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Intervention with costimulatory pathways as a therapeutic approach for graft-versus-host disease

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Abbreviations: aGVHD, acute graft-versus-host disease; AICD, activation-induced cell death; BDF1, (C57BL/6 x DBA/2)F1; cGVHD, chronic GVHD; DC, dendritic cell; GVL effect, graft-versus-leukemia effect; HSCT, hematopoietic stem cell transplantation; L, ligand; miHA, minor histocompatibility antigen; SLE, systemic lupus erythematosus; TCR, T-cell receptor; Treg cell, regulatory T cell

Abstract

Graft-versus-host disease (GVHD) is mediated by mature donor T cells contained in the hematopoietic stem cell graft. During the development of GVHD, signaling through a variety of costimulatory receptors plays an important role in allogeneic T cell responses. Even though delivery of costimulatory signals is a prerequisite for full activation of donor T cells in the phase of their interactions with host APCs, their involvement with GVHD might occur over multiple stages. Like many other aspects of GVHD, promise of therapeutic interventions with costimulatory pathways has been gleaned from preclinical models. In this review, I summarize some of the advances in roles of costimulatory molecules in GVHD pathophysiology and discuss preclinical approaches that warrant further exploration in the clinic, focusing on novel strategies to delete pathogenic T cells.

Keywords: antigens, CD86; cell death; graft vs host disease; immunotherapy; T-lymphocytes, regulatory

Introduction

GVHD is a complex disease resulting from donor T cell recognition of a genetically disparate recipient that is unable to reject the donor cells following allogenic hematopoietic stem cell transplantation (HSCT). Activation and expansion of T cells in response to alloantigens is central to GVHD pathophysiology. T cell activation following allogenic HSCT is a tightly regulated process involving multiple distinct but interrelated signals (reviewed in Welniak *et al*., 2007). As in other settings, the diversity of T-cell receptor (TCR) determines the specificity of an apparently limitless array of alloantigens by recognizing processed peptide-MHC complexes on APCs (reviewed in Schlomchik, 2007). Dendritic cells (DC) are the most important in allostimulating donor T cells. In the case of MHC-mismatched HSCT, donor T cells recognize the non-self MHC molecule loaded with host-derived peptides on host APCs. This process is known as the direct mode of Ag presentation. On the other hand, the non-self MHC molecule can be taken up and processed by donor APCs followed by presentation to donor T cells in the context of self-MHC molecules. In situations of MHC match but minor histocompatibility antigen (miHA) mismatch between the donor and the recipient, donor T cells can only recognize MHC-bound peptides derived from recipient miHAs that are originated in polymorphic gene products. Ag presentation to T-cell subsets depends upon the pathway for Ag processing and presentation in APCs. In the initiation phase, donor $CD8^+$ T cells recognize the peptide products of miHAs loaded onto MHC class I molecules of recipient APCs (class I pathway). Later, donor APCs are differentiated from donor hematopoietic stem cell grafts and activate donor $CD8^+$ T cells by cross-presenting exogenously acquired host-derived Ags on MHC class I molecules. In this case, donor APCs could re-prime donor $CD8^+$ T cells previously activated by recipient APCs against the same Ags expressed by recipient APCs (Shlomchik, 2007). It also is possible that donor APCs activate naïve donor $CD8^+$ T cells against new Ags derived from various types of tissues (epitope spreading). By contrast, donor $CD4^+$ T cells can be activated in response to Ags that are endocytosed by recipient- or donor-derived APCs and processed by the class II pathway. In contrast to donor $CD8^+$ T cells, donor $CD4^+$ T-cell activation is directed towards exogenously acquired non-hematopoietic Ags to induce GVHD.

During GVHD, donor T cells receive activation

and proliferation signals through their clonotypic TCR. This is called signal 1. However, signal 1 is not sufficient to trigger productive T-cell responses. Only in the presence of signal 2 that is delivered by costimulatory molecules, signal 1 drives clonal expansion, survival, and differentiation of donor T cells into distinct functional subsets. Early studies showed that the absence of costimulatory signals makes T cells fall into the state of unresponsiveness named anergy under the condition of TCR stimulation (Jenkins *et al*., 1990). This paradigm boosted a constellation of studies to block costimulatory signals to inhibit allo and auto-immune responses (Li *et al*., 2009). The best known costimulatory pathways are grouped into two families: the Ig superfamily, which includes the B7/CD28, CD2, and T-cell Ig and mucin (TIM) domain families, and the TNF/TNF receptor family.

