

# NEPHROLOGY

# Rounds®

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## T-Cell Costimulation Blockade: Immunoselective Maintenance Immunosuppression Versus Tolerance Induction in Transplantation

By NADER NAJAFIAN, MD

Transplantation is the only cure for end-stage organ failure. Transplanted tissues are usually recognized by the immune system as foreign and, in the absence of immunosuppression, are rapidly rejected. Transplants between genetically distinct individuals are termed allografts; allograft rejection is orchestrated by the activation of allospecific T cells. To prevent allograft rejection, current therapies suppress all T cells irrespective of their specificities and must be taken life-long, leaving patients with decreased defenses against infectious agents and cancers. Ideal therapies should be of short duration and target only alloreactive T cells, preserving other T cells to fight infections and cancers. Activation of T cells requires recognition by the T-cell receptor (TCR) of antigenic peptides presented within major histocompatibility complexes (MHCs) on the surface of antigen-presenting cells (APCs). In addition, concurrent engagement of costimulatory receptors on T cells by ligands on APCs is also required for optimal T-cell responses. Targeting costimulatory receptor/ligand pairs has been used effectively to induce allograft tolerance in specific rodent transplantation models. However, this strategy has been less effective in larger mammals, resulting in human trials using costimulatory blockade for selective immunosuppression rather than induction of tolerance. This issue of *Nephrology Rounds* summarizes the different reagents used to target the most widely studied and clinically relevant costimulatory molecules and the possible reasons limiting their efficacy in higher order mammals. A discussion of recent developments in the field outlines how they could help in the development of novel and more effective clinical strategies for using costimulatory blockade in organ transplantation.

### Evolution of the concept of T-cell costimulation in transplantation

Mice depleted of, or deficient in, T cells fail to acutely reject solid organ allografts. Thus, to understand acute rejection and design ways to prevent it, it is necessary to understand how T cells become activated.<sup>1</sup> T cells are stimulated upon engagement of their TCR to its specific antigenic peptide presented by MHC molecules on the surface of other cells (also called signal 1). T cells can be subdivided into CD4<sup>+</sup> and CD8<sup>+</sup> subsets, based on expression of the CD4 or CD8 coreceptors. These coreceptors also bind MHC molecules helping to stabilize TCR/MHC interactions and bringing signaling enzymes in close proximity to the TCR, initiating the signaling cascade. CD4<sup>+</sup> T cells recognize peptides presented by MHC class II molecules, whereas CD8<sup>+</sup> T cells recognize peptides presented by MHC class I molecules. Whereas all nucleated cells express MHC class I molecules and can therefore be recognized by CD8<sup>+</sup> T cells, few cell types express MHC class II molecules and can serve to activate CD4<sup>+</sup> T cells. B cells, macrophages, monocytes, and dendritic cells (DCs) constitute the main MHC class II-expressing cell types. Other cell types such as endothelial cells and human, but not mouse, CD4<sup>+</sup> T cells can upregulate MHC class II molecules upon activation.

In addition to signals induced upon TCR engagement, T-cell activation requires other signals that are triggered following ligation of costimulatory receptors on T cells by ligands on antigen-expressing cells (also called signal 2).<sup>2,3</sup> As detailed below, this simplistic 2-signal theory of T-cell activation has been challenged by novel emerging concepts as our knowledge about costimulation continues to grow.

