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The Signaling Networks of the Herpesvirus Entry Mediator (TNFRSF14) in Immune Regulation

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Summary

The tumor necrosis factor (TNF) receptor superfamily member herpesvirus entry mediator (HVEM) (TNFRSF14) regulates T-cell immune responses by activating both inflammatory and inhibitory signaling pathways. HVEM acts as both a receptor for the canonical TNF-related ligands, LIGHT [lymphotoxin-like, exhibits inducible expression, and competes with herpes simplex virus glycoprotein D for HVEM, a receptor expressed on T lymphocytes] and lymphotoxin- α , and as a ligand for the immunoglobulin superfamily proteins BTLA (B and T lymphocyte attenuator) and CD160, a feature distinguishing HVEM from other immune regulatory molecules. The ability of HVEM to interact with multiple ligands in distinct configurations creates a functionally diverse set of intrinsic and bidirectional signaling pathways that control both inflammatory and inhibitory responses. The HVEM system is integrated into the larger LT β R and TNFR network through extensive shared ligand and receptor usage. Experimental mouse models and human diseases indicate that dysregulation of HVEM network may contribute to autoimmune pathogenesis, making it an attractive target for drug intervention.

Keywords

T cells; asthma; autoimmunity; cancer; cytokines; inflammation

Introduction

The tumor necrosis factor receptor superfamily member (TNFRSF) herpesvirus entry mediator (HVEM) (TNFRSF14) acts as both receptor and ligand, properties that have been thoroughly exploited by viral pathogens. HVEM was originally identified in a functional screen for herpes simplex virus-1 (HSV-1) entry (1) and was recognized as one of two major routes of entry into human and mouse cells (2). The distinct ligand-binding profile of HVEM sets it apart from other members of the TNFR superfamily. In common with other TNFRSF members, HVEM binds the canonical TNF-related ligands, lymphotoxin- α (LT- α) and LIGHT (lymphotoxin-like, exhibits inducible expression, and competes with HSV glycoprotein D for HVEM, a receptor expressed by T lymphocytes) (TNFSF14); however, the distinguishing feature of HVEM is engagement of members of the immunoglobulin superfamily, B and T lymphocyte attenuator (BTLA) and CD160 (3), as well as the virion glycoprotein D of HSV (2). The ability of HVEM to interact with multiple ligands in distinct configurations creates a functionally diverse set of intrinsic and bidirectional signaling

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pathways (Fig. 1). The capacity to bind these different ligands resides in two different topographical regions in the extracellular domain of HVEM. These distinct sites impart the ability of HVEM to activate both pro-inflammatory and inhibitory pathways. The HVEM pathway is set within the larger TNF/LT network hardwired through the binding of LIGHT to LT β R, HVEM, and DcR3, and LT α to HVEM, TNFR1, and TNFR2 (4, 5) (Fig. 1). With HVEM at the nexus in several signaling pathways, it is not surprising that it plays important roles in the immune system, such as T-cell costimulation, regulation of dendritic cell (DC) homeostasis, autoimmune-mediated inflammatory responses, as well as host defense against pathogens. HVEM may also play significant roles outside the immune system, in the regulation of sensory neuron development (6) and adipocyte metabolism (7, 8). Substantial evidence from both experimental mouse models and human diseases indicate that dysregulation of LIGHT-HVEM-BTLA/CD160 cosignaling network may contribute to autoimmune diseases including rheumatoid arthritis, inflammatory bowel disease, and asthma, making the HVEM-BTLA/CD160 cosignaling system an attractive pathway for drug intervention. This review focuses on rectifying recent biophysical and cellular aspects of ligand-receptor interactions of HVEM with outcomes in relevant disease models.

HVEM and its ligands and coreceptors

HVEM functions as both a receptor with signal-transducing functions and as a ligand eliciting signaling. This functional dichotomy reflects the distinct ligand binding sites on HVEM. The HVEM gene contains 8 exons creating a type 1 transmembrane glycoprotein with four pseudo repeats of the cysteine-rich domain (CRD) (numbered 1-4 from the N-terminus) in its extracellular domain, characteristic of the TNFRSF (Table 1). The disulfide bonds in the CRD form an elongated, ladder-like structure. The signaling potential of HVEM resides in a relatively short cytoplasmic tail, with a binding site for the TNFR-associated factor (TRAF) family of ubiquitin E3 ligases that initiates activation of nuclear factor- κ B (NF- κ B) transcription factors critical for controlling genes involved in cell survival and inflammation (9, 10).

In its ecto domain, HVEM has two spatially distinct ligand-binding regions, one for the conventional ligands (LIGHT and LT α) and a second distinct site for the non-canonical ligands (BTLA, CD160, and HSV gD). Mutational analysis and molecular modeling implicate the LIGHT and LT α binding sites reside in CRD2 and CRD3, which is the characteristic for TNF receptors and their corresponding TNF-related ligands (11). In contrast, HVEM interacts with BTLA and CD160 in the CRD1 region. Structural-based mutagenesis and crystallography of the BTLA-HVEM and gD-HVEM complexes revealed CRD1 as the critical binding region (12-15) and through a conserved mechanism involving a short anti-parallel β strand (13). The DARC (gD and BTLA binding site on the TNFR HVEM in CRD1) region for BTLA-binding site is on the opposite face from the LIGHT-binding interface. CD160 and HSV-gD also bind HVEM in the CRD1 and show cross competitive binding profile indicating the binding sites are topographical close (9). The region where BTLA, CD160, and the viral ligand gD bind is known as the DARC side of HVEM (12). Soluble LIGHT is not competitive for BTLA or CD160 binding; however, membrane LIGHT displaces HVEM-BTLA interactions, presumably by steric hindrance due to constraints of positioning at the cell surface. The utilization of the LIGHT or the DARC binding regions of HVEM constitutes the basis for its ability to switch between activating and inhibitory signaling.

HVEM ligands

TNF-related ligands: LIGHT and LT α

LT α and LIGHT contain the TNF homology domain a β -sheet sandwich fold defining the conventionality of the TNF superfamily (16, 17). This β -sandwich structure readily assembles into trimers providing the basis for clustering of their cognate cell surface receptors that initiates signaling. Contributions of amino acid residues from adjacent subunits in the trimer form a receptor-binding site (18), with three equivalent sites in a homotrimer. The genes encoding LT α and LIGHT reside in major histocompatibility complex paralogous regions of the genome, with LT α in close association with LT β and TNF in the major histocompatibility complex (MHC), and LIGHT linked to CD27L and 41BBL (19) (Table 1).

The physical positions of the ligand in the membrane or in soluble form contribute to the direction and characteristic of the induced signals. Both LIGHT and LT α are positioned at the cell surface, but by distinct mechanisms. Most TNF-related cytokines including LIGHT contain an internal N-terminus (type II transmembrane protein), but proteases selectively cleave the ectodomain into a stable soluble form that retains receptor-binding and signal-activating properties. An alternate spliced form of LIGHT mRNA deletes the transmembrane domain directing this form into the cytosol with unknown function (19, 20). In contrast, LT α contains a conventional leader sequence leading to exclusive secretion of the LT α homotrimer. However, LT α also forms a heterotrimer with the related cytokine, LT β , which has a transmembrane domain, thus retaining LT α on the cell surface (21). The LT $\alpha\beta$ heterotrimer with a stoichiometry of 1:2 has a distinct receptor binding profile from LT α due to the changes in the interface of the adjacent LT β subunits (22).

Constitutive expression of LIGHT in T cells elicits a powerful proinflammatory response in mice (23, 24), and thus its expression is tightly controlled by multiple mechanisms including transcriptional regulation (25) with inducible, transient expression in T and B cells, NK cells, and immature DCs. Elevated serum levels of LIGHT are found in autoimmune diseases, which in humans can interact with a soluble decoy. Soluble decoy receptor-3 (DcR3) binds LIGHT to regulate bioavailability (26-28). The presence of DcR3 competes with HVEM and/or LT β R thus reducing the bioavailability of LIGHT. DcR3 is a secreted protein that is most closely related to osteoprotegerin, and identified as an overexpressed gene in human cancers (29-33). Although DcR3 is a non-signaling receptor of the TNFR superfamily, it binds to several TNF superfamily ligands, including LIGHT, FasL and TL1A (34-37). The DcR3 gene is absent in the mouse genome limiting a more complete understanding of its functional roles; however, DcR3 levels are elevated in serum in autoimmune diseases, suggesting DcR3 may have a pathological role in autoimmune disease-associated inflammation (38-41). The interaction of DcR3 and polymorphic forms of LIGHT suggested that DcR3 might have a role in regulating the bioavailability of its corresponding ligands in inflamed microenvironments (27).

Unconventional ligands: BTLA, gD, and CD160

Human BTLA (CD272) is an Ig superfamily member originally cloned from human and mouse T-cell cDNA libraries (42). BTLA is a type 1 glycosylated transmembrane protein with a single intermediate (I) type Ig extracellular domain (13, 14), a feature distinguishing BTLA from CD28/CTLA4-related signaling coreceptor family (Table 1).

