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Recent progress and new perspectives in studying T cell responses to allografts

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Abstract

Studies in the past decade advanced our understanding of the development, execution and regulation of T cell-mediated allograft rejection. The current review outlines recent progress and focuses on three major areas of investigation that are likely to guide the development of graft-prolonging therapies in the future. The discussed topics include the contribution of recently discovered molecules to the activation and functions of alloreactive T cells, the emerging problem of alloreactive memory T cells, and recently gained insights into the old question of transplantation tolerance.

INTRODUCTION

Adaptive immune responses to donor antigens are a potent barrier to successful transplantation. Allograft rejection is initiated and, in many cases, executed by T cells primed in peripheral lymphoid organs and recruited to the graft. Tremendous progress in our understanding of T cell mediated allograft rejection has been made in the last decade. A series of seminal studies has uncovered the nature of allorecognition, characterized the frequencies and cytokine profiles of T cells primed in response to transplantation, and indentified major effector mechanisms mediating allograft tissue injury. The successful blockade of the well-characterized CD28/CD80/CD86 and CD40/CD154 costimulatory pathways to prolong allograft survival in rodents launched multiple studies aimed at achieving long-term graft survival and, possibly, donor-specific tolerance. These studies were often influenced by knowledge simultaneously gained in the fields of infectious disease, autoimmunity and tumor immunology.

In this review of basic science investigation into mechanisms underlying cell-mediated allograft rejection, we have chosen to focus on three areas that rapidly advanced in the past ten years and that are likely to shape the field of transplantation immunology in the near future. First, we will discuss how recent advances in basic T cell immunobiology apply to the field of transplantation. Then, we will turn to studies revealing the role of alloreactive memory T cells as a major barrier to successful transplantation. Finally, we will consider recent progress in our understanding of transplantation tolerance and its mechanisms.

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NOVEL INSIGHTS INTO THE ACTIVATION AND EFFECTOR FUNCTIONS OF ALLOREACTIVE T CELLS

The hallmark features of T cell alloimmune responses are the numerous antigenic epitopes and the high numbers of reactive T cell clones. The two-signal concept of T cell activation afforded the potential to target multiple clones of alloreactive T cells without defining their specificity. Until recently, mainstream strategies for diminishing alloresponses and prolonging allograft survival have been directed at the “conventional” costimulatory pathways, CD28/CD80/CD86 and CD40/CD154. The identification of additional costimulatory molecules has prompted investigations of their roles during T cell alloresponses and the consequences of interfering with these pathways in hope to improve allograft outcomes and potentially achieve tolerance. The “alternative” costimulatory pathways include but are not limited to the members of the immunoglobulin superfamily: inducible T cell costimulator (ICOS) and programmed death-1 (PD-1); and, the molecules from the tumor necrosis factor receptor superfamily CD134 (OX40), CD27, CD137 (4-1BB) and CD30. It should be noted that the terms “conventional” and “alternative” do not presume functional hierarchy but rather reflect the chronology of discovery and, to some degree, their initially reported functions during primary and secondary T cell responses. While all of these pathways have been implicated in the processes of allograft rejection and/or acceptance (summarized in Table 1), the contributions of ICOS/B7RP-1 and CD134/CD134L signaling to these processes have been investigated in more detail.

The effects of recipient ICOS deficiency or ICOS blockade has been tested in many transplant models. Disruption of ICOS/B7RP-1 interactions modestly prolonged survival of heart, liver and islet allografts in fully MHC-mismatched rodent models (1–6). The prolonged survival was associated with the decreased expansion of donor-reactive T cells and with lower serum titers of donor-reactive alloantibody (7). ICOS blockade also promoted long term allograft survival in synergy with other graft-prolonging treatments, such as anti-CD154 mAb, CTLA4-Ig, cyclosporine and rapamycin, making it an attractive therapeutic candidate (1, 3, 5, 6).

