T Cell Costimulatory Blockade: New Therapies for Transplant Rejection

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Abstract. Optimal T cell responses occur when T cells receive both antigen-specific signals through the T cell receptor and non-antigen-specific costimulatory signals through accessory cell surface molecules. The best understood costimulatory receptor is CD28. Signals through the T cell receptor and CD28 cooperatively induce cytokine gene expression and promote T cell proliferation and survival. Negative signals delivered cooperatively induce cytokine gene expression and promote T cell proliferation and survival. Negative signals delivered through a related cell surface receptor, cytotoxic T lymphocyte antigen (CTLA-4), act to terminate immune responses and are required for normal immune homeostasis. This article reviews T cell costimulation, including the CD28/CTLA-4 system and other potential costimulatory pathways (such as CD40/CD154), the role of these pathways in normal immune responses, and the potential for the inhibition of these pathways to induce transplantation tolerance.

Although significant advances in tissue cross-matching, surgical techniques, immunosuppressive medications, antiviral and antifungal drugs, and ancillary services have led to dramatic improvements in short-term renal allograft survival rates in the past 20 yr, these improvements have not solved the problem of late allograft loss. In other words, among the grafts that are still functional at the 12-mo time point, there have been only minor improvements in the “half-life,” i.e., the time at which 50% of the grafts have ceased to function (1). This half-life was approximately 8 yr two decades ago, and it remains <10 yr.

The major cause of late allograft loss is chronic rejection, which, in the case of renal transplantation, manifests itself histologically as arteriosclerosis, glomerular sclerosis, and interstitial fibrosis. Clinically, these patients experience a gradual decline in renal function (usually over the course of years), often in association with proteinuria and hypertension. Chronic rejection is unlikely to be a single discrete entity but almost certainly involves the response of the host and the graft to a variety of factors, leading to pathologic and clinical end points that appear similar. Although there remains some debate regarding all of the possible causes of chronic rejection, there is general agreement that both immunologic and nonimmunologic factors contribute (2,3). Immunologic factors include tissue mismatching and episodes of acute rejection. Prominent nonimmunologic factors are cytomegalovirus infection, hypertension, and a history of delayed graft function. At least for experimental animals, immunologic factors are required for the occurrence of chronic rejection. Nonimmunologic factors may exacerbate chronic rejection and may even perpetuate it after immunologic factors have been eliminated, but they do not seem to be sufficient, by themselves, to cause chronic rejection. That is, in the absence of immunologic disparities, chronic rejection is observed rarely, if at all.

Therefore, it has been suggested that the key to preventing chronic rejection involves inducing immunologic tolerance to the graft. This view is based on the hypothesis that the best way to prevent late allograft loss is to remove what seems to be the key predisposing factor, i.e., the deleterious immune response of the host to the graft. Although, clearly, improved immunosuppression has the potential to yield improved long-term graft survival rates, it is unlikely that this would eliminate chronic rejection or solve the problems of susceptibility to opportunistic infections and malignancy and the occurrence of immunologic side effects (e.g., nephrotoxicity or diabetes). In contrast, the creation of transplantation tolerance would obviate the need for long-term immunosuppression and theoretically could prevent chronic rejection as well as many long-term complications currently associated with transplantation.

In the past several years, new insights into immune responses have led to the development of novel strategies for tolerance induction. Many of these have been tested only in small-animal models and are just beginning to be studied in nonhuman primate experiments and in clinical trials. One of the most promising new strategies is based on the discovery that optimal T cell activation requires both antigen-specific signals (signal 1) and non-antigen-specific signals (signal 2) (4). This article reviews general concepts of immunologic tolerance and the allogeneic response, analyzes in detail T cell costimulatory pathways, and discusses how blockade of these pathways might be used to modulate the allogeneic response and induce transplantation tolerance.

