

## Costimulation blockade

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# **Costimulation blockade: Current perspectives and implications for therapy**

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## **Abstract**

T cells must be activated before they can elicit damage to allografts, through interaction of their T cell receptor (TCR) with peptide-MHC complex, and through accessory molecules. Signalling through accessory molecules or costimulatory molecules is a critical way for the immune system to fine tune T cell activation. An emerging therapeutic strategy is to target selective molecules involved in the process of T cell activation using biological agents, which do not impact TCR signalling, thus only manipulating the T cells which recognise alloantigen. Costimulatory receptors and their ligands are attractive targets for this strategy and could be used both to prevent acute graft rejection as well as for maintenance immunosuppression. Therapeutic agents targeting costimulatory molecules, notably belatacept, have made the progression from the bench, through non-human primate studies and into the clinic. This Overview describes some of the most common costimulatory molecules, their role in T cell activation, and the development of reagents which target these pathways and their efficacy in transplantation.

## **T cell activation: a potential target for therapeutic blockade**

T cells play a central role in the immune response towards an allograft (1) therefore interfering with the process of T cell activation has the potential of prolonging allograft survival through modulation of the alloresponse. Naïve CD4<sup>+</sup> and CD8<sup>+</sup> T cells must become activated in order to acquire effector functions which can result in graft damage (2). Activation of a naïve T cell is a tightly regulated process which requires three distinct signals. Signal 1 determines the specificity of the immune response and involves the interaction between a given T cell receptor (TCR) on a T cell and a MHC-peptide complex on antigen presenting cells (APC) which generates a signal that is transmitted through the adjacent CD3 complex (3, 4). Additional, so-called second signals are generated through other cell surface molecular interactions, known as costimulation (5). The third signal is delivered from an APC to the T cell by means of cytokines. A number of cytokines have been implicated as providing signal 3, including IL-12 as it is able to promote Th1 differentiation (6).

The accumulation and integration of intracellular signalling molecules from these 3 pathways triggers T cell gene transcription including IL-2, anti-apoptotic molecules, such as Bcl-2, molecules. Together such signals lead to clonal expansion and survival of antigen-specific T cells and influence the effector functions acquired by T cells as they differentiate, such as granzyme B a molecule that plays a role in the function of cytotoxic T cells (CTL or Tc). Without costimulatory signals during the activation process, T cells become anergic and refractory to further stimulation by antigen (7). Therefore targeting costimulatory pathways in the setting of transplantation has the potential to alter the evolution of an immune response to an allograft and prevent rejection.

## **Costimulation**

Figure 1 shows examples of costimulation pathways involved in the T cell response. Although different costimulatory molecules can have over-lapping functions, which molecules act to co-stimulate T cell responses appears to depend on the stage of differentiation of the responding T cell as well as the availability of corresponding ligands (Table 1) (8). This feature highlights the high degree of redundancy which has evolved to enable effective regulation of an immune response. Given the significant role played by costimulation in T cell activation, costimulation blockade provides a promising adjunctive or alternative therapy to the currently licenced immunosuppressive drugs used to prevent graft rejection.

## **Diversity of costimulatory molecules**

Costimulatory molecules can be categorized by both their functional properties and structure. Based on functionality, costimulatory molecules can be divided into those participating in positive costimulatory pathways promoting T cell activation, survival and/or differentiation, or negative costimulatory pathways which antagonise signals from the TCR resulting in suppression of T cell activation and termination of the immune response. Classification by structure divides costimulatory molecules into four distinct groups: (1) immunoglobulin (Ig) superfamily members (e.g. CD28); (2) tumour necrosis factor receptor (TNFR) family members (e.g. CD40); (3) cell adhesion molecules or integrins (e.g. LFA-1); T cell Ig domain and mucin domain (TIM) molecules. Figure 2 and Table 1 shows a number of the best characterized costimulatory molecules in the Ig and TNFR superfamilies. These two families

will be the focus of this Overview, but it is important to note that this is not a definitive list of receptor-ligand pairs able to provide costimulation.

## **Therapeutic strategies in experimental models of transplantation**

Novel immunosuppressive drugs and strategies are required for the prevention of allograft rejection to reduce the side effects associated with current immunosuppressive drug therapeutic regimens and to provide better control of the low grade immune response that leads to late allograft loss. A number of new agents have been developed which target immunological pathways in rejection for example; 1) modulation of cell surface molecules such as costimulatory molecules 2) inhibition of signalling cascades 3) inhibition of T cell proliferation and 4) modulation of cell trafficking. There are many different reagents being developed to target these pathways (9), and this Overview will focus on the development of agents which impact the costimulatory pathways described above.

### **CD28/CTLA4:CD80/CD86 pathway**

The CD28-CD80/CD86 axis was the first costimulatory pathway to be defined and is therefore the best characterised to date (10-12) (Table 1; (13, 14)). After T cell activation, another receptor for CD80/CD86 is upregulated; CTLA4 (CD152) which although structurally homologous to CD28, has a ~20 fold higher affinity for CD80/CD86 and thus can out-compete CD28 for CD80/86 binding (Table 1; (15)). The CD28/CTLA4;CD80/CD86 axis has a dual function in the T cell response (Figure 3). In contrast, CTLA4 inhibits the T cell response by limiting CD28-CD80/CD86 interactions, decreasing IL-2 secretion and promoting indole-amine 2,3-dioxygenase (IDO) expression by APC upon ligation of CD80/86 that in turn promotes the expansion of regulatory T cells by modulating tryptophan

catabolism (16-18). Based upon these properties, a receptor Fc fusion protein, CTLA4-Ig, has been developed to block CD80/86 and thereby inhibit T cell costimulation (15, 19).

