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T cell activation and transplantation tolerance

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Abstract

Transplantation of allogeneic or “non-self” tissues stimulates a robust immune response leading to graft rejection, and therefore most recipients of allogeneic organ transplants require the lifelong use of immune suppressive agents. Excellent outcomes notwithstanding, contemporary immunosuppressive medications are toxic, are often not taken by patients, and pose long-term risks of infection and malignancy. The ultimate goal in transplantation is to develop new treatments that will supplant the need for general immunosuppression. Here we will describe the development and application of costimulation blockade to induce transplantation tolerance and discuss how the diverse array of signals that act on T cells will determine the balance between graft survival and rejection.

INTRODUCTION

A promising alternative to immunosuppression to enhance allograft survival in transplant recipients is the use costimulation blockade to induce immune tolerance specifically to allogeneic antigens [1; 2; 3; 4; 5; 6; 7; 8]. However exposure to infectious agents and inflammatory cytokines significantly reduces the effectiveness of current costimulation blockade protocols in preventing rejection of allografts [9; 10; 11]. Understanding the requirements for activation of alloreactive immune responses and how this is altered by exposure to pathogens and inflammatory agents is critical for the development of robust protocols to induce tolerance to alloantigens. Here we discuss the parameters that are essential for the activation of alloreactive T cells and induction of transplantation tolerance and how unexpected activation of innate and adaptive immune systems impact allograft survival.

T CELL ACTIVATION

T cells are a critical component of the immune response to allogeneic tissues, directly mediating rejection and graft-versus-host disease (GVHD) [12]. The activation of naïve T cells is a tightly regulated event and requires three distinct signals for the generation of an optimal response, including T cell receptor (TCR) engagement (signal 1), costimulation (signal 2), and cytokine stimulation (signal 3) [13]. T cells receiving the appropriate

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combination of these signals will initiate a programmed pathway of differentiation early during activation, and this will determine the magnitude and functionality of the ensuing response [14; 15; 16]. Most efforts to tolerize T cells in an antigen-specific manner have focused on delivering “signal 1” through the TCR in the absence of “signal 2”. Below, we will describe the importance of these signaling pathways in activation of alloreactive T cells (Figure 1A).

Signal 1: TCR engagement

The antigen binding component of the TCR complex is a heterodimer composed of an α and β chain [17] that recognizes proteolytically processed short peptides (8–15 amino acids) presented in the context of self major histocompatibility complex (MHC) on antigen-presenting cells (APC) [18; 19]. Zinkernagel and Doherty originally demonstrated that antigen-specific T cells recognize foreign peptides presented by self-MHC, by showing that cytotoxic T cells (CTL) lysed only virus-infected target cells that were matched at the MHC loci [20; 21]. Interestingly, a significant frequency of T cells also have the ability to recognize MHC molecules that are not present within the thymus during selection in a process referred to as allo-recognition, with both class I and II serving as targets [22]. These alloreactive T cells are present at significantly higher frequencies (100–1000 fold higher) than T cells specific for individual foreign peptides presented by self-MHC, allowing for the generation of strong primary immune responses to transplanted non-self tissues and rapid rejection of allografts [23; 24; 25].

Alloreactive T cells recognize alloantigens through two distinct pathways, the direct and the indirect pathways. In the direct pathway, T cells recognize an intact donor MHC antigen on the tissue allograft and in the indirect pathway, T cells recognize donor peptide antigens presented by self MHC [26; 27]. Two models have been proposed for direct allo-recognition [28; 29]. One is the high-density determinant model where alloreactive T cells recognize donor MHC molecules irrespective of the specific peptide presented. The alternative is the multiple-binary complex model, where alloreactive T cells recognize both the bound-peptide and the allogeneic MHC complex. For the indirect pathway, antigens from allogeneic cells are processed and presented in the context of self MHC molecules. Recipient APC can acquire these allogeneic peptides from dying donor APCs present within draining secondary lymphoid tissues or from donor cells directly at the graft site [30]. T cells recognizing alloantigens through either the direct or indirect pathways can mediate rejection of allografts [31].

