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High levels of soluble herpes virus entry mediator in sera of patients with allergic and autoimmune diseases

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Abbreviations: DC, dendritic cell; HVEM, herpes virus entry mediator; GST, glutathione S-transferase; MLR, mixed leukocyte reaction; RA, rheumatoid arthritis; sHVEM, soluble HVEM; SLE, systemic lupus erythematosus; TNFR, TNF receptor; TRAF, TNFR associated factor

Abstract

Herpes virus entry mediator (HVEM) is a newly discovered member of the tumor necrosis factor receptor (TNFR) superfamily that has a role in herpes simplex virus entry, in T cell activation and in tumor immunity. We generated mAb against HVEM and detected soluble HVEM (SHVEM) in the sera of patients with various autoimmune diseases. HVEM was constitutively expressed on $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells, $CD14^+$ monocytes, neutrophils and dendritic cells. In three-way MLR, mAb 122 and 139 were agonists and mAb 108 had blocking activity. An ELISA was developed to detect sHVEM in patient sera. sHVEM levels were elevated in sera of patients with allergic asthma, atopic dermatitis and rheumatoid arthritis. The mAbs discussed here may be useful for studies of the role of HVEM in immune responses. Detection of soluble HVEM might have diagnostic and prognostic value in certain immunological disorders.

Keywords: asthma; atopic; autoimmune diseases; dermatitis; inflammation mediators; rheumatoid arthritis; tumor necrosis factor

Introduction

Members of the tumor necrosis factor receptor (TNFR) superfamily share a similar architecture of their extracellular domain; this consists of a series of cysteine-rich segments containing 30-40 amino acids with six cysteines in each segment (Mallett *et al*., 1991). They are involved in the regulation of diverse immune functions. Some of the members of this family regulate signals leading to apoptosis, whereas others are involved in lymphocyte activation and differentiation (Locksley *et al*., 2001; Kwon *et al*., 2003). The open reading frame of herpes virus entry mediator (HVEM) encodes a 283 amino acid sequence, two perfect TNFR-like cysteine-rich domains, two imperfect cysteine-rich domains, and a short cytoplasmic tail similar to that seen in 4-1BB and CD40 (Montgomery *et al*., 1996; Kwon *et al*., 1997). Because an HVEM-Fc fusion protein inhibited proliferation in a mixed lymphocyte reaction using patient lymphocytes (Harrop *et al*., 1998), HVEM may either play a direct role in T cell stimulation or bind to a ligand that stimulates T cell proliferation. HVEM is a cellular mediator of the entry of herpes simplex virus type-1 (HSV-1) (Montgomery *et al*., 1996) and interacts directly with the HSV virion glycoprotein D (gD) required for entry into HVEM expressing cells (Whitbeck *et al*., 1997). Hsu *et al*. (1997) found that the cytoplasmic region of HVEM binds to several members of the TNFR-associated factor (TRAF) family, namely, TRAF1, TRAF2, TRAF3, and TRAF5, but not to TNFR4 or TRAF6, and that it activates transcription factors NF-κB and AP-1 (Marsters *et al*., 1997). As anti-HVEM mAb are needed for or in-depth studies

of the role of HVEM in immune responses and in clinical settings, we generated several mAbs and used them to characterize HVEM, and to detect soluble HVEM in clinical samples.

Materials and Methods

Cells and cell culture

Monocyte-like cell lines HL-60, U937, and THP-1, human $CD4^+$ T cell lines Jurkat and PM-1, human embryonic kidney cell line 293, and SP2/0 myeloma cell line were obtained from the American Type Culture Collection (ATCC, Manassas, VA). They were cultured in either RPMI 1640 or DMEM, supplemented with 10% FBS (Hyclone) and antibiotics. Growth was at 37°C with 5% CO_2/air.

