Foxp3 expression. The fact that such binding causes H4 acetylation and that such acetylation is only found in Foxp3$^+$ cells constitutes a good argument for this claim. However, these results do not rule out that there is a Smad3-independent and therefore TGF-β ligand–independent pathway of *Foxp3* induction. Despite this cautionary note, the authors seem to have established a pathway of TGF-β-dependent induction of *Foxp3* expression. It has been known for some time that *in vitro* TGF-β-dependent induction does not yield a very stable phenotype of Foxp3$^+$ T_{reg} cells and thus other factors may contribute *in vivo* as well. At this point, the results of Tone *et al.*[2] raise the question of whether there are Smad3-independent pathways of Foxp3 induction, which presumably can be tested by a 'knock-in' *Foxp3* allele with a mutated Smad3 binding site.

1. Brunkow, M.E. *et al. Nat. Genet.* **27**, 68–73 (2001).
2. Tone, M. *et al. Nat. Immunol.* **9**, 194–202 (2008).
3. Marie, J.C., Letterio, J.J., Gavin, M. & Rudensky, A.Y. *J. Exp. Med.* **201**, 1061–1067 (2005).
4. Huber, S. *et al. J. Immunol.* **173**, 6526–6531 (2004).
5. Kretschmer, K. *et al. Nat. Immunol.* **6**, 1219–1227 (2005).
6. von Boehmer, H. *J. Exp. Med.* **204**, 1737–1739 (2007).
7. Burchill, M.A., Yang, J., Vogtenhuber, C., Blazar, B.R. & Farrar, M.A. *J. Immunol.* **178**, 280–290 (2007).
8. Kim, H.P. & Leonard, W.J. *J. Exp. Med.* **204**, 1543–1551 (2007).
9. Chen, W. *et al. J. Exp. Med.* **198**, 1875–1886 (2003).
10. Wu, Y. *et al. Cell* **126**, 375–387 (2006).

CD160 and BTLA: LIGHTs out for CD4$^+$ T cells

Jonathan Kaye

The T cell costimulatory protein LIGHT and coinhbitory protein BTLA share a common ligand, HVEM. Now CD160 is also shown to bind HVEM and deliver a potent inhibitory signal to CD4 T cells.

Although the T cell antigen receptor (TCR) is responsible for imparting specificity to the initial activation of the cell, it is the assortment of additional cell surface proteins acting in a non–antigen-specific way that is central to the regulation of both the quantitative and qualitative aspects of the cell's response. Such modifiers can act in either a positive or a negative way in conjunction with TCR-mediated activation and thus have been called 'costimulators' or 'coinhibitors', respectively. Because T cells recognize foreign antigen in the form of peptides bound to self major histocompatibility complex (MHC) molecules on the surfaces of other cells, the T cell immune response can be 'fine tuned' on two levels: by the specific array of coinhibitory and costimulatory proteins expressed on the T cell surface and by the specific array of ligands for these receptors expressed by the antigen-presenting cell. In this issue of *Nature Immunology*, Freeman and colleagues add CD160 to the list of coinhibitory molecules and identify an unexpected dimension of an already complex network of competing activating and inhibitory signals received by T cells through a single ligand[1].

The monoclonal antibody BY55 was derived from mice immunized with a human leukemic natural killer (NK) cell line in an attempt to develop markers for cytotoxic cells[2]. Almost a decade ago, the gene was identified that encodes the protein now designated CD160 (ref. 3), which is recognized by BY55. Expression of CD160, a member of the immunoglobulin 'superfamily' of proteins, is reported to be expressed by NK cells, NKT cells, intraepithelial T cells, γδ TCR$^+$ T cells, and memory-phenotype, activated and effector CD8$^+$ T cells. In terms of function, work has centered on the role of CD160 in enhancing NK or CD8 T cell activation. Such effects have been attributed to the ability of CD160 to bind classical and nonclassical MHC class I molecules, although with apparent low affinity, requiring clustering of MHC class I molecules or overexpression of CD160 or MHC class I for detection of the interaction.

