

HVEM, a co-signaling molecular switch, and its interaction with BTLA, CD160 and LIGHT

***Jose-Ignacio Rodriguez-Barbosa*^{1,2,3*}, *Pascal Schneider*⁴, *Andreas Weigert*⁵, *Kyung-Mi Lee*^{6,7}, *Jose-Antonio Perez-Simon*^{3,8} and *Maria-Luisa del Rio*^{1,2,3}**

¹ *Transplantation Immunobiology Section, Research Institutes of the University of Leon, Campus of Vegazana s/n, 24071 Leon, Spain*

² *Leon Regional Transplantation Coordination Center, Leon University Hospital, Leon, Spain*

³ *Acción Estratégica en Salud, Consorcio CIBER-ONC, Seville, Spain*

⁴ *Department of Biochemistry, University of Lausanne, 1066 Epalinges, Switzerland*

⁵ *Institute of Biochemistry I, Faculty of Medicine, Goethe-University Frankfurt, 60590 Frankfurt, Germany*

⁶ *Department of Biochemistry and Molecular Biology, Korea University College of Medicine, Seoul 02841, Republic of Korea*

⁷ *Department of Biomedical Engineering, Center for Bio-Integrated Electronics, Simpson Querrey Institute for BioNanotechnology, Northwestern University, Evanston, IL 60208, USA*

⁸ *Department of Hematology, Virgen del Rocio University Hospital, Biomedical Institute of Sevilla, Sevilla, Spain*

*** To whom correspondence should be addressed:**

Dr. Jose-Ignacio Rodriguez-Barbosa, Institute of Biomedicine (Immunobiology),

University of Leon, Campus de Vegazana s/n, 24071 - Leon (Spain)

Phone: +34 987 293079, FAX: +34 987 291998

email: ignacio.barbosa@unileon.es

Running title: HVEM, a co-signaling molecular switch

Keywords: LIGHT, LT β R, HVEM, BTLA, CD160, co-inhibition, co-stimulation.

Abbreviations:

LIGHT: homologous to lymphotoxin, exhibits inducible expression and competes with HSV glycoprotein D for binding to herpesvirus entry mediator, a receptor expressed on T lymphocytes (also known as HVEM-L, TNFSF14, CD258);

HVEM: Herpesvirus entry mediator (TR2, HveA, TNFRSF14, CD270);

BTLA: B-and T lymphocyte attenuator (CD272)

LTbR: Lymphotoxin beta receptor

TCR: T cell receptor

TNF: Tumor necrosis factor

TNFSF: Tumor necrosis factor superfamily

TNFRSF: Tumor necrosis factor receptor superfamily

HSV1: Herpes simplex virus 1

NK: Natural killer cells

SALM5: Synaptic adhesion-like molecule 5

IFN: Interferon

ITIM: Immune receptor tyrosine inhibitory motif

NF- κ B: Nuclear factor kappa B

SHP1/2: Src homology domain 2 (SH-2) containing protein tyrosine phosphatases

EAE: Experimentally-induced hepatitis

ConA: Concanavalin A

WT: Wild type

KO: knock-out

CLL: Chronic lymphocytic leukemia

PI3K: Phosphoinositide 3-kinase

AKT: Protein kinase B (PKB), also known as Akt, is a serine/threonine-specific protein kinase

TRAF2: TNF receptor-associated factor 2

MAPK: Mitogen-activated protein kinases

Correspondence letter

Temporal and spatial expression of cosignaling receptors and their ligands regulates the early stages of T-cell activation (signal 1, T-cell receptor (TCR) signaling and signal 2, costimulation/coinhibition), clonal expansion and T-cell survival during their differentiation towards effector T cells. Once the inflammatory stimulus is eliminated, effector T cells return to homeostasis after undergoing a contraction phase by activation-induced cell death and the intervention of ligands for coinhibitory receptors, leaving a population of long-term memory T cells. The expression of ligands for coinhibitory receptors on hematopoietic cells and, more importantly, on non-hematopoietic cells of peripheral tissues is a key process in tuning the functional activity of effector T cells to prevent excess tissue inflammation that may lead to immunopathology and subsequent tissue dysfunction.