The number of costimulatory receptor/ligand pairs has grown significantly over the last decade.<sup>2,3</sup> These pairs can be traditionally divided into two main families: the immunoglobulin (Ig)-like CD28 family and the tumor necrosis factor (TNF)/TNF receptor (TNFR) family.<sup>4,5</sup> The CD28 family comprises the positive costimulatory receptors CD28 and induced costimulator (ICOS), as well as the inhibitors cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed death-1 (PD-1), B7-H3, and B7-H4. Members of the TNFR family expressed on T cells include the coactivating receptors CD27, CD134 (OX40), CD137 (4.1BB), CD30, herpesvirus entry mediator (HVEM), and membrane lymphotoxin (LT). T cells also express the TNF family members



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LIGHT and CD154 (CD40L). In addition to the traditional pathways, recent studies suggest that the T-cell Ig mucin (TIM) family of costimulatory receptors and ligands may play an important role in autoimmune diseases, as well as in allograft rejection and tolerance.<sup>6,7</sup> While redundancy ensures continuation of immune response in some cases, each pathway may have unique functions based on specific cell type (CD4/CD8/ natural killer [NK]), phenotypic differentiation (Th1/Th2), and timing of the immune response (naïve, effector, or memory). Some lines of evidence suggest that the costimulatory requirements for CD4<sup>+</sup> and CD8<sup>+</sup> T cells are distinct, CD4<sup>+</sup> T cells relying on CD28, CD154, and CD134 costimulation, while CD8<sup>+</sup> T cells are more dependent on CD27 and CD137.<sup>8</sup> This is one possible explanation why targeting CD28 or CD154 may be more effective in preventing acute rejection of organs whose elimination depends on CD4<sup>+</sup> cells than of those whose rejection can be effected by CD8<sup>+</sup> cells.<sup>9</sup> Furthermore, memory cells appear less susceptible to inhibition of costimulation, since proliferation and effector functions can occur following TCR engagement alone.<sup>10</sup> This may explain why costimulation-targeting therapies are less effective in nonhuman primates than in specific pathogen-free naïve inbred mice because the proportion of memory T cells is much higher in outbred animals.

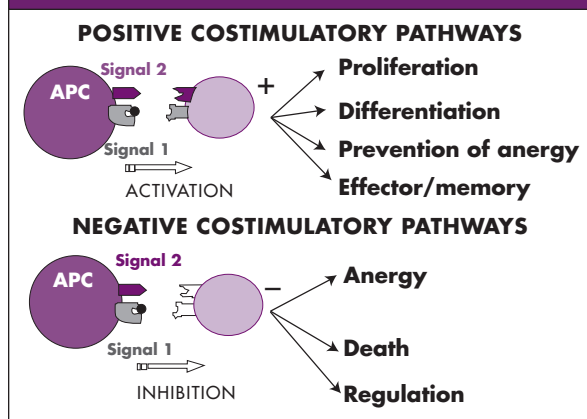
Another important recent finding is that costimulatory receptors can deliver signals to either enhance or inhibit T-cell activation, therefore modulating immune responses (Figure 1).<sup>11</sup> Thus, there are complex interactions among various costimulatory pathways, including positive and negative pathways, that ultimately determine the fate of the immune response. As a result, to induce tolerance it may be necessary not only to block the positive pathways, but also to enhance negative costimulatory signals.

Finally, costimulatory ligands are traditionally thought to be found on MHC class II-expressing cells, making these cells the most effective at stimulating T cells and supporting their referral as “professional” APCs. However, new data clearly demonstrate parenchymal expression of costimulatory ligands, thus providing a second regulation level of immune responses *in situ*.<sup>12</sup>

### Selective immunosuppression versus induction of tolerance

Currently, immunosuppressive therapies used in the clinic are aimed at inhibiting T-cell responses to prevent acute allograft rejection.<sup>13</sup> However, most existing drugs affect not only allograft-specific T cells, but also T cells of other specificities essential in combatting infectious agents or emerging cancer cells. In addition, the effect of these therapies is transient, such that transplanted patients need to keep taking these drugs for the rest of their lives, to prevent the acute rejection. Furthermore, some of these drugs are nephrotoxic and can contribute to chronic rejection, the most common cause of late allograft failure.<sup>14</sup> The mechanisms underlying chronic rejection are less well understood, but involve both immunological and nonimmunological pathways. Despite the improvement in 1-year allograft survival rates, the continued high incidence of chronic rejection has frustrated transplant physicians.<sup>15,16</sup> Episodes of acute rejection, drug-induced nephrotoxicity, infections, hypertension, and hyperlipidemia are all associated with an increased incidence of chronic rejection. Thus, the elimination or replacement of nephrotoxic immunosuppressive drugs would facilitate patient and graft survival, and reduce the incidence and severity of chronic rejection.