Signal 2: Costimulation

The second signal that is crucial for productive T cell activation is delivered by the engagement of one or more costimulatory molecules expressed by T cells with their ligands expressed on APC [32]. One of the earliest costimulatory molecules to be identified and extensively studied is CD28, which is expressed on the cell surface of all naïve CD4 and CD8 T cells. TCR stimulation in the absence of CD28 engagement results in abortive activation of T cells and anergy [33]. Binding of CD28 to its ligands (B7-1, CD80 and B7-2, CD86) on APC promotes optimal TCR signaling events that trigger IL-2 production, clonal expansion and generation of effector and memory T cells [34]. Following the discovery of CD28, cytotoxic T lymphocyte antigen-4 (CTLA-4, CD152) was identified based on structural homology to CD28, and a functional role for this molecule was suggested by the ability of a CD152-Ig fusion protein to inhibit T cell activation [35]. CD152 binds to CD80 and CD86 with much higher affinity than CD28, and in contrast to CD28, provides an inhibitory signal to T cells [36]. Recently, additional costimulatory molecules related to the CD28 immunoglobulin superfamily have been discovered, including the inducible costimulatory molecule (ICOS)-B7h and the programmed death (PD)-PD-L1/PD-L2

pathway [37]. ICOS is a positive costimulatory molecule that is present at low levels on resting T cells but is rapidly upregulated in a CD28-dependent manner during activation [38; 39]. One mechanism by which ICOS-B7h engagement augments T cell activation is by inducing the expression of CD40L (CD154) on T cells that in turn stimulates the up-regulation of CD80 and CD86 molecules on APCs and provides a positive feedback loop in sustaining CD28 costimulation [37]. The (PD)-PD-L1/PD-L2 pathway is a negative costimulatory pathway that has an important role in the maintenance of self-tolerance. PD-1 is expressed on activated CD4 and CD8 T cells, B cells, NK cells and macrophages [40]. PD-1 interacts with PD-L1 and PD-L2 that are expressed by APCs, endothelial cells and parenchymal cells [41; 42; 43]. A role for PD-1 in self-tolerance was revealed by the discovery that PD-1 deficient mice exhibit an autoimmune phenotype with lupus like-glomerulonephritis and progressive arthritis [44]. While many studies have demonstrated that PD-1 provides an inhibitory signal to T cells [45], PD-1 signaling also enhances T cell activation, stimulates proliferation in vitro, and accelerates autoimmunity and allograft rejection [46; 47; 48].

The tumor necrosis factor family-tumor necrosis factor family receptor (TNF-TNFR) superfamily members also provide costimulatory signals important in the generation of antigen-specific T cell responses. Members of the TNF-TNFR super family include CD40-CD154, OX40-40L (CD134-CD134L), 4-1BB-4-1BBL (CD137-CD137L), CD27-CD70, CD30-CD30L and HVEM-LIGHT (herpes-virus entry mediator - lymphotoxin like inducible protein that competes with glycoprotein D for HVEM on T cells) [49]. CD154 and CD40 were the first members of the TNF-TNFR superfamily that were demonstrated to provide costimulatory signals to T cells and have been extensively studied as targets to block for the induction of transplantation tolerance [50]. CD40 is constitutively expressed on B cells, dendritic cells (DCs), macrophages and thymic epithelium and is inducible on endothelial cells and fibroblasts during inflammation [51]. CD154 is expressed on activated T cells, NK cells and eosinophils [37]. The interaction of CD40-CD154 initiates signaling in the APCs that enhances antigen presentation and stimulates the up-regulation of CD80/86 costimulatory molecules [37]. The CD27-CD70 and HVEM-LIGHT pathways also provide positive costimulatory signals to T cells [32]. CD27 and HVEM are constitutively expressed on naïve T cells and are believed to contribute to the initial activation of T cells [52; 53]. These costimulatory molecules have been shown by several in vitro and in vivo studies to augment T cell responses. For example, blocking HVEM-LIGHT interactions in vitro can inhibit early T cell activation and cytokine secretion in allogeneic mixed-lymphocyte reactions [49; 54]. Blockade of CD27-CD70 interactions also inhibits proliferation and cytokine production by T cells [55; 56]. The CD134-CD134L and CD137-CD137L pathways also provide positive signals for T cells during activation [32]. Both CD134 and CD137 are absent or expressed only at low levels on resting T cells, and their expression is up-regulated following activation [49]. CD134 augments the generation of both Th1 and Th2 T cell responses and has an important role in the generation of memory CD4 T cells [57; 58; 59; 60]. CD137 signaling augments T cell proliferation and cytokine production and is critical for the generation of CD8 T cell responses to viral infections and peptide immunizations [61; 62]. The CD30-CD30L pathway has been shown to both promote Th2 immune responses in human PBMC and to negatively regulate effector CD8 T cell responses in autoimmunity and allograft rejection [63; 64; 65; 66]. Overall, there is a broad array of costimulatory pathways that either enhance or dampen T cell activation, and these pathways are all potential targets for blocking T cell activation and inducing T cell tolerance to alloantigens.

