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REVIEW

Costimulation blockade and its possible future use in clinical transplantation

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Summary

The nonimmune effects of currently used immunosuppressive drugs result in a high incidence of late graft loss due to nephrotoxicity and death of patients. As an immune-specific alternative to conventional immunosuppressants, new biotechnology tools can be used to block the costimulation signals of T-cell activation. Many experimental studies – particularly preclinical studies in nonhuman primates – have focused on blocking the 'classical' B7/CD28 and CD40/CD40L pathways, which are critical in primary T-cell activation. Here, we review the limitations, the recent advances and the first large-scale clinical application of the CTLA4-Ig fusion protein to block the B7/CD28 costimulation pathway. We also focus on new B7/CD28 and tumor necrosis factor (TNF)/TNF-R family costimulatory molecules that can deliver positive or negative costimulation signals regulating the alloimmune response. Strategies that use single agents to block costimulation have often proved to be insufficient. Given the diversity of the different costimulation molecules, future strategies for human transplantation may involve the simultaneous blockade of several selected pathways or the simultaneous use of conventional immunosuppressants.

Introduction

New immunosuppressive drugs have greatly decreased the frequency of graft failure due to acute rejection, but have had little effect on the incidence of late graft loss due to nephrotoxicity and death of patients [1]. This is largely due to the broad nonimmune effects of current immunosuppressive drugs, the targets of which are ubiquitous and nonspecific. Progress has recently been made in dissecting the T cell/antigen presenting cell (APC) interactions and, in particular, the costimulation pathways critical for T-cell activation. Biotechnology has developed new tools – monoclonal antibodies (mAb) and fusion proteins – targeting the critical molecules very precisely, facilitating specific action against immune cells.

Many experimental studies have focused on blocking the 'classical' B7/CD28 and CD40/CD40L pathways, which play a prominent role in primary T-cell activation. These molecules appear to be far less important in the generation and maintenance of memory and effector T-cell functions [2]. Memory T cells [2,3] and $CD8^+$ T cells [4], both of which mediate allograft loss, particularly in humans, have been shown to be less susceptible to classical costimulation blockade.

We review here the recent advances and the limitations of the classical costimulation pathway blockade, and the first large-scale application of costimulation blockade to human transplantation. We will also focus on novel costimulatory molecules of the B7/CD28 and tumor necrosis factor (TNF)/TNF-receptor (TNF-R) families, which deliver positive or negative costimulation signals regulating the alloimmune response. These molecules constitute alternative selective targets for achieving long-term allograft survival.

T-cell activation by three signals

During the alloimmune response, both naïve and memory alloreactive T cells are engaged by dendritic cells (DCs) of donor and recipient origin in secondary lymphoid organs

[5]. T-lymphocyte activation requires three signals. The first is antigen-specific and involves cognate T-cell receptor triggering by antigen on the surface of DCs or other APCs [6].

The second signal or 'costimulation signal' is not antigen-specific. Many molecules on the surface of T lymphocytes may receive a costimulation signal. Costimulatory molecules are diverse, with many different mechanisms of action. However, the B7/CD28 pathway is probably the most important and best characterized in T-cell activation. This costimulation signal is delivered when B7-1/CD80 and B7-2/CD86 on the surface of DCs engage CD28 on T cells [6]. These two signals activate three transduction pathways: the calcium–calcineurin pathway, the MAP-kinase pathway, and the NF-κB pathway [7]. These pathways trigger the production of many molecules, including interleukin-2 and the α-chain of its receptor CD25, and CD40 ligand. Interleukin-2 binding to its receptor activates the mTOR ('target of rapamycin') pathway – the third signal – resulting in cell cycle initiation and T-cell proliferation [7]. Proliferation and differentiation result in the generation of a large number of effector T cells.

Recent advances in our understanding of the B7/CD28/CTLA-4 pathway

This pathway is characterized by the dual specificity of two B7 family members, B7-1 and B7-2, for both the stimulatory receptor CD28 and the inhibitory receptor CTLA-4 (cytotoxic T lymphocyte-associated antigen 4/CD152). CD28 provides a T-cell activation signal, whereas CTLA-4 inhibits T-cell responses (Fig. 1a) [8]. CD28 is constitutively expressed on T cells, whereas CTLA-4 expression is rapidly upregulated following T-cell activation. CTLA-4 has a higher affinity receptor for both B7-1 and B7-2 than CD28.

A new regulatory role of DCs, involving B7/CTLA-4 reverse signaling, has been suggested [9,10]. A soluble form of CTLA-4 (CTLA-Ig) and a membrane-anchored form of CTLA-4 on regulatory T cells may bind to B7, thereby activating the immunosuppressive pathway of tryptophan catabolism in DCs [9]. This pathway results in the production by DCs of interferon-γ, which acts in an autocrine or paracrine manner to stimulate indole amine 2,3-dioxygenase (IDO), which degrades tryptophan. The resulting degradation products and tryptophan deprivation inhibit T-cell proliferation and promote apoptosis. These bidirectional B7:CTLA-4 interactions may be involved in the downregulation of T-cell responses.

Grohman *et al.* [9] have shown that the long-term survival of murine islet allografts induced by CTLA4-Ig depends on effective tryptophan catabolism and that this effect disappears in the presence of 1-methyltryptophan, a pharmacological inhibitor of IDO.