It is well established that APCs provide the critical costimulatory signals for turning on the GVHD process. Thus, an understanding of costimulation is of fundamental and therapeutic interest. In this review, I will summarize recent advances in this field, focusing on the possibility that costimulatory molecules are good therapeutic targets for GVHD.

Therapeutic approaches for costimulatory molecule targets in GVHD: blockade

It is no wonder that costimulatory molecules have been shown to promote GVHD, because costimulatory signals are required for fully activating alloreactive T cells during alloimmune responses. Most efforts have been devoted to understanding roles of costimulatory molecules in polarization or differentiation of T-cell subsets. However, evidence is accumulating that their roles are likely to be more complex. First, costimulatory receptors and their ligands have a distinct temporal and spatial expression pattern (Li *et al*., 2009). Second, functions of costimulatory molecules are not restricted to T cells and likely to be found in various types of cells including parenchymal cells (Keir *et al*., 2006). Third, identification of multiple pairings of between receptors and ligands or even between receptors and other receptors, coinhibitory receptors, and a reverse signaling indicates that there is a complicated immune network interconnecting receptors and their respective ligands (Sun *et al*., 2007; Sharpe, 2009). Lastly, costimulatory receptors have their own roles in immune responses or their roles are redundant with those of other receptors. It is possible that interactions of costimulatory receptors/their lignds participate in each of the five stages of GVHD evolution (cytoreductive conditioning, induction, donor T-cell expansion, recruitment, and effector phase) suggested by Welniak *et al*. (2007).

CD137

CD137 is a member of the TNF receptor family and functions as a costimulatory molecule for T cells. CD137 signals have been the most extensively studied in $CD8^+$ T cells (reviewed in Croft, 2009; Kwon, 2009). Ligation of CD137 on $CD8^+$ T cells has been shown to markedly increase their survival, proliferation, and CTL activities. Except for $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells, CD137 expression is inducible on lymphoid cells, including $CD4^+$ and $CD8^+$ T cells, NK cells, and NKT cells. Various myeloid cells such as DCs, neutrophils, mast cells, eosinophils, inflammatory macrophages, and activated myeloid progenitors seem to constitutively express CD137, even though its levels are low on the cell surface, its expression might be induced on granulocytes such as mast cells and eosinophils, and probably on inflammatory macrophages. Recent studies have identified expression of CD137 on nonhematopoietic cells under disease conditions (e.g., endothelial cells, smooth muscle cells, and cardiac myocytes. CD137L (CD137 ligand) is constitutively expressed on professional APCs (DCs, monocytes/macrophages, and B cells) and upregulated by their respective activation stimuli. Like CD137, CD137L can be expressed on myeloid cells, hematopoietic cells and other non-hematopoietic cells.

The parent→unirradiated F1 GVHD model is unique among GVHD models in that the development of GVHD is initiated only by alloreactivity under no inflammatory environment. This property is useful in dissecting various issues related to alloimmune responses. In this model, the genetic background of donor strains is critical in determining the outcome of GVHD. In a striking example, the infusion of donor T cells from the DBA/2 strain into an unirradiated (C57BL/6 x DBA/2)F1 (BDF1) mouse induces chronic GVHD (cGVHD), whereas the infusion of T cells of the other parent, C57BL/6, induces acute GVHD (aGVHD). The $CD4^+$ T cell of the DBA/2 strain activates and expands host B cells with an autoreactive potential, resulting in systemic lupus erythematosus (SLE)-like symptoms such as autoantibody production and glomerulonephritis. In aGVHD, not only do donor $CD8^+$ T cells eliminate host hematopoeietic cells, particularly host B cells, to induce massive engraftment of donor cells, but

