Cutting Edge: Selective Impairment of CD8+ T Cell Function in Mice Lacking the TNF Superfamily Member LIGHT

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Interactions of LIGHT and its receptors, herpesvirus entry mediator on T cells and lymphotxin β receptor on stromal cells, are implicated in the regulation of lymphoid organogenesis, costimulation of T cells, and activation of dendritic cells. In this work we report that LIGHT-deficient mice had normal lymphoid organs with T cells and APCs that normally responded to Ag stimulation and normally stimulated T cells. Although the number of Vß8+ T cells in naive LIGHT+/+ and LIGHT−/− mice was identical, Vß8+CD8+ T cell proliferation in response to staphylococcal enterotoxin B was significantly lower in LIGHT−/− mice. Consistently, induction and cytokine secretion of CD8+ CTL to MHC class I-restricted peptide was also reduced in LIGHT−/− mice. However, the proliferative response of Vß8+CD4+ T cells to staphylococcal enterotoxin B was comparable in LIGHT−/− and LIGHT+/+ mice. Our results suggest that LIGHT is required for activation of normal CD8+ T cells but not CD4+ T cells. The Journal of Immunology, 2002, 168: 4832–4835.

Molecules belonging to the TNF superfamily play a critical role in multiple facets of the immune system through the regulation of lymphoid organ formation, cell apoptosis, B cell activation, Ab class switching, T cell costimulation, and dendritic cell (DC) activation (1). LIGHT, a TNF superfamily molecule expressed on activated T cells and immature DC (2, 3), binds three receptors, herpesvirus entry mediator (HVEM), lymphotxin (LT)βR, and decoy receptor 3/TR6 (2, 4). By signaling through HVEM and LTβR, LIGHT profoundly participates in multiple immunological functions. First, LIGHT mediates apoptosis in some tumor cell lines, leading to growth suppression in vitro and in vivo (5, 6). Second, LIGHT serves as a costimulatory molecule for T cell activation, leading to enhanced proliferation, Th1-type cytokine production, and NF-κB translocation (3, 7). Gene transduction of LIGHT mediates tumor rejection through the generation of tumor-specific CTL (7), whereas blockade of LIGHT ameliorates acute graft-vs-host disease by activating host-specific CTL (7, 8). Third, LIGHT plays a role in T cell development as well as homeostasis of peripheral T cells. The mice with transgenic expression of LIGHT demonstrate an enhanced negative selection of immature thymocytes (9), enlarged secondary lymphoid tissues, inflamed organs, and activated phenotype of peripheral T cells with autoantibody production (10, 11). Fourth, HVEM signaling in immature DC drives the maturation of DC in cooperation with CD40 signaling, resulted in an increased expression of costimulatory molecules and cytokine production (12). Finally, LIGHT is predicted to participate in the generation of lymphoid organs. The mice deficient of LTβR completely lack the generation of all lymph nodes (LN) (13), whereas LTβ-deficient mice retain mesenteric and cervical LN (14), indicating that LTβ-independent, LTβR-dependent signals could play an important role in formation of this particular LN. LIGHT is considered as a candidate ligand for this interaction.

Accumulating data thus support an essential contribution of LIGHT in initiation, development, maintenance, and termination of immune responses. However, prior studies were conducted by using recombinant LIGHT protein or enforced expression of LIGHT by gene transfer, giving rise to an argument that it is more than a physiological situation. In addition, experiments with soluble fusion receptors of LIGHT, such as LTβR-Ig and HVEM-Ig, cannot exclude the possibility that these fusion proteins work through a blockade of receptors other than LIGHT, such as LTβ and LTα. To address these issues, we have established the mice by gene targeting to disrupt endogenous LIGHT expression. Immunological features of these mice were investigated in vitro and in vivo.