Role of B7/CTLA-4 interactions in regulator T-cells activity

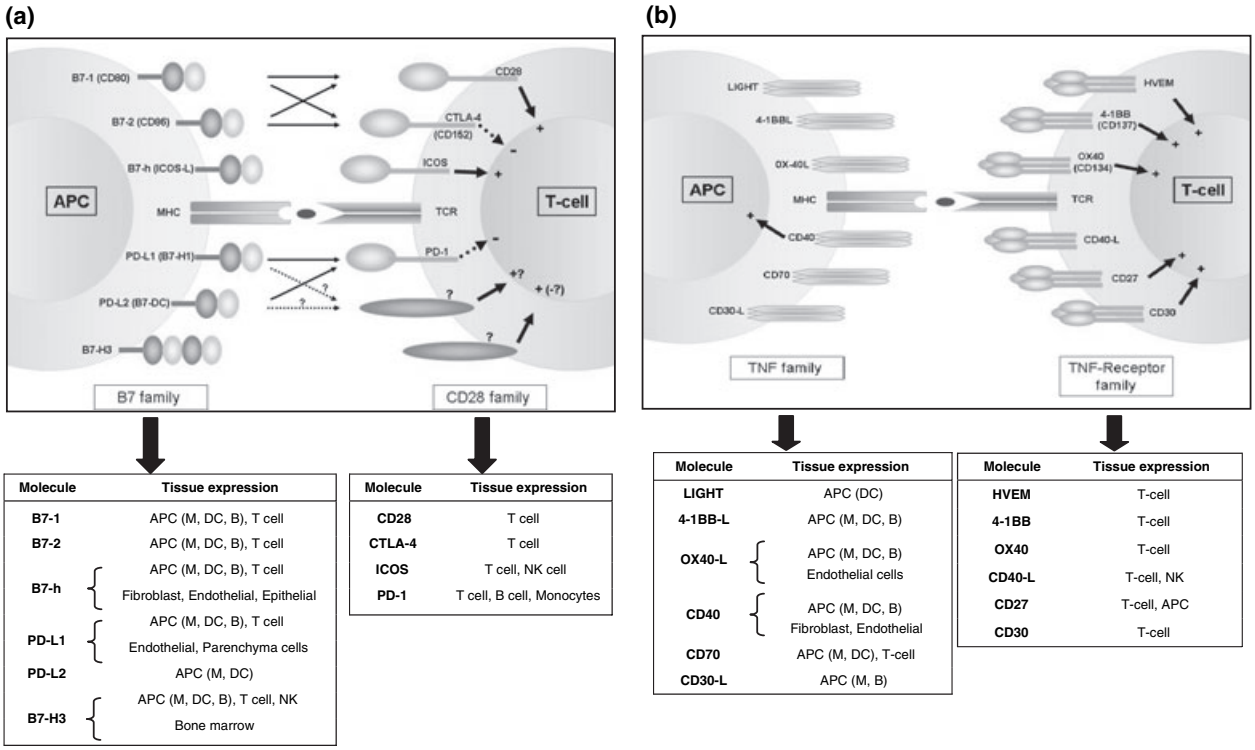
Recent experimental models of transplantation tolerance have highlighted the role of a specialized subgroup of $CD4^+CD25^+$ T lymphocytes, termed regulatory or suppressor T lymphocytes (T-reg) [11]. Clinical significance of T-reg is being precised in human transplantation, as shown by the detection in urinary cells from kidney-transplant patients of FOXP3 mRNA, a functional factor specific to T-reg [12].

B7-1 and B7-2 are required for the suppression exerted by $CD4^+CD25^+$ T cells, as their absence from the surface of effector cells results in lower susceptibility to suppression. Suppressor and effector cells may interact in two different ways *in vitro*. In the first, direct contact between suppressor and effector cells is required, through the binding of CTLA-4 on suppressor cells and B7-1/B7-2 on effector cells. In the second, suppression requires contact between a T-reg expressing CTLA-4 and a DC expressing B7, resulting in a negative effect mediated by tryptophan catabolism and directed at an effector cell bound to the same DC [13].

In vivo suppression also requires B7 expression on effector cells. B7-deficient and wild-type $CD4^+CD25^-$ T cells infusion into lymphopenic mice induced autoimmune disease, which was inhibited by $CD4^+CD25^+$ T-cell suppression in wild-type recipients only [14]. In a skin transplant model, CTLA-4 pathway blockade abolishes immunoregulation by $CD4^+CD25^+$ T cells, suggesting that CTLA-4 is required for tolerance [15].

The CD40/CD40L pathway

The CD40:CD40 ligand pathway, initially described as involved in B-cell activation, has also been shown to be critical for T-cell activation [16]. CD40 and CD40L belong to TNF and TNF-R superfamilies, respectively (Fig. 1b) [17]. CD40 is expressed on all APCs, including B cells and endothelial cells. The CD40 ligand (CD40L or CD154) is expressed on activated $CD4^+$ T cells and on a subset of $CD8^+$ T cells and natural killer (NK) cells. CD40 stimulation triggers important signals for antibody production by B cells and strongly induces B7 and MHC expression on APCs. The CD40/CD40L system thus increases antigen presentation and the antigen-specific signal, and plays an important role in costimulation [17].



Molecule	Tissue expression
B7-1	APC (M, DC, B), T cell
B7-2	APC (M, DC, B), T cell
B7-h	APC (M, DC, B), T cell Fibroblast, Endothelial, Epithelial
PD-L1	APC (M, DC, B), T cell Endothelial, Parenchyma cells
PD-L2	APC (M, DC)
B7-H3	APC (M, DC, B), T cell, NK Bone marrow

Molecule	Tissue expression
CD28	T cell
CTLA-4	T cell
ICOS	T cell, NK cell
PD-1	T cell, B cell, Monocytes

Molecule	Tissue expression
LIGHT	APC (DC)
4-1BB-L	APC (M, DC, B)
OX40-L	APC (M, DC, B) Endothelial cells
CD40	APC (M, DC, B) Fibroblast, Endothelial
CD70	APC (M, DC), T-cell
CD30-L	APC (M, B)

Molecule	Tissue expression
HVEM	T-cell
4-1BB	T-cell
OX40	T-cell
CD40-L	T-cell, NK
CD27	T-cell, APC
CD30	T-cell

Figure 1 Effects and expression of B7/CD28 (a) and TNF/TNF-R (b) family members. APC, antigen presenting cell; M, monocyte; DC, dendritic cell. Figure 1a adapted from Sharpe and Freeman [95].

Limitations of experimental 'classical' costimulation blockade

The B7/CD28 pathway

Costimulation blockade targeting the B7/CD28 pathway with CTLA4-Ig efficiently prevents acute heart or kidney rejection in many mouse and rat models [18–21]. However, this agent induces durable tolerance in only a few models of heart or kidney transplantation [20,22–24], and not in more stringent models, such as mouse skin and islet transplantation [25–27].

There may be several reasons for these limitations:

1 Costimulation blockade is less effective in memory T cells and $CD8^+$ T cells than in naïve T cells [3,27,28].
2 CTLA-4 plays a key role in downregulating T-cell responses, independently of CD28/B7 blockade. Two studies have shown that CTLA-4 blockade with CTLA4-Ig in CD28-deficient animals accelerates heart allograft rejection [22,29].
3 The timing of CTLA-4 manipulation is also crucial. CTLA-4 signaling is essential for initial engraftment in various transplant models, but its blockade during the maintenance phase does not precipitate the rejection of vascularized allografts [30,31].
4 As mentioned above, suppression of alloimmune response requires a functional CTLA-4/B7 pathway between T-reg and effector cells [14,15].

Some adjunctions may improve long-term graft survival and prevent chronic rejection: administration of CTLA4-Ig delayed to day 2 [20], prolonged administration [21], simultaneous donor-specific transfusion [DST; 29], simultaneous administration of donor antigen [19,32] and thymectomy [26].

The CD40/CD40L pathway

The CD40/CD40L pathway has been blocked with an anti-CD40L mAb, promoting long-term allograft survival in mice. However, as for CD28/B7 blockade, it rarely gave durable tolerance when used alone [32–35]. Effects of anti-CD40L mAb are reversible, so prolonged administration is often required [36]. As with CTLA4-Ig, the concomitant administration of donor antigens on splenocytes was required for long-term survival [35,37]. Simultaneous CD40/CD40L and B7/CD28 blockades have been shown to be synergic, promoting indefinite graft survival, even in stringent models such as mouse skin transplantation and xenotransplantation [33,38,39].