The importance of the CD28 costimulatory pathway in allogeneic responses was first demonstrated *in vitro* by using an anti-CD28 monoclonal antibody (19, 20) or CTLA4-Ig fusion protein (21, 22). However, using CD28<sup>-/-</sup> mice, Kawai *et al* demonstrated that the signals generated through CD28 were critical for the proliferation of alloreactive T cells *in vitro*, but that *in vivo* skin allograft rejection could occur in the absence of CD28 (23). In rodents, blockade of the CD28:CD80/CD86 pathway by CTLA-4-Ig, was shown to prevent acute allograft rejection, but this finding was found to be model and strain dependent (22, 24-26) due to the redundancy in the immune response. CTLA4-Ig also prevented the development of anti-donor antibody responses and resulted in long-term survival of islet, cardiac and renal transplants in rodent models (21, 27-29) (Figure 3). These data provide a rationale for combination therapies within the clinical setting.

### **CD40:CD154 pathway**

The role of the CD40:CD154 pathway in immunity became clear when hyper-IgM syndrome was found to be a direct result of a mutation in the gene encoding CD154 (30). The effects of CD40 on the immune response are mediated by a signalling cascade which is initiated when it binds its ligand CD154 (CD40L) (Table 1; (31, 32)); a CD28 independent event (33). Initial efforts were aimed at blocking the CD40:CD154 interaction by use of monoclonal antibodies specific for CD154; an approach that showed promise in transplantation models in rodents (34-36) and in non-human primates (NHP) (37-39). Anti-CD154 has a preferential impact on effector T cells by inhibiting their activation and therefore proliferation, while also enriching the Treg population (40).

In preclinical studies it was found that rhesus monkeys given anti-CD154 mAb for 5 months as part of an induction therapy followed by 5 further monthly doses accepted kidney allografts for over a year after treatment was discontinued. However, the allografts were eventually rejected suggesting that tolerance was not achieved (38, 39). In addition, anti-CD154 (IDEC-131) alone significantly prolonged cardiac allograft survival in cynomolgus monkeys, while graft survival was further extended with the introduction of anti-thymocyte globulin in addition to anti-CD154 but as in previous studies did not induce tolerance (41).

More recently, reagents which target CD40 rather than CD154 have been developed. Anti-CD40 was found to synergise with CTLA-4-Ig to promote long term allograft survival in mouse models of skin and bone marrow transplantation (42). Anti-CD40 (4D11) showed significant prolongation of renal allograft survival in NHPs and prevented the development of alloantibodies (43) suggesting that blockade of the CD40:CD154 pathway still may contain promise in humans (44).

### **ICOS:ICOSL pathway**

Another member of Ig superfamily is inducible costimulator (ICOS; CD278) (Table 1; (45-47)). In a full-MHC mismatch mouse cardiac allograft model Ozkaynak *et al* showed that blockade of ICOS in combination with either cyclosporine or anti-CD154 prevented chronic rejection (48). However, if donors and recipients were mismatched for minor histocompatibility antigens only, blockade of ICOS during the T cell priming phase accelerated rejection, while blockade during the effector phase of the alloimmune response prolonged graft survival (49). This may be explained by ICOS being critical for the function of effector/memory T cells as well as regulatory T cells (50). Co-blockade of ICOS:ICOSL



and CD40:CD154 (see above) results in indefinite cardiac allograft survival with a significant reduction in chronic allograft vasculopathy and therefore chronic rejection (51). These data suggest that preventing ICOS signals alone will be insufficient to induce long term allograft survival and tolerance, therefore combining interruption of ICOS-ICOSL interactions with blockade of other costimulatory pathways may be an important step forward if ICOS blockade is going to reach its full therapeutic potential.

### **PD-1:PD-L1/L2 pathway**

Like CTLA-4, PD-1 (CD279) is also a member of the Ig superfamily that has co-inhibitory activity (Table 1; (52)), and is important in suppressing T cell activation and preventing autoimmunity. PD-1<sup>-/-</sup> mice develop strain specific autoimmunity, demonstrating a role for PD-1 in negatively regulating the immune response (53, 54) and in maintaining peripheral tolerance to self-antigens.

Administration of blocking monoclonal antibodies against PDL1, but not PD-1 or PDL2, in a MHC Class II mismatched skin graft model, resulted in accelerated rejection due to selective prevention of T cell apoptosis, increased alloantigen driven T cell expansion and promotion of Th1 differentiation (55). Gao *et al* used a PDL1-Ig fusion protein and found that it prevented allograft rejection and allowed the induction of tolerance when combined with anti-CD154 or sub-therapeutic doses of rapamycin (56). These data suggest that the PD-1 ligands may mediate opposing effects as a result of their differential tissue distribution.

### **OX40:OX40L pathway**

The TNFR superfamily member OX40 (CD134) is transiently induced on both CD4<sup>+</sup> (57) and CD8<sup>+</sup> (58) T cells after activation (Table 1) which is involved in the late expansion phase

of effector T cells, as well as promoting memory T cell generation (59, 60). Blocking the OX40-OX40L pathway (using an OX40-Ig fusion protein) in a mouse model of cardiac transplantation was found to result in prolonged cardiac allograft survival when donor and recipient were mismatched at a minor histocompatibility antigen loci but not across a full MHC mismatch (61). In contrast, in a full-MHC mismatch model where TCR transgenic CD8<sup>+</sup> T cells were adoptively transferred into syngeneic T cell deficient recipients, anti-OX40 was able to significantly prolong skin graft survival, although tolerance was not achieved (62). OX40:OX40L has been suggested to be utilised differentially by effector and regulatory T cells (Treg). For example, blockade of OX40:OX40L inhibits the generation of an optimal effector T cell pool by promoting activation induced cell death (59, 62), whilst concomitantly aiding the induction of Treg (63, 64). These data provide a clear precedent for the utilisation of OX40-OX40L blockade in transplantation however this appears to be contingent on suboptimal or low frequency T cell responses.

