Transplant International

Transplant International ISSN 0934-0874

REVIEW

A more selective costimulatory blockade of the CD28-B7 pathway

Nicolas Poirier,[1] Gilles Blancho[2] and Bernard Vanhove[1,2]

1 TcL Pharma, Nantes, France

2 INSERM, UMR643, Nantes; CHU Nantes, Institut de Transplantation Urologie Néphrologie (ITUN), Nantes; Faculté de Médecine, Université de Nantes, Nantes, France

Keywords
autoimmunity, B7, CD28, costimulation, CTLA-4, immunointervention, regulatory T cells, transplantation.

Correspondence
Dr Bernard Vanhove, ITUN, INSERM U643, CHU Hôtel Dieu, 30 Bd Jean Monnet, 44093 Nantes, France. Tel.: 33 (0) 240 08 74 17; fax: 33 (0) 240 08 74 11; e-mail: bernard.vanhove@univ-nantes.fr

Received: 29 June 2010
Revision requested: 1 August 2010
Accepted: 20 September 2010
Published online: 21 October 2010

doi:10.1111/j.1432-2277.2010.01176.x

Summary

Progress from the last decade in the understanding of T-cell activation has led to new immunotherapeutic strategies for the treatment of immunological diseases. Since the discovery of costimulatory molecules in the 1980s, the field of T-cell costimulation blockade has literally exploded and now spanned 'from bench to bedside'. Such alternative therapies result in more selective effects specializing their action on Ag-experienced T lymphocytes. This can potentially prevent the progression of autoimmune diseases, allograft rejection and may even induce immune tolerance. In the 1990s, the CD28/B7/CTLA-4 pathway was identified as a crucial regulator of T-cell activation and tolerance induction. Here, we have summarized our current understanding of this complex costimulatory pathway involving co-stimulatory and co-inhibitory molecules and the way we can manipulate these molecules to inhibit, stimulate or kill target cells in experimental preclinical models as well as in clinical trials. We have also reviewed the role of costimulation in the biology of CD4+ CD25+ Foxp3+ regulatory T cells.

T lymphocytes are major players in immune responses following allotransplantation and in autoimmunity. T-cell activation is triggered by specific antigen recognition and reinforced by the engagement of costimulatory molecules that regulate T-cell differentiation into either pathogenic effector cells or anti-inflammatory regulatory cells. Among the series of costimulatory molecules identified these last decades, costimulation through the CD28/B7/CTLA-4 pathways helps determine this balance after initial antigen exposure. The current paradigm holds that constitutively expressed CD28 binds CD80/86 to provide a co-stimulatory signal that is important for sustaining T-cell activation, proliferation and a pro-inflammatory response [1]. CD28 enhances also the secretion and transcription/stability of interleukin (IL)-2 mRNA, which is necessary for effector T-cell and regulatory T-cell (Treg) expansion [2]. However, as Treg do not produce IL-2, they are dependent on IL-2 secretion by bystander activated T cells [3]. In addition, although CD28 signals are critical for Treg homeostasis and thymic generation [4], CD28 engagement by CD80/86 molecules inhibits Treg suppressive activity [5,6]. CTLA-4, the other CD80/86 ligand, delivers anti-proliferative signals to T cells [7], triggers indoleamine 2,3-dioxygenase (IDO) [8] production in antigen-presenting cells (APCs) and is essential for the suppressive function of Tregs [9] and the induction of tolerance to allografts [10,11]. Targeting the CD28-CD80/86 pathway in patients with CD80/86 antagonists (Abatacept, Belatacept, CD80/86 antagonists) is a promising alternative to current immunosuppressive treatments in autoimmunity [12,13] and renal transplantation [14]. However, CD80/86-specific blocking strategies inhibit CTLA-4 signals crucial to the function of Tregs, as well as CD80-mediated signal that have a role in the induction of adaptive Tregs [15]. Moreover, the recent discovery of the inhibitory interaction between PDL-1 and CD80 in mouse [16] and human [17] cells further suggests that immunointervention aimed at blocking ligand access to CD80 might deprive the system of significant physiological regulatory pathways. However, neither CD28-mediated

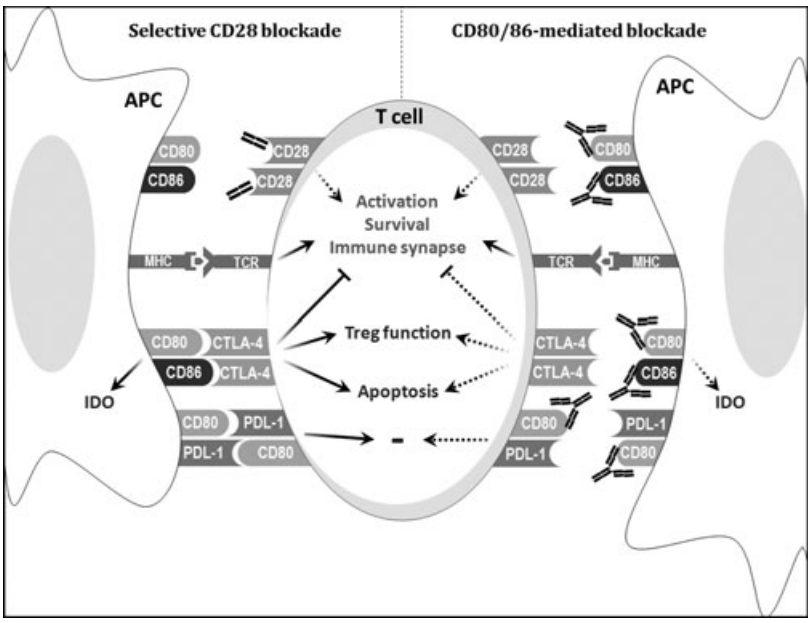

Figure 1 Costimulatory molecules and biological pathways implicated in the targeting of B7s versus CD28. Solid lines/arrows represent active signaling pathways after blockade of CD28 or B7. Dotted lines/arrows represent disrupted signaling pathways. APC, antigen-presenting cells; IDO, indoleamine 2,3-dioxygenase.

nor CD80/86-mediated strategies modify the inhibitory PDL-1/PD-1 pathway. In this review, we have revisited arguments that a more specific costimulation blockade that preserves regulatory signals is an effective strategy for modulating immune responses by preventing the maturation of pathogenic effectors while preserving the function of Tregs. One such proposed costimulation blockade is that of CD28 (see Fig. 1).

