Herpesvirus Entry Mediator Ligand (HVEM-L), a Novel Ligand for HVEM/TR2, Stimulates Proliferation of T Cells and Inhibits HT29 Cell Growth*

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Jeremy A. Harrop‡, Peter C. McDonnell§, Michael Brigham-Burke§, Sally D. Lyn§, Jayne Minton‡, K. B. Tan*, Kim Dede‡, Jay Spamanato‡, Carol Silverman‡, Preston Hensley‡, Rocco DiPrinzio‡, John G. Emery§, Keith Deen‡, Christopher Eichman‡, Marie Chabot-Fletcher‡, Alemseged Truneh‡, and Peter R. Young§ §

From the Departments of Immunopharmacology, Molecular and Cellular Immunology, Structural Biology, and Gene Expression Sciences, and Protein Biochemistry, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania 19406

Herpesvirus entry mediator (HVEM), a member of the tumor necrosis factor (TNF) receptor family, mediates herpesvirus entry into cells during infection. Upon overexpression, HVEM activates NF-κB and AP-1 through a TNF receptor-associated factor (TRAF)-mediated mechanism. Using an HVEM-Fc fusion protein, we screened soluble forms of novel TNF-related proteins derived from an expressed sequence tag data base. One of these, which we designated HVEM-L, specifically bound to HVEM-Fc with an affinity of 44 nM. This association was confirmed with soluble and membrane forms of both receptor and ligand. HVEM-L mRNA is expressed in spleen, lymph nodes, macrophages, and T cells and encodes a 240-amino acid protein. A soluble, secreted form of the protein stimulates proliferation of T lymphocytes during allogeneic responses, inhibits HT-29 cell growth, and weakly stimulates NF-κB-dependent transcription.

The members of the TNF family are defined as being structurally related to the first members of the family, TNFR1 and TNFR2. Each new member contains multiple copies of a cysteine-rich motif of approximately 40 amino acids that is known to provide the ligand recognition motif (1). While several members of this family are characterized by a cytoplasmic “death domain,” which is associated with the activation of apoptotic signaling pathways, other members stimulate the transcription factors NF-κB and AP-1, for which the cytoplasmic motif is less well defined. Those receptors in the family containing cytoplasmic domains currently number 14 and include TNFR1 (2, 3), TNFR2 (4), lymphotixin β receptor (LTβR) (5), nerve growth factor receptor (6), CD27 (7), CD30 (8), CD40 (9), OX40 (10), 4–1BB (11), DR4 (12), DR5/TRA1-22 (13–20), TACI (21), HVEM/TR2/ATAR (22–24), and RANK (25). Two additional receptors do not have cytoplasmic domains and are either secreted, such as OPG (26, 27), or linked to the membrane through a glycosphosphatidyl tail, such as TRID/DcR1/TRAIL-R3 (18, 19, 28). Additional secreted representatives are also encoded by viruses, which use them to block the endogenous ligand and circumvent its role in host defense (4, 29, 30).

One member of this family, HVEM, also known as TR2 (23) and ATAR (24), was shown to be used as a co-receptor for herpesvirus infection (22). Despite a very short cytoplasmic domain, overexpression studies suggested that it may activate NF-κB and AP-1 signaling pathways through engagement of TRAFs, in particular TRAF2 and TRAF5 (24, 31). Furthermore, a soluble form of the receptor, HVEM-Fc, was shown to inhibit a mixed lymphocyte reaction, suggesting a role for this receptor or its ligand in T lymphocyte proliferation (23).

With the exception of the nerve growth factor receptor, all of the known ligands for these receptors share significant sequence identity to TNF and are therefore believed to share a similar structure. TNF and its closest homologues form trimers and are believed to signal by bringing together two or more receptors as suggested by the co-crystal structure of the ligand/extracellular domain (32) and by the discovery that receptor monoclonal antibodies are often agonists. In order to further study the biological role of HVEM, we looked for potential ligands among novel TNF-like molecules discovered in an EST data base. A soluble form of one of these novel ligands, HVEM-L (for HVEM ligand), was shown to bind to a fusion protein of the extracellular domain of HVEM, HVEM-Fc, and subsequently this interaction was shown for membrane forms of the receptor as well. HVEM-L was further shown to activate NF-κB, stimulate the proliferation of T lymphocytes, and inhibit growth of the adenocarcinoma, HT-29.