The findings reported here[1] stem from the production of additional monoclonal antibodies to CD160 and a reevaluation of the pattern of CD160 expression on human peripheral blood lymphocytes. Thus, as with CD8$^+$ T cells, the authors find that a minor subset of CD4$^+$ T cells expresses CD160. The CD160$^+$ CD4$^+$ T cells include subsets of T cells with a memory or activated phenotype. Unexpectedly, however, they find that coimmobilization of anti-CD160 with an activating mix of monoclonal antibodies to CD3 and CD28 (targeting the TCR complex and the main costimulatory pathway) profoundly inhibits total CD4$^+$ T cell proliferation and cytokine production.

Paradoxically, the proliferation of even purified CD160$^-$ CD4$^+$ T cells was inhibited by antibody to CD160 (anti-CD160), although a higher concentration of inhibitory antibody was required. The investigators attribute this unexpected result to the upregulation of CD160 during T cell activation. However, this may not be the whole story. Although they did not find substantial staining with anti-CD160 on bulk CD4$^+$ T cells until 3 days after activation, much earlier events, such as induction of interleukin 2 mRNA and CD3ζ phosphorylation, were inhibited by anti-CD160. The interpretation of these results is complicated somewhat by the use of total CD4 T cells in these experiments. Thus, the contribution by subsets of CD160$^+$ CD4$^+$ T cells needs to be clarified, particularly as activated- and memory-phenotype cells could account for a disproportionate fraction of the total response. Nevertheless, whether a very small amount of CD160 on the naive CD4$^+$ T cell surface is nonetheless sufficient to deliver an inhibitory signal must also be considered. If confirmed, this raises a strong note of caution for the reliance on flow cytometry as the sole means of determining patterns of expression of proteins and, by extension, susceptibility to biological effects.

What is the basis for the CD160 inhibition of T cell activation? Using a global microarray analysis approach, these investigators show that CD160-mediated coinhibition inhibits the expression of many mediators of T cell activation, including cytokines, cytokine receptors, and nutrient transporters such as the amino acid exchanger SLC7A5. Notably, these results are reminiscent of similar properties of another coinhibitory cell surface protein, B and T lymphocyte attenuator (BTLA)[4]. Thus, high expression of CD160 on a subset of CD4$^+$ T cells, delayed

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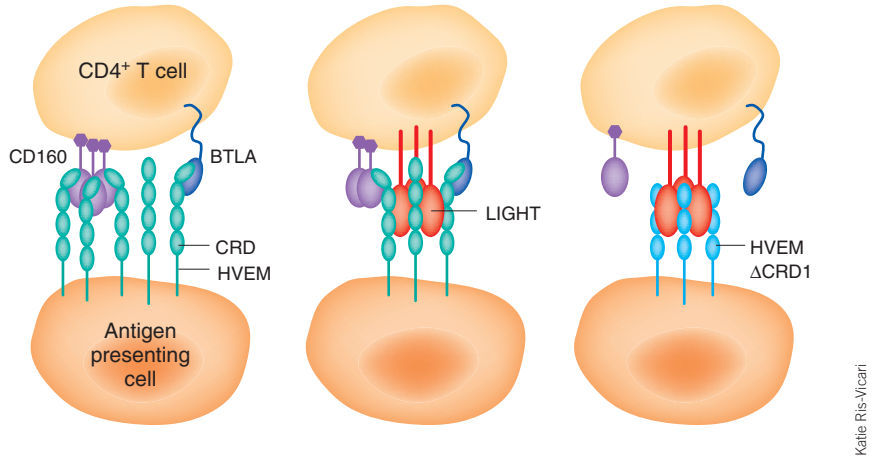

