

Evolutionarily divergent herpesviruses modulate T cell activation by targeting the herpesvirus entry mediator cosignaling pathway

Timothy C. Cheung^{*†}, Ian R. Humphreys^{*†}, Karen G. Potter^{*}, Paula S. Norris^{*}, Heather M. Shumway^{*}, Bonnie R. Tran^{*}, Ginelle Patterson^{*}, Rochelle Jean-Jacques^{*}, Miri Yoon[‡], Patricia G. Spear^{*§}, Kenneth M. Murphy[¶], Nell S. Lurain^{||}, Chris A. Benedict^{*§}, and Carl F. Ware^{*§}

^{*}Division of Molecular Immunology, La Jolla Institute for Allergy and Immunology, 10355 Science Center Drive, San Diego, CA 92121; [‡]Department of Microbiology and Immunology, The Feinberg School of Medicine, Northwestern University, Chicago, IL 60611; [¶]Department of Pathology and Center for Immunology, and Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO 63110; and ^{||}Department of Immunology and Microbiology, Rush University Medical Center, Chicago, IL 60612

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The herpesvirus entry mediator (HVEM), a member of the TNF receptor (TNFR) superfamily, can act as a molecular switch that modulates T cell activation by propagating positive signals from the TNF-related ligand LIGHT (TNFR superfamily 14), or inhibitory signals through the Ig superfamily member B and T lymphocyte attenuator (BTLA). Competitive binding analysis and mutagenesis reveals a unique BTLA binding site centered on a critical lysine residue in cysteine-rich domain 1 of HVEM. The BTLA binding site on HVEM overlaps with the binding site for the herpes simplex virus 1 envelope glycoprotein D, but is distinct from where LIGHT binds, yet glycoprotein D inhibits the binding of both ligands, potentially nullifying the pathway. The binding site on HVEM for BTLA is conserved in the orphan TNFR, UL144, present in human CMV. UL144 binds BTLA, but not LIGHT, and inhibits T cell proliferation, selectively mimicking the inhibitory cosignaling function of HVEM. The demonstration that distinct herpesviruses target the HVEM–BTLA cosignaling pathway suggests the importance of this pathway in regulating T cell activation during host defenses.

cytokines | immune evasion | T lymphocytes | costimulation

Efficient activation and differentiation of T cells depends on recognition of antigen and cooperating signals (cosignaling) that provoke either positive or inhibitory effects. Inhibitory pathways help maintain immune tolerance to self tissues. In the absence of inhibitory signals or with sustained positive cosignaling, tolerance can be overridden, leading to autoimmune responses. Two major groups of cosignaling receptors are recognized, those with an Ig-like fold, such as CTLA-4 (1), CD28 (2), PD1 (3), and B and T lymphocyte attenuator (BTLA) (4, 5), and those belonging to the TNF receptor (TNFR) superfamily, including OX40, 41BB, CD27, CD30, and herpesvirus entry mediator (HVEM; TNFR superfamily 14) among others (6–9). Generally, positive cosignaling receptors in the Ig family act by sustaining antigen receptor-associated kinase activity, whereas their inhibitory counterparts contain an immunoreceptor tyrosine-based inhibitory motif that recruits phosphatases [e.g., Src homology 2 (SH2) domain phosphatase-1; SH2 domain-containing inositol polyphosphate 5-phosphatase] attenuating antigen receptor signaling (1, 2, 10). By contrast, the cosignaling TNFRs activate serine kinases promoting expression of survival and proinflammatory genes through the transcription factors NF- κ B and activator protein 1, whereas some other TNFRs induce apoptosis, negatively regulating T cells by cellular elimination (6).

A unique inhibitory cosignaling pathway for T cells was recently defined (11, 12), which involves the engagement of BTLA by HVEM, connecting the Ig and TNFR cosignaling families. HVEM binding activates tyrosine phosphorylation of the immunoreceptor tyrosine-based inhibitory motif in BTLA and induces the associa-

tion with the protein tyrosine phosphatases Src homology domain 1 and 2 required for inhibitory signaling (13). However, HVEM can also act as a positive cosignaling receptor (reviewed in ref. 8) by binding TNF-related ligands LIGHT (TNF superfamily 14) and lymphotoxin α (LT α , TNF superfamily 2) (14). A fourth ligand of HVEM is envelope glycoprotein D (gD) of herpes simplex virus (HSV; α -herpesvirus) from which its name was derived (15, 16).