Targeting B7

Rodent studies

A first therapeutic strategy aimed at blocking the CD28/B7/CTLA-4 costimulatory pathway was the development of antibodies directed against CD80 and CD86 (commonly named B7.1 and B7.2) which are the receptors of CD28 and CTLA-4. Most studies in rodents evaluating the efficacy of anti-B7 antibodies showed that CD86 blockade was highly immunosuppressive *in vitro* and *in vivo*, whereas CD80 blockade alone produced little effect, and that a combination of these two antibodies caused a profound immunosuppression. Anti-B7 antibody-induced immunosuppression was effective in rescuing abortion-prone fetuses in sensitive mice by facilitating a Th2 bias at the maternal-fetal interface [18], in preventing graft-versus-host disease (GVHD) lethality by inhibiting donor CD4+ or CD8+ T-cell expansion [19], and in inducing long-term survival of skin and heart allografts by inhibiting acute cellular rejection [20,21]. However, chronic rejection was not inhibited after organ transplantation; its prevention necessitated an association with anti-CD40L antibodies [20,21]. Anti-B7 antibodies failed to induce tolerance *in vivo*. However, *ex vivo* anergic or regulatory T cells generated in mixed lymphocyte reactions (MLR) in the presence of anti-B7 antibodies were able to induce tolerance after transfer [22,23]. Anti-B7 antibodies also induce alternatively activated macrophages in MLR [24]. In autoimmune disease models, the discrepancy between CD80 and CD86 blockade is confusing. CD86 blockade alone prevented the development of diabetes in nonobese diabetic (NOD) mice but increased disease severity in experimental autoimmune encephalomyelitis (EAE). In contrast, anti-CD80 treatment alone accelerated the development of diabetes in female NOD mice and brought on diabetes in normally resistant male NOD mice but reduced EAE pathology [25–27]. The explanation is that anti-CD80 antibodies prevent CTLA-4 engagement by CD80, which is necessary for Treg function (see below).

An alternative strategy developed to block CD80/86 was the fusion protein CTLA4-Ig which combined the extracellular domain of CTLA-4 with the Fc portion of IgG1. For nearly 20 years, numerous studies described CTLA4-Ig as a potent inhibitor of CD28, binding to CD80 and CD86 (with a greater affinity for CD80) and therefore a potent *in vitro* and *in vivo* inhibitor of early T-lymphocyte activation (cellular cycle, proliferation, differentiation and survival). However, although theory and

initial *in vitro* observations concluded that CTLA4-Ig caused anergy of alloreactive T lymphocytes [28], this was not confirmed *in vivo*. In fact, *in vivo*, CTLA4-Ig induced a profound immunosuppression, but no T-cell-mediated regulation [29]. An exception was the induction of IDO and tolerogenic APC *in vivo* in rodent models of organ [30–32] but not bone marrow [33] transplantation. The induction of IDO could not be reproduced with the humanized versions of CTLA4-Ig [Abatacept, Belatacept; Bristol-Myers Squibb (BMS), New York, NY, USA], probably because the Fc domain was mutated to decrease Fc-mediated effector function [34,35]. CTLA4-Ig showed efficacy *in vivo* mainly by preventing acute long-term rejection in many transplant models (heart, kidney, skin, liver, limb and islets) but the ability to induce tolerance seemed to differ between organs and transplant models [10,36–41]. CTLA4-Ig also prevented chronic rejection in heart and kidney transplant models [42–45]. CTLA4-Ig efficacy was also demonstrated in GVHD [46], EAE [47], ischemia-reperfusion [48] and, by preventing the humoral response, in systemic lupus erythematosus [49] experimental models. However, similarly to anti-B7 antibodies, CTLA4-Ig reduced the number of CD4+ CD25+ Tregs and accelerated the development of diabetes in NOD mice [4,50]. Finally, CTLA4-Ig efficacy in transplantation was abrogated if initial alloreactive T-lymphocyte frequency was artificially raised [51] or under persistent viral infection [52]. The activity of CTLA4-Ig on memory T cells depends on their differentiation states: CD4+ effector memory T cells (CD62L low) are inhibited by CTLA4-Ig whereas central memory T cells (CD62L high) are not [53]. In addition, CTLA4-Ig is not modifying the proliferative renewal and maintenance of memory CD8 T cells. However, the magnitude of CD8 T-cell memory is reduced by CTLA4-Ig as it inhibits differentiation of resting into activated CD8+ T cells [54]. Another study showed nevertheless that antiviral memory T cells require costimulation to efficiently clear a persistent viral infection and that costimulatory pathways can be targeted to modulate the magnitude of an adoptive immunotherapeutic regimen [55]. Finally, further studies showed that CTLA-4 is also able to regulate CD4 and CD8 memory T cells [56,57]. Therefore CD80/86-mediated strategies, but not CD28-mediated blockade, could also prevent important physiological regulatory signals that kept memory T cells under control.

Nonhuman primate studies in transplantation

CTLA4-Ig and anti-B7 antibodies were tested in nonhuman primate (NHP) kidney allotransplantation and CTLA4-Ig was tested in islets allotransplantation. These studies confirmed that anti-CD86 antibodies were more effective in preventing acute rejection than anti-CD80 and that a combination of both antibodies was even more efficient in preventing acute cellular rejection, but without totally inhibiting alloantibodies and without inducing tolerance [58]. Association with rapamycin [59,60] or cyclosporin A (CsA) [61,62] did not improve these data. Combination induction therapy with anti-B7 and anti-CD154 or anti-CD40 antibodies inhibited alloantibodies but did not bring about survival prolongation as compared to anti-CD40L or anti-CD40 therapy alone [63,64]. It was concluded that anti-B7 antibodies are immunosuppressive but do not induce specific immune regulation and therefore do not fully inhibit inflammation [58,60,65].