EXPERIMENTAL PROCEDURES

Cloning of HVEM-L—A partial cDNA sequence encoding the extracellular domain of HVEM-L was identified through a BLAST search of an assembled EST data base for homologues of TNF, and this was confirmed by sequencing of a cDNA from an oxidized low density lipoprotein-treated macrophage cDNA library containing the most 5’ EST. The Gene Trapper Positive Selection (Life Technologies, Inc.) was used to obtain two full-length cDNAs from a human liver cDNA library in pcMvSpor, which differ in their noncoding regions.

mRNA Analysis—Multiple Tissue Northern blots were purchased from CLONTECH (Palo Alto, CA). Total RNA was extracted from cell lines (in exponential growth phase) and primary cells with TRIReagent (Molecular Research Center, Inc., Cincinnati, OH). Northern blotting,
labeled probe preparation, and hybridization have been previously described (27).

**Expression of Soluble HVEM-L (sHVEM-L)—**To express sHVEM-L in CHO cells, a mammalian expression vector was constructed that contained the cytomegalovirus promoter, T7 promoter sequence, 11 residues of the coding region of human HVEM, and the recognition site for BamHI/EcoRI sites, and a bovine growth hormone poly(A) site (pCTND1ID2). The extracellular domain of HVEM-L was engineered for expression through polymerase chain reaction with the primers 5'-CAGGATTGCGAGCAGTGAGAAGCCGGAAAGCTCGTTCGAGGTC-3' and 5'-AGAATCCGAGGGGATGGATTGACACACT-GATCTGATTGACAGTTCTAGGGCCAGAGAGTTTG-3'. DNA was digested with BamHI and EcoRI, and inserted into the above vector. One primer includes an enterokinase cleavage site after the histidine repeats for tag removal. The vector was transfected into CHO cells, and single colonies were selected in nucleotide- and nucleoside-free medium by limiting dilution. The highest expressing cell line was selected through analysis of supernatants using an anti-gp120 peptide monoclonal antibody.

**Purification of sHVEM-L and sHVEM—**sHVEM-L was captured by 28 liters of CHO conditioned media by S Sepharose chromatography and further purified by nickel-nitritotriacetic acid chromatography in 20 mM sodium phosphate, 150 mM sodium chloride, pH 7 (PBS, pH 7), followed by elution with 300 mM imidazole in PBS. The final product was purified by size exclusion chromatography (Superdex 200 column; Amersham Pharmacia Biotech) in 20 mM sodium phosphate, pH 7.

The purification of HVEM-Fc was described previously (23). When engineered with a factor Xa cleavage site between the HVEM extracellular domain and Fc region, sHVEM could be obtained from HVEM-Fc by cleavage with factor Xa and further protein A chromatography (33). The expression and purification of other Fc fusions was similar to HVEM-Fc (34).

**Binding Studies by Immunoprecipitation—**2 μg of HVEM-Fc receptor was incubated with 250 ng of various purified soluble ligands in 250 μl of 25 mM HEPES, pH 7.2, 0.25% bovine serum albumin, 0.01% Tween in RPMI 1640 (binding buffer) at 4 °C. Protein A capture and detection of bound ligand by Western blot with anti-gp120 peptide or anti-His repeat (CLONTECH) monoclonal antibodies has been previously described (34).