Thus, HVEM has the potential to serve as a molecular switch mediating either positive or inhibitory signaling, depending on which of its four ligands are bound. Moreover, the directionality of signaling is not clear, as the hierarchy of ligand occupancy and relative affinities of the interactors are not well defined. The N-terminal extracellular region of HVEM is composed of four pseudorepeats of a cysteine-rich domain (CRD), characteristic of the TNFR superfamily; each repeat contains three disulfide bonds that fold into complex loops depending in part on the spacing of the cysteines (17). Mutagenesis studies (18) and the conservation of LIGHT with LT α in the LT α –TNFR1 complex (19) indicate the second and third CRDs of HVEM contain the LIGHT-binding site. Crystallographic analyses (20) and mutagenesis studies (21) of the HVEM–gD complex revealed the viral protein bound primarily to CRD1 on the side opposite of the LIGHT binding site. gD contains an Ig-like fold with an extended N-terminal hairpin loop that binds HVEM (20). Thus, HVEM has at least two spatially distinct ligand binding regions, yet gD can competitively block the binding of membrane-bound LIGHT to HVEM (14). Domain-swapping experiments revealed the CRD1 of HVEM was sufficient to mediate BTLA binding (11). These observations raised the issue of the location of the BTLA binding site relative to gD and LIGHT.

Here, we identify a specific binding site for BTLA in CRD1 of HVEM that is distinct from the site occupied by LIGHT but overlaps with the gD binding site. CRD1 of HVEM is highly conserved in a previously orphaned member of the TNFR superfamily encoded by the UL144 ORF present in clinical isolates of human CMV (β herpesvirus) (22), a herpesvirus that is evolutionarily divergent from HSV-1 (α -herpesvirus). We show that UL144 binds BTLA, but not LIGHT, and inhibits T cell proliferation, selectively mimicking the inhibitory cosignaling function of HVEM.

Abbreviations: BTLA, B and T lymphocyte attenuator; hBTLA, human BTLA; mBTLA, mouse BTLA; BTLA-T, BTLA tetramer; CRD, cysteine-rich domain; gD, glycoprotein D; HSV, herpes simplex virus; HVEM, herpesvirus entry mediator; hHVEM, human HVEM; mHVEM, mouse HVEM; hLIGHT, human LIGHT; LIGHTt66, LIGHT truncated at G66; LT α , lymphotoxin α ; TNFR, TNF receptor.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. DQ100368 and DQ100369).

[†]T.C.C. and I.R.H. contributed equally to this work.

[§]To whom correspondence may be addressed. E-mail: p-spear@northwestern.edu, benedict@liai.org, or cware@liai.org.

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Materials and Methods

Fc Fusion Proteins, HVEM Mutants, and UL144 Variants. Fc fusion proteins were constructed between the ectodomain of the individual TNFR and the Fc region of human IgG1 as described in detail (22, 23). The extracellular domain of human BTLA (hBTLA) was synthesized by PCR using *pfu* DNA polymerase (Stratagene) and hBTLA cDNA as a template. A HindIII restriction site was introduced into the forward primer (5'-CCTGGCAAGCTTG-CCACCATGAAGACATTGCCTGCCAT-3'), and a SalI site was introduced into the reverse primer (5'-CGCTCGGTGCGACGCT-TGCCACTTCGTCCTTGA-3') to facilitate vector-insert ligation. The pCR3 vector (Invitrogen) containing the Fc region of human IgG1 (kind gift from J. Tschopp, University of Lausanne, Lausanne, Switzerland) was ligated with the BTLA insert. Human HVEM (hHVEM) and mouse HVEM (mHVEM)-Fc and LT β R-Fc were expressed in insect cells by using a baculovirus system; hBTLA-Fc and UL144-Fc were expressed in 293T cells. These Fc proteins were purified by protein G affinity chromatography. hHVEM-Fc was biotinylated by using the NHS-PEO₄-Biotin reagent according to the manufacturer's protocol (Pierce). The biotinylation reaction yielded a product of two biotin molecules per HVEM-Fc as determined by MS (SELDI, Ciphergen Biosystems, Fremont, CA). HSV-1 gD-Fc (rabbit IgG1) was produced in Chinese hamster ovary cells (24), and clarified supernatants were used in binding assays. Purified recombinant soluble gD (gD-1A 290-299) (25) was provided by G. Cohen (University of Pennsylvania, Philadelphia). Mouse BTLA tetramer (mBTLA-T) was made as described (11). Recombinant soluble human LIGHT (hLIGHT) truncated at G66 (LIGHTt66) was produced in 293T cells and purified as described (18). Purified human IgG (Gamma-gard, clinical grade, Baxter Health Care, Mundelein, IL) was used as a control for Fc fusion proteins.