Costimualtion blockade in nonhuman primates

Costimulation blockade in nonhuman primates (NHPs) has bridged the gap between small-animal models and clinical protocols. Adolescent rhesus monkeys display longer periods of kidney graft survival, from 8 to 30 days on CTLA4-Ig alone and up to 6 months with CTLA4-Ig plus humanized anti-CD40L antibody (hu-anti-CD40L) [40]. Survival was similar in monkeys receiving hu-anti-CD40L antibody alone, suggesting that CD40/CD40L pathway blockade is a critical step, but this effect was dose-dependent [41]. Long-term survival, but not tolerance, was achieved in this model. All animals developed donor-specific antibodies and had a focal perivascular infiltrate on routine day-28 biopsy [36]. In other NHP studies, treatment with CTLA4-Ig, anti-CD80 or anti-CD86 combined with hu-anti-CD40L neither induced durable tolerance nor antagonized the effect of anti-CD40L [42].

Some studies have recently explored new ways of improving the results of costimulation blockade in NHPs. Anergic T cells have suppressor activities *in vitro* and *in vivo* [43,44]. In human histoincompatible bone marrow transplantation (BMT), the treatment of donor bone marrow *ex vivo* with CTLA4-Ig and its co-culture with irradiated recipient cells to induce donor-specific anergic T cells, led to the reconstitution of hematopoiesis with a low risk of graft-versus-host disease (GVHD) [45]. Bashuda *et al.* investigated whether anergic T cells generated *ex vivo* could induce long-term kidney allograft survival. When splenic $CD4^+$ T cells from recipient rhesus monkeys were co-cultured with irradiated donor splenocytes in the presence of both anti-human CD80 and CD86 mAbs and injected into six recipient monkeys after 13 days of cyclosporine A (CsA) and cyclophosphamide treatment, three monkeys survived indefinitely [46]. Animals on the same regimen but injected with T cells activated with a third party died of acute rejection, suggesting that long-term survival depended on specifically anergized cells. The simplicity, safety, and efficacy of this protocol make it suitable for application to human transplantation.

Crossing the bridge to human clinical trials

As pointed out by Elster *et al.* [47], it is difficult to transpose experimental findings to human clinical trials, because NHPs lack 'heterologous immunity'. Indeed, NHPs raised in captivity may have limited exposure to nonself antigens and may therefore do not have an extensive repertoire of effector/memory cells.

The first agent used to block costimulation in human trials was huC58, a humanized anti-CD40L antibody. This agent was well tolerated in NHPs, but its development for human use was discontinued after seven patients suffered thromboembolic events [48]. This complication reflects the importance of costimulatory molecule expression by nonlymphoid cells, in this case the expression of CD40L by platelets and CD40 by endothelium cells. A chimeric anti-CD40 antibody, Chi220, has recently been developed to circumvent these adverse events. It has proved effective, in combination with CTLA4-Ig, for islet transplantation in NHPs [49]. B7/CD28 pathway blockade with a combination of anti-CD80/anti-CD86 antibodies reached the clinical trial stage. However, this agent was subsequently withdrawn from further development, despite its good safety profile, for financial reasons.

CTLA4-Ig (abatacept) is a fusion protein consisting of the extracellular domain of CTLA-4 and the Fc domain of IgG. CTLA4-Ig has a 200 times higher affinity for CD80 than for CD86 and is 100 times more potent for the blockade of CD80-dependent costimulation than for that of CD86-dependent costimulation [50]. Insufficient blockade of CD28/B7 interaction may partly account for the limited results obtained. A mutagenesis and screening strategy has been used to identify high-avidity mutants with slower dissociation rates [34]. Two amino-acid substitutions (L104E and A29Y) were identified as potentially useful, leading to the development of a new molecule, LEA29Y or belatacept.

First clinical trial with belatacept in renal transplantation

A large-scale study has been conducted to assess the efficacy of a strategy based upon costimulation blockade with belatacept in renal transplantation [51]. This phase 2 multicenter study included 218 adult recipients of a renal allograft, randomly assigned to groups receiving an intensive regimen of belatacept, a less-intensive regimen, or CsA. Both belatacept regimens included an early phase of frequent intravenous injections and a late phase of less frequent injections (at 4- or 8-week intervals). The early phase was longer for the intensive regimen. All patients received basiliximab induction therapy, mycophenolate mofetil and steroids. The primary noninferiority objective was reached, with the following incidences of acute rejection at 6 months: 6% (less-intensive belatacept), 7% (intensive belatacept) and 8% (CsA). Subclinical rejection at month-6 routine biopsy was more common with less-intensive belatacept (20%) than with intensive belatacept (9%) or cyclosporine (11%).

Glomerular filtration rate at 12 months was significantly higher in patients receiving belatacept than in those treated with CsA (66.3 and 62.1 vs. 53.5 ml/min/1.73 m^2). By month 12, the incidence of chronic allograft nephropathy was lower in patients receiving belatacept: 29% (less intensive) and 20% (intensive) vs. 44%,

respectively. The frequency of infection was similar in all three groups, at around 75%. Cancers occurred in two patients treated with intensive belatacept [one breast cancer and one post-transplantation lymphoproliferative disorder (PTLD)] and in two patients treated with CsA (one skin cancer and one thyroid cancer). However, PTLD developed in two additional patients treated with the intensive regimen 2 and 13 months after the replacement of belatacept with conventional immunosuppressive agents. All cases of PTLD were associated with primary Epstein–Barr virus infection or treatment with muromonab-CD3, both of which are known risk factors for the disorder. Approximately half the patients enrolled voluntarily in a long-term extension of the protocol after 1 year of treatment. Thus, the use of belatacept may allow patients to avoid the adverse renal, cardiovascular and metabolic effects of calcineurin inhibitors, whilst providing equally effective immunosuppression.

Exploring novel costimulatory pathways

It is now apparent that T-cell activation and transplant rejection may proceed in the absence of CD28/B7 and CD40/CD40L signaling. Memory T cells [2,3] and $CD8^+$ T cells [4], which play a major role in rejection, are less susceptible to this classical costimulatory blockade. Several other members of the CD28/B7 and TNF/TNF-R superfamilies deliver positive or negative costimulatory signals, early or late after encountering antigen. These signals are not limited to T-cell/APC interactions, but also involve relations between T cells and other T cells, B cells or parenchymal cells (Fig. 1a and b).

The induced costimulatory molecule:B7h pathway

Induced costimulatory molecule (ICOS) shares 20% homology with CD28 [52]. Unlike CD28, ICOS is not expressed on naïve T cells, but may be induced in $CD4^+$ and $CD8^+$ T cells within 48 h of activation, and persists in memory and effector T cells [53]. Thus, ICOS signaling is required for the activation and function of effector T cells, whereas CD28 primes naïve T cells. ICOS is also involved in T cell–B cell collaboration and immunoglobulin production [52,54]. B7h, the ligand of ICOS, is expressed on B cells, monocytes, DCs, nonprofessional APCs and in nonlymphoid tissues; it is also rapidly inducible [8].

