JLB

Review

HVEM/LIGHT/BTLA/CD160 cosignaling pathways as targets for immune regulation

M. L. del Rio, C. L. Lucas,† L. Buhler,†,‡ G. Rayat,§ and J. I. Rodriguez-Barbosa*,1*

*Laboratory of Immunobiology, Institute of Biomedicine, Campus de Vegazana s/n, Leon, Spain; †Bone Marrow Transplantation Section, Transplantation Biology Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA; ‡Surgical Research Unit, Department of Surgery, University Hospital Geneva, Geneva, Switzerland; and §Alberta Diabetes Institute, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

RECEIVED AUGUST 31, 2009; REVISED OCTOBER 26, 2009; ACCEPTED OCTOBER 29, 2009. DOI: 10.1189/jlb.0809590

ABSTRACT

Immunosuppression is currently the treatment of choice to attenuate the chronic deterioration of tissue function as a result of the effector mechanisms of the immunological response in transplant rejection and autoimmune diseases. However, global immunosuppression greatly increases the risk of acquiring life-threatening infections and is associated with organ toxicity when used long-term. Thus, alternative approaches that inhibit only the unwanted immune responses and preserve general immunity are highly desirable. The receptor/ligand pairs involved in the cross-talk between DC and T cells have been the focus of intense and exciting research during the last decade. The HVEM/LIGHT/BTLA/CD160 costimulatory/coinhibitory pathway has emerged as a potential target for the development of immune therapeutic interventions. Herein, we will summarize and discuss how blockade of the costimulatory HVEM/LIGHT interaction or agonist signaling through the inhibitory BTLA and CD160 receptors could contribute to the control of deleterious immune responses. *J. Leukoc. Biol.* 87: 223–235; 2010.

Introduction

Autoimmune diseases and transplant rejection cases are increasing enormously in modern societies as a direct consequence of the improvement in survival of patients afflicted with these ailments. The therapeutic approaches to control these chronic diseases are alike, and in both cases, global immunosuppression of the patient is the treatment of choice to reverse the symptoms of relapsing episodes of autoimmune disease and to abrogate the deleterious host anti-donor humoral and cellular immune responses in transplant patients. The T cell response against self-antigens or alloantigens is initiated when naïve T cells are primed by APCs in secondary lymphoid organs. Peptide recognition in the context of MHC on the cell surface of APC by TCR represents the first of two signals that trigger T cell activation and subsequently lead to differentiation of effector CD4 and CD8 T cells with proinflammatory and cytolytic activity, respectively [1–3]. Besides this initial TCR signal, a second signal through costimulatory molecules is required to promote T cell survival, cytokine-mediated clonal expansion, and progression to more advanced stages of functional specialization and maturation [4–6]. The critical role of costimulation in the process of T cell differentiation is demonstrated by the observation that in its absence, T cells become anergic and unresponsive to the antigen [7]. Once the antigenic stimulus of an immune response has been eliminated, the immune system must return to baseline levels to restore homeostasis. Coinhibitory molecules and transduction of their negative signals not only contribute to the attenuation of initial TCR-mediated activation but also modulate the process of T cell differentiation by limiting T cell proliferation and survival. Coinhibitory signaling contributes to the regulation of the contraction phase of the immune response by signaling directly into activated T cells and by promoting suppression indirectly by Tregs expressing the ligands for coinhibitory receptors. Positive and negative signals exchanged upon encounter of APC/T cells at various time-points during the immune response are the result of constitutive or induced expression of costimulatory and coinhibitory receptor/ligand pairs. However, it is an oversimplification to consider these costimulatory and coinhibitory signals as uniquely occurring during interactions between DC and T cells, as such signaling could also occur during the interaction of T cells with B cells, macrophages, or peripheral tissues, such as endothelial or parenchymal cells [8]. It remains to be studied in more depth how the balance of these competing interactions conditions the kinetics of the immune response in different cell types.

Abbreviations: ADCC=antibody-dependent cellular cytotoxicity, BTLA=B- and T-lymphocyte attenuator, CD40L=CD40 ligand, CRD=cysteine-rich domain(s), DC=dendritic cell(s), DcR3=decoy receptor 3, EAE=experimentally induced autoimmune encephalomyelitis, gD=glycoprotein D, GvHD=graft-versus-host disease, HVEM=Herpesvirus entry mediator, IEL=intestinal intraepithelial lymphocyte(s), KO=knockout, LIGHT=homologous to lymphotoxins, exhibits inducible expression, and competes with HSV gD for herpesvirus entry mediator, a receptor expressed by T lymphocytes, LTα=lymphotoxin α, LTβR: lymphotoxin β receptor, MOG=myelin oligodendrocyte glycoprotein, PD-1=programmed death-1, SC=stromal cell(s), SF=superfamily, SHP-1/2=Src homology 2-containing tyrosine phosphatase 1/2, Tregs=regulatory T cells, WT=wild-type

1. Correspondence: Institute of Biomedicine (Immunobiology), University of Leon, Campus de Vegazana s/n, 24071-Leon, Spain. E-mail: ignacio.barbosa@unileon.es

Coinhibitory and costimulatory molecules belong to two major families of cell surface-exposed proteins: the Ig SF, whose members contain Ig variable-like extracellular domains, and the TNF/TNFR SF [5, 9, 10]. Apart from CD28 and ICOS, which deliver costimulatory signals to T cells, other members of the Ig SF are involved in inhibiting or attenuating TCR-mediated T cell activation. Inhibitory Ig SF members include CTLA-4, PD-1, BTLA, and the recently discovered coinhibitory molecule CD160 (also termed BY55) [11–15]. TNFR SF comprises the other important group of molecules involved in costimulation/coinhibition of T cell responses. They are type I transmembrane proteins that adopt elongated structures using a scaffold of disulfide bridges. The disulfide bonds form extracellular CRD, which are a hallmark feature of this family of molecules [16]. The ligands for TNFR SF members are type II transmembrane proteins with intracellular N terminus domains [9, 16]. Several TNFR SF members, such as OX-40 (CD134), 4-1BB (CD137), and CD27, costimulate T cells during the late phase of T cell activation [9]. By contrast, HVEM is a TNFR SF member that displays dual functional activity by binding to coinhibitory receptors (such as BTLA or CD160) and attenuating TCR-mediated signaling or acting as a receptor of LIGHT and costimulating T cells [11, 14, 17–21] (**Fig. 1**).

In this review, we will describe the molecular structure, signal transduction, and expression pattern of several costimulatory and coinhibitory molecules on hematopoietic and parenchymal cells. We will focus primarily on the HVEM/LIGHT/BTLA/CD160 costimulatory/coinhibitory signaling pathway in the context of autoimmunity and cellular and organ transplantation. We will also include a proposal of the potential of antibody- and recombinant protein-based therapies to interfere with the stimulatory HVEM/LIGHT pathway and to potentiate the inhibitory HVEM/BTLA/CD160 pathway effectively for the overall purpose of attenuating undesirable T cell responses against self- and alloantigens.

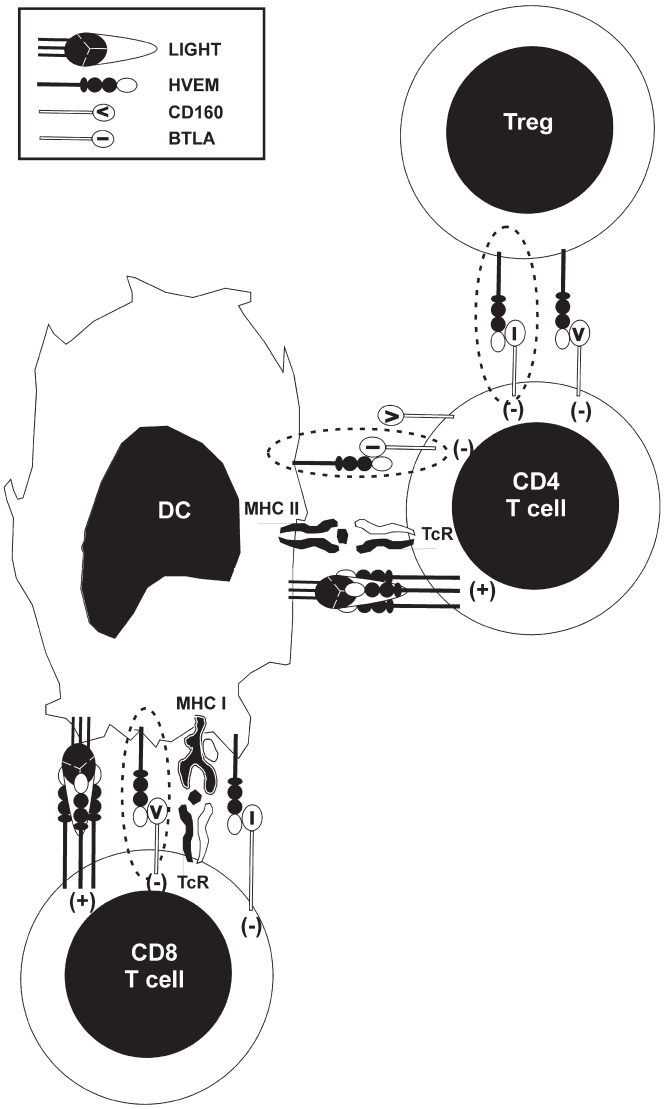

Figure 1. Engagement of HVEM on T cells by LIGHT costimulates T cell activation, whereas engagement of BTLA and CD160 by HVEM expressed on DC or Tregs coinhibits T cell signaling. The functional activity of T cells is regulated differentially by positive and negative signals exchanged between APC and T cells. HVEM/LIGHT interactions costimulate T cells. Engagement of HVEM on T cells by LIGHT expressed on DC costimulates mostly CD8 T cells but also CD4 T cell proliferation and differentiation. Negative signaling through CD160 upon binding by HVEM is probably more relevant on cytolytic T cells than on CD4 T cells as a result of the fact that CD160 is expressed more broadly on the former than on the latter. The HVEM/BTLA pathway, however, may down-modulate TCR-mediated signaling similarly in both T cell subsets. Lastly, expression of HVEM on Tregs has been shown to coinhibit effector CD4 T cell responses after engaging BTLA or possibly the CD160 receptor on effector T cells. (+) and (–), illustrate the type of signal that is transduced by a particular cell-surface receptor. Dashed ellipses surrounding the receptor/ligand interaction indicate the predominant pathway involved in the interaction between two cell types.

IMMUNOBIOLOGY OF THE HVEM/LIGHT PATHWAY: COSTIMULATING T CELL ACTIVATION

In this section, we will introduce the structure and expression pattern of the molecules involved in the HVEM/LIGHT pathway and the functional consequences of their interaction in different murine models of autoimmunity and transplantation.

Molecular structure and expression pattern of HVEM

HVEM (TNFRSF14) is a protein of the TNFR SF that serves as a shared ligand for the costimulatory and coinhibitory receptors discussed in this review. HVEM got its name based on the ability of Types 1 and 2 herpesvirus gD to bind to it for entry into the cell [22–24]. HVEM not only interacts with viral gD proteins but also binds receptors in the Ig SF (BTLA and CD160 [11, 14, 25]) and receptors in the TNF SF (LIGHT and LTα [9, 26, 27]). Human and murine HVEM are type 1 cell-surface proteins of 283 and 276 aa, respectively, with an extracellular domain composed of four CRD [22, 28]. CRD2 and CRD3 domains of HVEM interact with LIGHT [29, 30], and BTLA and CD160 bind to CRD1 and CRD2 of HVEM, competing with HSV gD binding [19, 31]. CRD1 is essential for inhibitory signaling induced by the recombinant HVEM-Ig fusion protein, as deletion of this domain results in costimulation by HVEM-Ig [11].