HVEM point mutants were made with a QuikChange site-directed mutagenesis kit (Stratagene). Incorporation of the correct amino acid substitution was confirmed by DNA sequencing of the entire coding region.

CMV genomic DNA was extracted from cells infected with CMV clinical strains representing each of the UL144 sequence groups, 1A, 1B, 1C, 2, and 3. The UL144 ORF was amplified by PCR from genomic templates representing each group by using the same set of primers. The forward primer contained a BamHI restriction site: 5'-ACGTGGATCCTCGTATTACAAACCGCGGAGAGGAT-3', and the reverse primer contained an XhoI restriction site: 5'-ACGTCTCGAGACTCAGACACGGTTCGTA-3'. The amplified UL144 products were cloned into the pND expression vector (gift of P. Barry, University of California, Davis) (26), and each cloned UL144 product was sequenced to verify the previously determined UL144 group sequence.

Flow Cytometry-Based Binding Assays. Flow cytometry-based binding assays were carried out as described (23, 27) and yield values for those ligands that match with other immobilized ligand binding assays (ELISA and plasmon resonance). Expression plasmids for BTLA, HVEM, HVEM mutants, and UL144 variants were transfected into 293T cells, and full-length hLIGHT was expressed in EL4 cells by retroviral vector transduction (pMIG, gift of D. Baltimore, California Institute of Technology, Pasadena, CA). BTLA-expressing human dermal fibroblasts (Clonetics, San Diego) were generated by transduction with hBTLA- or mBTLA-expressing retroviral vectors (11) that were generated by transient transfection of 293T cells (28, 29). For saturation binding and competition inhibition assays, graded concentrations of recombinant proteins [hHVEM-Fc, mHVEM-Fc, hBTLA-Fc, hLIGHTt66, gD-Fc, soluble gD, and mouse anti-hLIGHT recombinant "Omiclon" antibody (30)] were diluted in binding buffer (2% FBS in PBS, pH 7.4 with 0.02% Na₂S₂O₃) and incubated for 60 min at 4°C. Goat anti-human Fc fragment (IgG)-specific antibody conjugated

with R-phycoerythrin or goat anti-rabbit Ig antibody was used for detecting the Fc fusion proteins; anti-FLAG M2 mAb (Sigma) was used to detect hLIGHTt66, and phycoerythrin-conjugated streptavidin was used to detect biotinylated hHVEM-Fc. Specific mean fluorescence intensity was obtained by subtracting the background fluorescence staining of the nontransfected cells or isotype-matched control antibody (negative control) from the experimental group. The K_D values were calculated by nonlinear regression analysis with PRISM (version 4, GraphPad, San Diego), and the molecular mass of the purified protein was determined by MS.

T Cell Proliferation Assays. Human blood was obtained from healthy donors with ethical approval, and mononuclear cells were isolated by density gradient centrifugation. Flat-bottomed plates were incubated with varying concentrations of anti-CD3 (clone UCHT1, BD Pharmingen) and 5 μ g/ml anti-human IgG1 Fc antibody (Caltag, Burlingame, CA) overnight at 4°C. Human IgG or various TNFR-Fc proteins were preincubated at 37°C for 2 h with different concentrations. Purified CD4⁺ T cells obtained by negative immunomagnetic selection (Miltenyi Biotec, Auburn, CA) were added at a concentration of 2×10^6 cells per ml in DMEM with 5% heat-inactivated human AB serum, antibiotics, and 1 μ g/ml soluble anti-CD28 (R & D Systems) and cultured for 72 h with 1 μ Ci of [³H]thymidine added during the last 12 h.