HVEM was identified as a cellular ligand for BTLA by functional proteomic screens (15, 43). These studies also provided insight into the binding site of BTLA, indicating that the CRD1 region of HVEM was important for BTLA binding. The HVEM-BTLA cocrystal complex revealed the surface of interaction is large and the energetics of binding is through

a short anti-parallel β -strand interaction (13), a feature that is conserved in gD (44). BTLA expressed in membranes of viable cells forms homodimers as demonstrated by flow-based emission fluorescence resonance energy transfer (9), although the purified Ig domain forms monomers in solution interacting at a 1:1 stoichiometry with HVEM (13). The multimeric structure of BTLA at the cell surface provides a mechanism for clustering HVEM and thus initiating signaling.

BTLA mediates inhibitory signaling through recruitment of tyrosine phosphatases. The cytoplasmic tails of both human and mouse BTLA contain three conserved tyrosine-based signaling motifs [two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and a Grb-2 recognition consensus site] (45). The ITIMs in BTLA are important for the recruitment of phosphatases, Src homology domain phosphatase-1 (SHP-1) and SHP-2 (45, 46). Ligation of BTLA by HVEM resulted in the recruitment of SHP-1 and SHP-2 to the cytoplasmic tail of BTLA, providing a mechanism for limiting the stimulatory pathways activated by tyrosine kinases associated with T-cell (46, 47) and B-cell antigen receptors (48).

Substantial evidence implicating that BTLA functions as an inhibitory receptor of both B and T-cell signaling pathways. BTLA-deficient mice showed an enhancement of T-cell proliferation in responses to mitogen stimulation or to the activation with anti-CD3 antibodies (42, 49). Furthermore, these animals appear to be more susceptible to the development of autoimmune disorders, such as experimental autoimmune encephalomyelitis (42, 50). Antibodies to BTLA inhibited T-cell proliferation, resulting in downregulation of CD25 and the production of IL2, IL4, IL10, and IFN- γ (51, 52).

More recently, BTLA was recognized as a ligand for HVEM, inducing NF- κ B activation (9). This feature of BTLA demonstrates the HVEM-BTLA pathway is bidirectional and provides T-cell survival signals via HVEM activation of NF- κ B-dependent genes. The wide expression of BTLA in the hematopoietic compartment suggests a role as a bidirectional coreceptor in multiple situations including T and B cells, as well as myeloid cell lineages, for instance in regulating DC proliferation in secondary lymphoid organs (53). BTLA is constitutively expressed in naive T cells, T cells in the developing thymus, both naive and activated B cells, DCs, macrophages, NK cells, and NKT cells (42, 45, 49, 54). Finding that inhibitory signaling via BTLA can modulate immune responses *in vivo* suggests potential utility in treating autoimmune diseases and graft rejection (3, 55-57), and similarly, antagonists of BTLA signaling may enhance immunity to cancer (58-60).

CD160 (BY55) was originally identified by monoclonal antibody screening of an NK cell line (61). The mRNA encodes a membrane protein V-type Ig domain with two different anchors to the cell surface, a glycosphingolipid (GPI) anchor and a transmembrane form (62), generated by alternate splicing. CD160 may also be found as a soluble molecule as a result of cleavage of GPI-linked form by phospholipases.

CD160 engages both classical and nonclassical MHC class I molecules (63-65). HVEM was identified as the signaling ligand for CD160 by a proteomics screening of a human B-cell cDNA expression library using CD160-Ig (66). Like BTLA, CD160 also binds HVEM in the CRD1 region, and the binding sites of both BTLA and CD160 appear to be topographically close to each other (9). CD160 forms a disulfide-linked interchain homotrimers (61, 67) that can activate HVEM signaling, and thus like BTLA form a bidirectional signaling pathway (9).

Northern blot analysis showed that expression of human CD160 was highly restricted to circulating NK cells and T cells. Within subsets of NK cells, CD160 is expressed by CD56^{dim}CD16⁺ cytolytic NK cells, whereas in the subsets of T cells, its expression is found in $\gamma\delta$ TCR T cells, CD8⁺CD28⁻ effector T cells, intestinal intraepithelial lymphocyte (IEL)

T cells, and activated CD4⁺ T cells (61, 68). Furthermore, expression of CD160 was also detected in NKT cells (69). CD160 also showed an inducible pattern of expression with upregulation in activated CD8⁺ T cells and CD4⁺ T cells (66). Although CD160 is not expressed in naive or activated B lymphocytes, it is dramatically increased in B-cell malignancies (70, 71).

CD160 functions as an inhibitory receptor of both NK cell and T-cell signaling pathways but is also activating depending upon its specific form. Upon activation of CD4⁺ T cells, CD160 is upregulated, and engagement of CD160 by HVEM (expressed by APCs) blocks LIGHT-activating HVEM signaling that promotes cytokine release (66). Soluble CD160 blocks NK cell cytolytic activity (67). The CD160 transmembrane form has an activating role in NK cells as assessed by the increased CD107a surface mobilization observed upon its ligation through recruitment of Src-family kinase p56(lck) and Erk1/2 activation pathway (62). The enigmatic role of CD160 in immune function revealed to date may only reflect its multiple forms and how they engage their respective ligands and coreceptors.

HSV gD is a viral ligand of HVEM and allows the virion of HSV-1 and HSV-2 to initiate infection via multiple entry routes (2, 72-74). HSV gD is a type 1 transmembrane protein with receptor binding domain located at the N-terminus and the ectodomain contains three N-glycosylation sites and six cysteine residues forming three disulfide bonds. Although gD is a resident virion protein, expression of gD is also found in host cell membrane independent of the virion (75). Like BTLA, gD forms a dimer in the cellular membranes (9). Recent studies showed that gD may also serve as an activating ligand for HVEM, inducing NF- κ B activation in cells expressing HVEM. Stimulating HVEM-expressing THP-1 cells with soluble gD induced NF- κ B activation while inhibiting the binding between gD and HVEM neutralized the effect (76).

The HVEM-BTLA cocystal complex (13) revealed the surface and contact residues in HVEM are very similar to binding with HSV-gD (44), implicating gD as a viral mimic of BTLA. Even though the binding site of gD on HVEM is located in the CRD1 on the opposite face of HVEM from where LIGHT binds, gD is able to prevent HVEM from interacting with membrane LIGHT, BTLA, and CD160. The evolutionarily honed gD binding site on HVEM allows HSV gD to act as a multi-function inhibitor of HVEM's ligands.

BTLA also interacts with UL144, an HVEM ortholog present in the primate cytomegalovirus (β herpesvirus). The binding site of BTLA on UL144 is located at the CRD1 region of UL144 (12). Although HSV and CMV are evolutionarily divergent herpesviruses, the targeting of a common cytokine pathway, albeit by opposite mechanisms, implicates the HVEM-BTLA pathway is critically important for immune regulation (12).

Besides HVEM, gD binds Nectin1 and Nectin2, and O-sulfated glycans, all serving as potential entry routes for HSV to infect cells *in vitro* (77). However, using HVEM and Nectin1-deficient mice, HVEM was shown to play a secondary role to Nectin1 in the ability of HSV-2 to infect in the vaginal mucosal (78). Dual deficient mice showed no virus replication, although Nectin1-deficient mice supported infection albeit at an order of magnitude higher virus inoculum indicating HVEM functions as an entry route. Using a gD mutant selectively unable to bind HVEM, the early host response to HSV-2 as measured by chemokine expression was depressed, implicating gD-HVEM interactions are needed to suppress innate defenses (79). However, cellular immunity as measured by the initial clonal expansion of CD4⁺ and CD8⁺ T cells was equivalent with either gD, indicating gD-HVEM interaction is not altering adaptive responses. Another feature of the pathogenicity of HSV is its ability to infect neurons. The HVEM-gD entry route appears unessential in mice infected by direct inoculation into the hippocampus (80). In contrast, Nectin1 is critical for

intracranial infection and encephalitis, as no infection occurred in the Nectin1-deficient mice.

Trans- and cis-complexes of HVEM

The cellular context in which HVEM engages BTLA determines whether the cellular response is activating or inhibitory. The cellular expression patterns of HVEM and BTLA define the context for signaling. When expressed in different cells (*in trans*), HVEM binding BTLA (or CD160 or gD) is activating for HVEM and BTLA (9) (Fig. 1). HVEM-BTLA pathway is bidirectional in the *in trans* context: HVEM ligation turns on NF- κ B dependent survival genes, whereas BTLA signaling is inhibitory through SHP1/2 phosphatases. This result was surprising in view of interpretation of the cocrystal structure given by Compaan *et al.* (13), that BTLA was unable to induce HVEM activation.

