^{-/-} (gray) or *Rag1*^{-/-} (black) recipients. **(A)** Body weight was calculated as percentage of individual weight at the time of transfer. Average and SEM are shown from six independent experiments with 2–5 mice in each group in each experiment. **(B)** Frequency of CD4⁺ T cells in colon lamina propria 3–4 weeks after T cell transfer. **(C, D, E)** Cytokine expression in proximal colon tissue 3–4 weeks after transfer was assessed by real-time PCR and mRNA expression of **(C)** TNF, **(D)** IL-17A and **(E)** IL-6 mRNA is presented relative to L32

expression. Each symbol represents measurements from an individual mouse. ** $P < .01$,
*** $P < .001$

Figure 2. LIGHT-deficiency aggravates DSS-induced chronic colitis

Colitis was induced in wild-type (black) and LIGHT-deficient (*Tnfsf14*^{-/-}, gray) mice by administration of two or four cycles of 3% DSS in drinking water; control mice received normal drinking water without DSS. (A) Body weight and (B) survival were monitored (Log-rank test *** $P < .0001$). Body weight is presented as percentage of starting weight. (C, D) After 29 days large intestines were taken and compared for length. Averages and SEM of five independent experiments with 3–5 mice per group per experiment are presented. (E) Histology scores of colon and cecum were compared after two or four cycles of DSS

treatment. Averages and SEM of six independent experiments with 3–5 mice per group per experiment are shown. Mann Whitney test $**P<.01$, $***P<.001$ (F) H&E staining of sections from distal colon (upper panel) and cecum (lower panel). **e**=epithelial disruption, **i**=inflammatory infiltrate, **m**=lamina muscularis mucosae, **s**=submucosal swelling.

Figure 3. Cell infiltration in DSS-induced colitis

Colitis was induced with two or four cycles of DSS in *Tnfsf14*^{-/-} (gray) and wild-type (black) mice. Control mice were kept on regular drinking water without DSS. Frequencies of (A) CD4⁺ T cells, (B) neutrophils and (C) monocytes were analyzed in large intestine lamina propria after (A, B) two and four cycles, or (C) two cycles of DSS. Blood was taken after two cycles of DSS treatment. Average frequencies and SEM from (A, C) four or (B) five independent experiments are shown with 3–5 mice per group per experiment. Populations are presented as percentage of live cells expressing (A) CD4⁺TCRβ⁺, (B)

CD11b⁺Ly6G^{high} and (C) CD11b⁺Ly6G^{low}Ly6C^{high}. Each symbol represents measurements from an individual mouse. * $P < .05$, ** $P < .01$, *** $P < .001$ (D) Colitis was induced with four cycles of DSS and 7 μ m sections from distal colon or cecum were stained for total collagen (red) and non-collagen protein (green).

Figure 4. Expression of inflammatory mediators in the colon in DSS-induced colitis

Colitis was induced with two cycles DSS in wild-type (black) and *Tnfsf14*^{-/-} mice (gray). At day 12, distal colon tissue was assessed for mRNA expression of (A) cytokines and (B) chemokines by real-time PCR using a RT² ProfilerTM PCR array. Expression is presented relative to the average expression of *Gusb*, *Hprt1*, *Hsp90ab1* and *Gapdh*. Each symbol represents an individual mouse; combined data from two independent experiments are shown. * $P < .05$, ** $P < .01$

Figure 5. LTβR transduces the protective LIGHT-mediated signal

(A) Colitis was induced with three cycles of 3% DSS in wild-type (black squares) or HVEM-deficient (*Tnfrsf14*^{-/-}, gray triangles) mice. Control mice were kept on regular drinking water without DSS (black dotted line). Averages and SEM are presented from three independent experiments with: wild-type no DSS n=7, wild-type + DSS n=12, *Tnfrsf14*^{-/-} + DSS n=14. (C) Colitis was induced with three cycles of 3% DSS in wild-type (black lines) or *Tnfrsf14*^{-/-} (grey circles) mice. Wild-type mice were injected i.p. twice a week with anti-LTβR mAb (black triangles, dotted line) or rat IgG (black squares, solid line) starting at day

1. Averages and SEM are presented from two independent experiments with *Tnfsf14*^{-/-} (n=6), wild-type + IgG (n=7), wild-type + anti-LTβR (n=7). **(B, D)** Wild-type CD4⁺CD45RB^{high} T cells were transferred into *Tnfsf14*^{-/-}Rag1^{-/-} (gray) or Rag1^{-/-} (black) recipients. Rag1^{-/-} recipients received **(B)** anti-HVEM mAb (dotted line) or hamster IgG (solid line) or **(D)** anti-LTβR mAb (dashed line) or rat IgG (solid line) i.p. twice a week starting at the time of T cell transfer. **(E, F)** Anti-LTβR antibody was injected into C57BL/6 mice every 2–3 days starting at day 7 after the first cycle of DSS. Averages and SEM for **(B, E)** two independent experiments with total 7–10 mice per group or **(D)** 6 independent experiments with total 19–21 mice per group. Body weight is calculated as percentage of individual starting weight and statistical analysis was performed between the antibody treated compared to control IgG treated group; *P<.05, **P<.01, ***P<.001. **(F)** Symbols represent average histological scores of cecum and distal colon of individual mice, Mann Whitney test *P<.05.

Figure 6. LIGHT and LTβR expression in lamina propria populations

C57BL/6 mice were treated with two cycles of DSS or kept on regular water without DSS, and cell populations were sorted from large intestine lamina propria and assessed for expression of (A, B) LIGHT or (C, D) LTβR mRNA by real time PCR relative to L32 expression. Cells were sorted into (A) CD45⁺ and CD45⁻ populations, or (B, D) CD45⁺ cells were further divided into neutrophils (CD11b⁺Ly6G^{high}), the remaining CD11b⁺ and CD11b⁻ cells and compared to CD45⁻ cells. (C) From CD45⁻ cells, podoplanin⁺ (Pdpn⁺) fibroblasts were sorted (CD31⁻ and EpCAM⁻ cells) and compared to CD45⁺ cells. Each

symbol represents cells sorted from an individual mouse, except for neutrophils from mice without DSS, which were pooled from 3–4 mice.

Figure 7. Cellular source of cytokines

C57BL/6 mice were treated with two cycles of DSS, and the indicated cell populations were sorted from large intestine lamina propria and assessed for expression of (A) IL-6, (B) IL-1 β or (C) Osm by real time PCR relative to L32 expression. (D) NIH3T3 cells were cultured with or without 1ng/ml IL-1 β or 10ng/ml Osm or a combination of both and IL-6 mRNA expression was measured after 8h by real time PCR relative to L32 expression. One experiment is shown out of two performed with similar results.