Results

Spatially Distinct BTLA and LIGHT Binding Sites on HVEM. To determine the specificity and molecular topography of the HVEM-BTLA interaction we constructed Fc fusion proteins with the ecto domain of HVEM or BTLA as surrogates of their cell-bound receptors (23). hHVEM-Fc bound with a saturable profile ($K_D = 112$ nM) to hBTLA expressed in 293T cells as detected by flow cytometry (Fig. 1A), but failed to bind mBTLA over this concentration range. By contrast, mHVEM-Fc bound both hBTLA ($K_D = 27$ nM) and mBTLA ($K_D = 24$ nM) with similar affinities (Fig. 1B) in agreement with species restriction previously observed (11). Reciprocally, hBTLA-Fc-bound HVEM expressed in 293T cells ($K_D = 636$ nM), but less efficiently than when BTLA was positioned in the membrane (Fig. 1C). In a similar FACS assay, hHVEM-Fc-bound hLIGHT expressed in EL4 thymoma cells with a K_D of 11 nM (data not shown). A soluble form of recombinant hLIGHT (LIGHTt66) also bound with high affinity to cell-expressed hHVEM ($K_D = 13$ nM) (Fig. 1D) yet failed to inhibit binding of BTLA-Fc to HVEM, and as the concentration approached saturation (>60 nM) LIGHT enhanced BTLA-Fc binding to HVEM (Fig. 1E), suggesting the formation of a ternary complex, similar to results recently reported by Gonzalez *et al.* (12). In the mouse system, LIGHTt66 similarly did not block the binding of mHVEM to mBTLA-T (Fig. 1F), although mHVEM-Fc binding to membrane-expressed LIGHT was effectively competed. These results indicate that LIGHT and BTLA have substantially different binding affinities and occupy spatially distinct sites on HVEM.

A fourth reactant with HVEM, envelope gD from HSV-1, can bind both hHVEM and mHVEM (15, 24). With the BTLA site also located in the first CRD, we hypothesized that gD might serve as a useful tool to further probe the specific structural requirements for HVEM-BTLA interaction. A soluble deletion mutant of HSV-1 gD inhibited the binding of BTLA-T to cell-expressed mHVEM, yet also blocked binding of HVEM-Fc to membrane LIGHT with similar dose-response ($K_D = \approx 250$ nM) (Fig. 1G) (see also ref. 14). However, previous experiments have shown that gD did not block the binding of soluble LIGHT or LT α to HVEM-Fc in a plate binding format (31). This difference in competitive action of gD with soluble vs. transmembrane-anchored LIGHT indicates that the membrane position sterically restricts HVEM binding to LIGHT when gD is present. Similarly, BTLA-Fc inhibited the binding of HVEM-Fc to membrane LIGHT in a dose-dependent manner (Fig. 1H), suggesting that gD is a viral mimic of BTLA.