The expression of ICOS on effector T cells makes this molecule an alternative/complementary target of costimulation blockade. The key role of ICOS has been demonstrated by the significant prolongation of allograft survival with an anti-ICOS blocking mAb, although this effect was weaker than that with CTLA-4Ig or anti-CD40L mAb therapy [55]. In addition, a combination of CsA and anti-ICOS has been shown to act synergistically, inducing permanent allograft survival without the development of transplant arteriosclerosis.

In conjunction with DST, costimulation blockade with CTLA-4-Ig or CD40L mAb can induce permanent allograft survival [19,35]. Grafts in recipients treated with anti-CD40L antibody without DST have been shown to develop florid transplant arteriosclerosis associated with strong ICOS expression on infiltrating mononuclear cells. Grafts from animals treated with both anti-CD40L and anti-ICOS antibodies do not display these signs of chronic rejection. Similarly, anti-ICOS mAb plus CTLA4-Ig treatment prolongs rat to mouse islet-xenograft survival, with neither $CD8^+$ T-cell population expansion nor anti-rat antibody production, with respect to treatment with anti-ICOS antibody or CTLA4-Ig alone [56]. The therapeutic effect of ICOS blockade is significantly improved by the delayed timing, after T-cell priming [57].

The programmed death-1/PD-L1/PD-L2 pathway

Programmed death-1 (PD-1) [58] is related to CD28 and CTLA-4, but lacks the membrane proximal cysteine required for homodimerization. PD-1 is induced on peripheral $CD4^+$ and $CD8^+$ cells, NK cells, B cells, and monocytes, whereas the expression of other members of the CD28 family is restricted to T cells (Fig. 1a) [8]. PD-1 has two ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC) [59,60]. PD-L1 is expressed on resting APCs and T, B, and endothelial cells [61], and is upregulated on activation of these cells. However, unlike B7-1 and B7-2, they are also constitutively expressed on a large panel of nonlymphoid organs. This suggests that PD-L1 may regulate self-reactive T or B cells in peripheral tissues. PD-L2 is induced by IL-4 only on APCs. As for CTLA-4, PD-1 ligation transmits a potent inhibitory signal in the early stages of T-cell activation, resulting in a decrease in cytokine production and cell cycle arrest in the G_0/G_1 phase (also in B cells) [59,60,62,63].

Programmed death-1, PD-L1, and PD-L2 are expressed during the development of cardiac allograft rejection [64]. In cardiac allograft studies, PD-L1-Ig, but not PD-L2-Ig agonist fusion proteins, plus CsA significantly enhanced allograft survival over CsA or PD-L1-Ig alone. Similarly, PD-L1-Ig markedly reduces cardiac transplant arteriosclerosis and prolongs islet survival when given in conjunction with anti-CD40L mAb [64,65].

The exact mechanism by which PD-L1-Ig exerts its protective effects in these models is unclear; PD-L1-Ig may trigger a negative signal through PD-1 or block a positive signal for T-cell activation. However, this pathway was recently shown to play a more complex role than

initially thought. Transgenic islet allografts expressing PD-L1 display accelerated rejection, suggesting that PD-L1 may sometimes promote, rather than inhibit, T-cell responses [66]. The differential effects of PD-1 versus PD-L1 blockade support the existence of an inhibitory receptor for PD-L1 other than PD-1 (Fig. 1) [24]. Thus, the PD-1/PD-L1/PD-L2 pathway plays a critical role in regulating $CD4^+$ and $C8^+$ T-cell activation in peripheral tissues, but is not redundant with B7/CTLA-4.

The B7-H3 molecule

B7-H3 is a B7 homolog, undetectable in resting human lymphoid cells but inducible *in vitro* in DCs, monocytes, T, B, and NK cells [67,68]. A human B7-H3-Ig has been generated to characterize B7-H3 function [67]. This molecule binds a putative receptor, rapidly and transiently expressed on activated, but not resting human T cells. It acts as a costimulatory molecule, increasing $CD4^+$ and $CD8^+$ T-cell proliferation and inducing cytotoxic T lymphocytes [67]. However, the recently characterized B7-$H3^{-/-}$ mouse was shown to develop more severe autoimmune conditions than wild-type mice suggesting that B7-H3 may also suppress T-cell activation and functions [69].

Immunohistological analysis of renal biopsies from patients with acute allograft rejection showed B7-H3 expression in infiltrating mononuclear cells [70]. In transplantation models, hearts transplanted into B7-$H3^{-/-}$ or control mice were rejected equally and rapidly. However, whereas a brief course of CsA or rapamycin (RPM) in B7-$H3^{+/+}$ recipients extends cardiac allograft survival by only a few days, the use of the same protocol in B7-$H3^{-/-}$ recipients led to a prolonged (CsA) or indefinite survival (RPM). Similarly, anti-CD40L mAb induced permanent cardiac engraftment in B7-$H3^{-/-}$, but not wild-type recipients. In conclusion, B7-H3 is a potentially useful target for immunosuppression, particularly in association with the blockade of other pathways. Further studies are required to identify its ligands on T cells and to explore the effects of B7-H3 on human T-cell activation.

The TNF/TNF-R family

Simplistically, TNF-R-family members are expressed by T cells and their TNF-family ligands are expressed by APCs (Fig. 1b) [71]. TNF-R/TNF interactions are critical in the clonal expansion/effector phases of immune responses, and involve DC/T cell and B/T cell relations (the CD40/CD40L pair is discussed above).

Tumor necrosis factor-receptors may be either constitutively expressed by naïve T cells as for CD27 [72] and herpes virus entry mediator (HVEM) [73], or induced after antigen recognition, as for OX40, 4-1BB, CD30, and CD27 [74–77]. Unlike HVEM ligand (LIGHT), OX40L, 4-1BBL, CD70, and CD30L are not constitutively expressed by resting or immature APCs. They are induced simultaneously with their receptors on T cells, one to several days after activation [71]. CD70 is principally expressed by B-cells [78], whereas OX40L, 4-1BBL, and CD30L are expressed by a broad range of professional APCs.

The constitutive expression of HVEM/LIGHT suggests a role in the early activation of T cells and APCs. The inducible expression of CD27/CD70 indicates a second round of costimulation during the clonal-expansion phase of T-cell responses. The expression peak of OX40, 4-1BB, CD30, and their ligands, several days after antigen encounter, may help to sustain the ongoing response [71]. Despite variability in expression, binding to TNF-Rs increases cytokine secretion and the proliferation of T cells receiving the TCR signal [71].

Targeting of three TNF/TNF-R pathways in alloimmunity models

CD27/CD70 blockade prolongs the survival of fully mismatched cardiac allografts in wild-type murine recipients, inducing long-term survival in CD28-deficient mice while preventing the development of chronic allograft vasculopathy. This blockade has little effect on $CD4^+$ T-cell function but prevents CD28-independent $CD8^+$ T-cell-mediated rejection and expansion of the effector/memory $CD8^+$ T-cell populations [79]. These results have important implications for the development of new immunosuppressive strategies in primates and humans, as such strategies currently require $CD8^+$ T-cell depletion or suppression.

