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Transplant tolerance through costimulation blockade - are we there yet?

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1. ABSTRACT

Achieving a tolerant state specific to the transplanted graft without subjecting patients to the risks of non-specific immunosuppression is the goal of transplant immunologists. In spite of the success achieved with currently available immunosuppresive therapies over acute rejection, an ongoing T cell mediated alloimmune response still poses a major challenge to the health of an allograft through chronic rejection. Modulating these destructive alloresponses through T cell costimulation blockade is a promising area of interest. In this article, we review our current knowledge about the role of various positive and negative costimulatory pathways during an alloimmune response. The ultimate nature of that response depends on the complex interaction between these positive and negative costimulatory pathways. We discuss the progress that has been achieved so far, through targeting these individual pathways, their interaction with other costimulatory pathways and the currently available immunosuppressive agents in various organ transplant models.

2. INTRODUCTION

"Induction and paralysis of an immune response involve the recognition of one and two determinants on an antigen respectively." – Science (1970).

With these words, Bretscher and Cohn introduced the concept of co-stimulation in lymphocyte activation, in their landmark paper "A theory of self-nonself discrimination" (1). Lafferty and Cunningham (2) who expanded this work, proposed a model that "T cell receptor (TCR) recognition of an appropriately presented antigen will deliver signal 1 to the T cell and that a simultaneously delivered signal 2 *(the co-stimulatory signal)* is required to activate the cell. So if a T cell encounters its cognate MHC antigen but does not receive the costimulatory signal 2, then the T cell will either die or rendered resistant to activation in its future encounters with that antigen. Similarly a Signal 2 delivered in the absence of antigen recognition results in a neutral event for the T cell". The above principle suggested a novel way to prevent

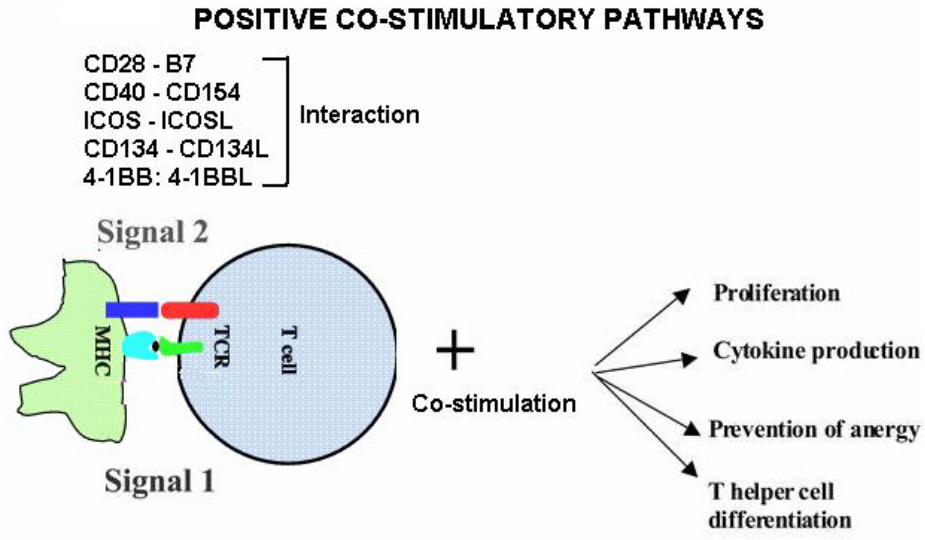

Figure 1. Interaction of the above-mentioned costimulatory molecules in concert with alloantigen-specific TCR engagement results in positive costimulation. Subsequent cascade of cellular signaling events leads to T cell proliferation, differentiation, activation and cytokine production.

alloimmune responses against the graft, without nonspecific immunosuppression, after therapeutic transplantation.

Transplant tolerance is defined as a selective lack of a destructive immune response to foreign antigens expressed by an allograft, leading to indefinite survival and acceptance of the graft, without the need for continuous non-specific immunosuppression. Why is this important? Organ transplantation is now firmly established as the therapy of choice for many forms of end-stage organ failure (3). Global demand for transplantable organs continues to exceed the available supply. Even after transplantation, despite the significant improvement in 1-year allograft survival rates, the incidence of graft loss due to chronic allograft dysfunction has remained stubbornly high (3, 4). A key factor driving the underlying pathophysiology of "Chronic rejection" in organ transplants is a persistent T cell mediated alloimmune response (5).

Moreover, currently available anti-rejection drugs cause overall immunosuppression by non-specifically blunting T-cell responses. For instance, glucocorticoids and calcineurin inhibitors impair TCR-mediated signaling (i.e. signal 1), whereas azathioprine and mycophenolate mofetil interfere with purine synthesis and agents such as antithymocyte globulin act by indiscriminate depletion of T cells. Thus current immunosuppressant therapy subjects the patients to non-specific immunosuppression thereby leading to substantial risk of opportunistic infections and cancer. Other non-immunosuppressive side effects include nephrotoxicity, diabetes, hyperlipidemia, and hypertension amongst others. So achieving a tolerant state would help not only to avoid the above side effects but also reduces the number of retransplants needed, thereby maximizing the use of the limited supply of donor organs.

3. COSTIMULATORY SIGNALS AND T-CELL ACTIVATION

Costimulation plays a critical role in deciding the fate of T-cell antigen interaction. Signal 1, delivered through the T-cell antigen receptor, is provided by the antigen itself and is responsible for the specificity of the immune response. The costimulatory signal 2, is not antigen specific and is dependent on the interaction of cell surface receptors with their ligands, typically on antigen presenting cells (APCs i.e. dentritic cells, B cells, monocytes and macrophages). Stimulation of the TCR without the second signal leads to T cell anergy for up to several weeks (6) or undergoes programmed cell death (apoptosis) (7). In contrast, delivery of "positive" costimulatory stimulus to the T cell triggers cytokine production, alloantigen-specific clonal expansion, and acquisition of a memory/effector phenotype capable of mediating a sustained immune response (See Figure 1).

The initial notion that costimulatory molecules play only a stimulatory role has had to undergo revision with the advent of negative costimulatory pathways. These negative costimulatory signals can lead to inhibition of T cell proliferation and cytokine production thereby causing anergy, apoptosis or induction of regulatory cells(8) (See Figure 2). Therefore, the ultimate fate of T cells and in turn immune responses appears to be mediated, at least in part, by the interplay between positive and negative T cell costimulatory pathways (9). It is increasingly recognized that these signaling events are not restricted to T cell-professional APC interactions, but also include T cell-to-T cell, T cell to B cell interactions and also T cell signaling from nonlymphoid cells in the periphery including endothelial cells and other parenchymal cells (10).

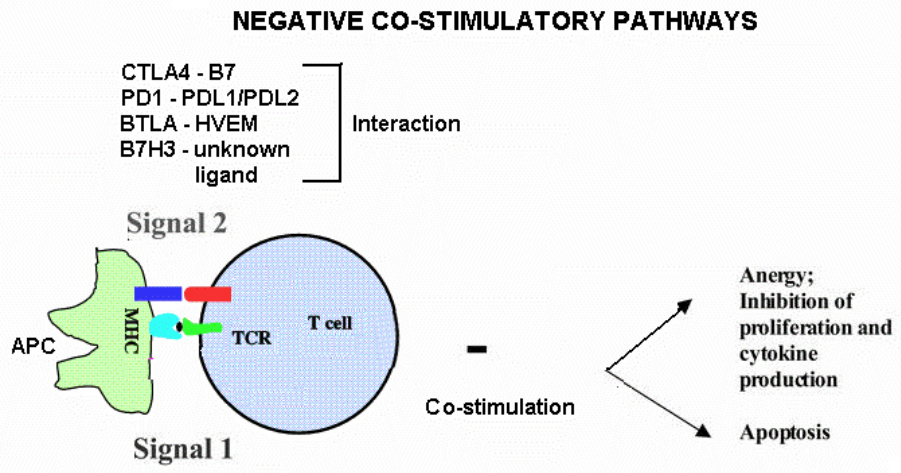

Figure 2. A negative costimulatory signal during TCR engagement with alloantigen specific T cell leads to T cell anergy/apoptosis, thereby limiting alloimmune response.