Immunologic Tolerance

The long-term goal in transplantation medicine is to induce tolerance to the engrafted organ. By tolerance, we mean a state in which the recipient, without the requirement for ongoing
medications or therapy, does not mount an inflammatory response to the allo- or xenograft. This does not suggest that the organ is not recognized by the immune system (immunologic ignorance) or that it fails to induce an immune response (immunologic nonresponsiveness) (5,6). Although these certainly occur and both can be mechanisms underlying immunologic tolerance, a growing body of data suggests that active immunoregulation may play a key role in the induction of transplantation tolerance. In these cases, the immune system clearly recognizes the allograft but the induced response is noninflammatory. In some instances, actively induced immunoregulatory cells (suppressor cells) may serve to inhibit proinflammatory cells (7,8).

Response to Alloantigens

Immune responses elicited by alloantigens are unique in many ways. Most notably, there are two distinct ways for alloantigens to activate T cells. In the so-called “direct” route of allore cognition, the donor MHC-peptide complex present on donor cells is recognized intact by host T cells (9). Alternatively, in the “indirect” pathway, donor MHC molecules are processed into peptides by host antigen-presenting cells (APC), and these peptides are then presented by host MHC molecules (10). The direct pathway is unique to the alloresponse, whereas indirect recognition is analogous to the “conventional” means of antigen presentation (11). It has been proposed that direct recognition is dominant during acute rejection and indirect recognition is more important for chronic rejection; however, the relative contributions of these two pathways to acute and chronic rejection remain poorly understood (12).

The capacity for direct recognition of alloantigens has an important implication for the alloimmune response. Although in the case of conventional antigens (including indirect allore cognition) it is estimated that the fraction of responsive T cells is approximately 1/10^5 to 1/10^6, approximately 1 to 10% of T cells respond to direct presentation of alloantigens (13). Therefore, the clonal compartment capable of alloreactivity is 1,000 to 10,000 times larger than for conventional antigens, including autoantigens. This suggests that modalities that might be sufficient to control normal immune responses or pathologic autoimmune responses may be unable to contain the quantitatively much larger alloimmune response.

T Cell/APC Interactions Involving the CD28 Pathway

Several mechanisms have evolved to protect the body from autoimmune reactions and to focus the response on foreign antigens. These include, for example, the homing of stimulated T cells to the inflammatory site and the requirement for antigen presentation by professional APC, such as dendritic cells, macrophages, and B cells (14). During antigen presentation, a variety of important bidirectional cognate interactions take place, with signaling to both the T cell and the APC (see below). The need for specific T cell/APC interactions stems from the fact that T cells require two signals to generate a productive immune response (4,15,16). The first signal is provided through the T cell receptor (TCR) by the MHC-peptide complex itself, and the second is a costimulatory signal that is necessary for the T cell to respond optimally to antigen. By their nature, costimulatory signals are not antigen-specific but, rather, amplify and synergize with the TCR ligation. Most cells (except red blood cells [RBC]) in the body express MHC-peptide complexes but are unable to provide costimulatory signals to T cells.

The best understood costimulatory signal is provided through the T cell surface molecule CD28, a member of the Ig gene superfamily (17). CD28 has two ligands, the homologous molecules CD80 (B7-1) and CD86 (B7-2); both are members of the Ig superfamily and are expressed on activated APC and some other cell types (17,18).

Ligation of CD28 optimizes T cell responses through two general mechanisms (Figures 1 and 2). First, CD28 costimulation enables T cells to respond to low levels of TCR ligation, supporting T cell responses even at low antigen concentrations (17,19). This effect is exerted through induction of expression of several cytokine genes (including interleukin-2 [IL-2], interferon-γ [IFN-γ], and tumor necrosis factor [TNF]) and stabilization of cytokine gene transcripts, with the result being greatly augmented production of cytokine proteins (17). Without CD28 costimulation, T cell responses are quantitatively weaker and are seen only with high antigen concentrations. Second, CD28 costimulation supports sustained T cell responses. In the absence of CD28 signaling, T cell responses are transient. There are two ways in which CD28 costimulation has been demonstrated to support sustained T cell responses, i.e., prevention of anergy induction and prevention of apoptosis (5,20).