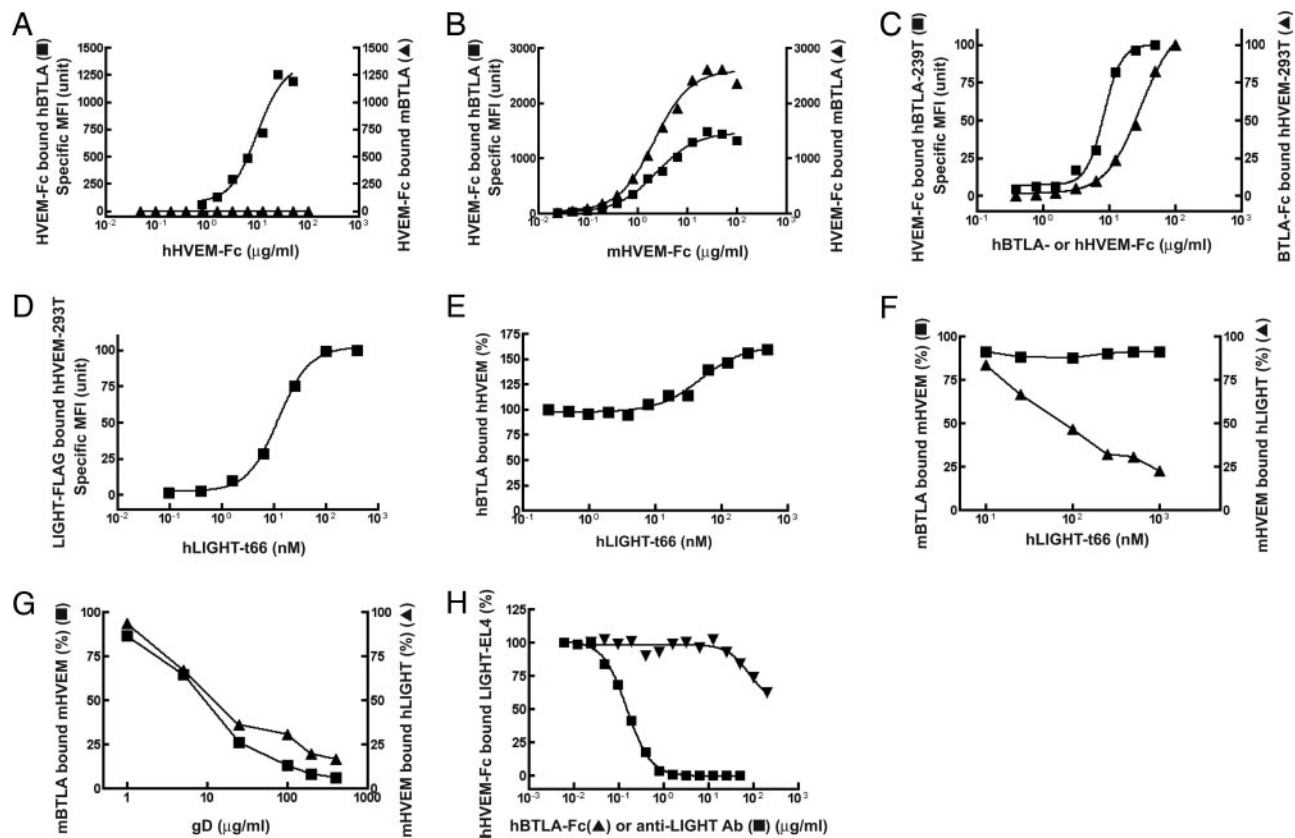


Fig. 1. Topography of BTLA, LIGHT, and gD binding to HVEM. (A and B) Dermal fibroblasts (2×10^4) stably expressing hBTLA or mBTLA were incubated with graded amounts of hHVEM (A) or mHVEM-Fc (B) in $50 \mu\text{l}$ of binding buffer for 60 min, washed, and stained with phycoerythrin (PE)-conjugated goat anti-human IgG, and fluorescence was detected by flow cytometry. MFI, mean fluorescence intensity. (C) HEK293 cells transfected with hHVEM or hBTLA expression plasmids were incubated with graded concentrations of either hBTLA-Fc or hHVEM-Fc as described above. (D) HEK293 cells transfected with hHVEM were incubated with graded concentrations of hLIGHTt66 (FLAG epitope), and bound ligand was detected with goat anti-FLAG-PE. (E) For competition binding assay, graded concentrations of LIGHTt66 were incubated with hHVEM-expressing HEK293 cells in the presence of $25 \mu\text{g}/\text{ml}$ of BTLA-Fc. (F) HEK293 cells stably transfected with mHVEM or hLIGHT-EL4 cells were incubated with graded concentrations of hLIGHTt66 in the presence of mBTLA-T ($1.4 \mu\text{g}/\text{ml}$) or mHVEM-Fc ($2 \mu\text{g}/\text{ml}$); detected with goat anti-human IgG-PE. Control for nonspecific staining with mBTLA-T was based on 293T cells. (G) Graded concentrations of soluble gD (gDt Δ 90-99) was used to compete for mBTLA-T ($1.4 \mu\text{g}/\text{ml}$) binding to mHVEM-HEK293 cells or mHVEM-Fc ($2 \mu\text{g}/\text{ml}$) to hLIGHT-EL4 cells as in F. (H) Graded concentrations of hBTLA-Fc or mouse anti-LIGHT Omniclon were incubated with hLIGHT-expressing EL4 cells in the presence of $6 \mu\text{g}/\text{ml}$ of biotinylated hHVEM-Fc. The parental EL4 cells were used as negative control (data not shown).

Together, these results indicate that LIGHT and BTLA occupy distinct sites on HVEM and identify the BTLA binding site as topographically close to the site occupied by gD in CRD1.