Costimulatory molecules are grouped broadly into CD28:B7 and the tumor necrosis factor: tumor necrosis factor receptor (TNF: TNFR) families based on their structural characteristics. All of the CD28 family members are type I transmembrane glycoproteins and are part of the immunoglobulin (Ig) superfamily (11). TNF family receptors contain one or more TIM (TRAF Interacting Motifs) in their cytoplasmic tails. Activation of TIM containing TNF receptors lead to recruitment of TRAF family members, and activation of multiple signal transduction pathways such as NF-KappaB (Nuclear Factor-KappaB), JNK (Jun N-terminal Kinase), p38, ERK (Extracellular Signal Regulated Kinase) and PI3K (Phosphoinisitide-3 Kinase). Among these molecules CD28/CTLA4-B7 and CD40-CD40L pathways have emerged as key players with higher functional significance.

Co-stimulatory blockade can prevent acute allograft rejection and can induce donor-specific tolerance in several rodent transplant models after various short-term treatments, although achieving tolerance is not possible in all models or strain combinations. This is due to the differences in the readiness to accept the graft by various strains of mouse. And also translating this success into higher mammals, non-human primates and particularly in humans has proven more difficult. There is also a hierarchy in the immunogenicity of the transplanted organs. Liver, kidney and heart are often readily accepted in some rodent strain combinations (12, 13) unlike primates, but skin grafts are more vigorously rejected by all species (14).

Hence the potency of the different types of costimulation blockade in various organ/tissue transplants and animal strain combinations needs to be interpreted in this context.

4. POSITIVE COSTIMULATORY PATHWAYS

Known positive costimulatory pathways include CD28:B7, ICOS-ICOSL in the CD28 family and CD40-CD154, CD134-CD134L, 4-1BB - 4-1BBL, CD27-CD70 in the TNF family.

4.1. CD28-B7 Pathway

CD28 is a key T cell surface costimulatory receptor and is the best characterized of all these molecules (15, 16). CD28 has two known ligands, B7-1 (CD80) and B7-2 (CD86). CD28 is constitutively expressed on naive T cells. B7-1 is generally absent on naïve cells and undergoes slower upregulation. In contrast though B7-2 is expressed at low levels on resting APCs, it undergoes rapid upregulation (17, 18). Thus B7-2 - CD28 interaction plays an important role in the induction of the immune response.

Upon activation T cells express B7-1 and CTLA4. CTLA4 is structurally similar to CD28 but more avidly (10-20 fold higher) binds to B7-1 and B7-2. This interaction results in a potent inhibitory signal that terminates the immune response. B7-1 seems to play a more important role in negative signaling than B7-2 through its interaction with CTLA4 for the following reasons. B7-1 favors binding to CTLA over CD28 by 20 - fold whereas B7-2 favors CTLA4 by only 8 fold. B7-1 is expressed on the cell surface as a dimer unlike B7-2, which is expressed as a monomer (19). CTLA4, also a dimer, can readily bind to two different B7 homodimers at a time to form a stable zipper like complex whereas CD28 can only bind one B7 homodimer (20).

Much has been learnt about the role of CD28:B7 pathway in transplantation through knockout models and by monoclonal blocking or signaling antibodies. In murine models, B7-1/B7-2 double knockout recipients are unable to reject cardiac allografts but could still acutely reject skin and islet allografts (21, 22). CD28 deficient recipients on the other hand do acutely reject cardiac allografts with CD8 T cells and NK cells playing a role in mediating this event (23, 24).

In CD28 intact wild type mice, administration of anti B7-1 monoclonal antibody has little effect whereas anti

B7-2 mAb prolong allograft survival. However in CD28 deficient mice, anti B7-1 actually shortens engraftment presumably by blocking an interaction with CTLA4 and surprisingly anti B7-2 prolonged engraftment (23). These findings strongly suggest that B7-2 interaction with CTLA4 is not as prominent as with B7-1. We have shown that anti CD28 mAb (25) and similarly combined anti B7-1/B7-2 mAb treatment prevented rejection and allowed long-term engraftment in rodent models. However in non-human primate vascularized transplant model, anti B7-1 & B7-2 either in combination with anti CD40L or with sirolimus has been shown to abrogate T-cell proliferation *in vitro*, thus preventing graft rejection and development of graft vascular disease (26).

However blocking this CD28:B7 pathway using the recombinant fusion protein CTLA4Ig has shown potent pro-tolerogenic effects and will be discussed further below.

4.2. CD40-CD154 (CD40L) Pathway

CD40 and its ligand CD154 (CD40L) were the first described and the most potent among the costimulatory molecules that belong to the TNFR-TNF superfamily. CD40 is constitutively expressed on APCs and also induced on endothelial cells and fibroblasts (27). CD154 is most abundant on activated CD4 T cells but has also been identified on CD8 cells, B cells, dendritic cells and most recently on activated platelets in the process of thrombus formation (28). Of note CD40: CD40L interaction initially provides costimulatory signal to the APC rather than T cell (reverse costimulation), thus leading to significantly augmented ability of the APC's antigen presenting ability. Thus indirectly it leads to strong T cell activation. In B cells, CD40 induced signal is essential for cellular proliferation, antibody production, isotype class switching and for the development of memory B cells.

Short courses of antibodies directed against CD154 have been shown to prevent acute allograft rejection in many rodent skin, cardiac, islet, marrow allografts and in non-human primate kidney transplant models (29-33). However, on its own it does not result in durable tolerance or successful prevention of chronic allograft rejection. This has been confirmed by the fact that CD154 deficient mice do not reject cardiac allografts but develop chronic allograft vasculopathy suggestive of other CD154 independent mechanisms of immune mediated allograft injury (34). However co-administration of bone marrow or donor specific transfusion with anti CD154 resulted in indefinite (usually defined as more than 100 days) or prolonged acceptance of the graft with donor-specific tolerance and prevention of chronic arteriopathy respectively (35-37). In non-human primates this strategy has not yet been tried in solid organ transplant models.

In both mice and non-human primates, rapamycin augments the ability of anti CD154 with or without CTLA4Ig, to promote allograft survival (38), whereas cyclosporine antagonizes this effect in some models (38, 39). Possible explanation of this could be that rapamycin augments whereas calcineurin inhibitors decrease activation induced cell death (AICD) by costimulation blockade. These impressive results in mice and primate transplant models led to the heightened expectation of its application in a clinical setting. However this enthusiasm was curbed by a high incidence of thromboembolic events complicating both nonhuman primate studies and phase 1 clinical human trials (40). Constitutive expression of CD40 and up regulation of adhesion molecules in vascular endothelium of various organs along with enhanced surface expression of the CD154 on activated platelets have been proposed as possible mechanisms for this (40). However the recent development of a chimeric anti CD40 mAb that synergizes with B7 blockade to prolong islet cell allograft survival in primates may help to circumvent this clinical problem(41).

4.3. ICOS-B7h (ICOSL) Pathway

Inducible costimulatory molecule (ICOS) is a CD28 homolog, not constitutively expressed but as the name implies is induced on activated T cells and also expressed on resting memory T cells (42). Its ligand, B7h is expressed at low levels on resting APCs, B cells and nonlymphoid tissues like endothelial cells, lung etc., but its expression is rapidly upregulated on cytokine triggered cellular activation (43). ICOS expression is stimulated on T cells by both TCR and CD28 signals but not solely dependent on CD28 signals, because blockade of ICOS in $CD28^{-/-}$ mice further inhibited Th1/Th2 differentiation (44). ICOS-ICOSL signaling has been shown to upregulate expression of CD40L which in turn triggers the CD28 signaling by enhanced surface expression of B7 on APCs, revealing the mutually beneficial interaction of these pathways.