Expression and purification of recombinant HVEM-GST and HVEM-Fc fusion proteins

We used PCR to amplify the cDNA sequence that encodes the ectodomain of HVEM. It was fused in frame with the GST-binding domain of glutathionine S-transferase (GST) or with human IgG_1 Fc (including hinge, CH_2 and CH_3 domains). Each of the in-frame fusions was confirmed by sequencing before being cloned into the PGEX-3 or pcDNA3 expression vector. pGEX-3-HVEM-transformed *Escherichia coli* were grown to an optical density of 0.6 (A_{590}) before protein expression was induced by the addition of 2 mM (final concentration) isopropyl thiogalactopyranoside (IPTG). The HVEM-GST fusion protein was purified by two-step chromatography with GSH-sepharose 4B. HVEM-Fc fusion protein was produced in NIH 3T3 cells and purified with a protein G column. An automatic peptide sequencer (ABI, Perkin-Elmer, Norwalk) was used to determine N-terminal amino acid sequences.

Immunization and fusion

A 6-8 week-old female Balb/c mouse (Harlan, Indianapolis, IN) was injected i.p. with 30 μg HVEM-GST fusion protein emulsified with an equal volume of Freund's complete adjuvant (Gibco-BRL, Gaithersburg, MD). After two weeks the mouse was injected IP with the same amount of HVEM-GST fusion protein emulsified with an equal volume of Freund's incomplete adjuvant. Two weeks later the mice received a third injection i.v. of 30 μg of HVEM-GST in PBS. Fusion of myeloma cells with splenocytes was performed as described by Kohler and Milstein (1975). SP2/0 were used as fusion partner during exponential growth, with more than 95% viability. Primed lymphocytes were mixed with myeloma cells at a ratio of 5:1 and fused in 50% (w/v) polyethylene glycol MW 1500 (Boehringer Mannheim, Indianapolis, IN). Cells were selected in HAT medium (Boehringer Manheim) using peritoneal macrophages as feeder cells. Antibody activity was determined after 10-14 days.

ELISA screening

We determined the anti-HVEM activity of supernatants of the hybridoma cells by ELISA (Jin *et al.*, 2001). ninety-six-well polypropylene microplates (Falcon, Oxnard, CA) were coated with 5 mg/ml HVEM-Fc in $NaHCO_3$ overnight at 4°C, blocked with 3% BSA, and incubated with the supernatants. Goat anti-mouse IgG conjugated with horseradish peroxidase (Sigma, St. Louis, CA) was used as secondary antibody. Plates were washed five times with PBS/Tween-20 before detecting bound antibody by incubation with ABT peroxidase substrate (Kirkegaard and Perry Lab, Inc., Gaithersburg, MD). Serum obtained from the mouse used to provide the splenocytes was used as a positive control. The Ig class and subclass of the resulting monoclonal antibodies was determined with the ImmunoPure Monoclonal Antibody Isotyping Kit (Pierce, Rockford, IL).

Purification of monoclonal antibodies

Balb/c mice were primed with Pristane and injected IP with subcloned hybridoma cells to induce ascites formation. The mAbs were purified from the ascites fluid by affinity column chromatography with protein G-Sepharose (Zymed Lab, Inc, South San Francisco, CA) (Coligan *et al.*, 1991). The three monoclonal antibodies (mAbs) used in this work, clones 108, 122 and 139, were of isotypes IgG1 (colnes, 108 and 122) or IgG2a (clone 139).

SDS-PAGE and Western blot analysis

HVEM-GST was purified by two-step chromatography with GST-sepharose 4B, and used as control for Western blot analysis. Lysates (5×10^7 cells/ml) of THP-1 and Jurkat cells were digested in lysis buffer [1% Triton X-100 in 150 mM NaCl and 20 mM Tris-HCl (pH 8.0)] at 4°C for 30 min. The lysates and HVEM-GST were denatured by boiling for 5 min with 0.5% SDS and 1% β-mercaptoethanol, subjected to SDS-PAGE, and transferred to nitrocellulose membranes. The membranes were blocked in reaction buffer (10 mM Tris [pH 7.4], 150 mM NaCl, 10% nonfat dried milk, 0.05% Tween-20) for 1 h and incubated with anti-HVEM mAb 108 diluted 1:5,000 in reaction buffer, followed by horse peroxidase-coupled goat anti-mouse IgG (Boehringer Mannheim) diluted 1:10,000. Secondary antibody was detected by incubation in ECL reagent (Amersham Biosciences, Piscataway, NJ) and exposure to X-ray film (Kodak,

Rochester, NY).