also donor $CD8^+$ T cells attack solid organs together with donor $CD4^+$ T cells. In the parent-into-unirradiated F1 aGVHD or cGVHD model, blockade of the CD137 costimulatory pathway, using anti-CD137L mAb, inhibits aGVHD, while exacerbating cGVHD (Nozawa *et al*., 2001). The effect of anti-CD137L mAb on GVHD is due to its inhibitory effect on donor $CD8^+$ T-cell activity in both models. Such an inactivation of donor $CD8^+$ T cells results in elevation of autoantibody levels in cGVHD, since they can not delete host B cells, including autoreactive B cells, compared with intact donor $CD8^+$ T cells. It is well known that donor $CD8^+$ T cells are transiently activated early after transfer into the host and that host B cells are very susceptible to the attack by activated donor $CD8^+$ T cells (reviewed in Via, 2010). In this model, anti-CD137L mAb has a minimal effect on donor $CD4^+$ T cells. However, blockade of CD137 in conditioned recipients reduces either $CD8^+$ T cell-mediated or $CD4^+$ T cell-mediated GVHD lethality (Blazar *et al*., 2001). Interestingly, treatment with agonistic anti-CD137 mAb increases a graft-versus leukemia (GVL) effect without inducing severe GVHD in mice that receive delayed infusion of a low donor splenocyte dose. The enhanced GVHD effect of anti-CD137 mAb seems to be caused by an augmented alloresponse by infused donor $CD8^+$ T cells rather than a leukemia-specific response by donor hematopoietic stem cell-derived T cells. The therapeutic effect of agonistic anti-CD137 mAb on GVHD will be discussed in detail in the next section.

Absence of CD137 signals in the recipient has been shown to provide resistance to GVHD induction by donor T cells (Kim *et al*., 2009). An adequate explanation for this observation is that CD137-deficient recipient DCs may not be able to allostimulate donor T cells (Choi *et al*., 2009). It is thought that blocking reagents such as CD137-Fc fusion protein and anti-CD137L mAb could be useful in inhibiting GVHD by decreasing the activity of recipient DCs as well as donor T cells.

CD28/ICOS/PD-1

CD28 is the prototype of T-cell costimulatory molecule and has extensively studied in various fields including transplantation. CD28 is constitutively expressed on naïve $CD4^+$ and $CD8^+$ T cells. It has two ligands, B7-1 and B7-2. B7-1 expression is inducible on APCs and B7-2 is constitutively expressed on APCs. Their expression is upregulated after activation. B7-1 and B7-2 have their high-affinity inhibitory receptor named CTLA-4 whose expression is induced on activated T cells. Reduction in GVHD lethality has been seen in mice treated with CTLA-4-Ig or infused with CD28-deficient donor T cells (Blazar *et al*., 1994; Hakim *et al*., 1995; Yu *et al*., 1998). The blocking effect of CTL-4-Ig fusion protein is not complete, probably because of redundant roles of CD28 with other costimulatory molecules, blockade of the negative signal transduction through CTLA-4, and inhibition of Treg cell proliferation. It might be desirable to enhance the blocking efficiency of CTLA-4-Ig by combining with blockade of other costimulatory pathways or current immunosuppressive drugs to inhibit GVHD. Considering clinical trials, rapamycin is an attractive drug to combine with CTLA-4-Ig in that these two reagents have a synergism in promoting apoptosis of alloreactive T cells (Li *et al*., 1999). The fact that CTLA-4-Ig can reverse SLE-like GVHD (Via *et al*., 1996) provides a rationale for human trials for SLE patients using recombinant CTLA-4-Ig fusion protein (abatacept or belatacept, a mutant form of CTLA-4-Ig that has markedly prolonged binding time for B7 molecules).

ICOS is a member of the CD28 family and expressed on activated T cells and memory T cells. The ligand for ICOS, B7h, is expressed on APCs and some parenchymal cells. Initial studies suggest that ICOS may play a more important role in regulating Th2 differentiation than in regulating initial T-cell proliferation. In the parent→F1 model of aGVHD or cGVHD, blocking anti-ICOS mAb inhibits cGVHD, whereas it enhances donor $CD8^+$ T-cell proliferation and cytotoxic activity in aGVHD (Ogawa *et al*., 2001). Similarly, ICOS-deficient donor $CD4^+$ T cells have an impaired ability of cytokine production, resulting in less severe GVHD morbidity and mortality in conditioned recipients (Yu *et al*., 2006). In contrast, ICOS-deficient donor $CD8^+$ T cells show elevated proliferation, survival and cytokine production in irradiated recipients. When GVHD is induced by transfer of unseparated donor T cells, the blockade or absence of ICOS diminishes GVHD associated with the gut and liver (Taylor *et al*., 2005; Hubbard *et al*., 2005). Inhibition of GVHD by ICOS blockade is associated with skewed differentiation towards Th2 cells in one study (Hubbard *et al*., 2005) and lower proliferation of donor T cells in another study (Taylor *et al*., 2005). It is not known why the discrepancy happens depending upon experimental models.