BTLA and gD Bind a Distinct, but Overlapping, Site on HVEM. To address whether BTLA occupies the gD binding site on HVEM, alanine/phenylalanine substitution mutations were introduced into hHVEM in residues within CRD1 and CRD2 (Fig. 2A). None of the mutants affected expression of HVEM on the cell surface (Fig. 2B) or total protein as detected with a polyclonal anti-HVEM in Western blots (data not shown). Mutations Y61F and K64A in CRD1 were particularly informative. The K64A, but not Y61F, mutation abolished binding to BTLA, yet both resulted in a complete loss of gD-Fc binding and virus infectivity as measured by gD expression and viral protein expression [Fig. 2B and data not shown; in concurrence with Connolly *et al.* (32)]. These mutants indicate that the BTLA binding site on HVEM is distinct from, but overlaps, that of gD. Saturation binding analysis of the HVEM mutants revealed decreased binding affinity of BTLA-Fc to HVEM mutants R62A and E65A (2- to 3-fold increase in K_D) and K64A, but not to several other mutants in CRD1 or CRD2 (Table 1). As expected, none of the HVEM mutants affected the affinity of LIGHTt66 binding, further indicating that the mutations were unlikely to have altered the global conformation of HVEM. These

results lead to a model in which the gD and BTLA binding sites are located primarily within CDR1, yet are topographically close, but distinct.

The BTLA Binding Site Is Conserved in CMV UL144. Mutational analysis indicated K64 is a major determinant in the ability of HVEM to engage BTLA with additional contributions from R62 and E65. These three residues form a charged ridge on the solvent-exposed surface of HVEM that is part of the loop formed by disulfide bonds C57-C75 and C67-C54 in CRD1 (Fig. 2A). The sequence of CRD1, including the positioning of the cysteines and the equivalent K64 residue, is highly conserved between hHVEM and mHVEM (62% overall identity in CRD1) (Fig. 3). Interestingly, a viral protein encoded by the UL144 ORF in human CMV showed significant homology to HVEM in CRD1 (Fig. 3). We previously described UL144 as a member of the TNFR family that contained only two CRDs, exhibiting the closest sequence homology to HVEM and TRAILR2; however, UL144 failed to bind any of the known members of the TNF ligand family, including LIGHT (22), thus its function remained elusive. The conservation of UL144 with HVEM in this region suggested the possibility that UL144 may function as a BTLA binding protein.

Sequence hypervariation exists in the ecto domain of UL144 from human CMV isolated from different clinical sources that can