Materials and Methods

Generation of LIGHT-deficient mice

The targeting construct containing 2.5 kb of 5’ homology and 3.1 kb of 3’ homology to genomic locus of LIGHT was linearized and used to electroporate 10^7 129/SvJ-derived embryonic stem (ES) cells. After positive and negative selection with G418 and FIAU, surviving ES clones were screened for homologous recombination with 18 U.S.C. Section 1734 solely to indicate this fact. 1

1 This work was supported in part by grants from the American Cancer Society (RPG-00-226), the National Institutes of Health (CA79915 and CA85721), and the Mayo Foundation, K.T. is the recipient of a U.S. Army Breast Cancer Research Program postdoctoral fellowship.

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3 Abbreviations used in this paper: DC, dendritic cell; HVEM, herpesvirus entry mediator; LT, lymphotxin; SEB, staphylococcal enterotoxin B; LM, lymph node; ES, embryonic stem; TNP, 2,4,6-trinitrophenyl.
of agouti-colored offspring were screened for germine transmission of the null allele. Heterozygous mice were interbred to generate F1 LIGHTnull mice. In addition, LIGHTnull mutation was bred onto a B6 background (designated B6LIGHT−/−) by at least five consecutive backcrosses to B6 mice. Expression of LIGHT was examined by RT-PCR analysis using total RNA isolated from Con A-activated T cells. An amplified 720-bp fragment of LIGHT cDNA was produced by a pair of forward (5′-CCATGTCTCA GAAAGCTTAGAGTGTTGATC-3′) and reverse (5′-TATCCG GATCCTCAGACCATGAAAGCTCCGA-3′) primers.

In vitro T cell assays

Total LN cells or CD4+ and CD8+ T cells purified from spleen and LN of BALB/c mice (H-2d) were incubated with 30-Gy-irradiated T cell-depleted spleen cells (5 × 10^6 cells/well) from B6LIGHT−/− or B6LIGHT+/− mice (H-2b) in a 96-well flat-bottom culture plate. In some wells, mCTLA4Ag (16) was included in the culture. BALB/c mice were purchased from the National Cancer Institute (Frederick, MD). For anti-CD3 mAb-induced proliferation assay, total LN cells or CD4+ and CD8+ T cells (2 × 10^5 cells/well) purified from spleen and LN cells of either B6LIGHT−/− or B6LIGHT+/− mice were incubated in a 96-well flat-bottom culture plate that was coated in advance with indicated doses of anti-CD3 mAb (clone 14-2C11; BD PharMingen, San Diego, CA). In all assays, proliferative activity was assessed by uptake of[^3]H]Trd (1 μCi/well) during the last 15 h of the 3-day culture. Depletion and purification of T cells were performed by VarioMACS (Miltenyi Biotec, Auburn, CA), as described previously (7).

In vivo administration of superantigen

LIGHT−/− or LIGHT+/− mice were injected i.v. with 50 μg of staphylococcal enterotoxin B (SEB; Toxin Technology, Sarasota, FL) on day 0. After 3 days, spleen and LN (inguinal and axillary) were harvested and stained with FITC-conjugated anti-TCR Vβ1/8.2, PE-conjugated anti-CD8, and CyChrome-conjugated anti-CD4 mAbs (BD PharMingen). Fluorescence was detected by a flow cytometry and analyzed with CellQuest software (BD Biosciences, Mountain View, CA), as previously described (7).