Studies using ICOS-deficient mice have indicated ICOS as an essential regulator of helper T cell activation, differentiation and effector function (45, 46). It plays a more important role in providing positive costimulatory boost to the activated or resting memory T cells than activating naïve T cells (47, 48). Studies using pathway antagonists, transgenic mice, and knockout mice have revealed the important role of ICOS in B cell differentiation, immunoglobulin class switching, germinal center formation, and memory B cell development(45).

ICOS blockade or ICOS deficiency in mismatched murine cardiac transplants, prolonged graft survival, although to a lesser degree than CTLA4Ig or anti-CD40L mAb therapy (49). But they did not prevent chronic rejection. We have shown that the timing of ICOS blockade is critical, since delayed ICOS blockade prolonged allograft survival into mismatched recipients compared with early ICOS blockade in which rejection was accelerated (50). Blocking ICOS: ICOSL pathway along with CD40: CD40L or CD28: B7 pathways (using anti CD154 or CTLA4Ig) showed synergistic effects in preventing acute rejection and also in inhibiting the development of chronic rejection (51). Similarly anti ICOS mAb synergized with cyclosporine in prolonging cardiac graft (49), tacrolimus in liver transplant (52) and rapamycin in prolonging islet allograft survival in rodent models (53). So ICOS pathway may be a new potential therapeutic target, with careful timing for manipulation along with combination co-stimulation

blockade. But further studies in higher mammals are warranted.

4.4. CD134: CD134L Pathway

CD134 (OX40), a TNFR superfamily member, is induced primarily during T cell activation and is expressed on activated $CD4^+$ T cells and on some $CD8^+$ T cells (54, 55). CD 134L (OX40L) is mainly expressed by antigen-presenting cells (APCs), such as activated B cells (55), dendritic cells(56), macrophages, as well as T cells (57) and endothelial cells. CD134 engagement with its ligand generates a positive costimulatory signal as potent as CD28 and is instrumental in promoting the effector and memory T cell responses. In addition it plays a key role in Treg cell development, homeostasis, and suppressive activity (58).

We have shown that blockade of CD134L alone does not prolong allograft survival, however in combination with CTLA4Ig it synergistically achieved long-term survival of rodent cardiac (59) and skin allografts. Most importantly, this combination has been shown to prevent rejection in presensitized rodent heart transplant model through its effect on primed/effector T cells. Similarly Vu *et al* have shown that memory T cell-mediated rejection resistant to CD28/CD154 blockade is sensitive to CD134 blockade in a stringent mice skin transplant model (60). Thus targeting this pathway in combination with other costimulatory blockade strategies in preventing memory T cell-mediated rejection may prove to be important in achieving transplantation tolerance.

4.5. 4-1BB: 4-1BBL Pathway

4-1BB (CD137), a TNFR family member, is expressed on activated CD4+ and CD8+ T cells, NK cells, and dendritic cells (61, 62). Its ligand, 4-1BBL, is expressed on antigen presenting cells (APCs) including dendritic cells, macrophages, and B cells (63). Though engagement of 4-1BB by its ligand provides both CD28-dependent and CD28-independent positive costimulatory signals to CD4 and CD8 T-cells, it is critical for CD8 T-cell survival (64). Major effect of 4-1BB appears to be sustaining activated T cell survival by enhancing cell division and preventing activation-induced cell death, particularly in the absence of CD28.

CD28 or 4-1BB deficient mice reject both minor histocompatibility and MHC discordant skin allografts, whereas CD28/4-1BB double knock out mice showed some prolongation in both minor and major histocompatibility mismatched models (65, 66). A signaling 4-1BB mAb accelerated CD8 mediated allograft rejection in murine cardiac, skin and small-bowel transplantation models (67, 68). However in graft versus host disease this pathway regulates both CD4 and CD8 mediated allorepsonses (69). Evidence of the crucial role of CD8 T-cells and NK cells in allograft rejection may explain CD28/CD154 co-stimulation blockade resistant rejection models (23, 70). So targeting this pathway may have a beneficial role in modulating the CD8 T cell mediated alloimmune responses in more stringent transplant models.

4.6. CD27:CD70 Pathway

CD27, a member of the TNFR superfamily, is constitutively expressed on naïve T cells and is upregulated after antigenic triggering but lost in the effector stage (71-73). On B cells, CD27 is expressed only after antigen-induced activation (74) and is considered a marker for memory B cells in humans (75). It is implicated in T cell activation, development and T-cell dependent antibody production by B cells (76). CD70 is rapidly induced on T and B cells on activation and also found in thymic epithelium.CD70 blockade prolonged mouse vascularized cardiac allograft survival in WT recipients and in CD28 deficient mice resulted in long-term graft survival without chronic rejection. CD70 blockade inhibits the proliferation and activation of CD8 T cells, hence prevents CD8 mediated rejection. Thus similar to 4-1BB pathway, CD27-70 may be an attractive target to improve strategies in inducing tolerance in models resistant to conventional T cell costimulatory blockade.

5. NEGATIVE CO-STIMULATORY PATHWAYS

5.1. CTLA4: B7 Pathway

CTLA4 is expressed after T cell activation and has a much higher affinity (10 to 20 fold) for B7-1/B7-2. CTLA4-B7 interaction delivers negative co-stimulatory stimulus to T cell thereby leading to either anergic state or apoptosis. CTLA4Ig, a recombinant fusion protein containing the extracellular domain of CTLA4 fused to an IgG heavy chain tail was developed to competitively antagonize CD28-B7 interaction. Taking advantage of its higher binding ability it out-competes CD28 for binding to B7-1 and B7-2 (77), and use of an IgG tail increases the half-life of the molecule.

CTLA4Ig prevents acute rejection and results in durable tolerance in some but not all mouse models of transplantation (78, 79). Though the exact mechanism of action is unknown, donor specific transfusion (DST) augments tolerance, but this effect particularly depends on the stringency of the model, degree of the immunological mismatch, and duration and the timing of CTLA-4 administration (80). Potency of CTLA4Ig could be augmented by administering this on day 2 of transplantation, perhaps by allowing time for the up regulation of B7-1 or T cell expressed CTLA4 to occur (78, 80). In both MHC class II and I mismatched mouse heart allograft models, long-term administration of CTLA4Ig on its own has been shown to overcome chronic rejection (81).

In nonhuman primate studies, targeting B7-1 and -2 either with blocking mAbs or with CTLA4Ig prolongs both renal and islet cell allograft survival (82-85). Notably, the effect requires the simultaneous blockade of CD40-CD154 signaling or the concomitant administration of rapamycin (82-86). Above combinations and anti-CD45 RB therapy have been shown to up regulate CTLA4 expression, thereby promoting tolerance (87, 88). LEA29Y (Belatacept), a high affinity variant of CTLA4Ig, has shown great promise as a combination agent in nonhuman primate studies (41, 86). It has synergistic effects when combined with anti-CD40 in both renal and islet cell transplantation(86, 89). Importantly, this agent has now entered stage II human clinical trials as a calcineurin-

sparing agent in human renal transplantation (90). At one-year post transplantation results show that Belatacept did not appear to be inferior to cyclosporine in preventing acute rejection but may preserve the glomerular filtration rate and reduce the rate of chronic allograft nephropathy through the elimination of the nephrotoxic effects of cyclosporine (91).