Flow cytometry

293 cells transfected with HVEM-pcDNA3 were stained 48 h after transfection. Cultured cells were also stained with or without stimulation with anti-CD3 (OKT3; 1 μg/ml) and LPS (100 ng/ml). They were washed twice, resuspended in 1% paraformaldehyde and analyzed by flow-cytometry with a FACScan (Becton Dickinson, San Jose, CA). Human PBMC were isolated from healthy donors by Ficoll-Paque Plus (Amersham Pharmacia, Oakville, Canada) at 300 *g* for 30 min. Neutrophils were isolated by polymorphprep (Nycomed Pharma AS, Oslo, Norway) gradient centrifugation according to the manufacturer's protocol. PBMC and neutrophils were washed in complete culture media. FITC-conjugated anti-HVEM mAb 122, PE-conjugated anti-CD4, CD8, CD19, and CD14 mAbs (BD PharMingen, San Diego, CA) were used to detect PBMC that expressed HVEM. Isotype-matched antibodies were used as controls.

Dendritic cell maturation

Dendritic cells (DC) were prepared from monocytes of PBMC as previously described (Brown *et al*., 2003). To obtain monocytes, non-adherent cells were removed following 2 h incubation at 37°C, and adherent cells scraped off culture dishes. The monocytes were further enriched by incubation with anti-CD14 magnetic beads (Miltenyi Biotec., Bergisch Gladbach, Germany). They were cultured in 6-well plates at 2×10^6 cells/well with GM-CSF (800 U/ml) and IL-4 (1,000 U/ml) for 6 days. Maturation of immature DCs (iDCs) was accomplished by transferring the cells to new 6-well plates and supplementating with monocyte- conditioned medium and TNF-α at final concentrations of 20% vol/vol and 10 ng/ml, respectively. Fresh GM-CSF and IL-4 were present throughout growth. The mature DCs (mDCs) were consistently made up of 90-95% $CD11c^+$ cells.

MLR-mediated PBMC proliferation

PBMC were isolated from three healthy adult volunteers by Ficoll gradient centrifugation at 400×*g* for 30 min. They were recovered, washed in RPMI 1640 (Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum and antibiotics, and adjusted to 1×10^6 cells/ml in the case of two of the donors, and to 2×10^5 cells/ml in the third. Fifty μl of each cell suspension was added to a 96-well (round bottom) plate (Falcon, Franklin Lakes, NJ) together with 50 ul of mAb 108, 122, 139 or unrelated control mAb. The plates were incubated at 37°C in 5% CO_2 for 96 h. One μCi of [^{3}H]methylthymidine (ICN Biomedicals, Costa Mesa, CA) was then added for an additional 16 h. The cells were harvested, and radioactivity counted.

Detection of soluble HVEM (sHVEM) in human serum

sHVEM was detected by ELISA using 108 as capturing mAb, and 122 as detecting mAb. A standard curve was constructed with HVEM-Fc (R & D system, Minneapolis, MN) as antigen. The ELISA plate was coated with 100 μg per well of mAb 108 (1 μg/ml), and blocked with 200 μg/well of 4% BSA. Samples of 100 μg/well were added, incubated at 37°C for 1 h and washed. Biotin-labeled mAb 122 was then added and incubation continued for another 1 h at 37°C. The plate was washed and streptavidin-conjugated horse-radish peroxidase added. TMB substrate solution (Endogen, Pierce biotechnology, Rockford, CA) was introduced and absorbance at 450 nm measured. The standard curve constructed with HVEM-Fc indicated that the sensitivity limit was 16 pg/ml. All assays were performed blind, and at the same time, on coded sterile samples.

Serum sHVEM data analysis

Comparisons of the sHVEM values of each patient and the healthy controls were assessed by one-way ANOVA. The statistical analyses were performed with the Microcal™ Orijin™ software package (Microcal-Software, Inc, Northampton, MA). A *P*-value of less than 0.05 was considered statistically significant.

Results

Anti-HVEM monoclonal antibodies

To produce mAbs that recognize HVEM, the HVEM-Fc fusion protein was used for ELISA screening since the HVEM-GST fusion protein had been used as immunogen. Specificity was confirmed by lack of cross-reaction with GST and other bacterial proteins. Flow cytometry was used to identify mAbs that stained native cell surface HVEM so that the mAbs could be used in future studies of the functions of HVEM in human immune system. Positive clones were subcloned at least twice by limiting dilution. The ELISA ImmunoPure Monoclonal Antibody Isotyping Kit showed that mAb 122 and 108 were IgG_1 subclass antibodies and mAb 139 was an IgG_{2a} subclass antibody.