Katie Ris-Vicari

Figure 1 HVEM can act as a coinhibitory ligand through binding to CD160 and BTLA or as a costimulatory ligand through binding to LIGHT. Both BTLA and CD160 bind the CDR1 domain of HVEM (left). The interaction of BTLA with HVEM is monomeric. CD160 is found on the cell surface as a disulfide-linked multimer, but whether it can cluster with HVEM remains to be determined. In addition, whether there are functional or physical interactions between BTLA and CD160 on the cell surface is unknown. Engagement of CD160 or BTLA in this context inhibits CD4+ T cell activation. Binding of LIGHT to CRD2-CDR3 of HVEM does not inhibit the binding of BTLA or CD160 (middle). For a T cell that expresses CD160, BTLA and LIGHT, the exact nature of the complexes formed with HVEM remains to be determined. However, the inhibitory signals mediated by CD160 and BTLA seem dominant. Removal of CDR1 from HVEM (HVEMΔCDR1) eliminates interaction with CD160 and BTLA and demonstrates the costimulatory activity of a LIGHT-HVEM interaction (right). Figure adapted from ref. 1.

kinetics of upregulation of CD160 on naive CD4+ T cells, inhibition of the expression of interleukin 2 and its receptor by anti-CD160 engagement, failure to induce apoptosis after crosslinking of CD160 and CD3, and even the inhibition of SLC7A5 upregulation by anti-CD160 are similar to expression of BTLA and the effects of engagement of BTLA by agonist monoclonal antibody, at least as reported for murine systems[4–6] (J.K., unpublished data). But the connection does not stop there. When Freeman and colleagues use expression cloning to search for a ligand for CD160, they identify herpesvirus entry mediator (HVEM), also the ligand for BTLA, and not MHC class I (**Fig. 1**).

In addition to its involvement in viral entry, HVEM (a member of the tumor necrosis factor receptor family) was originally characterized for its costimulatory function after binding to its ligand LIGHT ('homologous to lymphotoxin, shows inducible expression, competes with herpes simplex virus glycoprotein D, expressed by T cells'; also called TNFSF14) and as a receptor for lymphotoxin-α. The observation that T cell responses are enhanced in HVEM-deficient mice, however, suggests that the protein also acts as a negative regulator. The finding of an interaction between HVEM and the coinhibitory molecule BTLA provides a mechanistic foundation for this observation[7]. Thus, BTLA, HVEM, LIGHT and lymphotoxin-α (and the LIGHT receptor LTβR) are already known to constitute a complex regulatory network; now CD160 must be added to the picture.

HVEM can simultaneously bind BTLA and LIGHT, although the physiological function of such a trimolecular complex remains to be determined. Unlike LIGHT, which promotes trimeric clustering of HVEM, the BTLA-HVEM interaction is monomeric[8]. In an elegant series of experiments with soluble forms of BTLA, CD160 and LIGHT in conjunction with cells expressing wild-type or mutant HVEM, Freeman and colleagues show that CD160 and BTLA bind to the cysteine-rich domain 1 (CRD1) of HVEM at potentially overlapping sites. They find that binding of LIGHT to HVEM, previously mapped to CRD2-CRD3, is not inhibited by binding of CD160 or BTLA. LIGHT binding, however, does modestly enhance CD160 and BTLA binding. The potential exists, therefore, that clustering due to a LIGHT-HVEM interaction might promote the movement of CD160 and/or BTLA into an HVEM complex. These experiments also demonstrate the dominance of CD160-BTLA inhibition over LIGHT-mediated costimulation, as removal of CRD1 converts HVEM from a coinhibitor to a costimulator.

However, many questions remain. What is the exact stoichiometry of LIGHT, BTLA and CD160 on naive and activated T cells and in complex with HVEM? CD160 exists on the cell surface as a disulfide-linked multimer[3]; thus, like LIGHT, might CD160 cluster HVEM? Are there circumstances in which solely stimulatory HVEM targets (LIGHT) or solely inhibitory HVEM targets (BTLA and CD160) are expressed by CD4+ T cells? Are there functional differences between CD160- and BTLA-mediated inhibition? Indeed, is the inhibitory activity of CD160 dependent on BTLA? As shown in this work, HVEM-mediated inhibition of the activation of an alloreactive T cell clone could be reversed with either anti-CD160 Fab or anti-BTLA[1]. Nevertheless, at least in activated CD4+ T cells, immunofluorescence demonstrates distinct distributions of the two proteins. Experiments with BTLA-deficient mice (and future CD160-deficient mice) should help clarify the functional relationship between these proteins. How the glycosylphosphatidylinositol-linked CD160 protein signals also remains to be determined; although, phosphatidylinositol-3-OH kinase has been linked to CD160-mediated activation of NK cells[9]. Notably, phosphotyrosil-containing peptides from BTLA also recruit phosphatidylinositol-3-OH kinase[10]. BTLA has also been reported to associate with CD3ζ in lipid rafts in activated CD4+ T cells[11]. Whether such complexes also contain CD160 is therefore of interest. Finally, what is the relationship between the function of CD160 on NK cells and CD8+ T cells, which also have low expression of BTLA[5], and its function on CD4 T cells?