5.2. PD1-PDL1/PDL2 Pathway

PD1 is a CD28 homologue and this pathway, like CTLA4-B7, has been shown to play a critical and differential role in regulating $CD4^+$ and $CD8^+$ T cell mediated alloimmune responses within the graft. PD-1 is expressed by activated T cells, B cells, NK cells and macrophages (92, 93). PD1 interacts with PDL1 and PDL2. PDL1 (B7-H1) is expressed on all hemopoietic cells and many nonhemopoietic tissues like endothelia, heart etc., whereas PD-L2 (B7-DC) expression is restricted primarily to dendritic cells and macrophages (94-96). CTLA-4:B7 pathway provides a critical negative signal to alloreactive $CD8^+$ T cells, particularly in the presence of CD28 costimulation. In contrast, PD-1 receptor seems to down-regulate alloreactive $CD4^+$ T cells in the absence of CD28 costimulation (97).

Blockade of PD1 and its ligands are shown to play a differential role in alloresponses. Our group showed that in a murine cardiac allograft model, treatment with anti-PD-L1 mAb, but not anti-PD1 mAb, accelerates the time to rejection of fully allogenic cardiac allografts in wild-type recipients, whereas both antibodies accelerate rejection in the absence of CD28 costimulation (98). In a novel transgenic model of CD4-mediated skin allograft rejection, we also showed that blockade of PD1-PDL1 interaction but not PDL2 resulted in enhanced alloantigen-specific T cell expansion and activation. It coincided with reduced apoptosis of alloantigen specific T cells.

Administration of PD-L1Ig in $CD28^{-/-}$ recipients, or in conjunction with immunosuppression in fully MHC-disparate combinations, has been shown to markedly prolonged murine cardiac (99) and islet allograft survival, in some cases causing permanent engraftment Recent unpublished work from our group (100) shows that donor cardiac allografts deficient in PDL1 were rejected in an accelerated fashion in a partially mismatched mice model where wild type grafts survive indefinitely without any intervention. This novel finding demonstrates that PD-L1 expression, on the donor cardiac allograft tissue serves as an important negative regulatory mechanism for limiting alloimmune responses *in vivo*. Thus this pathway plays a significant role in regulating alloimmune responses but further studies are needed to exploit this further towards our goal of achieving transplant tolerance.

5.3. BTLA/LIGHT-HVEM Pathway

B and T lymphocyte attenuator (BTLA), a CD28 family member, is a novel costimulatory receptor that is induced by activated $CD4^+$ and $CD8^+$ T cells and also expressed by B cells, macrophages, and bone marrow-derived myeloid dendritic cells (13, 101, 102). The ligand for BTLA is herpes virus entry mediator (HVEM), which is expressed on naïve T and B cells. This is an unusual example of cross-talk between the CD28 family and the TNFR family and this interaction leads to negative costimulation. HVEM also has another binding partner LIGHT, a membrane-expressed TNF family member and this interaction culminates in positive costimulation (103).

Tao *et al* (104) showed that BTLA and HVEM are the major regulators of in-host allogeneic responses to class I- or class II-mismatched mouse cardiac allografts. However this effect is not seen in a fully MHC mismatched setting. Kosuge *et al* showed that blockade of the LIGHT-HVEM pathway using HVEMIg attenuated the development of graft arterial disease through suppression of cytokine expression and SMC proliferation (105). LIGHT deficient recipients show modest prolongation of cardiac allograft survival however this effect is significantly enhanced by cyclosporine (106). Thus, BTLA seems to exert its main effect when the strength of the immune response is weak. Hence targeting of this pathway in combination with other therapeutic approaches may be useful in preventing chronic rejection.

5.4. B7-H3 Pathway

B7 homolog 3 (B7-H3) is a new member of the B7 family of costimulatory molecules. It is broadly expressed in nonlymphoid tissues and is upregulated by inflammatory mediators (107-109). Although its receptor remains unknown, it is believed to be induced on activated T cells. In mice model, B7-H3 knock out recipients showed no survival advantage of their cardiac and islet allografts without any intervention. However administration of cyclosporin or rapamycin significantly improved the survival of the grafts in this group compared to WT recipients (110). Hence it was concluded that it is a positive costimulatory molecule whose expression contributed to the development of experimental acute and chronic allograft rejection. However there is data (107, 108)) to support both costimulatory & coinhibitory roles of B7-H3. So further studies are required to determine the role of B7-H3 pathway within the hierarchy of costimulatory molecules and to elucidate its interactions with the major costimulatory pathways such as the CD28-B7 and CD40-CD154 pathways.

6. CONCLUSION

Over the past 35 years, since the recognition of the indispensable role of co-stimulation in T cell activation, a number of costimulatory pathways have been identified. Though every member has been shown to play an important and unique function during the course of the immune response, several questions are unanswered. One has to wonder: is there a hierarchy with a unique and mutually beneficial role for all these pathways or is it a matter of redundancy? If one pathway is blocked or absent whether the others could take over the role of mounting immune responses against foreign antigens?

However, the ultimate nature of that response, at least partly, depends on the complex interaction between the various positive and negative costimulatory pathways. Targeting these pathways through timely intervention needs better understanding of their spatial and temporal expression. To achieve transplant tolerance,

future strategies will need to harness the regulation of alloimmune responses by negative costimulatory pathways in addition to blockade of positive costimulatory signals. Above all, a critical insight into the interactions between different costimulatory molecules and immunosuppressive agents is vital, along with carefully designed mechanistic studies, to translate what we learnt from rodents to humans.

7. ACKNOWLEDGEMENT

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8. REFERENCES

1. Bretscher, P. & M. Cohn: A theory of self-nonself discrimination. *Science*, 169, 1042-9 (1970)

2. Lafferty, K. J. & A. J. Cunningham: A new analysis of allogeneic interactions. *Aust J Exp Biol Med Sci*, 53, 27-42 (1975)

3. Sayegh, M. H. & C. B. Carpenter: Transplantation 50 years later--progress, challenges, and promises. *N Engl J Med*, 351, 2761-6 (2004)

4. Meier-Kriesche, H. U., J. D. Schold, T. R. Srinivas & B. Kaplan: Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. *Am J Transplant*, 4, 378-83 (2004)

5. Magee, C. & M. Pascual: The growing problem of chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med*, 349, 994-6 (2003)

6. Gimmi, C. D., G. J. Freeman, J. G. Gribben, G. Gray & L. M. Nadler: Human T-cell clonal anergy is induced by antigen presentation in the absence of B7 costimulation. *Proc Natl Acad Sci U S A*, 90, 6586-90 (1993)

7. Noel, P. J., L. H. Boise, J. M. Green & C. B. Thompson: CD28 costimulation prevents cell death during primary T cell activation. *J Immunol*, 157, 636-42 (1996)

8. Khoury, S. J. & M. H. Sayegh: The roles of the new negative T cell costimulatory pathways in regulating autoimmunity. *Immunity*, 20, 529-38 (2004)

9. Rothstein, D. M. & M. H. Sayegh: T-cell costimulatory pathways in allograft rejection and tolerance. *Immunol Rev*, 196, 85-108 (2003)

10. Kroczek, R. A., H. W. Mages & A. Hutloff: Emerging paradigms of T-cell co-stimulation. *Curr Opin Immunol*, 16, 321-7 (2004)

11. Sharpe, A. H. & G. J. Freeman: The B7-CD28 superfamily. *Nat Rev Immunol*, 2, 116-26 (2002)

12. Bickerstaff, A. A., J. J. Wang, R. P. Pelletier & C. G. Orosz: The graft helps to define the character of the alloimmune response. *Transpl Immunol*, 9, 137-41 (2002)

13. Gasser, M., A. M. Waaga-Gasser, J. E. Kist-van Holthe, X. Yuan, S. M. Lenhard, K. A. Abdallah & A. Chandraker: Chronic rejection: insights from a novel immunosuppressive-free model of kidney transplantation. *J Am Soc Nephrol*, 15, 687-94 (2004)