**Figure 1: Dual functions of costimulatory pathways**



Costimulatory pathways were originally believed to only transmit positive signals to T cells, leading to their proliferation and expansion. However, recent data suggest an important role for inhibitory costimulatory signals that can turn off the T cells. Thus, optimal targeting of both positive and negative costimulatory pathways may pave the way to inhibit detrimental T-cell alloresponses that limit organ allograft acceptance.

To understand how targeting costimulatory molecules may be able to achieve donor-specific tolerance,<sup>17</sup> it is necessary to review the mechanisms used by the human body to prevent autoreactive T cells from attacking its own organs. T cells develop in the thymus, where the majority of cells that recognize self-MHC molecules, as well as those recognizing MHC/peptide complexes with high affinity, are deleted by mechanisms termed death by neglect and negative selection, respectively (also called central tolerance).<sup>18</sup> In contrast, thymocytes that recognize MHC/peptide complexes with an intermediate affinity undergo positive selection, survive, mature, and populate secondary lymphoid organs. These T cells may recognize MHC/self peptides expressed on cells of the body, but the strength of this interaction is thought to be sufficient to maintain T-cell viability, but insufficient to trigger T-cell activation. Nevertheless, some T cells that recognize self-antigens with high affinity escape negative selection in the thymus. For instance, T cells reactive against melanin pigments or insulin components can be identified in healthy individuals, yet these people do not develop autoimmunity, suggesting that different peripheral mechanisms of tolerance other than negative selection in the thymus may be in place to prevent activation of these autoreactive T cells. These mechanisms of peripheral tolerance have been described, including anergy, clonal deletion, immune deviation, and regulation/ suppression (Figure 1).<sup>19</sup>

Engagement of the TCR in the absence of positive costimulatory signals, as well as signaling through the newly discovered negative costimulatory pathways (Figure 1) can lead to a state of T-cell hyporesponsiveness termed anergy. Anergic T cells do not proliferate or secrete the growth factor interleukin-2 (IL-2) upon restimulation, even in the presence of costimulation. Thus, because most parenchymal cells lack expression of positive costimulatory molecules or are able to express negative costimulatory ligands, it is possible that T cells encountering self-antigens *in vivo* on non-APCs become hyporesponsive.

Clonal deletion is the process resulting in the elimination of activated T cells of a defined specificity. Similar to anergy, this can occur because of a lack in engagement of positive costimulatory molecules or through signaling by negative costimulatory molecules (Figure 1). Finally, autoreactive

T cells can be kept in check by regulatory T cells (Tregs).<sup>20</sup> A subset of T cells, CD4<sup>+</sup>/CD25<sup>+</sup> Tregs develop in the thymus and serve to suppress responses of conventional T cells. These are referred to as innate or natural regulatory cells. Depletion of CD25<sup>+</sup> cells results in autoimmunity and enhanced antigen-specific T-cell responses. In addition to natural Tregs, different treatments have been shown to convert conventional T cells into cells with regulatory properties resulting in the suppression of immune responses *in vitro* and *in vivo*.

