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Promoting Immune Responses by LIGHT in the Face of Abundant Regulatory T Cell Inhibition

Yugang Wang,*† Mingzhao Zhu,*‡ Ping Yu, † and Yang-Xin Fu,*†

CD4+ regulatory T cell (Treg) populations are believed to play very important roles in the suppression of immune responses. Overriding Treg inhibition is necessary for initiating primary immune reaction upon inflammatory Ag stimulation. LIGHT, TNF superfamily member 14, has been shown to be a costimulatory molecule for effector T cells. Overexpression of lymphotoxin-related inducible ligand that competes for glycoprotein D binding to herpesvirus entry mediator on T cells (LIGHT) on T cells induces strong T cell-mediated experimental intestinal inflammation. How this process is initiated by LIGHT in suppressive intestinal environments remains incompletely understood. In this study, we assessed the effect of LIGHT on Tregs. Our results indicate that LIGHT can support the expansion and function of Tregs. However, when LIGHT was highly expressed, these abundant Tregs failed to suppress the development of T cell-mediated experimental colitis and antitumor immunity. We showed that this might be, in part, due to an ability of LIGHT to promote effector T cell proliferation and differentiation even in a Treg-abundant environment. Our data collectively suggest that LIGHT might be a critical cytokine involved in the development of autoimmune inflammatory diseases and that LIGHT-targeted immunotherapy might be useful in the treatment of these diseases. The Journal of Immunology, 2010, 184: 1589–1595.

The balance between effector T cells (Teffs) and regulatory T cells (Tregs) is critical for the control of immune homeostasis. A disruption of the balance is responsible for many inflammatory autoimmune diseases. Inflammatory bowel disease (IBD) is caused by reduced suppression or increased immune activation leading to gut inflammation when the gut immune homeostasis between the commensal microflora and the host immune system is broken (1, 2). Several mechanisms contribute to the maintenance of this immune homeostasis, and among these, CD4+ Treg populations are thought to play very important roles (3, 4). Transfer of naive CD4+ T cells that lack Tregs into a lymphopenic RAG-deficient host can cause IBD-like diseases. But transfer of Tregs not only can prevent the development of this colitis but also can cure the established disease in animal models (3, 4). Tregs’ development and functions are dependent on the transcription factor forkhead box protein 3 (FoxP3). Deficiency in Tregs by FoxP3 mutations can result in immunodysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome in human and similar phenotypes in mouse (5–8). The fate of the normal functional Treg population during the progression of IBD is unclear. To initiate immune activation during the process of IBD pathogenesis, FoxP3+ T cells (in this paper simply defined as Teffs) must override the negative inhibition by Tregs. Identifying the factor breaching Treg inhibition might shed new light on the treatment of IBD and other inflammatory autoimmune diseases.

Lymphotoxin-related inducible ligand that competes for glycoprotein D binding to herpesvirus entry mediator on T cells (LIGHT; TNF superfamily member 14) is a cytokine that belongs to the TNF superfamily (9–11). Overexpression of LIGHT on T cells in LIGHT transgenic (Tg) mice causes severe intestinal inflammation (12, 13). The pattern of disease in these mice resembles human Crohn’s disease and is ameliorated by neutralization of TNF (14). The LIGHT gene maps to 19p13.3, a known IBD susceptibility locus (15), and the role of LIGHT in the pathogenesis of IBD has been implied in previous studies (16, 17). Soluble lymphotoxin (LT) receptor that blocks LIGHT and LT can be effective in the treatment of experimental IBD (17).

The aim of this study was to determine whether LIGHT could expand Teffs and in the meantime suppress Tregs to induce IBD. In contrast to what we expected, the data showed that LIGHT expression on T cells could also promote Treg expansion. However, the net effect of LIGHT was to promote Teff proliferation and differentiation even in a Treg-enriched environment. Our data collectively implied that LIGHT might be able to play dual roles in the regulation of inflammation by promoting both Teffs and Tregs and that LIGHT might be a critical factor in initiating primary immune responses when Tregs are abundant.