Specificity of anti-HVEM

To determine the specificity of the anti-HVEM mAbs, the hybridoma cell culture supernatants were used to

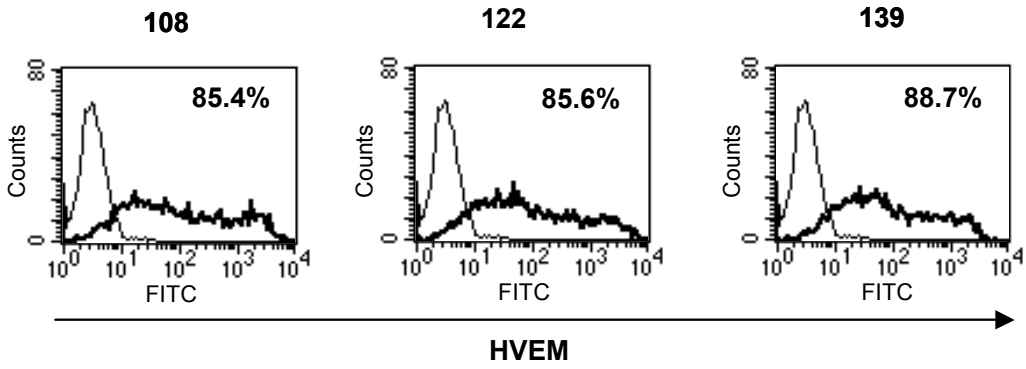

Figure 1. Binding of mAbs to 293 cells transfected with HVEM PcDNA3. 293 cells, transiently transfected with sense (heavy lines) or antisense (thin lines) HVEM pcDNA3 for 48 h, were incubated with hybridoma culture supernatants followed by FITC-conjugated goat anti-mouse IgG and subjected to cytometric analysis. All 3 mAbs, 122, 139, 108 recognized sense but not antisense transfectants.

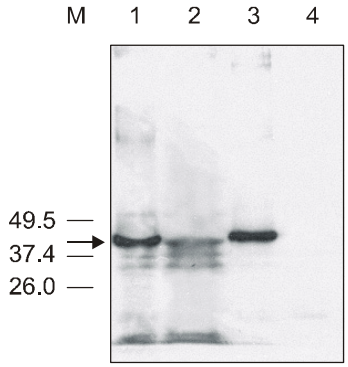

Figure 2. Western blot analysis of HVEM. Cell lysates and HVEM-GST samples were separated by SDS-PAGE, and analyzed by Western blotting with mAh108. Lane M; protein size markers, lane 1, THP-1 cells; lane 2, Jurkat cells; lane 3, HVEM-GST (0.2 μg/well); lane 4, GST. Arrow indicates HVEM.

stain 293 cells transfected with either sense or anti-sense HVEM cDNA. Goat anti-mouse-FITC conjugated antibody was used as secondary antibody. Flow cytometric analysis showed that all three selected mAbs recognized sense but not anti-sense HVEM. This indicates that all three mAbs are specific for surface expressed HVEM (Figure 1). The slight difference between the three mAbs in staining efficiency may result from: 1) different binding affinities, 2) recognition of different HVEM epitopes, or 3) different concentrations in the culture supernatants. Anti-HVEM mAbs 122, 108 and 139 recognized different epitopes on HVEM since they did not compete for Ag (data not shown), and therefore were used in ELISAs for sHVEM.

The specificity of the mAbs was further analyzed by Western blot analyses with mAb 108 from cell