In contrast to blocking CD28/CD80/CD86 or CD40/CD154 pathways, early administration of anti-ICOS antibody had little effect on allograft outcome in a model of murine vascularized cardiac transplantation. However, delayed (5-6 days post-transplant) ICOS blockade significantly prolonged graft survival suggesting that ICOS/B7RP-1 interactions are important for the effector stage of the response by previously activated T cells (7). The distinct patterns of ICOS expression and consequences of ICOS ligation on CD4 and CD8 subsets of pre-existing donor-specific memory T cells have been recently reported. In one study, treatment with blocking anti-ICOS mAb synergized with conventional costimulatory blockade and prolonged mouse cardiac allograft survival despite the presence of ICOS-expressing donor-reactive memory CD4 T cells. Interrupting ICOS/B7RP-1 costimulation did not inhibit the expansion of pre-existing memory CD4 T cells or the help provided for activation of donor-specific effector CD8 T cells. However, ICOS blockade diminished the recruitment of the activated memory and effector T cells into the graft and help provided by memory CD4 T cells for the production of donor-specific IgG antibody (8). In contrast to memory CD4 T cells, ICOS is not expressed on resting memory CD8 T cells but is rapidly up-regulated during cell division within the allograft. ICOS blockade reduced production of IFNγ and other proinflammatory functions of graft-infiltrating memory CD8 T cells (9). The diversity of ICOS-regulated functions suggests that therapies targeting the ICOS/B7RP-1 pathway may result in different patterns of alloimmune responses and allograft pathology depending on the composition of the memory T cell pool in a given transplant recipient.

Another example of how a single costimulatory pathway regulates distinct facets of the alloimmune response are CD134/CD134L interactions. Similar to ICOS, CD134 is not present on naïve T cells but is up-regulated upon activation and contributes to the effector and memory phases of the immune response. Studies in rat and mouse models showed that monotherapy with anti-CD134L antibody failed to significantly improve allograft survival. However, long-term graft survival was achieved when CD134L blockade was combined with therapies preventing conventional costimulation such as CTLA4-Ig and anti-CD154 Ab (10, 11). Importantly, this strategy was equally effective in preventing skin allograft rejection mediated by memory T cells (12). Further mechanistic insights into the role of the CD134 pathway were revealed using mice transgenically expressing either CD134L or a fluorescence reporter for FoxP3 gene transcription. These studies demonstrated that CD134 costimulation is required for survival of both effector and regulatory T cells but elicits opposite effects on their functions, enhancing expansion of effector/memory T cells but impairing suppressive abilities of Tregs (13, 14).

Recent studies of PD-1/PD-L1/2 signaling in transplant models provide an example of how negative costimulatory pathways may be harnessed for improving allograft outcome. Consistent with studies in autoimmunity and tumor immunology models, disruption of PD-1/PD-L1 interactions using anti-PD-L1 mAb augments T cell alloreactivity leading to accelerated rejection of murine MHC class II-mismatched skin allografts (15). Conversely, treatment with PD-L1-Ig fusion protein provides negative signaling through PD-1 and, in combination with anti-CD154 treatment, delays rejection of islet allografts (16).

In addition to the discovery of a wide array of T cell specific surface costimulatory molecules, it has been recently recognized that T cells can receive costimulation by complement, one of the major and best studied components of innate inflammation. The starting point in this rapidly developing field was the observation that the absence of donor C3 results in diminished priming of alloreactive T cells and in acceptance of allogeneic renal allografts in mice (17). In agreement with these findings, the genetic absence of the complement regulator decay accelerating factor (DAF) leads to enhanced T cell responses and DAF-deficient cardiac allografts are rejected with accelerated kinetics compared to wild type allografts (18, 19). It appears that during T cell activation, production of C3 and C5 complement components is up-regulated in both T cells and antigen presenting cells initiating loops of autocrine and paracrine stimulation. Binding of C3a to C3aR on dendritic cells limits the levels of intracellular cAMP, a potent negative regulator of inflammation. This in turn enhances the antigen presentation capacity of dendritic cells rendering them potent stimulators of allogeneic T cell responses (20, 21). On the other hand, C5aR signaling in T cells promotes survival of naïve T cells and their optimal expansion after TCR engagement (22). Analogously, complement derived from endothelial cells influences reactivation of primed T cells in a C5aR-dependent manner which may explain the predominant role of donor-derived complement during the effector phase of allograft rejection (23).