14. Monk, N. J., R. E. Hargreaves, E. Simpson, J. P. Dyson & S. Jurcevic: Transplant tolerance: models, concepts and facts. *J Mol Med*, 84, 295-304 (2006)

15. Linsley, P. S. & J. A. Ledbetter: The role of the CD28 receptor during T cell responses to antigen. *Annu Rev Immunol*, 11, 191-212 (1993)

16. June, C. H., J. A. Bluestone, L. M. Nadler & C. B. Thompson: The B7 and CD28 receptor families. *Immunol Today*, 15, 321-31 (1994)

17. McAdam, A. J., A. N. Schweitzer & A. H. Sharpe: The role of B7 co-stimulation in activation and differentiation of CD4+ and CD8+ T cells. *Immunol Rev*, 165, 231-47 (1998)

18. Alegre, M. L., K. A. Frauwirth & C. B. Thompson: T-cell regulation by CD28 and CTLA-4. *Nat Rev Immunol*, 1, 220-8 (2001)

19. Collins, A. V., D. W. Brodie, R. J. Gilbert, A. Iaboni, R. Manso-Sancho, B. Walse, D. I. Stuart, P. A. van der Merwe & S. J. Davis: The interaction properties of costimulatory molecules revisited. *Immunity*, 17, 201-10 (2002)

20. Stamper, C. C., Y. Zhang, J. F. Tobin, D. V. Erbe, S. Ikemizu, S. J. Davis, M. L. Stahl, J. Seehra, W. S. Somers & L. Mosyak: Crystal structure of the B7-1/CTLA-4 complex that inhibits human immune responses. *Nature*, 410, 608-11 (2001)

21. Szot, G. L., P. Zhou, A. H. Sharpe, G. He, O. Kim, K. A. Newell, J. A. Bluestone & J. R. Thistlethwaite, Jr.: Absence of host B7 expression is sufficient for long-term murine vascularized heart allograft survival. *Transplantation*, 69, 904-9 (2000)

22. Mandelbrot, D. A., A. J. McAdam & A. H. Sharpe: B7-1 or B7-2 is required to produce the lymphoproliferative phenotype in mice lacking cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). *J Exp Med*, 189, 435-40 (1999)

23. Yamada, A., K. Kishimoto, V. M. Dong, M. Sho, A. D. Salama, N. G. Anosova, G. Benichou, D. A. Mandelbrot, A. H. Sharpe, L. A. Turka, H. Auchincloss, Jr. & M. H. Sayegh: CD28-independent costimulation of T cells in alloimmune responses. *J Immunol*, 167, 140-6 (2001)

24. Maier, S., C. Tertilt, N. Chambron, K. Gerauer, N. Huser, C. D. Heidecke & K. Pfeffer: Inhibition of natural

killer cells results in acceptance of cardiac allografts in CD28-/- mice. *Nat Med*, 7, 557-62 (2001)

25. Dong, V. M., X. Yuan, A. J. Coito, A. M. Waaga, M. H. Sayegh & A. Chandraker: Mechanisms of targeting CD28 by a signaling monoclonal antibody in acute and chronic allograft rejection. *Transplantation*, 73, 1310-7 (2002)

26. Montgomery, S. P., H. Xu, D. K. Tadaki, A. Celniker, L. C. Burkly, J. D. Berning, F. Cruzata, E. A. Elster, G. Gray, R. L. Kampen, S. J. Swanson, D. M. Harlan & A. D. Kirk: Combination induction therapy with monoclonal antibodies specific for CD80, CD86, and CD154 in nonhuman primate renal transplantation. *Transplantation*, 74, 1365-9 (2002)

27. Larsen, C. P. & T. C. Pearson: The CD40 pathway in allograft rejection, acceptance, and tolerance. *Curr Opin Immunol*, 9, 641-7 (1997)

28. Henn, V., J. R. Slupsky, M. Grafe, I. Anagnostopoulos, R. Forster, G. Muller-Berghaus & R. A. Kroczek: CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature*, 391, 591-4 (1998)

29. Rossini, A. A., D. C. Parker, N. E. Phillips, F. H. Durie, R. J. Noelle, J. P. Mordes & D. L. Greiner: Induction of immunological tolerance to islet allografts. *Cell Transplant*, 5, 49-52 (1996)

30. Larsen, C. P., E. T. Elwood, D. Z. Alexander, S. C. Ritchie, R. Hendrix, C. Tucker-Burden, H. R. Cho, A. Aruffo, D. Hollenbaugh, P. S. Linsley, K. J. Winn & T. C. Pearson: Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature*, 381, 434-8 (1996)

31. Larsen, C. P., D. Z. Alexander, D. Hollenbaugh, E. T. Elwood, S. C. Ritchie, A. Aruffo, R. Hendrix & T. C. Pearson: CD40-gp39 interactions play a critical role during allograft rejection. Suppression of allograft rejection by blockade of the CD40-gp39 pathway. *Transplantation*, 61, 4-9 (1996)

32. Hancock, W. W., M. H. Sayegh, X. G. Zheng, R. Peach, P. S. Linsley & L. A. Turka: Costimulatory function and expression of CD40 ligand, CD80, and CD86 in vascularized murine cardiac allograft rejection. *Proc Natl Acad Sci U S A*, 93, 13967-72 (1996)

33. Blazar, B. R., P. A. Taylor, A. Panoskaltsis-Mortari, J. Buhlman, J. Xu, R. A. Flavell, R. Korngold, R. Noelle & D. A. Vallera: Blockade of CD40 ligand-CD40 interaction impairs CD4+ T cell-mediated alloreactivity by inhibiting mature donor T cell expansion and function after bone marrow transplantation. *J Immunol*, 158, 29-39 (1997)

34. Shimizu, K., U. Schonbeck, F. Mach, P. Libby & R. N. Mitchell: Host CD40 ligand deficiency induces long-term allograft survival and donor-specific tolerance in mouse cardiac transplantation but does not prevent graft arteriosclerosis. *J Immunol*, 165, 3506-18 (2000)

35. Wekerle, T., J. Kurtz, H. Ito, J. V. Ronquillo, V. Dong, G. Zhao, J. Shaffer, M. H. Sayegh & M. Sykes: Allogeneic bone marrow transplantation with co-stimulatory blockade induces macrochimerism and tolerance without cytoreductive host treatment. *Nat Med*, 6, 464-9 (2000)

36. Markees, T. G., N. E. Phillips, E. J. Gordon, R. J. Noelle, L. D. Shultz, J. P. Mordes, D. L. Greiner & A. A. Rossini: Long-term survival of skin allografts induced by donor splenocytes and anti-CD154 antibody in thymectomized mice requires CD4 (+) T cells, interferon-gamma, and CTLA4. *J Clin Invest*, 101, 2446-55 (1998)

37. Markees, T., N. Phillips, E. Gordon, R. J. Noelle, J. P. Mordes, D. L. Greiner & A. A. Rossini: Improved skin allograft tolerance induced by treatment with donor splenocytes and an extended course of anti-CD154 monoclonal antibody. *Transplant Proc*, 30, 2444-6 (1998)

38. Li, Y., X. C. Li, X. X. Zheng, A. D. Wells, L. A. Turka & T. B. Strom: Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance. *Nat Med*, 5, 1298-302 (1999)

39. Kirk, A. D., L. C. Burkly, D. S. Batty, R. E. Baumgartner, J. D. Berning, K. Buchanan, J. H. Fechner, Jr., R. L. Germond, R. L. Kampen, N. B. Patterson, S. J. Swanson, D. K. Tadaki, C. N. TenHoor, L. White, S. J. Knechtle & D. M. Harlan: Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. *Nat Med*, 5, 686-93 (1999)