Abatacept, a humanized form of CTLA4-Ig (BMS) was tested in NHP kidney allotransplantation but was ineffective in preventing acute rejection when administered as monotherapy (median survival time – MST – of 8 days) [66]. Abatacept was only efficient in association with a short treatment of anti-CD40L antibody [67] but still failed to induce long-term tolerance. A high affinity variant of CTLA4-Ig, LEA29Y (Belatacept; BMS), was evaluated in kidney allotransplantation and was efficient as monotherapy (MST of 45 days), as well as in association with a CNI (calcineurin inhibitor)-free regimen consisting of a continuous treatment of mycophenolate mofetil (MMF) plus steroids (MST of 155 days) [66]. In islet allotransplantation, the association of LEA29Y with an anti-rIL-2 antibody plus rapamycin was sufficient to induce long-term graft survival [68] and to prevent alloantibody production. However, tolerance induction was not achieved given that animals still showed *in vitro* donor-specific cellular reactivity and because treatment discontinuation resulted in rejection.

Clinical studies in transplantation

Anti-B7 antibodies did not enter the clinical development phase in transplantation. Belatacept on the other hand is still in clinical evaluation for kidney allotransplantation. A phase II study involving up to 200 patients of *de novo* renal allografts showed an equivalent efficacy between belatacept treatment in association with MMF plus anti-rIL-2 antibody as compared with CsA with MMF plus anti-rIL-2 antibody, a current gold standard immunosuppressant [14]. Importantly, belatacept-treated patients showed significantly better renal function and reductions in histological signs of chronic allograft nephropathy compared with cyclosporin-treated patients at 1 year, an effect that could be attributed to CNI-toxicity avoidance. However, the rate of acute rejection at 6 months tended to be higher in comparison with the CsA-group, in particular with the 'low' initial dose of Belatacept [14].

A phase III study, the Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial (BENEFIT), aimed at evaluating the low and high belatacept regimens already tested in phase II studies versus cyclosporin in kidney transplantation over 1 year [69]. The key findings of the BENEFIT trial confirmed the phase II studies: similar patient/graft survival, a superior renal function and better cardiovascular/metabolic profiles for belatacept-treated patients. The higher incidence and grade of acute rejection episodes already stressed in phase II studies were also confirmed (22% and 17% for high and low regimens vs. 7% with cyclosporin). By contrast, in an other study where marginal organ were transplanted (BENEFIT-EXT), this higher rate of acute rejection was not confirmed [70]. The finding that the low-dose regimen was associated with a lower rate of acute rejection compared with the higher regimen in this trial suggested that CD80/86 blockade with belatacept might interfere with the CTLA4-CD80/86 negative signaling that is required for maintaining the function of Tregs and that may participate in the control of alloresponses. However, whereas the CD28 pathway was critical for natural Tregs survival and thymic generation in rodents [4,50], belatacept-treated patients of the phase II study did not display any long-term differences in terms of circulating Tregs or Treg expansion when compared with the CNI-treated group [71,72].

Targeting CD28

Based on their stimulatory activity on T lymphocytes, antibodies directed against CD28 can be divided into two classes: firstly, 'superagonistic' anti-CD28 antibodies (CD28SA) induce a nonphysiological CD28 engagement and a complete activation of T lymphocytes even in the absence of T-cell receptor (TCR) stimulation [73]. Secondly, 'conventional' anti-CD28 antibodies (CD28CvA), cross-link CD28 and prompt a costimulatory signal only in synergy with a TCR stimulation.

Superagonistic anti-CD28 antibodies

CD28SA were defined by their capacity to cause a strong activation and proliferation of naive T lymphocytes in the absence of antigenic stimulation [74]. These antibodies were characterized to bind exclusively to the laterally exposed C''D loop of the immunoglobulin-like domain of CD28. CD28SA cross-link CD28 in such a way that it transmits an autonomous signal that activates the nuclear factor κB pathway without inducing any phosphorylation of either TCRζ or ZAP70 [73]. Of interest for transplant and autoimmune therapeutics, these CD28SA antibodies were also described to more efficiently activate and expand *in vitro* and *in vivo* natural Tregs than conventional T lymphocytes [3,75–77]. They elicit two qualitatively distinct waves of T-cell activation: a first phase of polyclonal activation of conventional T lymphocytes, followed by a second phase of Treg expansion dependent on paracrine IL-2 secretion from CD28SA-stimulated conventional T cells [78,79]. As a result, CD28SA were described in rodents as being efficient in preventing autoimmune disease such as EAE [80–82], protecting from GVHD [83] and as producing donor-specific tolerance in a kidney allograft model in rodents [76], whereas a monotherapy in cardiac allotransplantation only delayed acute rejection [77]. In these autoimmune and transplant models, *in vitro* CD28SA-activated Treg transfer was also able to confer protection *in vivo* [76,81,83]. On the other hand, T lymphocyte depletion and transient lymphopenia was also observed in rodent models with high doses of CD28SA [74,78,81]. In rodents, CD28SA-induced lymphopenia was well tolerated and not associated with a massive release of pro-inflammatory mediators [78,81], probably controlled and regulated by Treg expansion. For instance, Treg depletion in mice prior to CD28SA stimulation led to the systemic release of pro-inflammatory cytokines, indicating that in rodents, Tregs effectively suppress the inflammatory response [79].

Regrettably, the humanized CD28SA (TGN1412) did not behave similarly in man. The phase I clinical trial of this molecule as a potential immunotherapeutic for the treatment of chronic lymphocytic B-cell leukemia proved to be catastrophic because TGN1412, unexpectedly, provoked a rapid and massive cytokine storm that caused severe and life-threatening adverse effects [84]. No such toxicity was observed in preclinical studies in monkeys which received doses of up to 500 times higher. Monkeys showed only a weak pro-inflammatory cytokine induction after TGN1412 injection [such as IL-2, IL-4 and IL-5, but no release of interferon (IFN)-γ and tumor necrosis factor (TNF-α)] and a fourfold expansion of CD4+ and CD8+ peripheral T lymphocytes (associated with CD25 and CD69 activation markers) over approximately 20 days. The affinity of TGN1412 toward human and monkey CD28 receptor was found to be comparable [85], Fcγ receptor sequences demonstrated a high degree of similarity between these species and IgG4 binding to human and monkey Fcγ receptors was virtually the same [86]. The remaining question is therefore to ascertain why rodent and monkey T lymphocytes behave differently when compared with human T lymphocytes upon CD28SA stimulation. It was shown that TGN1412 induces calcium responses in human naive and memory CD4+ T lymphocytes but not in monkey T lymphocytes [87]. Although the TGN1412-binding region is perfectly conserved between human and monkey, monkey CD28

sequences diverge from human CD28 in three transmembrane residues [85], which could alter CD28 association with molecular partners, rendering TGN1412-mediated signaling weaker in monkeys. Finally, a more convincing explanation could be a relative loss of expression of inhibitory sialic acid-recognizing Ig-superfamily lectins (Siglec, CD33) on T and B lymphocytes during human evolution, in particular Siglec-5 [88,89]. In fact, human T and B cells were shown to be more reactive than chimpanzee T cells to a wide variety of stimuli (anti-TCR Abs, L-phytohemagglutin, *Staphylococcus aureus* superantigen, CD28SA and MLRs), suggesting that the CD28SA-induced cytokine storm is not related specifically to the CD28 target but rather to an overall over-reactivity of human lymphocytes in comparison with monkeys.