Two groups of proteins of the immunoglobulin superfamily (IgSF), CTLA-4/CD28/CD80/CD86 and PD-1/PD-L1/PD-L2, which function as cosignaling receptors and ligands, are the focus of intense research. The former group functions at the early phase of T-cell activation, and the latter group predominantly controls the effector phase of the immune response in peripheral tissues. In recent years, blockade of these immune checkpoints to potentiate effective antitumor responses has enormously advanced the field of cancer immunotherapy.¹ However, only a subgroup of patients responded to therapy with current immune checkpoint inhibitors. Therefore, other modulators of the T-cell response need to be explored.

Another area of recent intense research is the molecular switch HVEM (herpes virus entry mediator, TNFRSF14, CD270), a molecule broadly expressed in hematopoietic and non-hematopoietic cells. HVEM was first identified as the receptor of herpes simplex virus 1 (HSV-1), which binds via the viral gD protein. However, HVEM has other interaction partners that bind distinct HVEM sites; BTLA (B- and T-lymphocyte attenuator, CD272),

CD160 and gD compete for binding to the CRD1 and CRD2 domains of HVEM, whereas the TNF (tumor necrosis factor) family ligand LIGHT binds to HVEM at the CRD2 and CRD3 domains on the opposite side of the molecule. HVEM also binds to $LT\alpha$ (another member of the TNF superfamily) and to SALM 5, a molecule expressed on myeloid cells that regulates neuroinflammation. In addition to HVEM, LIGHT also binds to $LT\beta R$. A viral paralog of HVEM, UL144, can inhibit T cells via BTLA without activating natural killer (NK) cells expressing CD160.²

BTLA is a receptor of IgSF and was first identified as an inhibitory receptor. BTLA is expressed at different levels on the cell membrane of most hematopoietic cells of lymphoid lineage. BTLA is expressed at high levels in B cells, at lower levels in CD4 T cells and at even lower levels in CD8 T cells. BTLA is also expressed in NK cells, NKT cells and myeloid cells.^{3,4} BTLA regulates the homeostasis of $\gamma\delta$ T cells and innate lymphoid cells (ILCs), promotes the survival of effector T cells and contributes to their differentiation towards memory T cells.⁵ Within the myeloid lineage, BTLA in CD8 α + dendritic cells functions as an inhibitory receptor that regulates DC homeostasis in lymphoid tissues. Moreover, the binding of HVEM on T cells by BTLA expressed in CD8+DEC205+ dendritic cells can promote their differentiation towards peripheral Tregs through upregulation of CD5.⁶

The main isoform of CD160 is a GPI (glycosylphosphatidyl inositol)-anchored molecule that was discovered by expression screening of a cDNA library from an NK tumor cell line using a monoclonal antibody, clone BY55. In addition to HVEM, CD160 also binds with low affinity to classical and non-classical MHC class I, competing with CD8 for binding to the alpha 3 domain of MHC class I (Fig. 1, upper panel).⁷ CD160 expression is mainly, but not uniquely, restricted to cytotoxic cells, such as NKT cells and NK cells, and CD160 is also present in T cells, such as TCR $\alpha\beta$ + and TCR $\gamma\delta$ + intraepithelial

lymphocytes of the intestine and a subset of memory CD8 T cells in mice and humans. HVEM activates CD160 in NK cells, promoting signal transduction through the ERK1/2 and PI3K (phosphatidylinositol 3-kinase)–AKT (protein kinase B (PKB)) path-ways, leading to the production of IFN γ , whereas the binding of HVEM to CD160 in a subset of human CD4⁺ T cells delivers a coinhibitory signal. CD160 is also expressed in endothelial cells of newly formed blood vessels but not in endothelial cells of non-inflamed tissues. CD160 is also present in malignant B cells in chronic lymphocytic leukemia patients but not in normal B cells.

Cross-regulation and bidirectional signaling of the HVEM-LIGHT and HVEM-BTLA-CD160 network

The HVEM–LIGHT and HVEM–BTLA-CD160 network is a self-regulating ligand/receptor system that delivers bidirectional survival, proinflammatory and inhibitory signals to T cells,

NKT cells and other immune cells. Under homeostatic (non-inflammatory) conditions, HVEM and BTLA interact in cis to provide an intrinsic inhibitory signal that modulates T-cell activation. Upon activation, T cells rapidly and transiently express membrane-bound LIGHT, which binds to and internalizes HVEM. Disruption of the HVEM–BTLA complex leaves BTLA available to signal in trans through HVEM present in neighboring cells. Therefore, after T-cell activation and during the course of T-cell differentiation, the expression of HVEM decreases, while that of BTLA increases. This allows BTLA and transiently expressed LIGHT in activated T cells to interact with HVEM in trans in surrounding cells and deliver bidirectional costimulatory signals. Ligand-induced oligomerization of HVEM leads to the recruitment of TRAF2 (TNF receptor-associated

factor 2) and activation of downstream nuclear factor kappa B (NF- κ B) in T cells and other immune cells (Fig. 1, middle panel).⁸

