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Targeting lymphocyte activation through the lymphotoxin and LIGHT pathways

Carl F. Ware[1]

1*Division of Molecular Immunology, La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA*

Summary

Cytokines mediate key communication pathways essential for regulation of immune responses. Full activation of antigen-responding lymphocytes requires cooperating signals from the tumor necrosis factor (TNF)-related cytokines and their specific receptors. LIGHT, a lymphotoxin-β (LTβ)-related TNF family member, modulates T-cell activation through two receptors, the herpesvirus entry mediator (HVEM) and indirectly through the LT-β receptor. An unexpected finding revealed a non-canonical binding site on HVEM for the immunoglobulin superfamily member, B and T lymphocyte attenuator (BTLA), and an inhibitory signaling protein suppressing T-cell activation. Thus, HVEM can act as a molecular switch between proinflammatory and inhibitory signaling. The non-canonical HVEM-BTLA pathway also acts to counter LTβR signaling that promotes the proliferation of antigen-presenting dendritic cells (DCs) within lymphoid tissue microenvironments. These results indicate LTβ receptor and HVEM-BTLA pathways form an integrated signaling circuit. Targeting these cytokine pathways with specific antagonists (antibody or decoy receptor) can alter lymphocyte differentiation and activation. Alternately, agonists directed at their cell surface receptors can restore homeostasis and potentially reset immune and inflammatory processes, which may be useful in treating autoimmune and infectious diseases and cancer.

Keywords

cytokines; *autoimmune disease*; *T cells*; *TNF superfamily*

Tumor necrosis factor superfamily as regulators of T-cell activation

The activation and differentiation of T cells is dependent on T-cell receptor (TCR) engagement of antigen and cooperating signals mediated through several distinct receptor-ligand systems delivered by the antigen-presenting cell. In the absence of cosignaling systems, T-cell activation is not sustained. Antigen recognition together with multiple ‘cosignaling’ systems determines the quality of a T-cell response. Cosignaling systems can promote or inhibit T-cell activation, thus aiding in maintaining homeostasis of the immune system. Cosignaling systems may act by promoting efficient engagement of T-cell antigen receptor molecules to enhance initial activation, cell division, augment cell survival, or induce effector functions such as cytokine secretion or cytotoxicity. Inhibitory cosignals may eliminate cells via apoptosis, block the initial activation, or attenuate effector functions of T cells. Cosignaling can be quantitative, modifying thresholds of common signaling intermediates, or qualitative, involving signals distinct from other cosignaling systems or the TCR. Moreover, T-cell responses are dynamic processes that start with the activation of naive cells and transition through effector and memory

Correspondence to: Carl F. Ware.

Correspondence to: Carl F. Ware Division of Molecular Immunology La Jolla Institute for Allergy and Immunology La Jolla, CA 92037, USA Tel.: +1 858 752 6735 Fax: +1 858 752 6991 e-mail: cware@liai.org.

phases, reflecting expression of cosignaling receptors and ligands that can be upor downregulated depending on the stage of the T-cell response and the inflammatory milieu.

Two major functional groups of cell surface cosignaling regulators are recognized, those containing an immunoglobulin (Ig)-like fold in their ecto domains, such as cytotoxic T-lymphocyte antigen-4 (CTLA-4) (1), CD28 (2), programmed death 1 (PD1) (3), and B-T lymphocyte attenuator (BTLA) (4,5). The other cosignaling group belongs to the tumor necrosis factor receptor (TNFR) superfamily and includes, among others, DR3, Ox40, 41BB, CD27, CD30, and herpesvirus entry mediator (HVEM) (6-9).

Cosignaling systems are emerging as important targets to attenuate autoimmune diseases or enhance immune responses to tumors (10-14). However, cosignaling systems are complex networks that are, in general, inadequately defined in most disease processes. Thus, understanding the mechanisms and functional consequences of these cosignaling systems is of immediate clinical significance. This review focuses on the LIGHT [homologous to lymphotoxins (LTs), inducible expression, competes with herpes simplex virus glycoprotein D (HSV gD) for HVEM, a receptor expressed on T lymphocytes] and LTαβ pathways in their roles as cosignaling circuits involved in cellular immune responses.

TNFR paralogs

Multiple members of the TNF superfamily function as cosignaling systems for lymphocyte activation. The TNFR encoded on Chr 1p36 share a common function as cosignaling systems for T cells (reviewed in 7,8) (Table 1). This region in humans (1p36.33-1p36.21) contains gluococorticoid-induced TNFR, Ox40, HVEM, DR3, 41BB, CD30, and TNFR2 genes representing an expansion of the paralogous region on Chr 12p13 where genes encoding TNFR1, LTβR, and CD27 reside. The cognate ligands for these receptors reside in the paralogous regions on human Chr 6, 19, 1, and 9, revealing a striking conservation in gene structure and function linked to T-cell activation and the major histocompatibility complex (15). These paralogous TNFR superfamily members, such as CD27, 41BB, and OX40, function as costimulatory molecules enhancing T-cell activation and survival or induce elimination of activated T cells, e.g. TNFR1 and Fas (CD95) (8,16). The evolutionary conservation of the TNF-related ligands and receptors dedicated to T-cell homeostasis and linkage to antigen recognition molecules reflects their importance in fine-tuning of effector activation and maintenance of immune tolerance.

The immediate TNF family

Shared ligand-receptor binding interactions within members of the TNF superfamily indicate broader functional links exist between these individual systems. TNF, LTα, LTαβ, and LIGHT overlap in binding to four cognate cell surface receptors (Fig. 1). The immediate TNF family is probably best viewed as a network of signaling systems that can integrate to control multiple physiological processes including T-cell homeostasis (17).

The TNF-TNFR1 system is a critical sentinel signaling system that orchestrates inflammation induced by innate recognition systems as well as acting on adaptive immune cells. For instance, effector T cells persist in tissues in TNFR1-deficient mice following infection, implicating that TNF-mediated elimination of effector T cells aids in restoring homeostasis (18).

By contrast, the LTαβ-LTβR system controls embryonic development of secondary lymphoid organs (lymph nodes and Peyer's patches) and the maturation and maintenance in the adult of the microarchitecture of lymphoid organs through the differentiation of specialized stromal cells. Lymphocyte-stromal interactions via LTαβ-LTβR create microenvironments that route trafficking of lymphoid cells and promote cellular interactions (19-21). In mice, full

development of Peyer's patches and splenic microarchitecture require both TNFR1 and LTβR pathways. Although the mechanisms of integrating signals are not well understood, alteration in NF-κB transcriptional activity may be involved, as deficiencies in components of LTβR signaling share lymphoid organ phenotypes.

LIGHT (TNFSF14) displays a distinct but overlapping receptor binding profile with LTαβ (22) binding LTβR, HVEM, and decoy receptor 3 (DcR3) (reviewed in 23). LIGHT has emerged as a key factor in mediating strong T-cell inflammatory responses. The surprising feature of the HVEM (24), one of the receptors for LIGHT and LTα, is it also serves as a ligand for the non-canonical interaction with the Ig superfamily member BTLA, which provides inhibitory signaling (25). DcR3 lacks a traditional membrane anchor, suggesting its role as a soluble inhibitory factor by binding LIGHT and paralogs, Fas ligand and TL1A (26). This rather complicated web of ligands and receptors suggests redundancy in function; however, each individual cognate interaction provides signals for unique cellular differentiation patterns as evidenced by the constellation of distinct phenotypes in genedeficient mice. Our current challenge is to understand the full extent of how these individual cytokine signals integrate within the context of cellular responses and predict outcomes in disease processes.

Expression and signaling

TNFRs are prominently expressed by cells of hematopoietic origin but in some cases can be expressed in cells of nonlymphoid tissues, for example mucosal epithelial cells (e.g. LTβR, HVEM). Most nucleated cells express TNFR1, although TNFR2 is restricted to hematopoietic cells. T cells express most of the cosignaling TNFR at different stages of their differentiation, whereas antigen-presenting cells often express the corresponding TNF family cognates. T cells, in either their naive, activated, or memory state, may express some or all of these TNFRs but not the LTβR (27). For this reason, LTβR is not considered a direct cosignaling molecule. However, LTβR indirectly influences T-cell activation by modulating differentiation of antigen-presenting dendritic cells (DCs), mast cells, tissue macrophages, and stromal cells. T cells also express most of the cognate ligands, implicating these pathways in T cell-T cell communication.