Blockade of interactions between costimulatory molecules only affects T cells in the process of stimulation through their TCR and can lead to their death or inactivation, as detailed below, but it does not affect T cells that have not engaged a cognate antigen. At the time of transplantation, only alloreactive T cells should be undergoing activation, such that prevention of costimulatory signals during TCR engagement by alloantigen should desensitize only transplant-reactive T cells, unless there is inadvertent concurrent colonization by other foreign antigens. Therefore, insofar as the duration of the therapy is limited, subsequent infections or cancers should meet a normal immune response, while the graft remains protected. Two caveats exist for this postulate that may prevent long-term maintenance of tolerance: some T cells activated by subsequent infections may cross-react with alloantigens and promote allograft rejection; and newly developed T cells leaving the thymus may be activated by the graft unless alloantigens are expressed in the thymus to induce negative selection, or unless strong regulation has developed to inactivate these new thymic emigrants. Although initial results in rodent models suggested that costimulation blockade may provide an effective pathway toward induction of tolerance to a transplanted organ, thus far, nonhuman primate and human studies have failed to support this conclusion.<sup>21</sup> For these reasons, attention has recently shifted to using costimulatory blockade strategies for maintaining an immunosuppressed state rather than for inducing tolerance.<sup>22</sup>

### Strategies to target costimulatory molecules

Different approaches are used to target costimulatory molecules. The first involves the generation of fusion proteins between a costimulatory receptor or ligand and the Fc portion of an Ig, attached to increase the half-life of the molecule after injection *in vivo*. The goal of fusion proteins is to prevent receptor/ligand interactions, therefore blocking costimulatory signals during T cell activation. However, some of these soluble receptors or ligands can result in activation of intracellular signaling pathways upon binding to their partners. In addition, complement and FcR binding to the Fc tail may trigger elimination of the target cell, or activate complement receptor or FcR<sup>+</sup> cells that can, in turn, modulate immune responses. Therefore, it becomes apparent why these agents are referred to as costimulation-targeting therapies, rather than the more common “costimulation-blockade” therapies. The most famous example of an immunosuppressive fusion protein is CTLA-4-Ig.<sup>23</sup> Different versions of this fusion protein exist for use in animal models, including the initially reported human CTLA-4-human IgG1 and subsequent generations of mouse CTLA-4-mouse IgG2a, or the low FcR-binding version of mouse CTLA-4-mouse IgG3. CTLA-4-Ig has been shown to block the interaction of its ligands CD80/CD86 with CD28 on T cells, to induce CD80/CD86-mediated reverse signaling in DCs and, theoretically, may promote FcR- or complement-dependent death of APCs, although the possibility of this latter effect, in general, has not been investigated.

The second strategy to target costimulatory molecules has been the development of antibodies directed against a costimulatory receptor or ligand. As with fusion proteins, the theoretical goal for these antibodies is to block receptor/ligand interactions and prevent delivery of costimulatory signals to T cells. However, it is extremely difficult to predict whether such antibodies will be blocking or activating *in vivo*. Some may prevent receptor/ligand binding, while others may mimic the effect of the natural binding partner and deliver a costimulatory signal. Further, even when demonstrable results are produced *in vitro*, it is difficult to predict the *in vivo* effect of antibodies.

Novel emerging strategies to inhibit protein expression include introduction into relevant cells of different antisense oligonucleotides or ribonucleic acid (RNA) interference constructs. Although these approaches have not yet been reported for inhibition of costimulatory molecules in transplantation models, injection of DCs treated with CD40-, CD80- and CD86-specific antisense oligonucleotides has demonstrated a delay in the incidence of diabetes in susceptible mice.<sup>23</sup>

Another confounding factor in interpreting the effects of all costimulation-targeting therapies *in vivo* is the occasional existence of multiple ligands for a given receptor and/or of multiple receptors for a given ligand. Therefore, even with purely blocking reagents, it becomes extremely complex to predict the effect of costimulation-targeting therapies that may disrupt binding of several positive and negative costimulatory receptor/ligand pairs.