The intracellular domain of BTLA contains immunoreceptor tyrosine-based inhibition motifs (ITIMs) that, once phosphorylated, can recruit Src homology domain 2 (SH2)-containing protein tyrosine phosphatases (SHP-1 and SHP-2) to suppress antigen receptor signaling (Fig. 1, middle panel). Aged BTLA-deficient BALB/c mice are highly susceptible to spontaneous autoimmune hepatitis-like disease and to experimental autoimmune encephalitis (EAE) compared with their wild-type (WT) counterparts, suggesting a coinhibitory role of BTLA in T cells.⁴ CD160-deficient mice, however, show no alterations in lymphocyte development or cell numbers, although the control of NK-sensitive hematopoietic tumors (class I deficient cell line, RMA-S) is modestly compromised due to defective NK secretion of IFN- γ and the subsequent impairment of their effector function.⁹

However, BTLA is more than an inhibitory receptor. Stimulation of BTLA by HVEM is required for T-cell survival. Indeed, the survival of donor BTLA-deficient T cells is impaired after T-cell activation in a setting of allogeneic bone marrow transplantation, and the conversion of naive T cells to effector and memory cells is also impaired in BTLA-deficient mice.¹⁰

The interaction of HVEM with CD160 plays a role in the gut epithelium. Kronenberg's group, in a mouse model of adoptive transfer of pathogenic CD4⁺ CD45RB^{high} T cells to Rag1-deficient mice, was the first to focus on this interaction. In this model, colitis was greatly accelerated and exacerbated in Rag1-HVEM double-deficient mice compared with control Rag1-deficient mice. HVEM-deficient mice were also more susceptible to intestinal bacterial infections than WT mice. CD160 expressed on innate-like intraepithelial lymphocytes can bind to HVEM on epithelial cells in the gut and transduce

proinflammatory signals, promoting the release of antimicrobial peptides that provide protection against infection (Fig. 1, lower panel). However, in the intestine, the HVEM–LIGHT interaction appears to be less critical and of less relevance than the HVEM–CD160 interaction. This less relevant interaction has also been described by Kronenberg's group in two acute models of colitis induced by either adoptive transfer of CD4⁺CD45RB^{high} T cells into Rag1-deficient or LIGHT-Rag1 double-deficient recipient mice or by the administration of dextran sulfate sodium salt (DSS) to LIGHT-deficient or wild-type C57BL/6J mice. In these experimental settings, LIGHT deficiency in neutrophils regulated inflammation of the colon via interacting with LTβR expressed on non-hematopoietic cells rather than via HVEM.¹¹

The HVEM–BTLA–CD160 signaling network inhibits NKT cells in a mouse model of concanavalin A (ConA)-induced hepatitis, an autoimmune-like disease mainly promoted by liver invariant NKT cells positive for FasL, although systemic activation of T cells also occurs. ConA-induced hepatitis in BTLA- or CD160-deficient mice is more aggressive than in WT mice, suggesting that BTLA and CD160 function as negative regulators of NKT cells.^{12,13} In this same model, contrasting results have been reported in HVEM-deficient mice with either an increased or decreased liver pathology. One publication also showed increased susceptibility of HVEM-deficient mice to experimentally induced autoimmune encephalitis.¹⁴

In the HVEM network, interactions between two superfamilies of molecules (TNFRSF–TNFSF and immunoglobulin) occur, offering multiple targetable molecules for enhancing or attenuating immunological responses in immune-related diseases or cancer immunotherapy. Accordingly, anti-BTLA antibodies have shown effective antitumor activity in melanoma and mammary carcinoma models. Although HVEM has both stimulatory and inhibitory functions, the delivery of coinhibitory signals may be dominant

over proinflammatory signals. Thus, stimulating immune cells through LIGHT or NK cells through CD160 or relieving the inhibition imposed by HVEM and BTLA will be of great interest to enhance antitumor responses.