Expression of LIGHT and LTαβ may occur in different time and space. LTαβ expression is up during embryonic life and sustained thereafter (28), whereas LIGHT is on later, as the immune system matures. In the mouse, immature DCs from the spleen and lymph nodes express LIGHT, which is downregulated during the process of maturation (29). LTβ is also expressed in DCs but is upregulated during maturation (30). Lymphoid tissue inducer cells, the non-T/B lineage cells, express LTαβ during embryonic life, and it is particularly abundant in intestinal cryptopatches in the adult and less so in secondary lymphoid organs (31,32). Naive B cells are the major source of LTβ in the adult spleen and to a lesser extent $CD4^+$ T cells. The expression is relatively low but dramatically increased during antigen activation. B cell-stromal cell interactions via LTαβ-LTβR initiate expression of lymphoid tissue organizing chemokines (CXCL13), which sustains LTαβ expression during the maturation of lymphoid organs (33) (Fig. 2). $CD4^+$ T cells use LTβ in maintaining CCL21 expression segregating the T-cell zone from the B-cell follicles. The lymphocyte-stromal cell interaction mediated through the LTαβ to chemokine pathway sets up a reciprocating stimulation that allows lymphocytes to sense their position in the microarchitecture formed by the stromal cells.

LIGHT is involved late during neonatal life in processes forming mesenteric lymph nodes, but, in general, development is normal in $LIGHT^{-/-}$ mice (34). In humans, LIGHT expression is inducible via TCR signals in resting lymphocytes from peripheral blood, but in mucosal tissues $CD4^+$ T cells constitutively express LIGHT by a CD2-dependent mechanism (35). HVEM is broadly expressed in the lymphoid and myeloid compartments and is constitutively expressed

on naive $CD4^+$ and $CD8^+$ T cells but is transiently downmodulated during the initial phase of activation (36). HVEM is constitutively expressed on naive T cells, and LIGHT expression by immature DCs implies a possible role in the early events in T cell and antigen-presenting cell activation. Mucosal epithelial cells express HVEM, raising the possibility of immune regulation by parenchymal cells via lymphocyte-expressed BTLA. BTLA is broadly expressed in the hematopoietic compartment and coexpressed with HVEM on some cells, such as B cells, T cells, and DCs. In BALB/cJ mice, an expression polymorphism results in loss of BTLA expression in natural killer (NK) cells and macrophages, whereas in C57BL/6 T mice, it affects B cells, macrophages, NK cells, and DCs (37).

The paralogous TNFRs share common signaling motifs in their cytosolic domains. TNFR1 and DR3 contain death domains, which couple receptors to adapters through the death effector domain and caspase recruitment domain to the caspase pathways effecting apoptosis (38). The other cosignaling TNFRs contain short peptide motifs (39) that directly engage the TNFR-associated factor (TRAF) family of signaling adapters, which control serine kinases, like the NF-κB-inducing kinase (NIK) (40). Both death domain and TRAF signaling systems lead to the activation of NF-κB and activator protein 1 transcription factors, which are intimately linked to the transcription of genes involved in cell survival and apoptosis (41-43).

The mechanisms activating NF-κB by TNFR1 and LTβR provide insight into control of gene transcription accounting in part for the distinct cellular responses initiated by these receptors (Fig. 3). TNFR1 and LTβR activate two distinct forms of NF-κB. TNFR1 is a potent activator of the RelA:p50 form of NF-κB, whereas LTβR also activates the RelB:p52 complex through signal-inducible processing of p100 to p52 (44). These two forms of NF-κB regulate distinct sets of genes, imparting very different phenotypes in mice deficient in the genes encoding these two receptors. Two different kinase complexes initiate activation of NF-κB by phosphorylation of the inhibitors of κB (IκB). Serine phosphorylation of IκB couples to ubiquitin modification, leading to proteosome-dependent degradation of the IκB, which exposes the nuclear translocation sequence, allowing NF-κB to move into the nucleus. The IKKβ/γ (IKK2/NEMO) complex acts on IκBα, releasing the NF-κB RelA (p65):p50 heterodimer for nuclear translocation. By contrast, NIK and IKKα (IKK1) initiate degradation of p100, yielding p52, in complex with RelB as the active transcription factor. The TRAF family of adapters play an important role as inhibitors of kinases. The TRAF family consists of six members that participate and as subunits in ubiquitinylation reactions, a major feature of NF-κB regulation (45). TRAF3 is a key regulator of NIK as well as several other serine kinases involved in innate Toll-like receptor and innate interferon (IFN) responses (46). The synthesis of p100 is dependent on RelA linking the synthesis of this precursor/inhibitor to the RelB pathway, underscoring the complex network of regulatory processes that control the magnitude and duration of NF-κB response pathway. The number of target genes activated by the NF-κB family is up to several hundred and perhaps more (47) (see also www.nf-kb.org).

Structural features of the LIGHT-HVEM-BTLA pathway

LIGHT

LIGHT is a type II transmembrane protein containing a C-terminal TNF homology domain that folds into a β-sheet sandwich and assembles into a homotrimer (22,48) (Fig. 4). LIGHT engages two specific cellular receptors, the LTβR and HVEM. The quaternary structure as a trimer fits the canonical paradigm of the TNF family, a feature linked to the ability to the ligand to cluster cell surface receptors (49,50). Higher ordered structures may be needed for activating death receptors (51). Receptor clustering is the initiating step in activation of signaling pathways. Crystallographic analysis of several ligand-receptor complexes including LTα-TNFR1 (52), Ox40L-Ox40 (53), and TNF-related apoptosis-inducing ligand (TRAIL)-DR5 (54) show a trimeric ligand surrounded by three receptors, although some divergence from this

paradigm is present, for example in a proliferation-inducing ligand (APRIL)-transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) complex (55) and the structure of B-cell activating factor belonging to the TNF family (BAFF) (56). The crystal structure of the complex of LTα-TNFR1 revealed a receptor binding site forms as a composite of two adjacent subunits. In a paradigm-altering result, recent biophysical analyses revealed that the homotrimer of LIGHT, which should have three equivalent sites, contains only two high-affinity binding sites for the LTβR (57). The LTαβ2 heterotrimer also breaks the trimer paradigm, as the heterotrimer contains a single ββ and two unique αβ sites (58). Interestingly, membrane LTβ expressed alone binds LTβR but requires the LTα subunit for assembly into the stable heterotrimer (LTβ alone aggregates) (59). The only known receptor for LTαβ2 is the LTβR. However, in an artificial system, the heterotrimer can be created with the opposite stoichiometry. LTα2β, which has an αα site, binds both TNFR1 and LTβR. Thus, the binding interactions of the LTβR with LIGHT and LTαβ2 deviate from the TNF paradigm. The stoichiometric restriction of two receptor binding sites implicates dimerization of LTβR is sufficient for signaling, which may account for the capacity of bivalent antibodies to mimic signaling by LIGHT and LTαβ2. As discussed below, the ligand binding properties of HVEM further underscores the unorthodox binding behavior of the immediate TNF family.

TNF and LIGHT induce powerful proinflammatory reactions when constitutively expressed in T cells indicating the importance of mechanisms that limit signaling (Table 2). TNF and LIGHT are proteolytically cleaved (shed) into soluble forms that retain receptor binding activity, whereas LTβ is not shed, indicating cell to cell contact is essential to engage LTβR. LTα homotrimer has a signal sequence cleaved by signal peptidase and thus is exclusively secreted. The metalloproteinase (TACE/ADAM17) cleaves membrane TNF, whereas an undefined furin-like proteinase cleaves membrane LIGHT (60). Interestingly, an alternate transcript of LIGHT deletes the transmembrane region (LIGHTΔtm), which is made concurrent with the full-length transcript in activated T cells (61). The lack of stop transfer signal allows the nascent protein to enter the cytosol bypassing the glycosylation machinery in the endoplasmic reticulum. One possibility is that alternate splicing diverts transcripts encoding membrane LIGHT to an intracellular form, thus limiting expression of membrane LIGHT without shutting off transcriptional activity. Several additional mechanisms control the bioavailability of LIGHT including DcR3, a soluble ectodomain that binds LIGHT, FasL, and TL1A with a kDa in the low nM range (26,62). Levels of DcR3 detected in human plasma are 500 pg/ml range, which is well below saturating levels. The gene for DcR3 is not present in mice.

HVEM-BTLA

BTLA is single transmembrane glycoprotein containing an intermediate type Ig fold (63), making it structurally distinct from cosignaling molecules such as CD28, CTLA4, inducible costimulator (ICOS), or PD1 (64,65). The cytoplasmic domain of BTLA contains an inhibitory tyrosine-based motif that counteracts kinases via recruitment of tyrosine phosphatases [SH2 domain-containing phosphatase 1 (SHP-1) and SHP-2] attenuating proliferation signals in antigen-activated lymphocytes (13,66,67).

The ectodomain of HVEM contains three full cysteine-rich domains (CRD), the fourth C-terminal CRD has only two of the characteristic three disulfide bonds that form a CRD. Mutagenesis studies and molecular modeling predicts LIGHT contacts the elongated surface of HVEM spanning CRD2 and CRD3 (48). In contrast, BTLA binds HVEM in CRD1 on the opposite side of the receptor (36,68,69) in a region coined the ‘DARC’ side of HVEM, because it is also the attachment site for the HSV gD (63,69). These two sites on HVEM provide the basis for the molecular switch between positive and inhibitory signaling.