Signal 3: CD4 help and inflammation

In addition to signal 1 (TCR) and signal 2 (costimulation), naïve CD8 T cells also require a third signal for the optimal generation of effector and memory T cells [67]. Two examples of third signals enhancing the induction of antigen-specific CD8 T cell responses are CD4 T cell help [68; 69] and the presence of inflammatory cytokines [13]. The specific role of CD4 T cells in supporting the activation of CD8 T cell responses is dependent on the nature of the antigenic challenge. For example, CD8 T cells activated under conditions of limited inflammation are often critically dependent on CD4 T cell help for generation of a primary immune response [70; 71; 72]. One mechanism for this help is the maturation or “licensing” of APC by engagement of CD40 on the APC by CD154 expressed by activated CD4 T cells. In contrast, infection with pathogens that induce significant levels of inflammation, such as influenza virus, lymphocytic choriomeningitis virus (LCMV) or vesicular stomatitis virus, does not require CD4 help to induce primary CD8 T cell responses [68; 73]. However, the generation of functional CD8 memory after infection is dependent on CD4 T cell help received during the initial activation of the CD8 T cells [74; 75; 76]. A second mechanism by which CD4 T cells can provide help to CD8 T cell responses is by the production of IL-2 [67]. IL-2-signalling appears to have only a minimal role in the generation of primary CD8 T cell responses to viral infection or cellular antigens but is critical for the development of functional memory CD8 T cells [77; 78; 79]. Moreover, IL-2 production by CD4 T cells is necessary for the induction of effector and memory CD8 T cells following immunization with peptide-coated dendritic cells [80]. The generation of primary and memory alloreactive CD8 T cell responses and allograft rejection occurs in the absence of CD4 help, but optimal alloreactive CD8 T cell responses, in terms of frequency and functionality, require CD4 T cell help [81; 82; 83]. A recent study has also suggested that B cells are necessary for the development and survival of memory alloreactive CD4 and CD8 T cells during the rejection of skin allografts, although the exact mechanism(s) has not yet been identified [84].

Inflammatory cytokines also augment the generation of CD8 T cell responses [13]. In vitro studies first showed that the addition of IL12 and type-I interferon (IFN) during stimulation of naïve CD8 T cells with artificial APCs (microspheres coated with class-I MHC/peptide complex and CD80) resulted in strong clonal expansion and cytolytic function [85; 86; 87]. Moreover, T cells deficient in the cytokine receptors for type-I IFN or IL12 show reduced clonal expansion and generation of CD8 memory T cells during infection [88; 89; 90; 91]. These cytokines are thought to increase the expression of pro-survival molecules, such as B cell lymphoma-3 (Bcl-3), by CD8 T cells [92]. Engagement of Toll-like receptors (TLRs) on APCs triggers the production of type-I IFN and IL12 [93; 94]. For example, a recent study has shown that treatment of mice with polyinosinic:polycytidylic acid [poly(I:C)], which stimulates production of high levels of type-I IFN, will sensitize naïve phenotype CD8 T cells for rapid effector function, including production of IFN γ and increased levels of granzyme B [95]. This sensitization was driven by presentation of self antigens by MHC class I molecules and by indirect IFN-signaling, which resulted in up-regulation of eomesodermin (a T-box transcription factor) by the naïve CD8 T cells. Together these studies indicate the inflammatory milieu during T cell activation that will determine the ultimate fate of T cells.