#### **41BB:41BBL pathway**

Another inducible costimulatory molecule in the TNFR superfamily is 4-1BB (CD137), and ligation of 4-1BB to its ligand (41BBL) (Table 1; (65, 66)) preferentially promotes CD8<sup>+</sup> T cell proliferation, and survival compared to CD4<sup>+</sup> T cells (67). In a transplantation setting, agonistic 4-1BB monoclonal antibodies have been shown to accelerate cardiac and skin rejection (67). Furthermore, blockade of 4-1BB prolonged intestinal allograft survival where rejection was mediated by CD8<sup>+</sup> T cells but not where rejection was caused by CD4<sup>+</sup> T cells (68). Cho *et al* showed that 4-1BB<sup>-/-</sup> recipients had only a minor impairment in their ability to acutely reject fully-MHC mismatched cardiac allografts (69). These data suggest the 4-1BB-41BBL pathway could be targeted where CD8<sup>+</sup> T cells exercise CD28/CD154 independent rejection.

### **CD27:CD70 pathway**

The final TNFR family member to be discussed in this review is CD27 and its interaction with ligand CD70 (Table 1; (70)). CD70 expression is dependent on TCR stimulation and TLR stimulation (71). The CD27-CD70 interaction has been implicated in T cell development, T cell activation and T cell dependent antibody production by B cells (72). Blockade of CD27-CD70 pathway prolongs allograft survival in fully MHC mismatched cardiac allografts in wild type recipients (73). But in CD28<sup>-/-</sup> recipients CD70 blockade induced long term survival and prevented the development of chronic allograft vasculopathy (74). This synergy was mediated by the effector/memory alloreactive CD8<sup>+</sup> T cells, while little effect was seen on the CD4<sup>+</sup> T cell function.

### **LFA-1:ICAM and VLA-4:VCAM pathway**

Integrins have a number of roles in the immune response; T cell recirculation, migration into inflammatory sites, stabilisation of T cell-APC interactions and providing costimulatory signals. Experimental transplantation models have shown that blockade of the LFA-1-ICAM interactions with anti-LFA-1 monoclonal antibodies can result in prolonged survival of islet (75) and cardiac (76) allografts. In a murine cardiac allograft model, anti-VLA4 reduced the incidence and severity of arterial intimal thickening which is closely associated with chronic rejection (77). Anti-VLA4 and anti-LFA-1 administered together displayed potent synergy in a murine islet model, which resulted in significant graft prolongation compared with either of the monotherapies (78). Kitchens *et al* have shown anti-VLA4 or anti-LFA-1 can abrogate the resistance of memory T cells to costimulation blockade (79). Anti-VLA4 acts by blocking T cell trafficking to the graft, while anti-LFA-1 was also able to block T cell trafficking but also could impact memory T cell recall function (79).

## **TIM family**

TIM molecules are an emerging family of cell surface type 1 transmembrane glycoproteins which have recently been shown to have important immunological functions as costimulatory molecules (80). The TIM family regulate a wide variety of immune responses and can provide positive signals to T cells which can enhance T cell activation (81), proliferation (82) and cytokine production (81). In particular, costimulation via TIM-1 abolishes the generation and suppressor function of Treg by reducing FoxP3 expression. Agonistic TIM-1 mAb enhances Th17 differentiation, therefore suggesting TIM-1 plays a critical role in the delicate balance between Treg and Th17 (83) or autoimmunity and tolerance. Blockade of TIM family proteins may provide a viable strategy for the amelioration of autoimmune and inflammatory diseases. For example, anti-TIM-1 mAb and rapamycin synergise to prolong cardiac allograft survival by inhibiting Th1 responses (84). This strategy may also benefit from combination therapy with other costimulation blockade.

## **Obstacles to tolerance induction and clinical translation**

There is a growing body of evidence in rodent models to suggest that blocking costimulation can lead to tolerance induction (35, 85), however, data from NHP studies is more complex, and suggests that tolerance induction is more difficult to achieve in higher animals including humans (37-39, 86). These differences can be explained in part by the increased complexity of the higher vertebrate immune system and the increased diversity of pre-existing immunity due to exposure to environmental antigens (87). Although outbred laboratory animals have a wider diversity of exposure to such antigens, they are still less immunologically educated compared to humans. Differences can also result due to basic observations such as size;

vastly different number of total cells able to respond as well as differences in drug absorption and clearance. Despite these differences, large animal models remain the best way to gain knowledge before initiating a safe and ethically robust clinical trial.

## **The leap from bench-side to bedside**

Targeting costimulatory molecules has great potential in transplantation for preventing rejection as the therapy will only impact T cells undergoing activation. As a result the specificity of immunosuppression achieved using this therapeutic approach has the potential to be increased compared to current small molecule immunosuppressive drugs.

Belatacept (human CTLA4-Ig fusion protein; Nujolix®) has been approved for clinical use for the indication of prophylaxis of organ rejection in adult kidney transplant recipients (FDA in June 2011; EMA in April 2011). Belatacept was developed as a second generation CTLA4-Ig after another version of the fusion protein (abatacept) proved sub-optimal in NHP transplant models (88). Structurally belatacept and abatacept differ only in two amino acids, which allows for more potent binding to its ligands by belatacept resulting in more efficacious inhibition of T cell activation. The FDA has approved belatacept to be used in conjunction with basiliximab induction, mycophenolate mofetil (MMF) and corticosteroids as maintenance immunosuppression (89). The efficacy of belatacept in *de novo* kidney transplantation was assessed in two open label, randomised, multicentre phase II/III clinical trials, named BENEFIT (Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial). At 1 year, the incidence of acute rejection was higher in those patients treated with belatacept (90). The rates of infection and malignancy were comparable between both arms, while the metabolic and cardiovascular risk profiles were

better in the belatacept treated patients (91). Rejection episodes were associated with a failure of belatacept to suppress a subset of alloreactive T cells, i.e. memory T cells (92). Memory T cells have a reduced reliance on costimulation and therefore are more resistant to costimulation blockade (93-96). Development of reagents to target memory T cells (such as integrin antagonists (79)) in addition to costimulation blockade may provide a useful tool to overcome the increased incidence of rejection observed with belatacept. Memory T cells therefore pose a potential barrier to regimens such as costimulation blockade which hope to lead to tolerance induction and graft acceptance within the clinic.