The cytoplasmic domain of HVEM does not exhibit a death domain, a feature of only few members that belongs to the TNFR SF and therefore, is not involved in stimulating apoptosis [9]. HVEM activation is initiated by cognate TNF-ligand clustering of multiple HVEM molecules, which in turn, recruits the cytosolic TNFR-associated factor adaptor leading to serine kinase activation of the NF-κB system and subsequent cellular activation, differentiation, and survival signaling [9]. The cross-linking of HVEM induces NF-κB and AP-1 activation in human T cells and in a HVEM-transfected human embryo kidney-293 cell line [32]. Ligation of HVEM on T cells by membrane-bound LIGHT delivers positive signals through HVEM, whereas engagement of BTLA/CD160 by HVEM provides negative signals to T cells via BTLA/CD160 [11, 14, 18–21, 33] (Fig. 1).

HVEM receptor expression is distributed widely on hematopoietic and nonhematopoietic cells. Interestingly, the expression of LIGHT and HVEM is regulated reciprocally on the same cell [34]. HVEM expression is high in naïve and memory B cells but is not present on activated B cells in the germinal center [35]. Similarly to T cells, HVEM engagement on naïve B cells by LIGHT expressed on DC and T cells costimulates B cell proliferation and Ig secretion, thereby enhancing humoral immune responses [35] (**Fig. 2**). Besides T and B cells, HVEM is expressed on a wide range of other hematopoietic (DC, Tregs, monocytes, neutrophils, and NK cells) and nonhematopoietic cells (parenchymal cells). Triggering HVEM on these cell types leads to activation of their effector functions, increasing bactericidal activity and promoting NK cell activation [32, 36, 37].

The functional in vitro and in vivo characterization of HVEM-deficient mice brought some initial controversy to the field, as HVEM KO T cells were significantly more hyper-responsive to anti-CD3-mediated stimulation than WT control T cells, and this was interpreted to suggest that HVEM functioned as an inhibitory receptor on T cells [38]. However, almost simultaneously with the appearance of that report, Murphy and coworkers [25] identified BTLA as the first coinhibitory receptor of HVEM, which was consistent with the hyper-responsive phenotype of HVEM-deficient T cells. That is,

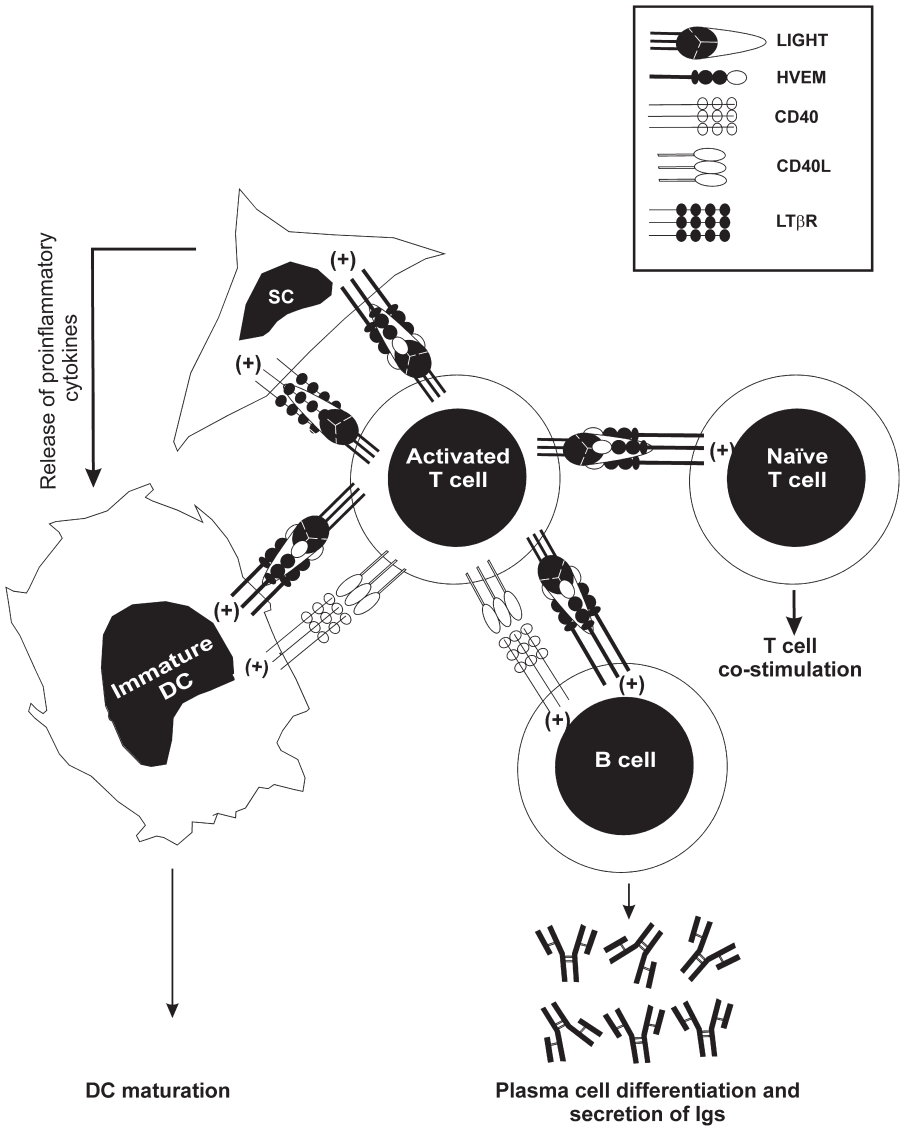

Figure 2. Central role of LIGHT expressed on activated T cells in the coordination of the immune response. LIGHT and HVEM are regulated reciprocally on DC and T cells, a phenomenon that promotes signaling in *trans* and minimizes *cis* interactions between these two molecules when expressed on the same cell. The time window for productive APC/T cell interaction involving these two receptors is therefore short and probably restricted to the early stages of the response, contributing mainly to DC maturation and initial costimulation of T cells. Once the immune response is initiated, signaling through HVEM/LIGHT is more likely to occur after T/T and T/B encounters than APC/T cell interactions. APC/T/SC collaboration: Engagement of HVEM on naïve T cells by *trans* interaction with LIGHT on DC or more likely, with LIGHT expressed on activated T cells costimulates initial T cell activation directly and promotes T cell survival once the immune response is established. Moreover, concurrent CD40L and LIGHT expression on activated T cells may also represent a maturation stimulus for immature DC, expressing low levels of CD40 and HVEM, acting as a recruitment mechanism to increase the size of the mature DC cell pool. Furthermore, the expression of LIGHT on activated T cells allows them to exchange information with SC expressing LTβR and/or HVEM. Thus, the engagement of HVEM and LTβR on SC by LIGHT expressed on activated T cells contributes directly to the release of cytokines in the peripheral tissues and to the maintenance of the inflammatory environment. T/T cell collaboration: LIGHT expressed on activated T cells costimulates naïve T cell activation by inducing HVEM signaling and thus, recruiting more lymphocytes to the pool of T cells responding to antigen. T/B collaboration: Engagement of HVEM on B cells by LIGHT expressed on activated T cells in combination with CD40 triggering provides efficient T cell help for B cell differentiation and secretion of antibodies (Igs). (+), symbol depicts an activating signal transduced by the indicated cell surface receptor.

in the absence of HVEM, coinhibition would be impaired, and consequently, T cell proliferation would be augmented. Moreover, in vitro studies with HVEM-Ig supported the role of HVEM as a ligand that induced negative signaling [11, 39]. Therefore, based on these experimental observations, it seems that in mouse models of disease, the role of HVEM as a ligand that induces coinhibitory signals through BTLA and CD160 might be dominant over its role in costimulating T cells through signaling induced upon LIGHT ligation. A summary of experimental approaches to dissecting the HVEM/LIGHT/BTLA/CD160 pathways and key results is provided (see **Table 1**).

Molecular structure, expression pattern, and function of LIGHT

LIGHT (TNFSF14, CD258) stands for homologous to LTs, exhibits inducible expression, and competes with HSV gD for herpesvirus entry mediator, a receptor expressed by T lymphocytes [26]. Except for LTβR (TNFRSF3), which is not expressed on T cells, the rest of the members of the TNF SF, including LIGHT, are expressed at distinct stages of T cell differentiation, and DC often express the corresponding ligands [16]. Structurally, LIGHT is a 240-aa type II transmembrane protein of 29 kDa that forms a cell-surface homotrimer. The trimeric structure of LIGHT enables it to cluster the cell-surface receptors with which it interacts, initiating activation of the costimulatory signaling pathways [28]. This is the key difference that enables LIGHT to induce stimulatory signaling through HVEM, while the other ligands for HVEM (namely, BTLA and CD160) cannot. Alternative splicing of mRNA generates two LIGHT isoforms: cytosolic LIGHT and membrane-bound LIGHT [34, 40]. LIGHT binds to three distinct receptors: HVEM, LTβR, and DcR3/TR6, which configure a complex molecular network, whose members in turn, bind to other molecules (LTβR binds to membrane LT, and HVEM binds weakly to LTα3) [9, 26, 41]. DcR3 (TNFRSF6B), which exists in humans but not in mice, is a molecule that lacks a membrane anchor and acts as a soluble inhibitory factor by binding LIGHT, Fas ligand, and TL1A [41]. Mutations in LIGHT at aa 119 and 174 disrupt its binding capacity to HVEM and LTβR [29]. LIGHT is susceptible to proteolytic cleavage and is released in a soluble form that can still recognize its receptor.

LIGHT has no evident signaling motifs within its intracellular domain [26]. Despite this, a CRD1-deleted membrane HVEM variant is able to costimulate T cell activation in vitro. This evidence is in agreement with the concept that LIGHT might also function as a costimulatory receptor on activated T cells, where this molecule is up-regulated. Two reports in the literature are in line with the claim that human and mouse LIGHT may indeed function as a costimulatory receptor, as they show that ligation of LIGHT by soluble TR6/DcR3 costimulated T cells by a MAPK activation-dependent mechanism [42, 43].

LIGHT is a cell-surface molecule widely expressed on hematopoietic cells (T cells, DC, NK cells, platelets, and B cells) at certain stages of cell differentiation. T cell activation up-regulates LIGHT expression more profoundly on CD8 T cells than on CD4 T cells, and the expression of this molecule is regulated reciprocally with HVEM on the same cell upon T cell activation or DC maturation [34] (Fig. 2). The reason for this coordinated regulation may be related to the fact that simultaneous expression of the two molecules on the same cell would facilitate *cis* interactions. This would prevent LIGHT from binding in *trans* with HVEM-expressing cells and therefore, preclude the opportunity for cell–cell interaction. On immature DC, contrary to naïve T cells, LIGHT is highly expressed, whereas HVEM protein expression is low. The process of DC maturation is associated with the declining of LIGHT expression and up-regulation of HVEM cell-surface protein [33] (Fig. 2). This pattern of expression suggests that the time window for potential HVEM/LIGHT interaction in DC/T cell clusters is rather narrow and restricted to early events surrounding initial T cell activation [33, 34].