Due to recent developments in T cell immunobiology, the interest of transplant immunologists has been drawn to identifying novel factors contributing to transplanted tissue injury. Donor-specific T cell responses elicited following transplantation are typically dominated by IFNγ-producing cells (24–26). However, IFNγ is dispensable for graft destruction indicating that other cytokines can contribute to the inflammation cascade and facilitate rejection (27–30). Furthermore, in some cases, IFNγ is required for long-term allograft acceptance (28, 30). The family of known effector T cell lineages has recently expanded with the identification of IL-17-producing T cells. IL-17 plays a critical role in host defense against bacterial and fungal pathogens and is involved in the pathogenesis of autoimmune diseases that were traditionally thought to be IFNγ- and Th1-dependent,

including experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis (31–34). Recent data from clinical and experimental transplantation suggest the involvement of IL-17 in allograft rejection. For example, IL-17 mRNA and protein expression are elevated in human renal and lung allografts during acute rejection episodes (35–38). In experimental transplantation, increased intragraft IL-17 levels have been observed in animal models of heart and renal allograft rejection (36). In addition, two groups have reported that IL-17-producing cells mediate cardiac allograft rejection in mice unable to mount Th1 alloimmune responses (39–41). The potential significance of IL-17 in transplantation is further underscored by findings that a neutralizing IL-17R-Ig fusion protein reduced intragraft production of IFNγ and prolonged survival of heart and aorta transplants in rodent models (42, 43). The contribution of IL-17 during allograft rejection by wild type recipients under normal physiological conditions can be diminished by the ability of IFNγ to inhibit differentiation of IL-17 secreting T cells (44). Nonetheless, the temporal appearance as well as the nature of cooperation between donor antigen-specific CD4 and CD8 T cells producing IL-17 and IFNγ remain unknown.

MEMORY T CELLS

Improved immunosuppression and successful control of T cells activated from naive precursors in response to allotransplantation, prompted the recognition of donor-reactive memory cells as a serious threat to transplanted organs. The high frequencies of previously antigen-exposed T cells in humans and the presence of donor-specific T cells with an effector/memory phenotype prior to transplantation or in heavily immunosuppressed transplant recipients suggested that the immune history of an individual can influence allograft outcome. Studies in the past decade identified several potential sources of alloreactive memory T cells, described multiple graft-damaging functions of these cells, and demonstrated the resistance of memory T cells to a number of currently used graft prolonging strategies.

It has been proposed that in addition to direct exposure to specific alloantigens, alloreactive T cells may become activated by pathogens and environmental antigens. This “heterologous immunity” may arise from dual TCR expression, bystander T cell activation during infection, or molecular resemblance between a microbial antigen/self MHC complex and an allogeneic MHC molecule. In mice, examples of pathogens eliciting T cell cross-reactivity to various H2 alleles include LCMV, influenza virus, vesicular stomatitis virus and Leishmania major (45–49). Similarly, human CD4 and CD8 T cell clones generated in response to EBV, HSV and CMV recognize allogeneic HLA molecules (50–53). Furthermore, a recent study demonstrated that a herpes virus-specific cytotoxic CD8 T cell clone can be re-activated by class II HLA molecules, further expanding the potential of TCR cross-reactivity in vivo (54). This chance cross-reactivity has important implications for several aspects of clinical transplantation. First, mouse studies convincingly demonstrate that even after the pathogen has been cleared, memory T cells cross-reactive to alloantigens thwart the effectiveness of costimulation blockade in inducing allograft tolerance (45, 48). Second, attempts by several groups to induce allograft tolerance during ongoing infection were unsuccessful (55–57). In these settings, the presence of activated effector T cells simultaneously reactive to pathogen and donor antigens may only partially account for allograft loss, as the negative effects of infection at this time can be mimicked by exposure to TLR agonists (58, 59). Finally, there is always a possibility of infection with a cross-reactive pathogen in recipients maintaining stable transplant function due to immunosuppression or established tolerance. Studies using LCMV showed that the deleterious effect of infection with a cross-reactive pathogen on allograft survival appears to decline with time after transplantation (60, 61). Nevertheless, the long term impact of cross-reactive effector T cells on allograft outcome remains to be determined.