In addition some patients who were treated with high dose belatacept also developed post-transplantation lymphoproliferative disorder (PTLD) (90, 97-99). The FDA approval of belatacept is therefore limited for use only in Epstein-Barrvirus (EBV) seropositive patients due to increased risk of PTLD in EBV-seronegative patients. The PTLD observed was mainly of the central nervous system (89). Belatacept has only been approved for use in adult kidney transplantation, as there was increased graft loss and mortality in liver transplant recipients (100).

Other reagents which target CD28 have also been developed (101, 102). A CD28-superagonist, TGN1412, showed enhanced expansion of regulatory T cells in preclinical studies and was taken into a Phase I clinical trial. However, 6 healthy volunteers treated with TGN1412 experienced a massive expansion of inflammatory T cells that resulted in a cytokine storm. The trial was terminated as all volunteers experienced multi-organ failure (103). Despite this, the development of antibody mediated selective costimulatory blockade remains a highly attractive therapeutic strategy as specifically blocking CD28 for example,

would still allow CTLA-4 signalling potentially enhancing T cell suppression. Monovalent Fab fragments (Fv) which selectively block CD28 have been developed to prevent signals through CD28 whilst allowing negative signals via CTLA-4 to remain intact. In a full MHC mismatch cardiac transplant mouse model, CD28 single chain Fv (scFv) fragments have been shown to significantly prolong allograft survival (104) and Poirier *et al* demonstrated that blocking CD28 in NHPs increased the number of Treg in the periphery as well as in the graft, which resulted in the prevention of renal allograft rejection (105).

The interruption of the CD40-CD154 interaction also has the potential to prevent rejection and promote long-term graft acceptance, as demonstrated by various rodent (32, 34, 36) and NHP studies (37, 38). Phase I-II trial evaluating anti-CD154 for the prevention of renal transplant rejection had to be terminated prematurely, due to thromboembolic complications as a result of activated platelets expressing CD154 (106). Therefore future plans for the development of anti-CD154 remain unclear. Antibodies targeting CD40 are being developed as they have been shown to have a similar efficacy in NHP kidney models without the thromboembolic side-effects seen with anti-CD40L mAbs (43, 107).

The immunomodulatory properties of integrins have encouraged the development of clinical antagonists to treat autoimmunity. In a phase I/II open label multi-centre trial, the efficacy and safety of efalizumab (anti LFA-1; humanised IgG1) was tested in renal transplant recipients that also received cyclosporine/rapamycin/steroids or cyclosporine/MMF/steroids. The patient and graft survival was comparable between the treatment arms at 6 months (108), but a significant subset of patients (30%) developed post-transplant lymphoproliferative disease (PTLD) (109), while chronic use resulted in progressive multifocal

leukoencephalopathy (PML) in some patients (110). This raises concerns over the safety and future uses of eflalizumab, particularly in the treatment of conditions such as psoriasis, although its short term use may be more acceptable in the field of transplantation. Indeed in a pilot trial in renal transplantation patients treated with eflalizumab had a low incidence of rejection (108). Also many of the current immunosuppressive agents used in transplant recipients carry similar risks for PML (110, 111).

## **Future opportunities**

The use of costimulation blockade in clinical transplantation has been accelerated by the clinical trial data for Belatacept, as well as its recent approval for renal transplantation. One of the important unmet goals for therapeutic strategies in transplantation is to reduce the toxic side effects of calcineurin inhibitors and other immunosuppressive drugs, while also lowering the risk of acute and delayed rejection. A number of new pathways and molecules have been investigated for this purpose including costimulation.

Blockade of costimulatory interactions have been found to modulate immune responses following T cell activation. However, specific pathways may or may not be utilised by particular subsets of T cells or are important only at specific stages in an immune response. Clearly this presents challenges to the 'one size fits all' concept for immunosuppression as it suggests that it may be necessary to use different combinations of therapeutic agents tailored to the immunological challenges faced by individual donor recipient pairs and that this may need to be modified as the immune response progresses. Therefore a combination of costimulation blockade may provide a therapeutic advantage over individual reagents. For



example anti-CD154, CTLA-4 Ig and an integrin antagonist (e.g. anti VLA-4 or anti-LFA-1) prolongs allograft survival in mice (79). Despite the success of costimulatory molecule blockade in rodent models, the key to success in the clinic will be to define the expression patterns of individual costimulatory molecules and their ligands within the allograft as well as in the secondary lymphoid tissue in order to understand the implications of targeting costimulatory molecules more precisely.

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1. Shelton MW, Walp LA, Basler JT, Uchiyama K, Hanto DW. Mediation of skin allograft rejection in scid mice by CD4<sup>+</sup> and CD8<sup>+</sup> T cells. *Transplantation* 1992; 54 (2): 278.
2. Wood KJ, Goto R. Mechanisms of rejection: current perspectives. *Transplantation* 2012; 93 (1): 1.
3. Zinkernagel RM, Doherty PC. Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytic choriomeningitis. *Nature* 1974; 251 (5475): 547.