LIGHT on activated T cells can deliver signals directly to other T cells expressing HVEM or can act indirectly through HVEM expressed on immature DC or on SC expressing HVEM and/or LTβR, stimulating these accessory cells to secrete proinflammatory factors (Fig. 2). Accumulating experimental evidence suggests that LIGHT expression on DC or T cells costimulates and enhances T cell proliferation and cytokine secretion [26, 33, 43]. Interestingly and in agreement with these reports, the addition of LIGHT-Ig recombinant fusion protein to T cells activates NF-κB and AP-1 and costimulates T cell proliferation and secretion of IFN-γ [32, 33, 39, 44, 45]. The observed enhancement in T cell activation was a result of HVEM-mediated signaling, as LTβR expression is absent on mature T cells [46]. LIGHT interaction with HVEM expressed on other cell types also triggers various functional activities. For instance, soluble or membrane-bound LIGHT, expressed on activated T cells, engaging HVEM expressed on immature DC when combined with the addition of CD40L-Ig, induced DC maturation and enhanced allogeneic, stimulatory activity [47] (Fig. 2). Likewise, stimulation of B cells with soluble LIGHT and CD40L-Ig increases B cell proliferation and secretion of Igs [35] (Fig. 2).

LIGHT expression on DC and more particularly, on activated T cells, therefore, plays a central role in costimulating effector functions on naïve T cells, B cells, DC, and SC (Fig. 2).

ROLE OF THE HVEM/LIGHT PATHWAY IN MURINE MODELS OF AUTOIMMUNE DISEASE AND TRANSPLANTATION

Information regarding the role of the HVEM/LIGHT pathway in autoimmunity and transplantation models is scarce. However, this pathway deserves more attention, as it may be a suitable molecular target for the control of autoreactive and alloreactive immune responses. Below, we will attempt to outline the physiological consequences of genetic deletion or antibody-mediated immune therapy triggering molecules implicated in the stimulatory HVEM/LIGHT pathway in murine models of autoimmunity and transplantation.

The role of the HVEM/LIGHT pathway in autoimmune diseases

The functional relevance of costimulatory molecules HVEM and LIGHT has been explored in murine models of autoimmune disease (see Table 1). The immediate consequence of LIGHT deficiency is the inability to deliver agonist costimula-

TABLE 1. Experimental Approaches Targeting the HVEM/LIGHT/BTLA/CD160 Pathway in Autoimmune Disease and Transplantation

Target	Approaches	Disease/setting	Outcome	References
HVEM	HVEM KO	EAE	Increased susceptibility	[38]
	HVEM KO	Con A-mediated autoimmune hepatitis	Increased susceptibility	[38]
	sHVEM-Ig	Spontaneous diabetes in NOD mice	Decreased susceptibility	[50]
	sHVEM-Ig	Collagen-induced arthritis in DBA1 mice	Increased susceptibility	[51]
	HVEM KO	GvHD: allogeneic BMTx and HVEM KO T cells across distinct MHC barriers	Increased survival	[53]
	Anti-HVEM (LBH1) mAb	GvHD: allogeneic BMTx and HVEM KO T cells across distinct MHC barriers	Increased survival	[53]
	sHVEM-Ig + cyclosporin A (low dose)	Heart allotransplantation BALB/c → B6	Increased survival	[48]
LIGHT	LIGHT transgene expression under the control T cell-specific promoter	Spontaneous systemic autoimmune disease	Increased susceptibility	[49, 50]
	LIGHT KO	GvHD: allogeneic BMTx and LIGHT KO T cells across distinct MHC barriers	Increased survival	[53]
	sLTβR-Ig + CTLA4-Ig	Islets allotransplantation BALB/c → diabetic B6	Tolerance to donor-type islets allograft	[54]
	sLTβR-Ig or anti-LIGHT polyclonal antibody	Parent to F1 GvHD model	Attenuation of the response	[52]
	LIGHT KO	Heart allotransplantation BALB/c → LIGHT B6 KO	Slight increased survival	[48]
BTLA	BTLA KO	Spontaneous autoimmune hepatitis	Increased susceptibility	[63]
	BTLA KO	EAE	Increased susceptibility	[14]
	BTLA KO	Heart allograft class 1 MHC-mismatched → BTLA KO	Decreased survival	[69]
	BTLA KO	Heart allograft class II MHC-mismatched → BTLA KO	Decreased survival	[69]
	BTLA KO	Fully MHC-mismatched heart allograft → BTLA KO	Moderate increased survival	[69]
	Anti-BTLA mAb (6A6)	Heart allograft class I MHC-mismatched → WT	Decreased survival	[69]
	Anti-BTLA mAb (6A6)	Heart allograft class II MHC-mismatched → WT	Decreased survival	[69]
	Anti-BTLA mAb (6A6)	Fully MHC-mismatched heart allograft	Modest increased survival	[69]
	PD-1/BTLA KO	Heart allograft class II MHC-mismatched → PD-1/BTLA KO	Accelerated rejection	[69]
	Depleting anti-BTLA mAb (6F7) + CTLA4-Ig	Islets allotransplantation BALB/c → diabetic B6	Indefinite graft survival	[75]
	Anti-BTLA (PJ196) mAb	Islets allotransplantation BALB/c → diabetic B6	Long-term survival	[74]
	BTLA KO	BTLA KO parent → nonirradiated F1 recipient	Increased survival of host and cell death of donor cells	[80]
	Anti-BTLA (6A6) mAb	Parent → nonirradiated F1	Increased survival of host and cell death of donor cells	[80]

s, Soluble; BMTx, bone marrow transplantation.

tory signals through HVEM and LTβR, which results in less T cell activation, particularly within the CTL compartment but also to a lesser extent, in the CD4 T cell compartment [45, 48]. Mice with a LIGHT transgene expressed under control of a T cell-specific promoter develop severe inflammation and are more predisposed to develop autoimmune diseases [49, 50]. Unexpectedly, immunization of HVEM-deficient mice with MOG emulsified in CFA triggers exacerbated, EAE, characterized by increased T cell proliferation and cytokine secretion [38]. Similarly, autoimmune hepatitis and its associated symptoms were also more pronounced in in vivo Con A-treated $HVEM^{-/-}$ mice than in similarly treated WT mice [38].

The in vivo use of HVEM-Ig fusion protein in spontaneous murine models of autoimmunity has produced opposite outcomes. In NOD mice, which spontaneously develop insulin-dependent diabetes through a T cell-dependent mechanism of

TABLE 2. Binding Affinities for Receptor/Ligand Interactions Involved in the HVEM/LIGHT/BTLA/CD160 Pathway: LIGHT, BTLA, and CD160 Have Substantially Different Binding Affinities and Occupy Spatially Distinct Sites upon Interaction with the HVEM Receptor

Mouse–mouse		Binding affinity (K_D)[a]	References
sHVEM-Ig	Membrane BTLA	24 nM	[31]
BALB/c sBTLA-Ig	B6 sHVEM-Ig	0.97 ± 0.19 μM	[95]
B6 sBTLA-Ig	B6 sHVEM-Ig	0.42 ± 0.06 μM	
sLIGHT-Ig	Membrane HVEM	No data	[b]Carl Ware and Diane Eaton, personal communications
Human–human		**Binding affinity (K_D)[a]**	**References**
sLIGHTt66	sHVEM-Ig	3.9 ± 3.9 nM	[29]
sHVEM-Ig	Membrane BTLA	5.5 nM	
sBTLA-Ig	Membrane HVEM	15 nM	[19]
sHVEM-Ig	Membrane LIGHT	7 nM	
sHVEM-Ig	Both membrane LIGHT/BTLA	500 pM	
sHVEM-Ig	Membrane BTLA	112 nM	[31]
sBTLA-Ig	Membrane HVEM	636 nM	
sLIGHT/sBTLA-Ig	Membrane HVEM	<636 nM	[31]
sHVEM-Ig	Membrane LIGHT	11 nM	
sLIGHTt66	Membrane HVEM	13 nM	

Mouse sHVEM-Ig binds to human BTLA (K_D: 27 nM), but human sHVEM-Ig does not bind mouse BTLA [31]. Cai et al. claimed that binding affinities of sBTLA-Ig to HVEM-expressing cells are similar to or slightly higher than that of sCD160-Ig to membrane HVEM [1, 27].

[a]The binding affinity value is expressed as the equilibrium dissociation constant value (K). Recombinant soluble fusion proteins are composed of the extracellular domain of the receptor bound to the Fc fragment of Ig and form dimers in solution. [b]Recombinant soluble murine LIGHT fusion protein tends to aggregate and becomes rather sticky, which makes it difficult to draw clear-cut conclusions from the binding assays.

destruction of insulin-producing β cells, blockade of the HVEM/LIGHT interaction by soluble HVEM-Ig fusion protein decreased the incidence of diabetes significantly [50]. On the contrary, in DBA1 mice, which are naturally prone to develop collagen-induced arthritis, the administration of recombinant HVEM-Ig fusion protein has been shown to aggravate the course of the disease in that there was an increased severity of histological destruction of joint tissue and an augmentation of host cellular and humoral responses against type II collagen in treated mice [51]. Therefore, the collagen-induced arthritis would suggest that the use of the HVEM-Ig fusion protein, at least in this mouse system, might not be an adequate therapeutic approach in autoimmune disease, possibly as a result of the blockade of the inhibitory HVEM/BTLA/CD160. This would suggest that soluble HVEM-Ig in vivo might not display the same agonist-inhibitory effect as the membrane-bound HVEM. However, this does not necessarily imply that a similar therapeutic strategy would cause identical, undesirable consequences in humans, as the costimulatory interaction between HVEM and LIGHT is of higher affinity than the coinhibitory interaction between HVEM and BTLA/CD160 (see **Table 2**),

The role of the HVEM/LIGHT pathway in transplantation

One of the first pieces of experimental evidence pointing out a role for the HVEM/LIGHT pathway in transplantation came from the observation that blockade of LIGHT by administration of the soluble decoy receptor LTβR-Ig or anti-LIGHT polyclonal antibody ameliorates GvHD in a murine model using bone marrow transplantation from parent into nonirradiated or irradiated (4 Gy) F1 recipients. The donor anti-host CTL response was abolished completely, and increased survival of treated recipient mice was observed when compared with untreated controls [52]. Similarly, anti-host CTL alloreactive responses were also abolished when donor HVEM KO or LIGHT KO T cells were adoptively transferred from parent to irradiated or nonirradiated F1 and across major and minor MHC histocompatibility barriers [53]. The prevention of GvHD in F1 mice receiving parental HVEM or LIGHT KO splenocytes is the consequence of impaired proliferation and subsequent cell death affecting CD8 T cells, which are more vulnerable to activation-induced cell death than CD4 T cells [53]. A reduction of GvHD severity that was associated with less proliferation and more cell death of anti-host CD8 T cells was obtained in a similar experimental GvHD setting with a blocking anti-HVEM mAb (Clone LBH-1) that precisely disrupts the interaction of HVEM with LIGHT and BTLA [53] (see Table 1).

In a murine model of islet allotransplantation, simultaneous blockade of HVEM/LIGHT and B7/CD28 with LTβR-Ig and CTLA-4-Ig fusion proteins led to long-term survival of islet allografts in diabetic mice and more importantly, promoted donor-type-specific tolerance [54] (see Table 1).

Fully MHC-mismatched cardiac allografts survived a few more days in LIGHT-deficient mice than in WT controls, reflecting that LIGHT deficiency in the hematopoietic compartment leads to diminished CTL activity and less secretion of IL-2 by CD4 T cells [48, 55]. The use of soluble HVEM-Ig fusion protein alone, which presumably disrupts costimulation through HVEM/LIGHT or strengthens negative signaling to

cells expressing BTLA or CD160, did not influence the course of heart allograft rejection. When HVEM-Ig was coadministered in combination with low-dose cyclosporine A, significantly improved heart allograft survival up to 21 days was observed compared with control mice that rejected the cardiac allografts in ~8 days [48] (see Table 1).