The use of a soluble recombinant viral HVEM analog, UL144, that only binds to BTLA and not to CD160 or soluble mutated HVEM-engineered molecules lacking the costimulatory binding site for LIGHT would represent promising biologics for the attenuation of immune responses.

Acknowledgments

This work was supported by grant FIS PI# 13/00029 (Fondo de Investigaciones Sanitarias, Ministry of Health, Spanish Government and cofunded by the European Union ERDF/ESF, “Investing in your future”), LE093U13 and Unit of Excellence Research UIC #012 (Department of Education of the Regional Government, Junta de Castilla y Leon) and Gerencia Regional de Salud (BIO/01/15), which were awarded to J.I.R.B. Miguel Servet National Grant (Health National Organization Research) CP12/03063, CPII17/00002 and FIS PI16/00002 (Instituto de Salud Carlos III and cofunded by European Union ERDF/ESF, “Investinginyourfuture”), and Gerencia Regional de Salud GRS963/A/2014, GRS1142/A/2015 and GRS1505/A/2017 funded the research of M.L.R.G. The National Network CIBERONC (Oncology Research Program, referenced as CB16/12/ 00480) also cofunded this work. P.S. is funded by grant 31003A-176356 of the Swiss National Science Foundation. A.W. is funded by the grants Deutsche Krebshilfe (70112451) and Deutsche Forschungsgemeinschaft (SFB1039, and FOR2438).

Author 's contributions

We apologize to the authors of relevant contributions not included in the text due to restrictions of space and the numbers of quotes. J.I.R.B. wrote the manuscript with the main support of M.L.R. and P.S. The other coauthors contributed their expertise in the field to the reading of the manuscript, suggesting the general scheme and the topics to be addressed and providing essential input in regard to the general considerations and corrections.

Additional information:

Competing interests: The authors declare no competing interests.

Legends to figures

Figure 1: HVEM is a bidirectional molecular switch that transduces positive and negative signals.

Upper panel: The soluble form of CD160 competes with CD8 for binding to the alpha 3 domain of MHC class I, inhibiting the cytotoxic function of CD8 T cells. The CD160-GPI isoform anchored to the cell membrane of NK cells can be enzymatically released by the functional activity of a metalloprotease. Although this soluble form of CD160 binds with weak affinity to the alpha 3 domain of MHC class I, it can compete with the beta subunit of the CD8 T-cell coreceptor for binding at the same site, preventing this interaction and, consequently, inhibiting the cytotoxic functional activity of CD8 T cells.¹⁵

Middle panel: HVEM is a bidirectional molecular switch that transduces positive and negative signals. As a receptor, HVEM can deliver proinflammatory and survival signals when engaged by BTLA or LIGHT. As a ligand, HVEM delivers coinhibitory signals through CD160 and BTLA in T cells and NKT cells, costimulatory signals to CD160-positive NK cells or prosurvival signals to BTLA-expressing effector and memory T cells. BTLA and CD160 compete for the same binding site within the CRD1–CRD2 region of HVEM, while LIGHT independently binds the opposite side of HVEM within the CRD2–CRD3 region. **a.** The cytoplasmic domain of BTLA contains three conserved

tyrosine-based signaling motifs, a proximal Grb-2 recognition consensus and two distal immunoreceptor tyrosine-based inhibitory motifs (ITIMs). The membrane-proximal tyrosine within the YDND motif binds to Grb-2, which interacts with the p85 subunit of phosphatidylinositol 3-kinase (PI3K), activating the PI3K/AKT survival pathway. The binding of HVEM to BTLA in a cis configuration delivers intrinsic inhibitory signals to naive T cells and prevents HVEM from binding in trans to BTLA or CD160. The intracellular ITIM motifs of BTLA, once phosphorylated, recruit SHP1/2 (Src homology domain 2 (SH-2) containing protein tyrosine phosphatases) phosphatases to dephosphorylate and inhibit downstream signaling molecules of early T-cell activation.