Materials and Methods

Mice and reagents

C57BL/6 (B6) and RAG-1−/− mice were purchased from the National Cancer Institute (Frederick, MD) or The Jackson Laboratory (Bar Harbor, ME). Lymphocyte cell-specific protein-tyrosine kinase-LIGHT Tg mice (B6 background) have been described previously (12). All of the mice were maintained under specific pathogen-free conditions. The animal protocols were reviewed and approved by the Institute Animal Care and Use Committee of the University of Chicago. The mice were used at 4–8 wk of age. Abs purchased from BD Pharmingen (San Diego, CA) consisted of anti-CD4 (GK1.5), anti-CD25 (PC61), and anti-CD28 (35.71). The PE anti-mouse/rat FoxP3 staining set (FJK-16s) was purchased from eBioscience (San Diego, CA). Anti-herpesvirus entry mediator (HVEM) blocking Ab (14C1.1) has been described previously (18).

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Abbreviations used in this paper: Ad-LIGHT, LIGHT-expression adenovirus; BMDC, bone marrow-derived dendritic cell; FoxP3, forkhead box protein 3; HVEM, herpes-virus entry mediator; IBD, inflammatory bowel disease; KO, knockout; LIGHT, lymphotoxin-related inducible ligand that competes for glycoprotein D binding to HVEM on T cells; LPL, lamina propria lymphocyte; LT, lymphotoxin; MOG, myelin oligodendrocyte glycoprotein; Tef, effector T cell; Tg, transgenic; Treg, regulatory T cell; WT, wild-type.
Purification of cells
CD4+, CD4+CD25−, and CD4+CD25+ T cells were purified from lymph node (inguinal, axillary, and mesenteric regions and spleen) cells using CD4 or CD4CD25 T cell isolation kits according to the manufacturer’s protocol (Miltenyi Biotec, Auburn, CA).

Flow cytometry
For the CFSE labeling assay, cells were labeled with 5 μM CFSE for 5 min at room temperature. FACS analysis was performed on a FACScan flow cytometer (BD Biosciences, San Jose, CA) using CellQuest software.

In vitro cell culture and proliferation assay
Cells were stimulated with 0.5–1 μg/ml soluble anti-CD3 Ab in the presence of wild-type (WT) bone marrow-derived dendritic cells (BMDCs) as APCs. In some experiments, CFSE-labeled Tregs or CFSE-labeled Teffs were cultured alone or cocultured with unlabeled Tregs or Teffs, respectively. After incubation for 48–72 h, the proliferation of CFSE-labeled cells was analyzed by flow cytometry.

Adoptive transfer of CD4 T cells
Lymphocytes were collected from WT littermates or LIGHT Tg mice (1–2 mo of age), and 1 × 106 CD4 T cells were i.v. transferred into RAG-1−/− mice (B6, at 6–7 wk of age). Disease progress was monitored daily by body weight. Mice that lost 15–20% of body weight or moribund mice were killed immediately.

Lamina propria lymphocyte purification
Lamina propria lymphocyte (LPL) purification was performed as previously described (14). Briefly, large bowel, including cecum and colon, was collected from the WT or Tg recipients of the adoptive transfer. Stool was removed, and samples were washed with RPMI 1640 (Mediatech, Manassas, VA). Then intestine was subject to EDTA digestion (1 mM EDTA in PBS) and serial collagenase digestions (1.5 mg/ml collagenase D and 0.5 mg/ml DNase I in RPMI 1640) (Sigma-Aldrich, St. Louis, MO). The supernatant of collagenase digestion was collected, resuspended in 40% Percoll, and loaded onto a 75% Percoll gradient (Sigma-Aldrich). The interface was collected after 20 min of centrifugation at room temperature with the brake off. The cells were washed and used for FACS analysis or intracellular cytokine staining. The cell number for LPLs is from the entire large intestine including cecum, descending colon, and rectum from either WT or Tg recipients.

Statistical analysis
Comparisons of data were analyzed by two-tailed Student t test using GraphPad Prism 4.0 (GraphPad, San Diego, CA). p < 0.05 was considered significant. For survival curves, statistics were done using the log-rank (Mantel-Cox) test.