Naïve $CD4^+$ T cells lacking OX40 show low levels of proliferation and die by apoptosis 4–5 days after activation [80]. Both naïve and activated $CD4^+CD25^+$ regulatory T cells express OX40, but the function of this molecule is unclear. In fully allogeneic murine BMT, GVHD was lethal unless regulatory T cells were co-injected with bone marrow and effector T cells. This effect was abolished by injecting anti-OX40 mAb [81].

Curry *et al.* used an OX40-Ig fusion protein to block the OX-40/OX-40L pathway in mouse cardiac allograft rejection across major histocompatibility (MHC) and minor histocompatibility (mHC) barriers. Heart survival for fully MHC-mismatched allografts was unaffected by OX40 blockade alone, but OX40-Ig treatment in the mHC-mismatched model resulted in long-term graft survival [82]. Furthermore, blocking OX40 costimulation and CD28/CD40L resulted in long-term skin allograft survival in CD4KO mice and CD8KO mice whereas mice

treated with the blockade of only one of these two pathways rapidly rejected skin allografts. Thus, $CD4^+$ and $CD8^+$ T-cell-mediated rejection under CD28/CD40L blockade is supported by OX40 costimulation [83]. However, the OX40 pathway is also important in CD28/CD40L-independent rejection, as blocking the OX40/OX40 ligand pathway (anti-OX40 ligand) markedly prolongs skin graft survival when combined with CD28/CD40L blockade [84].

As with OX-40, 4-1BBL blockade does not alter the initial proliferative response of $CD8^+$ T cells, but suppresses the accumulation of effector CTLs at the primary response peak, after 3–6 days [85]. In organ transplantation, Cho *et al.* have shown 4-1BB deficient mice displayed delayed heart allograft rejection compared with control mice. Moreover, treating wild-type mice with a blocking anti-4-1BBL mAb (TKS-1) resulted in substantial prolongation of heart allograft survival (median survival time = 42 days vs. 8 days for control), with 40% of the recipients displaying long-term (>60 days) survival. *In vitro*-mixed lymphocyte reactions show that blocking 4-1BB/4-1BBL interactions results in the inhibition of proliferation of both $CD4^+$ and $CD8^+$ T cells in response to allogeneic APCs [86]. Blockade of the 4-1BB pathway significantly inhibits intestinal mouse allograft rejection by $CD8^+$, but not $CD4^+$ T cells. Disruption of the 4-1BB pathway also impairs the priming of alloantigen-specific $CD8^+$ T cells. These data directly demonstrate an important role for 4-1BB in $CD8^+$ T-cell-mediated rejection [87].

Costimulation blockade-based combination therapies

Strategies with single agents blocking costimulation have proved to be insufficient in stringent models of transplantation. In particular, the results obtained with LEA29Y in NHPs kidney transplantation led investigators to combine this molecule for its clinical application with conventional immunosuppressive reagents to minimize the risk of acute rejection [34,51]. Given the diversity, redundancy and complementary nature of the different costimulation molecules, future strategies for human transplantation may involve the simultaneous blockade of several selected pathways or the concomitant use of currently approved conventional immunosuppressants. Table 1 summarizes a selection of studies reporting these two costimulation blockade-based strategies (Table 1).

It has been initially reported that the beneficial effects of costimulation blockade can be antagonized by certain conventional immunosuppressant. Calcineurin inhibitors and corticosteroids block alloimmune response by inhibiting early T-cell activation. This negative effect suggests that costimulation blockade require intact signaling through the T-cell receptor [88]. Li *et al.* have shown that activation-induced cell death is required for costimulation blockade-dependent peripheral tolerance. The addition of cyclosporine negates the beneficial effects of costimulation blockade by inhibiting proliferation, apoptosis, and subsequent deletion of alloreactive T cells [38]. In contrast to calcineurin inhibitors, rapamycin, which does not alter early TCR signaling and permits cell cycle-dependent apoptosis, synergizes with costimulation blockade to promote long-term allograft survival [38].

However, more recent studies describe the use of conventional agents in combination with a variety of costimulation blockade reagents resulting in synergistic activity in both murine and NHP models (Table 1). The initial clinical results with LEA29Y prove that this agent, as CsA, can be safely and efficiently associated with steroids and mycophenolate mofetil.

Concluding remarks

Costimulation blockade in clinical transplantation and in stringent experimental transplantation models has revealed that there exist CD28- and CD40-independent rejection mechanisms that are mediated by effector/memory T-cells. Several other members of the CD28/B7 and TNF/TNF-R superfamilies, differing in their effects and pattern of expression, deliver positive or negative costimulatory signals. Many of these molecules are expressed by nonlymphoid cells and may participate in modulating alloimmune responses. Experimental transplantation models using agonist or blocking reagents have already assessed most of these pathways, and all appear to be attractive targets for future clinical applications. Further studies are necessary to determine how important these new pathways are in the human alloimmune response. Future steps in the clinical development of costimulation blockade are to find the right combination of simultaneous blockade of these new pathways or to use simultaneously conventional immunosuppressants at a reduced level.

However, some issues have to be cautiously discussed before envisioning the widespread use of such agents. An advantage of classical oral immunosuppressive drugs is their short half-life and thus the relative reversibility of their effect, which is an important point in case of malignant or infectious complications. Future trials have to determine whether the long half-life of biological agents precludes their safety. In the belatacept trial, short-term infectious complications are observed with the same frequency as in the cyclosporine group. Nevertheless, the three PTLD observed under belatacept in this low-risk population were not expected and recall that this new

Table 1. Costimulation blockade-based combination therapies.

Combination therapy	Model	Results*	Reference
With other costimulation blockade agents			
Anti-ICOS + CTLA4-Ig	Rat to mouse/Islets	Prolongation of graft survival	[56]
Anti-ICOS + anti-CD40L	Mouse/heart	Prevention of chronic transplant arteriosclerosis	[55]
Agonist PD-L1-Ig + anti-CD40L	Mouse/heart	Prevention of chronic transplant arteriosclerosis	[64]
Agonist PD-L1-Ig + anti-CD40L	Mouse/Islets	Long-term graft survival	[65]
Anti-CD40L + CTLA4-Ig + anti-OX40-L	CD4KO or CD8KO mouse/skin	Prolongation of graft survival versus anti-CD40L + CTLA4-Ig	[83]
Anti-CD40L + CTLA4-Ig + anti-OX40-L	Mouse/skin	Prolongation of graft survival versus anti-CD40L + CTLA4-Ig	[84]
Anti-CD45RB + anti-CD40L	Mouse/skin	Prolongation of graft survival but late rejection	[30]
Anti-CD45RB + anti-CD40L	Mouse/skin, islet	Prolongation of graft survival	[25]
Anti-LFA-1 + anti-CD40L	Mouse/hepatocytes	Long-term survival	[89]
With conventional immunosuppressants			
CTLA4-Ig + CsA or RPM or ALS	Mouse/skin	Prolongation of graft survival with CsA or RPM, no effect with ALS	[90]
CTLA4-Ig + CsA	Rat/kidney	Indefinite allograft survival	[91]
Anti-ICOS + RPM	Mouse/islets	Prolongation of graft survival	[92]
Anti-ICOS + CsA	Mouse/heart	Indefinite allograft survival	[55]
Agonist PD-L1-Ig + subtherapeutic CsA	Mouse/heart	Prolongation of graft survival	[64]
Agonist PD-L1-Ig + RPM	Mouse/heart	Indefinite allograft survival?	[64]
CsA or RPM	B7-H3 KO mouse/heart	Increased (CsA) or indefinite (RPM) graft survival	[70]
Anti-CD45RB + anti-CD40L + RPM	CD8 KO mouse/skin	Long-term survival	[83]
Anti-CD40 + Anti-CD86 + CsA	NHP/kidney	Long-term survival	[93]
Anti-CD80 or Anti-CD86 + CsA	Rat to mouse/heart	Indefinite allograft survival with anti-CD86 but with anti-CD80	[94]