HVEM and BTLA can also be coexpressed in the same cell (*in cis*) (Fig. 2A). The intrinsic expression of HVEM and BTLA leads to formation of a *cis*-complex in which BTLA suppresses HVEM signaling of NF- κ B by all of its membrane-bound ligands (CD160, BTLA, and LIGHT) in *trans* (28). HVEM-BTLA forms a very stable complex at the cell surface, although each molecule is independently expressed. Several lines of evidence document the formation of an HVEM-BTLA *cis*-complex including co-association in viable cells by fluorescence resonance energy transfer (FRET) analysis and coimmunoprecipitation from T cells. The *cis*-complex appears to form via the same interaction sites as *in trans* binding as determined by mutagenesis of key residues. Cells expressing HVEM-BTLA *cis*-complex cannot bind HVEM-Fc or BTLA-Fc, at concentrations well beyond saturation, which provides a method for estimating the presence of the HVEM-BTLA *cis*-complex (28). In naive human T cells, we estimated that HVEM and BTLA are expressed at approximately 1:1 ratio with >85% in *cis*-complex. Changes in the ratio of HVEM to BTLA at the cell surface may add to the dynamics of the *cis*-complex, although the mechanisms regulating expression is not well defined. As the binding sites of BTLA, CD160, and gD on CRD1 of HVEM are topographically close to each other, we suggest that the formation of *cis*-complexes between HVEM and BTLA, CD160, or gD competitively (against BTLA, CD160, and gD) or non-competitively (against LIGHT and LT α) inhibits HVEM activation by ligands expressed in cells in the surrounding microenvironment.

The membrane form of LIGHT is the only cellular ligand capable of activating HVEM when expressed in the *cis*-complex. Indeed, dissociation of *cis*-complex with a monoclonal antibody to BTLA dramatically potentiated LIGHT-induced NF- κ B activation (28). We propose this interaction is a key mechanism that drives naive T cells into an activated state that is critical for cell survival signaling. The topographically distinct sites on HVEM for LIGHT and BTLA provide an important feature distinguishing between the membrane and soluble forms of LIGHT. LIGHT binds HVEM on CRD2 and CRD3, while BTLA binds HVEM on CRD1 at the DARC side. HVEM-BTLA *trans*-interactions are blocked when membrane LIGHT engages HVEM, which is likely due to steric hindrance. We suggest that the proximity of receptor-binding domain of LIGHT to the membrane surface could prevent or limit BTLA from engaging HVEM *in trans* when both LIGHT and BTLA reside on the same membrane (Fig. 2B). As there is a significant difference in the binding avidity of LIGHT and BTLA, the much higher avidity of LIGHT for HVEM may drive the uncompetitive dissociation of HVEM from BTLA. The stereo-geometry of membrane restricts LIGHT to *in trans* binding. Acting in a noncompetitive fashion, membrane LIGHT may disrupt inhibitory signaling by BTLA (Fig. 2B). However, it appears possible that CD160 can form a trimolecular complex with membrane LIGHT and HVEM, as the inherent flexibility of GPI linkage of CD160 is likely to accommodate the steric requirement

for the formation of a trimolecular complex. Thus, CD160 could act to inhibit BTLA from engaging HVEM and thus block inhibitory signaling.

The soluble forms of LIGHT and LT α were also unable to initiate HVEM signaling in cells expressing the *cis*-complex; indeed, the soluble ligands enhanced binding between HVEM and BTLA (28). The presence of soluble LIGHT or LT α in the trimolecular complexes may help stabilize the HVEM-BTLA *cis*- or *trans*-complexes (Fig. 2A,C). Thus, soluble LIGHT or LT α are predicted to function as agonists for BTLA inhibitory signaling via binding HVEM in the *cis*-complex. In cells that express HVEM without BTLA, such as mucosal epithelia cells, soluble LIGHT and LT α can act as agonists of HVEM driving an NF- κ B response alone or *in trans* with BTLA or CD160.

These results indicate that each molecule, LIGHT, HVEM, and BTLA (or CD160), has an inherent activating and inhibitory function depending on the context of engaging its 'ligand' in either *cis*- or *trans*-configurations and whether the ligand is soluble or transmembrane-anchored (LIGHT and CD160). These biophysical features of the HVEM systems are, to a first approximation, predictive of the behavior for T cells; other cell types remain to be evaluated. Substantial evidence has demonstrated that HVEM is a positive T-cell regulator. LIGHT-mediated HVEM signaling provides prosurvival signals and proliferative responses to T cells through the activation of NF- κ B transcriptional programs. In addition, ligation of BTLA, CD160, or gD to HVEM *in trans* also activated NF- κ B via a TRAF2-dependent pathway, providing a prosurvival signal for T cells (9). The HVEM signaling circuitry described here may provide the context for evaluating immune responses in physiological settings and pathogenic processes discussed in the following sections.

HVEM as a pro-inflammatory mediator in disease models

HVEM functions as a molecular switch between stimulatory or inhibitory inflammatory pathways by engaging two distinct groups of ligands and coreceptors. Some disease models indicate that HVEM functions primarily as an inhibitory ligand through BTLA, yet several other models indicate that HVEM via LIGHT promotes inflammation and disease pathogenesis (Table 2). The previous difficulties in the interpretation of some of the outcomes in mice genetically deficient in these molecules takes focus through the perspective of the *cis* and *trans* signaling modes now recognized for HVEM but viewed within the larger networks formed by LIGHT, LT $\alpha\beta$ -LT β R and LT α , TNF-TNFR and DcR3 pathways. This biophysical model can help interpret some complex phenotypes, yet additional experimentation and validation are needed, particularly in defining cell-to-cell interactions.

GVHD and transplantation

LIGHT, as a costimulator for T-cell function, has been found to play a critical role in graft-versus-host disease (GVHD), and it has been proposed as a potential target for therapy to reduce GVHD in transplant patients. In a non-irradiated parent-into-F1 mouse model of GVHD, LIGHT deficiency or blockade of LIGHT-HVEM interaction by LT β R-Fc inhibited alloreactive cytotoxic T-lymphocyte (CTL) generation and prolonged the survival of the host mice (81). In addition, *Hvem*^{-/-} donor cells failed to induce GVHD, suggesting that LIGHT signaling during GVHD appears to exert its influence through HVEM expressed in T cells (81). Interestingly, a related study using the non-irradiated parent-into-F1 graft revealed that blockade of the HVEM-BTLA interaction with an anti-BTLA antibody led to reduced CTL activity and GVHD response. Moreover, BTLA-deficient mice were unable to sustain the GVHD response compared to WT counterparts (82). Thus, during GVHD responses, HVEM interactions with LIGHT and/or BTLA promoted T-cell responses and

sustained CTL-mediated inflammation responsible for GVHD. Here, we suggest that both LIGHT and BTLA serve as activating ligands in *trans* for HVEM.

An inflammatory role for HVEM has also been described in solid organ transplantation models. Studies in mice with fully MHC-mismatched cardiac transplants suggested a role for the LIGHT-HVEM pathway in regulating T-cell-mediated transplant rejection (83). In addition, hosts deficient in both LIGHT and CD28 tolerated allogeneic skin grafts longer than WT control mice (84). Similarly, blockade of both LIGHT-HVEM and B7-CD28 interactions facilitated long-term islets allograft survival (85).

Tumor immunology

LIGHT-HVEM interaction plays an important role regulating host anti-tumor immune responses (86). Ectopic expression of LIGHT in certain tumors leads to the activation of T and NK cells and the surrounding tumor stromal tissue, resulting in an enhanced tumor-specific T-cell immune response and clearance of the tumor (86, 87). LIGHT signaling through HVEM on NK cells enhanced IFN γ production, whereas HVEM activation on T cells increased T-cell priming, tumor infiltration, and destruction of LIGHT-expressing tumors (87). The activation of LT β R by LIGHT also played an important role in these tumor models through induction of chemokines (e.g. CCL21) in stromal cells that form microenvironments supportive of immune responses.

LIGHT is crucial for airway remodeling and airway hyperresponsiveness in mouse models of chronic lung inflammation with features of asthma (88). LIGHT-deficient mice showed an impairment in fibrosis and smooth muscle accumulation, and pharmacological blockade of LIGHT binding to its receptors also reduced lung fibrosis, smooth muscle hyperplasia, and airway hyperresponsiveness. Contrarily, exogenous administration of recombinant soluble LIGHT to the airways induced fibrosis and smooth muscle hyperplasia in WT mice (88). Recombinant soluble LIGHT can bind to HVEM on vascular smooth muscle cells enhancing cell proliferation (89), and it is therefore possible that LIGHT induces hyperplasia by engaging HVEM on vascular smooth muscle cells. The ability of LIGHT to activate LT β R in smooth muscle cells cannot be ruled out. However, LIGHT-dependent tissue remodeling in these chronic asthma models seems to be related to an increased production in the lung of transforming growth factor- β (TGF- β) and interleukin-13 (IL-13), two cytokines implicated in remodeling. Whereas increased production of TGF- β appears to be triggered by LIGHT through the direct stimulation of LT β R expressed in lung macrophages, increased production IL-13 required HVEM expression in lung eosinophils. Eosinophils purified from the lung of asthmatic animals expressed high amounts of HVEM, but not LT β R, and *in vitro* stimulation with soluble recombinant LIGHT strongly promoted IL-13 production (88). Surprisingly, Th2 cells that also express substantial levels on HVEM were not affected by either LIGHT deficiency or LIGHT blockade, and their production of IL-13 and IL-5 was unaltered in comparison with controls (88). Although LIGHT did not regulate Th2 cell responses, in a different experimental setting, these authors reported a critical role for LIGHT-HVEM interaction in the generation and maintenance of long-lived memory Th1 and Th2 cells after recall response to soluble antigen (90). These findings demonstrate an HVEM requirement for cell activation, expansion, and/or survival that critically depends on the type of inflammatory response and varies within different immune cell subsets. The uncertainty is whether BTLA or CD160 coexpressed with HVEM may play a role in effector T cells or eosinophils regulating LIGHT-mediated cell activation and function.