Results
LIGHT-expression CD4+ T cell transfer into immunodeficient hosts induces colitis in the presence of Tregs
LIGHT Tg mice develop colitis (12, 13), but the mechanism is unclear. To determine whether overexpression of LIGHT on CD4 T cells alone is sufficient to cause colitis, naive total CD4 T cells (which included CD4+FoxP3+ Tregs) purified from 1- to 2-mo-old WT or LIGHT Tg mice were transferred into RAG-1−/− mice separately, and the development of colitis then was monitored as indicated (Fig. 1). WT CD4 T cell transfer into RAG-1−/− hosts did not induce colitis (Fig. 1A–C). The mice maintained or even increased their body weights (Fig. 1A), there were no obvious clinical signs of colitis, and the mice remained alive the whole time (Fig. 1B). This is as expected because it is known that cotransfer of Tregs with CD4+FoxP3+ Teffs can effectively prevent the development of colitis and removal of Tregs from the whole CD4 population is required to trigger the colitis (19, 20). However, whole LIGHT Tg CD4 T cell population transfer into RAG-1−/− hosts induced severe colitis 30 d after transfer, even though Tregs were present in the transferred populations (Fig. 1A–C). The LIGHT Tg CD4 T cell-transferred mice experienced severe diarrhea and sustained weight loss (Fig. 1A) and often died of this disease (Fig. 1B). Macroscopically, the colons of LIGHT Tg CD4 T cell-transferred hosts showed several signs of severe colitis, including pronounced thickening of the bowel wall, shortened colonic length, and unformed or absent stool (Fig. 1C). Microscopically, the colons of LIGHT Tg CD4 T cell-transferred hosts showed massive lymphocyte infiltration and epithelial damage (data not shown). Furthermore, LIGHT Tg CD4 T cell-transferred hosts showed increased numbers of CD4+FoxP3− Teffs as well as higher percentages of the differentiated CD4+ IFN-γ-producing Th1 cells (Figs. 1D, 4D), indicating ongoing immune activation. These data implied that Tregs failed to inhibit the development of effective immune responses in vivo when LIGHT was overexpressed on T cells.

LIGHT can help to expand Tregs
One hypothesis for LIGHT-induced colitis is that LIGHT can directly inhibit Tregs. Most previous studies focused primarily on
the role of LIGHT in the expansion of Teffs. We now tested whether LIGHT might regulate Treg expansion. WT CD4+/CD25+ Tregs were stimulated with anti-CD3 alone or anti-CD3 plus recombinant LIGHT in vitro. Tregs were insensitive to anti-CD3 alone stimulation; however, in the presence of LIGHT, Tregs showed proliferation, especially at relatively high anti-CD3 concentrations (Fig. 2A). This effect is relatively specific for LIGHT, because anti-CD3 plus anti-CD28 (without exogenous IL-2) did not cause Treg proliferation (Fig. 2A). Thus, it seems that LIGHT not only can promote Teff expansion but also can promote Treg response to Ag stimulation. To further confirm this, we used another in vitro culture system in which APCs were present. CD4+/CD25+ Tregs from WT or LIGHT Tg mice were mixed with WT BMDCs (as APCs) and stimulated with anti-CD3. CD4+/FoxP3+ real Treg proliferation then was determined by BrdU incorporation 2 d after incubation. LIGHT Tg Tregs had higher percentages of cells incorporating BrdU (Fig. 2B), which suggested that LIGHT indeed could promote Treg expansion in response to Ag stimulation.

Consistent with this notion, the numbers and percentages of CD4+/FoxP3+ Tregs were increased in LIGHT Tg mice compared with those in WT mice (Fig. 2C and data not shown). Furthermore, draining lymph node Tregs from LIGHT Tg mice showed higher percentages of cells incorporating BrdU after immunization with CFA plus myelin oligodendrocyte glycoprotein (MOG) peptide (Fig. 2D), which are consistent with a role for LIGHT in promoting Treg expansion during inflammation.

The receptor responsible for LIGHT-mediated costimulation of Treg then was tested in vitro by using a blocking Ab to HVEM, which is expressed on Tregs (21). Anti-HVEM Ab treatment diminished the costimulatory effect of LIGHT on Tregs (Fig. 2E), indicating a role for HVEM in mediating the effect of LIGHT on Tregs.

To determine whether LTβR that expressed on APCs can indirectly influence the LIGHT effect on Tregs, CD4+/CD25+ Tregs from WT mice were mixed with WT or LTβR knockout (KO) BMDCs and stimulated with anti-CD3 and LIGHT. Treg proliferation was similar between WT and LTβR KO groups (Fig. 2F), which suggested that LTβR signals probably played a minor role in LIGHT promoting Treg expansion.