Another source of alloreactive memory T cells is a consequence of T lymphocyte proliferation within lymphopenic conditions. Several approaches currently used as part of induction therapy prior to solid organ transplantation or as conditioning for bone marrow transplantation result in partial depletion of recipient lymphocytes. It has been noted that small numbers of naïve T cells rapidly expand in the lymphopenic host and repopulate peripheral lymphoid compartments. This process is distinct from normal T cell homeostasis as proliferating cells acquire the surface phenotype as well as functional characteristics of memory cells, namely rapid and strong recall responses and decreased requirements for costimulation. A portion of these “memory-like” cells may arise from allospecific precursors and be potentially dangerous for the transplanted organ. Consistent with this scenario, studies in mice demonstrated that memory T cells induced by homeostatic proliferation interfere with tolerance induction by costimulatory blockade (62).

Compared to their naïve counterparts, memory T cells are less susceptible to the deleterious effects of irradiation and depletion with antibodies or immunotoxins. Thus, murine CD4 T cells resistant to depletion with anti-lymphocyte serum express a memory cell phenotype (13). A study by Neujahr and colleagues (63) demonstrated that memory T cells are not only resistant to antibody-mediated depletion, but also undergo accelerated homeostatic proliferation. As a result, the residual T cell repertoire is skewed toward memory cells. In this light, reagents selectively targeting memory T cells would be more efficacious in promoting long-term allograft survival. A recent report by Weaver et al. indicates that a CD2-specific fusion protein LFA-3-Ig (alefacept) selectively eliminated effector memory T cells that express higher levels of CD2 compared to naïve and central memory T lymphocytes and in combination with CTLA4-Ig delayed renal allograft rejection in non-human primates (64).

Studies in non-human primates which, unlike laboratory rodents, contain high frequencies of endogenous memory T cells are consistent with the findings in mice. Thus, peripheral T cell ablation with anti-thymocyte globuline (ATG) led to the rapid reappearance of CD8 T cells with a memory phenotype in rhesus monkeys and interfered with the beneficial effects of costimulatory blockade on renal allograft survival (65). Similarly, rapid expansion of effector memory CD8 T cells was observed in cynomolgus monkeys that underwent delayed donor bone marrow transplantation to establish mixed chimerism and induce tolerance to previously transplanted kidney allografts (66). In these settings, the addition of anti-CD8 depleting Ab to the conditioning treatment led to improved bone marrow engraftment and kidney transplant survival.

The aftermath of lymphoablative therapies has been recently assessed in human transplant patients. As suggested by animal studies, T cells with an effector memory phenotype are prevalent in kidney transplant recipients after aggressive depletion with anti-CD52 Ab (alemtuzumab) or rabbit ATG (67). It remains unclear whether the increase in memory T cell numbers after lymphoblation is due to the expansion of non-depleted pre-existing memory cells or due to conversion of naïve T cells in the course of homeostatic proliferation. A study performed on a group of lung transplant patients demonstrated the persistence of CMV-specific memory T cells despite alemtuzumab treatment (68). A recent report by Sener et al. addressed this question in non-transplanted mice containing alloreactive memory T cells and treated with anti-lymphocyte serum (ALS) or with anti-thymocyte globulin (ATG). The results indicated that while the majority of memory T cells was depleted, the remaining memory T cells actively proliferated. In this study, no preferential expansion of memory T cells versus naïve T cells was detected early after depletion, however, the absence of transplant antigens could account for these observations (69).