4. Zinkernagel RM, Doherty PC. Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. *Nature* 1974; 248 (450): 701.
5. Mueller DL, Jenkins MK, Schwartz RH. Clonal expansion versus functional clonal inactivation: a costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. *Annu Rev Immunol* 1989; 7: 445.
6. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nature reviews. Immunology* 2003; 3 (2): 133.
7. Bretscher P, Cohn M. A theory of self-nonsel discrimination. *Science* 1970; 169 (950): 1042.
8. Li XC, Rothstein DM, Sayegh MH. Costimulatory pathways in transplantation: challenges and new developments. *Immunological reviews* 2009; 229 (1): 271.
9. Webber A, Hirose R, Vincenti F. Novel strategies in immunosuppression: issues in perspective. *Transplantation* 2011; 91 (10): 1057.
10. June CH, Bluestone JA, Nadler LM, Thompson CB. The B7 and CD28 receptor families. *Immunol Today* 1994; 15 (7): 321.
11. Jenkins MK. The ups and downs of T cell costimulation. *Immunity* 1994; 1 (6): 443.
12. Linsley PS, Ledbetter JA. The role of the CD28 receptor during T cell responses to antigen. *Annu Rev Immunol* 1993; 11: 191.
13. Fraser JD, Irving BA, Crabtree GR, Weiss A. Regulation of interleukin-2 gene enhancer activity by the T cell accessory molecule CD28. *Science* 1991; 251 (4991): 313.
14. Boise LH, Minn AJ, Noel PJ, et al. CD28 costimulation can promote T cell survival by enhancing the expression of Bcl-XL. *Immunity* 1995; 3 (1): 87.

15. Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA. CTLA-4 is a second receptor for the B cell activation antigen B7. *The Journal of experimental medicine* 1991; 174 (3): 561.
16. Walunas TL, Lenschow DJ, Bakker CY, et al. CTLA-4 can function as a negative regulator of T cell activation. *Immunity* 1994; 1 (5): 405.
17. Finger EB, Bluestone JA. When ligand becomes receptor--tolerance via B7 signaling on DCs. *Nature immunology* 2002; 3 (11): 1056.
18. Grohmann U, Orabona C, Fallarino F, et al. CTLA-4-Ig regulates tryptophan catabolism in vivo. *Nature immunology* 2002; 3 (11): 1097.
19. Tan P, Anasetti C, Hansen JA, et al. Induction of alloantigen-specific hyporesponsiveness in human T lymphocytes by blocking interaction of CD28 with its natural ligand B7/BB1. *The Journal of experimental medicine* 1993; 177 (1): 165.
20. Damle NK, Doyle LV, Grosmaire LS, Ledbetter JA. Differential regulatory signals delivered by antibody binding to the CD28 (Tp44) molecule during the activation of human T lymphocytes. *Journal of immunology* 1988; 140 (6): 1753.
21. Baliga P, Chavin KD, Qin L, et al. CTLA4Ig prolongs allograft survival while suppressing cell-mediated immunity. *Transplantation* 1994; 58 (10): 1082.
22. Turka LA, Linsley PS, Lin H, et al. T-cell activation by the CD28 ligand B7 is required for cardiac allograft rejection in vivo. *Proceedings of the National Academy of Sciences of the United States of America* 1992; 89 (22): 11102.
23. Kawai K, Shahinian A, Mak TW, Ohashi PS. Skin allograft rejection in CD28-deficient mice. *Transplantation* 1996; 61 (3): 352.
24. Pearson TC, Alexander DZ, Corbascio M, et al. Analysis of the B7 costimulatory pathway in allograft rejection. *Transplantation* 1997; 63 (10): 1463.

25. Reddy B, Gupta S, Chuzhin Y, et al. The effect of CD28/B7 blockade on alloreactive T and B cells after liver cell transplantation. *Transplantation* 2001; 71 (6): 801.
26. Glysing-Jensen T, Raisanen-Sokolowski A, Sayegh MH, Russell ME. Chronic blockade of CD28-B7-mediated T-cell costimulation by CTLA4Ig reduces intimal thickening in MHC class I and II incompatible mouse heart allografts. *Transplantation* 1997; 64 (12): 1641.
27. Judge TA, Tang A, Turka LA. Immunosuppression through blockade of CD28:B7-mediated costimulatory signals. *Immunologic research* 1996; 15 (1): 38.
28. Azuma H, Chandraker A, Nadeau K, et al. Blockade of T-cell costimulation prevents development of experimental chronic renal allograft rejection. *Proc Natl Acad Sci U S A* 1996; 93 (22): 12439.
29. Chandraker A, Azuma H, Nadeau K, et al. Late blockade of T cell costimulation interrupts progression of experimental chronic allograft rejection. *J Clin Invest* 1998; 101 (11): 2309.
30. Hayward AR, Levy J, Facchetti F, et al. Cholangiopathy and tumors of the pancreas, liver, and biliary tree in boys with X-linked immunodeficiency with hyper-IgM. *Journal of immunology* 1997; 158 (2): 977.
31. Blotta MH, Marshall JD, DeKruyff RH, Umetsu DT. Cross-linking of the CD40 ligand on human CD4+ T lymphocytes generates a costimulatory signal that up-regulates IL-4 synthesis. *Journal of immunology* 1996; 156 (9): 3133.
32. Larsen CP, Pearson TC. The CD40 pathway in allograft rejection, acceptance, and tolerance. *Current opinion in immunology* 1997; 9 (5): 641.
33. Blair PJ, Riley JL, Harlan DM, et al. CD40 ligand (CD154) triggers a short-term CD4(+) T cell activation response that results in secretion of immunomodulatory cytokines and apoptosis. *The Journal of experimental medicine* 2000; 191 (4): 651.