Therefore, the blockade of the HVEM/LIGHT interaction represents a potential target for immune intervention in transplantation with specific antagonistic mAb or noncytolytic HVEM-Ig fusion protein.

IMMUNOBIOLOGY OF THE HVEM/BTLA/CD160 PATHWAY: COINHIBITING T CELL ACTIVATION

Next, we will present the molecular structure, signaling mechanisms, and expression pattern of molecules in the coinhibitory HVEM/BTLA/CD160 pathway. The implications for therapeutic exploitation of this pathway in autoimmunity and transplantation will also be discussed.

BTLA structure, signaling, and pattern of expression

BTLA (CD272) is an I-set domain-containing member of the Ig SF. The extracellular domain suggests that it belongs to a different subset of Ig SF from that of the CD28-like family, and its cytoplasmic tail contains three conserved tyrosine motifs that are sites of phosphorylation capable of recruiting the tyrosine phosphatases SHP-1 and SHP-2 to attenuate TCR signaling [21, 56, 57]. Two of these three tyrosine residues have been identified: One is an ITIM, and the other is an immunoreceptor tyrosine-based switch motif [56]. This is consistent with BTLA functioning as a coinhibitory molecule capable of attenuating TCR signaling.

BTLA is a polymorphic molecule with three distinct allelic variants identified among 23 different strains of mice tested so far. The extracellular domain of BTLA differs by 10 aa when alleles from C57BL/6 and BALB/c mouse strains are compared [58]. BTLA expression appears in the thymus during positive selection at the same time as CD69 up-regulation. In the bone marrow, BTLA can be detected at low levels during pro-B and pre-B cell stages of development [18]. BTLA is constitutively expressed on peripheral naïve B cells and to a lesser extent, on naïve T cells, macrophages, DC, NKT cells, and NK cells [18, 58, 59]. Its expression is up-regulated quickly (within hours) on activated human Th1- and Th2-polarized CD4 T cells [60]. Anergic CD4 T cells express a low level of BTLA, which is nevertheless substantially higher than that on naïve or memory CD4 T cells [58]. BTLA is up-regulated on in vitro-differentiated bone marrow-derived DC [14, 18, 58]. However, unlike PD-1 and CTLA-4, BTLA is not expressed on Tregs [58]. The expression level of BTLA on CD8 T cells is slightly lower than on CD4 T cells, and T cells express less BTLA than B cells [58, 59].

The identification of the putative BTLA ligand was surrounded originally by some controversy. The first report postulated that BTLA interacted with the orphan B7 homologue, B7x. This assumption was based on binding studies of B7x-Ig fusion protein with spleen and lymph node cell suspensions from WT or BTLA-deficient mice [14, 61]. Nevertheless, this interpretation was refuted a few years later with the demonstration that TNFRSF14 (HVEM) was indeed the true BTLA ligand [25]. HVEM is the first TNFR SF member described to bind an Ig SF member [19, 25]. As mentioned above, HVEM is a ligand for the Ig SF members BTLA and CD160 and is also a receptor for the TNF SF members LIGHT and LTα [9, 10, 26, 27].

The experimental evidence implicating the BTLA receptor as a negative modulator of immune responses against self-antigens and alloantigens has been gathered from studies in BTLA-deficient mice reported by two different research groups [14, 18] and from in vitro observations obtained using agonist anti-BTLA antibodies and HVEM-Ig fusion proteins [35, 39, 62]. BTLA deficiency leads to a substantial increase in susceptibility to the development of autoimmune disorders compared with WT mice [14, 63]. In line with the observed in vivo phenotype of BTLA-deficient mice, in vitro studies indicate that BTLA-deficient T cells proliferate significantly more vigorously than WT T cells upon in vitro stimulation with anti-CD3 antibodies or peptide-loaded APCs [14, 18]. This increase in proliferation of BTLA-deficient T cells is likely primarily a result of an inhibitory effect of BTLA on CD8 T cell proliferation, as CD4 T cell proliferation was not affected [64]. Moreover, CD8 T cells in BTLA-deficient mice are more efficient at differentiating into memory CD8 T cells than CD8 T cells of WT mice. This correlates with the presence of a higher frequency of memory T cells in BTLA KO and also in HVEM KO mice compared with their WT counterparts [64]. Consistent with an inhibitory effect of BTLA, an agonist anti-BTLA mAb (Clone PK18) has been reported to deliver negative signals to T cells [62]. Moreover, treatment of DC with the HVEM-Ig fusion protein, which binds LIGHT on the DC and thereby blocks its interaction with HVEM on the surface of T cells when cocultured in vitro, impairs primary allogeneic T cell responses by preventing HVEM signaling into the T cell [35]. This is associated with a reduction of T cell proliferation and secretion of cytokines [39]. Lastly, the addition of the HVEM-Ig fusion protein to anti-CD3/anti-CD28-stimulated T cells also reduces T cell proliferation and the secretion of cytokines [39]. Paradoxically, intriguing in vitro findings in human T cells raise the possibility that BTLA and CD160 could also function as costimulatory ligands for HVEM, promoting NF-κB activation and cell survival and therefore, costimulating T cell function [65].

In summary, cell-surface BTLA is an I-set domain member of the Ig SF that binds HVEM and functions as a coinhibitory receptor with restricted expression on cells of the hematopoietic compartment. Analysis of the contribution of this receptor to cell function has thus far been confined to lymphocytes, leaving many aspects of BTLA biology unexplored. In light of the novel findings attributing a costimulatory function to BTLA and CD160 as ligands for HVEM, caution should be taken when interpreting the outcome of targeting this pathway in experimental disease models. Further studies are necessary to determine the role of BTLA in regulating activation of NK/NKT cells and myeloid cells. Below, we describe what is known about the role of BTLA in autoimmune diseases and in transplantation.

The role of BTLA in modulating autoimmune diseases

BTLA-deficient mice do not present any abnormalities in lymphoid organ structure or in leukocyte cell numbers, but they are hyper-responsive to in vitro stimulation with anti-CD3 [14, 18, 62, 64]. BTLA-deficient mice are more prone than WT mice to develop autoantibodies and autoimmune hepatitis spontaneously [63] and are more susceptible to the development of EAE when treated with a low dose (2 μg) of MOG emulsified in CFA [14]. Disease in BTLA-deficient mice was more severe with symptoms lasting longer than in their WT counterparts [14] (see Table 1). Similarly, BTLA-deficient mice are also susceptible to developing spontaneous, hepatitis-like disease with age. Disease development is accompanied by significant augmentation of serum IgG_1 and IgG_{2a}, along with autoantibodies against nuclear antigens [63]. This exacerbated humoral anti-self response has been associated with a substantial expansion of CD4 T cells and NKT cells in the liver parenchyma and portal areas and with endothelialitis and inflammation of bile ducts that progressed to liver dysfunction (significant increase in levels of the amino transferase enzymes AST and ALT) and premature death [63]. Of note, the immunoregulatory population of $CD8^+CD122^+$ T cells is also diminished in the liver of BTLA-deficient mice compared with WT mice. In addition to liver pathology, other organs such as lung, salivary glands, and pancreas showed inflammatory infiltrates [63] (see Table 1).

Taken together, these observations present clear evidence that in the absence of BTLA-mediated coinhibition, autoreactive T cells become more aggressive and cause autoimmune disease. Thus, BTLA plays a critical role in regulating the functional activity of natural and experimentally induced autoreactive T cells.

The function of BTLA in models of transplantation

The role of BTLA has also been explored thoroughly in the field of transplantation across MHC barriers (**Table 1**). In the absence of treatment, cardiac allografts across MHC class I or MHC class II barriers are accepted spontaneously and survive long-term (longer than 12 weeks), although with sufficient time, they develop artherosclerotic vascular lesions, a hallmark feature of chronic rejection [70–72]. Partially MHC-mismatched heart allografts placed into BTLA-deficient recipients are, however, rejected more acutely (8–12 weeks for MHC class I disparity or 2–3 weeks for MHC class II disparity) [66]. In line with these observations, in vivo administration of a putatively antagonistic anti-BTLA mAb (Clone 6A6) led to accelerated rejection of MHC class II-mismatched cardiac allografts in WT mice, which occurred at approximately 4 weeks after transplantation [66]. Of note, MHC class II-mismatched cardiac allografts were rejected even more rapidly in PD-1/BTLA double-deficient recipients than in PD-1- or BTLA-deficient mice [66]. These observations suggest that signaling through BTLA is critical in the negative regulation of T cell responses to partially MHC-mismatched cardiac allografts. In contrast, fully MHC-mismatched heart allografts in BTLA-deficient mice or in anti-BTLA-treated WT mice receiving blocking anti-BTLA mAb (Clone 6A6) survived slightly longer than in WT controls or isotype-treated WT mice, respectively [66]. This discrepancy may be a result of differences in the mechanism of rejection of partially versus fully MHC-mismatched grafts. The former is rejected mainly by CD4 Th2 T cells and eosinophils (MHC class II-mismatched) or CD4 Th1 T cells, CD8 T cells, and alloantibodies (MHC class I-mismatched) [73]. The absence of BTLA in this setting removes the inhibitory signal, which seems to be crucial for the control of CD4 or CD8 T cell-mediated allogeneic responses.

In a well-established murine model of allogeneic islet transplantation, fully MHC-mismatched islets transplanted from BALB/c mice into chemically induced diabetic C57BL/6 mice survived and normalized glycemia for 20–25 days post-transplantation [74, 75]. Significant prolongation of islet allograft function can be achieved by CTLA-4-Ig therapy given every other day for 10 days [76]. Remarkably, the concomitant administration of depleting anti-BTLA mAb (Clone 6F7) or a nondepleting antibody that down-modulates BTLA expression (Clone PJ196) with CTLA-4-Ig led to indefinite islet survival [67, 68, 77]. The reason for the different outcome in fully MHC-mismatched heart compared with fully MHC-mismatched islet transplantation experiments is likely a result of the fact that the heart transplantation experiments were performed with a blocking, nondepleting anti-BTLA mAb (Clone 6A6), whereas islet transplantation experiments were done with anti-BTLA antibodies that depleted BTLA-expressing cells (Clone 6F7) or down-modulated BTLA expression (Clone PJ196). The synergistic action of Clone PJ196 with CTLA-4-Ig in fully MHC-mismatched islet allografts could be explained by attributing to BTLA blockade a role in reducing effector T cell survival. Alternatively, the antibody may act as an agonist delivering negative signals through BTLA to T cells in vivo.

Bone marrow transplantation from parent into a nonirradiated F1 recipient is a suitable murine model to recreate acute and chronic GvHD. Depending on whether B6 or BALB/c splenocytes are used as a source of parental donor T cells, a Th1- or Th2-mediated immunopathology develops [78–80]. The role of BTLA has been explored in a murine model of acute GvHD that involves adoptive transfer of WT B6 donor or BTLA-deficient B6 donor splenocytes into nonirradiated B6 × BALB/c F1 recipients [69]. Although the authors anticipated that BTLA-deficient parental cells would exhibit an exacerbated alloreactive phenotype and cause a more severe GvHD (as observed when splenocytes from PD-1- or CTLA-4-deficient donors were adoptively transferred into WT MHC-mismatched recipients [81, 82]), they found unexpectedly that BTLA-deficient parental cells caused a short-lived GvHD that was readily resolved. The BTLA-deficient donor splenocytes showed an initial boost of proliferation similar to that of WT donor cells [69], but this proliferation was followed by rapid contraction and cell death, leading to resolution of the GvHD symptoms only in recipients of BTLA-deficient donor splenocytes and not in those receiving WT donor splenocytes [69]. In line with these findings, WT splenocytes from B6 mice adoptively transferred into blocking anti-BTLA-treated (6A6 mAb) F1 recipients led to similar results as those observed with BTLA-deficient donor cells [69]. This indicates that the interaction of BTLA with HVEM is required to promote cell survival of do-

nor anti-host T cells after initial expansion during the course of GvHD pathology.