The membrane-distal region of CRD1 domain of HVEM residues 26-33 form the 'tip' that leads to a β strand (residues 33-38) and together with the G° strand from BTLA, forms a short anti-parallel intermolecular β-sheet. The results suggest BTLA uses a unique binding surface, distinct from that used by coinhibitory receptors of the related CD28 family (70). Biophysical data indicate that the ectodomains of both BTLA and HVEM are monomeric and engage one another with a 1:1 stoichiometry (53). The binding affinity of the monotypic interaction was measured at ~1 μM, whereas binding of surrogate dimeric HVEM-Fc or BTLA-Fc constructs to their respective coreceptors expressed in cells were in the 5-30 nM range. These observations are in line with cell surface proteins that require surface clustering in their mechanisms of activation, which may well be important in HVEM-BTLA pathway.

LIGHT may serve as a key factor controlling the HVEM-BTLA switch between positive and inhibitory signaling (69,71,72). Binding studies indicate LIGHT in its membrane-anchored position disrupts the binding interaction between HVEM and BTLA (69). By contrast, soluble LIGHT failed to disrupt binding and actually enhanced HVEM-Fc binding to cell-bound BTLA. This interpretation is limited by how true the surrogate receptor-Fc constructs mimic their membrane counterparts. The induction of membrane LIGHT during T-cell activation and its occupancy of HVEM is predicted to displace BTLA and alleviate inhibitory signaling toward antigen receptor signals. As a post-translational control, LIGHT shedding should release the membrane restriction, allowing HVEM and BTLA to re-engage, and perhaps the newly generated soluble LIGHT may play an opposite role in enhancing HVEM-BTLA interactions. Both HVEM and BTLA are expressed on resting lymphocytes, albeit at low levels on naive CD4 T cells; thus, BTLA may act as a constitutive 'off' pathway for T cells. LIGHT and HVEM must be on juxtaposed membranes (*trans*) for binding to occur, with the N-terminus of HVEM proximal to the membrane in which LIGHT resides. However, the ability of HVEM to activate BTLA signaling when presented in *trans* from another cell suggests the juxtaposition of HVEM and BTLA in distinct membranes is sufficient for proper orientation (36) but does not exclude the possibility of an interaction in *cis* (Fig. 5). Indeed, LIGHT should also be able to disengage HVEM-BTLA in either *trans* or *cis* conformations. Thus, the transient expression of membrane LIGHT should function to turn off inhibitory HVEM-BTLA signaling, while simultaneously activating proinflammatory signaling via HVEM-NF-κB.

Recently, CD160, a glycosphingolipid-linked Ig domain protein, was identified as yet another HVEM ligand (73). CRD1 of HVEM was essential for binding CD160, implicating a binding site shared with BTLA. The role of CD160 in the LIGHT-HVEM-BTLA complex and its role in inhibitory signaling are certain to become an interesting new feature of this pathway.

Viral perspectives

Two viral proteins encoded by evolutionarily distinct herpesviruses provided key insight into the mechanisms regulating the LIGHT-HVEM-BTLA molecular switch (Fig. 6). The molecular interface of HSV gD with HVEM is a highly similar to BTLA, thus gD is likely to be an evolutionary descendent of BTLA. The non-competitive blockade of HVEM-LIGHT by gD paralleled the behavior of BTLA in blocking HVEM-Fc binding to membrane LIGHT (69). These results suggest the possibility that the proximity of the membrane sterically excludes BTLA from binding HVEM when gD occupies its binding site on the DARC side. Thus, HSV gD is a dual antagonist by competitive displacement of BTLA and non-competitive blockade of the binding of LIGHT.

The orphaned TNFR encoded by primate cytomegalovirus (CMV) UL144 orf directly binds BTLA but does not bind LIGHT (74). When constructed as a dimeric fusion protein with the Fc region of IgG, UL144-Fc was more efficient than HVEM-Fc in blocking T-cell proliferation, even though its binding affinity for BTLA was measurably less (~fivefold). One plausible

reason to account for the enhanced anti-proliferative activity of UL144 relative to HVEM is its insensitivity to displacement by LIGHT, resulting in continued engagement with BTLA even when LIGHT is expressed.

Herpesviruses are well adapted to their specific hosts, reflected in their ability to cause persistent infection without overt pathogenicity, yet immune control is essential to maintain this coexistence. What selective advantage does altering the LIGHT-HVEM-BTLA pathway have for herpesviruses?

HSV1 and HSV2 utilize two distinct entry receptors, HVEM and Nectin1, to infect cells (75). Although Nectin1 was shown to be the predominant route in a vaginal infection model in mice, HVEM was sufficient in the absence of Nectin1 (76). In addition to its presence in the virion, gD is also expressed in the membrane of the infected cell. HSV gD could potentially inhibit HVEM signaling by blocking engagement of both its ligands, LIGHT and BTLA, thus potentially nullifying this circuit. gD may represent an evolutionary descendent of BTLA, reflected by their common Ig domain structure and shared functional properties, including overlapping binding sites and uncompetitive blockade of LIGHT. Although perhaps obvious, blocking LIGHT-HVEM signaling would diminish proinflammatory signals in T cells, appearing as an advantage for the virus. However, when unchecked by LIGHT, the HVEM-BTLA pathway may maintain too much inhibitory signaling. In this case, the adaptation of gD to include blockade of the HVEM-BTLA pathway would counterbalance the loss of LIGHT.

UL144 orf in human CMV mimics only one function of the HVEM switch, the engagement of BTLA, which initiates inhibitory signaling without potential countering influence from LIGHT (69). Different clinical isolates of CMV exhibit relatively high-sequence variation in the ectodomain of UL144 (77), yet all retain BTLA binding activity. This result suggested significant immune pressure continuously sculpts the evolution of this molecule. Although UL144 is not required for virus replication, its role in the natural infection cycle of human CMV is unknown. Each evasion mechanism must be considered in the context of other immune-modifying functions of the pathogen; unfortunately this cannot be readily tested, as mouse CMV does not posses a UL144 ortholog.

Are viral entry routes clues to immune pressure? The first CRD in Ox40, TRAILR2, and NGFRp75 are used by retroviruses and lassavirus to infect cells (78). In each case, the viral envelope protein engages a region in the CRD1 equivalent in the TNFR. This evidence suggests that the selective pressures provided by this host-virus interface are an advantage for the virus. However, the result also raises the possibility that these receptors have functional properties in CRD1 analogous to HVEM. A functional role of the CRD1 regions of TRAIL receptor, NTRp75, and Ox40 is unknown in our current understanding of the biology of these receptors, but speculation predicts that additional HVEM-BTLA-like systems exist for these other TNFRs, and perhaps other TNFRs with a highly conserved CRD1.

These immune evasion mechanisms of viruses may provide critical new clues on how to modulate immunity without overt pathogenicity. The specific targeting of the LIGHT-HVEM-BTLA pathway by molecular mechanisms that act extracellularly provides evidence that targeting this pathway using biologics, such as antibody or decoy receptors, is feasible. However, caution is warranted, because the biologics are used at pharmacological doses and impact host physiologic systems ‘globally’, whereas expression of the viral immune evasion molecule may be limited to the microniche occupied by the infected cell.

Immunobiology of LIGHT-HVEM-BTLA

LIGHT

Substantial data from genetic and pharmacologic approaches implicate LIGHT-HVEM as a costimulatory signaling system, but the mechanisms remain inadequately understood (Table 3). Reports using soluble decoys LTβR-Fc or HVEM-Fc to inactivate their ligands *in vivo* hamper mechanistic interpretations because of the dual specificities of these reagents, which cannot distinguish a single target (LTαβ, LIGHT, or BTLA). Studies in gene-deficient mice have convincingly shown that LTαβ-LTβR pathway is important in formation and maintenance of lymphoid organ microarchitecture, homeostasis of DCs, formation of follicular DC networks in primary and secondary follicles, and germinal center formation in the spleen (20). That LTβR is not expressed in T or B cells points toward the LIGHT-HVEM system as the primary cosignaling pathway in T cells.

Blocking LIGHT can inhibit early T-cell proliferation and cytokine secretion in allogeneic mixed lymphocyte reaction (29,79,80) and in MHC-mismatched heart (81) and allogeneic skin grafts (34). This inhibitory effect may reflect an early suppression of T-cell expansion or cytokine production. These conclusions have been reinforced with the production of LIGHT transgenic mice, which showed increased numbers of activated T cells, higher proportions of memory and effector T cells, and signs of autoimmunity, particularly in the intestine and reproductive organs (82,83). In a more recent study, contrasting data were obtained *in vitro* and *in vivo*. LIGHT-deficient antigen-presenting cells were unimpaired in their ability to stimulate proliferation of wildtype $CD4^+$ or $CD8^+$ T cells in an allogeneic mixed lymphocyte reaction, whereas *in vivo* responses in LIGHT-deficient mice demonstrated defective expansion of superantigen-reactive $CD8^+$ T cells and defective cytotoxic T-lymphocyte generation after peptide priming (84,85). LIGHT interacting with T-cell-expressed HVEM can be costimulatory; a role for LIGHT-HVEM is only apparent when LIGHT expression on T cells was also blocked. Evidence for LIGHT transmitting costimulatory signals to T cells has been obtained *in vitro* (86). These data are consistent with a role for HVEM and LIGHT in activating naive T cells and in regulating clonal expansion.