**LIGHT can increase FoxP3 expression level**

We next addressed whether LIGHT stimulation could affect the amount of FoxP3 in Treg cells. It is known that FoxP3 expression can be increased by anti-CD3 plus anti-CD28 stimulation in vitro (22). As shown in Fig. 3A, FoxP3 expression was also increased by anti-CD3 plus LIGHT stimulation. In vivo, slightly increased FoxP3 expression could be found in the mesenteric lymph nodes in the LIGHT Tg mice that had not gone through any passive immunization manipulation (Fig. 3B). Furthermore loss of LIGHT expression on T cells leads to a reduction of FoxP3 expression during inflammation. This was demonstrated in Fig. 3C; as we transferred WT or LIGHT/−/− T cells into RAG-1/−− mice, respectively, and then challenge the mice with CFA plus MOG, LIGHT/−/− T cells had reduced FoxP3 expression 7 d after immunization (FoxP3 mean fluorescence intensity; LIGHT/−/− 367.25 ± 38.03 versus WT 581 ± 67.02). Thus, LIGHT stimulation could upregulate FoxP3 expression.

**Inhibitory function of LIGHT-expanded Tregs is not reduced**

To test the functional impact of LIGHT on Tregs, we assessed the ability of WT and LIGHT Tg CD4+/CD25+ Tregs to suppress the proliferation of WT CD4+CD25+ Teffs in vitro. Tregs from LIGHT Tg mice exhibited potent inhibitory activity, and the capacity for suppression from LIGHT-expanded Tregs was similar to (or slightly stronger than) that of WT Tregs (Fig. 4A). We also tested the ability of LIGHT Tg Tregs to suppress the proliferation of WT or Tg Teffs. LIGHT Tg Tregs showed similar capability to suppress both LIGHT Tg Teffs and WT Teffs (Fig. 4B). These results implied that the inhibitory function of LIGHT-expanded Tregs was normal and not reduced.

**LIGHT-expanded Tregs distribute normally**

To test whether LIGHT stimulation could influence Treg migration ability, we checked the Treg distribution pattern 20 d after WT or LIGHT Tg CD4+ T cell transfer to RAG-1/−− mice. Similar patterns

**FIGURE 2.** LIGHT can help to expand Tregs. A, CD4+/CD25+ Treg populations from naive WT mice were stimulated with anti-CD3 alone, anti-CD3 plus recombinant LIGHT, or anti-CD3 plus anti-CD28 at the indicated concentrations. The proliferation was determined by [3H]thymidine incorporation. Tregs proliferated significantly at relatively high concentrations of anti-CD3 with LIGHT compared with anti-CD3 alone (*p < 0.05, **p < 0.005). B, WT or LIGHT Tg CD4+/CD25+ Tregs were mixed with WT BMDCs and stimulated with anti-CD3 (1 μg/ml) for 2 d. The proliferation of Tregs was monitored by pulsing BrdU during the last 12–14 h of incubation. C, Blood and spleen cells from WT or LIGHT Tg mice were stained with anti-CD4 and anti-FoxP3, and the percentages of CD4+/FoxP3+ Tregs were increased in LIGHT Tg mice compared with those in WT mice (Fig. 2C and data not shown). Furthermore, draining lymph node Tregs from LIGHT Tg mice showed higher percentages of cells incorporating BrdU after immunization with CFA plus myelin oligodendrocyte glycoprotein (MOG) peptide (Fig. 2D), which suggested that LIGHT indeed could promote Treg expansion in response to Ag stimulation.

Consistent with this notion, the numbers and percentages of CD4+/FoxP3+ Tregs were increased in LIGHT Tg mice compared with those in WT mice (Fig. 2C and data not shown). Furthermore, draining lymph node Tregs from LIGHT Tg mice showed higher percentages of cells incorporating BrdU after immunization with CFA plus myelin oligodendrocyte glycoprotein (MOG) peptide (Fig. 2D), which are consistent with a role for LIGHT in promoting Treg expansion during inflammation.