To date, the requirement for a third signal in the generation of alloreactive CD8 T cell responses has not been firmly established [96]. Early studies exploring the importance of innate immunity in the rejection of allografts focused on the myeloid differentiation marker 88 (MyD88), an adaptor molecule involved in signaling through many TLRs and the receptors for IL1 and IL18. For example, minor antigen mismatched skin grafts were not rejected in a system where both the host and donor were MyD88 deficient, and this was attributed to the inability to generate an effective T cell response to the minor antigens [97]. In contrast, the rejection of fully MHC mismatched skin and cardiac grafts occurs in the

absence of MyD88 signaling, even though the frequency of IFN- γ and IL2 producing alloreactive T cells is decreased [98]. A more recent study by Lakkis and colleagues evaluated the role of type-I IFN in the induction of alloreactive immune responses and in the rejection of both minor antigen and fully MHC mismatched skin grafts [99]. Type-I IFN receptor deficient mice generated both primary and memory alloreactive T cells and were able to reject skin allografts. Together these studies suggest that there are redundant third signals for the generation of alloreactive T cell responses.

INDUCTION OF TRANSPLANTATION TOLERANCE BY COSTIMULATION BLOCKADE

A central goal for transplantation immunologists has been to exploit mechanisms of self-tolerance to induce specific tolerance to allogeneic tissues during transplantation (Figure 1B). Blockade of costimulatory signals during the activation of alloreactive T cells has proven to be an effective method to induce tolerance to allografts in mice and non-human primates [2; 100]. The effectiveness of costimulation blockade is based on the abortive activation of antigen-specific T cells following TCR engagement (signal 1) in the absence of costimulation (signal 2) [101; 102].

The CD28-CD80/86 and the CD40-CD154 pathways (Table 1) have been the most rigorously tested in blockade protocols for the ability to induce transplantation tolerance [32]. Blockade of CD28 engagement on recently activated T cells can be accomplished efficiently using a fusion molecule comprised of the extracellular domain of CD152 and the Fc fragment of IgG [103]. CD152-Ig prevents the *in vitro* activation of alloreactive T cells in mixed leukocyte cultures [104; 105]. *In vivo* blockade of the CD28 pathway using CD152-Ig prevents rejection of cardiac, renal and islet allografts in rodent models [106; 107; 108; 109]. In addition, the combination of this CD28 blockade and an infusion of donor specific cells (donor specific transfusion or DST) induces a more robust allograft tolerance [110; 111]. However CD28 blockade is not as efficient in preventing allograft rejection in non-human primate models [112].

Blockade of CD40-CD154 interactions has also shown promise for the induction of transplantation tolerance. Blocking of CD40-CD154 interaction using anti-CD154 monoclonal antibody (mAb) in combination with an infusion of DST significantly prolongs skin, cardiac, islet and bone marrow allografts survival in mouse models [6; 7; 8; 113; 114; 115]. Treatment with anti-CD154 mAb is also effective in prolonging skin, islet and renal allografts in non-human primate models [112; 116; 117; 118; 119]. Unfortunately, clinical trials with anti-CD154 antibodies led to thromboembolic complications likely due to expression of CD154 on activated platelets [120; 121]. However, there is still enthusiasm for targeting this pathway using novel reagents and for understanding mechanisms of tolerance induction that may be translatable to other immunomodulatory agents. For example the use of anti-CD40 antibodies to block the CD40-CD154 pathway has shown promise in non-human primates for kidney transplants and in mice for islet transplants [122; 123; 124]. Finally, combination blockade of CD28 signaling using CD152-Ig and CD40 signaling using anti-CD154 or anti-CD40 mAb also induces a robust tolerance to alloantigens, including tolerance to skin allografts [122; 125; 126; 127; 128]. In addition to these classic models, the ICOS, CD134, CD137 and CD27 costimulation pathways are being evaluated as targets of blockade for the induction of transplantation tolerance [37].

MECHANISM OF TOLERANCE INDUCTION DURING COSTIMULATION BLOCKADE

As described above, costimulation blockade is an effective means to induce transplantation tolerance. There are several features of transplantation tolerance that are synonymous to self-tolerance. For example, both phenomena require tolerization to large number of antigens [100] and can occur through central or peripheral mechanisms. Central tolerance is induced by injection of donor bone marrow into conditioned hosts to establish allogeneic hematopoietic chimerism [129; 130; 131]. This chimerism results in the clonal deletion of alloreactive T cells and B cells during their development. Peripheral tolerance requires the deletion of mature alloreactive T and B cells residing in the periphery through mechanisms such as deletion, anergy and regulation [132; 133]. Below we briefly describe mechanisms of tolerance induction that includes deletion, anergy and regulation in the context of transplantation tolerance.