Studies during the past few years provided further mechanistic insights into how donor-reactive memory T cells inflict damage to the transplanted organ. The general notion that "memory T cells expand after re-exposure to the antigen, migrate into the graft and cause tissue injury" has been reconsidered with the identification of specific functions of donor-reactive T cell subsets. The most important function of memory CD4 T cells appears to be providing help for the rest of the alloimmune response including CD8 T lymphocytes and the production of donor-reactive alloantibody by B cells (70). Due to these helper functions, memory CD4 T cells are capable of initiating rejection even if they are confined to the secondary lymphoid organs and can not reach the graft themselves (71). In contrast, memory CD8 T cells have been shown to infiltrate into cardiac allografts within hours after transplantation. After crossing the endothelial barrier, memory CD8 T cells proliferate and facilitate allograft inflammation through up-regulation of chemokines and adhesion molecules. This early inflammation is a critical step in the recruitment of neutrophils, macrophages and recently activated effector T cells into the graft (9, 72, 73). The heterogeneity of alloreactive memory T cells and the multitude of their functions undermine the possibility of universal therapies targeting memory in bulk. Depending on the composition of the memory T cell repertoire, different therapeutic approaches may be required to prevent their pathogenic functions in individual sensitized transplant patients.

TOLERANCE

In the past decade, the induction of specific tolerance to donor antigens has remained a desirable yet elusive goal of transplant immunology. While tolerance-inducing approaches have evolved over time, the deletion of alloreactive T cells, immune deviation, anergy and regulation are still recognized as the mechanisms underlying donor-specific hyporesponsiveness and indefinite allograft acceptance. During the last several years, substantial progress has been made in our understanding of regulation. Various cell types were shown to have regulatory properties, including $CD4^{+}CD25^{hi}$, $CD8^{+}$, $CD4^{-}CD8^{-}$, γδ T cells and NKT cells. The discovery of FoxP3, a transcription factor restricted to T cells with suppressive functions and critical for differentiation of these cells, spurred multiple studies on regulatory T cells (Tregs) in the context of transplantation. An important distinction has been made between naturally occurring Tregs (nTregs) and T cells with regulatory properties that differentiate after exposure to donor antigens, or inducible Tregs (iTregs).

The significance of nTregs in transplantation is not as obvious as in controlling autoimmune responses, in that the presence of nTregs in a healthy individual does not commonly result in spontaneous allograft acceptance. However, a study of single MHC class II disparate heart allograft rejection demonstrated that naturally occurring Tregs limit the expansion of donor-reactive effector T cells with low precursor frequencies and prevent allograft rejection (74). The classic example of induced regulation is the phenomenon of infectious tolerance achieved with non-depleting anti-CD4 antibody. More recent costimulatory blockade-based strategies such as donor specific transfusion/anti-CD154 Ab treatment also elicit immune regulation among other tolerance-promoting mechanisms (75). Temporal analysis of alloimmune responses revealed that in some cases Tregs may act as a bridge to other mechanism of tolerance. Thus, transient regulation by $CD25^{-}$ CD4 T cells is required for the induction of mixed chimerism and tolerance after bone marrow transplantation in recipients conditioned with low dose irradiation and anti-CD154 antibody (76). The suppressive functions of iTregs often depend on the secretion of immunomodulatory cytokines IL-10 and TGFβ and on the expression of Treg cell surface markers CTLA-4 and GITR (75, 77-79). Analogous to findings in rodents, trans-vivo DTH assays using PBMCs from human transplant recipients maintaining stable graft function in the absence of immunosuppression demonstrated donor antigen-linked TGFβ or IL-10 dependent regulation (80).

It is still unclear whether specificity for donor antigens is required for suppression by either natural or inducible Tregs. On one hand, Tregs reactivated with specific donor alloantigens promote the acceptance of third party allografts. Moreover, the ability to prolong allograft survival in such a bystander fashion extends to Tregs generated in response to a model antigen (81). In contrast, a study using TCR transgenic CD4 T cells demonstrated that antigen-specific Tregs are superior to polyclonal Tregs in suppressing alloantigen-driven responses and that the expression of relevant epitopes in the graft is critical for Treg activity (82). It has been proposed that regulatory CD4 T cells maintaining transplantation tolerance have indirect rather than direct alloreactivity (83). In support of this, CD4 T cell responses activated through the indirect pathway are necessary for prolonged allograft survival induced by costimulatory blockade (84). Furthermore, Tregs genetically modified to recognize alloantigens through both direct and indirect pathways are more efficient in promoting allograft acceptance than Tregs responding to donor MHC class II molecules exclusively through the direct pathway (85).