Intestinal inflammation

A particularly important role for the LIGHT-HVEM interaction seems to take place in the intestine where this pathway has been associated with inflammation and pathogenesis of

inflammatory bowel disease (IBD) (91-93). In transgenic mice, constitutive expression of LIGHT in T lymphocytes exacerbated T cell activation resulting in multi-organ inflammation, particularly in severe intestinal inflammation with tissue destruction (23, 94). The intestine in these mice had signs of chronic inflammation with substantial mononuclear cell infiltrates dominated by activated T lymphocytes. The vast majority of these T cells were conventional, activated, or memory-type $\text{TCR}\alpha\beta^+$ CD4^+ and $\text{CD8}\alpha\beta^+$ T cells and were found in the intraepithelial compartment and the *lamina propria* of the small and large intestine (23). Mucosal T cells from LIGHT transgenic mice showed enhanced $\text{IFN}\gamma$ production, indicating a predominant Th1 response in the gut. However, these phenotypes may be additionally associated with Th17 response because the Th17 assays were not fully established at that time. Interestingly, activated T cells in the mesenteric lymph nodes (MLNs) of LIGHT transgenic mice expressed higher amounts of the $\alpha_4\beta_7$ integrin (95). The $\alpha_4\beta_7$ integrin is involved in mucosal homing (96), and it allows T cells to efficiently attach to the vascular addressin MAdCAM-1, which is expressed in intestinal micro vessels (97). Therefore, overexpression of LIGHT may cause severe intestinal inflammation by enhancing the recruitment of activated Th1 and potentially Th17 cells to the intestine. It is likely but not fully resolved that constitutively expressed LIGHT induces inflammation by boosting HVEM and $\text{LT}\beta\text{R}$ -mediated pathways. It has been proposed that the sustained binding of LIGHT to the $\text{LT}\beta\text{R}$ induces the transformation of the gut mucosa to promote the formation of pathological lymphoid structures (93). $\text{LT}\beta\text{R}$ is not expressed in T or B lymphocytes or NK cells, thus most of the T-cell-mediated pathology observed in the LIGHT transgenic animals likely depends on HVEM or indirectly through $\text{LT}\beta\text{R}$.

Schaer *et al.* (98) recently described a role for HVEM in disease pathogenesis in the dextran sulfate sodium (DSS) colitis model, an epithelial injury model of intestinal inflammation. The administration of DSS to WT mice led to severe weight loss and intestinal inflammation, yet DSS treatment in HVEM-deficient animals induced significantly less weight loss and attenuated intestinal immunopathology, suggesting that HVEM contributes to the development of disease in this model (98). Although the participation of T cells in the inflammatory response triggered by DSS is controversial, it is generally accepted that DSS-induced colitis largely depends on innate immune cells, and therefore it could be possible that HVEM expression in innate immune cells contributes to intestinal pathology after DSS challenge. However, we found HVEM dispensable for disease development in the DSS colitis model (99) with equivalent weight loss and comparable histological scores (99). Furthermore, DSS experiments performed in lymphopenic $\text{Rag}^{-/-}$ and HVEM-deficient $\text{Rag}^{-/-}$ ($\text{Hvem}^{-/-}\text{Rag}^{-/-}$) mice to directly address the role of HVEM expressed in innate immune cells, independently of T or B lymphocytes, revealed similar results (M. Steinberg, unpublished observations). The reasons for the different outcomes from the two studies using HVEM-deficient mice in the DSS model are not clear. There is evidence that the genetic background of different mouse strains influences the susceptibility to DSS-induced colitis, as does commensal intestinal microflora in the animals, which varies from one animal facility to another.

A role for HVEM in both activating and inhibitory signaling pathways in intestinal inflammation emerged from CD4^+ T-cell adoptive transfer models. Wang and colleagues (93) demonstrated that the transfer of LIGHT transgenic lymph node cells into $\text{Rag}^{-/-}$ animals induced a consistent, rapid, and synchronized intestinal inflammation. However, the transfer of LIGHT transgenic/ $\text{Hvem}^{-/-}$ lymphocytes cells into $\text{Rag}^{-/-}$ recipients failed to fully induce disease development. These data indicate that HVEM expression by lymph node cells, likely T lymphocytes, promotes intestinal inflammation and contributes to the immunopathology caused by constitutive expression of LIGHT. In the $\text{CD4}^+\text{CD45RB}^{\text{high}}$ T-cell colitis transfer model, HVEM expression in donor T cells is required for the development of colitis, but only to a limited extent (99). Although $\text{Rag}^{-/-}$ recipient mice

transferred with *Hvem*^{-/-} T cells unquestionably became ill, the animals had lower mononuclear cell infiltrates in the proximal and distal colons than recipients transferred with WT T cells. In addition, recipients of HVEM-deficient T cells had a higher number of goblet cells in the distal colon, indicating that inflammation in these animals was milder than that observed in animals transferred with WT T cells. We also found similar results when *Rag*^{-/-} mice were recipients of *Light*^{-/-}CD4⁺CD45RB^{high} T cells (99). These findings were recently confirmed by Schaer *et al.* (98), where reduced disease pathogenesis in *Rag*^{-/-} animals was observed after the transfer of either *Hvem*^{-/-} or *Light*^{-/-} donor CD4⁺ T cells. Therefore, T-cell-derived LIGHT interacting with HVEM expressed by an adjacent T cell may be required for the full Th1 and/or Th17 differentiation of gut-homing effector T cells driving colitis in the transfer model.

Studies with human cells also suggested a role for LIGHT and HVEM in intestinal disease pathogenesis. LIGHT-HVEM interaction was shown to induce activation of human intestinal mucosal T cells and IFN γ production (91). Moreover, high levels of LIGHT were detected in mature Th1 CD4⁺ T cells and mucosal T lymphocytes isolated from patients with IBD (91), and LIGHT expression was significantly increased in the intestine of active Crohn's disease patients (93). Genome-wide screens have linked HVEM to celiac disease, an intestinal autoimmune disease triggered by hyper reactivity to glutens (100, 101) and LIGHT maps within the *IBD6* susceptibility region on Chr19p13.3. Polymorphic variants in the coding region of *LIGHT* increase receptor binding to LT β R and decrease affinity for the soluble DcR3 enhancing LIGHT signaling potential. However, as yet, there is no direct mechanistic link between these genes and disease pathogenesis. All together, these findings are consistent with a costimulatory or inflammation-promoting role for LIGHT and HVEM when both molecules are expressed by T lymphocytes during T-T cell encounters (81, 91).

HVEM promotes T-cell survival

As mentioned earlier, the engagement of HVEM by its ligands in *trans* recruits TRAF2 and activating NF- κ B and JNK/AP-1 transcription factors (9), which induce genes contributing to T-cell survival *in vivo* (102, 103). Early evidence for a role of HVEM in T-cell survival comes the demonstration that expression of HVEM in T cells is required for the maintenance of normal pools of alloreactive CD4⁺ and CD8⁺ T cells formed during the first 10 day period in a GVHD model (81). Similarly, LIGHT expression in donor T cells was also required for their survival and CTL activity against the host (81). In a recent study, Soroosh *et al.* (90) demonstrated that the blockade of the LIGHT-HVEM interaction or the genetic absence of HVEM or LIGHT in T cells severely reduced the persistence of antigen-reactive memory T-cell pools after secondary expansion following antigen recall. In co-transfer experiments with WT and *Light*^{-/-} CD4⁺ T cells, comparable numbers of long-lived T cells accumulated; however, a profound defect in the accumulation of *Hvem*^{-/-} T cells occurred when WT and knockout T cells were co-transferred. These data indicate an intrinsic requirement for HVEM in the activated T cell for their survival. The defect in the survival of HVEM-deficient T cells correlated with reduced activity of protein kinase B (*Akt*) and an inability to maintain expression of the anti-apoptotic molecule Bcl-2 (90). More evidence for a role of HVEM in T-cell survival comes from studies in the T-cell transfer model of colitis. *Hvem*^{-/-} CD4⁺ naive T cells transferred into *Rag*^{-/-} recipients accumulated significantly less than WT T cells and they were not able to sustain intestinal inflammation (98). In this study, HVEM deficiency did not alter the ability of CD4⁺ T cells to expand during the first weeks following their transfer into *Rag*^{-/-} mice; however, a substantial reduction in the number of *Hvem*^{-/-} T cells, compared to control WT T cells, was observed in the MLNs and intestine of the *Rag*^{-/-} recipients 50 days post-transfer (98). These data are in agreement with previous observations demonstrating that in this colitis model, the absence of HVEM or LIGHT in donor CD4⁺ T cells resulted in milder intestinal

inflammation and reduced disease in the transferred *Rag*^{-/-} recipients (99). These findings indicate that the LIGHT-HVEM interaction promotes T-cell survival during inflammatory responses.