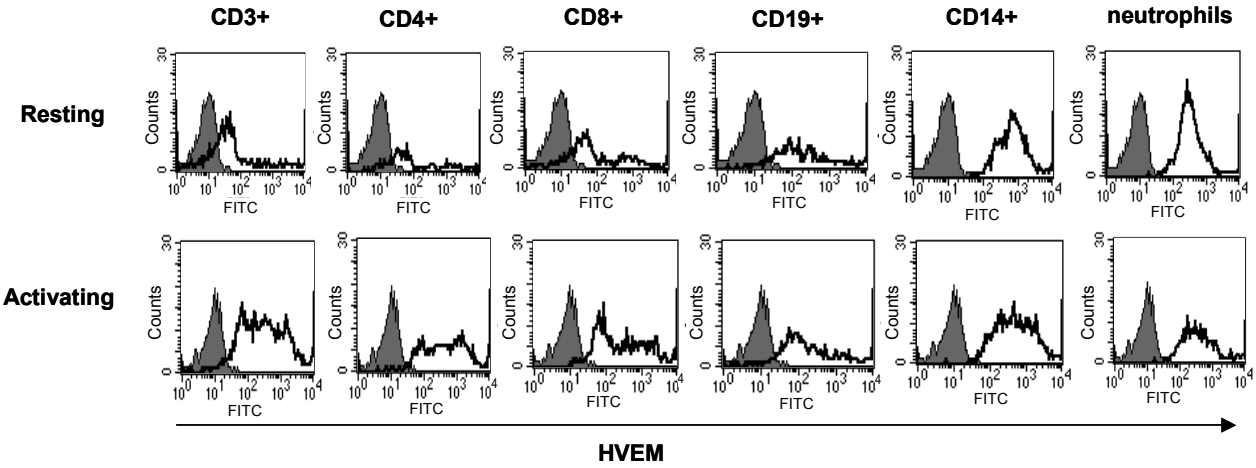

Figure 3. HVEM expression and distribution on human peripheral blood mononuclear cells detected with mAb 122. Human PBMC were isolated from healthy adult volunteers as described in Materials and Methods, and incubated with mIgG1 (filled peaks) or mAb 122 (clear peaks), followed by FITC-conjugated goat anti-mouse IgG. In addition, the PBMC were subjected to a second color fluorescence with Tric-conjugated anti-CD4, CD8, CD14 or CD19 mAbs. Analysis was performed with a FACscan.

lysates and HVEM-GST to detect protein fragments (Figure 2). Lysates from intact cells showed a major band at 40.0 kDa with some smaller fragments between 32.0 and 39.0 kDa (Figure 2, lane 1 and 2). HVEM-GST gave a single band of 42.2 kDa recognized by the anti-HVEM (Figure 2, lane 3). Thus, it is likely that natural form of HVEM is 40.0 kDa glycoprotein.

HVEM expression

Immunostaining with mAb 122 (mIgG_1) yielded generally the highest intensity of staining and the lowest non-specific background. Flow cytometric analysis of human PBMC using two color analysis, showed that HVEM was constitutively expressed on $CD3^+$ T lymphocytes, including both $CD4^+$ and $CD8^+$ subsets, and was also highly expressed on $CD19^+$ B lymphocytes, $CD14^+$ monocytes and neutrophils (Figure 3). Expression on T cells increased marginally after 24 h stimulation. As previously shown [5], freshly isolated peripheral T cells expressed high levels of HVEM. When cells were activated for 3 days, expression decreased slightly (data not shown). HVEM may be constitutively expressed in T cells in the absence of stimulation.

The expression pattern of HVEM was investigated on myeloid cells such as dendritic cells (DCs). HVEM was constitutively expressed at a high level on peripheral monocyte-derived immature DCs (Figure 4). Expression barely changed after maturation with an inflammatory cytokine such as TNF-α (Figure 4A). Expression was also detected on $CD34^+$ DCs derived from cord blood cells (Figure 4B), but activation of these mature DCs with LPS had little effect on expression (data not shown).

Effect of anti-HVEM mAbs on MLR proliferation responses

In order to examine the stimulatory or inhibitory capacity of the mAbs, their effect on a three-way mixed leukocyte reaction (MLR) were tested. As shown in Figure 5, mAb 122 strongly enhanced PBMC proliferation; mAb 139 also had a stimulatory effect, whereas mAb 108 blocked the MLR-mediated PBMC proliferation. We conclude that 122 and 139 are agonistic mAbs, and 108 is an antagonistic mAb.

sHVEM in human serum

Apart from some decoy receptors, molecules of the TNF receptor superfamily are cell surface molecules. However, they can be cleaved from the cell membrane in some clinical situations, generating soluble derivatives. We investigated sHVEM levels in sera from healthy donors, and patients with certain immunological diseases (atopic dermatitis; AD, psoriasis;