CsA, Cyclosporine A; RPM, Rapamycin; ALS, anti-lymphocytes serum; ICOS, induced costimulatory molecule.
*Significantly different versus single agent therapy.

agent do not provide a specific immunosuppression and may also block immune surveillance of tumors. These patients have to be carefully followed up because the long-term safety of belatacept is not known.

More practically, even if some patients are satisfied to take fewer tablets each day, the intravenous route of administration makes more complicated the ambulatory follow-up of transplant patients. The development of subcutaneous delivery systems is an essential step to reach a widespread use of these agents.

The way to use costimulation blocking agents in the long-term appears to be an essential point to explore in the future, as far as their efficacy and safety are concerned. We have to address whether costimulation blockade has an effect, positive or negative, on the process of accommodation and whether it is possible to give lower and lower doses over time as with classical drugs. We have to learn how to adapt the dose and rhythm of administration to the organ grafted and to the immunological risk of patients.

To reach these objectives, we need monitoring tools adapted to these costimulation blocking agents to measure their efficacy. To find the best assays, we have to first perform correlation studies between these assays and the efficacy of the agent, assessed by the incidence of acute rejection. Routine monitoring of blood drug level is the first evident assay, as for all classical immunosuppressants. For example, monitoring of belatacept blood level is available and further studies are necessary to find if there is a correlation with the incidence of acute rejection. We can also speculate on the way and the interest to detect in patients a direct effect of costimulation blockade, which is the generation of anergic T cells. These anergic cells have suppressor activities *in vitro* and *in vivo* as mentioned above [43–46]. Thus, it would be interesting to study by cytometric flow analysis whether patients treated with costimulation blockade display more circulating regulatory $CD4^+$ $CD25^+$ T cells than control patients.

In conclusion, a short course of costimulation blocking agent to achieve drug-free antigen-specific tolerance does not appear to be a realistic endpoint in the immediate future of clinical transplantation. However, costimulation blockade appears as a powerful tool to design calcineurin-inhibitor-free or nonnephrotoxic immunosuppressive protocols. It is clear that actually, a single therapy cannot be used, probably because of the redundancy of the various costimulation pathways. Consequently, costimulation blocking agents are actually envisioned as a part of a

combination therapy involving classical drugs, as anti-metabolites, mTOR inhibitors or even calcineurin inhibitors at reduced doses, allowing the reduction of nonimmune side effects of current protocols.

References

1. Meier-Kriesche H-U, Schold JD, Kaplan B. Long-term renal allograft survival have we made significant progress or is it time to rethink our analytic and therapeutic strategies? *Am J Transplant* 2004; **4**: 1289.
2. London CA, Lodge MP, Abbas AK. Functional responses and costimulator dependence of memory CD4+ T cells. *J Immunol* 2000; **164**: 265.
3. Valujskikh A, Pantenburg B, Heeger PS. Primed allospecific T cells prevent the effects of costimulatory blockade on prolonged cardiac allograft survival in mice. *Am J Transplant* 2002; **2**: 501.
4. Ensminger SM, Witzke O, Spriewald BM, *et al.* CD8+ T cells contribute to the development of transplant arteriosclerosis despite CD154 blockade. *Transplantation* 2000; **69**: 2609.
5. von Andrian UH, Mackay CR. T-cell function and migration. Two sides of the same coin. *N Engl J Med* 2000; **343**: 1020.
6. Bromley SK, Iaboni A, Davis SJ, *et al.* The immunological synapse and CD28–CD80 interactions. *Nat Immunol* 2001; **2**: 1159.
7. Halloran PF. Immunosuppressive drugs for kidney transplantation. *N Engl J Med* 2004; **351**: 2715.
8. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol* 2005; **23**: 515.
9. Grohmann U, Orabona C, Fallarino F, *et al.* CTLA-4-Ig regulates tryptophan catabolism in vivo. *Nat Immunol* 2002; **3**: 1097.
10. Finger EB, Bluestone JA. When ligand becomes receptor-tolerance via B7 signaling on DCs. *Nat Immunol* 2002; **3**: 1056.
11. Graca L, Thompson S, Lin CY, Adams E, Cobbold SP, Waldmann H. Both CD4(+)CD25(+) and CD4(+)CD25(−) regulatory cells mediate dominant transplantation tolerance. *J Immunol* 2002; **168**: 5558.
12. Muthukumar T, Dadhania D, Ding R, *et al.* Messenger RNA for FOXP3 in the urine of renal-allograft recipients. *N Engl J Med* 2005; **353**: 2342.
13. von Boehmer H. Mechanisms of suppression by suppressor T cells. *Nat Immunol* 2005; **6**: 338.
14. Paust S, Lu L, McCarty N, Cantor H. Engagement of B7 on effector T cells by regulatory T cells prevents autoimmune disease. *Proc Natl Acad Sci USA* 2004; **101**: 10398.
15. Kingsley CI, Karim M, Bushell AR, Wood KJ. CD25+CD4+ regulatory T cells prevent graft rejection: CTLA-4- and IL-10-dependent immunoregulation of alloresponses . *J Immunol* 2002; **168**: 1080.
16. Durie FH, Foy TM, Masters SR, Laman JD, Noelle RJ. The role of CD40 in the regulation of humoral and cell-mediated immunity. *Immunol Today* 1994; **15**: 406.
17. van Kooten C, Banchereau J. Functions of CD40 on B cells, dendritic cells and other cells. *Curr Opin Immunol* 1997; **9**: 330.
18. Turka LA, Linsley PS, Lin H, *et al.* T-cell activation by the CD28 ligand B7 is required for cardiac allograft rejection in vivo. *Proc Natl Acad Sci USA* 1992; **89**: 11102.
19. Sayegh MH, Zheng XG, Magee C, Hancock WW, Turka LA. Donor antigen is necessary for the prevention of chronic rejection in CTLA4Ig-treated murine cardiac allograft recipients. *Transplantation* 1997; **64**: 1646.
20. Sayegh MH, Akalin E, Hancock WW, *et al.* CD28-B7 blockade after alloantigenic challenge in vivo inhibits Th1 cytokines but spares Th2. *J Exp Med* 1995; **181**: 1869.
21. 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**: 1641.
22. Yamada A, Kishimoto K, Dong VM, *et al.* CD28-independent costimulation of T cells in alloimmune responses. *J Immunol* 2001; **167**: 140.
23. Lenschow DJ, Zeng Y, Thistlethwaite JR, *et al.* Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4lg. *Science* 1992; **257**: 789.
24. Ito T, Ueno T, Clarkson MR, *et al.* Analysis of the role of negative T cell costimulatory pathways in CD4 and CD8 T cell-mediated alloimmune responses in vivo. *J Immunol* 2005; **174**: 6648.
25. Rothstein DM, Livak MF, Kishimoto K, *et al.* Targeting signal 1 through CD45RB synergizes with CD40 ligand blockade and promotes long term engraftment and tolerance in stringent transplant models. *J Immunol* 2001; **166**: 322.
26. Sho M, Kishimoto K, Harada H, *et al.* Requirements for induction and maintenance of peripheral tolerance in stringent allograft models. *Proc Natl Acad Sci USA* 2005; **102**: 13230.
27. Trambley J, Bingaman AW, Lin A, *et al.* Asialo GM1(+) CD8(+) T cells play a critical role in costimulation blockade-resistant allograft rejection. *J Clin Invest* 1999; **104**: 1715.
28. Onodera K, Chandraker A, Schaub M, *et al.* CD28-B7 T cell costimulatory blockade by CTLA4Ig in sensitized rat recipients: induction of transplantation tolerance in association with depressed cell-mediated and humoral immune responses. *J Immunol* 1997; **159**: 1711.
29. Lin H, Rathmell JC, Gray GS, Thompson CB, Leiden JM, Alegre M-L. Cytotoxic T lymphocyte antigen 4(CTLA4) blockade accelerates the acute rejection of cardiac allografts in CD28-deficient mice: CTLA4 can function independently of CD28. *J Exp Med* 1998; **188**: 199.