Emerging evidence indicates that despite its generally inhibitory role, BTLA can also initiate prosurvival signals for effector T cells. Murphy and colleagues (82) previously reported in a GVHD mouse model that BTLA expression in donor T cells promotes the long-term survival of activated T lymphocytes. More recently, a similar study also demonstrated that the transfer of BTLA-deficient donor T cells results in an impaired donor anti-host T-cell response, and this is primarily caused by a reduced survival of the knockout T cells (104). Interestingly, these authors found that the expression of a truncated form of BTLA lacking the intracellular signaling domain in *Btla*^{-/-} donor T cells was sufficient to prolong their survival, suggesting that BTLA may promote T-cell survival acting principally as a ligand for HVEM (104). These data are in agreement with our previous findings showing that BTLA-Fc fusion protein can specifically activate NF- κ B-RelA pathway in transfected cell lines expressing HVEM, and enhance the survival of *Btla*^{-/-} T cells undergoing proliferation in culture following stimulation with an anti-CD3 ϵ antibody (9).

Further evidence suggesting a role for BTLA-HVEM interaction in T-cell survival comes from *in vivo* studies performed in the colitis T-cell transfer model. In this model, *Btla*^{-/-} CD4⁺CD45RB^{high} T cells transferred into *Rag*^{-/-} hosts failed to accumulate, with reduced number of effector T cells in the recipients, when compared to WT donors (99). Furthermore, when equal numbers of WT CD4⁺ T cells and congenically marked *Btla*^{-/-} or *Hvem*^{-/-} CD4⁺ T cells were cotransferred into *Rag*^{-/-} mice, higher percentages of the WT T cells accumulated in spleens, MLNs, and the intestine of transferred animals. The cotransfer of WT T cells with *Btla*^{-/-}*Hvem*^{-/-} double deficient T cells yielded similar results (9). Interestingly, equivalent results were obtained with cells cotransferred into *Hvem*^{-/-}*Rag*^{-/-} and *Btla*^{-/-}*Rag*^{-/-} recipients, a situation where the expression of HVEM and BTLA was restricted specifically to the donor T cells. The fact that neither the host environment nor WT T cells influenced the accumulation of the cotransferred HVEM- and/or BTLA-deficient T cells indicated a requirement for the expression of both molecules intrinsically, implicating the HVEM-BTLA *cis* configuration. BTLA in the *cis* configuration limits activation of NF κ B-dependent survival by HVEM in naive T cells. However, the prosurvival effect of BTLA-HVEM interaction observed in this model seems to be mediated by BTLA signaling. Therefore, in the GVHD model, BTLA appears to promote T-cell survival, acting primarily as a ligand in *trans* for HVEM. In the inflammatory conditions taking place during the development of colitis in the transfer model, BTLA may enhance T-cell survival acting as a signaling receptor in *cis* with HVEM. The intracellular region of BTLA contains conserved tyrosine residues that conform to a binding site for growth-factor receptor-bound protein 2 (Grb2) and the p85 subunit of the phosphatidylinositol 3-kinase (PI3K), and it has been suggested these downstream molecules may lead to enhanced T-cell survival (3). Although it is not well defined at present how BTLA signals when engaged in *cis* configuration, we could speculate that its binding to HVEM in *cis* preferentially activates Grb-2 and PI3K pathways leading to the upregulation of prosurvival genes. The manner by which HVEM-BTLA *cis* interaction regulates T-cell activation and survival during different types of immune responses represents an area of active research.

In summary, besides its role as a costimulatory receptor, HVEM can also serve as a prosurvival molecule for T lymphocytes. In some situations HVEM works as the primary signaling element that promotes T-cell survival after its engagement in *trans* by LIGHT or BTLA expressed in neighboring cells. In other situations, HVEM may enhance T-cell survival delivering signals through BTLA in the *cis* configuration.

Whereas most of these studies indicate that HVEM works principally as pro-survival molecule for T cells, it has been proposed that in the inflamed lung microenvironment, HVEM-BTLA interaction may also increase sensitivity to apoptosis. During airway inflammation, *Btla*^{-/-} CD4⁺ T cells showed decreased apoptosis associated with a prolonged airway inflammation (105, 106). Interestingly, prolonged T-cell survival in inflamed lungs correlated well with induction of HVEM in the airway epithelium, suggesting that HVEM-BTLA signaling may directly control T-cell apoptosis, a topic worthy of further investigation.

HVEM triggers inhibitory signals in immune cells

The concept that HVEM functions strictly as a costimulatory receptor for T cells was incongruent with the inhibitory phenotype emerging from investigation of *Hvem*^{-/-} mice. The surprising discovery demonstrating that the inhibitory receptor BTLA engages HVEM provided a mechanistic explanation for the functional duality of HVEM in T-cell responses. A growing number of studies have explored the role for HVEM-BTLA interaction in the immune system. It has been shown that upon TCR stimulation, BTLA is able to co-cluster with the CD3 ζ chain at the immunological synapse, where, in turn, it can regulate molecules essential for T-cell signal transduction (107). After engagement with HVEM, BTLA becomes tyrosine phosphorylated and associates with both the TCR complex and the phosphatases SHP-1 and SHP-2, which are thought to attenuate TCR-downstream signaling pathways. *In vitro* studies have shown that HVEM-deficient as well as BTLA-deficient T cells are hyper-responsive after TCR stimulation with anti-CD3 monoclonal antibodies (42, 49, 108). In addition, HVEM-deficient T cells showed increased proliferation and enhanced production of cytokines compared with WT T cells, in response to concanavalin A (ConA) *in vitro* stimulation (108). In an antigen-specific priming system, HVEM expression in CHO cells reduced T-cell proliferation when BTLA was expressed in the T lymphocytes, demonstrating that HVEM-BTLA interaction is inhibitory (15). In addition, in non-inflammatory conditions, both BTLA- and HVEM-deficient mice have increased number of memory T cells, suggesting that the BTLA-HVEM interaction negatively regulates the homeostatic expansion of memory CD4⁺ and CD8⁺ T cells *in vivo* (109). These data indicate that BTLA engagement by HVEM triggers inhibitory signals leading to the attenuation of T-cell responses.

BTLA-HVEM interaction has been also involved with the attenuation of B-cell responses (48). BTLA was found to associate with the B-cell receptor (BCR) and to recruit SHP-1 and SHP-2 in B cells, thereby attenuating B-cell activation by targeting the downstream signaling molecules Syk, BLNK, PLC γ 2, as well as NF- κ B (48). Although at present the targets of SHP-1 and SHP-2 phosphatases associated with BTLA in T cells are unknown, by analogy with the events that occur in B lymphocytes, it is likely that they dephosphorylate signaling intermediates downstream of the antigen receptors.

Most studies addressing the role of HVEM-BTLA interaction have been done with a focus on T and B lymphocytes; however, the relatively wide distribution of these molecules throughout the hematopoietic compartment indicates that the HVEM-BTLA pathway is not limited to inhibitory signaling in lymphocytes. For example, both HVEM- and BTLA-deficient mice have altered proportion of DC subsets compared to WT animals (53). Moreover, mixed bone marrow chimeras experiments revealed a competitive advantage for *Hvem*^{-/-} and *Btla*^{-/-} DCs in repopulating the spleens of chimeric mice. The HVEM-BTLA pathway restricts the growth of CD8 α ⁻ DC subsets within secondary lymphoid tissues, but DC subsets are normal in the bone marrow. Indeed, the HVEM-BTLA system counteracts the proliferative action of the LT α β -LT β R pathway in regulating the cellularity of lymphoid

tissue DCs, supporting the idea these cytokines form a regulatory 'network'. The molecular intersection of these pathways that achieves DC homeostasis is not yet known.