Generation of E7-specific CTL responses in vivo

E7 peptide (RAHYNIVTF), an H-2Dd-restricted CTL epitope derived from human papillomavirus-16 E7 protein, was synthesized and purified by HPLC (>95% purity) in the Mayo Protein Core Facility (Rochester, MN). On day 0, E7 peptide (100 μg/mouse) mixed with IFA was inoculated s.c. into the flank of LIGHT−/− or LIGHT+/− mice. On day 7, draining inguinal and axillary LN were harvested and the cells were incubated at 2.5 × 10^6 cells/ml in the presence of 100-Gy-irradiated EL4E7 cells (2 × 10^6 cells/ml). EL4E7, a human papillomavirus-16 E7-transduced EL4 lymphoma cell line, was kindly provided by Drs. G. J. P. Fernando and I. delian (5). We injected correctly targeted mice with HSV-TK and S represent HSV thymidine kinase and neomycin-resistant gene (Neo). Digestion with SacI yields a 6.5-kb and a 5.5-kb fragment from the wild-type gene and the mutated allele, respectively. HSV-TK and S represent HSV thymidine kinase and S represent neomycin-resistant gene (Neo). Digestion with SacII digested the 3′ flanking probe used for Southern blot analysis (filled box) are shown. The targeting vector was designed to replace the exon 1 with a neomycin-resistant gene (Neo). Digestion with SacI and hybridized to the 3′ flanking probe. The 6.5-kb wild-type fragment and the 5.5-kb mutant fragment are indicated. C, RT-PCR analysis of LIGHT. Spleen cells (5 × 10^6/ml) from LIGHT+/+, LIGHT+/−, or LIGHT−/− mice were stimulated with 3 μg/ml Con A. After 3 days, total RNA was purified from the activated cells and applied to RT-PCR with LIGHT-specific primers. Amplified LIGHT cDNA was detected as a 720-bp band. As control, murine GAPDH cDNA was amplified by specific primers that yielded a 1-kb band.

Results and Discussion

We generated LIGHTnull allele in ES cells by deleting the exon 1 via homologous recombination (Fig. 1A). We injected correctly targeted ES cell lines into B6 blastocysts and obtained chimeric mice that passed the LIGHTnull allele through the germline. F1 heterozygotes on a 129/B6 background were intercrossed and LIGHT−/− mice were found among the offspring at a normal Mendelian ratio (Fig. 1B). In addition, F2 heterozygous mice on the 129/B6 background were backcrossed to B6 mice for at least five generations to create LIGHT−/− mice B6 background. Loss of LIGHT expression was demonstrated by RT-PCR analysis of RNA isolated from activated T cells (Fig. 1C). LIGHT−/− mice were fertile, and healthy in gross appearance irrespective of their genetic background.

The hallmark of LTβR and LTβ knockout mice is the deficiency on the development of secondary lymphoid organs (13, 14). In addition to LTβ, LIGHT is also shown to bind LTβR (2), although its role in the formation of lymphoid organs is not yet known. We examined lymphoid organs by visual and histological examination. LIGHT−/− mice showed normal sizes and appearance of lymphoid tissues including thymus, spleen, LN, and Peyer’s patches. Histological analysis indicated that LIGHT−/− mice retain normal microarchitecture of secondary lymphoid tissues. In addition, there is no difference in the total cell number and the composition of lymphocyte subsets in thymus, spleen, and LN between LIGHT+/+ and LIGHT−/− littermates (data not shown). Therefore, in contrast to the lack of secondary lymphoid organs in LTβ knockout mice, endogenous LIGHT does not appear to be required for this function. The reason for this observation is unclear, but the expression pattern of LIGHT vs LTβ may be attributed to this difference. It has been shown that LIGHT is not expressed constitutively in normal tissues/cells and fetal tissues (2). Therefore, there may be a lack of interaction between LIGHT and LTβR during early development of lymphoid organs.

LIGHT could be detected on both DC and activated T cells (2, 3), and LIGHT-HVEM interaction is shown to provide a costimulatory signal for the activation of T cells and cytokine secretion in the presence of antigenic signal (7). Furthermore, LIGHT is also required for monocyte-derived human DC in the stimulation of allogeneic T cells (3) and anti-CD3 stimulated, APC-independent T cell activation (10). To examine the role of LIGHT on APC, we first used LIGHT−/− APC in allogeneic MLR. As shown in Fig. 2, T cell-depleted spleen cells from LIGHT−/− mice in B6 background were capable of stimulating allogeneic BALB/c T cells similar to APC from LIGHT+/+ mice (Fig. 2A). Inclusion of
FIGURE 2. APC and T cells from LIGHT−/− mice in allogeneic MLR and anti-CD3-induced proliferation in vitro. A-C, Allogeneic MLR was performed by incubating BALB/c T cells as responder cells and T cell-depleted B6LIGHT+/+ (□) or B6LIGHT−/− (■) spleen cells as stimulator cells. Indicated numbers of total LN cells (A), purified CD4+ T cells (B), or CD8+ T cells (C) were used as responder cells. In A, mCTLA4Ig (1 μg/ml) was added into the wells with B6LIGHT+/+ (□) and B6LIGHT−/− (■) stimulator cells. D-F, Total LN cells (D), purified CD4+ T cells (E), or CD8+ T cells (F) from B6LIGHT+/+ (□) or B6LIGHT−/− (■) were incubated in the presence of indicated doses of immobilized anti-CD3 mAb. In all assays, the cells were incubated for 3 days and proliferative activity was measured by [3H]Tdr incorporation during the last 15 h. Results are expressed as the mean ± SD of triplicate wells.