The HVEM/BTLA interaction has also been implicated in the function of Tregs. Although naïve T cells and natural Tregs express similar amounts of HVEM, activation augments HVEM expression on Tregs. This increased HVEM expression on Tregs may serve to restore T cell homeostasis after the antigenic stimulus has disappeared. In agreement with this notion, $HVEM^{-/-}$ Tregs display diminished capacity to suppress naïve T cells, and WT Tregs are unable to modulate the function of $BTLA^{-/-}$ effector T cells [83] (Fig. 1).

In summary, these observations point to the view that engagement of BTLA on T cells by HVEM expressed on DC or Tregs is required for the inhibition of T cell function and survival of alloreactive T cells in the parent-into-F1 GvHD model and for the inhibitory function of Tregs. The discrepancy of the results observed in murine models of transplantation, when different clones of anti-BTLA mAbs were administered, is related directly to the distinct effector functions of the antibodies used in the different studies.

Molecular structure, expression, and functional activity of CD160

Human CD160 was first identified after an extensive screening of mAb raised against a human NK leukemia cell line [84]. A hybridoma, termed BY55, was generated, and the secreted mAb was used for cloning the cDNA of the gene encoding the cell-surface protein recognized by the mAb. The identified gene was later assigned to cluster of differentiation CD160 [85], which contains a single IgV-like domain that is present on the cell surface as a result of a GPI anchor that recruits it to lipid rafts. It is released easily as a soluble protein from activated lymphocytes following cleavage by a metalloprotease [85]. Recently, a transmembrane form of CD160, which is generated by alternative splicing and has a slightly different structure, has been discovered [86]. Importantly, CD160 may form tightly disulfide-linked multimers, suggesting that a trimeric form of this molecule may exist [85].

Human CD160 mRNA and protein are expressed mainly in cytotoxic cells such as $CD56^{dim}$ $CD16^{+}$ NK cells, NKT cells, γδT cells, $CD8^{+}CD28^{-}$ T cells, IEL, and a small subset of CD4 T cells, but it is not expressed on B cells or myeloid cells [84–86]. Human CD160 expression is up-regulated on CD8 T cells that lose CD28 expression; however, the $CD8^{+}CD28^{+}$ T cell subset only up-regulates expression of CD160 after several weeks of antigen-driven T cell activation. Although CD160 is only expressed on 5% of human peripheral blood CD4 T cells, a population of $CD4^{+}CD160^{+}$ cells is found frequently in inflammatory lesions of the skin [87]. In mice, the pattern of expression of CD160 is similar to that in humans (detectable in all NKT cells, CD8 IEL, and half of CD4 IEL), and CD160 is up-regulated rapidly after CD8 T cell activation. Moreover, ~10% of memory CD8 T cells, few CD4 T cells, and ~20% of NK cells express CD160 in the spleen [15]. Exhausted CD8 T cells in the late stage of chronic viral infection, which coincides with loss of CTL function, exhibit a specific augmentation of CD160 mRNA expression compared with naïve or even memory CD8 T cells [88–90].

It has been known since 1999 that human CD160 was able to weakly bind to MHC class I molecules [15, 91–93]. Given that anti-CD160 mAb inhibited human CD4 T cell activation, the first approach to test whether this action was mediated by MHC class I binding to CD160 was to block MHC class I with specific antibodies. MHC class I blockade, however, did not reverse the inhibitory effect of antibody anti-CD160. This prompted the authors to search for an alternative ligand of CD160. To that end, they used a human B cell cDNA library expressed on the COS cell line and CD160-Ig recombinant fusion protein as a tag for fishing the receptor. Thus, HVEM was identified as the ligand for the CD160 receptor [11, 27].

Functional studies to determine the role of CD160 in different murine models of disease are still lacking, as mice deficient in CD160 have not yet been developed. Therefore, most of the information available about the function of CD160 comes from studies with human T cells. In vitro stimulation of human CD4 T cells with anti-CD3 or anti-CD3/anti-CD28 up-regulates CD160 expression at Day 3 with peak expression at Day 4 [11]. Cross-linking of human CD160 with mAb inhibits anti-CD3/anti-CD28-induced CD4 and CD8 T cell activation profoundly [11]. The CD160-mediated inhibition of human T cell proliferation has not been reproduced in murine T cells using rat anti-mouse CD160 mAb developed in two different laboratories [15, 94], as no inhibitory activity of CD160 on murine T cell proliferation was observed. However, anti-CD160 treatment reduced IFN-γ production in a NK cell line transfected with murine CD160 and stimulated with anti-NK1.1 antibodies [15, 94]. Another function attributed to CD160 is its ability to bind nonclassical (e.g., CD1d) and classical MHC class I molecules weakly. The consequence of this interaction is enhancement of NK and T cell-mediated cytotoxic activity [91–93]. This is nevertheless puzzling considering that CD160 receptor engagement by HVEM has been reported to deliver negative signals, suggesting that CD160 may act as a molecular switch in some cell types. Thus, depending on the extracellular domain of the protein that is involved in the interaction, negative or positive signals could be transduced.

Taking into account that the pattern of CD160 expression is restricted to cytolytic T cells and NK cells, therapeutically inducing negative signals through CD160 may be beneficial in attenuating autoimmune disease and graft rejection. Complementing therapies in use for the control of CD4 T cell-mediated responses combined with CD160 signaling to inhibit cytotoxic cells may prove to be an effective therapeutic approach.

THE BALANCE OF INHIBITORY AND STIMULATORY SIGNALS OF THE HVEM/LIGHT/BTLA/CD160 PATHWAY

The ligation of coinhibitory receptors BTLA and/or CD160 on T cells with HVEM expressed on DC or Tregs transduces negative signals into T cells that are counterbalanced by costimulatory signals delivered after direct engagement of HVEM on T cells by LIGHT expressed on DC or more likely, on other activated T cells (T–T cell cooperation; Figs. 1 and 2). The predominance of the interaction of HVEM with BTLA and CD160 over the HVEM/LIGHT pathway or vice versa might be the result of

differences in ligand/receptor affinity and the differential expression pattern of these molecules on cell types at different stages of cell differentiation (Figs. 1 and 2, and see Table 2). LIGHT, BTLA, and CD160 have substantially different binding affinities and occupy spatially distinct sites upon interaction with the HVEM receptor, which enables HVEM to function as a molecular switch. The net effect of the LIGHT/HVEM and HVEM/BTLA/CD160 interaction, when these different receptors and ligands are simultaneously present, determines the outcome of the response.

Surface plasmon resonance-based Biacore experiments along with immunoprecipitation assays and cell-binding assays have provided valuable information about the stoichiometry and affinity of HVEM interacting with its various binding partners. Existing biophysical data demonstrate that the extracellular domains of BTLA and HVEM are monomeric and that they engage each other at 1:1 stoichiometry in solution [20]. However, other indirect experimental evidences suggest the opposite, and given that the sites on HVEM for interaction with BTLA/CD160 and LIGHT are not overlapping, binding of LIGHT to HVEM would cluster three molecules on the same complex, and this would facilitate the binding and clustering of BTLA around this molecular complex. This is supported by immunoprecipitation assays in which LIGHT is pulled down with BTLA-Ig fusion protein by protein A sepharose only in the presence of HVEM. In agreement with this idea, soluble LIGHT enhances BTLA-Ig binding to membrane HVEM. The reverse interaction is also true, as soluble HVEM-Ig interacts with much higher affinity with cells transfected with LIGHT and BTLA than with cells transfected with BTLA or LIGHT individually (**Table 2**) [19, 31]. A more recent report by Cheung et al. [65] suggests that BTLA and CD160 may also operate as activating ligands of HVEM, promoting NF-κB activation and cell survival. This would support a model in which HVEM and BTLA may configure a pathway of coinhibitory and costimulatory signals involved in the regulation of T cell activation and cell survival.

These observations highlight the importance of the differences in ligand/receptor-binding affinity and the differential expression pattern of these molecules on the same cell or dif-