Another controversy in the field of immune regulation is the location at which Tregs become reactivated and express suppressor functions. While T cells with regulatory phenotype are often observed at the graft site, lymph node entry appears to be critical for the development and function of alloreactive $FoxP3^+$ Tregs (86). A recent study by Zhang and colleagues offers a complex model to reconcile previous observations. In this model, regulatory cells initially infiltrate into the graft and inhibit trafficking of donor-derived antigen-presenting cells thus attenuating priming of alloreactive T cells. Then, activated Tregs migrate from the graft into secondary lymphoid tissues where they directly suppress the activation and expansion of effector T cells (87).

Tolerance was originally defined as antigen-specific unresponsiveness, with specificity being a characteristic feature of adaptive immune responses. However, it is increasingly clear that in addition to numerous subsets of Tregs, cells of the innate immune system actively participate in tolerance induction and maintenance. The dynamic interplay between Tregs and mast cells has been recently described in a model of skin allograft tolerance induced via costimulatory blockade. Despite the acknowledged proinflammatory properties of mast cells, this cell subset was required during the tolerance induction phase (88). Interleukin-9 secreted by Tregs has been implicated as a cytokine eliciting the immunosuppressive properties of mast cells, although IL-10, TGFβ and OX40/OX40L may also be involved in this process (reviewed in (89)). The mechanisms through which mast cells facilitate tolerance are not entirely clear and possibly include secreting anti-inflammatory cytokines, such as IL-10 and TGFβ, as well as presenting donor antigen via class II MHC molecules to function as immunommodulatory antigen presenting cells. However, the effect of mast cell activation on allograft outcome may be reversed depending on the timing, strength and character of stimuli. Thus, chemically induced mast cell degranulation results in the loss of Treg function and allograft rejection after tolerance has been established (90).

Natural killer (NK) cells represent another example of innate immunity involvement in allograft rejection and tolerance. Even though NK cells can not initiate allograft rejection on their own, their role in promoting acute and chronic allograft pathology is well documented. In contrast to the graft-undermining functions of NK cells, recent studies by two independent groups showed that NK cells have important regulatory properties that promote tolerance induction to skin and pancreatic islet allografts in mice. The data indicate that NK cells rapidly destroy MHC-disparate allogeneic dendritic cells, most likely in perforin-dependent fashion, thus decreasing the magnitude of direct alloresponses and increasing the time window for the induction of Tregs (91, 92).

RECENT FINDINGS THAT MAY INFLUENCE CURRENT UNDERSTANING OF T CELL MEDIATED TRANPLANT REJECTION

We would like to mention several important developments in transplantation immunology beyond the scope of this review. A recent series of studies has established the link between autoimmunity and transplantation suggesting that immune responses to self antigens expressed by the allograft may contribute to rejection. The traditional view of T cell priming by graft and host dendritic cells following transplantation has been revisited to accommodate recent findings on the role of B cells in antigen presentation. Contrary to predictions and earlier observations, therapeutic manipulation of the chemokine network has been shown to have rather modest effect on T cell recruitment into the graft tissue but instead alter the priming of donor-specific T cells. Another interesting line of investigation is how cells of the innate immune system, namely macrophages and neutrophils, cooperate with donor-specific T cells to inflict allograft tissue injury. Deeper understanding of these issues and investigating their impact in clinical transplantation are likely to result in the development of novel therapeutic strategies that may work in synergy with existing approaches for improving allograft function and survival.