30. Sho M, Yamada A, Najafian N, *et al.* Physiological Mechanisms of regulating alloimmunity: cytokines, CTLA-4, CD25+ cells, and the alloreactive T cell clone size. *J Immunol* 2002; **169**: 3744.
31. Judge TA, Wu Z, Zheng X-G, Sharpe AH, Sayegh MH, Turka LA. The role of CD80, CD86, and CTLA4 in alloimmune responses and the induction of long-term allograft survival. *J Immunol* 1999; **162**: 1947.
32. 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. *Proc Natl Acad Sci USA* 1995; **92**: 9560.
33. 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**: 434.
34. Larsen CP, Pearson TC, Adams AB, *et al.* Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. *Am J Transplant* 2005; **5**: 443.
35. 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 USA* 1996; **93**: 13967.
36. 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**: 686.
37. Parker D, Greiner D, Phillips N, *et al.* Survival of mouse pancreatic islet allografts in recipients treated with allogeneic small lymphocytes and antibody to CD40 ligand. *PNAS* 1995; **92**: 9560.
38. Li Y, Li XC, Zheng XX, Wells AD, Turka LA, Strom TB. 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* 1999; **5**: 1298.
39. Elwood ET, Larsen CP, Cho HR, *et al.* Prolonged acceptance of concordant and discordant xenografts with combined CD40 and CD28 pathway blockade. *Transplantation* 1998; **65**: 1422.
40. Kirk AD, Harlan DM, Armstrong NN, *et al.* CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc Natl Acad Sci USA* 1997; **94**: 8789.
41. Elster EA, Xu H, Tadaki DK, *et al.* Treatment with the humanized CD154-specific monoclonal antibody, hu5C8, prevents acute rejection of primary skin allografts in nonhuman primates. *Transplantation* 2001; **72**: 1473.
42. Kirk AD, Tadaki DK, Celniker A, *et al.* Induction therapy with monoclonal antibodies specific for CD80 and CD86 delays the onset of acute renal allograft rejection in nonhuman primates. *Transplantation* 2001; **72**: 377.
43. Lombardi G, Sidhu S, Batchelor R, Lechler R. Anergic T cells as suppressor cells in vitro. *Science* 1994; **264**: 1587.
44. Schwartz RH. A cell culture model for T lymphocyte clonal anergy. *Science* 1990; **248**: 1349.
45. Guinan EC, Boussiotis VA, Neuberg D, *et al.* Transplantation of anergic histoincompatible bone marrow allografts. *N Engl J Med* 1999; **340**: 1704.
46. Bashuda H, Kimikawa M, Seino K, *et al.* Renal allograft rejection is prevented by adoptive transfer of anergic T cells in nonhuman primates. *J Clin Invest* 2005; **115**: 1896.
47. Elster EA, Hale DA, Mannon RB, Cendales LC, Swanson SJ, Kirk AD. The road to tolerance: renal transplant tolerance induction in nonhuman primate studies and clinical trials. *Transpl Immunol* 2004; **13**: 87.
48. Kawai T, Andrews D, Colvin RB, Sachs DH, Cosimi AB. Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. *Nat Med* 2000; **6**: 114.
49. Adams AB, Shirasugi N, Jones TR, *et al.* Development of a chimeric anti-CD40 monoclonal antibody that synergizes with LEA29Y to prolong islet allograft survival. *J Immunol* 2005; **174**: 542.
50. Linsley PS, Greene JL, Brady W, Bajorath J, Ledbetter JA, Peach R. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. *Immunity* 1994; **1**: 793.
51. Vincenti F, Larsen C, Durrbach A, *et al.* Costimulation blockade with belatacept in renal transplantation. *N Engl J Med* 2005; **353**: 770.
52. Hutloff A, Dittrich AM, Beier KC, *et al.* ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. *Nature* 1999; **397**: 263.
53. Coyle AJ, Lehar S, Lloyd C, *et al.* The CD28-related molecule ICOS is required for effective T cell-dependent immune responses. *Immunity* 2000; **13**: 95.
54. Yoshinaga SK, Whoriskey JS, Khare SD, *et al.* T-cell co-stimulation through B7RP-1 and ICOS. *Nature* 1999; **402**: 827.
55. Ozkaynak E, Gao W, Shemmeri N, *et al.* Importance of ICOS-B7RP-1 costimulation in acute and chronic allograft rejection. *Nat Immunol* 2001; **2**: 591.
56. Nabeyama K, Yasunami Y, Toyofuku A, *et al.* Beneficial effects of costimulatory blockade with anti-inducible costimulator antibody in conjunction with CTLA4Ig on prevention of islet xenograft rejection from rat to mouse. *Transplantation* 2004; **78**: 1590.
57. Harada H, Salama AD, Sho M, *et al.* The role of the ICOS-B7h T cell costimulatory pathway in transplantation immunity. *J Clin Invest* 2003; **112**: 234.
58. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J* 1992; **11**: 3887.
59. Freeman GJ, Long AJ, Iwai Y, *et al.* Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000; **192**: 1027.
60. Latchman Y, Wood CR, Chernova T, *et al.* PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol* 2001; **2**: 261.