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TGF-β/liveration was still evident even in the presence of high amounts of Ag levels are high. The effect of LIGHT in promoting T cell proliferation in a Treg-enriched environment. We first used anti-CD3 (shaded area) or LIGHT KO (black line) mice were analyzed by FACS. Immunization, draining lymph node CD4 T cell FoxP3 levels from WT CD4+CD25+ Tregs. Indeed, Teffs had higher percentage of cells with TGF-β/liveration ability in the presence of LIGHT Tg Teffs by [3H]thymidine incorporation. Data are representative of two experiments. B, Tg CD4+CD25+ Tregs were mixed with BMDCs and WT or Tg Teffs at the indicated ratios, then stimulated with anti-CD3 (1 μg/ml) for 72 h. The proliferation was determined by [3H]thymidine incorporation. The percentage of suppression was calculated by the following formula: (the cpm value without Tregs – the cpm value with Tregs)/the cpm value without Tregs × 100. C, Naive WT or LIGHT Tg total CD4 T cells were transferred into RAG-1−/− mice (n = 3–4 per group). Twenty days after transfer, the numbers of CD4+FoxP3+ Tregs in the indicated tissues in each group then were determined by FACS analysis. Data are representative of two experiments.

FIGURE 3. LIGHT can increase the FoxP3 expression level. A, CD4+ CD25+ Teff populations from naive WT mice were stimulated with anti-CD3 alone (Ctrl), anti-CD3 plus recombinant LIGHT, or anti-CD3 plus anti-CD28 overnight, respectively. The FoxP3 level was then monitored by FACS analysis. B, FACS analysis of CD4 T cell FoxP3 level from 2-mo-old WT (shaded area) or LIGHT Tg (black line) mesenteric lymph nodes. C, Four million WT or LIGHT KO T cells were transferred to RAG-1−/− mice, respectively (n = 4 per group); the mice were then challenged s.c. with 100 μg per mouse of MOG peptide in CFA. Seven days after immunization, draining lymph node CD4 T cell FoxP3 levels from WT (shaded area) or LIGHT KO (black line) mice were analyzed by FACS.

LIGHT-mediated CD4+FoxP3+ Teff proliferation in a Treg-enriched environment

Because LIGHT can promote immune responses in the presence of Tregs but does not inhibit Treg inhibitory function and distribution, we then assessed whether LIGHT can instead promote CD4+FoxP3+ Teff proliferation in a Treg-enriched environment. We first used anti-CD3 alone or anti-CD3 plus recombinant LIGHT to stimulate CFSE-labeled WT CD4+CD25− Teffs in vitro in the presence of APCs and WT CD4+CD25+ Tregs. Indeed, Teffs had higher percentage of cells in the CFSElow population in the presence of LIGHT, indicative of better proliferation (Fig. 5A). We also tested WT Teff and LIGHT Tg Teff proliferation ability in the presence of LIGHT Tg Tregs by [3H] thymidine incorporation. Impressively, even in the presence of 30–40% Tregs in the coculture system, LIGHT Tg Teffs could still proliferate reasonably well, whereas WT Teffs proliferated poorly (Fig. 5B). This is probably due to a costimulatory function of LIGHT on Teffs, which raises the basal level of proliferation on Teffs. This would make it difficult for Tregs to inhibit them completely, especially when Ag levels are high. The effect of LIGHT in promoting T cell proliferation was still evident even in the presence of high amounts of TGF-β (2 ng/ml) in vitro, although the magnitude of the proliferation was much lower than that without TGF-β (compare Fig. 5C, right panel with left panel). This small amount of T cell activation by LIGHT in a strong suppressive environment correlates well with the relatively slow manifestation of colitis in the LIGHT Tg mice, in which it takes ~4–5 mo after birth for the development of pathological obvious colitis (12).

To further confirm that LIGHT can promote CD4+FoxP3− Teff proliferation in vivo, we repeated the WT and LIGHT Tg naive CD4 T cell transfer experiment. Indeed, the LIGHT Tg Teff number transferred into RAG-1−/− hosts increased dramatically (Fig. 5D), even in the presence of an expanded Treg population. These results together implied that LIGHT could promote Teff proliferation even in a Treg-abundant environment.

Tregs are known to be a dominant inhibitory factor for preventing effective tumor immunity, and it is practically very difficult to reactivate cytotoxic T cells in the presence of a large number of pre-existing intratumoral Tregs. The ability of LIGHT to promote immune activation in the presence of pre-existing Tregs was then explored in a tumor setting. Ag104Ld is a fibrosarcoma tumor line, in which Tregs contributed significantly to the tumor development, and depletion of Tregs in the tumor led to tumor rejection (23). Intratumoral treatment of the Ag104Ld tumor by injection of LIGHT-expression adenovirus (Ad-LIGHT) led to an expansion of lymphocyte populations (Fig. 6A, upper panel) and thus accordingly increased Treg numbers inside the tumors at day 3 post-treatment. Furthermore, the FoxP3 expression level was also higher in the Ad-LIGHT-treated group than that in the control group at day