To examine binding of HVEM-Fc to full-length HVEM-L, HEK 293 cells were transfected with HVEM-L DNA or vector control using lipofectamine (Life Technologies, Inc.). 48 h after transfection, regular growth medium was replaced with methionine, cysteine-free Dulbecco's modified Eagle's medium (Life Technologies, Inc.) for 1 h followed by the addition of 1.5 μCi of radiolabeled [35S]Met/Cys (ICN Biomedicals, Inc.) and 2% dialysed fetal bovine serum in buffer B. After a 2 h rinsing, the cell lysates, precipitation with HVEM-Fc, and analysis by SDS-polyacrylamide gel electrophoresis have been previously described (34).

**Binding Studies by Surface Plasmon Resonance—**The association and dissociation rates of the interaction of HVEM-L with captured HVEM-Fc was determined by surface plasmon resonance using a BIAcore 1000 (BIAcore Inc., Piscataway, NJ). The capture surface was a protein A (Pierce)-modified CMS sensor chip (35). Sensorgrams were collected at 25 °C and a flow rate of 30 μl/min of buffer blank were collected and analyzed by nonlinear regression (36). Buffer blank were collected at 25 °C and a flow rate of 30 μl/min of buffer blank were collected and analyzed by nonlinear regression (36). Buffer blank were collected at 25 °C and a flow rate of 30 μl/min of buffer blank were collected and analyzed by nonlinear regression (36).

**Flow Cytometry—**Surface staining was carried out using staining buffer consisting of PBS and 0.1% sodium azide. Cells were preincubated with 10 μg of human IgG (Organon Teknika Corp., Durham, NC) for 10 min to block nonspecific Fc binding. Biotinylated soluble receptor-Ig or unconjugated mouse mAb was then incubated with cells for 30 min at 4 °C. Cells were washed and binding was detected using streptavidin/phycoerythrin for biotinylated receptor-Ig or goat anti-mouse antibody conjugated with fluorescein isothiocyanate (Sigma). After a further 30 min incubation at 4 °C, cells were washed, fixed in 2% formaldehyde for 20 min at 4 °C, and analyzed on a FACSort (Becton Dickinson Corp., San Jose, CA).

**ELISAs—**Immunopurified ELISA plates (Dynatech Laboratories, Inc., Chantilly, VA) were coated with PBS containing receptor-Fc fusion protein at 10 μg/ml overnight at 4 °C. Plates were washed in buffer A (0.05% Tween 20, 0.02% sodium azide in PBS). Blocking buffer B (0.1% gelatin, 0.02% sodium azide in PBS) for 1 h, and washed with buffer A. sHVEM-L was then added to wells in buffer C (buffer A containing 0.1% gelatin) and incubated for 60 min at 37 °C. HVEM-L binding was detected by incubating wells with 10 μg/ml mouse anti-gp120 mAb to the epitope tagged on sHVEM-L in buffer C for 1 h at 37 °C. Cells were then washed, incubating with goat anti-mouse antibody conjugated with alkaline phosphatase (1:1000) in buffer C, and detecting with 1 mg/ml p-nitrophenyl phosphate. Absorbance was read at 405 nm using a Dynatech microplate reader (Dynatech Laboratories, Chantilly, VA).

**Three-way Mixed Lymphocyte Reaction (MLR)—**MLR proliferation assays were carried out as described previously (23). Dilutions of HVEM-L were added to wells of 96 well flat bottomed plates (Falcon Labware, Franklin Lakes, NJ) together with 50 units/ml human interferon-γ (R & D Systems, Minneapolis, MN). Cells were incubated with sHVEM-L for 90 h before the addition of [3H]thymidine for the last 6 h of culture. Cells were harvested (Skatron Instruments, Chantilly, VA), and β-scintillation counting was carried out as described previously (23).

