Divide and conquer

Blocking graft versus host but not graft versus leukemia T cells with agonist BTLA co-inhibitory signals

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One of the main objectives in allogeneic hematopoietic stem cell transplantation (aHSCT) research is the prevention of graft versus host disease (GVHD) while maintaining the graft versus leukemia/lymphoma (GVL) effect. Whether these two responses generated by donor T cells can be sufficiently separated and controlled remains controversial. While various approaches have been tested to achieve this goal, success has been relatively limited. Lymphocyte responses are negatively regulated by a series of receptors that function along with antigen receptors to deliver co-inhibitory signals. B and T lymphocyte associated (BTLA) is a novel co-inhibitory molecule expressed by activated T cells, B cells and other immune cells. A study by Albring et al. has now shown in a murine model that a single injection of agonistic anti-BTLA monoclonal antibody can inhibit GVHD long-term while maintaining GVL responses and immunity to infection. These studies suggest that future development of biologics to harness the function of co-inhibitory signals will be an important approach in the prevention of autoimmunity and GVHD and in protocols to achieve transplantation tolerance.

Allogeneic hematopoietic stem cell transplantation is an attractive treatment to cure various types of cancers and in the future it may even provide the solution for immune tolerance in organ transplantation. However, its application is primarily limited by the occurrence of graft versus host disease, despite the discovery of

GVHD over a half a century ago.¹ Thus, a major goal is the development of conditioning regimens that allow donor cell engraftment without GVHD. The ideal aHSCT regimen would not only prevent GVHD but also maintain or even increase the GVL effect. Various treatments have been used to control GVHD in clinical settings by either depleting donor T cells² or using immunosuppressive agents.^{3,4} The problem with these approaches is that they also reduce the GVL response. Thus it is not clear whether the GVHD and GVL can be sufficiently divided and controlled. Numerous other approaches have been investigated recently in experimental models, approaches based on our current understanding of the signals and cells controlling tolerance in T cells. T-cell tolerance is promoted by antigen encounter in the absence of co-stimulatory signals and by the presence of a T-cell subset with suppressive capacity called regulatory T cells (Treg). Thus, blockade of co-stimulatory signals⁵ and addition of Treg^{6,7} are potential approaches to limit GVHD. However, like immunosuppression, these strategies may not be good at sparing the GVL response, although some success in maintaining GVL while preventing GVHD has been achieved in certain model systems. It can be anticipated that co-stimulation blockade treatments may be needed on a repetitive basis over an extended period in order to induce sufficient tolerance, and Treg therapy may only hold GVHD causing T cells at bay as long as the Treg continue to be maintained and activated. It is not yet clear whether Treg will induce tolerance in the anti-host T cells that cause

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Abbreviations: GVL, graft versus leukemia/lymphoma; GVH, graft versus host; GVHD, graft versus host disease; aHSCT, allogeneic hematopoietic stem cell transplantation

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GVHD or simply maintain them in a non-activated state similar to 'ignorance'. A much less studied therapeutic approach to tolerance induction takes advantage of the existence of negative co-signaling receptors on lymphocytes,⁸ the co-inhibitors that counterbalance co-stimulation. It has been known for some time that co-inhibitory molecules play a role in the maintenance of self-tolerance,⁹⁻¹³ as originally postulated forty years ago.¹⁴ Development of agonist approaches triggering co-inhibitory signals may have an advantage if the tolerogenic signals can be delivered over a short period of time and lead to long-term unresponsiveness in the anti-host T-cell population. Testing these ideas has been limited by the lack of agonists of co-inhibitory receptors. However, many monoclonal antibodies have been developed to a particular co-inhibitor called BTLA (CD272), including agonists.¹⁵ Recently, Murphy and colleagues substantially moved this idea forward by demonstrating the ability of targeting BTLA to control GVHD using a non-depleting agonistic monoclonal anti-BTLA antibody (6A6).¹⁶ Among co-inhibitory molecules, BTLA is a member of the CD28 family,¹⁷ that binds its ligand, herpes virus-entry mediator (HVEM), to provide negative signals to lymphocytes.¹⁸⁻²⁰ It is part of a complex system that can include receptor interactions on the same cell, in cis and trans interactions between BTLA and HVEM on different cells.²¹

Murphy and colleagues have shown that a single administration of agonistic anti-BTLA antibody, at the time of aH SCT, is enough to prevent GVHD. Disease was completely prevented in a non-lethal GVHD model, and in a lethal GVHD model the majority of the mice survived with strongly lowered disease scores. However, delayed administration of 6A6 (two weeks post aH SCT) did not reverse GVHD. Using BTLA^{-/-} and HVEM^{-/-} donors they showed the effect of 6A6 was dependent on the expression of BTLA in donor cells and was not due to blocking of the interaction of BTLA with HVEM on donor cells. Although they found no significant difference in IL-2 production or CD8 T-cell proliferation and accumulation between anti-BTLA and control groups, 6A6 treatment

substantially reduced CD4 T-cell proliferation and numbers. The lack of effect on CD8 T-cell numbers contrasts with an earlier study by the same group using a different GVHD model that does not include irradiation²² which showed that 6A6 treatment reduced the number of both CD4 and CD8 T cells. BTLA has also been shown to regulate CD8 T cells in other settings.^{20,23}