BTLA

The discovery that HVEM and BTLA form an inhibitory signaling pathway (36) provided an initial explanation to the paradox presented by the distinct phenotypes of the LIGHT- and HVEM-deficient mice. LIGHT-deficient T cells proliferated poorly in response to TCR stimulation, as expected; however, $HVEM^{-/-}$ T cells exhibited an enhanced activation profile, phenocopied by $BTLA^{-/-}$ T cells. Adding a twist to an already Gordian knot, in a graft rejection model, BTLA emerged as a positive signaling system in which T effector cells required BTLA for survival (87). This result implicates a positive role for BTLA in certain stages of the T-cell life cycle. Resolution of this Gordian knot may require more than the cutting edge of a Greek sword.

Polymorphic variants of BTLA could contribute to disease. A single polymorphism in human BTLA-coding region has been identified that may be linked to rheumatoid arthritis (88); however, there are abundant polymorphisms in the intergenic regions. Three allelic variants of BTLA, which bind HVEM, exist in common mouse strains but are not linked to pathology (4). Mice deficient in BTLA show normal lymphocyte development. T cells from these animals are hyperresponsive to anti-CD3 antibody stimulation, and reciprocally, anti-BTLA antibody can inhibit T-cell activation (89). BTLA-deficient T cells show increased proliferation, and BTLA-deficient mice have increased specific antibody responses and enhanced sensitivity to experimental autoimmune encephalomyelitis (4) and airway hypersensitivity (90), consistent with a proposed role as an inhibitory cosignaling molecule.

Integrated signaling network

The LIGHT-HVEM-BTLA and the LTαβ-LTβR pathways control cellular processes required for adaptive immunity (17), but little evidence indicated how these pathways might integrate in cosignaling events. LTβR is involved indirectly in T-cell activation by modulating the life cycle of DCs within lymphoid organs, which provides a model for investigating pathway integration. DCs play a crucial role bridging innate and adaptive immune responses through the activation of naive antigen-specific T cells (91). Within secondary lymphoid organs like the spleen, three subsets of $CD11c^{hi}$-expressing DCs are defined as $CD8\alpha^+$, $CD4^+$, or $CD8\alpha^-CD4^-$, the latter two forming the $CD8\alpha^-$ DC subset. These DC subsets are distinct from the type 1 IFN-secreting plasmacytoid DCs, which express low levels of CD11c ($B220^+CD11b^-$) (92,93). DCs emigrate from bone marrow, enter lymphoid tissues, and divide locally (94,95). The daughter cells maintain their ability to present antigen; however, each subset performs distinct functions. The $CD4^+$ and $CD8\alpha^-CD4^-$ DC subsets principally reside in the marginal zone bridging channels, whereas the $CD8\alpha^+$ DCs are found in the T-cell-rich area in the white pulp (96).

LTβR signaling specifically regulates the proliferation of the $CD8\alpha^-$ DC subsets (97). LTβR-deficient mice have normal bone marrow DC subsets (98) but greatly diminished numbers of $CD8\alpha^-$ DCs in the spleen. Typically the ratio of CD8α/CD4 subsets in C57Bl/6 mice is 0.5, whereas the ratio inverts (1.8) in LTβR-deficient mice. Mice deficient in LTα, LTβ, and NIK, but not LIGHT display this same phenotype, revealing a pathway involving LTαβ-LTβR to NIK and RelB as a critical components in the proliferation of DCs (99,100) (Fig. 7).

In striking contrast, increased numbers of $CD4^+$ and $CD8\alpha^-$ $CD4^-$ DCs are present in the spleens of $HVEM^{-/-}$ and $BTLA^{-/-}$ mice, suggesting that the HVEM-BTLA pathway is an inhibitory checkpoint for DC accumulation in the spleen (99). A majority (~70%) of the resident DCs in the adult mouse spleen are under dynamic control by the LTαβ-LTβR pathway. Treatment of wildtype mice with the LTβR-Fc decoy specifically reduced $CD8\alpha^-$ DCs, whereas an agonist LTβR antibody increased the same subsets. Interestingly, a reduced basal level of DCs were maintained in the spleen in the absence of LTβ, LIGHT, and HVEM, indicating a second distinct mechanism operates to control DC populations in the spleen. Competitive bone marrow chimeras, a mixture of wildtype and gene-deleted cells transplanted to repopulate irradiated recipients, revealed a striking competitive advantage of HVEM- or BTLA-deficient DCs, a phenotype expected for cells alleviated from an inhibitory pathway. Interestingly, the genotype of the stromal cells in the recipient mice modulated the extent that DCs competitively repopulated the spleen. $HVEM^{-/-}$ DCs repopulated the spleen more efficiently in a wildtype recipient than in an $HVEM^{-/-}$ recipient. Thus, HVEM and BTLA signals provided by the splenic stromal microenvironment influence inhibitory signaling involved in maintaining DCs in lymphoid tissues. Interestingly, wildtype DCs were also impacted in the mixed chimeras reflected by the increased $CD8\alpha^-$ DC subsets (ratio = 0.3) independently of recipient background. This effect of HVEM or BTLA deficiency on wildtype cells is consistent with cellular interactions in *trans* with neighboring DCs that provide inhibitory signaling regulating proliferation and accumulation. Thus, DC interactions with other DCs and with the stromal microenvironment provide sources of inhibitory signaling, although the directional flow of signals between these various cell types requires further elucidation.

BTLA deficiency negatively regulated antigen-independent homeostatic expansion of both $CD4^+$ and $CD8^+$ T cells but additionally impacted the homeostasis of T cells and memory cell differentiation (101). HVEM- or BTLA-deficient mice exhibited an increased number of memory $CD8^+$ T cells, with BTLA expression required in memory $CD8^+$ T cells. Naive $BTLA^{-/-}$ $CD8^+$ T cells were more efficient than wildtype cells at generating memory in a competitive antigen-specific system, an affect independent of the initial expansion of the responding antigen-specific T-cell population.

Surprisingly, T cells may utilize survival signals dependent on BTLA (87,102). In a graft-versus-host disease model using non-irradiated parent into F1 recipients, HVEM-deficient donor cells could not sustain a rejection response. BTLA$^{-/-}$ cells showed a similar phenotype. HVEM-deficient donor cells underwent initial activation and expansion *in vivo* following transfer but failed to survive past 10-11 days, undergoing cell death and exhibiting failure to re-express interleukin-7R. Expression of HVEM and BTLA was required in the parental effector cells. However, mixed transfers of wildtype donor cells rescued the survival of BTLA-deficient donor cells, indicating a non-T-cell intrinsic action of BTLA. Thus, HVEM-BTLA-dependent regulation of T cells and DC subsets involves both intrinsic and extrinsic signaling.

The mechanisms involved in regulating DC growth and homeostasis by the LT-related cytokines are minimally understood. However, in a broader context, these results lend credence to the idea that LTαβ-LTβR and the LIGHT-HVEM-BTLA systems form an integrated circuit controlling intercellular communication between T cells and DCs (17). In this case, the context is homeostatic control. One scenario envisions naive T and B cells expressing LTβ serve as a low level stimulus activating LTβR, thus maintaining a steady state of DC proliferation within lymphoid organs. DCs also express LTβ (30), which may serve as an additional source of ligand. HVEM-BTLA serves to check this proliferation.

Could DC regulation during immune and inflammatory responses result from the amplification of the positive homeostatic signals (LTαβ and LIGHT) and suppression of the inhibitory pathways (HVEM-BTLA)? Perhaps the cellular source of these ligands may determine the context of DC proliferation during inflammation. Recent evidence indicates that T cells expressing LTαβ are required for maximal expression of CD86 on antigen-bearing DCs and for efficient priming of CD4^{+} and CD8^{+} T cells. Moreover, conditioning of DCs for optimal T-cell proliferation and cytokine secretion required LTβ expression on antigen-specific T cells (103). Thus, activated T cells have the potential to induce local DC proliferation through LTαβ signaling. B cells also have the potential as demonstrated by enforced expression of LTα in B cells (97). This process could be exceptionally important at sites of chronic inflammation, which often contain lymphoid cell aggregates with features of lymphoid structures (21). LIGHT may also serve this role as an LTβR ligand expressed by activated T cells (104). Activated and naive T cells and B cells, NK cells, and DCs can express LTβ, LIGHT, as well as LTβR and HVEM-BTLA, revealing a complex relationship in signaling that demands detailed examination.