### Targeting CD28 family members in transplantation

**CD28/CD80/CD86:** CD28 is expressed on all mouse T cells, on 90% of human CD4<sup>+</sup> T cells and 50% of human CD8<sup>+</sup> T cells.<sup>2</sup> It is the only known costimulatory molecule of this family to be constitutively expressed on naïve T cells and, therefore, is crucial for costimulation of naïve T cells upon an initial antigen encounter. Its ligands, CD80 and CD86, are expressed on T cells and APCs, and their expression increases following activation. *In vitro*, CD28 engagement promotes T-cell activation by enhancing TCR-mediated T-cell proliferation, IL-2 transcription, messenger (m) RNA stability, and T-cell survival, and preventing TCR-dependent induction of T-cell anergy. CD80/CD86 engagement promotes costimulation by binding to CD28, but it also initiates reverse signaling, resulting in upregulation of MHC class II on APCs, increased production of IgG and IgE by B cells, and production of IL-6 by DCs, which in turn enhances T-cell proliferation. However, CD80/CD86 can also inhibit T-cell responses or induce tolerance by binding the coinhibitor CTLA-4, thereby promoting T-cell cycle arrest.

The most widely used reagent to target CD28/CD80/CD86 costimulation is CTLA-4-Ig.<sup>23,25</sup> Because CTLA-4 has a higher affinity for CD80/CD86 than CD28, CTLA-4-Ig binds B7 family members and prevents CD28 engagement on T cells. In transplantation, CTLA-4-Ig has been shown to promote long-term acceptance of islet and cardiac allografts in mice for many, but not all, strain combinations.<sup>2</sup> CD28-deficient mice retain the capacity to reject cardiac allografts,<sup>26</sup> demonstrating that CTLA-4-Ig does more than merely prevent CD28 engagement, although it is possible that the reduction in numbers of Tregs in CD28-deficient mice also facilitates allograft rejection. Recently, a mutant version of CTLA-4-Ig – LEA29Y – has been generated, which displays

enhanced affinity for B7 family members.<sup>27</sup> This compound is more effective than CTLA-4-Ig at inhibiting alloresponses *in vitro* and has been shown in nonhuman primates to promote indefinite islet allograft survival when combined with sirolimus/tacrolimus.

**CTLA-4 (CD152)/CD80/CD86:** Although many anti-CTLA-4 antibodies have been generated over the years, these have all turned out to be blocking reagents, that, when administered *in vivo*, result in enhanced T-cell responses. Although this outcome is desirable to increase T-cell function in tumor settings, and clinical trials are testing the efficacy of such approaches in cancer patients, it is not useful for transplantation settings. Engineering the expression of single-chain, membrane-bound anti-CTLA-4 antibodies (scCTLA-4-Fv) on allogeneic tumor cells has been tried to cross-link CTLA-4 *in vivo* and mimic its coinhibitory effects.<sup>28</sup> Transplanted cells expressing scCTLA-4-Fv appeared to promote energy of alloreactive T cells, a proof of principle that this approach may facilitate donor-specific tolerance. However, the efficacy of this strategy has not yet been confirmed using transplanted organs engineered to express these constructs.

**ICOS: B7h pathway:** ICOS shares 20% homology with CD28 and was first cloned in a screen for unique molecules expressed on human T cells following activation.<sup>29</sup> Unlike CD28, ICOS is not expressed on naïve T cells; instead, its expression is upregulated after T-cell activation and persists on effector and memory T cells. This difference suggests that ICOS signaling may be more important for regulating activated T cells, whereas CD28 functions to prime naïve T cells. The expression pattern of ICOS on T cells in germinal centers indicates a role for ICOS in collaboration between T and B cells.<sup>3</sup> ICOS- and B7h-deficient mice exhibit profound deficits in Ig isotype class switching and germinal center formation after immunization with model protein antigens under most conditions.<sup>4</sup> Common variable immunodeficiency (CVID) patients exhibit a deficiency in ICOS.<sup>30</sup> Due to a homozygous deletion of ICOS and the resulting impaired T-cell help for B cells, ICOS-deficient humans develop an adult-onset immunodeficiency characterized by reduced numbers of B cells, lack of memory B cells, and low serum Igs. However, the T cells from these patients are normal with regard to subset distribution, activation, cytokine production, and proliferation. The phenotype of human ICOS deficiency suggests a critical involvement of ICOS in T cells, help for late B-cell differentiation, class switching, and memory B-cell generation.

