The regulation of T cell homeostasis and autoimmunity by T cell–derived LIGHT

See related Commentary on pages 1741–1742.

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Costimulatory molecules on antigen-presenting cells (APCs) play an important role in T cell activation and expansion. However, little is known about the surface molecules involved in direct T-T cell interaction required for their activation and expansion. LIGHT, a newly discovered TNF superfamily member (TNFSF14), is expressed on activated T cells and immature dendritic cells. Here we demonstrate that blockade of LIGHT activity can reduce anti-CD3–mediated proliferation of purified T cells, suggesting that T cell–T cell interaction is essential for this proliferation. To test the in vivo activity of T cell–derived LIGHT in immune homeostasis and function, transgenic (Tg) mice expressing LIGHT in the T cell lineage were generated. LIGHT Tg mice have a significantly enlarged T cell compartment and a hyperactivated peripheral T cell population. LIGHT Tg mice spontaneously develop severe autoimmune disease manifested by splenomegaly, lymphadenopathy, glomerulonephritis, elevated autoantibodies, and severe infiltration of various peripheral tissues. Furthermore, the blockade of LIGHT activity ameliorates the severity of T cell–mediated diseases. Collectively, these findings establish a crucial role for this T cell–derived costimulatory ligand in T cell activation and expansion; moreover, the dysregulation of T cell–derived LIGHT leads to altered T cell homeostasis and autoimmune disease.

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Introduction

Members of the TNF/TNF receptor (TNF/TNFR) superfamily play multiple roles in the activation and homeostasis of immune cells. First, TNF/TNFR members coordinate the lymphoid microenvironment required for immune responses. Lymphotoxin (LT) and TNF are essential in the development and organization of secondary lymphoid tissues and ectopic lymphoid neogenesis (1–7). Second, TNF/TNFR members participate in the regulation of immune responses to inflammation, infection, and tumor (4, 8–10). Third, TNF/TNFR members also function as mediators of cell death. The Fas/Fas ligand (Fas/FasL) system is probably the most striking example of a pathway directly involved in the elimination of autoreactive lymphocytes by apoptosis (10–12). Fourth, several of the TNF/TNFR members have been shown to enhance the proliferation of different subsets of lymphocytes in conjunction with antigen receptor stimulation (13–15). For example, BAFF, a newly identified TNF family member (16), also known as TALL-1 (17), THANK (18), and BlyS (19), is a cytokine affecting proliferation, activation, and homeostasis of B cells (14, 19–22). CD40-CD154–mediated contact–dependent signals between B and T cells are required for the generation of thymus-dependent humoral immune responses (13, 23). Therefore, upregulation of TNF family members may provide a costimulatory mechanism to increase the proliferation of lymphocytes in an autocrine or paracrine fashion upon stimulation with antigen.

The well characterized costimulatory pathway for optimal T cell activation involves the T cell surface molecule CD28, which responds to the costimulatory molecules B7-1 (CD80) and B7-2 (CD86) expressed on activated antigen-presenting cells (APCs) (24). Previous studies have shown that murine B7 molecules could costimulate with anti-CD3 mAb or concanavalin A (ConA) to induce T cell activation (25–27). $CD28^{-/-}$ mice have impaired responsiveness to ConA, suggesting that the interaction of B7 and CD28 is critical for the APC-dependent T cell activation (28). Cytotoxic T-lymphocyte antigen-4–Ig (CTLA4-Ig), a soluble receptor for B7, could block ConA and anti-CD3 mAb–induced proliferation in splenocytes or lymph node (LN) cells (28–30). However, cultures of T cells that had been rigorously depleted of accessory cells were found to proliferate in a B7-independent manner (29). These experiments, therefore, raise two possibilities: that an APC-derived costimulatory signal may be unnecessary under some circumstances, such as direct cross-linking of the TCR, or that T cells may be able to provide costimulation to each other via the ligand(s) and receptor(s) expressed on T cells themselves. However, it is unclear whether such

additional costimulatory molecules are present and whether the ligation of these molecules by T cell–derived costimulatory ligand(s) is required for further activation and/or expansion of T cells.

LIGHT, a recently identified TNF family member, is upregulated on activated T cells and downregulated on mature dendritic cells (DCs) (31–33). LIGHT can bind two receptors: LTβR, expressed on stromal cells and nonlymphoid hematopoietic cells (2, 34), and herpes virus entry mediator (HVEM), expressed on T, B, and other hematopoietic cells (31, 35–37). Recombinant LIGHT has been shown to costimulate T cell proliferation in combination with anti-CD3 mAb in vitro (33, 38). In particular circumstances, LIGHT can induce the apoptosis of certain tumor cell lines via LTβR and HVEM (32, 39).

The potential role of T cell–derived LIGHT in T cell homeostasis and T cell–associated diseases was explored in this study. Our data suggest an essential role for T cell–derived LIGHT in the activation and expansion of T cells in vitro. LIGHT transgenic (Tg) mice expressing LIGHT under the control of a T cell lineage–specific promoter and enhancer spontaneously develop fatal autoimmune disease caused by hyperactivity of T lymphocytes. Furthermore, the blockade of LIGHT activity ameliorates the severity of T cell–mediated diseases, including spontaneous autoimmune diabetes. These findings reveal that a T-T cell interaction through LIGHT is required for the complete expansion of T cells, and that the dysregulation of LIGHT activity results in the disturbance of T cell homeostasis and ultimately in the breakdown of peripheral tolerance.

Methods

Transgenic mice. LIGHT cDNA was initially cloned by RT-PCR into pCDNA3.1, then inserted into the AscI site of plck.E2 (a generous gift from T. Hettmann, The University of Chicago), which contains the proximal *lck* promoter, human growth hormone gene (polyadenylation site), and locus control region elements from the human CD2 gene. An 8-kb fragment was excised by NotI and used for subsequent microinjection performed by the University of Chicago Cancer Research Center Transgenic Mice Facility. PstI-digested tail DNA from mice was hybridized to a radiolabeled 0.7-kb LIGHT-specific probe. Four positive founders were generated with approximate copy numbers of 19, 5, 16, and 6 copies determined by Southern blot, two in a C57BL/6 (B6) background and the other two in a C3H background. Four independent lines were generated from four founders, and the extent of the phenotypes was correlated with the copy number of the transgene. All the mice used in this study were in B6 background, and the representative picture shown was from the B6 founder with a higher copy number. LIGHT protein expression was detected in thymocytes, splenocytes, and LN cells from Tg mice by FACS with LTβR-Ig (40) and anti-LIGHT antibody (33).