PD-1, another member of the CD28 family, is expressed on activated $CD4^+$ and $CD8^+$ T cells, activated B cells, NK cells and macrophages. PD-1 ligands, PD-1-L1 and PD-1-L2, are expressed on APCs after cellular activation. Absence of PD-1 on

donor cells accelerates GVHD (Blazar *et al*., 2003a). It seems that engagement of PD-1 on donor $CD8^+$ T cells by PD-1-L2 plays a important role in inhibiting GVHD by suppressing IFN-γ production (Blazar *et al*., 2003a; Habicht *et al*., 2007). PD-1 signals also are important in inhibiting GVHD by Treg cells (Kitazawa *et al*., 2007).

CD40 and other TNF receptor family members

Critical immune responses are governed by interactions of CD40 expressed on DCs and B cells and CD40L on activated $CD4^+$ T cells. CD40 signals activate DCs and activated DCs in turn promote the activity of $CD8^+$ T cells (DC licensing) as well as $CD4^+$ T cells. CD40 plays a critical role in Ab class switch. Reflecting this, early studies showed that blockade of the CD40 pathway inhibit cGVHD and aGVHD in the parent→unirradiated aGVHD or cGVHD model (Durie *et al*., 1994). In conditioned GVHD models, endogenous CD40/CD40L interaction increases aGVHD lethality (Blazar *et al*., 1997) by promoting both direct $CD4^+$ T cell-mediated tissue destruction and $CD4^+$ T-cell expansion (Buhlmann *et al*., 1999). CD40 on donor APCs are required for the development of intestinal GVHD mediated by donor $CD4^+$ T cells (Anderson *et al*., 2005), indicating that blockade of CD40 pathway may have a therapeutic effect during the evolution of GVHD. It is of note that tolerized $CD4^+$ T cells generated in *in vitro* mixed leukocyte reaction (MLR) by adding anti-CD40L mAb poorly induce GVHD (Blazar *et al*., 1998). Those tolerized T cells also have potent immune regulatory activity (Taylor *et al*., 2002).

Other TNF receptor family members, blockade of whose pathway have been shown to inhibit GVHD, include OX40, HVEM (herpes virus entry mediator) and CD30. OX40/OX40L and CD30/CD30L interactions play a role in $CD4^+$ T cell-mediated GVHD (Blazar *et al*., 2003b, 2004), while HVEM is involved in $CD8^+$ T cell-mediated GVHD (Tamada *et al*., 2002). It should be emphasized that coblockade of several costimulatory pathways are more effective in protection of GVHD than inhibition of any single pathway (Saito *et al*., 1998; Tamada *et al*., 2002; Blazar *et al*., 2003b).

Therapeutic approaches for costimulatory molecule targets in GVHD: stimulation

CD137

Since the conceptualization of costimulation, it has been postulated that agonistic mAbs to co-stimulatory receptors have the potential to enhance immunity and thus can be used to eradicate tumors and virus-infected cells. However, numerous reports have shown that in vivo ligation of costimulatory molecules has the paradoxical consequence to not only enhance but also inhibit immune responses. To my knowledge, the first mAb which has been shown to have a dual function is one raised against CD137. Early observations showed that the same agonistic mAb to CD137 that have a potent anti-tumor effect (Melero *et al*., 1997) can abrogate T cell-dependent Ab responses (Mittler *et al*., 1999). Since this phenomenon was reported, an explosion of literature have demonstrated that stimulation of CD137 results in strong suppression of a variety of autoimmune or inflammatory diseases that are believed to be mediated mainly by $CD4^+$ T cells (Sun *et al*., 2002a, 2002b, 2006; Foell *et al*., 2003, 2004; Seo *et al*., 2004; Fukushima *et al*., 2005; Kim *et al*., 2005, 2007; Shao *et al*., 2005; Cho *et al*., 2006; Polte *et al*., 2006). Mechanisms of action behind anti-CD137-mediated immunosuppression still remain to be fully elucidated but seem to depend upon the context of inflammatory processes involved in the development of diseases. For example, agonistic anti-CD137 mAb inhibits autoantibody production almost without exception, subsequently blocking autoantibody-mediated autoimmune diseases such as SLE and rheumatoid arthritis (Sun *et al*., 2002b; Foell *et al*., 2003, 2004; Seo *et al*., 2004; Kim *et al*., 2005). CD137 ligation also serves as a powerful therapeutic mediator for Th2-mediated diseases, including asthma (Fukushima *et al*., 2005; Cho *et al*., 2006; Polte *et al*., 2006; Sun *et al*., 2006). By contrast, agonistic anti- CD137 mAb exacerbates diseases accompanying acute inflammation (Kim *et al*., 2009).