The ICOS ligand, B7h, is expressed on B cells, monocytes, and DCs. It is also expressed on nonprofessional APCs such as fibroblasts, endothelial cells, renal tubular epithelial cells, hematopoietic progenitors, and embryonic stem cells.<sup>3,31</sup> The expression pattern of B7h further suggests that this ligand is involved in the regulation of effector, rather than priming, T-cell functions via DCs.

In the transplant setting, parenchymal ICOS expression is increased in rejecting cardiac allografts, and ICOS deficiency/blockade prevents the development of vascularized cardiac allograft rejection.<sup>32</sup> Also, ICOS-B7h costimulation blockade prevents the development of

chronic rejection after CD40-CD154 blockade and anti-ICOS mAb synergizes with CTLA4Ig to induce donor-specific tolerance.<sup>33</sup> Interestingly, ICOS blockade has disparate effects on immune responses depending on the timing of blockade in various model systems.<sup>34</sup> This study showed that ICOS-B7h blockade induced prolongation of allograft survival more effectively during the effector/differentiation phase than in the priming phase of the alloimmune response. Given these results, manipulation of the ICOS-B7h signaling pathway in order to prolong graft survival may be best initiated after T-cell priming.

Recent studies indicate the potential of blockade by ICOS/B7h along with immunosuppressive drugs and/or B7/CD154 costimulation blockade regimen to promote transplantation tolerance.<sup>11</sup> Determining whether such approaches are feasible in the clinic will undoubtedly be the focus of future studies in primates and ultimately, in humans.

**PD-1/PD-L pathway:** The PD-1 receptor and its ligands, PDL1 (B7-H1) and PDL2 (B7-DC), have recently been characterized.<sup>35</sup> PD-1 is induced on peripheral T cells, B cells, and myeloid cells upon activation. The broader expression of PD-1 contrasts with the expression of other CD28 homologues, which are restricted to T cells.

Perhaps the best evidence to support a negative regulatory role of the PD-1 pathway *in vivo* is the phenotype of PD-1-deficient mice. These mice develop an autoimmune-like phenotype, but delayed in onset as compared to CTLA4<sup>-/-</sup> mice. PD-1 ligands are expressed not only on bone marrow (BM)-derived cells (APCs and T cells), but also on other cell types. Northern blot analyses have detected PDL1 and PDL2 in nonlymphoid organs such as heart, placenta, and lung in both humans and mice.<sup>36</sup> Parenchymal expression of PD-1 ligands may serve to regulate autoreactive T- or B-cell responses in peripheral tissues, and/or may serve to regulate inflammatory responses at these sites. In the transplant setting, recent data from Hancock's group demonstrated that in the context of submaximal TCR or positive costimulatory signal blockade, targeting of PD-1 can block allograft rejection and modulate T- and B-cell-dependent pathologic immune responses *in vivo*.<sup>37,38</sup> In a murine cardiac allograft model, treatment with anti-PD-L1 mAb, but not anti-PD-1 mAb, accelerates the time to rejection of fully allogeneic cardiac allografts in wild-type recipients, whereas both antibodies accelerate rejection in the absence of CD28 costimulation.<sup>26</sup> Finally, recent work has demonstrated that blockade of the interaction between PD-1 and PDL1 can accelerate cardiac allograft vasculopathy (CAV).<sup>39</sup> Recently, our group demonstrated that, while an intact recipient PD-1-PDL1 pathway is essential for induction and maintenance of the tolerance induced by CTLA4-Ig in a murine cardiac allograft model, the expression of PDL1 in donor cardiac allograft tissue is essential to prevent the development of CAV in allografts that survive long-term after the same treatment.<sup>12</sup> Together, these results demonstrate that PD-1/PDL interactions are of significant importance in alloimmune responses. Fusion proteins against PD-1 that transduce negative signals may be available in the future and used to study the effects of enhancing signals delivered through PD-1 *in vivo*.