**RESULTS—**Identification of a Novel TNF Homologue—To identify potential ligands of HVEM and other novel TNF receptor homologues, we searched an assembled EST database for homologues of TNF. One novel homologue was identified from several overlapping ESTs and was confirmed from sequencing of the cDNA containing the most 5' EST. Screening of a human liver cDNA library subsequently yielded a 1.49-kilobase pair cDNA, which encoded a complete open reading frame of 240 amino acids. This protein was designated HVEM-L based on

$$ A_{nat} = A_{nat} + \exp \left( \frac{M_1 - v_p \rho_m (v^2 - r_m^2)}{2RT} \right) + \text{base} $$

Here, \( A_{nat} \) is the total absorbance, and \( A_{nat} \) is the absorbance of the protein at the meniscus; \( M \) and \( v \) are the molecular mass and partial specific volume of the protein; \( \rho \) is the solvent density; \( w \) is the angular velocity; \( r_m \) and \( r_p \) are the reference radial positions; and \( R \) and \( T \), respectively, are the gas constant and the absolute temperature. "Base" is a term that signifies absorbing material that is not sedimenting.

The partial specific volume was estimated by the method of Cohn and Edsall for sHVEM-L to be 0.720 ml/g, and the solvent density (150 mM NaCl, 20 mM sodium phosphate, pH 7.4) was estimated to be 1.005 ml/g (38). The molecular mass for the sHVEM-L monomer determined by matrix-assisted laser desorption/ ionization mass spectrometry was 21,687 Da. The predicted mass from the sequence was 19,791 Da. The difference corresponded to ~11 mol of hexosyl/monomer. Carbohydrate was incorporated into the determination of \( v \) as suggested by Laue (38). The test for equilibrium was the superposition of data sets taken 4 h apart.

**NF-κB-Driven Luciferase Reporter Assay—**Using three transfectants with a plasmid encoding a luciferase reporter gene under the control of an IL-8 promoter (39) were seeded into 96-well plates at a density of 2 × 10^5 cells/well. HVEM-L, sHVEM-L preincubated for 15 min with a 10-fold excess of HVEM-Fc or DR3-Fc, or PBS alone was added to different wells and incubated at 37 °C for 5 h. After the removal of supernatant, sets of six analyte concentrations, 5.5–700 nM, and an antibody driven to the epitope tag expressed on sHVEM-L in buffer C for 1 h at 37 °C. Cells were washed and incubating with goat anti-mouse antibody conjugated with alkaline phosphatase (1:1000) in buffer C, and detecting with 1 mg/ml p-nitrophenyl phosphate. Absorbance was read at 405 nm using a Dynatech microplate reader (Dynatech Laboratories, Chantilly, VA).

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subsequent studies (see below). Like other TNF homologues, the predicted protein is a type II membrane protein with a 37-amino acid intracellular domain, a single transmembrane-spanning region, and a 181-amino acid extracellular domain (Fig. 1A). There is one predicted N-linked glycosylation site at residue 102. Alignment of the extracellular domain of HVEM-L with members of the TNF family shows that it is closest to LTβ and the closely related TNFα and LTα (Fig. 1, B and C).

Expression of Soluble TNF Homologue—We tested the receptor binding properties of this novel TNF homologue by expressing...
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A and HVEM-L was passed over this surface. Analysis of the sensorgrams (Fig. 4B) shows that the association phase is monophasic with \( k_{a} = 5.4 \times 10^{5} \pm 0.7 \) (m \(^{-1} \) s \(^{-1} \)), whereas the dissociation phase is biphasic with two dissociation rates: \( k_{d1} = 0.024 \pm 0.003 \) s \(^{-1} \) and \( k_{d2} = 2.42 \times 10^{-4} \pm 0.88 \) s \(^{-1} \). The latter is due to saturation of HVEM-Fc on the surface by high concentrations of HVEM-L, which drives all of the binding toward a monovalent interaction. As HVEM-L dissociates from the surface, additional receptors are available to interact with a single HVEM-L trimer, and this decreases the dissociation rate. The intrinsic dissociation constant of HVEM-L trimer binding to HVEM-Fc calculated from the ratio \( k_{d1}/k_{d2} \) is 44 nM, and the avidity is approximately 100-fold tighter.