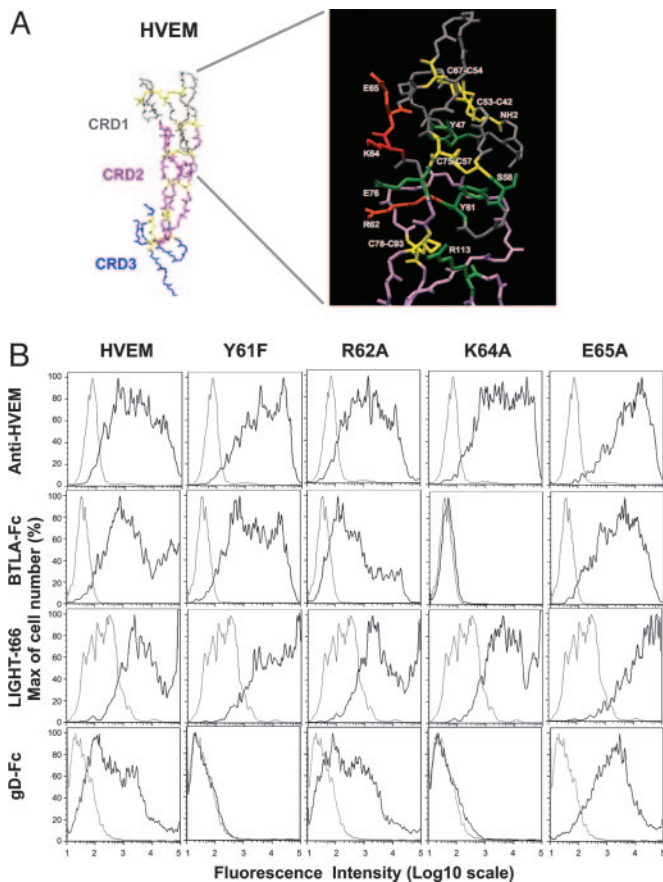


Fig. 2. Binding analyses of BTLA-Fc, soluble LIGHT, and gD to HVEM mutants. (A) Location of site-directed mutations in the structure of hHVEM (Protein Data Bank ID code 1JMA, Swiss-Pdb Viewer). The α -carbon backbone of hHVEM with side chains of mutated amino acids is shown. (Left) CRD1, gray; CRD2, purple; CRD3, blue; cysteine residues, yellow. (Right) Mutated amino acid residues: Arg-62 (R62), Lys-64 (K64), and Glu-65 (E65), red; Tyr-47 (Y47), Ser-58, (S58), Y61, E76, and R113 (green); some side chains are not shown for clarity. (B) 293T cells transfected with the expression plasmids of WT hHVEM or individual substitution mutants were stained with anti-HVEM antibody, hBTLA-Fc (100 μ g/ml), soluble hLIGHT (400 nM), and gD-Fc (0.4 μ g/ml). Binding analyses were performed by flow cytometry. Binding profiles of HVEM ligands to HVEM-293T cells (dark lines) and mock-transfected 293T parental cells (light lines) are shown.

be categorized into five major groups: 1A, 1B, 1C, 2, and 3 (33) (Fig. 3). Expression plasmids encoding representatives of each UL144 group were transfected into 293T cells, and the binding of

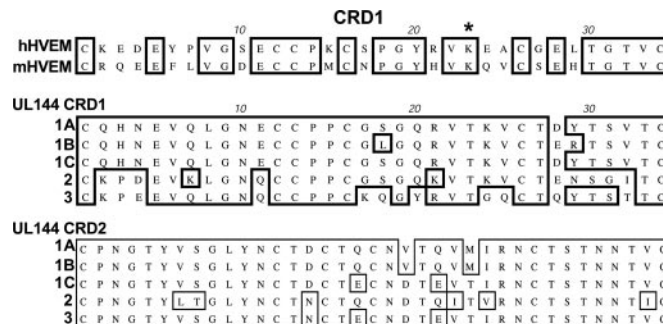


Fig. 3. Sequence alignment of HVEM and UL144 CRD1. hHVEM and mHVEM CRD1 alignment and representative sequences from the five subtypes of UL144 were aligned with hHVEM (CLUSTALW, PAM350 series, MACVECTOR 7). The asterisk denotes Lys-64 in hHVEM that is critical for binding BTLA. The GenBank accession nos. for the UL144 sequences are group 1A, DQ100368; 1B, AF085003; 1C, AF179208; 2, DQ100369; and 3, AF084982.

hBTLA-Fc was examined by flow cytometry. Binding profiles revealed specific interactions between hBTLA-Fc with cells transfected with each of the UL144 variants from human CMV (Fig. 4A). Reciprocally, UL144-Fc generated from the Fiala(F) strain of human CMV (a group 3 sequence) (22) specifically bound hBTLA, but not mBTLA (data not shown). Somewhat surprisingly, hBTLA-Fc bound to cell-expressed UL144 from each group with similar affinity ($K_D = 2\text{--}4 \mu\text{M}$) despite the sequence variation in CRD1, although binding was weaker than that seen for HVEM (≈ 5 -fold). hHVEM-Fc effectively competed with cell-expressed UL144(1C) for binding BTLA-Fc (Fig. 4B), indicating they engage a spatially related interaction site on BTLA.

The functional similarity of UL144 and HVEM was observed in the ability of UL144-Fc to inhibit the proliferation of human CD4⁺ T cells when activated with limiting amounts of anti-CD3 and anti-CD28 in the presence of immobilized fusion proteins. HVEM-Fc and UL144-Fc, but not LT β R-Fc, were effective at inhibiting proliferation (Fig. 5A); however, UL144-Fc was significantly more potent than HVEM-Fc in this assay (Fig. 5B). Both HVEM-Fc and UL144-Fc were most potent in blocking T cell proliferation when immobilized, indicating that crosslinking is probably needed for those proteins to be effective. In contrast to hHVEM and mHVEM, UL144(F) did not function as an entry factor for HSV-1 and did not bind LIGHT (22).