Deletion

A central barrier to the induction of tolerance to allogeneic tissues is the high precursor frequency of alloreactive T cells [23; 25]. Thus, an important parameter for establishment of transplantation tolerance is the deletion of alloreactive T cells through either passive or active death pathways [134; 135]. One example of an active mechanism for the deletion of peripheral T cells is activation induced cell death (AICD), which involves the death of activated T cells that have been re-stimulated by TCR engagement and that have received signals through cell death receptors such as Fas (CD95) and TNF-receptors [136]. The importance of active cell death in the deletion of alloreactive T cells was demonstrated in CD95-deficient mice, which are resistant to AICD [137]. In this study, blockade of CD28 and CD40 during bone marrow transplantation did not reduce the levels of alloreactive CD4 T cells in CD95 deficient mice.

Passive cell death is triggered by extracellular stress, growth factor deprivation, cytotoxic drugs or irradiation and is regulated by the members of the Bcl-2 family that consists of pro-survival proteins such as Bcl-2, Bcl-xL (bcl-211), bcl-w (bcl-212), Mcl1 and other pro-apoptotic members such BAX, BAK, BAD and BIM [138]. A role for passive cell death in the deletion of alloreactive T cells during the induction of tolerance has been shown in mice over-expressing Bcl-xL in T cells. Bcl-xL is a pro-survival factor and over-expression in T cells makes them resistant to passive cell death [139]. Transgenic expression of Bcl-xL abrogates induction of tolerance by blockade of CD28-CD80/86 or CD40-CD154 interactions and leads to chronic rejection of cardiac allografts [135] and acute rejection of bone marrow transplants [140].

Anergy

Even though a significant frequency of alloreactive T cells are deleted during the induction of transplant tolerance by costimulation blockade, a low level of these antigen-specific T cells remain detectable in the periphery [134; 141; 142]. These alloreactive T cells that escape deletion during tolerance induction appear anergic or unresponsive [50; 143]. Anergic T cells fail to undergo activation following TCR engagement due to a reduced ability to mediate signaling events downstream of the TCR [144]. Moreover, anergic T cells are functionally unresponsive to IL2 signaling, produce reduced levels of IL2 and fail to undergo proliferation [145; 146]. Anergic CD4 T cells have also been shown to promote a tolerogenic environment by inhibiting the maturation of DCs by an undefined cell contact-dependent mechanism [147].

Regulatory T cells

One of the major mechanisms essential for the induction of transplantation tolerance is CD4⁺ CD25⁺ FoxP3⁺ regulatory T cells, or Tregs [148; 149]. Tregs are essential to maintain immune homeostasis and to prevent the development of autoimmunity by suppressing effector T cells and the function of APCs [150; 151]. During costimulation blockade the balance of Tregs to effector T cells determines if allografts are maintained or rejected. The ability of Tregs to regulate immunity can be attributed to a number of mechanisms, including the secretion of soluble factors such as IL10, TGF- β , IL35 and galactin-1, the cell surface expression of immune modulatory proteins, such as CD152, LAG-3 (CD223) and CD39, the direct lysis of immune cells by perforin- and granzyme-dependent pathways and the consumption of cytokines such as IL2 [152]. The importance of Tregs for transplantation was demonstrated in studies showing that Tregs reduced mouse GVHD and could adoptively transfer tolerance to mouse allografts [27; 153; 154]. In humans, an important role for Tregs has been suggested by the positive correlation with Tregs number and graft survival in individuals receiving lung, kidney, liver and islet allografts [155; 156; 157; 158; 159]. In addition, recent studies in mouse models engrafted with functional human immune systems have demonstrated the ability of Tregs to suppress rejection of human arterial and skin allografts by human immune systems [160; 161].