**Binding of HVEM-Fc to HVEM-L-transfected COS Cells**—To see if HVEM bound to the membrane form of HVEM-L, a vector containing the entire open reading frame of HVEM-L (Fig. 1A) was transfected into COS cells, and binding of HVEM to HVEM-L was examined by flow cytometry. Using a monoclonal antibody raised to sHVEM-L, high levels of HVEM-L surface expression were detected on 26% of transfected cells (Fig. 5C) compared with an isotype control mAb (Fig. 5D). No binding was detected on mock-transfected cells (data not shown). Similarly, HVEM-L-transfected COS cells bound significant levels of biotinylated HVEM-Fc (Fig. 5A), which was completely blocked by preincubation of HVEM-L-transfected cells with a 10-fold molar excess of unlabeled HVEM-Fc or sHVEM-L (Fig. 5, C and D, respectively). Furthermore, control Fc-biotin did not bind to HVEM-L-transfected cells (Fig. 5B), and biotinylated HVEM-Fc did not bind to mock-transfected cells (data not shown), confirming the specificity of this binding.

When the transfected COS cells were metabolically labeled with \(^{35}\)S)methionine/cysteine, HVEM-Fc was able to precipitate a 50-kDa protein from HVEM-L-transfected but not mock-transfected cells (Fig. 3C). Binding was competed with unlabeled HVEM. The molecular mass is higher than the predicted 45 kDa, again suggestive of glycosylation. These data indicate that HVEM can bind to both membrane and secreted forms of HVEM-L.

**FIG. 1—continued**

**Identification of the Ligand for HVEM**—We examined the binding of the soluble TNF receptor family members by surface plasmon resonance. The protein bound to HVEM-Fc in a specific manner and so was named HVEM-L. When incubated with a panel of TNFR homologue fusions such as OPG-Fc, HVEM-Fc, DR3-Fc, DR4-Fc, and TRID-Fc and an unrelated Fc fusion, IL-5R-Fc, only HVEM-L was able to precipitate sHVEM-L as detected by Western blot with anti-gp120 mAb (Fig. 3A). Furthermore, the binding was competed with sHVEM-L (Fig. 3B). Similar specific binding could be demonstrated by an ELISA in which HVEM-Fc but not OPG-Fc was able to capture sHVEM-L as detected by anti-gp120 mAb (Fig. 4A).

The kinetic rate constants and the intrinsic dissociation constant were determined by surface plasmon resonance. HVEM-Fc was captured onto the sensor surface using protein A, and HVEM-L was passed over this surface. Analysis of the sensorgrams (Fig. 4B) shows that the association phase is monophasic with \( k_{a} = 5.4 \times 10^{5} \pm 0.7 \) (m \(^{-1} \) s \(^{-1} \)), whereas the dissociation phase is biphasic with two dissociation rates: \( k_{d1} = 0.024 \pm 0.003 \) s \(^{-1} \) and \( k_{d2} = 2.42 \times 10^{-4} \pm 0.88 \) s \(^{-1} \). The latter is due to saturation of HVEM-Fc on the surface by high concentrations of HVEM-L, which drives all of the binding toward a monovalent interaction. As HVEM-L dissociates from the surface, additional receptors are available to interact with a single HVEM-L trimer, and this decreases the dissociation rate. The intrinsic dissociation constant of HVEM-L trimer binding to HVEM-Fc calculated from the ratio \( k_{d1}/k_{d2} \) is 44 nM, and the avidity is approximately 100-fold tighter.

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ability of HVEM-L to activate NF-κB using an IL-8 promoter-driven luciferase gene transfected into U937 cells. U937 cells have been previously shown to express HVEM on their surface by flow cytometry (33). In response to TNF (5 ng/ml), this U937 cell line gives a 20-fold increase in luciferase gene expression (data not shown). The addition of increasing amounts of HVEM-L to these U937 cells led to a concentration-dependent increase in luciferase expression with an EC50 of 130 nM, which saturated at 2-fold above the unstimulated level (Fig. 6). While small, this increase was specific, since it was completely blocked by the addition of excess HVEM-Fc but not DR3-Fc. Similar results were obtained with reporters containing either the IL-8 or the human immunodeficiency virus NF-κB promoter element (data not shown). Thus, HVEM-L can weakly stimulate NF-κB-driven transcription.