34. Hancock WW, Sayegh MH, Zheng XG, Peach R, Linsley PS, Turka LA. Costimulatory function and expression of CD40 ligand, CD80, and CD86 in vascularized murine cardiac allograft rejection. *Proc Natl Acad Sci U S A* 1996; 93 (24): 13967.
35. Larsen CP, Elwood ET, Alexander DZ, et al. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature* 1996; 381 (6581): 434.
36. Parker DC, Greiner DL, Phillips NE, et al. Survival of mouse pancreatic islet allografts in recipients treated with allogeneic small lymphocytes and antibody to CD40 ligand. *Proceedings of the National Academy of Sciences of the United States of America* 1995; 92 (21): 9560.
37. Kenyon NS, Chatzipetrou M, Masetti M, et al. Long-term survival and function of intrahepatic islet allografts in rhesus monkeys treated with humanized anti-CD154. *Proceedings of the National Academy of Sciences of the United States of America* 1999; 96 (14): 8132.
38. Kirk AD, Burkly LC, Batty DS, et al. Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. *Nat Med* 1999; 5 (6): 686.
39. Kirk AD, Harlan DM, Armstrong NN, et al. CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc Natl Acad Sci U S A* 1997; 94 (16): 8789.
40. Meng L, Wu Z, Wang Y, et al. Differential impact of CD154 costimulation blockade on alloreactive effector and regulatory T cells in murine renal transplant recipients. *Transplantation* 2008; 85 (9): 1332.

41. Azimzadeh AM, Pfeiffer S, Wu G, et al. Alloimmunity in primate heart recipients with CD154 blockade: evidence for alternative costimulation mechanisms. *Transplantation* 2006; 81 (2): 255.
42. Gilson CR, Milas Z, Gangappa S, et al. Anti-CD40 monoclonal antibody synergizes with CTLA4-Ig in promoting long-term graft survival in murine models of transplantation. *Journal of immunology* 2009; 183 (3): 1625.
43. Imai A, Suzuki T, Sugitani A, et al. A novel fully human anti-CD40 monoclonal antibody, 4D11, for kidney transplantation in cynomolgus monkeys. *Transplantation* 2007; 84 (8): 1020.
44. Li XL, Menoret S, Le Mauff B, Angin M, Anegon I. Promises and obstacles for the blockade of CD40-CD40L interactions in allotransplantation. *Transplantation* 2008; 86 (1): 10.
45. Dong C, Juedes AE, Temann UA, et al. ICOS co-stimulatory receptor is essential for T-cell activation and function. *Nature* 2001; 409 (6816): 97.
46. Tafuri A, Shahinian A, Bladt F, et al. ICOS is essential for effective T-helper-cell responses. *Nature* 2001; 409 (6816): 105.
47. Dong C, Temann UA, Flavell RA. Cutting edge: critical role of inducible costimulator in germinal center reactions. *Journal of immunology* 2001; 166 (6): 3659.
48. Ozkaynak E, Gao W, Shemmeri N, et al. Importance of ICOS-B7RP-1 costimulation in acute and chronic allograft rejection. *Nature immunology* 2001; 2 (7): 591.
49. Harada H, Salama AD, Sho M, et al. The role of the ICOS-B7h T cell costimulatory pathway in transplantation immunity. *The Journal of clinical investigation* 2003; 112 (2): 234.
50. Burmeister Y, Lischke T, Dahler AC, et al. ICOS controls the pool size of effector-memory and regulatory T cells. *Journal of immunology* 2008; 180 (2): 774.

51. Guillonneau C, Aubry V, Renaudin K, et al. Inhibition of chronic rejection and development of tolerogenic T cells after ICOS-ICOSL and CD40-CD40L co-stimulation blockade. *Transplantation* 2005; 80 (2): 255.
52. Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. *Immunity* 2007; 27 (1): 111.
53. Nishimura H, Okazaki T, Tanaka Y, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* 2001; 291 (5502): 319.
54. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 1999; 11 (2): 141.
55. Sandner SE, Clarkson MR, Salama AD, et al. Role of the programmed death-1 pathway in regulation of alloimmune responses in vivo. *Journal of immunology* 2005; 174 (6): 3408.
56. Gao W, Demirci G, Strom TB, Li XC. Stimulating PD-1-negative signals concurrent with blocking CD154 co-stimulation induces long-term islet allograft survival. *Transplantation* 2003; 76 (6): 994.
57. Calderhead DM, Buhlmann JE, van den Eertwegh AJ, Claassen E, Noelle RJ, Fell HP. Cloning of mouse Ox40: a T cell activation marker that may mediate T-B cell interactions. *J Immunol* 1993; 151 (10): 5261.
58. al-Shamkhani A, Birkeland ML, Puklavec M, Brown MH, James W, Barclay AN. OX40 is differentially expressed on activated rat and mouse T cells and is the sole receptor for the OX40 ligand. *Eur J Immunol* 1996; 26 (8): 1695.