Antibodies and flow cytometric analysis. The following antibodies were used for double or triple color staining: anti-CD3-PE, anti-CD3-FITC, anti-CD19-PE, anti-B220-FITC, anti-CD62L-PE, anti-CD44-Cyc, anti-CD69-FITC, anti-CD8-FITC, anti-CD8-PE, anti-CD4-FITC, anti-CD4-Cyc, anti-CD11b-biotin, and anti-Gr-1-biotin (PharMingen, San Diego, California, USA). The single-cell suspensions of splenocytes and LN cells were washed in PBS plus 0.1% NaN_3, and analyzed on a FACScan and Cell Quest Software (both from Becton Dickinson Immunocytometry Systems, Mountain View, California, USA). For intracellular cytokine staining, single-cell suspensions from LNs were stimulated with 50 ng/ml PMA plus 500 ng/ml ionomycin for 4 hours at 37°C in the presence of 20 μg/ml brefeldin A. After fixation in 4% formaldehyde, the cells were stained intracellularly for IFN-γ or IL-4 (PharMingen) in the presence of 0.5% saponin for cell permeabilization, followed by staining of the surface marker CD3.

ELISPOT. ELISA spot plates (Cellular Technology, Cleveland, Ohio, USA) were coated with rat anti-mouse IFN-γ antibodies (2 μg/ml) (BD PharMingen, San Diego, California, USA). Plates were blocked with PBS/0.1% BSA and washed with PBS. Splenocytes from wild-type (WT) and Tg mice were added at various concentrations (0, 2×10^5, 5×10^5, and 1×10^6 per well) and cultured for 16 hours at 37°C. Detecting antibodies (biotin-conjugated rat anti-mouse IFN-γ; BD PharMingen) were added and incubated for 2 hours at room temperature, followed by incubation with anti-biotin–alkaline phosphatase (anti-biotin-AP). Color development was performed with nitroblue tetrazolium substrate solution (Sigma Chemical Co., St. Louis, Missouri, USA).

Fusion proteins and in vitro proliferation assays. Mouse HVEM-Ig fusion protein was generated by RT-PCR amplification of a cDNA encoding the HVEM extracellular domain from mouse splenocytes (the sense primer 5′-ACGCGGAATTCTTCTTGATCAAGAAAATGGAACCTCTC-3′, the antisense primer 5′-GTAGATAGATCTTGGGAGGAGCAGGTGGTGTCTGT-3′). The PCR fragment was inserted into pMIgV vector containing cytomegalovirus promoter, the Fc portion of murine IgG2a, and a dihydrofolate reductase selection marker. The construct was transfected into Chinese hamster ovary cells. The transfectants were then used for the generation of fusion protein produced by National Cell Culture Center (Minneapolis, Minnesota, USA). LTβR-Ig used in this study has been described previously (40). In brief, cDNA encoding the extracellular domain of murine LTβR was fused with the Fc portion of human IgG1, then transfected into BHK/VP16 cells. CTLA4-Ig consists of murine CTLA4 extracellular domain fused with murine IgG2a Fc portion (a generous gift from Ken Newell, Emory University, Atlanta, Georgia, USA). Control Ig's used in this study were murine IgG and human IgG (Sigma Chemical Co.). Both control Ig's behaved similarly in the proliferation assay, and the data from the murine IgG group are shown in Figure 1. Anti-CD3 mAb is the hamster mAb 145-2C11 (a generous gift from Jeff Bluestone, University of California at San Francisco, San Francisco, California, USA). LN cells or splenocytes from WT B6 mice (2×10^5 per well) were

stimulated with ConA (1.5 μg/ml; Sigma Chemical Co.) in the presence of control Ig, HVEM-Ig, LTβR-Ig, or CTLA4-Ig at concentrations of 10, 30, or 100 μg/ml for 48 hours, pulsed with 1 μCi of [^{3}H]thymidine for 16 hours, and then harvested for liquid scintillation counting. For the purified T cell proliferation assay, splenocytes and LN cells were pooled and passed through a nylon wool column (Polysciences Inc., Warrington, Pennsylvania, USA). The eluted cells from the nylon wool column (~80% T cells) were further enriched by immunomagnetic column by a negative depletion method (Stem Cell Technologies Inc., Vancouver, British Columbia, Canada). The purity of the enriched T cells reached more than 99% consistently as determined by staining with anti-CD3-FITC antibody. The purified T cells (2×10^5 per well) were stimulated with immobilized anti-CD3 mAb (1 μg/ml) in the presence of control Ig, CTLA4-Ig, or HVEM-Ig (30 or 100 μg/ml), pulsed, and harvested as described above. For GM-CSF responder proliferation assay, the splenocytes (2×10^5 per well) from WT littermates or Tg mice were cultured with GM-CSF at the concentration of 2, 10, or 50 U/ml (R&D Systems Inc., Minneapolis, Minnesota, USA) for 48 hours, pulsed, and harvested as described above.

Histological, immunohistochemical, and immunofluorescence staining. Indicated tissues were fixed with 10% formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin (H-E) or periodic acid-Schiff (PAS) by standard methods. Immunohistochemical staining was performed as previously described (41). For immunofluorescence studies of deposition of Ig's, kidneys were embedded in OCT compound (Miles Inc., Elkhart, Indiana, USA) and snap-frozen at –70°C. Four- to five-micrometer sections were air-dried and fixed with acetone, then pretreated with goat serum and stained with FITC-conjugated goat anti-mouse IgG (H+L) (Caltag Laboratories Inc., Burlingame, California, USA) or FITC-conjugated goat anti-mouse Ig light chain (Sigma Chemical Co.).

Detection of autoantibodies and rheumatoid factors. For detection of anti-DNA autoantibodies, ELISA plates (Dynex Technologies, Chantilly, Virginia, USA) were coated with DNA (250 μg/ml) from herring sperm (Sigma Chemical Co.). Plates were washed with PBS/0.05% Tween-20 and blocked with PBS/0.1% BSA. Serum samples were diluted at various concentrations and bound antibodies were detected with AP-conjugated goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, Alabama, USA). The OD was measured at 405 nm by a spectrophotometer (Molecular Devices, Menlo Park, California, USA). For the detection of total IgG, goat anti-mouse Ig (H+L) (Southern Biotechnology Associates) was coated and the same detecting antibody, goat anti-mouse IgG-AP, was used. For the detection of rheumatoid factor, a panel of purified mouse IgG1, IgG2a, IgG2b, and IgG3 was used for coating and AP-conjugated goat anti-mouse IgM was used as a detecting antibody (Southern Biotechnology Associates).