Discussion

The potential of HVEM to serve as a molecular switch for positive or inhibitory signaling during T cell activation will depend on which of its four ligands is engaged. We defined the hierarchy of occupancy of HVEM by BTLA and LIGHT, which

Table 1. Binding analysis of BTLA, LIGHT, and gD to HVEM

Binding partners	HVEM mutants								
	HVEM	Y47F	S58A	Y61F	R62A	K64A	E65A	E76A	R113A
BTLA-Fc (K_D ; nM)*	636	520	551	753	1453	NB	1686	381	626
LIGHTt66 (K_D ; nM)*	13	14	19	17	17	14	18	22	18
gD-Fc [†]	+	+	+	-	+	-	+	+	+

293T cells were transfected with WT or mutant HVEM expression plasmids. Binding analyses were performed on day 3 after transfection. BTLA-Fc, the extracellular domain of hBTLA was fused to Fc of human IgG; LIGHT-t66, FLAG epitope-tagged soluble LIGHT. The amino acid residues in HVEM are numbered from the first amino acid of the translation product before signal peptidase cleavage. NB, not bound.

*Saturation binding analysis measured by flow cytometry-based assay was used to estimate the equilibrium binding constant (K_D) as described in *Materials and Methods* (representative of two experiments).

[†]gD of HSV was fused to Fc of rabbit Ig and used in the binding assays at 0.4 μ g/ml.

BTLA in blocking HVEM-Fc binding to membrane LIGHT. These results suggest the possibility that the proximity of the membrane sterically excludes HVEM from binding LIGHT when gD or BTLA occupies its binding site in the DARC (gD and BTLA binding site on the TNFR HVEM in CRD1) region (noncompetitive behavior). Promoted by high-affinity binding, the LIGHT–HVEM complex, may in turn, sterically exclude membrane BTLA from binding HVEM, thus acting in a noncompetitive fashion to disrupt inhibitory signaling by BTLA.

A third line of evidence supporting the ability of LIGHT to act as a noncompetitive inhibitor of the HVEM–BTLA complex is provided by UL144. Surprisingly, UL144-Fc was far more efficient than HVEM-Fc in blocking T cell proliferation, even though its binding affinity for BTLA was measurably less (5-fold). One possibility to account for the enhanced antiproliferative activity of UL144 relative to HVEM could be its inability to bind LIGHT, resulting in continued engagement with BTLA even when LIGHT is expressed.

BTLA may serve as a constitutive “off” pathway for T cells because both HVEM and BTLA are expressed on resting lymphocytes, albeit at low levels on naïve CD4⁺ T cells (36). The induction of LIGHT during T cell activation (14) and occupancy of HVEM may displace BTLA and diminish inhibitory action on antigen receptor signals as one potential mechanism regulating the ability of HVEM to act as a molecular switch. Temporal expression of LIGHT may also influence inhibitory signaling. In addition, signals induced through these pathways may lead to differential regulation of the cellular ligands for HVEM. LIGHT may inhibit BTLA activity indirectly by promoting maturation and/or activation of dendritic cells via its alternate receptor LTβR (37). Furthermore, exogenous factors such as decoy receptor 3 or proteolysis of LIGHT may also act as mechanisms regulating the HVEM–BTLA pathway.

Herpesviruses are well adapted with respect 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? Our results indi-

cate that gD could potentially inhibit HVEM signaling by blocking engagement of both its ligands, LIGHT and BTLA, thus potentially nullifying this circuit. It is tempting to speculate that 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. By contrast, human CMV mimics only one function of the HVEM switch, the engagement of BTLA, and initiates inhibitory signaling without potential countering influence from LIGHT. The relatively high sequence variation in the ectodomain of UL144 displayed by different clinical isolates of CMV (33), yet retention of BTLA binding activity by all isolates, suggests significant immune pressure is sculpting the evolution of this molecule, which is supported by the finding that specific antibody responses to UL144 are detected in humans (22). Each mechanism must be viewed in the context of other immune-altering functions that have shaped unique niches by each herpesvirus. That evolutionarily divergent α and β herpesviruses target the LIGHT–HVEM–BTLA pathway, although by distinct mechanisms, implicates the importance of this cytokine circuit in immune regulation. These immune evasion mechanisms of herpesviruses may provide critical clues on how to modulate immunity without overt pathogenicity.

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