Conventional anti-CD28 antibodies

Agonistic bivalent antibodies

As a result of its homodimeric nature, the degree of cross-linking of CD28 is directly correlated to activation. CD28 transmits a molecular signal through its association with phosphatidylinositol 3-kinase (PI3-kinase) via the cytoplasmic domain [90] and consequently to T-cell activation and proliferation in conjunction with TCR stimulation [91]. Antibodies being also divalent, the action of CD28CvA on T cells usually results in CD28 cross-linking and T-cell costimulation. The process appears to be only partially dependent on Fc engagement by Fc receptors as 'silenced' CD28CvA, with an Fc domain displaying reduced binding capacities to Fc receptors, still costimulate T cells [92]. On the one hand, several CD28CvA were as immunosuppressive as B7 blockade in rodent models of GVHD [93,94], EAE [95], experimental autoimmune uveoretinitis [96] and in a model of *in vivo* T-cell responses to superantigen [93], and therefore behaved like antagonists. These antibodies actually induced *in vivo* a selective depletion of T lymphocytes that recognized alloantigens by an IFN-γ dependent apoptosis mechanism [94]. On the other hand, other anti-CD28 antibodies in their IgG format displayed both agonist and antagonist properties [93]. They could enhance human T-cell transendothelial migration *in vitro* and induce migration of memory, but not naive, T cells to extra-lymphoid tissue independently of TCR-derived signals or homing-receptors [97].

Bivalent antibody with modulating activity

The mouse anti-rat CD28 monoclonal antibody JJ319 was described to stimulate T lymphocyte activation *in vitro*, but it acted as an antagonist *in vivo*, as it brings about a down modulation of the CD28 receptor on the surface of T lymphocyte without inducing T-cell depletion [98–100]. Consequently, JJ319 was functionally antagonist *in vivo* and prevented acute rejection of heart [98,99,101] and liver [102] allografts. In renal transplantation, JJ319 monotherapy was sufficient to prevent chronic rejection and to induce donor-specific tolerance [103,104]. By contrast, in heart and liver transplantation, an association with CsA or with a donor-specific blood transfusion or with a blockade of the CD40/CD40L pathway was necessary to induce tolerance and to inhibit chronic rejection [99,101,102]. JJ319 was also efficient in GVHD by blocking expansion of alloreactive T cells and promoting their apoptosis after few divisions [100]. In addition, whereas very few studies described the induction of regulatory cells after B7 blockade in transplantation, most of the reports that assessed selective CD28 blockade described regulatory cells such as CD4+ CD25+ Foxp3+ [6,104], CD4+ CD45RC− Foxp3− T lymphocytes [102], CD3− IDO+ cells [101] and CD3− B7+ INOS+ myeloid-derived suppressor cells [105].

Fc-silent bivalent antibodies

The binding of CD28CvA to Fcγ receptors (FcγR) reinforces their agonist activity. Therefore, Fc-silent anti-CD28 antibodies were designed by introducing mutations into the Fc fragment to reduce or prevent the cross-linking of CD28 through Fc/FcγR interactions. A hamster-mouse chimeric Fc-silent anti-mouse CD28 antibody (anti-CD28-PV1-IgG3) enabled long-term survival of heart allografts in rats by reducing the activation of alloantigen-mediated key signaling events in T cells [106]. FK734, a humanized Fc-silent anti-human CD28 antibody, reduced T-cell-mediated skin allograft rejection in a humanized severe combined immunodeficient (SCID) model [92] and reduced epidermis thinning and HLA-DR-positive lymphocytic infiltrates of human psoriasis plaques transplanted into SCID mice [107]. However, this humanized Fc-silent antibody still generated residual agonistic signals leading to T-cell activation and cytokine release. *In vitro*, it enhanced proliferation, IL-2 and IFN-γ secretion of CD4+ or CD8+ T lymphocytes when stimulated with monocytes or human endothelial cells [92], probably as a result of the mechanical cross-linking of CD28 homodimers by the antibody. However, in the presence of CD86-transfected monocytes, this Fc-silent antibody inhibited proliferation and cytokine secretion in T lymphocytes, a phenomenon that could be attributed to the engagement by CD86 of the negative costimulatory CTLA-4 on responding T cells.

Antagonistic monovalent anti-CD28 molecules

Monovalent fragments from CD28CvA can inhibit CD28/B7 interactions without stimulating CD28. They can be used as true antagonists to inhibit proliferation and cytokine secretion in T lymphocytes [108] and can induce

anergy *in vitro* [28]. *In vitro*, it was recently found [6] that in the presence of a CD28 antagonist, T cells cannot become activated not only because they lack the CD28-mediated costimulatory signal but also because their CTLA-4 negative costimulators become engaged and this inhibits the TCR-induced stop signal that otherwise allows T-cell immobilization [109]. In the presence of a CD28 antagonist, therefore, T cells stay motile and cannot form stable immunological synapses with CD80/86+ APCs [6]. In this respect, selective CD28 blockade differs from B7-mediated blockade (anti-B7 and CTLA4-Ig) that also prevents CD28-mediated costimulation but does not inhibit cell arrest in APC [6]. Shorter T-cell arrest times when encountering APC has been associated with tolerant states *in vivo* by biphotonic studies in rodents [110] whereas the formation of stable synapses and T-cell immobilization was associated with immune responses. Moreover, as Treg suppression activity is dependent on CTLA-4 [9,111–114], but not CD28 [111,115,116], selective CD28 targeting does not actually inhibit suppression by Treg [6]. *In vivo*, monovalent antagonist anti-CD28 antibodies delayed acute rejection when given as monotherapy and synergized with CNIs to prevent acute and chronic allograft rejection in kidney and heart transplant models in NHPs [6]. Although CNI decrease the function/generation of Treg cells [117], the acquisition of post-transplant donor-specific hyporesponsiveness was observed and Tregs were found to be increased in the periphery and accumulated in the allograft where molecular signatures of immune regulation (HO-1, IDO, TGF-β, etc.) were observed. Therefore, from the experimental perspective, the theoretical benefits of selective CD28 blockade depicted in Fig. 1 appear to be a fact. Whether they also spell out any clinical advantage, however, remains a subject for future investigation.