In vivo treatment protocol. For the insulin-dependent diabetes mellitus (IDDM) model, 5- to 6-week-old female NOD mice were given 100 μg of HVEM-Ig or control Ig (murine IgG; Sigma Chemical Co.) intraperitoneally each week for 3 weeks. The glucose concentration in blood obtained from a tail vein was measured weekly using SureStep strips (Johnson & Johnson, Milpitas, California, USA). Animals were considered diabetic after two consecutive measurements of ≥ 250 mg/dl.

Results

T cell–derived LIGHT functions as a costimulatory molecule for expansion of T cells. ConA has been used extensively as a T cell–specific mitogen to study T cell activation, which induces T cell activation via an APC-dependent mechanism (42, 43). To test whether the interaction between LIGHT and its receptor(s) is required for the activation/expansion of T cells, splenocytes or LN cells from WT mice were cultured with ConA in the presence of HVEM-Ig, LTβR-Ig, CTLA4-Ig, or control Ig. Interestingly, the inclusion of HVEM-Ig or LTβR-Ig into the culture system significantly inhibited the T cell response to ConA stimulation as compared with control Ig (Figure 1a). Consistent with previous studies (28, 30), CTLA4-Ig profoundly blocked the ConA-induced T cell proliferation (Figure 1a). In addition, the T cell responses to anti-CD3 mAb were also substantially inhibited by HVEM-Ig and LTβR-Ig (data not shown). These results suggest that LIGHT is required for an optimal T cell response.

Anti-CD3 mAb can directly cross-link the T cell receptor (TCR) complex and stimulate T cell proliferation in an APC-independent way. To directly evaluate the role of T cell–derived LIGHT in regulating the response of T cells, WT T cells that had been rigorously depleted of APC were used to test their ability to respond to an optimal dose of anti-CD3 (Figure 1b). Interestingly, the blockade of LIGHT by HVEM-Ig dramatically reduced T cell proliferation in our APC-free system even in the presence of a strong TCR stimulus (Figure 1c). In addition to purified T cells, similar results were obtained using thymocytes (data not shown). These data suggest that T cell–derived LIGHT plays an essential role in the activation and subsequent expansion of T cells, which probably involves T-T cell interactions via LIGHT and its receptor. In contrast, CTLA4-Ig did not show an inhibitory impact on the proliferation of T cells in our APC-free system (Figure 1c), which was also shown by a previous study (29). Taken together, our results are consistent with the notion that LIGHT from T cells is required for T cell activation/expansion via T-T cell–dependent interactions, whereas B7-1 and B7-2 from APCs are more important for inducing T cell responses through APC–T cell interactions.

Generation of LIGHT Tg mice in the T cell lineage. LIGHT is selectively expressed on immature DCs or activated T cells (31, 38). It is difficult to discriminate the contribution of immature DC-derived or T cell–derived

LIGHT to the homeostasis and responses of T cells in vivo, since both cell types express the costimulatory molecule. To investigate the role of T cell–derived LIGHT in the expansion of T cells in vivo, we generated Tg mice that constitutively express the LIGHT protein under the control of the proximal *lck* promoter and CD2 enhancer, which gives rise to a T cell lineage–specific expression of LIGHT (44, 45). Higher LIGHT protein expression was observed in the splenocytes and LN cells of Tg mice compared with WT mice when studied by flow cytometry using LTβR-Ig and anti-LIGHT antibody (data not shown). Lines 24 and 27 in a B6 background were selected for further investigation. Similar phenotypes were observed in both lines, and the results described here are therefore not distinguished between them.

T cell–derived LIGHT is sufficient to promote the expansion of T cells in vivo, leading to the severe enlargement of secondary lymphoid tissues. Striking phenotypes were observed in LIGHT Tg mice by 5–6 months old, and these mice spontaneously developed lymphoproliferative disorder manifested by splenomegaly and lymphadenopathy (Figure 2a). The size of the spleen was increased in LIGHT Tg mice (88.5 ± 17.9 mg in WT vs. 339.6 ± 82.4 mg in Tg mice, $n = 7$, $P < 0.001$), and similar phenotypes were seen in the Tg LNs. The total number of splenocytes was also increased ($78.5 \times 10^6 \pm 13.5 \times 10^6$ in WT vs. $151.6 \times 10^6 \pm 27.7 \times 10^6$ in Tg mice, $P < 0.01$), and a more significant increase in the cell number was observed in LNs (sevenfold increase in Tg compared with WT mice) (Figure 2b). In contrast to mice transgenic for BAFF, another TNF family member, which had enlarged secondary lymphoid tissues caused by the expanded B cell compartment (14, 16, 46), most expansion occurred in the T cell compartment of LIGHT Tg mice. The ratio of T to B cells was abnormally increased in the spleen and LNs of LIGHT Tg mice, suggesting a significantly enlarged T cell compartment in Tg mice (Figure 2c). Immunohistochemical analysis with T and B cell–specific markers showed that the T cell zone increased remarkably in the LNs of Tg mice (Figure 2d). Thus, LIGHT Tg mice clearly showed the signs of an expanded peripheral T cell compartment, suggesting that T cell–derived LIGHT is sufficient to cause the expansion of peripheral T cells in vivo.

Hyperactivation of T lymphocytes mediated by T cell–derived LIGHT. Lymphocytes isolated from Tg mice showed overall blastogenic activation as evidenced by an increase in the forward light scattering properties of these cells compared with WT lymphocytes. To investigate whether overexpression of LIGHT in the T cell lineage could affect the activation status of peripheral T cells, we examined the peripheral T cells in Tg mice by flow cytometry using the activation markers for T cells. CD69 and CD25 (IL-2Rα) are considered to be early activation markers for T cells. CD62L (L-selectin) is expressed at high levels on naive T lymphocytes, and its expression is reduced on activated T cells, whereas CD44 is upregulated on activated cells (47). Therefore, $CD62L^{low}CD44^{high}$ T cells are thought to be activated effector cells. Double and triple stainings were performed using antibodies against these activation markers in conjunction with antibodies against T cell markers such as CD3 or CD4/CD8. The expression of CD69 and CD25 was significantly upregulated in LIGHT Tg T cells, which indicated that Tg T cells were continuously undergoing activation (Figure 3a and data not shown). Moreover, a significant increase of $CD62L^{low}CD44^{high}$ T cells (69.27% in Tg vs. 19.25% in WT) was observed in LIGHT Tg mice (Figure 3b). Taken together, these results demonstrate that constitutive expression of LIGHT on T cells is sufficient for the activation and expansion of peripheral T cells in vivo.