Targeting LIGHT-HVEM-BTLA

The extracellular position of the ligands and receptors in the TNF family provides a direct opportunity to use biologic-based therapeutics. TNF inhibitors of the antibody (infliximab) or decoy receptor (etanercept) class have proven their worth in alleviating symptoms of inflammation associated with autoimmune diseases and provide the current paradigm for therapeutics directed at the TNF superfamily, although other approaches are being considered. Biologic-based therapeutics can function as antagonists, agonists, or both. Currently both antibodies to the ligands and cellular receptors converted into soluble decoys provide functional antagonism of the ligand-receptor interaction, whereas antibodies directed to the receptors can be either agonists or antagonists or both. By definition an antagonist blocks ligand binding without eliciting receptor activation. Anti-receptor antibody can be an antagonist, competitively blocking the cellular ligand from binding, and simultaneously an agonist, as the bivalent antibody mimics the multivalent TNF ligand activating the receptor.

Side effects from targeting TNF superfamily members are a significant but solvable problem. A major undesirable consequence from targeting TNF family members is increased susceptibility to infections. Susceptibility to infections in mice deficient in LTαβ or LIGHT

pathways is not overt but rather selective in the type of pathogen (Table 4). CMV seems to stand out among the herpesviruses for increased virulence in LTαβ/LIGHT-deficient mice, which likely reflects the ability of murine CMV to alter several LT regulated responses. In part, increased susceptibility reflects a role for LTαβ-LTβR in regulating the early type 1 IFN (IFNαβ) response (105). The early IFNαβ response originates in LTβR-expressing splenic stromal cells infected with murine CMV and significantly the IFNαβ response is Toll-like receptor independent. LTαβ expressed by naive B cells provides the stimulus for LTβR signaling in the splenic stroma. Interestingly, murine CMV transiently disrupts the microarchitecture in the spleen of wildtype mice through specific suppression of CCL21 in stromal cells (106). LTβR signaling is required for the differentiation of these specialized stromal cells producing tissue organizing chemokines CCL21 and CXCL13. The expectation of using LTαβ/LIGHT antagonists, similar to TNF blockade, may increase susceptibility to selected viral and bacterial pathogens.

Antibodies and receptor-Fc proteins have other functional properties in addition to their ligand (antigen) binding specificities that may impact clinical efficacy. Secondary effects of antibodies include activating effector systems like complement and cellular cytotoxicity, which may be advantageous in eliminating disease-causing cells that express ligands/antigens on their surface. Receptor-Fc fusion proteins do not activate antibody effector systems.

By contrast, antibody directed to individual receptors may be useful as agonists to activate specific receptors, in contrast to natural polygamous ligands. For instance, agonist antibodies to LTβR induce resistance to CMV in human (107) and mouse models (108). The mechanisms of action may include restoring early IFN response as well as enhancing DC subsets. This approach suggests agonists could be useful in treating infectious diseases (109).

The discovery of the LIGHT-HVEM-BTLA switch provides three novel targets for modulating immunity that may be useful in treatment of autoimmune diseases, cancer, and infections. LIGHT has three known mechanisms of action: activation of the LTβR, activation of HVEM, and disruption of HVEM-BTLA inhibitory pathway. Thus, blockade of LIGHT will inhibit signaling through the LTβR and HVEM but leave intact the inhibitory HVEM-BTLA pathway and homeostatic signaling by LTαβ. Whether LTαβ or LIGHT or both are necessary for pathogenesis in autoimmune disease is an unresolved question. Here the relative dominance of LIGHT or LTαβ as the most important target may require empirical resolution (110,111).

Studies in patients with inflammatory bowel disease support experimental animal models that LIGHT provides a critical proinflammatory signal in mucosal tissues (112,113) and visceral organs (114,115). Human mucosal T cells and NK cells and a subpopulation of gut-homing $CD4^+$ T cells in the peripheral blood, not naive T cells in blood, express membrane LIGHT (35,116). LIGHT mRNA is elevated in biopsies from small bowel in inflamed sections of tissue, suggesting LIGHT as a mediator of mucosal inflammation. Chr19p13.3 contains a susceptibility locus for inflammatory bowel disease (*IBD6*), and although this region is gene dense, the status of *LIGHT* as disease candidate is significant and is consistent with observations in experimental animal models.

Agonists to BTLA may be useful in limiting inflammation in autoimmune diseases and reestablishing tolerance. Agonists directed at BTLA may be useful in preventing activation of initial immune responses, for instance in allograft rejection or graft-versus-host disease. Recent evidence indicates that combination of anti-BTLA agonist with CTLA4-Fc established tolerance to allogeneic pancreatic islets to correct diabetes in mice (117). The islet allografts showed intact islets and insulin production despite a host cellular response, with local accumulation of regulatory T cells ($Foxp3^+$). More strikingly, these mice accepted second donor-specific islet graft without further treatment yet rejected third party grafts (118). Thus,

agonists directed to inhibitory receptors represent an alternate mechanism to alleviate inflammatory responses and establish immune tolerance.

Breaking tolerance may be the key for effective therapy against tumors. Redirecting immune responses toward malignant cells by expression of LIGHT in the tumor or local microenvironment may overcome developmentally established tolerance to self (119,120). In this context, membrane LIGHT was essential in activating a cellular immune response to tumors (119). Delivery systems based on viral vectors are viewed as a major safety hurdle. A delivery system that directs LIGHT expression to the tumor or its microenvironment in a safe and effective fashion is critically needed. Moreover, the method should focus the immune response on the tumor antigens in such a way as to avoid breaking tolerance to normal tissues.

With a similar perspective, LTβR agonists may be useful in treating persistent infectious diseases, such as herpesvirus, through modulation of innate defenses and alteration of microenvironments suitable for enhancement or reconstitution of immune cells. Stromal elements that express LTβR are not likely to induce cytokine storms, which may limit the use of agonists directed at strong T-cell expressed cosignaling receptors (e.g. CD28). LTβR-dependent expansion of DC subsets by agonist antibodies may be useful in providing more efficient presentation of endogenous antigens in infected individuals or through preestablishing immunity by vaccination.

Historically, no single criterion stood out as a predictor of the relevant clinical indication to apply TNF modulators; however, clinical experiences overcome that barrier in using therapeutics targeting TNF family members. The biology linked to the LTαβ-LTβR and LIGHT-HVEM-BTLA pathways appear to offer multiple approaches to targeting human diseases.

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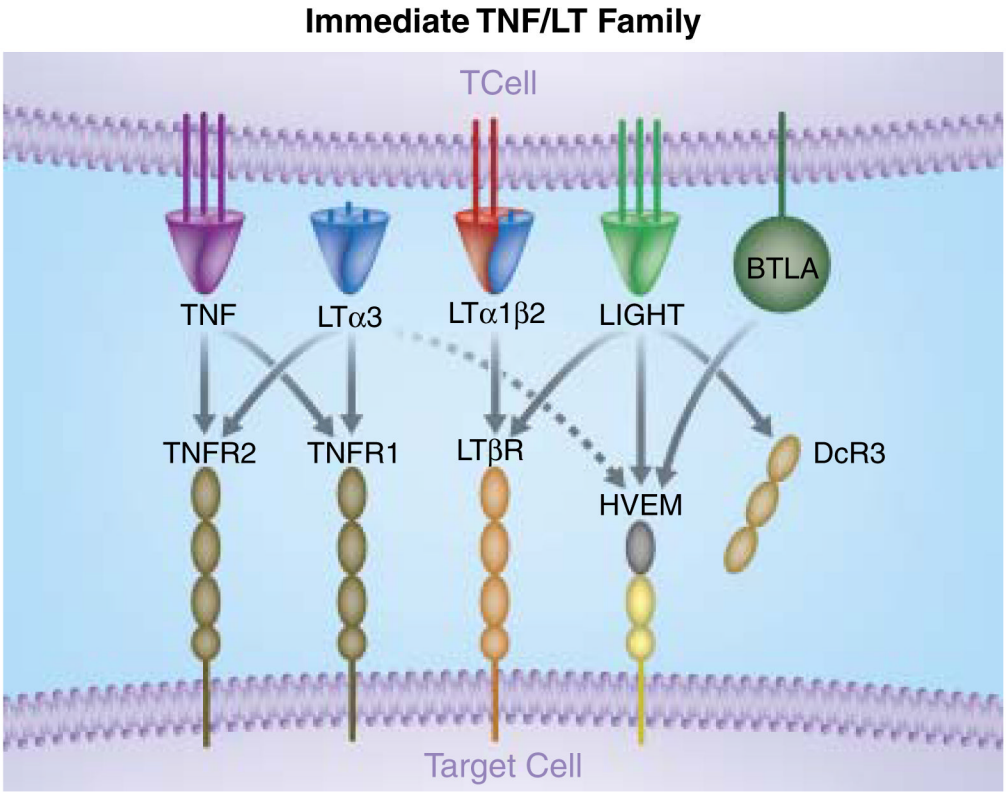

Fig. 1. Members and binding interactions of the immediate TNF family

Ligands shown at the top are depicted as trimers with transmembrane anchors. Cellular receptors are shown on the bottom. Arrows indicate the receptor-ligand binding specificity of the various members. Solid lines indicate high affinity interactions; dashed line refers to weak interactions.