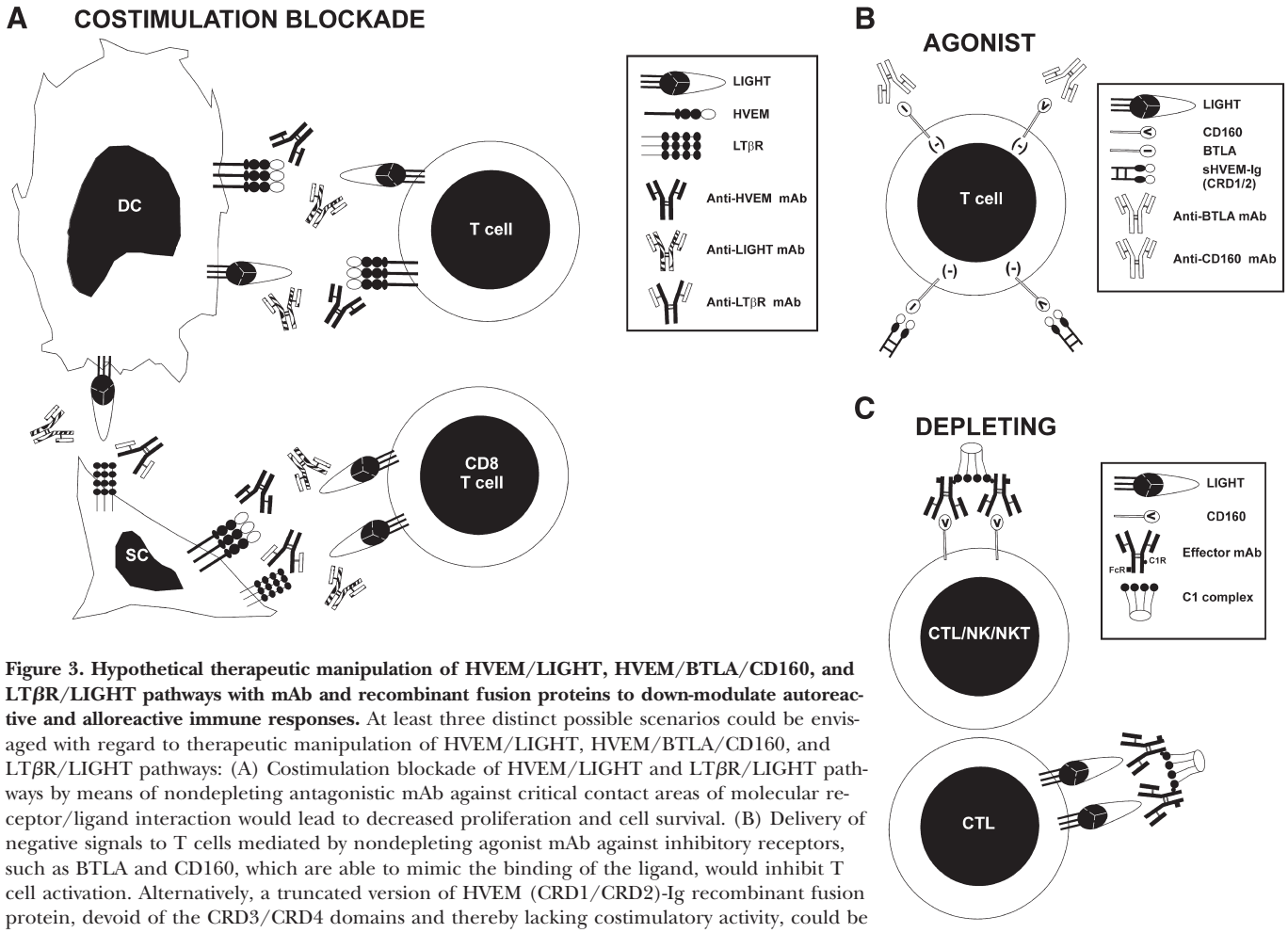

Figure 3. Hypothetical therapeutic manipulation of HVEM/LIGHT, HVEM/BTLA/CD160, and LTβR/LIGHT pathways with mAb and recombinant fusion proteins to down-modulate autoreactive and alloreactive immune responses. At least three distinct possible scenarios could be envisaged with regard to therapeutic manipulation of HVEM/LIGHT, HVEM/BTLA/CD160, and LTβR/LIGHT pathways: (A) Costimulation blockade of HVEM/LIGHT and LTβR/LIGHT pathways by means of nondepleting antagonistic mAb against critical contact areas of molecular receptor/ligand interaction would lead to decreased proliferation and cell survival. (B) Delivery of negative signals to T cells mediated by nondepleting agonist mAb against inhibitory receptors, such as BTLA and CD160, which are able to mimic the binding of the ligand, would inhibit T cell activation. Alternatively, a truncated version of HVEM (CRD1/CRD2)-Ig recombinant fusion protein, devoid of the CRD3/CRD4 domains and thereby lacking costimulatory activity, could be used for inhibiting T cell function upon binding to BTLA or CD160. (C) Depletion or physical elimination of CD8 T cells and other cytolytic cells such as NK and NKT cells that preferentially express CD160 with specific anti-CD160 mAb and elimination of activated CD8 T cells with anti-LIGHT mAb able to mediate complement or ADCC effector function activity would be an effective approach to be combined with current proven strategies targeting CD4 T cell-mediated responses.

ferent cell types at distinct stages of cell differentiation. Future studies are necessary to refine more clearly the working model of how the balance of these stimulatory and inhibitory pathways affects immunological outcomes in vivo.

FUTURE THERAPEUTIC PERSPECTIVES

The extracellular localization of the receptor/ligand pairs described in this review makes these cell-surface proteins excellent targets for therapeutic intervention. Blockade of the stimulatory HVEM/LIGHT pathway or enhancement of the inhibitory HVEM/BTLA/CD160 pathway with antibodies or recombinant proteins, in combination with immunosuppressive drugs, may assist clinicians in down-modulating and efficiently suppressing T cell-mediated immune responses, while reducing the load of immunosuppressants required for reversing relapsing episodes of autoimmune disease and achieving appropriate protection of a transplanted graft. Given that these strategies interfere with the natural physiological communication and exchange of information between APCs and T cells, donor-specific tolerance may be accomplished more easily by manipulating these pathways.

Current biological-based therapeutic drugs can act as antagonist or agonist of the receptor/ligand pair interaction. An antagonist drug compound by definition prevents the binding of the ligand to its receptor without signaling through the receptor, as for instance, with soluble decoy receptors. By contrast, an agonist drug compound exerts its effect on the target cell by mimicking the function of the ligand when it binds to its specific receptor. Additionally, selective removal of a cell population that is expressing the receptor or the ligand is sometimes a desirable consequence of the therapy. This can be achieved by taking advantage of antibody-mediated effector functions (by administration of an antibody or a receptor/ligand Fc fusion protein), such as complement-mediated lysis and ADCC.

Recombinant soluble decoy LTβR-Ig and HVEM-Ig fusion proteins are often used as molecules for the blockade of biological functions involving interactions among LTβ/LTβR, LIGHT/LTβR, HVEM/LIGHT, or HVEM/BTLA/CD160. However, it turns out to be difficult to draw accurate conclusions from experiments performed with these soluble decoy receptors, as several pathways are blocked simultaneously. Therefore, we favor the concept that blocking mAb raised against critical protein domains that disrupt unique receptor/ligand interactions would provide a more efficient manner to dissect specifically the involvement of the different pathways in the pathogenesis of diseases. This would be translated in the long-term into a wiser and more sensible pharmacological intervention to tackle the deleterious actions of destructive autoimmune and alloreactive responses. We can postulate at least three theoretical approaches of suitable therapeutic interventions aiming at attenuating the course of the immune response:

- Antibody-mediated blockade of LIGHT with antagonistic anti-LIGHT mAb (which do not elicit receptor activation) would impede signaling through LTβR on SC or costimulation through HVEM engagement on T cells, leaving the interaction between HVEM and BTLA or CD160 untouched and productive in delivering inhibitory signals. Costimulatory blockade of HVEM with anti-HVEM antagonistic antibodies would be a second feasible approach to preclude the binding of LIGHT to HVEM-expressing cells. However, with this approach, the HVEM domains implicated in binding the inhibitory BTLA and CD160 receptors must be left accessible (**Fig. 3A**). A recent report added an extra level of complexity to the network of signals involved in the HVEM/LIGHT/BTLA/CD160 pathway. It appears that BTLA and CD160 may also function as costimulatory ligands for HVEM [65]. From a therapeutic point of view, any strategy aiming at blocking costimulation delivered by BTLA and CD160 upon binding with HVEM would also prompt the unavoidable and undesirable effect of preventing coinhibitory signaling. Therefore, additional studies are needed to elucidate whether HVEM/BTLA/CD160 coinhibitory axis or BTLA/CD160/HVEM costimulatory axis is the predominant pathway. This is nevertheless a new avenue of research that needs to be explored further in controlled animal models of disease.
- Agonist antibodies against BTLA and CD160, able to mimic the function of HVEM binding to these receptors, would represent an approach to deliver negative signals to T cells exclusively (Fig. 3B).
- Lastly, if the antibodies used can mobilize effector functions, such as complement-mediated cytotoxicity or ADCC, elimination of the target cells expressing the receptors or ligands could also be accomplished easily. Of particular interest would be the removal of activated cytolytic CD8 T cells by targeting CD160 or LIGHT combined with the use of current therapies that effectively inhibit CD4 T cell-mediated responses (Fig. 3C).

ACKNOWLEDGMENTS

This work has been supported by grant FIS PI-050021 of Fondo de Investigaciones Sanitarias (Ministerio de Sanidad y Consumo, Spanish Government, Spain) and grant LE012A08 of Consejeria de Educación, Junta de Castilla y Leon to J. I. R-B. We thank Dr. David Sancho (London Research Institute, UK) and Dr. Yong Zhao (State Key Laboratory of Biomembrane and Membrane Biotechnology, China) for suggestions and critical reading of the manuscript.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Cai, G., Anumanthan, A., Brown, J. A., Greenfield, E. A., Zhu, B., Freeman, G. J. (2008) CD160 inhibits activation of human CD4+ T cells through interaction with herpesvirus entry mediator. *Nat. Immunol.* **9,** 176–185.
2. Abbas, A. K., Murphy, K. M., Sher, A. (1996) Functional diversity of helper T lymphocytes. *Nature* **383,** 787–793.
3. Parish, I. A., Kaech, S. M. (2009) Diversity in CD8(+) T cell differentiation. *Curr. Opin. Immunol.* **21,** 291–297.
4. Zhou, L., Chong, M. M., Littman, D. R. (2009) Plasticity of CD4+ T cell lineage differentiation. *Immunity* **30,** 646–655.