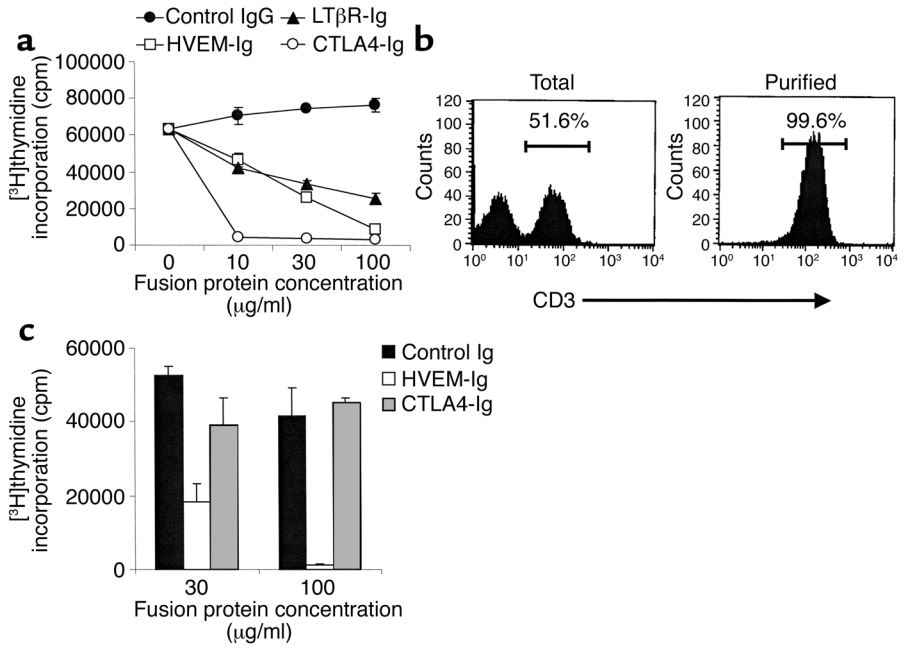

Figure 1

T cell–derived LIGHT functions as a costimulatory ligand in a T-T cell–dependent fashion. (**a**) Splenocytes were collected from WT B6 mice (5–6 weeks old) and cultured with CTLA4-Ig, LTβR-Ig, HVEM-Ig, or control Ig at various concentrations in the presence of ConA (1.5 μg/ml). (**b**) Splenocytes and LN cells were pooled and T cells were purified and stained with anti-CD3-FITC. The percentages of CD3-positive cells are indicated. (**c**) Highly purified T cells were stimulated with immobilized anti-CD3 mAb (1 μg/ml) in the presence of HVEM-Ig, CTLA4-Ig, or control Ig. Proliferation was measured by [^{3}H]thymidine incorporation. The results are representative of three experiments.

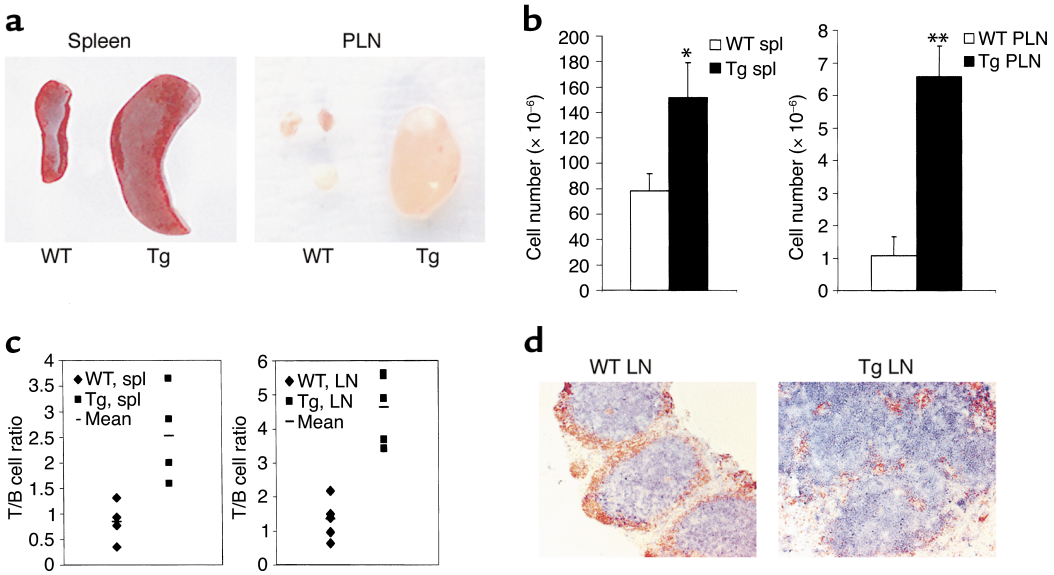

Figure 2

T cell–derived LIGHT is sufficient to cause the expansion of peripheral T cells in vivo. (**a**) Splenomegaly and lymphadenopathy were observed in LIGHT Tg mice at 5–8 months of age. Spleen and axillary LNs were shown. Pictures (×4) are representative of seven mice analyzed for each group. (**b**) The total cell number was increased in the spleens (left panel, $*P < 0.01$) and peripheral LNs (right panel, $**P < 0.001$) of Tg mice. The results were representative of seven mice analyzed in each group. (**c**) FACS analysis of the ratio of T ($CD3^+$) to B cells ($CD19^+$) in the spleen (left panel) and LNs (right panel) of WT (diamonds) and Tg (squares) mice ($n = 5$). (**d**) The enlargement of the T cell zone in LIGHT Tg LNs. Immunohistochemical staining was performed using anti-Thy1.2-Bio for T cells (blue) and anti-B220-FITC for B cells (brown). Representative pictures (×10) are shown. PLN, peripheral LN.

Increased cytokine production and expansion of granulocyte-macrophage lineage in LIGHT Tg mice. One of the principal responses of activated T lymphocytes is the production of cytokines. To evaluate the impact of T cell–derived LIGHT on the function of T cells in vivo, we analyzed the cytokine production of Tg T cells by intracellular staining. LN cells were stimulated in vitro for 4 hours with PMA and ionomycin, stained with antibodies specific for IFN-γ or IL-4, and analyzed by flow cytometry. More than a fivefold increase in IFN-γ–producing T cells was detected in Tg mice compared with WT controls (Figure 4a). The frequency of IFN-γ–producing cells was also examined by ELISPOT. Consistently, there were more IFN-γ–producing cells in Tg splenocyte populations ($73 \pm 6/10^6$ in Tg vs. $14 \pm 3/10^6$ in WT). Tg mice showed more IL-4–producing T cells than did WT mice, as determined by flow cytometry analysis (1.35% in Tg vs. 0.19% in WT).