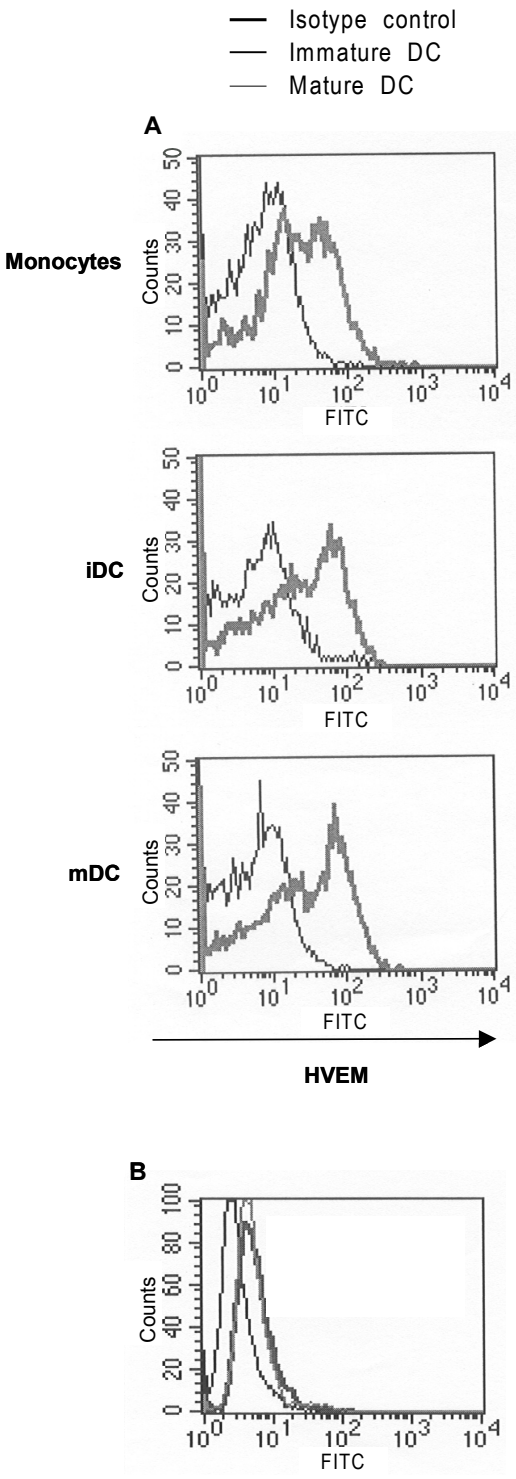

Figure 4. HVEM expression on monocytes, iDC and mDC. DC were prepared from PBMC (A), or from cord blood $CD34^+$ cells (B) as described in Materials and Methods. iDC were harvested on day 5 of culture and incubated for an additional 2 days with TNF-α, to produce mDC. DC were stained with anti-CD11c-PE and anti-HVEM-FITC and analyzed by flow cytometry. HVEM was detected by mAb122 (heavy lines or by control IgG_1 (light line) in (A). Shigted heavy (mature DC) and light (immature DC) lines indicate HVEM expression.

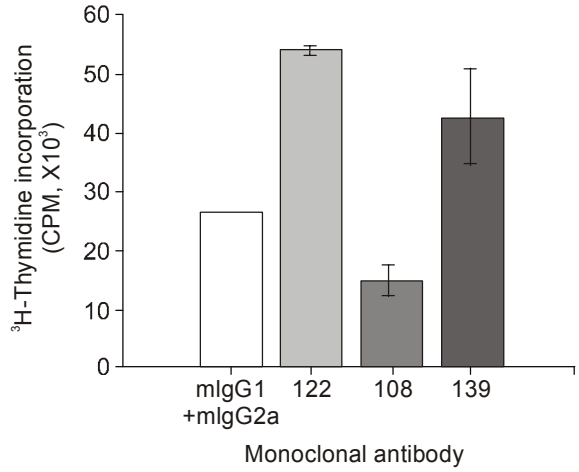

Figure 5. Effect of mAbs on a mixed leukocyte reaction (MLR). PBMC from two donors were adjusted to 1×10^6 cells/ml. Fifty microliters of each cell suspension was added in quadruplicate to 96-well round bottom plates, together with 4 μg/well of anti-HVEM mAb or control mAbs. Total volume of each well was adjusted to 200 μl/well. The plate was incubated for 3 days and [^{3}H]thymidine was added to each well for an additional 12 h. The three anti-HVEM mAbs 122 (IgG_1), 108 (IgG_1) and 139 (IgG_{2a}) were used. $mIgG_1$ and $mIgG_{2a}$ are Ig isotype controls. [^{3}H]thymidine incorporation was measured as an indication of proliferation. Values are mean±SD.