In the DBA/2→unirradiated BDF1 cGVHD model, agonistic anti-CD137 mAb is highly effective in inhibiting cGVHD by deleting donor $CD4^+$ T cells which are required for breaking host B-cell tolerance (Kim *et al*., 2005). The deletion of donor $CD4^+$ T cells by agonistic anti-CD137 mAb is due to the activation-induced cell death (AICD). In this model, administration with agonistic anti-CD137 mAb also results in the production of high levels of IFN-γ by donor $CD8^+$ T cells and IFN-γ in turn deletes host B cells. However, anti-CD137-mediated inhibition of autoantibody production occurs mainly through deletion of donor $CD4^+$ T cells and is irrelevant to whether or not B cells are depleted, because host B-cell self-tolerance can not be broken without donor $CD4^+$ T-cell help. By contrast, agonistic anti-CD137 mAb ameliorates advanced cGVHD by deleting both alloreactive

donor $CD4^+$ T cells and host B cells, including autoreactive B cells. It seems that there are two different mechanisms regarding deletion of $CD4^+$ T cells and B cells by agonistic anti-CD137 mAb: It is likely that the Fas death pathway is responsible for deletion for $CD4^+$ T cells (Kim *et al*., 2007), as discussed below, whereas IFN-γ plays a key role in B-cell apoptosis in a macrophage-dependent (Sun *et al*., 2002b) or -independent manner (Kim *et al*., 2005).

The SLE-like cGVHD model has been doubted as a clinically relevant model for human cGVHD based on preparative regimens, composition of the donor graft, genetic backgrounds of donor and host animals, and posttransplant events (reviewed in Chu and Gress, 2008). Another model that has been extensively used in the study of cGVHD has clinical and pathological manifestations similar to human autoimmune scleroderma. The most common strain combination utilizes unfractionated splenocyte or purified $CD4^+$ T cell populations from B10.D2 into Balb/c hosts (MHC-matched and miHA-mismatched). Surprisingly, agonistic anti-CD137 mAb reverses skin fibrosis, ulceration, and alopecia, a dominant feature of cGVHD, ultimately improving a general health condition (Kim *et al*., 2007). The reversal is associated with markedly reduced $CD4^+$ T-cell cytokines and increased apoptosis of donor $CD4^+$ T cells. The Fas death pathway (for AICD of alloreactive donor $CD4^+$ T cells) is required for ameliorating cGVHD by anti-CD137 mAb. It should be noticed that when administered early after irradiation, agonistic anti-CD137 mAb can induce acute lethal toxicity in which donor T cells and host cells are involved (Kim *et al*., 2009). It should be defined what type of non-T cells is responsible for anti-CD137-mediated toxicity. This issue is important, because it is likely that an inflammatory environment interferes with T-cell apoptosis induced by anti-CD137 mAb (Kim *et al*., 2009).