59. Gramaglia I, Weinberg AD, Lemon M, Croft M. Ox-40 ligand: a potent costimulatory molecule for sustaining primary CD4 T cell responses. *J Immunol* 1998; 161 (12): 6510.
60. Rogers PR, Song J, Gramaglia I, Killeen N, Croft M. OX40 promotes Bcl-xL and Bcl-2 expression and is essential for long-term survival of CD4 T cells. *Immunity* 2001; 15 (3): 445.
61. Curry AJ, Chikwe J, Smith XG, et al. OX40 (CD134) blockade inhibits the co-stimulatory cascade and promotes heart allograft survival. *Transplantation* 2004; 78 (6): 807.
62. Kinnear G, Wood KJ, Marshall D, Jones ND. Anti-OX40 prevents effector T-cell accumulation and CD8+ T-cell mediated skin allograft rejection. *Transplantation* 2010; 90 (12): 1265.
63. Chen M, Xiao X, Demirci G, Li XC. OX40 controls islet allograft tolerance in CD154 deficient mice by regulating FOXP3+ Tregs. *Transplantation* 2008; 85 (11): 1659.
64. Valzasina B, Guiducci C, Dislich H, Killeen N, Weinberg AD, Colombo MP. Triggering of OX40 (CD134) on CD4(+)CD25+ T cells blocks their inhibitory activity: a novel regulatory role for OX40 and its comparison with GITR. *Blood* 2005; 105 (7): 2845.
65. Pollok KE, Kim YJ, Zhou Z, et al. Inducible T cell antigen 4-1BB. Analysis of expression and function. *Journal of immunology* 1993; 150 (3): 771.
66. Langstein J, Michel J, Fritsche J, Kreutz M, Andreesen R, Schwarz H. CD137 (ILA/4-1BB), a member of the TNF receptor family, induces monocyte activation via bidirectional signaling. *Journal of immunology* 1998; 160 (5): 2488.
67. Shuford WW, Klussman K, Tritchler DD, et al. 4-1BB costimulatory signals preferentially induce CD8+ T cell proliferation and lead to the amplification in vivo



- of cytotoxic T cell responses. *The Journal of experimental medicine* 1997; 186 (1): 47.
68. Wang J, Guo Z, Dong Y, et al. Role of 4-1BB in allograft rejection mediated by CD8<sup>+</sup> T cells. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2003; 3 (5): 543.
69. Cho HR, Kwon B, Yagita H, et al. Blockade of 4-1BB (CD137)/4-1BB ligand interactions increases allograft survival. *Transpl Int* 2004; 17 (7): 351.
70. Hendriks J, Xiao Y, Borst J. CD27 promotes survival of activated T cells and complements CD28 in generation and establishment of the effector T cell pool. *The Journal of experimental medicine* 2003; 198 (9): 1369.
71. Tesselaar K, Xiao Y, Arens R, et al. Expression of the murine CD27 ligand CD70 in vitro and in vivo. *Journal of immunology* 2003; 170 (1): 33.
72. Gravestein LA, Borst J. Tumor necrosis factor receptor family members in the immune system. *Semin Immunol* 1998; 10 (6): 423.
73. Shariff H, Greenlaw RE, Meader L, et al. Role of the Fc region in CD70-specific antibody effects on cardiac transplant survival. *Transplantation* 2011; 92 (11): 1194.
74. Yamada A, Salama AD, Sho M, et al. CD70 signaling is critical for CD28-independent CD8<sup>+</sup> T cell-mediated alloimmune responses in vivo. *Journal of immunology* 2005; 174 (3): 1357.
75. Nicolls MR, Coulombe M, Yang H, Bolwerk A, Gill RG. Anti-LFA-1 therapy induces long-term islet allograft acceptance in the absence of IFN-gamma or IL-4. *Journal of immunology* 2000; 164 (7): 3627.

76. Paul LC, Davidoff A, Benediktsson H, Issekutz TB. The efficacy of LFA-1 and VLA-4 antibody treatment in rat vascularized cardiac allograft rejection. *Transplantation* 1993; 55 (5): 1196.
77. Molossi S, Elices M, Arrhenius T, Diaz R, Coulber C, Rabinovitch M. Blockade of very late antigen-4 integrin binding to fibronectin with connecting segment-1 peptide reduces accelerated coronary arteriopathy in rabbit cardiac allografts. *The Journal of clinical investigation* 1995; 95 (6): 2601.
78. Yang H, Issekutz TB, Wright JR, Jr. Prolongation of rat islet allograft survival by treatment with monoclonal antibodies against VLA-4 and LFA-1. *Transplantation* 1995; 60 (1): 71.
79. Kitchens WH, Haridas D, Wagener ME, et al. Integrin antagonists prevent costimulatory blockade-resistant transplant rejection by CD8(+) memory T cells. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2012; 12 (1): 69.
80. Freeman GJ, Casanovas JM, Umetsu DT, DeKruyff RH. TIM genes: a family of cell surface phosphatidylserine receptors that regulate innate and adaptive immunity. *Immunological reviews* 2010; 235 (1): 172.
81. Umetsu SE, Lee WL, McIntire JJ, et al. TIM-1 induces T cell activation and inhibits the development of peripheral tolerance. *Nature immunology* 2005; 6 (5): 447.
82. Meyers JH, Chakravarti S, Schlesinger D, et al. TIM-4 is the ligand for TIM-1, and the TIM-1-TIM-4 interaction regulates T cell proliferation. *Nature immunology* 2005; 6 (5): 455.
83. Degauque N, Mariat C, Kenny J, et al. Immunostimulatory Tim-1-specific antibody deprograms Tregs and prevents transplant tolerance in mice. *The Journal of clinical investigation* 2008; 118 (2): 735.

84. Ueno T, Habicht A, Clarkson MR, et al. The emerging role of T cell Ig mucin 1 in alloimmune responses in an experimental mouse transplant model. *The Journal of clinical investigation* 2008; 118 (2): 742.
85. Lenschow DJ, Zeng Y, Thistlethwaite JR, et al. Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4lg. *Science* 1992; 257 (5071): 789.
86. Xu H, Tadaki DK, Elster EA, et al. Humanized anti-CD154 antibody therapy for the treatment of allograft rejection in nonhuman primates. *Transplantation* 2002; 74 (7): 940.
87. Kirk AD. Crossing the bridge: large animal models in translational transplantation research. *Immunological reviews* 2003; 196: 176.
88. Larsen CP, Pearson TC, Adams AB, et al. Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2005; 5 (3): 443.
89. belatacept Falf.
90. Vincenti F, Charpentier B, Vanrenterghem Y, et al. A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2010; 10 (3): 535.
91. Vanrenterghem Y, Bresnahan B, Campistol J, et al. Belatacept-based regimens are associated with improved cardiovascular and metabolic risk factors compared with cyclosporine in kidney transplant recipients (BENEFIT and BENEFIT-EXT studies). *Transplantation* 2011; 91 (9): 976.