GM-CSF can also be produced by activated T cells. GM-CSF promotes hematopoiesis and results in the enlargement of the spleen with the preferential increase of GM lineages and activation of mature macrophages and granulocytes. We tested the production of GM-CSF in the culture supernatants of splenocytes and found that there was an approximately threefold increase in the GM-CSF production in Tg mice. Furthermore, more progenitor cells in GM lineage in Tg mice were revealed by histological analysis, characterized by their larger size and eccentrically located nucleus and cytoplasm containing many azurophilic granules (data not shown). Consistent with this notion, the splenocytes from Tg mice were dramatically more responsive to GM-CSF than WT splenocytes were (Figure 4b). The splenocytes were recovered from in vitro culture and analyzed by flow cytometry to identify the different populations of cells expanded by GM-CSF. As we expected, the major populations recovered were macrophages ($CD11b^+$) and granulocytes ($Gr\text{-}1^+$) (data not shown). Similarly, bone marrow (BM) cells from Tg mice were also more responsive to GM-CSF than were those of WT mice (data not shown), suggesting increased GM-CSF–responding precursors in the BM of Tg mice. Our results indicated that there was an increase in GM-CSF production, leading to increased systemic hematopoiesis in the GM lineage, probably due to LIGHT-mediated T cell activation in Tg mice.

Consistent with the above observation, we also identified a distinct non-T and non-B cell population in Tg spleen by flow cytometry analysis. Further analysis of this population showed that most of these cells were $CD11b^+$ or $Gr\text{-}1^+$, indicating an expansion of macrophages and granulocytes in Tg mice (Figure 4, c and d). Therefore, T cell–derived LIGHT is sufficient to cause the expansion of macrophages and granulocytes. Macrophages and granulocytes are the major effector cells of the immune response; together with the participation of these cells, activated T cells may cause the destruction of the peripheral tissues leading to autoimmunity.

LIGHT Tg mice developed severe autoimmune manifestations. Striking phenotypes were consistently observed in LIGHT Tg mice beginning at 5 months, suggesting the potential role of LIGHT in the induction of

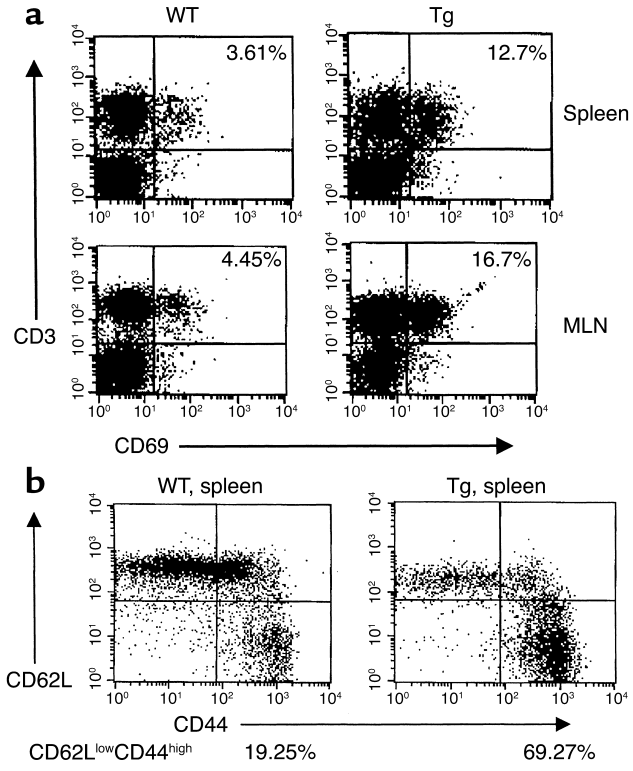

Figure 3

Hyperactivation of T lymphocytes mediated by T cell–derived LIGHT. (**a**) Splenocytes or LN cells from WT and Tg mice were stained with antibodies against CD3 and CD69. Numbers indicate the percentage of $CD3^+CD69^+$ cells. (**b**) Splenocytes from WT and Tg mice were stained with antibodies against CD3, CD62L, and CD44. The dot plots represent cells gated on the $CD3^+$ population. The results are representative of five experiments. WT and Tg mice at the age of 5–8 months were used. MLN, mesenteric LN.

autoimmunity. Interestingly, the examination of seven pairs of Tg and WT mice at the age of 5–8 months showed diffuse thickening of the intestinal wall up to 0.5 cm in the Tg mice, while that of the WT controls showed an average thickness of 0.1 cm. Microscopic examination revealed the dramatic inflammatory cell infiltrate in the lamina propria and submucosa with prominent germinal center formation in a diffuse pattern, which was never present in the control mice (Figure 5a). The infiltrate consisted of predominantly lymphoplasmacytic cells with occasional mast cells and neutrophils (Figure 5a). Scattered plaque-like cutaneous lesions ranging from 0.3 cm to 2 cm along with ulceration and scar formation were observed in virtually all the Tg mice starting at 5 months of age, whereas the control mice of the same age never displayed such phenotypes; ten pairs of Tg and WT mice were carefully examined. Histological sections demonstrated conspicuous mixed acute and chronic inflammatory cell infiltrate extending from epidermis to subcutis (Figure 5b) and aberrant hair follicular proliferation in LIGHT Tg mice. No apparent resemblances were found between the skin lesions in LIGHT Tg mice and human scleroderma, an autoimmune disease caused by the malfunction of the vascular and immune systems resulting in the overproduction of collagen. Such intestinal and cutaneous lesions seem to be unique to B6 LIGHT Tg mice, as they have never been observed in B6-*lpr/lpr* mice, which often begin to develop lymphoproliferative disorder at this age. The inflammatory cell infiltrate was most evident in the gut and skin, but also present in the skeletal muscle, pulmonary interstitia, and portal areas of the liver (data not shown).

More intriguing phenotypes were revealed by renal pathological analysis in Tg mice that spontaneously developed diffuse global proliferative glomerulonephritis involving over 80% of the glomeruli (Figure 6, a and b). The involved glomeruli were diffusely enlarged, demonstrating mesangial prominence and intracapillary and extracapillary proliferation with obliteration of the capillary lumina (Figure 6b). Focal areas of necrosis containing fragmented nuclei (hematoxylin bodies) were also present along with leukocytic infiltration and segmental sclerosis. The significantly thickened basement membrane in Tg mice was highlighted in the PAS staining (Figure 6, c and d). The characteristic diffuse proliferation under light microscopy closely simulates type IV lupus nephritis in systemic lupus erythematosus (SLE) patients. Consistent with this observation, immunofluorescence staining revealed strong diffuse IgG deposition in a coarsely granular pattern in Tg mice (Figure 6, e and f), similar to what is often observed in type IV lupus patients. Immunofluorescence staining against total Ig light chains showed comparable positive staining in a pattern similar to that of IgG (Figure 6, g and h). These findings in the kidneys of Tg mice were further substantiated by the presence of subendothelial and mesangial deposits observed under electron microscopy (data not shown), which is pathognomonic of lupus nephritis. The phenotypes observed in LIGHT Tg mice resemble those in MRL-*lpr/lpr* mice, an established murine model for lupus.