40. Kawai, T., D. Andrews, R. B. Colvin, D. H. Sachs & A. B. Cosimi: Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. *Nat Med*, 6, 114 (2000)

41. Adams, A. B., N. Shirasugi, T. R. Jones, M. M. Durham, E. A. Strobert, S. Cowan, P. Rees, R. Hendrix, K. Price, N. S. Kenyon, D. Hagerty, R. Townsend, D. Hollenbaugh, T. C. Pearson & C. P. Larsen: Development of a chimeric anti-CD40 monoclonal antibody that synergizes with LEA29Y to prolong islet allograft survival. *J Immunol*, 174, 542-50 (2005)

42. Yoshinaga, S. K., J. S. Whoriskey, S. D. Khare, U. Sarmiento, J. Guo, T. Horan, G. Shih, M. Zhang, M. A. Coccia, T. Kohno, A. Tafuri-Bladt, D. Brankow, P. Campbell, D. Chang, L. Chiu, T. Dai, G. Duncan, G. S. Elliott, A. Hui, S. M. McCabe, S. Scully, A. Shahinian, C. L. Shaklee, G. Van, T. W. Mak & G. Senaldi: T-cell co-stimulation through B7RP-1 and ICOS. *Nature*, 402, 827-32 (1999)

43. Beier, K. C., A. Hutloff, A. M. Dittrich, C. Heuck, A. Rauch, K. Buchner, B. Ludewig, H. D. Ochs, H. W. Mages & R. A. Kroczek: Induction, binding specificity and function of human ICOS. *Eur J Immunol*, 30, 3707-17 (2000)

44. Kopf, M., A. J. Coyle, N. Schmitz, M. Barner, A. Oxenius, A. Gallimore, J. C. Gutierrez-Ramos & M. F. Bachmann: Inducible costimulator protein (ICOS) controls T helper cell subset polarization after virus and parasite infection. *J Exp Med*, 192, 53-61 (2000)

45. McAdam, A. J., R. J. Greenwald, M. A. Levin, T. Chernova, N. Malenkovich, V. Ling, G. J. Freeman & A. H. Sharpe: ICOS is critical for CD40-mediated antibody class switching. *Nature*, 409, 102-5 (2001)

46. Tafuri, A., A. Shahinian, F. Bladt, S. K. Yoshinaga, M. Jordana, A. Wakeham, L. M. Boucher, D. Bouchard, V. S. Chan, G. Duncan, B. Odermatt, A. Ho, A. Itie, T. Horan, J. S. Whoriskey, T. Pawson, J. M. Penninger, P. S. Ohashi & T. W. Mak: ICOS is essential for effective T-helper-cell responses. *Nature*, 409, 105-9 (2001)

47. Coyle, A. J., S. Lehar, C. Lloyd, J. Tian, T. Delaney, S. Manning, T. Nguyen, T. Burwell, H. Schneider, J. A. Gonzalo, M. Gosselin, L. R. Owen, C. E. Rudd & J. C. Gutierrez-Ramos: The CD28-related molecule ICOS is required for effective T cell-dependent immune responses. *Immunity*, 13, 95-105 (2000)

48. Coyle, A. J. & J. C. Gutierrez-Ramos: The expanding B7 superfamily: increasing complexity in costimulatory signals regulating T cell function. *Nat Immunol*, 2, 203-9 (2001)

49. Ozkaynak, E., W. Gao, N. Shemmeri, C. Wang, J. C. Gutierrez-Ramos, J. Amaral, S. Qin, J. B. Rottman, A. J. Coyle & W. W. Hancock: Importance of ICOS-B7RP-1 costimulation in acute and chronic allograft rejection. *Nat Immunol*, 2, 591-6 (2001)

50. Salama, A. D., X. Yuan, A. Nayer, A. Chandraker, M. Inobe, T. Uede & M. H. Sayegh: Interaction between ICOS-B7RP1 and B7-CD28 costimulatory pathways in alloimmune responses *in vivo*. *Am J Transplant*, 3, 390-5 (2003)

51. Kosuge, H., J. Suzuki, R. Gotoh, N. Koga, H. Ito, M. Isobe, M. Inobe & T. Uede: Induction of immunologic tolerance to cardiac allograft by simultaneous blockade of inducible co-stimulator and cytotoxic T-lymphocyte antigen 4 pathway. *Transplantation*, 75, 1374-9 (2003)

52. Guo, L., X. K. Li, S. Enosawa, N. Funeshima, S. Suzuki, H. Kimura, Y. Sugawara, K. Tezuka & M. Makuuchi: Significant enhancement by anti-ICOS antibody of suboptimal tacrolimus immunosuppression in rat liver transplantation. *Liver Transpl*, 10, 743-7 (2004)

53. Nanji, S. A., W. W. Hancock, C. C. Anderson, A. B. Adams, B. Luo, C. D. Schur, R. L. Pawlick, L. Wang, A. J. Coyle, C. P. Larsen & A. M. Shapiro: Multiple combination therapies involving blockade of ICOS/B7RP-1 costimulation facilitate long-term islet allograft survival. *Am J Transplant*, 4, 526-36 (2004)

54. Paterson, D. J., W. A. Jefferies, J. R. Green, M. R. Brandon, P. Corthesy, M. Puklavec & A. F. Williams: Antigens of activated rat T lymphocytes including a molecule of 50,000 Mr detected only on CD4 positive T blasts. *Mol Immunol*, 24, 1281-90 (1987)

55. Calderhead, D. M., J. E. Buhlmann, A. J. van den Eertwegh, E. Claassen, R. J. Noelle & H. P. Fell: Cloning of mouse Ox40: a T cell activation marker that may mediate T-B cell interactions. *J Immunol*, 151, 5261-71 (1993)

56. Ohshima, Y., Y. Tanaka, H. Tozawa, Y. Takahashi, C. Maliszewski & G. Delespesse: Expression and function of OX40 ligand on human dendritic cells. *J Immunol*, 159, 3838-48 (1997)

57. Miura, S., K. Ohtani, N. Numata, M. Niki, K. Ohbo, Y. Ina, T. Gojobori, Y. Tanaka, H. Tozawa, M. Nakamura & et al.: Molecular cloning and characterization of a novel glycoprotein, gp34, that is specifically induced by the human T-cell leukemia virus type I transactivator p40tax. *Mol Cell Biol*, 11, 1313-25 (1991)

58. Takeda, I., S. Ine, N. Killeen, L. C. Ndhlovu, K. Murata, S. Satomi, K. Sugamura & N. Ishii: Distinct roles for the OX40-OX40 ligand interaction in regulatory and nonregulatory T cells. *J Immunol*, 172, 3580-9 (2004)

59. Yuan, X., A. D. Salama, V. Dong, I. Schmitt, N. Najafian, A. Chandraker, H. Akiba, H. Yagita & M. H. Sayegh: The role of the CD134-CD134 ligand costimulatory pathway in alloimmune responses *in vivo*. *J Immunol*, 170, 2949-55 (2003)

60. Vu, M. D., M. R. Clarkson, H. Yagita, L. A. Turka, M. H. Sayegh & X. C. Li: Critical, but conditional, role of OX40 in memory T cell-mediated rejection. *J Immunol*, 176, 1394-401 (2006)

61. Kwon, B. S. & S. M. Weissman: cDNA sequences of two inducible T-cell genes. *Proc Natl Acad Sci U S A*, 86, 1963-7 (1989)

62. Pollok, K. E., Y. J. Kim, Z. Zhou, J. Hurtado, K. K. Kim, R. T. Pickard & B. S. Kwon: Inducible T cell antigen 4-1BB. Analysis of expression and function. *J Immunol*, 150, 771-81 (1993)