mCTLA4Ig to block B7-CD28 costimulation inhibited the activation of BALB/c T cells, regardless of the source of APC from LIGHT+/+ and LIGHT−/− mice. Purified CD4+ (Fig. 2B) and CD8+ (Fig. 2C) T cells also showed a comparable expansion in response to LIGHT+/+ and LIGHT−/− APC. To examine the role of T cell-associated LIGHT, we prepared highly purified T cells from LIGHT−/− mice and stimulated them by immobilized anti-CD3 mAb as mimic antigenic signal. LIGHT−/− T cells proliferated in response to anti-CD3 in a comparable level to that of LIGHT+/+ T cells in a wide range of doses (Fig. 2D). Both subsets of CD4+ and CD8+ T cells from LIGHT−/− mice also demonstrated similar levels of proliferation compared with LIGHT+/+ T cells (Fig. 2E and F). In addition, there was no significant difference in IFN-γ production of activated T cells from either LIGHT−/− or LIGHT+/+ mice (data not shown). Our results suggest that endogenous LIGHT is not required for APC-dependent and -independent responses of T cells in our systems.

Our results are different from previous observations that endogenous LIGHT on DC and T cells is involved in the activation of T cells. Several explanations can be offered at this time. A redundant mechanism that substitutes costimulatory functions of LIGHT may develop in LIGHT-deficient mice. For example, other ligands for HVEM rather than LIGHT may exist or become dominant in LIGHT-deficient mice. It is also possible that LIGHT may contribute the process of T cell activation indirectly through unknown mechanisms. Therefore, the effect of LIGHT could not be evaluated entirely by in vitro T cell culture system. To test these possibilities, we examined the role of LIGHT in T cell responses in vivo. We first used SEB, a bacterial superantigen secreted by Staphylococcus aureus, which causes vigorous expansion and subsequent deletion of Vβ8+ T cells in both CD4+ and CD8+ T cell subsets in an MHC class II-dependent manner (17). Before SEB injection, there was no significant difference among major Vβ frequencies including Vβ8 in peripheral CD4+ and CD8+ T cells between LIGHT+/+ and LIGHT−/− mice (data not shown). Three days after SEB injection, at the time when expansion of Vβ8+ T cells was most prominent, the total number of spleen cells was similar in LIGHT+/+ and LIGHT−/− mice, whereas LN cellularity of LIGHT−/− mice was slightly reduced in LIGHT+/+ mice (Fig. 3). Interestingly, SEB-induced expansion of CD8+ Vβ8+ T cells in spleen and LN was significantly reduced in LIGHT−/− mice compared with LIGHT+/+ mice. In contrast, expansion of CD4+ Vβ8+ T cells was similar between LIGHT+/+ and control mice. Deletion of CD4+ Vβ8− and CD8+ Vβ8− T cells 10 days after SEB injection was comparable in both LIGHT+/+ and LIGHT−/− mice (data not shown), suggesting that LIGHT is not necessary for SEB-induced apoptosis. Taken together, our results suggest that LIGHT plays a role in the activation of CD8+, but not CD4+, in vivo.