Similar to BTLA, engagement of CD160 by HVEM appears to inhibit the activity of immune cells. Evidence from experiments performed with human CD4⁺ T cells indicates that the HVEM-CD160 interaction inhibits T-cell activation by interfering with the TCR signaling pathway (66). Indeed, expression of HVEM on transfected NIH3T3 cells used as artificial APCs in T-cell cultures led to potent inhibition of T-cell proliferation and cytokine production (66). Moreover, a monomeric anti-CD160 Fab that acts as a blocking reagent reversed the HVEM-mediated inhibition of T-cell proliferation (66). Similarly, during an antigen-specific memory T-cell response *in vitro*, T-cell proliferation and IFN γ production were augmented when the HVEM-CD160 interaction was disrupted with the anti-CD160 Fab (66). Although BTLA and CD160 can both trigger inhibitory signals for T cells, the intracellular events taking place following their engagement by HVEM are different. Crosslinking of CD160 reduced tyrosine phosphorylation of several substrates, particularly the CD3 ζ chain. However, contrarily to BTLA, these events are independent of SHP-1 and SHP-2 since engagement of CD160 did not induce phosphorylation of these tyrosine phosphatases (66). The precise mechanism whereby CD160 engages signaling pathways is poorly understood and further investigation is required to determine how the HVEM-CD160 interaction inhibits T-cell activation.

Anti-inflammatory role of HVEM in disease models

Although in some situations HVEM can function as a costimulatory receptor for some adaptive and innate immune cells, *Hvem*^{-/-} animals have increased susceptibility to several experimental autoimmune diseases. These results indicate the importance of the cellular context in HVEM signaling. Accordingly, a growing number of studies have recently highlighted important anti-inflammatory functions for HVEM in different experimental disease models (Table 2).

HVEM attenuates ConA-induced hepatitis

In the concanavalin A (ConA)-induced hepatitis model, injection of this plant lectin into WT mice leads to a polyclonal T-cell activation and T-cell-mediated, rapid onset autoimmune-like hepatitis. *Hvem*^{-/-} mice were highly susceptible to ConA-induced hepatitis, and they exhibited increased mortality that appeared to be associated with hyper-reactive immune cells, and higher and prolonged levels of pro-inflammatory cytokines (108). Enhanced activation of *Hvem*^{-/-} T cells following ConA stimulation was detected both *in vitro* and *in vivo*, and elevated serum levels of pro-inflammatory cytokines including IL-6, TNF, and IFN γ were detected in HVEM-deficient animals (108). Interestingly, *Btla*^{-/-} mice treated with ConA have a similar phenotype. Following ConA challenge, BTLA-deficient animals had increased mortality and morbidity (110, 111). In these mice, severe liver injury correlated with elevated levels of pro-inflammatory cytokines primarily produced by dysregulated *Btla*^{-/-} NKT cells (111). Whereas BTLA-HVEM interaction may directly regulate NKT cell activation and cytokine production, increased mortality and/or liver injury in ConA-treated *Hvem*^{-/-} or *Btla*^{-/-} mice may be associated with impaired tolerance or dysregulated regulatory T-cell (Treg) function. A role for HVEM in Treg function has been proposed based on the observation that *Hvem*^{-/-} Treg had decreased suppressive activity as compared with WT Tregs (112). Similarly, BTLA was shown to play a critical role in the induction of peripheral tolerance of both CD4⁺ and CD8⁺ T cells (56). These results indicate that HVEM-BTLA interaction may control the functional activity of NKT cells or other cell types such as Tregs to prevent autoimmunity in the ConA-induced hepatitis model.

HVEM inhibits T-cell-mediated encephalopathy

Experimental autoimmune encephalopathy (EAE) is a well characterized model to study T-cell-mediated autoimmunity. In this model, administration of myelin oligodendrocyte glycoprotein (MOG) peptides into mice induces disease pathogenesis characterized by inflammation of the CNS, demyelination, and paralysis of the animals. The role of HVEM in the pathogenesis of EAE has been previously addressed (108). WT and *Hvem*^{-/-} mice immunized with MOG peptide developed disease to a similar extent. However, HVEM-deficient animals had an earlier onset of disease and a prolonged duration (108). Moreover, recovery from disease was significantly delayed in *Hvem*^{-/-} mice compare to WT animals. Increased susceptibility of HVEM-deficient mice to EAE correlated with an increased frequency of antigen-specific T cells in the draining lymph nodes and a more activated phenotype of T cells. Indeed, *Hvem*^{-/-} T cells restimulated *in vitro* with MOG peptide showed enhanced proliferation and higher production of TNF and IFN γ (108). Similar to *Hvem*^{-/-} mice, BTLA-deficient animals also showed higher incidence, earlier onset, and prolonged duration of EAE compared to WT animals (42). These findings suggest that in EAE, HVEM negatively regulates T-cell-mediated inflammatory responses triggering inhibitory signals through BTLA.

HVEM prevents accelerated colitis induced by T cells

HVEM clearly revealed its role as an inhibitory signaling system with BTLA in the mouse colitis model. In the CD4⁺CD45RB^{high} T cells into *Rag*^{-/-} mice model, HVEM expression is required in the radioresistant stroma to protect from colitis (99). Although as noted the absence of HVEM in the donor CD4⁺CD45RB^{high} T lymphocytes slightly reduced the severity of colitis development, the opposite effect was observed for HVEM expression in the RAG-deficient hosts. Unexpectedly, we found that the transfer of WT T cells into *Hvem*^{-/-}*Rag*^{-/-} mice led to a dramatic acceleration of colitis and severe intestinal inflammation. In these animals, accelerated disease was characterized by the accumulation of activated Th1 and Th17 cells in the colon that correlated with extremely rapid weight loss and elevated histological scores in the large intestine (99). Therefore, severe colitis in transferred *Hvem*^{-/-}*Rag*^{-/-} mice is likely the result of enhanced and dysregulated pro-inflammatory responses mediated by CD4⁺ T cells in the intestine of these animals indicating the predominant effect of HVEM in this colitis model system is anti-inflammatory. Indeed, when *Hvem*^{-/-} or *Light*^{-/-} CD4⁺CD45RB^{high} T cells were transferred into *Hvem*^{-/-}*Rag*^{-/-} mice, the disease-accelerating effects HVEM loss in the *Rag*^{-/-} hosts was dominant over the more subtle disease-delaying effects when HVEM was not expressed by the donor T cells (99). These findings depended critically on the power of the T-cell transfer model of colitis in analyzing cell-cell interactions and the interplay of the innate and adaptive immune systems, where the pro-inflammatory and anti-inflammatory effects of HVEM expressed in donor T cells or *Rag*^{-/-} host cells could be separated.

Strikingly, transfer experiments in bone marrow chimeric *Rag*^{-/-} recipients revealed that HVEM prevented accelerated colitis when expressed in a radio-resistant cell population in the *Rag*^{-/-} hosts. Indeed, CD4⁺CD45RB^{high} T-cell transfer into irradiated *Rag*^{-/-} mice reconstituted with *Hvem*^{-/-}*Rag*^{-/-} bone marrow cells (*Hvem*^{-/-}*Rag*^{-/-} $\wedge$ *Rag*^{-/-}), did not lead to accelerated disease. In sharp contrast, the transfer of T cells in the opposite chimeras (*Rag*^{-/-} $\wedge$ *Hvem*^{-/-} *Rag*^{-/-}) led to a dramatic acceleration of disease and severe intestinal inflammation (99). The critical radio-resistant cell subset responsible for HVEM-mediated colitis inhibition has not been yet identified. This is a particularly difficult task, since HVEM is broadly expressed in immune and somatic cells that are radio-resistant. There are several possible candidates including gut-resident APCs, mucosal DCs, intestinal macrophages, and colonic epithelial cells (113). Interestingly, we found that intestinal

epithelia cells constitutively express HVEM (99). Not only do intestinal epithelial cells provide a point of contact for enteric antigens, but they also play a direct role in mucosal immunity, particularly by regulating T-cell responses to enteric antigens (114). Thus, expression of HVEM by host epithelium could be essential for engagement of BTLA or CD160 in effector T cells to avoid an exaggerated T-cell activation.

BTLA is expressed in activated CD4⁺ T cells isolated from spleen, MLN, and *lamina propria* of *Rag*^{-/-} recipients, leading to the notion that the interaction between HVEM in *Rag*^{-/-} host and BTLA in T cells could alter the acceleration of colitis. Consistent with this hypothesis, treatment of *Hvem*^{-/-}*Rag*^{-/-} recipients of CD4⁺CD45RB^{high} T cells with an anti-BTLA monoclonal antibody (clone 6F7) with agonistic properties reversed the accelerated colitis but only when the T cells expressed BTLA. *Hvem*^{-/-}*Rag*^{-/-} mice transferred with *Btla*^{-/-} T cells developed a rapid onset disease that was not affected by treatment with the anti-BTLA monoclonal antibody (99). In immunocompetent mice, the mouse anti-BTLA monoclonal antibody clone 6F7 functioned as a depleting antibody (55, 115), and in particular for B cells (57). We did not observe significant cell depletion when the antibody was administered into transferred *Hvem*^{-/-}*Rag*^{-/-} mice, and CD4⁺ T-cell numbers in these animals were essentially equivalent to those observed in IgG control-treated recipients (M. Steinberg, unpublished observation). These results lead to the conclusion that the anti-BTLA monoclonal antibody treatment prevented accelerated colitis principally working as an agonist for BTLA expressed in transferred T cells.