Conclusion

T-cell costimulation plays a major role in the molecular interactions between T cells and APC leading to T-cell activation, proliferation, survival and cytokine secretion. While standard immunosuppression (including CNI) inhibits these features with considerable effectiveness, it does not induce immune regulation and displays serious toxicity, predominantly affecting the kidney, the cardiovascular system and lipid metabolism. The unmet clinical need therefore is an alternative less toxic and more selective immunosuppression strategy. However, whether inducing antigen-specific regulation is an advantage in clinical organ transplantation remains to be demonstrated. B7-mediated blockade, now in the clinic, has been developed as a nontoxic alternative to CNI. This advantage is offset, however, by a higher incidence of rejection episodes. As described above, in addition to affecting the CD28-mediated pathway of costimulation, reagents targeting B7 also inhibit CTLA-4 and PDL-1 inhibitory signals of T lymphocytes and prevent Treg function which requires intact CTLA-4/B7 interactions. Finding out whether selective CD28 blockade bears any significant practical advantages in relation to B7 targeting, as predicted in theory, is a matter that requires formal testing. However, in this respect, the efficacy of CD28 antagonists in rodents and preclinical models to induce immune-regulation and inhibit acute and chronic allograft rejection can be deemed as promising.

References

1. Takahashi T, Kuniyasu Y, Toda M, *et al.* Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int Immunol* 1998; **10**: 1969.
2. Powell JD, Ragheb JA, Kitagawa-Sakakida S, Schwartz RH. Molecular regulation of interleukin-2 expression by CD28 co-stimulation and anergy. *Immunol Rev* 1998; **165**: 287.
3. Hünig T. Manipulation of regulatory T-cell number and function with CD28-specific monoclonal antibodies. *Adv Immunol* 2007; **95**: 111.
4. Salomon B, Lenschow DJ, Rhee L, *et al.* B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 2000; **12**: 431.
5. Zheng Y, Manzotti CN, Liu M, *et al.* CD86 and CD80 differentially modulate the suppressive function of human regulatory T cells. *J Immunol* 2004; **172**: 2778.
6. Poirier N, Azimzadeh AM, Zhang T, *et al.* Inducing CTLA-4-dependent immune regulation by selective CD28 blockade promotes regulatory T cells in organ transplantation. *Sci Transl Med* 2010; **2**: 17ra10.
7. Waterhouse P, Penninger JM, Timms E, *et al.* Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science* 1995; **270**: 985.
8. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol* 2004; **4**: 762.
9. Wing K, Onishi Y, Prieto-Martin P, *et al.* CTLA-4 control over Foxp3+ regulatory T cell function. *Science* 2008; **322**: 271.
10. Zheng XX, Markees TG, Hancock WW, *et al.* CTLA4 signals are required to optimally induce allograft tolerance with combined donor-specific transfusion and anti-CD154 monoclonal antibody treatment. *J Immunol* 1999; **162**: 4983.
11. Tsai M, Ho H, Chien H, Ou-Yang P, Lee C, Lee P. The role of B7 ligands (CD80 and CD86) in CD152-mediated

allograft tolerance: a crosscheck hypothesis. *Transplantation* 2004; **77**: 48.
12. Alegre ML, Frauwirth KA, Thompson CB. T-cell regulation by CD28 and CTLA-4. *Nat Rev Immunol* 2001; **1**: 220.
13. Kremer JM, Westhovens R, Leon M, *et al.* Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. *N Engl J Med* 2003; **349**: 1907.
14. Vincenti F, Larsen C, Durrbach A, *et al.* Costimulation blockade with belatacept in renal transplantation. *N Engl J Med* 2005; **353**: 770.
15. Perez N, Karumuthil-Melethil S, Li R, Prabhakar BS, Holterman MJ, Vasu C. Preferential costimulation by CD80 results in IL-10-dependent TGF-beta1(+) -adaptive regulatory T cell generation. *J Immunol* 2008; **180**: 6566.
16. Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. *Immunity* 2007; **27**: 111.
17. Butte MJ, Peña-Cruz V, Kim M, Freeman GJ, Sharpe AH. Interaction of human PD-L1 and B7-1. *Mol Immunol* 2008; **45**: 3567.
18. Zhu X, Zhou Y, Wang M, Jin L, Yuan M, Li D. Blockade of CD86 signaling facilitates a Th2 bias at the maternal-fetal interface and expands peripheral CD4+CD25+ regulatory T cells to rescue abortion-prone fetuses. *Biol Reprod* 2005; **72**: 338.
19. Blazar BR, Sharpe AH, Taylor PA, *et al.* Infusion of anti-B7.1 (CD80) and anti-B7.2 (CD86) monoclonal antibodies inhibits murine graft-versus-host disease lethality in part via direct effects on CD4+ and CD8+ T cells. *J Immunol* 1996; **157**: 3250.
20. 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.
21. Woodward JE, Bayer AL, Chavin KD, Blue ML, Baliga P. T-cell alterations in cardiac allograft recipients after B7 (CD80 and CD86) blockade. *Transplantation* 1998; **66**: 14.
22. Taylor PA, Noelle RJ, Blazar BR. CD4(+)CD25(+) immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade. *J Exp Med* 2001; **193**: 1311.
23. 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.
24. Tzachanis D, Berezovskaya A, Nadler LM, Boussiotis VA. Blockade of B7/CD28 in mixed lymphocyte reaction cultures results in the generation of alternatively activated macrophages, which suppress T-cell responses. *Blood* 2002; **99**: 1465.
25. Kuchroo VK, Das MP, Brown JA, *et al.* B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. *Cell* 1995; **80**: 707.
26. Miller SD, Vanderlugt CL, Lenschow DJ, *et al.* Blockade of CD28/B7-1 interaction prevents epitope spreading and clinical relapses of murine EAE. *Immunity* 1995; **3**: 739.
27. Lenschow DJ, Ho SC, Sattar H, *et al.* Differential effects of anti-B7-1 and anti-B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse. *J Exp Med* 1995; **181**: 1145.
28. Tan P, Anasetti C, Hansen JA, *et al.* Induction of alloantigen-specific hyporesponsiveness in human T lymphocytes by blocking interaction of CD28 with its natural ligand B7/BB1. *J Exp Med* 1993; **177**: 165.
29. Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. *Immunol Rev* 2008; **224**: 166.
30. Grohmann U, Orabona C, Fallarino F, *et al.* CTLA-4-Ig regulates tryptophan catabolism *in vivo*. *Nat Immunol* 2002; **3**: 1097.
31. Guillot C, Ménoret S, Guillonneau C, *et al.* Active suppression of allogeneic proliferative responses by dendritic cells after induction of long-term allograft survival by CTLA4Ig. *Blood* 2003; **101**: 3325.
32. Séveno C, Coulon F, Haspot F, *et al.* Induction of regulatory cells and control of cellular but not vascular rejection by costimulation blockade in hamster-to-rat heart xenotransplantation. *Xenotransplantation* 2007; **14**: 25.
33. Pree I, Bigenzahn S, Fuchs D, *et al.* CTLA4Ig promotes the induction of hematopoietic chimerism and tolerance independently of Indoleamine-2,3-dioxygenase. *Transplantation* 2007; **83**: 663.
34. Davis PM, Nadler SG, Stetsko DK, Suchard SJ. Abatacept modulates human dendritic cell-stimulated T-cell proliferation and effector function independent of IDO induction. *Clin. Immunol* 2008; **126**: 38.
35. Carman JA, Davis PM, Yang W, *et al.* Abatacept does not induce direct gene expression changes in antigen-presenting cells. *J Clin Immunol* 2009; **29**: 479.
36. 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.
37. Lin H, Bolling SF, Linsley PS, *et al.* Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA4Ig plus donor-specific transfusion. *J Exp Med* 1993; **178**: 1801.
38. Yin D, Fathman CG. Induction of tolerance to heart allografts in high responder rats by combining anti-CD4 with CTLA4Ig. *J Immunol* 1995; **155**: 1655.
39. Akalin E, Chandraker A, Russell ME, Turka LA, Hancock WW, Sayegh M. CD28-B7 T cell costimulatory blockade by CTLA4Ig in the rat renal allograft model: inhibition of cell-mediated and humoral immune responses *in vivo*. *Transplantation* 1996; **62**: 1942.
40. Zheng XX, Sayegh MH, Zheng XG, *et al.* The role of donor and recipient B7-1 (CD80) in allograft rejection. *J Immunol* 1997; **159**: 1169.