## Targeting of TNF/TNFR family members in transplantation

CD40 is expressed on B cells, macrophages, DCs, thymic epithelial cells, activated endothelial cells, and activated fibroblasts.<sup>2</sup> Its ligand, CD154, is induced on activated T cells with greater expression in CD4<sup>+</sup> than CD8<sup>+</sup> T cells, NK cells, and eosinophils. *In vitro*, CD40 cross-linking has been demonstrated to induce upregulation of CD80 and CD86, CD44 and intercellular adhesion molecule-I (ICAM-I), and to result in enhanced B-cell survival in a nuclear factor (NF)- $\kappa$ B-dependent mechanism. *In vivo*, CD40 engagement leads to germinal center formation, antibody isotype class switching, and generation of memory B cells. Ligation of CD40 on APCs can replace the requirement for CD4 to help in activating CD8<sup>+</sup> T cells.

Anti-CD154 has been widely used in transplantation to inhibit activated T cells. Treatment with anti-CD154 (MR1) has been shown to induce long-term acceptance of cardiac and pancreatic islet allografts in mice, although transplants are eventually rejected chronically.<sup>40</sup> Similarly, CD154-deficient mice accept cardiac allografts long-term, but eventually develop chronic rejection. Targeting of the CD40/CD154 pathway was shown to synergize with administration of a donor-specific transfusion, resulting in donor-specific tolerance and absence of chronic rejection. A similar synergy has been described with the combination of anti-CD154 and CTLA-4-Ig, which also prevents chronic rejection of cardiac allografts in mice and induces long-term acceptance of skin grafts in some strain combinations.<sup>41</sup> In rhesus monkeys, anti-CD154 administered for 5 months post-transplant resulted in acceptance of kidney and pancreatic islet allografts for more than 1 year.<sup>42</sup> However, grafts were progressively rejected at the cessation of treatment, indicating lack of induction of transplantation tolerance.

Finally, combination therapies of anti-CD154 and global immunosuppressants have been explored for the purpose of clinical translation. Administration of cyclosporine A (CsA) was shown to prevent anti-CD154-mediated long-term graft acceptance; this was attributed to CsA inhibiting activation-induced cell death (AICD) and CTLA-4 upregulation of T cells. In contrast, treatment with rapamycin potentiated anti-CD154-mediated graft acceptance via super-induction of AICD.<sup>43</sup>

As detailed below, clinical trials with anti-CD154, however, have been disappointing and were halted because of life-threatening thromboembolic side effects.<sup>44</sup> One approach has been to target CD40 rather than CD154; experiments in nonhuman primates using blocking anti-CD40 mAbs appear very promising.<sup>45</sup>

### Clinical trials

While CTLA4-Ig can suppress rejection in rodents, it lacked efficacy in primate transplant models. Thus, 2 amino-acid substitutions gave rise to a novel modified version of CTLA4-Ig, LEA 29Y (belatacept).<sup>46</sup> The increased avidity resulted in a 10-fold increase in potency *in vitro* and a significant prolongation of renal allograft survival in a preclinical primate model.<sup>27</sup> In a recently, and to date only, published clinical trial involving renal transplant recipients, belatacept was shown to be noninferior to CsA in preventing acute rejection and was very well tolerated.<sup>47</sup> Renal transplant recipients (N=218)

were randomized to receive intensive belatacept therapy, less-intensive belatacept therapy, or CsA; all patients received basiliximab, mycophenolate mofetil (MMF), and corticosteroids. The incidence of acute rejection at 6 months was similar among all groups. However, patients taking belatacept had a significantly higher glomerular filtration rate measured at 12 months than those given CsA; the belatacept group also had a lower incidence of chronic allograft nephropathy on biopsy and a tendency toward improved cardiovascular and metabolic profiles. The frequency of infection was similar for all groups.