IMPACT OF INFECTION AND INFLAMMATION ON COSTIMULATION BLOCKADE

Many studies have demonstrated the efficacy of costimulation blockade to induce transplantation tolerance in naïve mice. However, humans are exposed regularly to infectious agents and to a wide array of environmental antigens that stimulate both the innate and adaptive arms of the immune system. More recent studies have evaluated the impact of infection and inflammation on the ability to induce transplantation tolerance in mouse models (Figure 1C). Our laboratory first demonstrated that acute infection of mice with LCMV and Pichinde virus abrogated tolerance to skin allografts induced by costimulation blockade and resulted in rejection [162]. Interestingly, allograft tolerance was broken by viral infection only during the early phase of costimulation blockade (days 1 to 15), as mice infected at later time points did not reject their skin allografts. LCMV infection was subsequently shown to prevent the deletion of alloreactive T cells during costimulation blockade [163]. Extending these findings, others and we showed that active and persistent viral infections abrogated the induction of tolerance by costimulation blockade [142; 164; 165; 166; 167]. In addition to viruses, acute bacterial and protozoan infections also impair the induction of transplantation tolerance by costimulation blockade [168; 169; 170; 171]. Finally, an individual's past viral infections may also impact their ability to be tolerized to alloantigens, as virus-immune mice are refractory to the induction of tolerance by costimulation blockade [172; 173; 174]. Together, these studies demonstrate the challenges for the use of costimulation blockade in the clinic.

Two hypotheses have been proposed to explain the ability of infection to block the induction of tolerance by costimulation blockade. One hypothesis is that the pathogen-specific T cell responses generated by infection cross-react with alloantigens and are able to mediate allograft rejection even in the presence of costimulation blockade [175]. The cross-reactive hypothesis is supported by studies showing that virus-specific CD8 T cells from both mice and humans directly recognize allogeneic cells [173; 174; 176; 177; 178; 179] and by the detection of memory alloreactive T cells in individuals never exposed to alloantigens [180]. A second hypothesis for the abrogation of tolerance by infection is that the inflammatory environment generated by the infection prevents the deletion of alloreactive T cells by costimulation blockade [181]. We have shown that infection with LCMV enhances the

activation of alloreactive T cells during exposure to allogeneic cells in vivo and prior to the activation of virus-specific T cells [182]. Moreover exposure to TLR ligands, which strongly activate the innate immune system and stimulate inflammatory cytokine release, also prevents the induction of tolerance to allografts by costimulation blockade [114; 142; 183; 184]. Mice undergoing costimulation blockade and being simultaneously treated with LPS or poly(I:C) produce significant quantities of type-I IFN (signal 3), which prevents the deletion of the alloreactive T cells and directly stimulates CD8 T cell cytotoxic activity [184]. Exposure to the TLR-9 agonist CpG, also breaks tolerance induced by costimulation blockade [114; 142], and the mechanism for this has been attributed to reduced Treg functionality [185] and to the production of IL6 and IL17 [186]. It is therefore possible that T cell cross-reactivity and inflammation operate concurrently to abrogate transplantation tolerance after infection.

CONCLUSIONS

Costimulation blockade is an appealing approach to induce tolerance to allografts and has enjoyed a high level of success in pre-clinical models. However, for this approach to translate to the clinic, we need to overcome the barriers presented in “real world” scenarios, such as infection and exposure to inflammatory agents. The continued investigation of the diverse array of signals that influence the immune response to alloantigens will be critical for success of costimulation blockade in transplant recipients.

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Figure 1.

Activation and tolerization of alloreactive T cells. A) Optimal T cell activation requires 3 signals, including: TCR engagement (Signal 1), costimulation (Signal 2) and cytokine stimulation (Signal 3). B) Costimulation blockade induces transplantation tolerance and prevents allograft rejection. C) Exposure to infectious agents or inflammatory stimuli during costimulation blockade abrogates the induction of tolerance.

Table 1

Strategies to Blockade CD28-B7 and CD40-CD154 Pathways for the Induction of Transplantation Tolerance.

Targeted Pathway	Tissues Successfully Transplanted	References
CD28-B7		
-CD152-Ig (CTLA4-Ig)	Rodent heart	[106; 107; 108; 109;110; 111; 125; 187]
	Rodent islets	
	Rodent kidney	
-anti-CD80/86 mAb	Non-human primate kidney	[188; 189]
-anti-CD28 mAb (agonistic)	Rodent renal	[190; 191]
CD40-CD154		
-anti-CD154	Rodent heart	[6; 7; 8; 113; 114; 115]
	Rodent islets	
	Rodent kidney	
	Rodent skin	
	Rodent bone marrow	
	Non-human primate skin	[112; 116; 117; 118; 119]
	Non-human primate islets	
	Non-human primate kidney	
	Porcine islets	[192; 193]
-anti-CD40	Rodent islets	[122; 127]
	Rodent skin	
	Rodent bone marrow	[123; 128]
	Nonhuman primate kidney	
	Porcine islets	[124]