Figure 1. Distinct effects of CD28 costimulation. The ability of CD28 signaling to support immune responses can be attributed both to the induction of cytokine genes (such as interleukin-2 [IL-2]), which induce T cell proliferation and prevent anergy, and to the induction of cell survival genes, which prevent apoptotic death of T cells. AICD, activation-induced cell death; TCR, T cell receptor.
Anergy is defined as a state of nonresponsiveness, occurring even when the cell receives appropriate signals (those that would normally induce a response). When T cells are stimulated by antigen without receiving a costimulatory signal (i.e., TCR ligation alone without CD28 costimulation), a state of anergy is induced (15), these anergic T cells become refractory to antigen, even if CD28 signals are provided at the time of restimulation. Anergy seems to occur even if the initial TCR signaling was sufficient to induce IL-2 production and proliferation, indicating that anergy avoidance is separable from mitosis (21). The duration of the anergic state is not known but may last for at least 3 to 4 wk (22). Anergy can be broken by provision of exogenous IL-2 at the time of TCR restimulation and possibly also by stimulation of the CD2 accessory molecule.

In addition to preventing anergy, CD28 costimulation provides important survival signals to T cells (23,24). Activated T cells require growth factors for cell division and, as with many rapidly dividing cells, in the absence of these growth factors they not only fail to divide but also undergo programmed cell death (25,26). This apoptotic response to cytokine withdrawal has been termed passive cell death, to differentiate it from activation-induced cell death (AICD). The best known example of AICD occurs when the Fas molecule (CD95) is engaged on activated T cells (26,27). CD28 costimulation prevents apoptosis from cytokine withdrawal in at least two ways. First and most directly, CD28 costimulation prevents the expression of the cell survival gene bcl-xL in antigen-activated T cells. Bcl-xL, a member of the Bcl-2 family, is a potent survival factor for T cells and prevents apoptosis even with growth-factor deprivation (25). Bcl-xL may also be able to inhibit Fas-mediated AICD, although this issue remains somewhat controversial (23,24). In addition to its direct effects in upregulating Bcl-xL, CD28 costimulation acts indirectly to prevent passive apoptosis through its ability to induce and augment cytokine secretion. This effect is mediated at least in part by the ability of IL-2 to upregulate the expression of Bcl-2 in T cells (28). Interestingly, although the ability of CD28 costimulation to induce IL-2 expression is important for T cell clonal expansion and T cell survival, IL-2 also has a counter-regulatory function; T cells are able to undergo AICD in response to Fas ligation only if they have been primed by prior exposure to IL-2 (Figure 3) (29). This effect of IL-2 is possibly mediated by increased expression of Fas ligand (FasL) and decreased expression of FLICE-like inhibitory protein (FLIP), an inhibitor of Fas signaling (29). In the absence of IL-2, T cells are refractory to AICD, resulting in unchecked lymphoid expansion and severe autoimmune syndromes (30). Therefore, it is possible that CD28 costimulation may be important for normal homeostatic regulation of the immune compartment.

CTLA-4 Receptor

Several years after the identification of the CD28 molecule and the recognition of its role in T cell activation, it was recognized that cytotoxic T lymphocyte antigen (CTLA-4) (CD152), a previously described molecule, is highly homologous to CD28 (17). Both molecules are members of the Ig gene superfamily and are located on the same chromosome (within 30 kb of each other) in mouse and human genes, making it highly likely that one arose as a duplication of the other. CTLA-4 binds the same ligands (CD80 and CD86) as does CD28. However, rather than sending a costimulatory signal into the T cell interior, it is generally agreed that CTLA-4 transduces a negative regulatory signal (Figure 4) (31–33). In the case of resting T cells first induced to enter the cell cycle, CTLA-4 ligation blocks the expression of IL-2 and its receptor
and leads to cell cycle arrest (31). In the case of previously activated T cells, it has been suggested that CTLA-4 activation can lead to apoptosis (34).

Although CD28 is expressed at high surface density on resting T cells, CTLA-4 is only minimally expressed on resting cells and is primarily detected after T cell activation (17). However, it is important to note that CTLA-4 has a higher affinity for CD80 and CD86 than does CD28. A model has been proposed in which CD28, a low-affinity but relatively abundant receptor, is preferentially able to bind CD80 and CD86 during the first 1 to 2 d after T cell activation, delivering costimulatory signals to support the immune response. Within 2 to 3 d after activation, upregulation of CTLA-4 occurs; this higher affinity receptor can effectively compete for CD80 and CD86 and turn off the immune response.