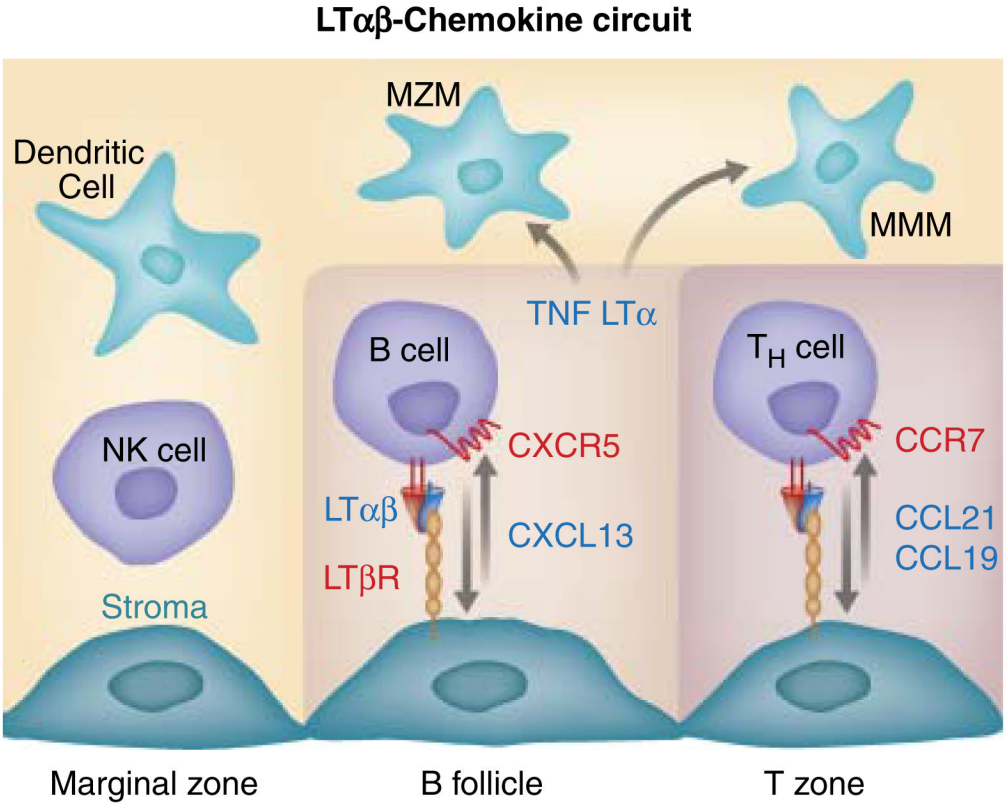

Fig. 2. Microarchitecture of the spleen is regulated by LTβR-dependent chemokine expression

Chemokine circuits form between lymphocytes and stroma. Depicted are cellular interactions in the architecture of white pulp in the spleen dependent on LT/TNF signaling. The marginal zone contains marginal zone macrophages (MZM) and metallophilic macrophages (MMM). DCs require LT signaling for local proliferation in the spleen. B and T lymphocytes are compartmentalized in discrete areas in the white pulp (B-cell follicle and T-cell zone) through the reciprocal induction of LT expression on lymphocytes by chemokines and chemokine expression by stromal cells via the LTβR.

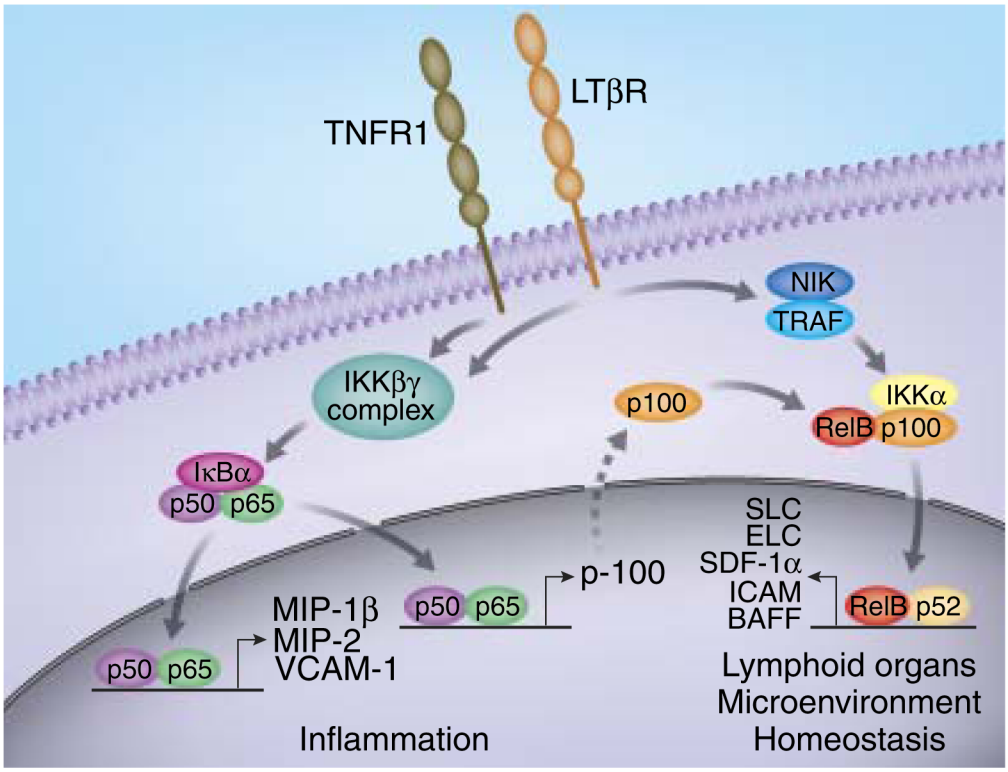

Fig. 3. TNFR and LTβR signaling pathways

Components in the TNFR1 and LTβR pathways for NF-κB activation. TNFR1 can access TRAF2 to activate the canonical NF-κB pathway RelA(p50/p65) via IκB degradation. This pathway controls many inflammatory genes and p100 synthesis. The LTβR induces both the canonical and the RelB NF-κB pathway via the NIK-IKKα-mediated processing of p100 and activation of p52/ RelB target genes.