5. Salomon, B., Bluestone, J. A. (2001) Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation. *Annu. Rev. Immunol.* **19,** 225–252.
6. Li, X. C., Rothstein, D. M., Sayegh, M. H. (2009) Costimulatory pathways in transplantation: challenges and new developments. *Immunol. Rev.* **229,** 271–293.
7. Weaver, T. A., Charafeddine, A. H., Kirk, A. D. (2008) Costimulation blockade: towards clinical application. *Front. Biosci.* **13,** 2120–2139.
8. Schwartz, R. H. (2003) T cell anergy. *Annu. Rev. Immunol.* **21,** 305–334.
9. Kroczek, R. A., Mages, H. W., Hutloff, A. (2004) Emerging paradigms of T-cell co-stimulation. *Curr. Opin. Immunol.* **16,** 321–327.
10. Watts, T. H. (2005) TNF/TNFR family members in costimulation of T cell responses. *Annu. Rev. Immunol.* **23,** 23–68.
11. Adams, A. B., Larsen, C. P., Pearson, T. C., Newell, K. A. (2002) The role of TNF receptor and TNF superfamily molecules in organ transplantation. *Am. J. Transplant.* **2,** 12–18.
12. Walunas, T. L., Lenschow, D. J., Bakker, C. Y., Linsley, P. S., Freeman, G. J., Green, J. M., Thompson, C. B., Bluestone, J. A. (1994) CTLA-4 can function as a negative regulator of T cell activation. *Immunity* **1,** 405–413.
13. Ishida, Y., Agata, Y., Shibahara, K., Honjo, T. (1992) Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J.* **11,** 3887–3895.
14. Watanabe, N., Gavrieli, M., Sedy, J. R., Yang, J., Fallarino, F., Loftin, S. K., Hurchla, M. A., Zimmerman, N., Sim, J., Zang, X., Murphy, T. L., Russell, J. H., Allison, J. P., Murphy, K. M. (2003) BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1. *Nat. Immunol.* **4,** 670–679.
15. Maeda, M., Carpenito, C., Russell, R. C., Dasanjh, J., Veinotte, L. L., Ohta, H., Yamamura, T., Tan, R., Takei, F. (2005) 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.
16. Locksley, R. M., Killeen, N., Lenardo, M. J. (2001) The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* **104,** 487–501.
17. Croft, M. (2005) The evolving crosstalk between co-stimulatory and co-inhibitory receptors: HVEM-BTLA. *Trends Immunol.* **26,** 292–294.
18. Han, P., Goularte, O. D., Rufner, K., Wilkinson, B., Kaye, J. (2004) An inhibitory Ig superfamily protein expressed by lymphocytes and APCs is also an early marker of thymocyte positive selection. *J. Immunol.* **172,** 5931–5939.
19. Gonzalez, L. C., Loyet, K. M., Calemine-Fenaux, J., Chauhan, V., Wranik, B., Ouyang, W., Eaton, D. L. (2005) A coreceptor interaction between the CD28 and TNF receptor family members B and T lymphocyte attenuator and herpesvirus entry mediator. *Proc. Natl. Acad. Sci. USA* **102,** 1116–1121.
20. Compaan, D. M., Gonzalez, L. C., Tom, I., Loyet, K. M., Eaton, D., Hymowitz, S. G. (2005) Attenuating lymphocyte activity: the crystal structure of the BTLA-HVEM complex. *J. Biol. Chem.* **280,** 39553–39561.
21. Chemnitz, J. M., Lanfranco, A. R., Braunstein, I., Riley, J. L. (2006) B and T lymphocyte attenuator-mediated signal transduction provides a potent inhibitory signal to primary human CD4 T cells that can be initiated by multiple phosphotyrosine motifs. *J. Immunol.* **176,** 6603–6614.
22. Hsu, H., Solovyev, I., Colombero, A., Elliott, R., Kelley, M., Boyle, W. J. (1997) ATAR, a novel tumor necrosis factor receptor family member, signals through TRAF2 and TRAF5. *J. Biol. Chem.* **272,** 13471–13474.
23. Montgomery, R. I., Warner, M. S., Lum, B. J., Spear, P. G. (1996) Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. *Cell* **87,** 427–436.
24. Whitbeck, J. C., Peng, C., Lou, H., Xu, R., Willis, S. H., Ponce de Leon, M., Peng, T., Nicola, A. V., Montgomery, R. I., Warner, M. S., Soulika, A. M., Spruce, L. A., Moore, W. T., Lambris, J. D., Spear, P. G., Cohen, G. H., Eisenberg, R. J. (1997) Glycoprotein D of herpes simplex virus (HSV) binds directly to HVEM, a member of the tumor necrosis factor receptor superfamily and a mediator of HSV entry. *J. Virol.* **71,** 6083–6093.
25. Sedy, J. R., Gavrieli, M., Potter, K. G., Hurchla, M. A., Lindsley, R. C., Hildner, K., Scheu, S., Pfeffer, K., Ware, C. F., Murphy, T. L., Murphy, K. M. (2005) B and T lymphocyte attenuator regulates T cell activation through interaction with herpesvirus entry mediator. *Nat. Immunol.* **6,** 90–98.
26. Mauri, D. N., Ebner, R., Montgomery, R. I., Kochel, K. D., Cheung, T. C., Yu, G. L., Ruben, S., Murphy, M., Eisenberg, R. J., Cohen, G. H., Spear, P. G., Ware, C. F. (1998) LIGHT, a new member of the TNF superfamily, and lymphotoxin α are ligands for herpesvirus entry mediator. *Immunity* **8,** 21–30.
27. Cai, G., Freeman, G. J. (2009) The CD160, BTLA, LIGHT/HVEM pathway: a bidirectional switch regulating T-cell activation. *Immunol. Rev.* **229,** 244–258.
28. Bodmer, J. L., Schneider, P., Tschopp, J. (2002) The molecular architecture of the TNF superfamily. *Trends Biochem. Sci.* **27,** 19–26.
29. Rooney, I. A., Butrovich, K. D., Glass, A. A., Borboroglu, S., Benedict, C. A., Whitbeck, J. C., Cohen, G. H., Eisenberg, R. J., Ware, C. F. (2000) The lymphotoxin-β receptor is necessary and sufficient for LIGHT-mediated apoptosis of tumor cells. *J. Biol. Chem.* **275,** 14307–14315.
30. Ware, C. F. (2008) Targeting lymphocyte activation through the lymphotoxin and LIGHT pathways. *Immunol. Rev.* **223,** 186–201.
31. Cheung, T. C., Humphreys, I. R., Potter, K. G., Norris, P. S., Shumway, H. M., Tran, B. R., Patterson, G., Jean-Jacques, R., Yoon, M., Spear, P. G., Murphy, K. M., Lurain, N. S., Benedict, C. A., Ware, C. F. (2005) Evolutionarily divergent herpesviruses modulate T cell activation by targeting the herpesvirus entry mediator cosignaling pathway. *Proc. Natl. Acad. Sci. USA* **102,** 13218–13223.
32. Marsters, S. A., Ayres, T. M., Skubatch, M., Gray, C. L., Rothe, M., Ashkenazi, A. (1997) Herpesvirus entry mediator, a member of the tumor necrosis factor receptor (TNFR) family, interacts with members of the TNFR-associated factor family and activates the transcription factors NF-κB and AP-1. *J. Biol. Chem.* **272,** 14029–14032.
33. Tamada, K., Shimozaki, K., Chapoval, A. I., Zhai, Y., Su, J., Chen, S. F., Hsieh, S. L., Nagata, S., Ni, J., Chen, L. (2000) LIGHT, a TNF-like molecule, costimulates T cell proliferation and is required for dendritic cell-mediated allogeneic T cell response. *J. Immunol.* **164,** 4105–4110.
34. Morel, Y., Schiano de Colella, J. M., Harrop, J., Deen, K. C., Holmes, S. D., Wattam, T. A., Khandekar, S. S., Truneh, A., Sweet, R. W., Gastaut, J. A., Olive, D., Costello, R. T. (2000) Reciprocal expression of the TNF family receptor herpes virus entry mediator and its ligand LIGHT on activated T cells: LIGHT down-regulates its own receptor. *J. Immunol.* **165,** 4397–4404.
35. Duhen, T., Pasero, C., Mallet, F., Barbarat, B., Olive, D., Costello, R. T. (2004) LIGHT costimulates CD40 triggering and induces immunoglobulin secretion; a novel key partner in T cell-dependent B cell terminal differentiation. *Eur. J. Immunol.* **34,** 3534–3541.
36. Gavrieli, M., Sedy, J., Nelson, C. A., Murphy, K. M. (2006) BTLA and HVEM cross talk regulates inhibition and costimulation. *Adv. Immunol.* **92,** 157–185.
37. Fan, Z., Yu, P., Wang, Y., Wang, Y., Fu, M. L., Liu, W., Sun, Y., Fu, Y. X. (2006) NK-cell activation by LIGHT triggers tumor-specific CD8+ T-cell immunity to reject established tumors. *Blood* **107,** 1342–1351.
38. Wang, Y., Subudhi, S. K., Anders, R. A., Lo, J., Sun, Y., Blink, S., Wang, J., Liu, X., Mink, K., Degrandi, D., Pfeffer, K., Fu, Y. X. (2005) The role of herpesvirus entry mediator as a negative regulator of T cell-mediated responses. *J. Clin. Invest.* **115,** 711–717.
39. Harrop, J. A., McDonnell, P. C., Brigham-Burke, M., Lyn, S. D., Minton, J., Tan, K. B., Dede, K., Spampanato, J., Silverman, C., Hensley, P., DiPrinzio, R., Emery, J. G., Deen, K., Eichman, C., Chabot-Fletcher, M., Truneh, A., Young, P. R. (1998) Herpesvirus entry mediator ligand (HVEM-L), a novel ligand for HVEM/TR2, stimulates proliferation of T cells and inhibits HT29 cell growth. *J. Biol. Chem.* **273,** 27548–27556.
40. Wan, X., Zhang, J., Luo, H., Shi, G., Kapnik, E., Kim, S., Kanakaraj, P., Wu, J. (2002) A TNF family member LIGHT transduces costimulatory signals into human T cells. *J. Immunol.* **169,** 6813–6821.
41. Shi, G., Luo, H., Wan, X., Salcedo, T. W., Zhang, J., Wu, J. (2002) Mouse T cells receive costimulatory signals from LIGHT, a TNF family member. *Blood* **100,** 3279–3286.
42. Granger, S. W., Butrovich, K. D., Houshmand, P., Edwards, W. R., Ware, C. F. (2001) Genomic characterization of LIGHT reveals linkage to an immune response locus on chromosome 19p13.3 and distinct isoforms generated by alternate splicing or proteolysis. *J. Immunol.* **167,** 5122–5128.
43. Yu, K. Y., Kwon, B., Ni, J., Zhai, Y., Ebner, R., Kwon, B. S. (1999) A newly identified member of tumor necrosis factor receptor superfamily (TR6) suppresses LIGHT-mediated apoptosis. *J. Biol. Chem.* **274,** 13733–13736.
44. Granger, S. W., Rickert, S. (2003) LIGHT-HVEM signaling and the regulation of T cell-mediated immunity. *Cytokine Growth Factor Rev.* **14,** 289–296.
45. Scheu, S., Alferink, J., Potzel, T., Barchet, W., Kalinke, U., Pfeffer, K. (2002) Targeted disruption of LIGHT causes defects in costimulatory T cell activation and reveals cooperation with lymphotoxin β in mesenteric lymph node genesis. *J. Exp. Med.* **195,** 1613–1624.
46. Force, W. R., Walter, B. N., Hession, C., Tizard, R., Kozak, C. A., Browning, J. L., Ware, C. F. (1995) Mouse lymphotoxin-β receptor. Molecular genetics, ligand binding, and expression. *J. Immunol.* **155,** 5280–5288.
47. Morel, Y., Truneh, A., Sweet, R. W., Olive, D., Costello, R. T. (2001) The TNF superfamily members LIGHT and CD154 (CD40 ligand) costimulate induction of dendritic cell maturation and elicit specific CTL activity. *J. Immunol.* **167,** 2479–2486.
48. Ye, Q., Fraser, C. C., Gao, W., Wang, L., Busfield, S. J., Wang, C., Qiu, Y., Coyle, A. J., Gutierrez-Ramos, J. C., Hancock, W. W. (2002) Modulation of LIGHT-HVEM costimulation prolongs cardiac allograft survival. *J. Exp. Med.* **195,** 795–800.
49. Shaikh, R. B., Santee, S., Granger, S. W., Butrovich, K., Cheung, T., Kronenberg, M., Cheroutre, H., Ware, C. F. (2001) Constitutive expression of LIGHT on T cells leads to lymphocyte activation, inflammation, and tissue destruction. *J. Immunol.* **167,** 6330–6337.
50. Wang, J., Lo, J. C., Foster, A., Yu, P., Chen, H. M., Wang, Y., Tamada, K., Chen, L., Fu, Y. X. (2001) The regulation of T cell homeostasis and autoimmunity by T cell-derived LIGHT. *J. Clin. Invest.* **108,** 1771–1780.
51. Pierer, M., Schulz, A., Rossol, M., Kendzia, E., Kyburz, D., Haentzschel, H., Baerwald, C., Wagner, U. (2009) Herpesvirus entry mediator-Ig treatment during immunization aggravates rheumatoid arthritis in the collagen-induced arthritis model. *J. Immunol.* **182,** 3139–3145.