In two preclinical models of cGVHD, now it is clear that agonistic anti-CD137 mAb has the ability to delete pathogenic alloreactive $CD4^+$ T cells and autoreactive B cells. However, there is evidence showing that agonistic anti-CD137 mAb can delete Ag-specific $CD8^+$ T cells as well as $CD4^+$ T cells *in vivo* (Zhang *et al*., 2007, 2010). Earlier treatment with agonistic anti-CD137 mAb maintains elevated levels of TNF-α in LCMV (lymphocytic choriomeningitis virus)-infected mice, leading to Fas expression on activated $CD8^+$ T cells and this in turn results in Fas-mediated T-cell apoptosis (Zhang *et al*., 2007). Even though Fas-mediated death signal is not sufficient to delete LCMV antigen-specific $CD8^+$ T cells, STAT3 activation by signaling through CD137 in DCs is required for their complete AICD (Zhang *et al*., 2010). In the C57BL/6→unirradiated BDF1 aGVHD model, agonistic anti-CD137 mAb can completely delete not only donor $CD4^+$ T cells but also donor $CD8^+$ T cells (our unpublished data). As seen in cGVHD (Kim *et al*., 2005; Kim *et al*., 2007), it is likely that, in the condition of strong allostimulation (i.e., MHC-disparity between the donor and the recipient), engagement of CD137 provides strong costimulatory signaling leading to AICD of donor T cells within 5 days after transfer of donor cells. It remains to be elucidated whether other host hematopoietic and/or nonhematopoietic cells are needed for AICD of donor T cells by agonistic anti-CD137 mAb in GVHD.

One important issue to be solved with regards to CD137-targeted immunotherapy is whether agonistic anti-CD137 mAb can form a tolerogenic environment. In rheumatoid arthritis, $CD8^+$ T cells have been shown to play such a role by inducing the expression of indoleamine 2,3-dioxyginase (IDO) in APCs in an IFN-γ-dependent manner (Seo *et al*., 2004). However, this mechanism of action has not been shown to be involved in the immunosuppression by agonistic anti-CD137 mAb in other disease models (Kim *et al*., 2005; Polte *et al*., 2006; Sun *et al*., 2006; Kim *et al*., 2007). Interestingly, CD137 is constitutively expressed on Treg cells and CD137 signals can promote their proliferation and prosurvival *in vitro*, especially in the presence of IL-2 (Zheng *et al*., 2004; Elpek *et al*., 2007). Agonistic anti-CD137 mAb can expand Treg cells *in vivo* (our unpublished data). The expansion of Treg cells by agonistic anti-CD137 mAb is dependent upon IL-2 which is secreted by memory T cells (Zhu *et al*., 2007; Narazaki *et al*., 2010). Our unpublished results showed that Treg cells primed by anti-CD137 mAb have a higher ability to suppress the proliferation and cytokine production of $CD4^+$ T cells. Furthermore, aGVHD is not induced in host mice which were primed by anti-CD137 mAb 3 weeks before donor cell transfer. These results indicate that agonistic anti-CD137 mAb shares common features with CD28 superagonists in that they expand Treg cells *in vivo*, as discussed in the next section.

CD137 is inducible in activated T cells which consist of Ag-specific cells exposed to Ags. In fact, CD137 has been shown to be the most reliable marker for alloreactive human $CD8^+$ T cells and depletion of CD137-expressing $CD8^+$ T cells is an efficient tool to delete alloreactive T cells (Wehler *et al*., 2007). A soluble form of CD137L chimeric with streptoavidin can be internalized into DCs and thus used to a vehicle to deliver tumor antigens

(Sharma *et al*., 2010). Similarly, anti-CD137 mAb and a trimeric form of CD137L can be internalized into the cell after their binding to the cell surface CD137 (our unpublished data). In addition, toxin-conjugated anti-CD137 mAb is effective in depleting alloreactive T cells and CD137-expressing cells *in vitro* and *in vivo*. Since agonistic anti-CD137 mAb shows toxicity especially in an inflammatory condition, antagonistic mAbs that do not have the ability to trigger the CD137 signal transduction is recommendable for this approach.

CD28

Agonistic anti-CD28 mAb can prevent aGVHD by selectively depleting alloantigen-specific donor T cells (Yu *et al*., 2004). The depletion of activated donor T cells mediated through CD28 does not depend upon the Fas or TNF receptor type I or II death pathway but requires donor-derived IFN-γ (Yu *et al*., 2004). Rapamycin has synergism with agonistic anti-CD28 mAb in preventing lethal aGVHD (Albert *et al*., 2005).