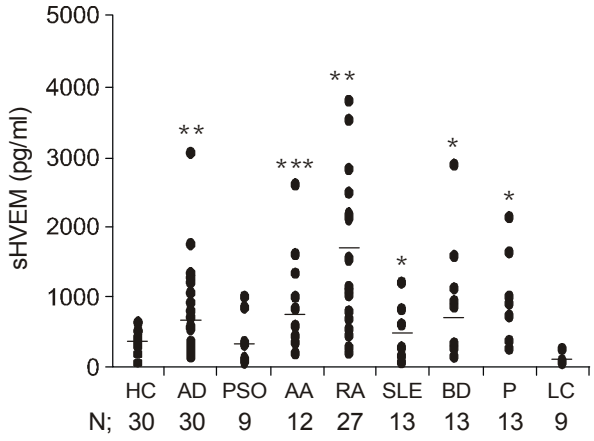

Figure 6. Soluble HVEM (sHVEM) levels in sera of patients and healthy donors determined by ELISA. The data are means of triplicates, and horizontal bars depict means. ***$P<0.001$, **$P<0.01$, and *$P<0.05$ compared with the control group. HC, healthy controls; AD, atopic dermatitis; PSO, psoriasis; AA, allergic asthma; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; BD, Bechet's disease; P, pneumonia; LC, lung cancer, and N, number of donors in each group.

PSO, allergic asthma; AA, rheumatoid arthritis; RA, systemic lupus erythematosus; SLE, Bechet's disease; BD) and with pneumonia (P) and lung cancer (LC) using a sandwich ELISA technique (Figure 6).

sHVEM was significantly elevated in sera of patients with AD (mean±SD; 718±617 pg/ml; $P<0.01$), with AA (871±712 pg/ml; $P<0.001$), with RA (1638±1366 pg/ml; $P<0.001$), with SLE (544±395 pg/ml; $P<0.05$), with BD (755±790 pg/ml; $P<0.05$), and with pneumonia (808±557 pg/ml; $P<0.05$) compared with those of healthy controls (304±184 pg/ml). Sera from patients with LC had very low levels (100±57 pg/ml) of sHVEM. Thus our data indicate that significantly higher levels of sHVEM are present in sera of patients with immunological disorders such as AA, and RA than in healthy control. Although AD and SLE show a trend of high serum concentration of sHVEM, a conclusion can not be drawn because of small sample size.

Discussion

Earlier study showed that HVEM expression at the mRNA level was most prominent in lymphoid tissues such as thymus, spleen and peripheral blood leukocytes (PBLs), although it had a wide tissue distribution; HVEM mRNA was also abundant in both resting and activated $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells, and monocytes (Kwon *et al*., 1997). Consistent with those results, present study confirmed that there is cell surface expression of HVEM on primary T cells, B cells and monocytes. In addition, HVEM was detected on other immune cells such as neutrophils, monocytes and $CD34^+$ cord blood cell-derived DCs. This indicates that HVEM is widely expressed on immune cells and is not restricted to any subpopulations of PBLs. This wide distribution seems to be unique to HVEM, since expression of most TNF receptor superfamily members is restricted to particular immune cells (Gruss, 1996; Lotz *et al*., 1996). The expression pattern of HVEM suggests that it may play a role in multiple aspects of the immune response. Since the expression of LIGHT (the HVEM ligand) on T cells is induced after activation, T cell activation through HVEM signaling may involve interaction of T cells with each other. Indeed, blockade of HVEM-LIGHT interactions inhibits proliferation of highly purified T cells in response to TCR stimulation (Shaikh *et al*., 2001). Thus, co-stimulation via HVEM depends on the presence of LIGHT on T cells (Morel *et al*., 2000; Scheu *et al*., 2002). Interestingly, Morel *et al*. (2002) have provided evidence that HVEM expression is down-regulated by stimulation of LIGHT on activated T cells, suggesting that the HVEM-LIGHT system is self-regulating (Morel *et al*., 2002). In sum, co-stimulation of T cells *via* HVEM signaling is likely to be reinforced by LIGHT expression following TCR engagement, and turned off after down-regulation of cell surface HVEM by LIGHT.

