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Analysis and Commentary: Attenuation by Targeting the B and T Cell Attenuator (BTLA)

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BTLA is a member of the immunoglobulin family thought to serve as a negative costimulatory molecule on activated T cells. Several recent studies, including the one published in this issue by del Rio and colleagues, have used BTLA-deficient mice and/or anti-BTLA antibodies to investigate the role of BTLA in regulating graft versus host reaction (GvHR) (1–3). Curiously, targeting BTLA either via genetic deletion, or blocking antibody, or agonistic antibody, all resulted in amelioration of GvHR. This suggests that BTLA may have several functions. Indeed, if BTLA's function were solely inhibitory, agonistic antibody cross-linking should inhibit T cell function, reducing GvHR, whereas genetic deletion or antibody blockade should enhance T cell function, exacerbating GvHR.

BTLA is expressed on activated T cells, B cells, dendritic cells, macrophages and some NK cells. Supporting its inhibitory effects, BTLA-deficient T cells display increased proliferation upon mitogenic stimulation and BTLA-KO mice are more susceptible to some autoimmune diseases. BTLA binds the TNFR family member herpes virus entry mediator (HVEM), which can also bind the coinhibitory receptor CD160 and the coactivating ligand LIGHT. The similar phenotype observed in HVEM-deficient and BTLA-deficient mice suggests that BTLA/HVEM is the predominant interaction on T cells (4).

To interpret experiments using antibodies that target a surface molecule, it is essential to know whether a given antibody can drive depletion of the targeted cells, block the epitope for the endogenous ligand, or trigger signaling by the targeted molecule. To study the role of BTLA in GvHR, the Murphy group used BTLA-deficient mice as well as the non-depleting and presumably blocking anti-BTLA antibody 6A6 in a hematopoietic stem cell transfer model in which irradiated F1 recipients received parental bone marrow and/or splenocytes (2). Their experiments demonstrated reduced recovery of donor T cells with blocked or

genetically absent BTLA, with the protective effect of 6A6 requiring direct engagement of BTLA on donor T cells. Sakoda et al., in a model of GvHR induced by transfer of donor splenocytes into either non-irradiated or lethally irradiated F1 recipients, used their own agonistic, non HVEM-blocking anti-BTLA mAb, BYK-1, and also BTLA-deficient mice (3). Similar to the Murphy model, reduced GvHR observed after transfer of BTLA-KO T cells was associated with diminished recovery of donor T cells whereas BYK-1 did not prevent T cell engraftment. Moreover, these authors demonstrated that reconstitution of BTLA-deficient T cells with a signaling-deficient BTLA molecule restored T cell survival. These 2 studies suggest that T cell-BTLA can serve as a ligand to the receptor HVEM that then delivers a pro-survival signal to T cells. Sakoda also showed that agonistic targeting of BTLA resulted in reduced cytotoxicity and cytokine production against allogenic stimuli (3). In the current study, del Rio and colleagues used their own anti-BTLA antibody, 4G12b, in non-irradiated F1 recipients of parental splenocytes, and confirmed the prevention of GvHR with no reduction in numbers of donor CD4⁺ and CD8⁺ T cells (1). Rather, they reported markedly reduced *in vivo* lysis of host cells and reduced production of cytokines by donor CD8⁺ T cells in response to alloantigen, suggesting that this agonistic antibody is preventing an alloreactive and cytotoxic pathway independently of any effects on T cell survival. Interestingly, the 6A6 used by the Murphy group and the 4G12b antibody used by del Rio recognize an overlapping epitope on BTLA, but binding of 4G12b to BTLA does not prevent BTLA from engaging HVEM (5), therefore presumably allowing BTLA to drive HVEM-mediated pro-survival effects. Moreover, their distinct Fc portions (hamster IgG for 6A6 versus rat IgG for 4G12b and BYK-1) may trigger different FcR- and complement-mediated signals which may contribute to their distinct consequences on T cell survival *in vivo*. Together, these 3 studies suggest that targeting BTLA is capable of reducing GvHR by alternative mechanisms depending on whether the endogenous HVEM-BTLA interaction is preserved or not (Figure 1).

Although these studies clarify the dual role of BTLA on T cells, a few issues remain unresolved. For instance, donor B cells express BTLA and may produce alloantibodies in this model. The agonistic anti-BTLA treatment may inhibit B cells either directly or indirectly by reducing T cell help such that reduced production of alloantibodies could contribute to the diminished *in vivo* cytotoxicity in anti-BTLA-treated animals. In addition, the contribution of interactions by remaining receptor/ligand pairs in the HVEM pathway needs to be considered. This may be challenging given the evidence of bi-directional signaling downstream of the BTLA/HVEM interaction as well as the potential contributions of HVEM binding to CD160 and LIGHT.

Abbreviations

BMT	bone marrow transplantation
BTLA	B and T lymphocyte attenuator
CTL	cytotoxic T lymphocyte
GvHR	graft versus host reaction

HVEM	herpes virus entry mediator
TNFR	tumor necrosis factor receptor

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Figure 1. Schematic model of the dual effects of BTLA engagement

Top panel: During TCR-mediated T cell activation, HVEM can serve as a ligand for BTLA and CD160 that can both deliver inhibitory signals to T cells. In addition, HVEM can serve as a receptor for both LIGHT, resulting in coactivation of T cells, and BTLA, resulting in enhanced T cell survival. **Middle panel:** Blocking antibodies to BTLA (or its genetic absence) abolish both the negative signaling by BTLA and the pro-survival effects of HVEM. **Lower panel:** Agonistic, non-blocking antibodies to BTLA drive the inhibitory signals of BTLA while allowing endogenous BTLA to engage HVEM and support T cell survival.