Regardless of the answers to those questions, the CD160-BTLA-HVEM network is a promising target for the development of therapeutics. Data indicate that targeting BTLA might affect diverse immune-mediated responses, including allergic airway inflammation[12], transplant rejection[13], cerebral malaria[14], graft-versus-host disease[15] and CD8 T cell memory formation[16]; the last is of potential utility for vaccine development. Related to this, in a recent paper in *Nature Medicine*, Ertl and colleagues demonstrate that insertion of antigens into herpesvirus glycoprotein D, thus targeting them to the BTLA-binding site of HVEM, substantially enhances T cell and B cell responses to the same antigens[17]. The data indicate blockade of the inhibitory BTLA-HVEM interaction, rather than increased LIGHT costimulation or dendritic cell maturation, as the likely cause of the enhancement. With the identification of CD160 as another partner for HVEM, and one whose binding site may

overlap with that of BTLA, determining if CD160 is involved in the function of these vaccines and the potential of this cell surface protein as a therapeutic target are now of great interest.

1. Guifang, C. *et al. Nat. Immunol.* **9**, 176–185 (2008).
2. Maiza, H. *et al. J. Exp. Med.* **178**, 1121–1126 (1993).
3. Anumanthan, A. *et al. J. Immunol.* **161**, 2780–2790 (1998).
4. Watanabe, N. *et al. Nat. Immunol.* **4**, 670–679 (2003).
5. Han, P., Goularte, O.D., Rufner, K., Wilkinson, B. & Kaye, J. *J. Immunol.* **172**, 5931–5939 (2004).
6. Krieg, C., Han, P., Stone, R., Goularte, O.D. & Kaye, J. *J. Immunol.* **175**, 6420–6427 (2005).
7. Sedy, J.R. *et al. Nat. Immunol.* **6**, 90–98 (2005).
8. Compaan, D.M. *et al. J. Biol. Chem.* **280**, 39553–39561 (2005).
9. Rabot, M. *et al. Int. Immunol.* **19**, 401–409 (2007).
10. Gavrieli, M. & Murphy, K.M. *Biochem. Biophys. Res. Commun.* **345**, 1440–1445 (2006).
11. Wu, T.H., Zhen, Y., Zeng, C., Yi, H.F. & Zhao, Y. *Immunol. Cell Biol.* **85**, 590–595 (2007).
12. Deppong, C. *et al. J. Immunol.* **176**, 3909–3913 (2006).
13. Truong, W. *et al. Transplantation* **84**, 1368–1372 (2007).
14. Lepenies, B. *et al. J. Immunol.* **179**, 4093–4100 (2007).
15. Hurchla, M.A., Sedy, J.R. & Murphy, K.M. *J. Immunol.* **178**, 6073–6082 (2007).
16. Krieg, C., Boyman, O., Fu, Y.X. & Kaye, J. *Nat. Immunol.* **8**, 162–171 (2007).
17. Lasaro, M.O. *et al. Nat. Med.* advance online publication, doi: 10.1038/nm1704 (13 Jan 2008).

IL-17 drives germinal center B cells?

David Tarlinton

The inflammatory cytokine interleukin 17, normally considered a T cell–associated factor, is now reported to be the central participant driving the development of germinal center–derived autoantibodies in a model disease setting.