Tissue-specific Expression of HVEM-L—The expression of HVEM-L mRNA was examined in several tissues, cells, and cell lines by Northern blot. The 2.7-kilobase pair HVEM-L mRNA was abundantly expressed in spleen and lymph nodes, and lower expression was detected in peripheral blood lymphocytes, colon, small intestine, bone marrow, thymus, and lung (Fig. 7A). Low levels of expression were also detected in total RNA extracted from freshly isolated macrophages and myeloid cell lines such as KG1a and THP-1, but not PLB-985, HL60, U937, Jurkat, HUT78, Molt 3, K562, HEL, TF274, MG63, MCP7, HT29, monocytes, and coronary arterial endothelial cells (data not shown). Consistent with the high spleen and lymph node expression, HVEM-L mRNA expression was abundantly expressed in spleen and lymph nodes, and lower expression was detected in peripheral blood lymphocytes, colon, small intestine, bone marrow, thymus, and lung (Fig. 7A). Low levels of expression were also detected in total RNA extracted from freshly isolated macrophages and myeloid cell lines such as KG1a and THP-1, but not PLB-985, HL60, U937, Jurkat, HUT78, Molt 3, K562, HEL, TF274, MG63, MCP7, HT29, monocytes, and coronary arterial endothelial cells (data not shown). Furthermore, HVEM-L ESTs were found predominantly from...
activated T cells and oxidized low density lipoprotein-induced macrophage cDNA libraries. These data suggest that HVEM-L expression is highly regulated in immune cells and might play a role in immune cell regulation.

Effect of sHVEM-L on an MLR—Both HVEM-L mRNA and HVEM mRNA and protein are expressed in T cells. Since HVEM-Fc (also known as TR2-Fc (23)) and HVEM monoclonal antibodies partially inhibit the proliferation of stimulated T cells and an MLR (23, 33), it was possible that they did so by blocking endogenous HVEM-L activity. Hence, we examined the effect of sHVEM-L in a three-way mixed lymphocyte reaction, in which three donor cells are mixed together, and the resulting allogeneic proliferation was measured. sHVEM-L alone caused an enhancement of proliferation with an EC_{50} of \sim 0.1 ng/ml, but at high doses this was reversed, suggesting down-regulation at high concentrations (Fig. 8). The enhanced proliferation was inhibited by excess HVEM-Fc (data not shown) as seen in the absence of sHVEM-L. The stimulatory effect of HVEM-L is consistent with the previously observed inhibitory effects of HVEM-Fc and HVEM monoclonal antibodies in this assay.

sHVEM-L Inhibits Proliferation of HT29 Adenocarcinoma Cells—The relatively close homology between HVEM-L and the lymphotoxins (Fig. 1C) suggested that these cytokines may have overlapping activities. To address this, the capacity of sHVEM-L to reduce proliferation of HT29 adenocarcinoma cells was examined. sHVEM-L inhibited HT29 cell proliferation in a dose-dependent manner with an IC_{50} of 5 \mu M (Fig. 9A). In contrast, IL-4 had no activity. In a second experiment, HT29 cells were incubated with 500 pg/ml of sHVEM-L in the presence of varying concentrations of HVEM-Fc or control DR5-Fc (Fig. 9B). HVEM-Fc dose-dependently blocked sHVEM-L inhibition of proliferation, whereas DR5-Fc had no effect, indicating a specific receptor ligand interaction. The activity of HVEM-L was comparable in activity and magnitude with TNF\alpha (IC_{50} 2 \mu M) and stronger than LT\alpha (IC_{50} 100 \mu M) (Fig. 9C).

DISCUSSION

HVEM was originally identified as a co-receptor for herpesvirus infection (22) and subsequently shown to activate NF-\kappaB and AP-1 upon transfection in HEK293 cells (24, 31). Furthermore, an HVEM-Fc fusion protein was shown to inhibit a mixed
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HVEM-L, which is a novel member of the TNF family. Like other TNF family members, HVEM-L is expressed as a type II membrane protein and, based on the characteristics of a secreted form of the protein, forms a trimer. As expected from previous studies of HVEM, HVEM-L stimulates NF-κB-dependent transcription and proliferation in an MLR.