There is abundant evidence supporting a physiologic role for
CTLA-4 in regulating immune homeostasis. Mice with a targeted deletion of CTLA-4 die within a few weeks after birth, with massive lymphoid expansion and infiltration of critical parenchymal organs such as the heart (35,36). This probably is not an autoimmune response, with selective expansion of autoreactive T cells. More likely, this represents T cells that proliferate normally in response to environmental antigens, which now lack the regulatory signals that stop clonal expansion (and perhaps induce apoptosis). This syndrome of lymphoid expansion can be prevented by blockade of CD28 signals and is absent in recombination-deficient mice bearing a transgenic TCR that does not react with normal self or environmental antigens (37). These results demonstrate that CTLA-4 blocks T cell proliferation, which normally occurs in response to antigen-specific activation plus CD28 costimulation. It is also clear that the negative regulatory capacity of CTLA-4 is not exerted solely through inhibition of CD28 signals, and CTLA-4 ligation can suppress T cell responses even in CD28-deficient mice. It should be noted that some investigators have reported a positive signaling capacity for CTLA-4; however, this result has not been uniformly observed and is highly controversial (38).

**Signaling Pathways Through the TCR and CD28**

TCR ligation induces the expression of many cytokines by T cells. Some of these are used in an autocrine and/or paracrine manner by T cells themselves, and others direct the growth and differentiation of distinct cell types, such as B cells, macrophages, and natural killer cells. Among the cytokines that are important for T cells themselves, a predominant role has been ascribed to IL-2 (39). In vitro, this seems to be the major soluble growth factor for T cells and is also responsible for preventing anergy and inhibiting apoptosis (see above). Neutralizing antibodies to the cytokine itself or blocking antibodies to its receptor typically inhibit virtually all T cell proliferation in most in vitro systems. In vivo, the situation is probably more complex, because other cytokines, such as IL-4 and IL-7 (whose receptors share common signaling components with the IL-2 receptor), are able to substitute for IL-2 in supporting many immune responses (40).

TCR gene signaling and CD28 gene signaling cooperate to induce IL-2 gene expression (Figure 5). Transcription of the IL-2 gene is tightly regulated and cannot be induced by a single signaling pathway. Rather, multiple transcription factors, such as nuclear factor of activated T cells (NF-AT), activator protein-1 (AP-1) (Jun/Fos), nuclear factor-κB (NF-κB), and octamer-1 (Oct-1), are involved. This is complicated even further by the presence of a negative regulatory factor (see below).

Ligation of the TCR activates multiple intracellular signaling events. The earliest events include removal of inhibitory phosphate groups by CD45 tyrosine phosphatase, resultant activation of the Lck and Fyn tyrosine kinases associated with the CD4/CD8 coreceptor and TCR-CD3 complex, respectively, and phosphorylation of the TCR ζ-chain. This phosphorylation recruits and activates the ζ-associated protein-70 (ZAP-70), which results in three important signaling events. Two of these involve activation of phospholipase C-γ, which cleaves phosphatidylinositol into diacylglycerol and inositol trisphosphate. Diacylglycerol activates protein kinase C, which activates the transcription factor NF-κB. Inositol trisphosphate increases intracellular calcium concentrations, which activates the phosphatase calcineurin, inducing the transcription factor NF-AT. The third important signaling event is the activation of Ras. Kinases downstream of Ras, including extracellular-regulated kinase (ERK), ultimately induce and activate Fos, a component of the AP-1 transcription factor (19).

In the past several years, it has become clear that CD28 delivers a signal into T cells that does more than simply amplify the downstream effects of the TCR-CD3 complex. CD28 delivers biochemically distinct signals that synergize with TCR signals to promote IL-2 gene expression. CD28 has an intracellular motif (YMNM) that is highly conserved among species and that, when phosphorylated, serves as a binding motif for phosphatidylinositol-3-kinase (19). The phosphorylation of this intracellular motif most likely involves Lck (CD4-p56lck complex) and/or Fyn (TCR-CD3-p59fyn complex) protein tyrosine kinases. Despite the clear evidence that CD28 induces phosphatidylinositol-3-kinase, it is uncertain to what extent phosphatidylinositol-3-kinase induction is responsible for the costimulatory effects of CD28 (19,41).