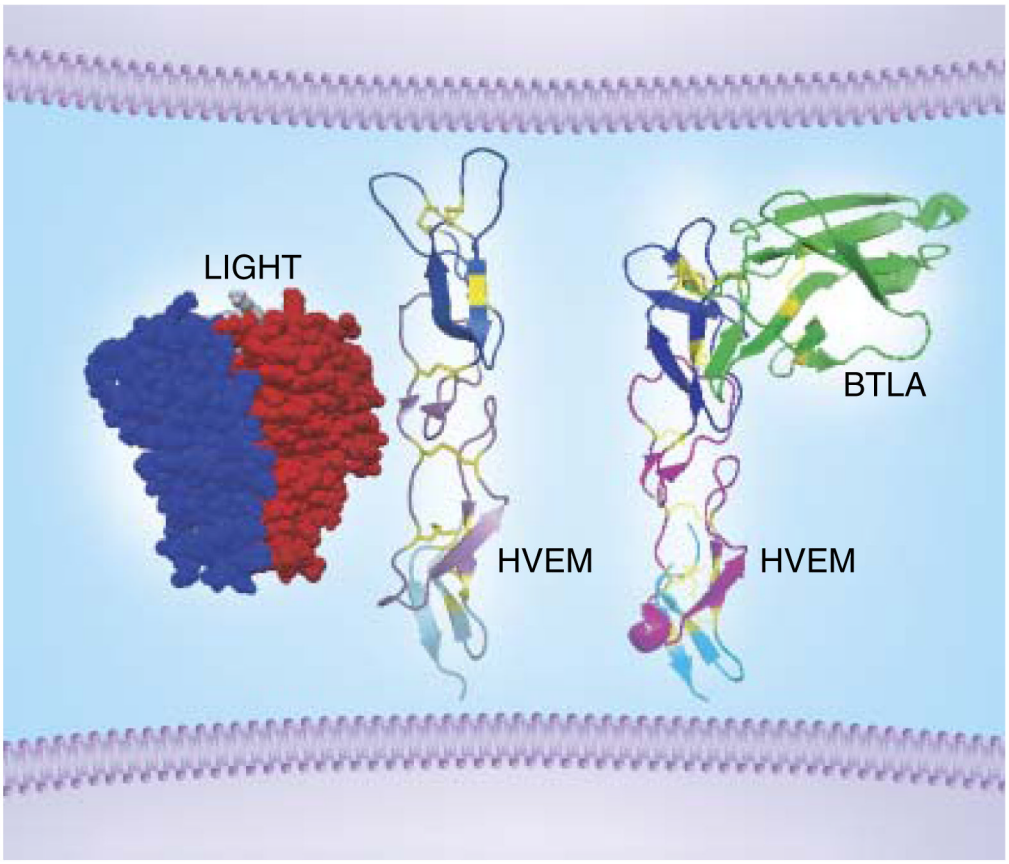

Fig. 4. Structural models of LIGHT-HVEM and HVEM-BTLA complex

The molecular model of trimeric LIGHT in space filling mode was generated by SwissModel and encompasses amino acids Ser103 to Val240. Subunits are represented in red, blue, and gray (not fully visible); the transmembrane domains of each subunit would anchor in the top membrane. The LIGHT is adjacent to CRD2 and CRD3 in HVEM, predicted to contain the LIGHT binding site. The structure of the ectodomain of HVEM is in ribbon format showing CRD1 (blue), CRD2 (magenta), CRD3 (gray), and disulfide bonds (yellow) in all structures. The C-terminus is oriented towards the bottom, where it would transverse the membrane. The HVEM-BTLA complex is viewed from the side (middle panel) showing BTLA (green) (from 2AW2.pdb). Structures were drawn using PyMOL (http://www.pymol.org).

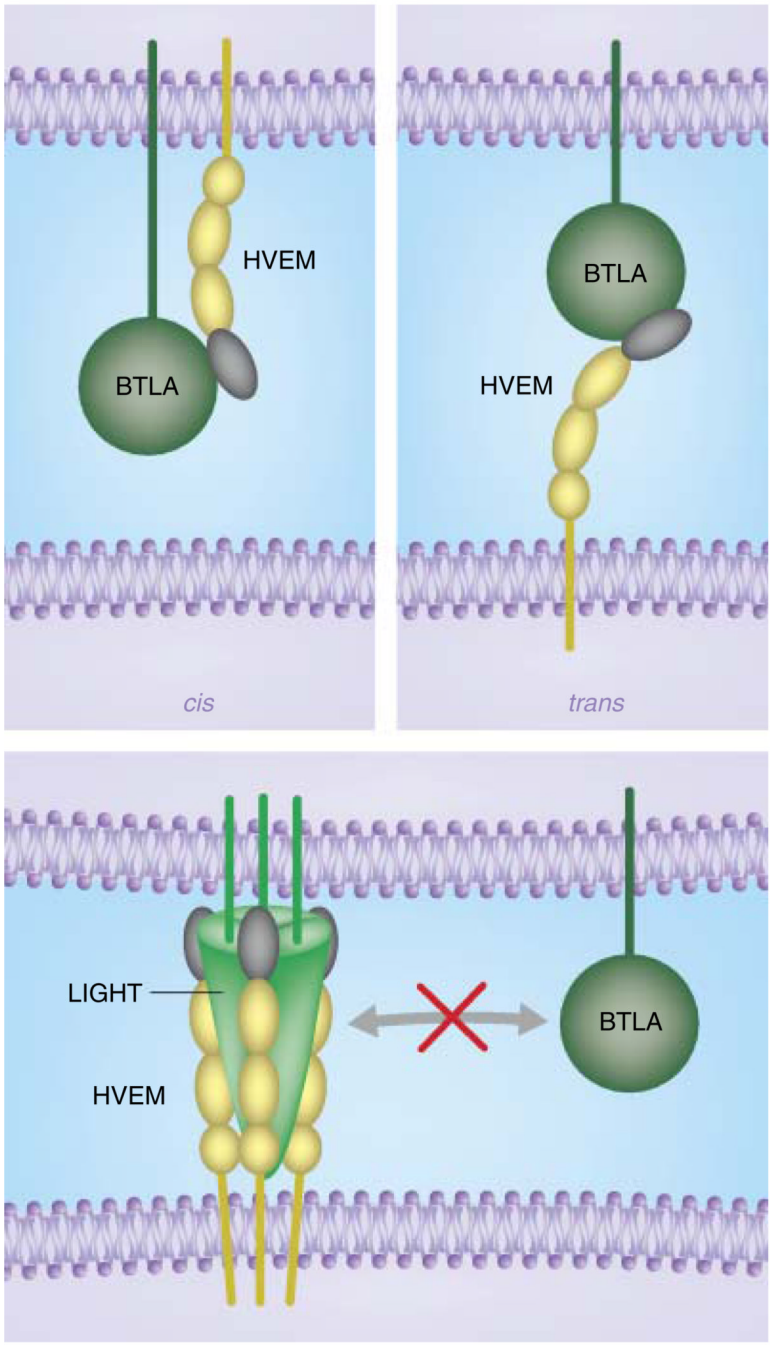

Fig. 5. The LIGHT-HVEM-BTLA switch

HVEM and BTLA interact when expressed in the same cell (*cis*) or between adjacent cells (*trans*). Conformation flexibility of HVEM or BTLA may be required when in *cis*, based on structural analysis (63). Induction of membrane LIGHT, which interacts in *trans* with HVEM, excludes BTLA from binding HVEM, turning off inhibitory signaling.

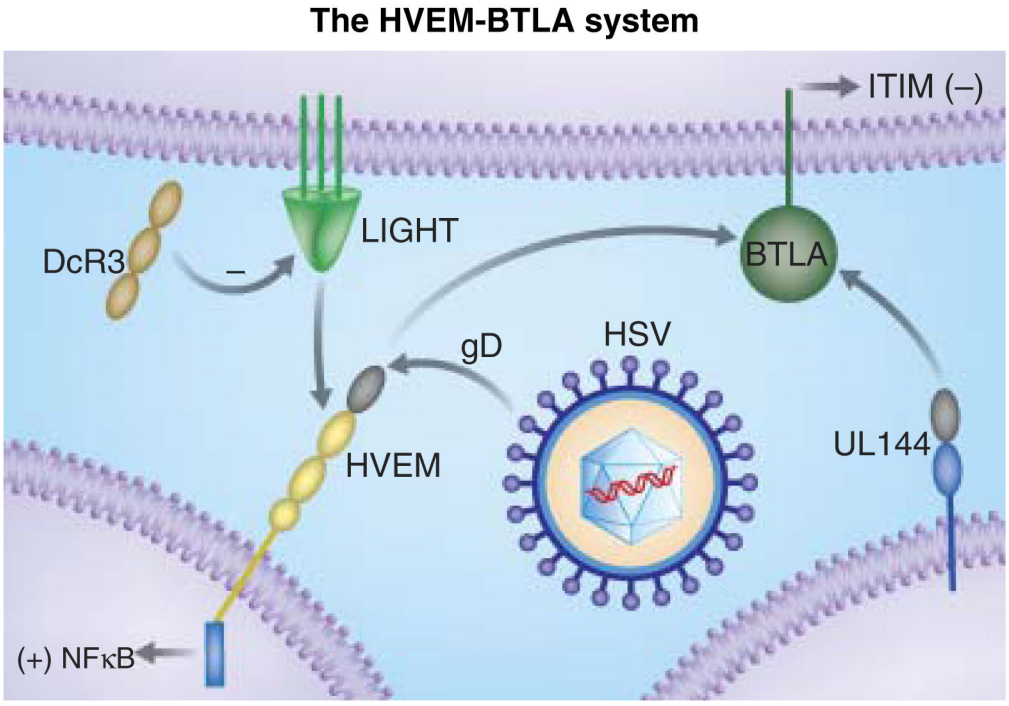

Fig. 6. The HVEM switch is targeted by herpesvirus

The depicted interactions involving HVEM that initiate positive cosignaling through LIGHT-HVEM interaction or inhibitory signaling through HVEM binding BTLA. LIGHT bound to HVEM activates (+) TRAF-dependent activation of NF-κB, whereas HVEM-BTLA acts through an immunoreceptor tyrosine-based inhibitory motif of BTLA to recruit the phosphatase SHP-2, attenuating kinases activated by TCR signaling. The HSV virion envelope protein gD attaches to HVEM, acting as an entry step for infection. The binding of gD to HVEM competitively blocks BTLA binding and non-competitively prevents LIGHT binding, inhibiting both intercellular communication pathways. UL144 of human CMV binds to BTLA but not LIGHT, selectively mimicking the inhibitory pathway of HVEM-BTLA. DcR3 is a soluble TNFR family member that binds to LIGHT, acting as a circulating inhibitor of LIGHT-HVEM signaling. The CRD1 of each protein is shown as a gray oval (78).