The recombinant inbred BXD2 mouse strain develops an erosive arthritis accompanied by circulating immune complexes and progressive glomerulonephritis. These symptoms are correlated with increased titers of autoantibodies reactive with nucleosome proteins, metabolic enzymes and both structural and heat shock proteins. Transplantation of hybridomas secreting such autoantibodies induces pathogenic responses, including autoimmune disease, in recipients independently of CD4 T cell help[1]. The development of autoantibodies in intact BXD2 mice, however, is T cell dependent, as it is abolished by treatment with a CD28 antagonist. Detailed analysis of the variable (V) regions of such pathogenic antibodies has shown enhanced somatic hypermutation (SHM) and class-switch recombination, which together with increased expression of activation-induced cytidine deaminase in BXD2 B cells suggests a contribution of germinal centers (GCs) to disease development[2]. In this issue of *Nature Immunology*, Mountz and colleagues put forward a challenging model linking interleukin 17 (IL-17) with GC activity to explain the spontaneous development of antibody-mediated autoimmunity in BXD mice[3]. They report that the GC phenotype of the BXD2 mice is 'driven' by the direct action of IL-17 on BXD2 B cells to produce GCs of excess frequency, duration and activity. These data represent the first example of an effect of IL-17 on B cell activity and function in GCs; although the model proposed by the authors is certainly intriguing, it remains formally untested.

Consideration of the model by Mountz and colleagues of how IL-17 promotes autoimmunity requires some understanding of how key molecules operate in the GC, particularly the chemomokine receptors CXCR4 and CXCR5 (**Fig. 1a**). Some time ago, differential expression of CXCR5 and CXCR4 on B cells in the various zones of the GC and the upregulation of CXCR5 on *in vivo*–activated $CD4^+$ T cells were described[4,5]. It was reported that B cells in the GC dark zone have high expression of CXCR4, which is the receptor for CXCL12 that is also associated with the localization of pre–B cells and plasma cells. Conversely, CXCR5, the receptor for CXCL13 that is also required for the follicular localization of B cells, predominates on B cells in the GC light zone.

Traditionally, specific GC activities have been partitioned into the two zones, with proliferation and SHM being elements of the dark zone, and selection and differentiation being considered more functions of the light zone[6]. The inference has been that movement between zones is an important component of normal GC function, allowing rounds of mutation and selection to occur. The extent of V-gene mutation is thus used as a measure of time spent in the GC. In the absence of either CXCR4 or CXCR5 on B cells, partitioning of GC B cells is decidedly abnormal[4]. Additional consequences of loss of either receptor, such as transit time in the GC and formation of memory, remain to be investigated—a deficiency emphasized by the model of Mountz and colleagues[3].

It should also be borne in mind that modulating chemokine receptor expression is not the only means by which the chemotaxis of lymphocytes, or any cells, for that matter, can be regulated. Chemotaxis via both CXCR4 and CXCR5 can be rendered moot by means that leave surface expression unaffected. One such process is through the 'regulator of G protein signaling' (RGS) proteins that accelerate the Gα GTPase reaction. Most GC B cells have high expression of RGS1 and RGS13 (ref. 7) yet have not lost the ability to migrate toward CXCL13 and migrate more strongly toward CXCL12 than do follicular B cells[4]. GC T cells, in contrast, express RGS16 yet retain the ability to migrate toward CXCL13 (ref. 8). It would seem, therefore, that expression of RGS proteins is not necessarily predictive of migration capacity.

Mountz and colleagues now convincingly show that BXD2 mice are predisposed to produce IL-17-secreting T helper cells, that exogenous IL-17 accelerates GC formation and autoantibody formation in young BXD2 mice, and that treating older BXD2 mice with an IL-17 antagonist reduces GC formation[3]. Although this study establishes a relationship between IL-17 and aspects of the B cell phenotype in these mice, these results do not prove causality.

The direct action of IL-17 on B cells is demonstrated with chimeras in which only a fraction of the mature B cells are IL-17 receptor (IL-17R) sufficient. These B cells, introduced into an IL-17R-deficient BXD2 host and boosted with IL-17-producing adenovirus

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