Elevation of autoantibodies both serves as a criterion for the clinical diagnosis of autoimmune disease and has been shown to be characteristic of MRL-*lpr/lpr* mice (48). We therefore tested the serum of LIGHT Tg mice and control littermates by ELISA for autoantibodies. LIGHT Tg mice demonstrated anti-DNA autoantibody levels elevated up to eightfold from those of the control littermates, whereas the level of total IgG in Tg mice was only slightly increased compared with the level in control littermates (Figure 6i). Tg mice also displayed elevated levels of rheumatoid factors, another commonly detected autoantibody in chronic inflammation and autoimmune diseases (Figure 6j). The findings of lupuslike glomerulonephritis and increased inflammatory cell infiltrate in multiple organs, along with elevations of serum autoantibodies, indicated the establishment of autoimmunity in LIGHT Tg mice. Therefore, the overproliferation and hyperactivation of T cells mediated by T cell–derived LIGHT resulted in the breakdown of B cell tolerance, supporting the

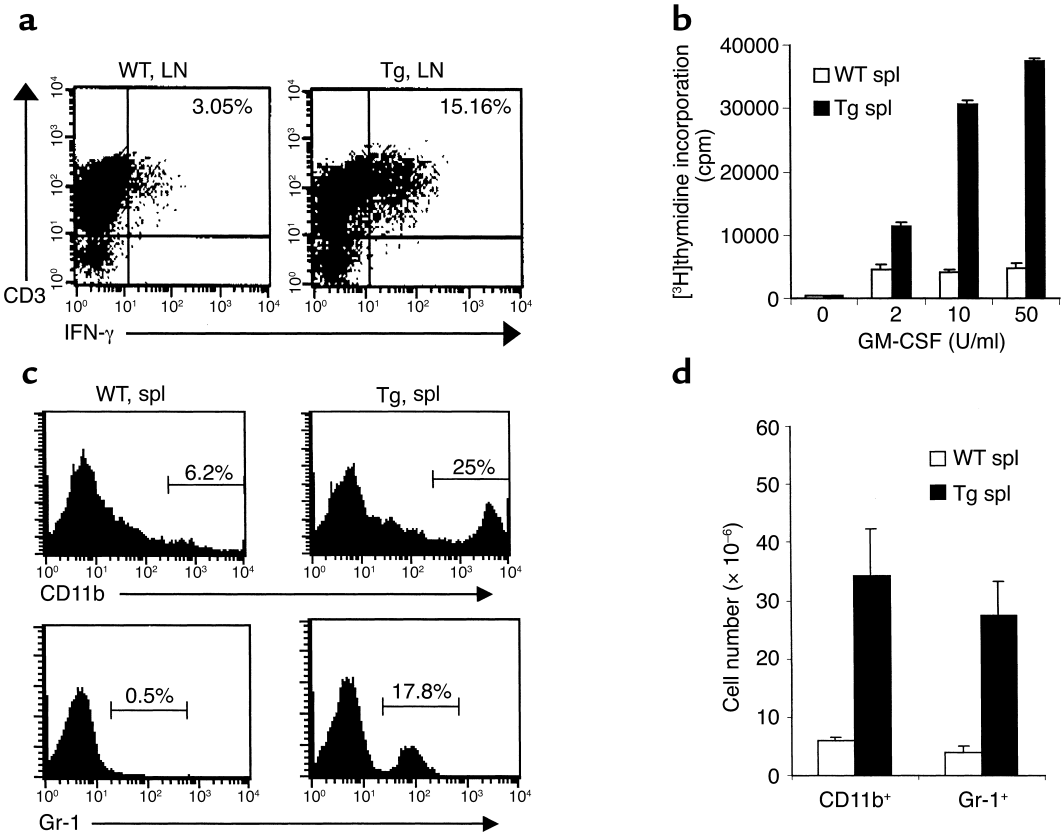

Figure 4
Enhanced cytokine production and expanded macrophage and granulocyte populations in LIGHT Tg mice. (**a**) Surface CD3 and intracellular IFN-γ expression was determined by double color staining of LN cells from WT and Tg mice after 4 hours of in vitro stimulation with PMA (50 ng/ml) and ionomycin (500 ng/ml). Numbers indicate the percentage of IFN-γ–producing T cells ($CD3^+$). Data are representative of three experiments. (**b**) GM-CSF–responding precursors were increased in Tg mice. Splenocytes from WT and Tg mice were cultured with different concentrations of GM-CSF; proliferation was measured by [^{3}H]thymidine incorporation. (**c**) FACS analysis of macrophage and granulocyte populations in Tg mice. Splenocytes (spl) from WT and Tg mice were stained with antibodies against CD11b or Gr-1. Percentage of $CD11b^+$ and $Gr\text{-}1^+$ cells is indicated. (**d**) Expanded populations of macrophages and granulocytes in Tg mice. Splenocytes were isolated from WT and Tg mice and cell number was determined, and then cells were stained with CD11b-Bio or Gr-1-Bio antibodies. Absolute cell numbers were calculated. These results are representative of three experiments. WT littermates and Tg mice at 5–8 months of age were used.

notion that the dysregulation of LIGHT expression may be a critical element in the induction of both T and B cell autoimmunity and in the pathogenesis of autoimmune diseases.

The participation of LIGHT in T cell–associated diseases. To study whether LIGHT is involved in the development of T cell–mediated diseases, we chose spontaneous autoimmune diabetes as our model. IDDM is a T cell–mediated autoimmune disease in which the insulin-producing β cells are selectively destroyed by autoreactive T cells, and the nondiabetic (NOD) mouse is the well-established model for studies of IDDM (49, 50). To examine the potential role of LIGHT in IDDM, 5- to 6-week-old female NOD mice were treated weekly with HVEM-Ig (100 μg per mouse) for a short term (3 weeks), and the glucose level was monitored starting at 8–9 weeks of age. HVEM is a receptor for LIGHT and does not bind to membrane LT (31). At the age of 6–7 weeks, many islets in NOD mice were already infiltrated with autoreactive T cells, and treatment with HVEM-Ig at this time significantly prevented the development of IDDM. More than 80% of mice in the control group developed IDDM, whereas only 25% of the mice in the treated group developed the disease (Figure 7). These results suggest that the blockade of LIGHT prevents the pathogenesis of IDDM and that LIGHT may play a critical role in the development of T cell–associated diseases.