Further downstream of the TCR-ζ-CD3 and CD28 signals, the mitogen-activated protein kinases c-Jun N-terminal kinase (JNK) and ERK are activated (19). Signaling downstream of TCR alone, via Ras, activates ERK but acts in synergy with CD28 to activate JNK, which is dependent on another GTPase, Rac (42). JNK then acts to phosphorylate c-Jun and to increase the transcriptional activity of AP-1 (Jun-Fos). The final event in the promotion of IL-2 gene transcription is the binding of the transcriptional factors generated downstream of TCR and CD28 to the promoter region 5’ of the IL-2 gene.

The IL-2 promoter is composed of two Oct-1 binding sites, four adjacent CD28 response elements (CD28RE) (made up of NF-AT/AP-1 binding sites), an additional NF-AT binding site, and an NF-κB binding site. All CD28RE must be bound for IL-2 gene transcription to occur. Both TCR-CD3 and CD28 signaling are therefore required. TCR-CD3 signaling leads to NF-AT entry into the nucleus, and both TCR-CD3 and CD28 contribute to the formation of AP-1. NF-AT and AP-1 then form a complex to bind the CD28RE and promote IL-2 gene transcription.

CD28 also regulates IL-2 production at the posttranscriptional level. IL-2 mRNA contains AU-rich elements (ARE) in its 3’ untranslated region that confer instability to the mRNA (43). Furthermore, ARE-binding proteins most likely target these AU-containing mRNA for degradation. Costimulation increases the half-life of IL-2 mRNA, which is inversely correlated with these ARE-binding proteins (43). Recently, the 3’ untranslated region was shown to contain at least two regions that stabilize IL-2 mRNA through activation of the JNK signaling pathway (44). It should also be noted that the transcriptional and mRNA-stabilizing effects of CD28 are not exerted...
on the IL-2 gene alone. A number of other cytokine genes, including TNF and IFN-γ, are similarly regulated by CD28.

**Signaling and Negative Regulators in Anergic T Cells**

A number of recent studies have characterized signaling events in anergic T cells, as part of an effort to understand the biochemical events that can regulate T cell nonresponsiveness. These studies demonstrated failure to activate Ras and decreased activation of downstream JNK and ERK, which can account for the defective AP-1 response and IL-2 production (45,46). It was recently shown that CD28 regulates the association of protein tyrosine kinases with the TCR-CD3 signaling complex (47). CD28 may prevent anergy by facilitating the effective association of TCR-ζ and CD3ε with the critical protein tyrosine kinase Lck (which also activates the intracellular motif of CD28) and the subsequent recruitment of ZAP-70 (47).

CD28 signaling has also been shown to inhibit transcription of negative regulators of IL-2. Binding of the zinc finger E box-binding protein (ZEB) (Nil-2-a) to a nucleotide sequence element (negative regulatory element-A [NRE-A]) 5’ of the IL-2 gene has been shown to inhibit IL-2 gene expression (48,49). There is increased binding of ZEB to the nucleotide response element-A site in anergic cells, compared with resting T cells, and this binding can block AP-1-induced activation of IL-2 gene transcription (49,50). Consistent with these observations, it has been shown that mutations in the region up-
stream of the IL-2 gene (positions −150 to −180) completely prevent anergy induction (51).

**Other Costimulatory Interactions**

**CD40/CD154 System**

The above discussion focused on the CD28 surface receptor as the primary mediator of costimulatory signals to T cells. However, many other molecules also have been proposed to provide costimulation. In most instances, it has been shown that these molecules (e.g., lymphocyte function associated antigen-1) are comitogenic (i.e., they support proliferation) and do so by augmenting the biochemical signals transduced through the TCR (43). These pathways do not transduce distinct biochemical signals and do not prevent anergy/apoptosis. In these ways, their effects are distinct from those of CD28, and they fall short of meeting strict criteria for the definition of costimulation.