41. Fan K, Wang H, Wei H, *et al.* Blockade of LIGHT/HVEM and B7/CD28 signaling facilitates long-term islet graft survival with development of allospecific tolerance. *Transplantation* 2007; **84**: 746.
42. Russell ME, Hancock WW, Akalin E, *et al.* Chronic cardiac rejection in the LEW to F344 rat model. Blockade of CD28-B7 costimulation by CTLA4Ig modulates T cell and macrophage activation and attenuates arteriosclerosis. *J Clin Invest* 1996; **97**: 833.
43. Azuma H, Chandraker A, Nadeau K, *et al.* Blockade of T-cell costimulation prevents development of experimental chronic renal allograft rejection. *Proc Natl Acad Sci USA* 1996; **93**: 12439.
44. Chandraker A, Azuma H, Nadeau K, *et al.* Late blockade of T cell costimulation interrupts progression of experimental chronic allograft rejection. *J Clin Invest* 1998; **101**: 2309.
45. Kim KS, Denton MD, Chandraker A, *et al.* CD28-B7-mediated T cell costimulation in chronic cardiac allograft rejection: differential role of B7-1 in initiation versus progression of graft arteriosclerosis. *Am J Pathol* 2001; **158**: 977.
46. Blazar BR, Taylor PA, Linsley PS, Vallera DA. *In vivo* blockade of CD28/CTLA4: B7/BB1 interaction with CTLA4-Ig reduces lethal murine graft-versus-host disease across the major histocompatibility complex barrier in mice. *Blood* 1994; **83**: 3815.
47. Cross AH, Girard TJ, Giacoletto KS, *et al.* Long-term inhibition of murine experimental autoimmune encephalomyelitis using CTLA-4-Fc supports a key role for CD28 costimulation. *J Clin Invest* 1995; **95**: 2783.
48. Takada M, Chandraker A, Nadeau KC, Sayegh MH, Tilney NL. The role of the B7 costimulatory pathway in experimental cold ischemia/reperfusion injury. *J Clin Invest* 1997; **100**: 1199.
49. Finck BK, Linsley PS, Wofsy D. Treatment of murine lupus with CTLA4Ig. *Science* 1994; **265**: 1225.
50. Tang Q, Henriksen KJ, Boden EK, *et al.* Cutting edge: CD28 controls peripheral homeostasis of CD4+CD25+ regulatory T cells. *J Immunol* 2003; **171**: 3348.
51. Ford ML, Koehn BH, Wagener ME, *et al.* Antigen-specific precursor frequency impacts T cell proliferation, differentiation, and requirement for costimulation. *J Exp Med* 2007; **204**: 299.
52. Williams MA, Onami TM, Adams AB, *et al.* Cutting edge: persistent viral infection prevents tolerance induction and escapes immune control following CD28/CD40 blockade-based regimen. *J Immunol* 2002; **169**: 5387.
53. Ndejembi MP, Teijaro JR, Patke DS, *et al.* Control of memory CD4 T cell recall by the CD28/B7 costimulatory pathway. *J Immunol* 2006; **177**: 7698.
54. Suresh M, Whitmire JK, Harrington LE, *et al.* Role of CD28-B7 interactions in generation and maintenance of CD8 T cell memory. *J Immunol* 2001; **167**: 5565.
55. Garidou L, Heydari S, Truong P, Brooks DG, McGavern DB. Therapeutic memory T cells require costimulation for effective clearance of a persistent viral infection. *J Virol* 2009; **83**: 8905.
56. Metz DP, Farber DL, Taylor T, Bottomly K. Differential role of CTLA-4 in regulation of resting memory versus naive CD4 T cell activation. *J Immunol* 1998; **161**: 5855.
57. Hokey DA, Yan J, Hirao LA, *et al.* CLTA-4 blockade *in vivo* promotes the generation of short-lived effector CD8 T cells and a more persistent central memory CD4 T cell response. *J Med Primatol* 2008; **37**(Suppl. 2): 62.
58. 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 non-human primates. *Transplantation* 2001; **72**: 377.
59. Bîrsan T, Hausen B, Higgins JP, *et al.* Treatment with humanized monoclonal antibodies against CD80 and CD86 combined with sirolimus prolongs renal allograft survival in cynomolgus monkeys. *Transplantation* 2003; **75**: 2106.
60. Boulday G, Ashton-Chess J, Bernard P, *et al.* Association of rapamycin and co-stimulation blockade using anti-B7 antibodies in renal allotransplantation in baboons. *Nephrol Dial Transplant* 2004; **19**: 1752.
61. Ossevoort MA, Ringers J, Kuhn EM, *et al.* Prevention of renal allograft rejection in primates by blocking the B7/CD28 pathway. *Transplantation* 1999; **68**: 1010.
62. Hausen B, Klupp J, Christians U, *et al.* Coadministration of either cyclosporine or steroids with humanized monoclonal antibodies against CD80 and CD86 successfully prolong allograft survival after life supporting renal transplantation in cynomolgus monkeys. *Transplantation* 2001; **72**: 1128.
63. Montgomery SP, Xu H, Tadaki DK, *et al.* Combination induction therapy with monoclonal antibodies specific for CD80, CD86, and CD154 in nonhuman primate renal transplantation. *Transplantation* 2002; **74**: 1365.
64. Haanstra KG, Ringers J, Sick EA, *et al.* Prevention of kidney allograft rejection using anti-CD40 and anti-CD86 in primates. *Transplantation* 2003; **75**: 637.
65. Hale DA, Dhanireddy K, Bruno D, Kirk AD. Induction of transplantation tolerance in non-human primate preclinical models. *Philos Trans R Soc Lond B Biol Sci* 2005; **360**: 1723.
66. 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.
67. 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.
68. Adams AB, Shirasugi N, Durham MM, *et al.* Calcineurin inhibitor-free CD28 blockade-based protocol protects allogeneic islets in nonhuman primates. *Diabetes* 2002; **51**: 265.