FIGURE 4. LIGHT-expanded Treg inhibitory function is not reduced. A, WT CD4+CD25− naive Teffs were mixed with BMDCs and WT or Tg CD4+CD25+ Tregs and stimulated with anti-CD3 (1 μg/ml) for 72 h. The proliferation was determined by [3H]thymidine incorporation. Data are representative of two experiments. B, Tg CD4+CD25+ Tregs were mixed with BMDCs and WT or Tg Teffs at the indicated ratios, then stimulated with anti-CD3 (1 μg/ml) for 72 h. The proliferation was determined by [3H]thymidine incorporation. The percentage of suppression was calculated by the following formula: (the cpm value without Tregs – the cpm value with Tregs)/the cpm value without Tregs × 100. C, Naive WT or LIGHT Tg total CD4 T cells were transferred into RAG-1−/− mice (n = 3–4 per group). Twenty days after transfer, the numbers of CD4+ FoxP3+ Tregs in the indicated tissues in each group then were determined by FACS analysis. Data are representative of two experiments.
FIGURE 5. LIGHT can promote Teff proliferation in a Treg-enriched environment. A, CFSE-labeled naive WT CD4+CDSF5− Tcells were stimulated with anti-CD3 alone (dashed line) in the presence of WT BMDCs as APCs or further mixed with WT CD4+CDSF5− Tregs (Teffs/Tregs = 2:1) and stimulated with anti-CD3 (1 μg/ml) alone (shaded area) or anti-CD3 plus LIGHT (black line) for 2 d; CFSE dilution was monitored by FACS analysis. B, WT or LIGHT Tg CD4+CDSF5− T cells were mixed with BMDCs and with or without LIGHT Tg CD4+CDSF5− Tregs (Teffs/Tregs = 2:1) and stimulated with anti-CD3 (1 μg/ml) for 3 d. The proliferation was determined by [3H] thymidine incorporation. (p < 0.05) C, Naive WT spleen cells were stimulated with anti-CD3 at the indicated concentrations with or without recombiant LIGHT (1 μg/ml) and in the absence (left panel) or the presence (right panel) of TGF-β (2 ng/ml). The proliferation was determined by [3H] thymidine incorporation 3 d after incubation. D, Purified naive WT or LIGHT Tg CD4 T cells from 1- to 2-mo-old animals were transferred into Rag-1−/− mice (n = 3-4 per group); total numbers of CD4+FoxP3− Tcells were determined 20 d after transfer. Data are representative of two experiments.

3 posttreatment (Fig. 6A, lower panel), which confirms the ability of LIGHT to influence FoxP3 expression levels. However, this transient local LIGHT stimulation did not preferentially expand Tregs, because the percentage of Tregs in the Ad-LIGHT-treated tumor was similar to that in the control group at day 3 posttreatment (Fig. 6A, lower panel). Interestingly, the percentage of Tregs inside the tumor kept increasing with time and reached 18–20% among tumor-infiltrating lymphocytes in the Ad-Null-treated control group at day 9 posttreatment, possibly due to a favorable intratumoral environment for Treg development, whereas the percentage of Tregs remained unchanged in the Ad-LIGHT-treated tumor (Fig. 6B). In contrast, CD4+ Tcells that could secrete IFN-γ were dramatically increased at this time in the Ad-LIGHT-treated tumor (Fig. 6B), indicating an ability of LIGHT to promote Teff proliferation and differentiation in the presence of abundant Tregs. More importantly, IFN-γ-secreting CD8+ tumor-killing T cells also were expanded greatly by the Ad-LIGHT treatment (Fig. 6C), which might contribute directly to the effect of Ad-LIGHT treatment in inducing this tumor rejection as we reported previously (24). Thus, we provide a scenario in which LIGHT could promote antitumor immunity in a suppressive tumor environment.