61. Liang SC, Latchman YE, Buhlmann JE, *et al.* Regulation of PD-1, PD-L1, and PD-L2 expression during normal and autoimmune responses. *Eur J Immunol* 2003; **33**: 2706.
62. Carter L, Fouser LA, Jussif J, *et al.* PD-1:PD-L inhibitory pathway affects both CD4(+) and CD8(+) T cells and is overcome by IL-2. *Eur J Immunol* 2002; **32**: 634.
63. Chemnitz JM, Parry RV, Nichols KE, June CH, Riley JL. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. *J Immunol* 2004; **173**: 945.
64. Ozkaynak E, Wang L, Goodearl A, *et al.* Programmed death-1 targeting can promote allograft survival. *J Immunol* 2002; **169**: 6546.
65. 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**: 994.
66. Subudhi SK, Zhou P, Yerian LM, *et al.* Local expression of B7-H1 promotes organ-specific autoimmunity and transplant rejection. *J Clin Invest* 2004; **113**: 694.
67. Chapoval AI, Ni J, Lau JS, *et al.* B7-H3: a costimulatory molecule for T cell activation and IFN-[gamma] production. *Nat Immunol* 2001; **2**: 269.
68. Steinberger P, Majdic O, Derdak SV, *et al.* Molecular characterization of human 4Ig-B7-H3, a member of the B7 family with four Ig-like domains. *J Immunol* 2004; **172**: 2352.
69. Suh W-K, Gajewska BU, Okada H, *et al.* The B7 family member B7-H3 preferentially down-regulates T helper type 1-mediated immune responses. *Nat Immunol* 2003; **4**: 899.
70. Wang l, Fraser C, Kikly K, *et al.* B7-H3 promotes acute and chronic allograft rejection. *Eur J Immunol* 2005; **35**: 428.
71. Croft M. Co-stimulatory members of the TNFR family: keys to effective T-cell immunity? *Nat Rev Immunol* 2003; **3**: 609.
72. Borst J, Sluyser C, De Vries E, Klein H, Melief CJ, Van Lier RA. Alternative molecular form of human T cell-specific antigen CD27 expressed upon T cell activation. *Eur J Immunol* 1989; **19**: 357.
73. Morel Y, Schiano de Colella J-M, Harrop J, *et al.* Reciprocal expression of the TNF family receptor herpes virus entry mediator and its ligand LIGHT on activated T cells: LIGHT down-regulates its own receptor. *J Immunol* 2000; **165**: 4397.
74. 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**: 6510.
75. Pollok KE, Kim SH, Kwon BS. Regulation of 4-1BB expression by cell–cell interactions and the cytokines, interleukin-2 and interleukin-4. *Eur J Immunol* 1995; **25**: 488.
76. Ellis T, Simms P, Slivnick D, Jack H, Fisher R. CD30 is a signal-transducing molecule that defines a subset of human activated CD45RO+ T cells. *J Immunol* 1993; **151**: 2380.
77. de Jong R, Loenen W, Brouwer M, *et al.* Regulation of expression of CD27, a T cell-specific member of a novel family of membrane receptors. *J Immunol* 1991; **146**: 2488.
78. Hintzen R, Lens S, Beckmann M, Goodwin R, Lynch D, van Lier R. Characterization of the human CD27 ligand, a novel member of the TNF gene family. *J Immunol* 1994; **152**: 1762.
79. Yamada A, Salama AD, Sho M, *et al.* CD70 signaling is critical for CD28-independent CD8+ T Cell-mediated alloimmune responses in vivo. *J Immunol* 2005; **174**: 1357.
80. 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**: 445.
81. 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**: 2845.
82. Curry AJ, Chikwe J, Smith XG, *et al.* OX40 (CD134) blockade inhibits the co-stimulatory cascade and promotes heart allograft survival. *Transplantation* 2004; **78**: 807.
83. Vu MD, Amanullah F, Li Y, Demirci G, Sayegh MH, Li XC. Different costimulatory and growth factor requirements for CD4+ and CD8+ T cell-mediated rejection. *J Immunol* 2004; **173**: 214.
84. Demirci G, Amanullah F, Kewalaramani R, *et al.* Critical role of OX40 in CD28 and CD154-independent rejection. *J Immunol* 2004; **172**: 1691.
85. Cooper D, Pansal-Bakala P, Croft M. 4-1BB (CD137) controls the clonal expansion and survival of CD8 T cells in vivo but does not contribute to the development of cytotoxicity. *Eur J Immunol* 2002; **32**: 521.
86. Cho HR, Kwon B, Yagita H, *et al.* Blockade of 4-1BB (CD137)/4-1BB ligand interactions increases allograft survival. *Transpl Int* 2004; **17**: 351.
87. Wang J, Guo Z, Dong Y, *et al.* Role of 4-1BB in allograft rejection mediated by CD8+ T cells. *Am J Transplant* 2003; **3**: 543.
88. Smiley ST, Csizmadia V, Gao W, Turka LA, Hancock WW. Differential effects of cyclosporine A, methylprednisolone, mycophenolate, and rapamycin on CD154 induction and requirement for NFkappaB: implications for tolerance induction. *Transplantation* 2000; **70**: 415.
89. Lunsford KE, Koester MA, Eiring AM, Horne PH, Gao D, Bumgardner GL. Targeting LFA-1 and cd154 suppresses the in vivo activation and development of cytolytic (CD4-Independent) CD8+ T cells. *J Immunol* 2005; **175**: 7855.
90. Hale DA, Gottschalk R, Maki T, Monaco AP. Use of CTLA4-Ig in combination with conventional

immunosuppressive agents to prolong allograft survival. *Transplantation* 1997; **64**: 897.
91. Perico N, Imberti O, Bontempelli M, Remuzzi G. Toward novel antirejection strategies: in vivo immunosuppressive properties of CTLA4Ig. *Kidney Int* 1995; **47**: 241.
92. Nanji SA, Hancock WW, Anderson CC, *et al.* Multiple combination therapies involving blockade of ICOS/B7RP-1 costimulation facilitate long-term islet allograft survival. *Am J Transplant* 2004; **4**: 526.
93. Haanstra KG, Sick EA, Ringers J, *et al.* Costimulation blockade followed by a 12-week period of cyclosporine A facilitates prolonged drug-free survival of rhesus monkey kidney allografts. *Transplantation* 2005; **79**: 1623.
94. Hosiawa KA, Wang H, DeVries ME, *et al.* CD80/CD86 costimulation regulates acute vascular rejection. *J Immunol* 2005; **175**: 6197.
95. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. *Nat Rev Immunol* 2002; **2**: 116.