69. Vincenti F, Charpentier B, Vanrenterghem Y, *et al.* A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). *Am J Transplant* 2010; **10**: 535.
70. Durrbach A, Pestana JM, Pearson T, *et al.* A phase III study of belatacept versus cyclosporine in kidney transplants from extended criteria donors (BENEFIT-EXT study). *Am J Transplant* 2010; **10**: 547.
71. Chavez H, Beaudreuil S, Abbed K, *et al.* Absence of CD4CD25 regulatory T cell expansion in renal transplanted patients treated *in vivo* with Belatacept mediated CD28-CD80/86 blockade. *Transpl Immunol* 2007; **17**: 243.
72. Bluestone JA, Liu W, Yabu JM, *et al.* The effect of costimulatory and interleukin 2 receptor blockade on regulatory T cells in renal transplantation. *Am J Transplant* 2008; **8**: 2086.
73. Lühder F, Huang Y, Dennehy KM, *et al.* Topological requirements and signaling properties of T cell-activating, anti-CD28 antibody superagonists. *J Exp Med* 2003; **197**: 955.
74. Tacke M, Hanke G, Hanke T, Hünig T. CD28-mediated induction of proliferation in resting T cells *in vitro* and *in vivo* without engagement of the T cell receptor: evidence for functionally distinct forms of CD28. *Eur J Immunol* 1997; **27**: 239.
75. Lin C, Hünig T. Efficient expansion of regulatory T cells *in vitro* and *in vivo* with a CD28 superagonist. *Eur J Immunol* 2003; **33**: 626.
76. Azuma H, Isaka Y, Li X, *et al.* Superagonistic CD28 antibody induces donor-specific tolerance in rat renal allografts. *Am J Transplant* 2008; **8**: 2004.
77. Kitazawa Y, Fujino M, Sakai T, *et al.* Foxp3-expressing regulatory T cells expanded with CD28 superagonist antibody can prevent rat cardiac allograft rejection. *J Heart Lung Transplant* 2008; **27**: 362.
78. Müller N, van den Brandt J, Odoardi F, *et al.* A CD28 superagonistic antibody elicits 2 functionally distinct waves of T cell activation in rats. *J Clin Invest* 2008; **118**: 1405.
79. Gogishvili T, Langenhorst D, Lühder F, *et al.* Rapid regulatory T-cell response prevents cytokine storm in CD28 superagonist treated mice. *PLoS ONE* 2009; **4**: e4643.
80. Schmidt J, Elflein K, Stienekemeier M, *et al.* Treatment and prevention of experimental autoimmune neuritis with superagonistic CD28-specific monoclonal antibodies. *J Neuroimmunol* 2003; **140**: 143.
81. Beyersdorf N, Gaupp S, Balbach K, *et al.* Selective targeting of regulatory T cells with CD28 superagonists allows effective therapy of experimental autoimmune encephalomyelitis. *J Exp Med* 2005; **202**: 445.
82. Tischner D, Weishaupt A, van den Brandt J, *et al.* Polyclonal expansion of regulatory T cells interferes with effector cell migration in a model of multiple sclerosis. *Brain* 2006; **129**: 2635.
83. Beyersdorf N, Ding X, Hünig T, Kerkau T. Superagonistic CD28 stimulation of allogeneic T cells protects from acute graft-versus-host disease. *Blood* 2009; **114**: 4575.
84. Suntharalingam G, Perry MR, Ward S, *et al.* Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* 2006; **355**: 1018.
85. Ohresser M, Olive D, Vanhove B, Watier H. Risk in drug trials. *Lancet* 2006; **368**: 2205.
86. Schraven B, Kalinke U. CD28 superagonists: what makes the difference in humans? *Immunity* 2008; **28**: 591.
87. Waibler Z, Sender LY, Merten C, *et al.* Signaling signatures and functional properties of anti-human CD28 superagonistic antibodies. *PLoS ONE* 2008; **3**: e1708.
88. Soto PC, Stein LL, Hurtado-Ziola N, Hedrick SM, Varki A. Relative over-reactivity of human versus chimpanzee lymphocytes: implications for the human diseases associated with immune activation. *J Immunol* 2010; **184**: 4185.
89. Nguyen DH, Hurtado-Ziola N, Gagneux P, Varki A. Loss of Siglec expression on T lymphocytes during human evolution. *Proc Natl Acad Sci USA* 2006; **103**: 7765.
90. Damle NK, Doyle LV, Grosmaire LS, Ledbetter JA. Differential regulatory signals delivered by antibody binding to the CD28 (Tp44) molecule during the activation of human T lymphocytes. *J Immunol* 1988; **140**: 1753.
91. Gross JA, Callas E, Allison JP. Identification and distribution of the costimulatory receptor CD28 in the mouse. *J Immunol* 1992; **149**: 380.
92. Shiao SL, McNiff JM, Masunaga T, Tamura K, Kubo K, Pober J. Immunomodulatory properties of FK734, a humanized anti-CD28 monoclonal antibody with agonistic and antagonistic activities. *Transplantation* 2007; **83**: 304.
93. Beyersdorf N, Ding X, Blank G, Dennehy KM, Kerkau T, Hünig T. Protection from graft-versus-host disease with a novel B7 binding site-specific mouse anti-mouse CD28 monoclonal antibody. *Blood* 2008; **112**: 4328.
94. Yu X, Albert MH, Martin PJ, Anasetti C. CD28 ligation induces transplantation tolerance by IFN-gamma-dependent depletion of T cells that recognize alloantigens. *J Clin Invest* 2004; **113**: 1624.
95. Perrin PJ, June CH, Maldonado JH, Ratts RB, Racke MK. Blockade of CD28 during *in vitro* activation of encephalitogenic T cells or after disease onset ameliorates experimental autoimmune encephalomyelitis. *J Immunol* 1999; **163**: 1704.
96. Silver PB, Hathcock KS, Chan CC, Wiggert B, Caspi RR. Blockade of costimulation through B7/CD28 inhibits experimental autoimmune uveoretinitis, but does not induce long-term tolerance. *J Immunol* 2000; **165**: 5041.
97. Mirenda V, Jarmin SJ, David R, *et al.* Physiologic and aberrant regulation of memory T-cell trafficking by the costimulatory molecule CD28. *Blood* 2007; **109**: 2968.
98. Dengler TJ, Szabo G, Sido B, *et al.* Prolonged allograft survival but no tolerance induction by modulating CD28