CD28 superagonist mAbs have been shown to selectively expand Treg cells and be effective in treating experimental autoimmune encephalomyelitis in rats (Beyersdorf *et al*., 2005). In 2006, when the fully humanized CD28 superagonist was first given to six healthy male volunteers, it triggered an immediate and severe cytokine release syndrome (Suntharalingam *et al*., 2006). This may be a typical instance showing that reagents tested in mice or large animals could have different functional effects on humans due to distinct species-specific expression patterns or targeted epitopes (Hansel *et al*., 2010).

It is not known how IFN-γ contributes to depletion of alloantigen-specific T cells after CD28 ligation in murine GVHD models. As discussed above, agonistic CD28 mAb can result in transplantation tolerance by deletion of alloantigen-specific T cells and expansion of Treg cells. These two mechanisms could be interrelated by IFN-γ, since IFN-γ secreted by Treg cells are important for suppressing alloreactive T-cell activity (Sawitzki *et al*., 2005). However, it should be addressed how Treg cells induce transplantation tolerance by deleting alloreactive T cells.

CD40 and GITR

There is some evidence showing that CD40 stimulation has an inhibitory effect on GVHD. In the DBA/2→unirradiated BDF1 SLE-like cGVHD model, ligation of CD40 prevents the evolution of cGVHD (Kim *et al*., 2008; Puliaev *et al*., 2008). Agonistic anti-CD40 mAb induces activation of donor $CD8^+$ T cells. In the normal condition, most of donor $CD8^+$ T cells are rapidly removed after transfer into the recipient and residual ones fall into an anergic state (Kim *et al*., 2006). Once activated, donor $CD8^+$ T cells kill donor hematopoietic cells including potential autoreactive host B cells (Kim *et al*., 2006). This lymphodepletion is the same phenotype that is seen in the C57BL/6→unirradiated BDF1 aGVHD model. However, activation of donor $CD8^+$ T cells by agonistic anti-CD40 mAb does not result in damage of typical aGVHD target organs such as the large intestine and liver. This is in contrast with agonistic anti-GITR mAb which can completely convert cGVHD towards aGVHD in which intestine and liver damage is apparent (Kim *et al*., 2006). The difference between anti-CD40 and anti-GITR mAb is in their ability to generate the pool size of activated alloreactive donor $CD8^+$ T cells. Since agonistic anti-CD40 mAb shows a potent GVL effect in cGVHD, it seems that the pool size of alloreactive donor $CD8^+$ T cells is a determining factor for segregating GVHD from a GVL effect.

In an MHC-mismatched aGVHD model, agonistic anti-GITR mAb differentially regulates the development of GVHD induced by $CD4^+$ versus $CD8^+$ T cells (Muriglan *et al*., 2004). While GITR stimulation with donor $CD8^+$ T cells increases GVHD morbidity and mortality, it induces a significant decrease in GVHD induced by donor $CD4^+$ T cells. In this model, allostimulated donor $CD4^+$ T cells undergo increased apoptosis in a Fas-dependent manner in the presence of GITR stimulation.

Currently it seems to be too early to discuss the possibility of clinical application for agonistic anti-CD40 or anti-GITR mAb in GVHD. There is a case where agonistic anti-CD40 mAb has a therapeutic effect on experimental autoimmune encephalomyelitis (Mauri *et al*., 2000). A possibility could not be excluded that mAbs against CD40 or GITR have a beneficial effect on GVHD when combined with immunosuppressants.

Conclusion

To date, the majority of therapies for autoimmune diseases and transplantation-related complications primarily focus on the global inhibition of immune inflammatory activity. The goal of ongoing research in GVHD is to develop therapies that inhibit or eliminate activated alloreactive T cells as well as alloantigen-specific treatments, which allow for the directed blockade of the deleterious effects of

alloreactive immune cell function. Theoretically, this concept could be realized to treat GVHD, since GVHD evolution is thought to be maintained by activated APCs continuously presenting alloantigens to alloreactive T cells. Costimulatory molecules are good targets for this purpose. Currently, it is possible to induce peripheral tolerance to some extent by deleting alloreactive donor T cells, using either costimulatory blockers or costimulatory activators. To translate this field into the clinic, it is urgent to develop novel methods to target currently-appreciated costimulatory pathway and deeply understand GVHD pathophysiology involving costimulatory molecules.

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