The transfer of WT T cells into BTLA-deficient *Rag*^{-/-} recipients surprisingly also led to accelerated colitis, suggesting that the HVEM-BTLA interaction may also inhibit inflammation induced by innate immune cells. Although disease acceleration in *Btla*^{-/-}*Rag*^{-/-} mice was comparable to that observed in HVEM-deficient host, *Btla*^{-/-}*Rag*^{-/-} mice had slightly less weight loss and less severe histological scores than *Hvem*^{-/-}*Rag*^{-/-} recipients (99). Contrary to the situation in *Hvem*^{-/-}*Rag*^{-/-} recipients, in the transfer of WT CD4⁺CD45RB^{high} T cells to *Btla*^{-/-}*Rag*^{-/-} animals, there was no apparent defect in the signaling of BTLA in T cells, and therefore a somewhat different mechanism, perhaps one more dependent on alterations in the behavior of innate host cells, could be operating to accelerate disease in the *Btla*^{-/-}*Rag*^{-/-} recipients (113).

These findings together indicate that in the colitis transfer model, HVEM can function as a potent anti-inflammatory molecule when expressed in non-lymphoid cells in the *Rag*^{-/-} hosts. Moreover, HVEM attenuates intestinal inflammation and severe colitis by triggering inhibitory signals through BTLA expressed in T lymphocytes and innate immune cells.

Potential inhibitory role of HVEM in collagen-induced arthritis

The role of HVEM in the development of autoimmune arthritis was investigated in the collagen-induced arthritis (CIA) mouse model. In this model, arthritis is induced after immunization of DBA/1J mice with type II collagen in Freund's adjuvant. Administration of an HVEM-Fc fusion protein immediately after immunization of the animals led to more severe arthritis and significantly increased histologic joint destruction. Accordingly, serum levels of IFN γ and IL-6 were elevated in mice treated with HVEM-Fc, and collagen-induced *in vitro* T-cell-proliferation and IFN γ production were also augmented in mice treated with the fusion protein (116). Treatment of collagen-immunized mice with LT β R-Fc, which can interfere with the binding of LIGHT and LT α 1 β 2 to their receptors, did not aggravate arthritis, suggesting that the HVEM-Fc-mediated disease enhancement may occur independently of LIGHT (116). Therefore, HVEM-Fc could cause enhanced T-cell responses and more severe disease acting through BTLA or CD160. One possible explanation is that HVEM-Fc blocks critical inhibitory signals triggered by HVEM-BTLA

or HVEM-CD160 interactions contributing to exacerbated T-cell responses (116). Another possibility that has been proposed is that HVEM-Fc engagement of BTLA on T cells enhances the survival of effector T cells and consequently may lead to increased T-cell-mediated inflammation (116). The aggravation of experimental arthritis by HVEM-Fc is an intriguing observation, but the mechanism is unresolved.

Anti-inflammatory role of HVEM in infection models

Herpesvirus entry mediator serves as one of several receptors for HSV gD that allows the virus to gain entry into cells (117-119). Similar to BTLA, HSVgD binding to HVEM involves CRD1 (15) and BTLA binding to HVEM could be blocked by the addition of soluble HSVgD protein (12, 43). Based on this property of HSVgD, studies have revealed that vaccines expressing HIV, HPV, (120), or influenza A (121) antigens fused into the C-terminal domain of gD increased vaccine-specific immune responses. In these studies, vaccines with the HSVgD induced markedly higher antigen-specific T-cell responses compared to vaccines containing only the viral antigens without gD. The effect of HSVgD enhancing adaptive immune responses to a variety of viral antigens depended on gD binding to HVEM (120, 121). Therefore, more potent antigen-specific T-cell responses elicited by vaccines containing gD was attributed to the ability of gD to block HVEM-BTLA or HVEM-CD160 inhibitory interactions, resulting in enhanced CD8⁺ T-cell activation and proliferation. These data suggest that during infection, HVEM plays an anti-inflammatory role attenuating antigen-specific T-cell immune responses. Another possible interpretation of these results, however, is that the more robust T-cell responses observed with vaccines containing gD were caused by enhancement of antigen-specific T-cell survival due to the engagement of HVEM by gD. It is thought that gD-HVEM interaction mediates the NF κ B-dependent anti-apoptotic effects seen for HSV-1 (28, 76). Indeed, gD can function as an unconventional ligand for HVEM that activates the NF κ B-RelA signaling pathway either in *cis* or *trans* configurations in contrast to the cellular ligands (9). The ability of HSVgD to activate HVEM and upregulate the NF- κ B pathway and prosurvival genes in host cells may provide this virus with a selective advantage early during infection. The remarkable diversity in viral mimicry of the HVEM-BTLA complex (122) suggests that this pathway serves as a key selective pressure driving evolution of host defenses (118).

Anti-inflammatory roles for HVEM have also been described in experimental models of bacterial infections. Using *Listeria monocytogenes* as an infectious agent, a recent study in mice demonstrated that HVEM-BTLA interaction regulates early host protective immunity. Both *Hvem*^{-/-} and *Btla*^{-/-} mice but not *Light*^{-/-} mice were able to eliminate infectious bacteria much better than control animals. Similarly, WT mice treated with an antagonist anti-BTLA monoclonal antibody also had less bacteria when compared with mice treated with control antibody (123). These findings suggest that blockade of the HVEM-BTLA interaction enhances immunity to eliminate infectious bacteria. Additional experiments using a chimeric HVEM-Fc protein capable of triggering BTLA signaling showed that mice treated with the HVEM-Fc were more susceptible to *L. monocytogenes* infection and had higher bacterial burdens in spleen and liver. This *in vivo* effect of HVEM-Fc was independent of LIGHT and was mediated by BTLA signaling (123). These results suggest that the HVEM-Fc treatment triggers inhibitory BTLA signaling, which dampens or inhibits immune responses that are required for bacteria clearance. It remains to be determined, however, what cell type and what immune response are negatively regulated by BTLA signaling during the host immune response to *L. monocytogenes*.

In this study HVEM-BTLA interaction appeared to temper innate immune responses to *Listeria*, yet a different study showed that HVEM activation in innate cells can also promote cell-mediated bacteria killing (124). The binding of LIGHT to HVEM expressed in human

monocytes and neutrophils enhanced effector functions of these cells, independently of LT β R. Indeed, LIGHT engagement of HVEM expressed in myeloid cells enhanced the production of IL-8, TNF, nitric oxide (NO), and reactive oxygen species, and increased the bactericidal activity against *L. monocytogenes* and *Staphylococcus aureus* (124). This result indicates that the LIGHT-HVEM interaction can activate subsets of innate immune cells during immune responses to infectious bacteria. The discrepancy between these two findings may be due to *in vitro* approach by Heo and colleagues (125) and *in vivo* experiments conducted by Sun *et al.* (123).

An inhibitory role for HVEM-BTLA pathway has also been described during the inflammatory response induced by *Plasmodium berghei* ANKA. Infection of mice with *P. berghei* ANKA induced infiltration of leukocytes in brain capillaries that is associated with a pathology that recapitulates some features of cerebral malaria in humans. In this model, HVEM and BTLA expression in the brain of infected mice was substantially increased and correlated with the accumulation of both CD8⁺ and CD4⁺ T cells (126). Furthermore, administration of an anti-BTLA antibody with presumably agonistic activity significantly reduced incidence of disease and decreased numbers of T cells sequestered in the brain after infection (126). These results indicate that HVEM-BTLA interaction can negatively regulate T-cell-mediated brain inflammation and disease development caused by *P. berghei* ANKA infection. These findings suggest a potential role for the HVEM-BTLA pathway in regulating development of severe malaria in humans.

Concluding remarks

Receptor and ligand interactions in the TNF superfamily are frequently non-monogamous, and HVEM is an example of a promiscuous mediator with at least five different ligands, and decidedly different outcomes. HVEM holds a place of distinction in engaging molecules in both the TNF and immunoglobulin superfamilies encoded in mammalian and viral genomes. This distinction places HVEM at a nexus of several signaling networks in the immune system.

HVEM can either enhance or prevent inflammation depending on the ligand or coreceptor it interacts with and in distinct cellular configurations, in *cis* or in *trans* (Fig. 3). In some situations, and in particular when expressed in T lymphocytes, HVEM functions mainly as a pro-inflammatory molecule by acting as a receptor for LIGHT (Fig. 3A). However, the involvement of HVEM-LIGHT interactions in promoting inflammation is clearly more complex than as a simple unidirectional signaling pathway. The functional outcomes of membrane and soluble LIGHT are opposite depending on the presence of BTLA or CD160. Bidirectional signaling between LIGHT and HVEM remains to be rigorously established (127) (Fig. 3A).