Humanized anti-B7.1 (h1F1) and anti-B7.2 (h3D1) mAbs have been shown to be safe and effective in a phase I trial when administered in combination with CsA, MMF, and corticosteroids. A blocking anti-CD28 mAb is also under development. This drug would have the advantage over anti-B7 mAbs to inhibit engagement of the activating CD28, but not the inhibitory CTLA-4 receptor.

Despite the reported efficacy of anti-CD154 in rodents and higher order models, its future clinical use is uncertain due to reported thromboembolic events in clinical trials. It is postulated that these were due to the expression of CD154 in human, but not mouse platelets. Targeting of the CD40/CD154 pathway appeared to be inapplicable to human transplantation following these clinical trials. However, the strong evidence for anti-CD154-mediated induction of regulation and the potential for donor-specific tolerance repeatedly demonstrated in mouse models have led investigators to revisit new strategies to target this axis without inducing thromboembolic side effects. To circumvent this potential complication, several investigators have developed a chimeric Ab that targets CD40 as an alternative to CD154. While efficacy in humans will require further future clinical studies, the combination with LEA29Y in a nonhuman primate model of islet transplantation facilitated long-term survival.<sup>45</sup>

### Causes for failure of costimulation-targeting therapies

Costimulation-targeting therapies have failed to induce transplantation tolerance in several instances in murine models, but even more frequently in nonhuman primates. Several causes for resistance have been identified. First, naïve CD4<sup>+</sup> T cells appear easier to suppress by targeting the CD28 or CD154 axes than naïve CD8<sup>+</sup> T cells.<sup>48</sup> Often, failure of CD28/CD154-targeting therapies has been ascribed to residual CD8<sup>+</sup> T-cell function and depletion of CD8<sup>+</sup> T cells has significantly improved transplant outcome. It seems logical, therefore, to add to these therapies reagents that appear to inhibit CD8<sup>+</sup> T cells efficiently.

Secondly, memory T cells are also less susceptible to inhibition by CTLA-4-Ig and anti-CD154.<sup>10</sup> Although most transplant recipients have not been previously exposed to antigens from their donor, they may possess memory T cells that recognize donor antigens. Because TCR recognizes peptide/MHC complexes as 3-dimensional structures, cross-reactivity between special structures that share similarities is theoretically possible. Indeed, infections with specific viruses or parasites have been shown to give rise to memory T cells that cross-react with alloantigens in mouse models. As humans

are continually exposed to environmental antigens, subclinical and clinical infections, transplanted organs may encounter many memory alloreactive T cells that can resist inhibition of the CD28/CD154 axes. However, more research is needed with murine and primate T cells to identify the signaling pathways of memory cells more definitively.

Finally, numerous positive and negative costimulatory pathways have been discovered over the last decade;<sup>2</sup> while blocking some positive costimulatory signals may be ineffective simply due to redundancy, it is becoming increasingly apparent that promoting or at least maintaining negative costimulatory signals are also essential to attenuate or even eliminate alloreactivity.

## Conclusions and future directions

Costimulation blockade represents an exciting new method for preventing allograft rejection and inducing tolerance in experimental models. Progress in bringing costimulation blockade to the clinic has been slow, but success with agents such as belatacept suggests that clinicians may expect reports on greater use of costimulation blockade to minimize or eliminate exposure to nephrotoxic agents (eg, calcineurin inhibitors) and even possibly to induce donor-specific tolerance. Major hurdles include the need to better understand the expression and functionality of various positive and negative costimulatory molecules *in vivo* during the course of alloimmune response.

*Dr. Najafian is an Assistant Professor of Medicine, Harvard Medical School, Boston, MA.*

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