92. Krummey SM, Ford ML. Heterogeneity within T Cell Memory: Implications for Transplant Tolerance. *Frontiers in immunology* 2012; 3: 36.
93. Brook MO, Wood KJ, Jones ND. The impact of memory T cells on rejection and the induction of tolerance. *Transplantation* 2006; 82 (1): 1.
94. Valujskikh A, Pantenburg B, Heeger PS. Primed allospecific T cells prevent the effects of costimulatory blockade on prolonged cardiac allograft survival in mice. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2002; 2 (6): 501.
95. Zhai Y, Meng L, Gao F, Busuttil RW, Kupiec-Weglinski JW. Allograft rejection by primed/memory CD8+ T cells is CD154 blockade resistant: therapeutic implications for sensitized transplant recipients. *Journal of immunology* 2002; 169 (8): 4667.
96. Croft M, Bradley LM, Swain SL. Naive versus memory CD4 T cell response to antigen. Memory cells are less dependent on accessory cell costimulation and can respond to many antigen-presenting cell types including resting B cells. *Journal of immunology* 1994; 152 (6): 2675.
97. Vincenti F, Blacho G, Durrbach A, et al. Five-year safety and efficacy of belatacept in renal transplantation. *Journal of the American Society of Nephrology : JASN* 2010; 21 (9): 1587.
98. Larsen CP, Grinyo J, Medina-Pestana J, et al. Belatacept-based regimens versus a cyclosporine A-based regimen in kidney transplant recipients: 2-year results from the BENEFIT and BENEFIT-EXT studies. *Transplantation* 2010; 90 (12): 1528.
99. Grinyo J, Charpentier B, Pestana JM, et al. An integrated safety profile analysis of belatacept in kidney transplant recipients. *Transplantation* 2010; 90 (12): 1521.
100. Klintmalm GB FS, Lake JR, Vargas HE, Wekerle T, Meadows-Shropshire S. Belatacept-based immunosuppression in de novo liver transplant recipients: 1-year

- experience from a phase II study [oral abstract #355]. . *American journal of transplantation* 2011; 11 (s2): 137.
101. Shiao SL, McNiff JM, Masunaga T, Tamura K, Kubo K, Pober JS. Immunomodulatory properties of FK734, a humanized anti-CD28 monoclonal antibody with agonistic and antagonistic activities. *Transplantation* 2007; 83 (3): 304.
  102. Graves SS, Stone DM, Loretz C, et al. Antagonistic and agonistic anti-canine CD28 monoclonal antibodies: tools for allogeneic transplantation. *Transplantation* 2011; 91 (8): 833.
  103. Suntharalingam G, Perry MR, Ward S, et al. Cytokine storm in a phase I trial of the anti-CD28 monoclonal antibody TGN1412. *The New England journal of medicine* 2006; 355 (10): 1018.
  104. Zhang T, Zhou H, Wu G, O'Hara RJ, Pierson RNI, Azimzadeh AM. Selective Ligation of Cd28 By A Single-Chain Fv Inhibits T Cell Proliferation and Promotes Allograft Survival. *Transplantation* 2004; 78 (2): 569.
  105. Poirier N, Blancho G, Vanhove B. CD28-Specific Immunomodulating Antibodies: What Can Be Learned From Experimental Models? *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2012.
  106. Couzin J. Drug discovery. Magnificent obsession. *Science* 2005; 307 (5716): 1712.
  107. Badell IR, Thompson PW, Turner AP, et al. Nondepleting anti-CD40-based therapy prolongs allograft survival in nonhuman primates. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2012; 12 (1): 126.
  108. Vincenti F, Mendez R, Pescovitz M, et al. A phase I/II randomized open-label multicenter trial of efalizumab, a humanized anti-CD11a, anti-LFA-1 in renal

transplantation. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2007; 7 (7): 1770.

109. Kitchens WH, Larsen CP, Ford ML. Integrin antagonists for transplant immunosuppression: panacea or peril? *Immunotherapy* 2011; 3 (3): 305.
110. Carson KR, Focosi D, Major EO, et al. Monoclonal antibody-associated progressive multifocal leucoencephalopathy in patients treated with rituximab, natalizumab, and efalizumab: a Review from the Research on Adverse Drug Events and Reports (RADAR) Project. *The lancet oncology* 2009; 10 (8): 816.
111. Neff RT, Hurst FP, Falta EM, et al. Progressive multifocal leucoencephalopathy and use of mycophenolate mofetil after kidney transplantation. *Transplantation* 2008; 86 (10): 1474.

## **FIGURE LEGENDS:**

**Figure 1:** Diversity of costimulatory molecules in the different stages on the immune response.

**Figure 2:** A simplified diagram of the costimulatory molecules which are important in providing signal 2 for T cell activation. TNFR and Ig superfamily co-stimulatory signals appear to overlap in the activation of MAP kinase cascades, PKB, and activation of the transcription factor NF- $\kappa$ B. Concomitant activation of the transcription factors NF- $\kappa$ B, AP-1

and NFAT are critical for the transcription of genes that promote T cell activation such as IL-2.

**Figure 3:** Proposed model of the mechanism action of CTLA-4-Ig.

**Table 1:** Expression and function of costimulatory pairs in the immune response to an allograft