The inhibitory effect of HVEM appears to be dominant over its pro-inflammatory function, since HVEM-deficient animals show higher susceptibility to inflammatory diseases in several experimental models. Accumulating evidence has indicated that HVEM-BTLA signaling represents an important immune regulator during different types of immune responses (113, 128). Engagement of BTLA by HVEM triggers inhibitory signals in immune cells, particularly T lymphocytes (Fig. 3B), critical for the homeostasis of the immune system. In many cases, the absence of HVEM-BTLA signaling results in exaggerated immune cell activation that often leads to dysregulated inflammation and autoimmune disease.

Studies with BTLA-deficient mice or agonistic antibodies against BTLA have demonstrated that BTLA acts as an inhibitory receptor and its engagement by HVEM attenuates adaptive and innate cell immune responses. With bidirectional signaling in *trans*, HVEM-BTLA

engagement can induce HVEM-mediated NF- κ B activation important for survival genes in T cells and other immune cells (Fig. 3A). Simultaneously, HVEM can induce inhibitory signaling in lymphocytes and innate immune cells via BTLA (Fig. 3B).

The model of LIGHT-HVEM-BTLA interactions in the *cis* and *trans* configurations allows interpretation of complex outcomes between multicellular interactions. Substantial data support the predictive value of this model based on T-cell responses. The challenge to the model, of course, comes with predicting outcomes in other cellular interaction systems, given the wide expression patterns of HVEM and BTLA. Outcomes in B cells, NK cells, macrophages, and other cells remain to be investigated. Identifying the best potential targets for the development of therapeutics for inflammatory disorders is a more substantial challenge, mainly because each component, LIGHT, HVEM and BTLA, inherently have both activating and anti-inflammatory actions. Accurately predicting the outcomes from the blocking different molecular interactions involving HVEM and its ligands will be necessary for the development of safe and effective therapeutics.

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Fig. 1. Ligand-receptor specificity in the HVEM network

The arrows connecting the TNF related and the Ig ligands (upper panel) to their cognate receptors (lower panel) define the ligand-receptor specificity in the HVEM network. Ligation of HVEM by any of its ligands in *trans* leads to NF κ B activation. The immunotyrosine inhibitory motif (ITIM) in BTLA recruits tyrosine phosphatases (SHP1/2) limiting antigen receptor signals. Herpes simplex virus envelope glycoprotein D (gD) engages HVEM as an entry route and expressed in the infected cell. UL144 is encoded by human cytomegalovirus and binds BTLA. $LT\alpha$ and decoy receptor-3 are soluble, and CD160 is shown in its GPI linkage to the membrane.

Fig. 2. Molecular model of the activating and inhibitory pathways of HVEM

(A) Membrane LIGHT displaces BTLA from binding with HVEM due to membrane restriction. (B) Cooperative binding between soluble LIGHT, LTα with HVEM and BTLA. A trimolecular complex of LIGHT/LTα–HVEM–BTLA is composed of three molecules of soluble LIGHT/LTα, HVEM and BTLA. (C) Formation of HVEM-BTLA/CD160/gD *cis*-complexes. *Cis*-interaction between HVEM, BTLA, CD160 and HSV gD inhibits HVEM binding to its ligands in *trans*.

Fig. 3. HVEM Interactions regulating T-cell immune responses

(A). Engagement of HVEM by membrane LIGHT during T-T cell interactions leads to the activation of the NFκB-RelA, AP-1, and Akt pathways that enhances cell proliferation, cytokine production, and survival of HVEM-expressing cells. Similarly, engagement of HVEM by BTLA in *trans* induces NF-κB activation and promotes T-cell survival. Alternative interactions of HVEM with BTLA, e.g. in *cis* configuration, also promotes T-cell survival by a mechanism that has not been fully elucidated. (B). Engagement of BTLA on T cells by HVEM expressed in antigen-presenting cells (APCs) induces ITIM phosphorylation on BTLA facilitating the recruitment of phosphatases SHP-1 and SHP-2, which once activated, downregulates the TCR signaling pathway resulting in reduced cellular activation, proliferation, and cytokine production. Similarly, HVEM binding to CD160 expressed in T cells leads to reduced phosphorylation of the CD3ζ chain and intracellular proteins what result in decreased T-cell activation and proliferation. The mechanism by which CD160 engagement by HVEM attenuates T-cell responses remains unknown. In naive T cells, the HVEM and BTLA *cis* configuration negatively regulates NF-κB signaling pathway that may sustain the naive phenotype by blocking ligands in the surrounding microenvironment.

Table 1

Features of HVEM, its ligands, and interactors

	Alternate names	Identifying Domain	Chromosome position (human)	SNP (coding region polymorphic variants)	Expressing cells
Receptor					
HVEM	TNFRSF14, HveA, ATAR, CD270	TNFR	1p36.32	R17K, F29Y, T117A, R135C, I241V	Hematopoietic: most lineages, epithelial cells; sensory neurons
LT β R	TNFRSF3, TNF-R-III, CD18	TNFR	12p13	C92W, F146S, R182S, S229A, I274V, S287F, S296P, R332Q, 344-frame shift, R398G	Stromal and parenchymal cells, myeloid, but not lymphoid
TNFR1	TNFRSF1A, p55-60, CD120a	TNFR	12p13.2	R35G, L75P, T305P, C426R,	Broadly expressed, all nucleated cells
TNFR2	TNFRSF1B, p75-80, CD120b	TNFR	1p36.22	T11A, M187V, R196M, K232E, T236A, P264L, P269T, R295Q, R301P, P318H, M353V, F370S, R375H	Hematopoietic lineages, activated T and B cells;
UL144		TNFR			Human CMV infected cells
Ligands					
LIGHT	TNFSF14, HVEM-L, LT γ , TL4		19p13.3	L32S, Q87R, V120L, E214K	Activated T cells, NK cells, Immature DC, granulocytes and monocytes
LT α	TNFSF1, LT		6p21.3	R13C, R34G, P51H, K123R,	Activated T and B cells, NK
LT β	TNFSF3, p33		6p21.3	V92L, F97S, Y98N, 108-frame shift, I137V	B cells; upregulated on activated T cells and Th1 cells; DC and NK cells
TNF	TNFSF2, TNF α		6p21.3	N52H, L64P, L84P, T94A, N194I	Activated myeloid and lymphoid cells, somatic cells
BTLA	BTLA1, CD272	Ig	3q13.2	V124I, T149N, L219P	Hematopoietic cells
CD160	BY55	Ig	1q21.1	V91I, A98T, 70-frame shift	NK cells, NKT cells
HSV gD					Virion and Infected cells

Table 2

Pro- and anti-inflammatory roles of HVEM in experimental disease models.

Pro-inflammatory		
Disease Model	Findings	Ref.
GVHD ¹	Blockade of LIGHT-HVEM interaction prolonged host survival and <i>Hvem</i> ^{-/-} donor cells fail to induce GVHD.	(81)
Cardiac Transplantation ²	LIGHT-deficient mice tolerated fully MHC-mismatched cardiac transplants and treatment of mice with Hvem:Ig and CsA prolonged graft survival.	(83)
Islet Transplantation ³	Blockade of both LIGHT-HVEM and B7-CD28 interactions facilitated long-term islets allograft survival	(85)
Tumor Immunology	Enhanced tumor-specific T cell response and tumor clearance induced by LIGHT-expressing tumors	(86, 87)
Asthma	LIGHT-induced airway remodeling partly dependent on IL13 produced by HVEM-expressing eosinophils.	(88)
IBD ⁴	Transfer of <i>Hvem</i> ^{-/-} LIGHT-transgenic MLN or <i>Hvem</i> ^{-/-} CD4 ⁺ CD45RB ^{high} T cells into <i>Rag</i> ^{-/-} hosts led to reduced disease pathogenesis.	(93, 98, 99)
Anti-inflammatory		
ConA-induced hepatitis	<i>Hvem</i> ^{-/-} mice are highly susceptible to disease, and HVEM-deficient mice shown enhanced T-cell activation and pro-inflammatory cytokines in serum	(108)
MOG-induced EAE	Earlier onset of disease and a prolonged duration in <i>Hvem</i> ^{-/-} mice correlates with increased frequency of activated antigen-specific T cells	(108)
IBD ⁴	Accelerated colitis and severe intestinal immunopathology in <i>Hvem</i> ^{-/-} <i>Rag</i> ^{-/-} mice transferred with WT CD4 ⁺ T cells	(99)
Collagen-induced arthritis	HVEM:Fc administration enhances T-cell responses, cytokine production, and aggravates disease	(116)

¹ non-irradiated parent-into-F1 mouse model.² fully MHC-mismatched cardiac transplant model³ islet allografts⁴ CD4⁺CD45RB^{high} T-cell transfer model

Abbreviations: GVHD, graft-versus host disease; CsA, cyclosporine A; ConA, concanavalin A; MOG, myelin oligodendrocyte glycoprotein; EAE, experimental autoimmune encephalomyelitis; IBD, inflammatory bowel disease.