63. Pollok, K. E., Y. J. Kim, J. Hurtado, Z. Zhou, K. K. Kim & B. S. Kwon: 4-1BB T-cell antigen binds to mature B cells and macrophages, and costimulates anti-mu-primed splenic B cells. *Eur J Immunol*, 24, 367-74 (1994)

64. Takahashi, C., R. S. Mittler & A. T. Vella: Cutting edge: 4-1BB is a bona fide CD8 T cell survival signal. *J Immunol*, 162, 5037-40 (1999)

65. Cannons, J. L., P. Lau, B. Ghumman, M. A. DeBenedette, H. Yagita, K. Okumura & T. H. Watts: 4-1BB ligand induces cell division, sustains survival, and

enhances effector function of CD4 and CD8 T cells with similar efficacy. *J Immunol*, 167, 1313-24 (2001)

66. DeBenedette, M. A., T. Wen, M. F. Bachmann, P. S. Ohashi, B. H. Barber, K. L. Stocking, J. J. Peschon & T. H. Watts: Analysis of 4-1BB ligand (4-1BBL)-deficient mice and of mice lacking both 4-1BBL and CD28 reveals a role for 4-1BBL in skin allograft rejection and in the cytotoxic T cell response to influenza virus. *J Immunol*, 163, 4833-41 (1999)

67. Shuford, W. W., K. Klussman, D. D. Tritchler, D. T. Loo, J. Chalupny, A. W. Siadak, T. J. Brown, J. Emswiler, H. Raecho, C. P. Larsen, T. C. Pearson, J. A. Ledbetter, A. Aruffo & R. S. Mittler: 4-1BB costimulatory signals preferentially induce CD8+ T cell proliferation and lead to the amplification *in vivo* of cytotoxic T cell responses. *J Exp Med*, 186, 47-55 (1997)

68. Wang, J., Z. Guo, Y. Dong, O. Kim, J. Hart, A. Adams, C. P. Larsen, R. S. Mittler & K. A. Newell: Role of 4-1BB in allograft rejection mediated by CD8+ T cells. *Am J Transplant*, 3, 543-51 (2003)

69. Blazar, B. R., B. S. Kwon, A. Panoskaltsis-Mortari, K. B. Kwak, J. J. Peschon & P. A. Taylor: Ligation of 4-1BB (CDw137) regulates graft-versus-host disease, graft-versus-leukemia, and graft rejection in allogeneic bone marrow transplant recipients. *J Immunol*, 166, 3174-83 (2001)

70. Akiba, H., H. Oshima, K. Takeda, M. Atsuta, H. Nakano, A. Nakajima, C. Nohara, H. Yagita & K. Okumura: CD28-independent costimulation of T cells by OX40 ligand and CD70 on activated B cells. *J Immunol*, 162, 7058-66 (1999)

71. de Jong, R., W. A. Loenen, M. Brouwer, L. van Emmerik, E. F. de Vries, J. Borst & R. A. van Lier: Regulation of expression of CD27, a T cell-specific member of a novel family of membrane receptors. *J Immunol*, 146, 2488-94 (1991)

72. van Lier, R. A., J. Borst, T. M. Vroom, H. Klein, P. Van Mourik, W. P. Zeijlemaker & C. J. Melief: Tissue distribution and biochemical and functional properties of Tp55 (CD27), a novel T cell differentiation antigen. *J Immunol*, 139, 1589-96 (1987)

73. Hamann, D., P. A. Baars, M. H. Rep, B. Hooibrink, S. R. Kerkhof-Garde, M. R. Klein & R. A. van Lier: Phenotypic and functional separation of memory and effector human CD8+ T cells. *J Exp Med*, 186, 1407-18 (1997)

74. Kobata, T., S. Jacquot, S. Kozlowski, K. Agematsu, S. F. Schlossman & C. Morimoto: CD27-CD70 interactions regulate B-cell activation by T cells. *Proc Natl Acad Sci U S A*, 92, 11249-53 (1995)

75. Klein, U., K. Rajewsky & R. Kuppers: Human immunoglobulin (Ig)M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. *J Exp Med*, 188, 1679-89 (1998)

76. Yamada, A., A. D. Salama, M. Sho, N. Najafian, T. Ito, J. P. Forman, R. Kewalramani, S. Sandner, H. Harada, M. R. Clarkson, D. A. Mandelbrot, A. H. Sharpe, H. Oshima, H. Yagita, G. Chalasani, F. G. Lakkis, H. Auchincloss, Jr. & M. H. Sayegh: CD70 signaling is critical for CD28-independent CD8+ T cell-mediated alloimmune responses *in vivo*. *J Immunol*, 174, 1357-64 (2005)

77. Linsley, P. S., W. Brady, M. Urnes, L. S. Grosmaire, N. K. Damle & J. A. Ledbetter: CTLA-4 is a second receptor for the B cell activation antigen B7. *J Exp Med*, 174, 561-9 (1991)

78. Sayegh, M. H., E. Akalin, W. W. Hancock, M. E. Russell, C. B. Carpenter, P. S. Linsley & L. A. Turka: CD28-B7 blockade after alloantigenic challenge *in vivo* inhibits Th1 cytokines but spares Th2. *J Exp Med*, 181, 1869-74 (1995)

79. Turka, L. A., P. S. Linsley, H. Lin, W. Brady, J. M. Leiden, R. Q. Wei, M. L. Gibson, X. G. Zheng, S. Myrdal, D. Gordon & et al.: T-cell activation by the CD28 ligand B7 is required for cardiac allograft rejection *in vivo*. *Proc Natl Acad Sci U S A*, 89, 11102-5 (1992)

80. Lin, H., S. F. Bolling, P. S. Linsley, R. Q. Wei, D. Gordon, C. B. Thompson & L. A. Turka: Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA4Ig plus donor-specific transfusion. *J Exp Med*, 178, 1801-6 (1993)

81. Glysing-Jensen, T., A. Raisanen-Sokolowski, M. H. Sayegh & M. E. Russell: 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*, 64, 1641-5 (1997)

82. Izawa, A., M. H. Sayegh & A. Chandraker: The antagonism of calcineurin inhibitors and costimulatory blockers: fact or fiction? *Transplant Proc*, 36, 570S-573S (2004)

83. Kirk, A. D., D. K. Tadaki, A. Celniker, D. S. Batty, J. D. Berning, J. O. Colonna, F. Cruzata, E. A. Elster, G. S. Gray, R. L. Kampen, N. B. Patterson, P. Szklut, J. Swanson, H. Xu & D. M. Harlan: Induction therapy with monoclonal antibodies specific for CD80 and CD86 delays the onset of acute renal allograft rejection in non-human primates. *Transplantation*, 72, 377-84 (2001)

84. Kirk, A. D., D. M. Harlan, N. N. Armstrong, T. A. Davis, Y. Dong, G. S. Gray, X. Hong, D. Thomas, J. H. Fechner, Jr. & S. J. Knechtle: CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc Natl Acad Sci U S A*, 94, 8789-94 (1997)

85. Birsan, T., B. Hausen, J. P. Higgins, R. W. Hubble, J. Klupp, M. Stalder, A. Celniker, S. Friedrich, R. M. O'Hara

& R. E. Morris: Treatment with humanized monoclonal antibodies against CD80 and CD86 combined with sirolimus prolongs renal allograft survival in cynomolgus monkeys. *Transplantation*, 75, 2106-13 (2003)

86. Adams, A. B., N. Shirasugi, M. M. Durham, E. Strobert, D. Anderson, P. Rees, S. Cowan, H. Xu, Y. Blinder, M. Cheung, D. Hollenbaugh, N. S. Kenyon, T. C. Pearson & C. P. Larsen: Calcineurin inhibitor-free CD28 blockade-based protocol protects allogeneic islets in nonhuman primates. *Diabetes*, 51, 265-70 (2002)