At present, it is not known what function HVEM

has on B cells. Data presented here, and elsewhere, show that B cells express high levels of HVEM, suggesting that it may be involved in B cell function (Harrop *et al*., 1998). Since LIGHT is expressed on activated T cells, and transgenic mice that express LIGHT in their T cell lineage produce elevated levels of auto-antibodies (showing a systemic lupus erythematosus phenotype), HVEM signaling on B cells may play a role in antibody production (Wang *et al*., 2001). The HVEM-LIGHT system could influence antibody production in two ways. First, the engagement of HVEM by LIGHT on activated T cells might enhance B cell activities. Second, stimulation of DC HVEM by LIGHT on activated T cells might activate DCs such that they in turn enhance B cell functions.

The expression and distribution of HVEM on various established cell lines varied according to the proportion of cells used and their state of activation. It was constitutively expressed on the monocyte-like cell lines THP-1 and U937, whereas only very low levels were found on HL-60, human $CD4^+$ T cells, Jurkat cells, PM-1, and the B-cell line Frev. Expression on HL-60, THP-1, U937, and Jurkat cells was significantly increased after 3 days stimulation with PMA (unpublished observation). Expression pattern of HVEM protein was similar to the mRNA expression data previously published by our laboratories (Lee *et al*., 2001) but there were differences in expression level that might be due to: 1) post-translational modification, 2) configuration and presentation of epitopes recognized by the mAbs, 3) mAb sensitivity.

The effect of the anti-HVEM monoclonal antibodies was examined in a mixed lymphocyte reaction (MLR). As shown in Figure 5, mAb 122 was stimulatory and mAb 108 inhibitory. MAb 139 was also stimulatory although less than mAb 122. These data can be interpreted as that HVEM and HVEM ligand are involved in cell-cell interaction in the MRL reaction and that an anti-HVEM mAb can behave in two different ways: MAb 122 and mAb 139 may be signaling mAbs that can activate lymphocytes by cross-linking HVEM, whereas mAb 108 is a blocking mAb which binds to HVEM but does not generate a signal. These monoclonal antibodies will be useful in determining the role of HVEM in T cell co-stimulation.

Processes mediated by members of the TNF receptor superfamily can be modified by proteolytic cleavage and shedding of cell surface TNF receptors. It is thought that soluble TNF receptors can compete with surface forms of the receptors, thereby decreasing the efficacy of their ligands (Salih *et al*., 2001). This may affect cell-cell interactions and responses to receptor ligands, or it may provoke more distal responses to cellular activation (McGeehan *et al*., 1994). This study showed that considerable amounts of soluble HVEM exist in sera of patients with certain diseases, and that it is significantly elevated in patients with allergic diseases (atopic dermatitis and allergic asthma), autoimmune disease (rheumatoid arthritis), and inflammatory disease (pneumonia). According to previous reports (Cheng *et al*., 1994; Michel *et al*., 1998; Schneider *et al*., 1998), there are high levels of soluble (s) Fas (sFas) and s4-1BB in sera of patients with multiple sclerosis and rheumatoid arthritis. The functions of sHVEM are not known but the present study suggests a more specific role in certain diseases. It will be important to determine whether the level of sHVEM in patient serum is correlated with progress and outcome of the disease (Sharief, 2002).

In sum, this study demonstrated HVEM expression in a variety of immune cells, including T cells, B cells, monocytes, neutrophils and DCs. Like other members of the TNF receptor superfamily, soluble HVEM exists in sera of patients with immunological disorders. The levels of sHVEM in the serum of lung cancer patients were even lower than that in the serum of normal controls. Lung cancer samples were employed as a disease control in this study. We therefore do not know whether the low serum level of sHVEM in lung cancer patients reflects any immunological significance or immune competency of the patients. It certainly warrants further studies.

Acknowlegement

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