One pathway that has received significant attention and is clearly important both in T cell costimulation and in transplantation is that mediated by CD40 and its ligand CD154 (formerly known as CD40L). CD154, a member of the TNF family of molecules, is expressed on activated T cells, primarily CD4+ T cells (52). CD40 is a member of the TNF receptor family of molecules and is expressed on a variety of cell types, including APC, endothelial cells, and keratinocytes. In the case of B cells, ligation of CD40 provides important cell survival signals, augments Ig production, and induces an Ig isotype switch from IgM to IgG and IgE (52). The CD40/CD154 interaction is extremely important for humoral immunity. In fact, these molecules first came to widespread attention with the identification of CD154 as the site of the genetic defect responsible for X-linked hyper-IgM syndrome in human subjects, a syndrome that is characterized by profound defects in humoral immune responses.

More recently, it has become clear that the CD40/CD154 pathway is important in T cell responses as well (see below), although the mechanisms of this effect are not completely understood. Some experimental evidence suggests that CD154 directly transduces a positive signal to the cell interior, although it presently seems doubtful that this accounts for most of the observed in vivo effects of this pathway in cellular immune responses. Rather, the bulk of the data indicates that CD40 ligation of APC (and other cells) promotes their ability to induce and support T cell responses. This may be accomplished in any or all of several ways. First, in the case of all APC, engagement of CD40 induces the expression of B7 molecules, particularly B7-1 (53). Therefore, it has been suggested that part of the mechanism of action of the CD40/CD154 pathway in supporting T cell immune responses is indirect, i.e., through induction of CD28 ligands and resultant activation of the CD28 pathway. Second, in addition to B7, other molecules important for T cell responses are induced in CD40-activated APC; these molecules include adhesion molecules such as intercellular adhesion molecule-1 and CD44H, both of which may also have stimulatory effects on T cells (54). Third, CD40 stimulation of macrophages and dendritic cells signals for production of IL-12 (52). This cytokine supports IFN-γ production and proinflammatory Th1 immune responses. Finally, in certain cell types, such as endothelial cells, CD40 ligation induces the expression of cell adhesion molecules such as CD62E, CD54, and CD106, which are important for lymphocyte migration to inflammatory sites, as well as stimulation and effector functions (52).

**4-1BB (CD137) and Heat-Stable Antigen (HSA)**

In addition to the CD28/B7 and CD40/CD154 interactions just described, two other T cell pathways deserve special note, namely those involving 4-1BB ligand (4-1BBL) and HSA. Like CD154, 4-1BB (CD137) is a member of the TNF receptor family gene and is expressed on activated T cells (55). Its ligand, 4-1BBL, is a TNF family member and is found on activated APC (56). In murine experimental systems in which the CD28 pathway is blocked (using monoclonal antibodies or fusion proteins) or genetically targeted (using CD28-knockout mice), it has been shown that 4-1BBL can “replace” CD28 and provide important signals to T cells, supporting immune responses (57). Blockade of 4-1BB in situations in which CD28 costimulation is not available strongly inhibits both T helper and T cytotoxic immune responses. We do not yet know, however, the extent to which 4-1BB might be used during physiologic immune responses. Published data support the prediction that the primary role of 4-1BB would be in responses in which CD28 costimulation is not available; however, we do not know which, if any, in vivo responses occur in the absence of CD28 signals.

HSA is a molecule that is expressed early during T cell development in the thymus. Recently, several studies have suggested that HSA could serve as a T cell costimulatory molecule (58). As with 4-1BB, a primary role for HSA has been found in situations where CD28 costimulation is blocked, so the physiologic relevance of this pathway is unknown. It has also been suggested that HSA might be a preferential costimulatory pathway for memory T cells, with CD28 being reciprocally important for naive T cells. Many studies have shown that the costimulatory requirements for naive T cells differ from those for memory T cells, but it has not been clear whether this difference is quantitative, qualitative, or both. If HSA is relatively specific for memory T cells, then this would have important implications for strategies designed to block immune responses in primed hosts, a situation that is often quite refractory to intervention.