The current study addressed a critical question about the effect of 6A6 on regulatory T cells (Tregs) in the current model. Co-inhibitors are present on both T effectors (Teff) and Tregs. Whether BTLA is regulating Teff or Tregs is an interesting and important question. An earlier study demonstrated that Tregs expressed lower levels of BTLA compared to activated T cells, even post TCR activation.²⁴ By using Foxp3^{EGFP} reporter and congenic wild type mice, Albring et al. provided evidence that the 6A6 mediated inhibition of CD4. Teff expansion allowed the expansion of donor derived pre-existing Treg, resulting in a concomitant increase in the ratio of Treg/Teff cells.¹⁶ Increased Treg was not due to BTLA somehow acting as a positive signal in Tregs. In fact 6A6 could inhibit Treg; however, the low levels of BTLA expressed on Treg together with reduced competition due to a loss of CD4 Teff with 6A6 treatment led instead to increased numbers of Treg. Although the effect of 6A6 is primarily mediated through the inhibition of Teff, Tregs were also required to prevent severe clinical GVHD. The ability of anti-BTLA to substantially alter the balance between Teff and Treg occurred only in the setting of T cells responding to antigen and not in the setting of syngeneic hematopoietic cell transplants. This supports the concept that co-inhibitory signals, and targeting of these pathways, will primarily affect T cells encountering antigen, leaving naïve T cells unaffected. These studies demonstrate the complexity in evaluating whether co-inhibitors are functioning in Treg or Teff cells and indicate that direct functions in Teff cells can tip the balance in favour of Treg.

In studies on human cells, BTLA expression can be preferentially maintained on anti-tumor T cells,²⁵ raising the important question of whether

BTLA targeting might block anti-tumor responses. Interestingly, the current study showed that anti-BTLA could inhibit GVHD without abrogating GVL.¹⁶ One potential explanation for this outcome could be differential effects of anti-BTLA on T-cell subsets mediating GVHD and GVL.²⁶ The persistence of anti-tumor activity in anti-BTLA treated recipients could be due to CD8 T cells, as anti-BTLA did not affect donor CD8 T-cell proliferation and numbers; instead it inhibited donor CD4 T cells that are considered to be critical players in GVHD.²⁷ However, CD4 responses are often necessary, in addition to any role of CD8 responses, for effective elimination of malignant cells,²⁸ reducing the likelihood that differential effects on T-cell subsets is the basis for maintenance of the GVL response. A more likely explanation for the differential effects on GVHD and GVL is based on the finding that just one injection of anti-BTLA is effective and therefore inhibition of the donor T cell response will depend on whether the donor T-cells are encountering their cognate antigen during the relatively short period that antibody is present (Fig. 1). For most T cells that are causing the GVHD, their target cognate antigens will be widespread host antigens so that all or almost all GVHD causing T cells will encounter their cognate antigen while anti-BTLA is present and therefore will receive 'tolerizing' BTLA signals via 6A6. In contrast, at least some of the donor anti-lymphoma response will be to more tissue restricted tumor antigens, and not all of these tumor antigen specific T cells will be encountering their cognate antigen during the short period when 6A6 is present at sufficiently high levels. While these cells are likely to express BTLA²⁵ and be bound by 6A6, BTLA doesn't provide any negative signal due to a lack of a concomitant TCR signal. These T cells are free (not tolerized) to kill lymphoma cells when they eventually do encounter the tumor antigens at a time when 6A6 levels have declined. This latter explanation for the maintenance of the GVL response implies that it will be important to reduce the tumor burden as much as possible prior to anti-BTLA treatment in order to avoid inducing tolerance in T cells mediating the GVL effect. In addition to the tumor model, the authors

Figure 1. A model to explain how triggering co-inhibitory signals can control GVHD while maintaining GVL. Donor T cells express co-inhibitory receptors such as BTLA, and while some will have T cell receptors (TCR) specific to widely distributed host antigens (GVH or GVH + GVL specificity), a fraction will be specific to more localized antigens present primarily on cancerous cells (GVL specificity). The ability of agonist anti-BTLA antibody to block GVH disease while maintaining the GVL response may be due to the wide spread nature of antigens targeted in GVH disease and the more localized presentation of tumor antigens. Most GVH T cells receive the requisite TCR and BTLA negative co-signals that induce tolerance. In contrast, many GVL T cells do not receive the TCR signal when a high level of anti-BTLA (6A6) is present and therefore remain competent to respond when they encounter their antigen at later times and 6A6 has been cleared.

have used bacterial and viral pathogen models to show that 6A6 does not cause long term immunosuppression.

Consistent with the results of Albring et al. another recent study reported that GVHD can be prevented by using a newly generated agonistic monoclonal anti-BTLA antibody and the effect was dependent on BTLA signals in donor T cells.²⁹ Overall, these studies have shown that targeting co-inhibitors such as BTLA is an important new strategy to prevent GVHD without inducing a generalized immunosuppression and should be examined in the clinical setting to facilitate aHSCT. A major question remaining for clinical translation is the effect of immunosuppressants currently employed in aHSCT, such as calcineurin inhibitors, on the effectiveness of co-inhibitory signals. The tolerance induced by BTLA and other similar receptors have been termed co-inhibitory precisely because both the antigen receptor and inhibitory receptor co-operate to provide the

inhibitory signal. Immunosuppressive blockade of antigen receptor signals may prove counterproductive to the generation of tolerance mediated by agonistic antibodies to co-inhibitory receptors such as BTLA. Given the central role of co-inhibitory signals in immune regulation, 'forced' co-inhibitory signals should be broadly applicable to all situations of unwanted adaptive immune responses. Therapeutic approaches applying agonists of co-inhibitors such as BTLA are in their infancy, and increased focus on this area can be anticipated given the relative ease of development compared to cellular therapy.

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