Discussion

Previous studies of T cell activation have mainly focused on the costimulation pathway mediated by the interaction of T cells and APCs, such as the CD28/B7 system (24, 51, 52). However, little is known about the surface molecules that are involved in T-T cell interaction required for clonal expansion of T cells and T cell–mediated diseases. Here, we showed that the blockade of LIGHT by its soluble receptor HVEM-Ig dramatically reduced the anti-CD3–mediated T cell proliferation in the absence of APCs, indicating that LIGHT can function as a costimulatory

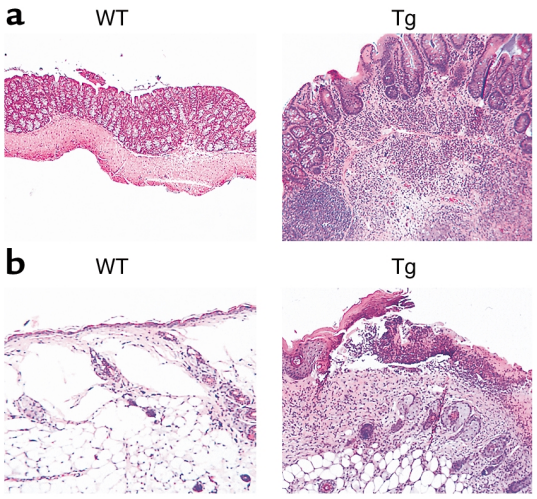

Figure 5
Induction of autoimmunity by T cell–derived LIGHT. Histology of intestine (**a**) and skin (**b**) from WT and Tg mice at 5–8 months of age. (**a**) Sections of colon from WT and Tg mice were stained with H-E. A severalfold increase of the thickness of intestine wall was prominently observed in Tg mice compared with control littermates, as well as dense inflammatory cell infiltration. (**b**) In the skin lesions of Tg mice, conspicuous mixed acute and chronic inflammatory cell infiltrate extending from epidermis to subcutis was observed in LIGHT Tg mice, accompanied by the destruction of skin appendage and aberrant hair follicular proliferation. Representative pictures are shown. Original magnification: ×10.

molecule for the complete expansion of peripheral T cells in a T-T cell–dependent manner. In contrast to reagents that block LIGHT activity, CTLA4-Ig did not show any impact on the proliferation of T cells in our APC-free system (Figure 1c). These results are consistent with the notion that CD28 interactions with the B7 family of costimulatory ligands are essential for inducing T cell activation via an APC-dependent mechanism (24, 51, 52), while LIGHT might be important for T-T cell interaction. Taken together, these results support our hypothesis that LIGHT from T cells is required for T cell expansion via T-T cell interaction whereas B7-1/B7-2 from APCs are probably more important for initiating T cell responses during the early priming phase.

It has been very difficult to test the in vivo activity of T cell–derived costimulatory molecules in the homeostasis and function of peripheral T cells. Therefore, we generated Tg mice for LIGHT in a T cell lineage to address this issue. Our results not only suggest that T cell–derived LIGHT is sufficient to promote the activation and expan-

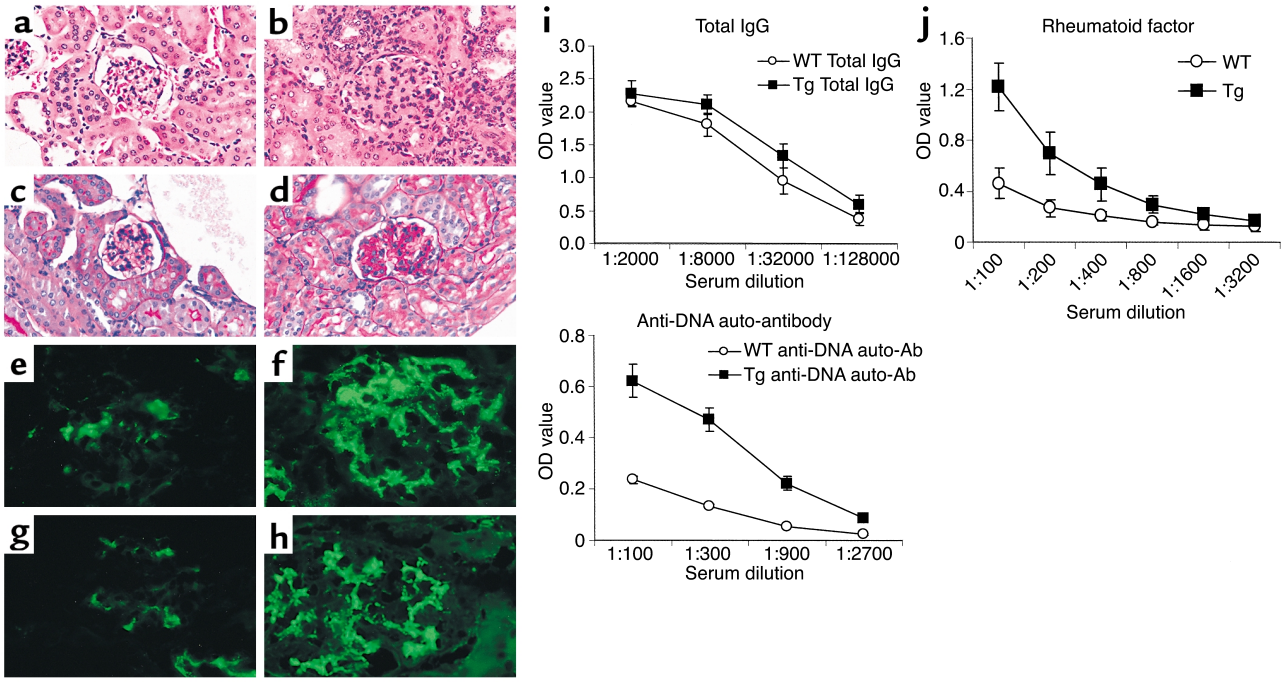

Figure 6
Renal pathological analysis and the elevated autoantibodies and rheumatoid factor levels in LIGHT Tg mice. Representative pictures from WT (**a**, **c**, **e**, and **g**) and Tg (**b**, **d**, **f**, and **h**) mice at 5–8 months of age are shown. (**a** and **b**) H-E staining; (**c** and **d**) PAS staining; (**e**–**h**) immunofluorescence staining. Original magnification is as follows: **a**–**d**, ×40; **e**–**h**, ×63. Glomeruli of Tg mice at 5–8 months of age were enlarged and lobulated with increased cellularity and inflammatory cell infiltration (**b**). The deposition of PAS-positive material was observed along the capillary wall and in mesangium in Tg mice (**d**). Sections of kidneys from WT and Tg mice were stained with FITC-conjugated goat anti-mouse IgG or total Ig. Strong Ig deposits were observed in the glomeruli of Tg mice (**f** and **h**). The serum levels of anti-DNA autoantibody (**i**, bottom panel) and total IgG (**i**, top panel) were determined in parallel by ELISA using the same set of WT (open circles) and Tg (filled squares) mice (n = 5). The serum level of rheumatoid factor in WT and Tg mice (n = 7) was determined by ELISA (**j**). Serum dilutions are indicated and data are means ± SD.