**Induction of Transplantation Tolerance by Blockade of Costimulatory Signals**

In the past several years, a variety of laboratories have shown that blockade of T cell costimulatory signals can improve long-term allograft survival rates and induce transplantation tolerance. Most of these studies have used either CTLA4Ig, a fusion protein of CTLA-4 and human Ig that competitively binds CD80 and CD86, or a blocking monoclonal antibody to CD154 (59). Because this area has been the subject of a recent review (9), in this article we limit ourselves to noting that, from the many studies that have been performed
to date, several important principles emerge. First, this strategy is effective in many models. Costimulatory blockade has been successful in mouse and rat models of cardiac, hepatic, islet, renal, lung, and bone marrow transplantation (60,61). Second, only a short-term course of therapy is needed. In most instances, single doses or at most a 7-d course of treatment can achieve maximal results. Third, although a single agent alone, such as CTLA4Ig or anti-CD154 antibody, can improve long-term graft survival rates, these agents by themselves are unlikely to yield indefinite graft survival; late allograft loss resulting from chronic rejection is the rule. Most commonly, either a transduction of donor-specific lymphocytes or the combination of CTLA4Ig and anti-CD154 is required for long-term survival, with or without tolerance (62,63). Fourth, this strategy seems to be safe. No adverse side effects have been reported. Fifth, the usual limitations of tolerogenic strategies apply. For example, skin transplants, even in mice, are more resistant to tolerance induction. Furthermore, the results in nonhuman primates are not as good as those in rodent models. To date, there have been three reported studies of nonhuman primates, one with islet allografts and two with renal allografts (64–66). Although the numbers of animals are small, one can tentatively conclude that CTLA4Ig alone seems to be inadequate therapy and the combination of CTLA4Ig and anti-CD40L antibody is additive.

An important issue regarding the use of costimulatory blockade in clinical transplantation is how this blockade would interact with currently used immunosuppressive drugs. In murine models in which CTLA4Ig and/or anti-CD40 antibody has been used to induce tolerance, it has been shown that concomitant administration of cyclosporine prevents tolerance induction (63). It seems that the induction of tolerance in T cells deprived of costimulatory signals is an active process involving TCR signaling events that are sensitive to cyclosporine. Therefore, in the presence of cyclosporine, tolerance cannot be achieved by this means. On the other hand, in situations in which CTLA4Ig prolongs graft survival but does not induce tolerance, e.g., in nonhuman primates, cyclosporine has an additive effect (66). Therefore, the effect of cyclosporine on costimulatory blockade seems to depend on whether the regimen is inherently tolerogenic or merely immunosuppressive.

Interestingly, the negative regulatory signals transduced by CTLA-4 may be critical for tolerance induction (33). In a protocol in which the combination of CTLA4Ig, given 2 d after transplantation, and donor-specific lymphocytes is used to induce cardiac allograft tolerance in mice, blockade of CTLA-4 at the time of transplantation prevents tolerance induction and leads to early rejection (67). Therefore, early CTLA-4 signals may be permissive for some T cell tolerogenic/inhibitory strategies; without these signals, it may prove difficult to turn off the immune response. Similarly, in murine models of autoimmune disease, blockade of CTLA-4 exacerbates the duration and severity of the illness (68–70). Because agents such as CTLA4Ig prevent CD80 and CD86 from binding to both CD28 and CTLA-4, they have both the potential to block positive signals (through CD28) and the undesired ability to block negative signals (through CTLA-4). It is tempting to speculate that agents that would specifically prevent CD28 ligation but would permit CTLA-4 to bind to its ligands would prove even more effective.

**Other Uses of Costimulatory Blockade in Transplantation**

Although most attention has been focused on the use of costimulatory blockade to induce tolerance, recent studies suggest an additional potential use, i.e., prevention of ischemia-reperfusion injury (71,72). Perhaps surprisingly, recent studies demonstrated a T cell–mediated component to ischemia-reperfusion injury. Additional work showed that this component can be blocked by inhibition of costimulatory signals. In animal models, the ability of CTLA4Ig to prevent ischemia-reperfusion injury is correlated with prevention of chronic rejection, a finding that may have important implications for human studies.

**Summary**

The discovery of T cell costimulatory pathways and the development of agents to block these pathways have provided a new set of strategies to induce transplantation tolerance. Although these approaches are extremely promising, the potential of these agents to improve patient and allograft survival rates can be determined only by rigorous clinical trials.

**References**