Discussion
Tregs play a key role in negative regulation of host immune responses. Overexpression of LIGHT on T cells breaks down the Treg-mediated immunosuppressive state and induces strong T cell-mediated intestinal inflammation (12, 13). How this process is initiated by LIGHT in such suppressive intestinal environments is enigmatic. It was hypothesized that LIGHT might suppress Treg function or expansion. In this paper, we reported that overexpression of LIGHT could actually support the expression of FoxP3 and Treg expansion. The expanded Tregs by LIGHT could still suppress Teffs efficiently. Intriguingly, overexpression of LIGHT also provided Teffs a means of expansion and differentiation even in a Treg-enriched environment. Thus, the net effect of overexpression of LIGHT is immune activation. It has been a puzzle why large numbers of Tregs can be found at sites of chronic inflammation, where Tregs are supposed to inhibit immune activation (25, 26). Our findings could potentially explain this paradox and implicated that a costimulatory molecule probably plays a role in promoting both Tregs and Teffs at the sites of inflammation.

It should be mentioned that nonimmunized naive LIGHT KO mice did not have reduced Tregs (data not shown), which indicated a nonessential role for LIGHT in Treg homeostasis during the naive condition. However, this does not necessarily rule out a biological role for LIGHT on Tregs in vivo, especially considering possible compensatory mechanisms by other cytokines. Furthermore, under lymphopenic conditions transferred LIGHT KO Tregs had
significantly reduced FoxP3 expression (Fig. 3C), which arguably indicated a role for LIGHT on Tregs at least during some pathological or pharmacological conditions. The roles for LIGHT on Tregs in various disease models remain to be determined.

The costimulatory effect of LIGHT on Tregs is probably mediated through HVEM, because anti-HVEM can block this effect in vitro. However, HVEM also can act as a ligand on Tregs to interact with the B and T lymphocyte attenuator expressed on Teffs to suppress Teff activation (21). Thus, HVEM plays an important role in regulating various Treg functions dependent on which cell and molecular interactions are formed. Interestingly, HVEM KO mice had more Tregs than the WT control mice after CFA and MOG challenge (data not shown), which suggested that the interaction of HVEM with B and T lymphocyte attenuator was dominant in vivo.

Our data indicate that LIGHT costimulation on conventional T cells overcomes the suppression by Tregs and TGF-β. The potential mechanisms remain to be fully investigated. It does not seem to be through reduced TGF-β signaling, because Teffs activated by TCR stimulation expressed more TGF-β than naive T cells and LIGHT costimulation did not further influence its expression level (data not shown). Our current model is that LIGHT costimulation could dramatically reduce the activation threshold for Teffs and make Tregs less efficient in completely shutdown of Teff activation. Accumulation of activated Teffs chronically in the intestine might ultimately lead to colitis in LIGHT Tg mice.

Selective depletion of Tregs has been suggested to be an attractive therapeutic method in promoting tumor immunotherapy (27). However, many technical and theoretical challenges remain. Our study implied that overexpression of LIGHT inside a tumor could be a very promising way to improve tumor immunity even without depleting Tregs. In our Ag104L4 tumor model where Tregs are able to inhibit tumor immunity with tumor progress (23), activation of TLR by LPS inside the tumor failed to unmask tumor immunogenicity and induce tumor rejection (P. Yu, Y. Wang, and Y. Fu., unpublished data), although it has been reported that TLR activation can block Treg inhibition and promote adaptive immune activation (28). However, overexpression of LIGHT inside the tumor can effectively induce tumor rejection (24), revealing that LIGHT has interesting properties in promoting immune activation in a Treg-enriched environment. There might be multiple reasons for this. First, it is possible that LIGHT can work through dendritic cells in initiating an immune response. Indeed, earlier studies indicated that LIGHT can promote dendritic cell expansion and maturation, especially when in cooperation with CD40L (29, 30). Second, LIGHT can induce the expression of chemokines by stromal cells to attract T cells migrating into the tumors (31). Third, LIGHT can promote Teff expansion and differentiation, even in a Treg-enriched environment, as we have shown in this paper. Thus, LIGHT can bridge innate cells and adaptive cells to promote immune activation. This property makes LIGHT an intriguing tool for vaccine development and tumor immunotherapy. However, blocking LIGHT also might be effective in preventing the devastating immune responses that occur during autoimmune inflammatory diseases.

In summary, these data provide evidence that overexpression of LIGHT can help immune activation even in a highly Treg-enriched environment. LIGHT thus is likely to play important roles in IBD pathogenesis and could be a very attractive candidate for a therapeutic intervention. Understanding the role of LIGHT involvement in various pathogeneses thus will provide us with more effective ways to control autoimmunity, tumor immunity, and other inflammatory responses.

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Disclosures
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References


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