To provide further evidence for selective impairment of CD8+ compartment in LIGHT−/− mice, we investigated Ag-specific T cell responses in vivo. We first examined CD4+ T cell functions by 2,4,6-trinitrophenyl (TNP)-specific Ab generation induced by TNP-KLH immunization in which anti-TNP Ab production is dependent on Th responses to KLH. We detected no significant differences in all Ig subtypes including IgM, IgG1, IgG2a, IgG2b,
Ag-specific cell-dependent humoral responses are independent of LIGHT. This is not the case in our system because both activated CD4+/H11001/ and CD8+/H9253/ release assay. B, The culture supernatants were harvested from days 2 to 5, and the amount of IFN-γ was measured by specific ELISA. Results are expressed as the mean ± SD of triplicate wells.

FIGURE 4. Impaired CTL and cytokine responses to class I Ag in LIGHT+/− mice. E7 peptide emulsified with IFA (100 μg/mouse) was injected s.c. into either LIGHT+/− (□) or LIGHT−/− (■) mice on day 0. After 7 days, draining LN cells were harvested and incubated with 100-Gy-irradiated EL4E7 cells. A, The cells were harvested and CTL activity against EL4 and EL4E7 was determined in a standard 51Cr release assay. A, The light micrograph shows a lymph node from a Light−/− mouse. B, The light micrograph shows a lymph node from a Light+/− mouse. C, The light micrograph shows a lymph node from a Light+/+ mouse.

and IgG3 among LIGHT+/+, LIGHT−/−, and LIGHT−/− mice (data not shown), suggesting that LIGHT has a minimal effect on activation of Th cells, B cell maturation, and Ig class switch. We next assessed CD8+ T cell functions by CTL generation and cytokine production in response to E7-derived H-2Db-restricted peptide. It was reported that induction of CD8+ CTL in this system is independent on Th cells (18). CTL activity induced by E7 immunization was significantly lower in LIGHT−/− than in control mice (Fig. 4A). These CTL activities were specific to E7, because CTL did not kill parental EL4 target cells. In addition, production of IFN-γ (Fig. 4B) and TNF-α (data not shown) from E7-immunized LIGHT−/− lymphocytes was significantly decreased compared with those from LIGHT+/+ lymphocytes. Production of IL-4 and IL-10 was undetectable in both LIGHT+/+ and LIGHT−/− lymphocytes (data not shown). These results support our conclusion that LIGHT selectively plays a role in CD8+ T cell activation, and show that Th cell-dependent humoral responses are independent of LIGHT.

Earlier in vitro studies have shown that LIGHT can enhance Ag-specific T cell responses in both CD4 and CD8 subsets (Ref. 7 and K. Tamada, unpublished data). Furthermore, overexpression of LIGHT by T cells in transgenic mice is known to stimulate proliferation of both CD4+ and CD8+ autoreactive T cells (10, 11). These studies clearly indicate a function for LIGHT in T cell activation and suggest a role for aberrantly expressed LIGHT in autoimmune onset. Our current knockout study rather addresses the function of LIGHT expressed endogenously and shows a selective requirement of LIGHT for CD8+ T cell compartment. Although a disproportional expression of HVEM between activated CD4+ and CD8+ T cells was reported in the human system (19), this is not the case in our system because both activated CD4+ and CD8+ T cells express similar levels of HVEM (K. Tamada, unpublished data). Therefore, it is highly likely that LIGHT participates in the activation process of CD8+ T cells in vivo by an indirect mechanism rather than by direct interaction with HVEM on T cells. Expression of LTβR and HVEM are found on stroma cells (20) and endothelial cells (21), respectively. Activation of these cells may provide additional support for T cell activation in tissues. Many molecules of the TNF superfamily are reported to affect migration of T cells by regulating expression of chemokines and their receptors (22). It is tempting to speculate that LIGHT may also play a role in T cell migration to lymphoid organs in the course of immune responses. In summary, our findings have revealed the important role of LIGHT in the generation and maintenance of CD8+ T cell responses in vivo.

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References