Several other TNF-like ligands can costimulate T cells, including TNFα, LTA, CD27L, CD30L, 4-1BB, and OX40L (42–45). Like several of these, especially TNFα and LTA, we found that the expression of HVEM-L is highly induced after activation of myeloid or T cells, but HVEM-L is not present in non-activated cells. In contrast, HVEM is expressed constitutively in a number of cell types, and its expression is only weakly up-regulated during T cell activation (23), whereas several other TNF receptor homologues are more substantially regulated by activation (46–49). There may also be differences in the kinetics of expression, subcellular localization, and response to specific stimuli that will need to be the subject of further investigation.

The weak activation of NF-κB-dependent transcription by HVEM-L was surprising given the significant activation of NF-κB obtained by overexpression after transfection into HEK293 cells (24, 31). The transcripational activation did not depend on the particular NF-κB promoter used, which suggests that the low level of activation is not due to differential stimulation of NF-κB subunits. There is more than one possible explanation for these differences. The level of NF-κB activation may depend upon the level of surface HVEM expression, which in the U937 cells used was low but detectable, or it may reflect a weak coupling of HVEM to NF-κB in U937 cells versus HEK293 cells. Whether either reflects the physiology of natural cells will need to be explored further. The weak signaling might also reflect stimulation of additional HVEM-L receptor subunits. Indeed, while this paper was under review, another group published the identification of HVEM-L, which they called LIGHT (50) and showed that it bound to both HVEM and LTb receptor.

We showed that HVEM-L inhibits proliferation of HT29 cells much like its closest homologues, LTA, TNFα, and LTAβ (2). Since HT29 cells express LTβR and HVEM, it is not yet known which receptor(s) are responsible for the effects seen with HVEM-L. Like LTβR, HVEM does not contain an intracellular

lymphocyte proliferation assay (23), and more recent experiments have shown that both HVEM-Fc and monoclonal antibodies against HVEM are able to inhibit the proliferation, activation, and cytokine production of T cells (33). Consequently, it has been of great interest to determine how the regulation of T cell activity by this receptor compares and contrasts with that of other TNF receptor-related proteins expressed on these cells, such as TNFR1, TNFR2, Fas, CD27, CD30, OX40, and 4-1BB (1). A key part of this understanding is the identification of the cognate ligand and where and how its expression is regulated.

In this paper, we have identified a ligand for HVEM,
cells were cultured in 96-well flat bottomed plates with 50 units/ml interferon-γ in the presence of shHVEM-L or IL-4 (A) or shHVEM-L (500 pg/ml) and varying concentrations of either HVEM-Fc or DR5-Fc (B). A comparison of the relative potency of TNFα, LTα, and HVEM-L in the presence of 50 units/ml interferon-γ is shown in C. In all assays, cells were cultured for 96 h, and proliferation was monitored during the last 6 h of culture, by the addition of 1 μCi of [3H]thymidine (B). Cells were harvested, and β-scintillation counting was carried out as described under “Experimental Procedures.” These experiments are representative of three similar assays.

FIG. 9. sHVEM-L inhibits the proliferation of HT29 cells. HT29 cells were cultured in 96-well flat bottomed plates with 50 units/ml interferon-γ in the presence of shHVEM-L or IL-4 (A) or shHVEM-L (500 pg/ml) and varying concentrations of either HVEM-Fc or DR5-Fc (B). A comparison of the relative potency of TNFα, LTα, and HVEM-L in the presence of 50 units/ml interferon-γ is shown in C. In all assays, cells were cultured for 96 h, and proliferation was monitored during the last 6 h of culture, by the addition of 1 μCi of [3H]thymidine (B). Cells were harvested, and β-scintillation counting was carried out as described under “Experimental Procedures.” These experiments are representative of three similar assays.

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