52. Tamada, K., Shimozaki, K., Chapoval, A. I., Zhu, G., Sica, G., Flies, D., Boone, T., Hsu, H., Fu, Y. X., Nagata, S., Ni, J., Chen, L. (2000) Modulation of T-cell-mediated immunity in tumor and graft-versus-host disease models through the LIGHT co-stimulatory pathway. *Nat. Med.* **6,** 283–289.
53. Xu, Y., Flies, A. S., Flies, D. B., Zhu, G., Anand, S., Flies, S. J., Xu, H., Anders, R. A., Hancock, W. W., Chen, L., Tamada, K. (2007) Selective targeting of the LIGHT-HVEM costimulatory system for the treatment of graft-versus-host disease. *Blood* **109,** 4097–4104.
54. Fan, K., Wang, H., Wei, H., Zhou, Q., Kou, G., Hou, S., Qian, W., Dai, J., Li, B., Zhang, Y., Zhu T., Guo Y. (2007) Blockade of LIGHT/HVEM and B7/CD28 signaling facilitates long-term islet graft survival with development of allospecific tolerance. *Transplantation* **84,** 746–754.
55. Liu, J., Schmidt, C. S., Zhao, F., Okragly, A. J., Glasebrook, A., Fox, N., Galbreath, E., Zhang, Q., Song, H. Y., Na, S., Yang, D. D. (2003) LIGHT-deficiency impairs CD8+ T cell expansion, but not effector function. *Int. Immunol.* **15,** 861–870.
56. Gavrieli, M., Watanabe, N., Loftin, S. K., Murphy, T. L., Murphy, K. M. (2003) Characterization of phosphotyrosine binding motifs in the cytoplasmic domain of B and T lymphocyte attenuator required for association with protein tyrosine phosphatases SHP-1 and SHP-2. *Biochem. Biophys. Res. Commun.* **312,** 1236–1243.
57. Vendel, A. C., Calemine-Fenaux, J., Izrael-Tomasevic, A., Chauhan, V., Arnott, D., Eaton, D. L. (2009) B and T lymphocyte attenuator regulates B cell receptor signaling by targeting Syk and BLNK. *J. Immunol.* **182,** 1509–1517.
58. Hurchla, M. A., Sedy, J. R., Gavrieli, M., Drake, C. G., Murphy, T. L., Murphy, K. M. (2005) B and T lymphocyte attenuator exhibits structural and expression polymorphisms and is highly induced in anergic CD4+ T cells. *J. Immunol.* **174,** 3377–3385.
59. Del Rio, M. L., Kaye, J., Rodriguez-Barbosa, J. I. (2009) Detection of protein on $BTLA^{low}$ cells and in vivo antibody-mediated down-modulation of BTLA on lymphoid and myeloid cells of C57BL/6 and BALB/c BTLA allelic variants. *Immunobiology*, Epub ahead of print.
60. Otsuki, N., Kamimura, Y., Hashiguchi, M., Azuma, M. (2006) Expression and function of the B and T lymphocyte attenuator (BTLA/CD272) on human T cells. *Biochem. Biophys. Res. Commun.* **344,** 1121–1127.
61. Zang, X., Loke, P., Kim, J., Murphy, K., Waitz, R., Allison, J. P. (2003) B7x: a widely expressed B7 family member that inhibits T cell activation. *Proc. Natl. Acad. Sci. USA* **100,** 10388–10392.
62. Krieg, C., Han, P., Stone, R., Goularte, O. D., Kaye, J. (2005) Functional analysis of B and T lymphocyte attenuator engagement on CD4+ and CD8+ T cells. *J. Immunol.* **175,** 6420–6427.
63. Oya, Y., Watanabe, N., Owada, T., Oki, M., Hirose, K., Suto, A., Kagami, S., Nakajima, H., Kishimoto, T., Iwamoto, I., Murphy, T. L., Murphy, K. M., Saito, Y. (2008) Development of autoimmune hepatitis-like disease and production of autoantibodies to nuclear antigens in mice lacking B and T lymphocyte attenuator. *Arthritis Rheum.* **58,** 2498–2510.
64. Krieg, C., Boyman, O., Fu, Y. X., Kaye, J. (2007) B and T lymphocyte attenuator regulates CD8+ T cell-intrinsic homeostasis and memory cell generation. *Nat. Immunol.* **8,** 162–171.
65. Cheung, T. C., Steinberg, M. W., Oborne, L. M., Macauley, M. G., Fukuyama, S., Sanjo, H., D'Souza, C., Norris, P. S., Pfeffer, K., Murphy, K. M., Kronenberg, M., Spear, P. G., Ware, C. F. (2009) Unconventional ligand activation of herpesvirus entry mediator signals cell survival. *Proc. Natl. Acad. Sci. USA* **106,** 6244–6249.
66. Auchincloss Jr., H., Mayer, T., Ghobrial, R., Winn, H. J. (1989) T-cell subsets, bm mutants, and the mechanisms of allogeneic skin graft rejection. *Immunol. Res.* **8,** 149–164.
67. Yamada, A., Laufer, T. M., Gerth, A. J., Chase, C. M., Colvin, R. B., Russell, P. S., Sayegh, M. H., Auchincloss Jr., H. (2003) Further analysis of the T-cell subsets and pathways of murine cardiac allograft rejection. *Am. J. Transplant.* **3,** 23–27.
68. Corry, R. J., Winn, H. J., Russell, P. S. (1973) Primarily vascularized allografts of hearts in mice. The role of H-2D, H-2K, and non-H-2 antigens in rejection. *Transplantation* **16,** 343–350.
69. Tao, R., Wang, L., Han, R., Wang, T., Ye, Q., Honjo, T., Murphy, T. L., Murphy, K. M., Hancock, W. W. (2005) Differential effects of B and T lymphocyte attenuator and programmed death-1 on acceptance of partially versus fully MHC-mismatched cardiac allografts. *J. Immunol.* **175,** 5774–5782.
70. Le Moine, A., Goldman, M., Abramowicz, D. (2002) Multiple pathways to allograft rejection. *Transplantation* **73,** 1373–1381.
71. Prowse, S. J., Lafferty, K. J., Simeonovic, C. J., Agostino, M., Bowen, K. M., Steele, E. J. (1982) The reversal of diabetes by pancreatic islet transplantation. *Diabetes* **31** (Suppl. 4), 30–38.
72. Wang, Y., Pontesilli, O., Gill, R. G., La Rosa, F. G., Lafferty, K. J. (1991) The role of CD4+ and CD8+ T cells in the destruction of islet grafts by spontaneously diabetic mice. *Proc. Natl. Acad. Sci. USA* **88,** 527–531.
73. Benhamou, P. Y. (2002) Immunomodulation with CTLA4-Ig in islet transplantation. *Transplantation* **73,** S40–S42.
74. Truong, W., Plester, J. C., Hancock, W. W., Kaye, J., Merani, S., Murphy, K. M., Murphy, T. L., Anderson, C. C., Shapiro, A. M. (2007) Negative and positive co-signaling with anti-BTLA (PJ196) and CTLA4Ig prolongs islet allograft survival. *Transplantation* **84,** 1368–1372.
75. Truong, W., Plester, J. C., Hancock, W. W., Merani, S., Murphy, T. L., Murphy, K. M., Kaye, J., Anderson, C. C., Shapiro, A. M. (2007) Combined coinhibitory and costimulatory modulation with anti-BTLA and CTLA4Ig facilitates tolerance in murine islet allografts. *Am. J. Transplant.* **7,** 2663–2674.
76. Truong, W., Hancock, W. W., Anderson, C. C., Merani, S., Shapiro, A. M. (2006) Coinhibitory T-cell signaling in islet allograft rejection and tolerance. *Cell Transplant.* **15,** 105–119.
77. Ferrara, J. L., Cooke, K. R., Pan, L., Krenger, W. (1996) The immunopathophysiology of acute graft-versus-host-disease. *Stem Cells* **14,** 473–489.
78. Shlomchik, W. D. (2007) Graft-versus-host disease. *Nat. Rev. Immunol.* **7,** 340–352.
79. Tschetter, J. R., Mozes, E., Shearer, G. M. (2000) Progression from acute to chronic disease in a murine parent-into-F1 model of graft-versus-host disease. *J. Immunol.* **165,** 5987–5994.
80. Hurchla, M. A., Sedy, J. R., Murphy, K. M. (2007) Unexpected role of B and T lymphocyte attenuator in sustaining cell survival during chronic allostimulation. *J. Immunol.* **178,** 6073–6082.
81. Blazar, B. R., Carreno, B. M., Panoskaltsis-Mortari, A., Carter, L., Iwai, Y., Yagita, H., Nishimura, H., Taylor, P. A. (2003) Blockade of programmed death-1 engagement accelerates graft-versus-host disease lethality by an IFN-γ-dependent mechanism. *J. Immunol.* **171,** 1272–1277.
82. Habicht, A., Kewalaramani, R., Vu, M. D., Demirci, G., Blazar, B. R., Sayegh, M. H., Li, X. C. (2007) Striking dichotomy of PD-L1 and PD-L2 pathways in regulating alloreactive CD4(+) and CD8(+) T cells in vivo. *Am. J. Transplant.* **7,** 2683–2692.
83. Tao, R., Wang, L., Murphy, K. M., Fraser, C. C., Hancock, W. W. (2008) Regulatory T cell expression of herpesvirus entry mediator suppresses the function of B and T lymphocyte attenuator-positive effector T cells. *J. Immunol.* **180,** 6649–6655.
84. Maiza, H., Leca, G., Mansur, I. G., Schiavon, V., Boumsell, L., Bensussan, A. (1993) A novel 80-kD cell surface structure identifies human circulating lymphocytes with natural killer activity. *J. Exp. Med.* **178,** 1121–1126.
85. Anumanthan, A., Bensussan, A., Boumsell, L., Christ, A. D., Blumberg, R. S., Voss, S. D., Patel, A. T., Robertson, M. J., Nadler, L. M., Freeman, G. J. (1998) Cloning of BY55, a novel Ig superfamily member expressed on NK cells, CTL, and intestinal intraepithelial lymphocytes. *J. Immunol.* **161,** 2780–2790.
86. Giustiniani, J., Bensussan, A., Marie-Cardine, A. (2009) Identification and characterization of a transmembrane isoform of CD160 (CD160-TM), a unique activating receptor selectively expressed upon human NK cell activation. *J. Immunol.* **182,** 63–71.
87. Abecassis, S., Giustiniani, J., Meyer, N., Schiavon, V., Ortonne, N., Campillo, J. A., Bagot, M., Bensussan, A. (2007) Identification of a novel CD160+ CD4+ T-lymphocyte subset in the skin: a possible role for CD160 in skin inflammation. *J. Invest. Dermatol.* **127,** 1161–1166.
88. Barber, D. L., Wherry, E. J., Masopust, D., Zhu, B., Allison, J. P., Sharpe, A. H., Freeman, G. J., Ahmed, R. (2006) Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* **439,** 682–687.
89. Okazaki, T., Honjo, T. (2006) Rejuvenating exhausted T cells during chronic viral infection. *Cell* **124,** 459–461.
90. Day, C. L., Kaufmann, D. E., Kiepiela, P., Brown, J. A., Moodley, E. S., Reddy, S., Mackey, E. W., Miller, J. D., Leslie, A. J., DePierres, C., Mncube, Z., Duraiswamy, J., Zhu, B., Eichbaum, Q., Altfeld, M., Wherry, E. J., Coovadia, H. M., Goulder, P. J., Klenerman, P., Ahmed, R., Freeman, G. J., Walker, B. D. (2006) PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* **443,** 350–354.
91. Agrawal, S., Marquet, J., Freeman, G. J., Tawab, A., Bouteiller, P. L., Roth, P., Bolton, W., Ogg, G., Boumsell, L., Bensussan, A. (1999) Cutting edge: MHC class I triggering by a novel cell surface ligand costimulates proliferation of activated human T cells. *J. Immunol.* **162,** 1223–1226.
92. Le Bouteiller, P., Barakonyi, A., Giustiniani, J., Lenfant, F., Marie-Cardine, A., Aguerre-Girr, M., Rabot, M., Hilgert, I., Mami-Chouaib, F., Tabiasco, J., Boumsell, L., Bensussan, A. (2002) Engagement of CD160 receptor by HLA-C is a triggering mechanism used by circulating natural killer (NK) cells to mediate cytotoxicity. *Proc. Natl. Acad. Sci. USA* **99,** 16963–16968.
93. Barakonyi, A., Rabot, M., Marie-Cardine, A., Aguerre-Girr, M., Polgar, B., Schiavon, V., Bensussan, A., Le Bouteiller, P. (2004) Cutting edge: engagement of CD160 by its HLA-C physiological ligand triggers a unique cytokine profile secretion in the cytotoxic peripheral blood NK cell subset. *J. Immunol.* **173,** 5349–5354.
94. Tsujimura, K., Obata, Y., Matsudaira, Y., Nishida, K., Akatsuka, Y., Ito, Y., Demachi-Okamura, A., Kuzushima, K., Takahashi, T. (2006) Characterization of murine CD160+ CD8+ T lymphocytes. *Immunol. Lett.* **106,** 48–56.
95. Nelson, C. A., Fremont, M. D., Sedy, J. R., Norris, P. S., Ware, C. F., Murphy, K. M., Fremont, D. H. (2008) Structural determinants of herpesvirus entry mediator recognition by murine B and T lymphocyte attenuator. *J. Immunol.* **180,** 940–947.

KEY WORDS:
coinhibition · costimulation · transplantation · autoimmunity