b. Soluble LIGHT proteolytically released from activated T-cells binds and stabilizes the HVEM/BTLA complex in the cis configuration without activating HVEM. **c.** Upon T-cell activation, membrane-bound LIGHT is transiently expressed on T cells. Binding of LIGHT to the HVEM–BTLA complex on the same cell displaces BTLA and promotes HVEM internalization, which terminates negative signaling through BTLA and decreases the threshold of T-cell activation. Then, membrane-bound BTLA is available to interact in trans with HVEM expressed in surrounding T cells and other immune and non-immune cells. The binding of BTLA by HVEM can also deliver proinflammatory and prosurvival signals to T cells through activation of the survival PI3K–AKT signaling pathway. Trans interactions between LIGHT and HVEM result in bidirectional proinflammatory and survival signals in T cells. Lower panel: Bidirectional signaling upon HVEM–CD160 interactions. **a.** Binding of HVEM in T cells by CD160 expressed on cytotoxic cells leads to NF- κ B activation and the promotion of T-cell survival. **b.** The binding of HVEM in the intestinal epithelium by CD160 of gamma/delta T cells or innate lymphoid cells activates STAT-3, leading to the secretion of antimicrobial peptides that prevent infection. **c.** In vitro addition of soluble BTLA.Ig or soluble CD160.Ig to HVEM-transfected HEK293T cells activates the nuclear factor kappa B (NF- κ B) transcription factor and leads to the subsequent expression of proinflammatory genes. **d.** Binding of the GPI-anchored isoform of CD160 co-inhibits NKT cells and human CD4 T cells by an uncharacterized mechanism. **e.** The binding of CD160 in activated NK cells by HVEM increases their functional activity and the secretion of IFN- γ . **f.** HVEM delivers prosurvival signals to chronic lymphocytic leukemia (CLL) cells expressing CD160.

References

1. van Elsas, A., Hurwitz, A. A. & Allison, J. P. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. *J. Exp. Med.* 190, 355–366 (1999).
2. Bossen, C. et al. Interactions of tumor necrosis factor (TNF) and TNF receptor family members in the mouse and human. *J. Biol. Chem.* 281, 13964–13971 (2006).
3. Rodriguez-Barbosa, J. I., Fernandez-Renedo, C., Moral, A. M. B., Buhler, L. & Del Rio, M.L. T follicular helper expansion and humoral-mediated rejection are independent of the HVEM/BTLA pathway. *Cell Mol. Immunol.* 14, 497–510 (2017).
4. Watanabe, N. et al. BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1. *Nat. Immunol.* 4, 670–679 (2003).
5. Bekiaris, V., Sedy, J. R., Macauley, M. G., Rhode-Kurnow, A. & Ware, C. F. The inhibitory receptor BTLA controls gammadelta T cell homeostasis and inflammatory responses. *Immunity* 39, 1082–1094 (2013).
6. Jones, A. et al. Immunomodulatory functions of BTLA and HVEM govern induction of extrathymic regulatory T cells and tolerance by dendritic cells. *Immunity* 45, 1066–1077 (2016).
7. Maeda, M. et al. Murine CD160, Ig-like receptor on NK cells and NKT cells, recognizes classical and nonclassical MHC class I and regulates NK cell activation. *J. Immunol.* 175, 4426–4432 (2005).
8. Murphy, T. L. & Murphy, K. M. Slow down and survive: enigmatic immunoregulation by BTLA and HVEM. *Annu. Rev. Immunol.* 28, 389–411 (2010).
9. Tu, T. C. et al. CD160 is essential for NK-mediated IFN-gamma production. *J. Exp. Med.* 212, 415–429 (2015).
10. Hurchla, M. A., Sedy, J. R. & Murphy, K. M. Unexpected role of B and T lymphocyte attenuator in sustaining cell survival during chronic allostimulation. *J. Immunol.* 178, 6073–6082 (2007).

11. Giles, D. A. et al. The tumor necrosis factor superfamily members TNFSF14 (LIGHT), lymphotoxin beta and lymphotoxin beta receptor interact to regulate intestinal inflammation. *Front. Immunol.* 9, 2585 (2018).
12. Miller, M. L., Sun, Y. & Fu, Y. X. Cutting edge: B and T lymphocyte attenuator signaling on NKT cells inhibits cytokine release and tissue injury in early immune responses. *J. Immunol.* 183,32–36 (2009).
13. Kim, T.J.P. et al. CD160 serves as a negative regulator of NKT cells in acute hepatic injury. *Nat. Commun.* (2019).
14. Wang, Y. et al. The role of herpesvirus entry mediator as a negative regulator of T cell-mediated responses. *J. Clin. Invest.* 115, 711–717 (2005).
15. Giustiniani, J., Marie-Cardine, A. & Bensussan, A. A soluble form of the MHC class I-specific CD160 receptor is released from human activated NK lymphocytes and inhibits cell-mediated cytotoxicity. *J. Immunol.* 178, 1293–1300 (2007).