antibody JJ319 after high-responder rat heart transplantation. *Transplantation* 1999; **67**: 392.
99. Dong VM, Yuan X, Coito AJ, Waaga AM, Sayegh MH, Chandraker A. Mechanisms of targeting CD28 by a signaling monoclonal antibody in acute and chronic allograft rejection. *Transplantation* 2002; **73**: 1310.
100. Haspot F, Villemain F, Laflamme G, *et al.* Differential effect of CD28 versus B7 blockade on direct pathway of allorecognition and self-restricted responses. *Blood* 2002; **99**: 2228.
101. Guillonneau C, Séveno C, Dugast A, *et al.* Anti-CD28 antibodies modify regulatory mechanisms and reinforce tolerance in CD40Ig-treated heart allograft recipients. *J Immunol* 2007; **179**: 8164.
102. Urakami H, Ostanin DV, Hünig T, Grisham MB. Combination of donor-specific blood transfusion with anti-CD28 antibody synergizes to prolong graft survival in rat liver transplantation. *Transplant Proc* 2006; **38**: 3244.
103. Laskowski IA, Pratschke J, Wilhelm MJ, *et al.* Anti-CD28 monoclonal antibody therapy prevents chronic rejection of renal allografts in rats. *J Am Soc Nephrol* 2002; **13**: 519.
104. Haspot F, Séveno C, Dugast A, *et al.* Anti-CD28 antibody-induced kidney allograft tolerance related to tryptophan degradation and TCR class II B7 regulatory cells. *Am J Transplant* 2005; **5**: 2339.
105. Dugast A, Haudebourg T, Coulon F, *et al.* Myeloid-derived suppressor cells accumulate in kidney allograft tolerance and specifically suppress effector T cell expansion. *J Immunol* 2008; **180**: 7898.
106. Jang M, Pan F, Erickson LM, *et al.* A blocking anti-CD28-specific antibody induces long-term heart allograft survival by suppression of the PKC theta-JNK signal pathway. *Transplantation* 2008; **85**: 1051.
107. Raychaudhuri SP, Kundu-Raychaudhuri S, Tamura K, *et al.* FR255734, a humanized, Fc-Silent, Anti-CD28 antibody, improves psoriasis in the SCID mouse-psoriasis xenograft model. *J Invest Dermatol* 2008; **128**: 1969.
108. Vanhove B, Laflamme G, Coulon F, *et al.* Selective blockade of CD28 and not CTLA-4 with a single-chain Fv-alpha1-antitrypsin fusion antibody. *Blood* 2003; **102**: 564.
109. Schneider H, Downey J, Smith A, *et al.* Reversal of the TCR stop signal by CTLA-4. *Science* 2006; **313**: 1972.
110. Hugues S, Fetler L, Bonifaz L, Helft J, Amblard F, Amigorena S. Distinct T cell dynamics in lymph nodes during the induction of tolerance and immunity. *Nat Immunol* 2004; **5**: 1235.
111. Takahashi T, Tagami T, Yamazaki S, *et al.* Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med* 2000; **192**: 303.
112. Read S, Malmström V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. *J Exp Med* 2000; **192**: 295.
113. Onishi Y, Fehervari Z, Yamaguchi T, Sakaguchi S. Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells *in vitro* and actively inhibit their maturation. *Proc Natl Acad Sci USA* 2008; **105**: 10113.
114. Friedline RH, Brown DS, Nguyen H, *et al.* CD4+ regulatory T cells require CTLA-4 for the maintenance of systemic tolerance. *J Exp Med* 2009; **206**: 421.
115. Sakaguchi S, Sakaguchi N, Shimizu J, *et al.* Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev* 2001; **182**: 18.
116. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol* 2005; **23**: 515.
117. Zeiser R, Nguyen VH, Beilhack A, *et al.* Inhibition of CD4+CD25+ regulatory T-cell function by calcineurin-dependent interleukin-2 production. *Blood* 2006; **108**: 390.