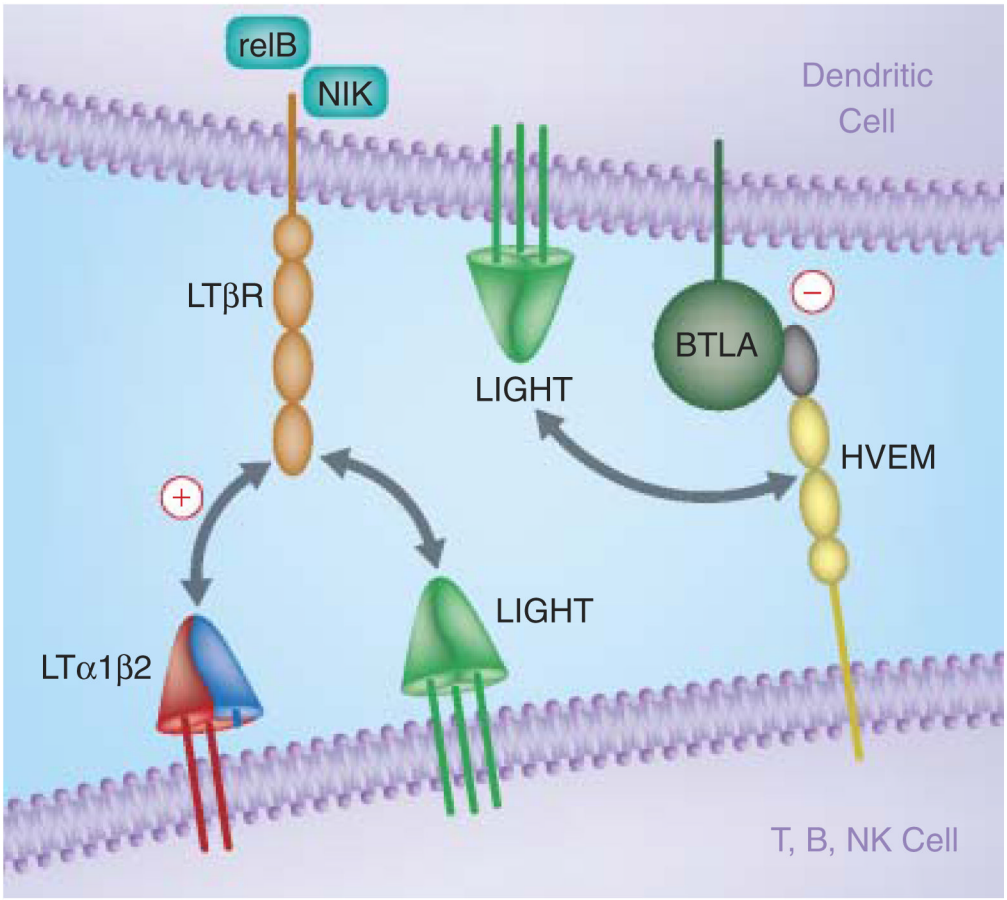

Fig. 7. Integrated signaling by LTαβ-LTβR and HVEM-BTLA pathways regulates DC homeostasis

LTαβ specifically regulates the proliferation of CD8α$^-$ DC subsets in the spleen through a LTβR-NIK-RelB-dependent pathway during homeostasis. LIGHT expression in activated T cells can also increase DC proliferation through LTβR. Signaling through HVEM-BTLA provides inhibitory signaling that limits LTαβ-dependent proliferation of the CD8α$^-$ DC subsets. HVEM-BTLA expression in DCs and in stromal cells contributes to limiting DC proliferation (99).

Table 1

Cosignaling TNF receptors

TNFR*	Locationw†	Expression‡	Signaling§	Function in T cells¶
TNFR1	6F3	Induced	D D	Clonal contraction- apoptosis
LTβR	6F3	None	TRAF	Microenvironments
CD27	6F3	Constitutive	TRAF	Survival - early expansion
GITR	4E2	Constitutive	TRAF	Positive cosignaling - T Regulatory cells
Ox40	4E2	Induced	TRAF	Survival - CD4 T Effector/memory
HVEM	4E2	Constitutive	TRAF	Positive - LIGHT Negative - BTLA
DR3	4E2	Induced	D D	Negative selection/apoptosis
41BB	4E2	Induced	TRAF	Survival - effector/memory
CD30	4E1	Induced	TRAF	TH2 survival
TNFR2	4E1	Induced	TRAF	Survival

*Numerical nomenclature for genomic localization (www.genenames.org).

†Chromosomal position in *Mus musculus*.

‡Expression on naive T cells from spleen.

§Cytoplasmic domain: DD, death domain; TRAF, TNFR-associated factors.

¶From studies with gene-deficient mice.

Table 2

Regulatory mechanisms of membrane LIGHT

Regulatory feature	References
Inducible transcriptional regulation controls LIGHT synthesis	(22,35)
Alternate splicing of LIGHT mRNA redirects protein compartmentalization to cytosol	(61)
Shedding membrane LIGHT alters membrane position	(61)
Downregulation of receptor expression	(36)
Decoy receptor 3 blocks LIGHT interaction with LTβR and HVEM	(26)

Table 3

Blockade of LIGHT and LTαβ in experimental pathogenesis models

Model*	Result	References
Transgenic LIGHT		
T cells	Acute onset, autoimmune like disease. Inflamed intestines, reproductive organs, skin and liver; abnormal lymphoid tissues	(82,83)
T-cell transfer	Atherosclerosis	(121)
T-cell transfer	Inflammatory bowel disease	(122)
Tumor transgene†	Tumor rejection by $CD8^+$ T cells	(123)
$LIGHT^{-/-}$ mice		
Cardiac allograft rejection	Rejection minimized	(81)
GVHD	Reduced inflammation	(102)
Superantigen	$CD8^+$ T-cell proliferation defect	(84)
Mitogen-induced hepatitis	Increased survival and decreased hepatic inflammation mediated by CD4 T	(115)
Biologics		
GVHD (MHC II)	HVEM-Fc or LTβR-Fc decreased inflammation	(124,125)
EAE	LTβR-Fc suppressed paralysis	(111)
Cuperizone-induced demyelination	LTβR-Fc decreased demyelination and enhanced remyelination	(126)
CIA	LTβR-Fc suppressed	(110)
Islet allograft	Anti-BTLA/CTLA4Ig suppressed promoted graft acceptance	(117,127)

GVHD, graft-versus-host disease; MHC, major histocompatibility complex; EAE, experimental autoimmune encephalitis; CIA, collagen-induced arthritis.

* LIGHT expressed as a transgene in T cells.

† LIGHT expressed as a transgene in the tumor.

Table 4

Lymphotoxins in experimental host defense models

Pathogen*	Mouse model†	Susceptibility	Mechanism	References
Herpesvirus				
MHV68	LTα$^{-/-}$	Minimal	ND	(128)
HSV-1	LTα$^{-/-}$	Increased	Decreased effector CD8^{+} T cells	(129)
MCMV	LTα$^{-/-}$	Increased	PoorIFN-β	(108)
MCMV	LTβR-Fc Tg	Increased	Poor innate defenses	(107)
MCMV	LIGHT$^{-/-}$	Minimal	ND	(108)
MCMV	HVEM$^{-/-}$	No change		(108)
LCMV	LTβ$^{-/-}$;LTα$^{-/-}$	Increased	Defective architecture	(130,131)
LCMV	LTβR-Fc	Decreased	Decreased CD8^{+}/IFN-γ	(132)
Theiler's virus	LTα$^{-/-}$ LTβR-Fc	Increased	Defective architecture	(133)
Influenza	LTα$^{-/-}$	Minimal	ND	(134)
M. tuberculosis	LTβR$^{-/-}$	Increased	NO_2 synthase decreased	(135)
M. tuberculosis	LTα$^{-/-}$	Increased	No T cells in granuloma	(136)
M. bovis	LTβR-Fc	Increased	Poorgranuloma formation	(137)
Listeria m.	LTβR$^{-/-}$	Increased	ND	(135)
Leishmania m.	LTβ$^{-/-}$	Increased	Defective architecture	(138)
Leishmania m.	LIGHT$^{-/-}$	Increased	Impaired IL-12p40 production by DCs	(139)
Toxoplasma g.	LTα$^{-/-}$	Increased	NO_2 synthase decreased	(140)
Plasmodium b.	LTα$^{-/-}$	Decreased	Decreased LTα-dependent inflammation	(141)

*Studies conducted in gene-deficient mice ($^{-/-}$); LTβR-Fc Tg, mice expressing LTβR-Fc as a transgene; LTβR-Fc, mice injected with protein.

†Pathogens: virus: mouse γ-herpesvirus-68 (MHV68), herpes simplex virus (HSV1, α-herpesvirus), mouse cytomegalovirus (MCMV), lymphocytic choriomeningitis virus (LCMV); bacteria: *Mycobacterium, Listeria monocytogenes;* parasite: Leishmania major, Toxoplasma gondii, Plasmodium berghei.