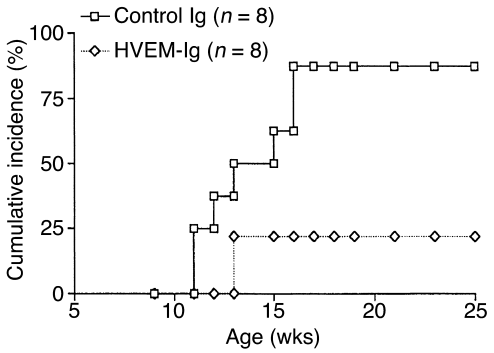

Figure 7
Blockade of LIGHT activity ameliorated the severity of spontaneous autoimmune diabetes. A soluble receptor of LIGHT prevented the development of IDDM. NOD female mice (5–6 weeks old) were treated with HVEM-Ig weekly for 2 weeks. Blood glucose levels were measured weekly starting from 9 weeks of age (n = 8), and animals were considered diabetic after two consecutive measurements of ≥250 mg/dl.

sion of peripheral T cells, which subsequently results in the development of autoimmune disease, but also show that LIGHT may have an essential role in T cell–dependent diseases such as IDDM. Our findings with LIGHT provide an example of a T cell–derived costimulatory ligand that is sufficient to induce a program of downstream events leading to T cell activation, breakdown of peripheral tolerance, and induction of autoimmunity (summarized in Figure 8). Although LIGHT can potentially bind three receptors (31, 53), HVEM is probably the receptor responsible for T-T cell interaction, as LTβR is not found on T cells (2, 34) and DcR3/TR6 is a decoy receptor that lacks a transmembrane domain (53). In addition, it is possible that LIGHT may have an unidentified receptor expressed on T cells. Due to the upregulation of LIGHT upon T cell activation, the simultaneous presence of both the ligand and the receptor could provide a stimulatory mechanism for the clonal expansion of peripheral T cells in an autocrine or paracrine fashion, as LIGHT can be secreted. These data suggest that T cell–derived LIGHT plays an essential role in the activation and subsequent expansion of T cells, which requires T-T cell interaction probably via the LIGHT/HVEM axis.

TNF family members play multiple critical roles in the homeostasis of lymphocytes. For example, transgenic mice for BAFF, a recently identified TNF family member, have an elevated number of B lymphocytes in the periphery, secrete autoantibodies, and develop an SLE-like condition leading to glomerulonephritis and breakdown of peripheral tolerance (14, 16, 46, 54). Similarly, LIGHT plays a major role in T cell homeostasis, as dysregulation of LIGHT leads to the breakdown of self-tolerance and development of autoimmune diseases. Therefore, LIGHT may be a counterpart of BAFF in T cell biology with the clear ability to break peripheral tolerance when dysregulated. We have not excluded the possibility that overexpression of LIGHT in Tg mice may directly and indirectly lead to the phenotypes observed in both T cells and other immune cells.

LIGHT may contribute to T cell–associated diseases. Treatment with HVEM-Ig, a soluble receptor for LIGHT, significantly prevented the development of IDDM, suggesting that LIGHT may play a critical role in the pathogenesis of IDDM. HVEM does not bind to membrane LT and has been shown to bind weakly to soluble $LT\alpha_3$ in vitro (31). The expression of LIGHT in resting T cells is low, but the increased expression of LIGHT can be found in our graft-versus-host disease–like (GVHD-like) autoimmune model (up to 17.5% T cells positive for anti-LIGHT antibody staining). In fact, LIGHT plays an essential role in the development of GVHD (33). Overall, these observations indicate that T cell–derived LIGHT is likely to be involved in T cell–mediated diseases and that its dysregulation may trigger the abnormal activation of T cells, spawning severe tissue destruction and autoimmune manifestations.

Interestingly, LIGHT Tg mice appear to have active mononuclear cellular infiltration in many organs, including the intestine and the skin, which show the most evident pathological manifestations, including ulceration and scar formation in the skin and massive infiltrate in the intestine. It is possible that the generalized expansion of activated T cells is harmless to the host except in tissues that interface with the external environment, where excessive T cell response to microbial or environmental antigens results in local pathology. In other words, the autoimmune disease may represent dysregulated homeostasis in response to environmental antigens.

Our results demonstrate that LIGHT is an important costimulatory molecule functioning in a T-T cell–dependent manner required for the complete expansion of peripheral T cells. The dysregulation or overexpression of LIGHT may play an important role in the pathogenesis of T cell–mediated inflammation and autoimmunity. Furthermore, our transgenic model indicates that LIGHT is sufficient to cause the activation and expansion of peripheral T cells that subsequently lead to the breakdown of peripheral tolerance. This transgenic model brings new insight into the pathogenesis of various autoimmune disorders and

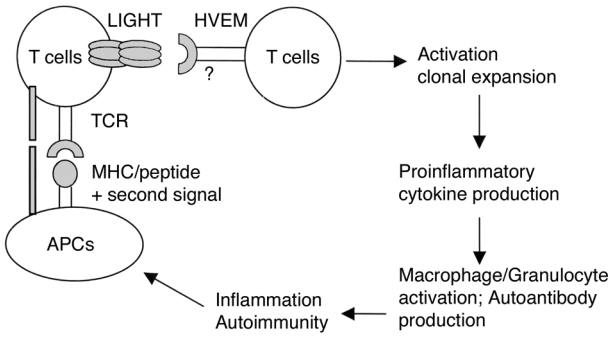

Figure 8
Proposed model for the LIGHT-induced autoimmunity. Question mark means other unidentified receptor(s).

provides an interesting framework for studying the mechanisms regulating T cell activation, immune tolerance, and the induction of autoimmunity.

Acknowledgments

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