87. Sho, M., H. Harada, D. M. Rothstein & M. H. Sayegh: CD45RB-targeting strategies for promoting long-term allograft survival and preventingchronic allograft vasculopathy. *Transplantation*, 75, 1142-6 (2003)

88. Rothstein, D. M., M. F. Livak, K. Kishimoto, C. Ariyan, H. Y. Qian, S. Fecteau, M. Sho, S. Deng, X. X. Zheng, M. H. Sayegh & G. P. Basadonna: Targeting signal 1 through CD45RB synergizes with CD40 ligand blockade and promotes long term engraftment and tolerance in stringent transplant models. *J Immunol*, 166, 322-9 (2001)

89. Pearson, T. C., J. Trambley, K. Odom, D. C. Anderson, S. Cowan, R. Bray, A. Lin, D. Hollenbaugh, A. Aruffo, A. W. Siadak, E. Strobert, R. Hennigar & C. P. Larsen: Anti-CD40 therapy extends renal allograft survival in rhesus macaques. *Transplantation*, 74, 933-40 (2002)

90. Vincenti, F.: New monoclonal antibodies in renal transplantation. *Minerva Urol Nefrol*, 55, 57-66 (2003)

91. Vincenti, F., C. Larsen, A. Durrbach, T. Wekerle, B. Nashan, G. Blancho, P. Lang, J. Grinyo, P. F. Halloran, K. Solez, D. Hagerty, E. Levy, W. Zhou, K. Natarajan & B. Charpentier: Costimulation blockade with belatacept in renal transplantation. *N Engl J Med*, 353, 770-81 (2005)

92. Agata, Y., A. Kawasaki, H. Nishimura, Y. Ishida, T. Tsubata, H. Yagita & T. Honjo: Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. *Int Immunol*, 8, 765-72 (1996)

93. Vibhakar, R., G. Juan, F. Traganos, Z. Darzynkiewicz & L. R. Finger: Activation-induced expression of human programmed death-1 gene in T-lymphocytes. *Exp Cell Res*, 232, 25-8 (1997)

94. Dong, H., G. Zhu, K. Tamada & L. Chen: B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat Med*, 5, 1365-9 (1999)

95. Freeman, G. J., A. J. Long, Y. Iwai, K. Bourque, T. Chernova, H. Nishimura, L. J. Fitz, N. Malenkovich, T. Okazaki, M. C. Byrne, H. F. Horton, L. Fouser, L. Carter, V. Ling, M. R. Bowman, B. M. Carreno, M. Collins, C. R. Wood & T. Honjo: Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med*, 192, 1027-34 (2000)

96. Latchman, Y., C. R. Wood, T. Chernova, D. Chaudhary, M. Borde, I. Chernova, Y. Iwai, A. J. Long, J. A. Brown, R. Nunes, E. A. Greenfield, K. Bourque, V. A. Boussiotis, L. L. Carter, B. M. Carreno, N. Malenkovich, H. Nishimura, T. Okazaki, T. Honjo, A. H. Sharpe & G. J. Freeman: PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol*, 2, 261-8 (2001)

97. Ito, T., T. Ueno, M. R. Clarkson, X. Yuan, M. M. Jurewicz, H. Yagita, M. Azuma, A. H. Sharpe, H. Auchincloss, Jr., M. H. Sayegh & N. Najafian: Analysis of the role of negative T cell costimulatory pathways in CD4 and CD8 T cell-mediated alloimmune responses *in vivo*. *J Immunol*, 174, 6648-56 (2005)

98. Sandner, S. E., M. R. Clarkson, A. D. Salama, A. Sanchez-Fueyo, C. Domenig, A. Habicht, N. Najafian, H. Yagita, M. Azuma, L. A. Turka & M. H. Sayegh: Role of the programmed death-1 pathway in regulation of alloimmune responses *in vivo*. *J Immunol*, 174, 3408-15 (2005)

99. Ozkaynak, E., L. Wang, A. Goodearl, K. McDonald, S. Qin, T. O'Keefe, T. Duong, T. Smith, J. C. Gutierrez-Ramos, J. B. Rottman, A. J. Coyle & W. W. Hancock: Programmed death-1 targeting can promote allograft survival. *J Immunol*, 169, 6546-53 (2002)

100. Yang, J., X. Yuan, N. Vadivel, T. Shin, M. H. Sayegh & A. Chandraker: The role of donor versus recipient PD-L1, a novel costimulatory molecule, in allograft rejection. *American Society of Nephrology,* 38th Annual Renal Week Meeting, Philadelphia (2005)

101. Watanabe, N., M. Gavrieli, J. R. Sedy, J. Yang, F. Fallarino, S. K. Loftin, M. A. Hurchla, N. Zimmerman, J. Sim, X. Zang, T. L. Murphy, J. H. Russell, J. P. Allison & K. M. Murphy: BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1. *Nat Immunol*, 4, 670-9 (2003)

102. Hurchla, M. A., J. R. Sedy, M. Gavrieli, C. G. Drake, T. L. Murphy & K. M. Murphy: B and T lymphocyte attenuator exhibits structural and expression polymorphisms and is highly Induced in anergic CD4+ T cells. *J Immunol*, 174, 3377-85 (2005)

103. Croft, M.: The evolving crosstalk between co-stimulatory and co-inhibitory receptors: HVEM-BTLA. *Trends Immunol*, 26, 292-4 (2005)

104. Tao, R., L. Wang, R. Han, T. Wang, Q. Ye, T. Honjo, T. L. Murphy, K. M. Murphy & W. W. Hancock: Differential effects of B and T lymphocyte attenuator and programmed death-1 on acceptance of partially versus fully MHC-mismatched cardiac allografts. *J Immunol*, 175, 5774-82 (2005)

105. Kosuge, H., J. Suzuki, T. Kakuta, G. Haraguchi, N. Koga, H. Futamatsu, R. Gotoh, M. Inobe, M. Isobe & T. Uede: Attenuation of graft arterial disease by manipulation

of the LIGHT pathway. *Arterioscler Thromb Vasc Biol*, 24, 1409-15 (2004)

106. Ye, Q., C. C. Fraser, W. Gao, L. Wang, S. J. Busfield, C. Wang, Y. Qiu, A. J. Coyle, J. C. Gutierrez-Ramos & W. W. Hancock: Modulation of LIGHT-HVEM costimulation prolongs cardiac allograft survival. *J Exp Med*, 195, 795-800 (2002)

107. Suh, W. K., B. U. Gajewska, H. Okada, M. A. Gronski, E. M. Bertram, W. Dawicki, G. S. Duncan, J. Bukczynski, S. Plyte, A. Elia, A. Wakeham, A. Itie, S. Chung, J. Da Costa, S. Arya, T. Horan, P. Campbell, K. Gaida, P. S. Ohashi, T. H. Watts, S. K. Yoshinaga, M. R. Bray, M. Jordana & T. W. Mak: The B7 family member B7-H3 preferentially down-regulates T helper type 1-mediated immune responses. *Nat Immunol*, 4, 899-906 (2003)

108. Chapoval, A. I., J. Ni, J. S. Lau, R. A. Wilcox, D. B. Flies, D. Liu, H. Dong, G. L. Sica, G. Zhu, K. Tamada & L. Chen: B7-H3: a costimulatory molecule for T cell activation and IFN-gamma production. *Nat Immunol*, 2, 269-74 (2001)

109. Sun, M., S. Richards, D. V. Prasad, X. M. Mai, A. Rudensky & C. Dong: Characterization of mouse and human B7-H3 genes. *J Immunol*, 168, 6294-7 (2002)

110. Wang L., C.C. Fraser, K. Kirkley, A. D. Wells, R. Han, A. J. Coyle, L. Chen & W. W. Hancock: B7-H3 promotes acute and chronic allograft rejection. *Eur J Immunol*, 35 (2), 428-38 (2005)

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