CONCLUDING REMARKS

Studies of the past decade actively pursued the ultimate goal of transplant immunology - long-term organ transplant survival with minimal or no immunosuppression. While donor-specific hyporesponsiveness or immune tolerance can be achieved in experimental animals, translation of these findings into the clinic is likely to encounter several hurdles. First, even under ideal laboratory conditions, successful immunosuppression-free engraftment is rarely achieved in all recipients, which is unacceptable in human patients. Second, current strategies to induce transplantation tolerance fail to control pre-existing donor-reactive memory T cells. Third, recent studies in animal transplant models further revealed the redundancy of costimulatory pathways, cytokine networks and effector mechanisms mediating graft tissue injury. It is becoming increasingly clear that therapeutic approaches to successful immunosuppression-free transplantation will have to include multiple agents targeting various components of the donor-reactive immune response and that such therapies need to be tailored based on the recipient’s immunologic profile. Further mechanistic insights into the complex events leading to transplant rejection and into the role of alloreactive memory T cells in this process should facilitate the development of diagnostic and therapeutic tools for clinical organ transplantation.

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Table 1

Costimulatory pathways and their role in transplantation (reviewed in 93–96).

Receptor/ Ligand(s)	Expression	Function	Role in transplantation
CD28/ CD80 and CD86	CD28–all naïve T cells CD80 – inducible on APCs CD86 – constitutive on APCs, up-regulated upon activation	Key costimulatory pathway regulating proliferation, survival and effector functions of activated T cells.	Targeting this pathway prolongs allograft survival in multiple rodent models. CD80 and CD86 blockade with CTLA4-Ig-like reagents improves allograft outcome in pre-clinical primate models and is currently in clinical trial.
CD154/ CD40	CD154 – activated CD4 T cells, NK cells, platelets CD40 – constitutive on APCs, can be induced on endothelial and parenchymal cells	Amplifies T cell responses through activation of dendritic cells, provides helper signals to B cells.	Anti-CD154 antibody treatment efficiently prevents allograft rejection in naïve but not sensitized rodents. Tromboembolic side effects of anti-CD154 antibody in non-human primates and humans has prompted the development of CD40-targeting reagents.
ICOS/ B7RP-1	ICOS – activated T cells, resting memory CD4 T cells B7RP-1 – constitutive on APCs, B cells, parenchymal and endothelial cells; up- regulated upon activation	Effector functions of activated T cells, Th2 differentiation, antibody production, transendothelial migration of T lymphocytes.	ICOS blockade is beneficial for allograft survival in rodent models, especially when combined with other costimulation-blocking reagents. Targeting ICOS/B7RP-1 decreases production of donor-reactive alloantibody, inhibits infiltration of effector T cells into the graft and prevents early intragraft cytokine production by pre-existing memory CD8 T cells.
CD134/ CD134L	CD134 – activated T cells, $Foxp3^+$ regulatory T cells CD134L – dendritic cells, B cells, activated endothelial cells	Co-stimulates T cell activation and differentiation; promotes generation and survival of memory T cells; inhibits suppressive functions of regulatory T cells.	Anti-CD134L antibody treatment synergizes with CD28/CD80 and CD86 and with CD40/ CD154 costimulatory blockade to prolong heart and islet allograft survival in rodents and to prevent skin allograft rejection mediated by memory T cells.
CD27/ CD70	CD27– naïve T cells, B cells, NK cells CD70- APCs, activated T and B cells	Supports T cell development, activation, T/B cell interaction and antibody production, anti-viral NK cell function.	In the absence of CD28 costimulation, CD70 blockade prolongs survival of murine cardiac allografts by inhibitng activation of alloreactive CD8 T cells.
PD-1/ PD-L1/2	PD-1 – activated T and B cells, NK cells macrophages PD-L1/2 – up-regulated on activated APCs; PD-L1 is constitutively expressed on parenchymal cells and induced on activated endothelial cells.	Negatively regulates T cell expansion and effector functions.	Anti-PD-L1 antibody treatment leads to enhanced alloresponses and accelerated allograft rejection. Conversely, signaling through PD-1 works in synergy with anti- CD154 therapy to prevent murine islet allograft rejection.
4-1BB/ 4-1BBL NK cells	4-1BB – activated T cells (especially CD8 T cells) and NK cells 4-1BB – dendritic cells, macrophages, activated B cells	Promotes T cell survival, differentiation and effector functions, may enhance functions of memory CD8 T cells.	Blocking 4-1BB pathway delays small bowel allograft rejection mediated by CD8 T lymphocytes. In contrast, stimulating signals through 4-1BB accelerate graft rejection in this model.