The blockade of T-cell co-stimulation as a therapeutic stratagem for immunosuppression: Focus on belatacept

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Abstract: The development of immunosuppressive drugs has in recent years been focused on prevention of acute rejection. This has led to an increase in one-year allograft survival. However, these drugs have non-immune effects which contribute to the high incidence of late graft loss, as a consequence of chronic allograft nephropathy, and the death of patients. As an immune-specific alternative to conventional immunosuppressants, new biotechnology tools have been developed; they target the costimulation signal of T-cell activation, particularly by the "classical" B7/CD28 and CD40/CD40L pathways. Here, we review the limitations of current immunosuppressive protocols, the benefits of classical B7/CD28 costimulation blockade, and the first large-scale clinical application of this strategy to human transplantation with belatacept. We will also consider novel costimulatory molecules of the B7/CD28 and TNF/TNF-R families, which appear to be important for the functions of memory and effector T-cells.

Keywords: kidney transplantation, immunosuppressants, costimulation, belatacept, chronic allograft nephropathy, fusion protein

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 chronic allograft nephropathy or on patient deaths (Meier-Kriesche et al 2004). This is largely due to the broad non immune effects of current immunosuppressive drugs, the targets of which are ubiquitous and non specific. 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 and fusion proteins – targeting the relevant molecules very precisely.

Here we review the limitations of current immunosuppressive protocols, the benefits of classical B7/CD28 costimulation blockade, and its first large-scale application with belatacept. We will also address novel costimulatory molecules of the B7/CD28 and TNF/TNF-R families, which appear to be particularly important for the functions of memory and effector T-cells. These categories of T-cells play important roles in allograft rejection and are less susceptible to classical costimulation blockade (London et al 2000).

Limitations of current immunosuppressive protocols

Because there is a relationship between acute rejection and late allograft failure, the recent development of immunosuppressive drugs has been focused on the prevention of acute rejection. Thus, with the introduction of cyclosporine in the early 1980s and newer immunosuppressive drugs in the 1990s (tacrolimus, mycophenolate mofetil or MMF

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Table 1 Main non-immune side effects of immunosuppressive maintenance drugs used in kidney transplantation

Drugs	Side effects
Steroids	Diabetes, Hypertension, Hyperlipidemia, Obesity
Calcineurin Inhibitors (Cyclosporine, Tacrolimus)	Hypertension[1], Hyperlipidemia[1], Diabetes[2], Nephrotoxicity, BK virus nephropathy[2]
Anti-proliferative Drugs (Azathioprine, Mycophenolate Mofetil, Sodium Mycophenolate)	Hematological (Neutropenia, Anemia) Diarrhea[3], BK virus nephropathy[3]
mTOR[4] inhibitors (Sirolimus, Everolimus)	Delayed wound healing, Hyperlipidemia, Hematological disorders (Anemia, Thrombopenia, Leucopenia), Pneumopathy (sirolimus), Increase of CNI nephrotoxicity

[1]More frequent with cyclosporine.
[2]More frequent with tacrolimus.
[3]More frequent with prodrugs of mycophenolic acid.
[4]Mammalian.
Abbreviations: TOR, Target of Rapamycin.

and sirolimus), the incidence of acute rejection has substantially decreased to 10 to 30% (Denton et al 1999). Consequently, one-year allograft survival has increased: according to the OPTN/SRTR report, the adjusted one-year graft survival for recipients of deceased non-extended criteria donor kidneys was 91% (Cohen et al 2006). However, contrary to the optimistic projections for the 1988–1995 period (Hariharan et al 2000), actuarial analysis has revealed only a small increase in graft half-lives: from 6 years in 1988 to 8 years in 1995 (Meier-Kriesche et al 2004). Chronic allograft nephropathy (CAN) and death of transplant patients with a functioning graft are the main causes of late allograft failure. The broad non immune effects of current immunosuppressive drugs (see Table 1) contribute to both CAN and the risk of patient death.

Death of patients with functioning graft

Death with a functioning graft accounts for 50% of late allograft failure (Pascual et al 2002), and 43% of such cases are due to cardiovascular events (West et al 1996). The following factors are frequently encountered in renal transplant patients and contribute to cardiovascular diseases: older age, obesity, hypertension, diabetes mellitus, hyperlipidemia, smoking, long-term chronic renal failure and dialysis. Patients may present these factors before transplantation, but some are frequent side effects of currently used immunosuppressive drugs (Table 1). Other causes of death include infection and cancer, often associated with a generally high immunosuppressive burden rather than the direct effect of a single molecule. An exception is the lower incidence of cancers observed in patients treated with mTOR inhibitors – sirolimus and everolimus – because of their anti-proliferative effect on several types of malignancies (Kauffman et al 2005).

Chronic allograft nephropathy

CAN is a pathological finding on kidney biopsy; it consists of fibrosis and tubular atrophy, and the clinical presentation is often a gradual decrease in GFR associated with proteinuria and hypertension (Pascual et al 2002). CAN is not a single pathogenic entity but most frequently the result of combined immunological and nonimmunological manifestations (Table 2).

The immunological factors include episodes of acute cellular rejection and also sub-clinical acute rejections defined as histological evidence of acute cellular rejection from routine kidney biopsies (those performed not because of a deterioration of renal function). The recent histological characterization of antibody-mediated rejection (AMR) with C4d staining and the detection of donor-specific antibodies (DSA) with sensitive techniques have indicated the role of humoral immunity in acute and chronic rejection (Snanoudj et al 2005). Circulating DSA are present in many (100% in some series) patients with histological evidence of chronic rejection and in 50% of cases, these antibodies appear de novo after transplantation (Lee et al 2002). Moreover, among patients with de novo DSA, the rate of graft failure after one year is 8.6%, whereas it is only 3.0% for patients without these antibodies (Terasaki and Ozawa 2004).

The non immunological features include classic conditions that increase the rate of decline of renal function in all nephropathies (diabetes, hypertension, hyperlipidemia, obesity). The "quality" of the donor is becoming a critical issue. Indeed, because of the shortage of organs and the growing demand, kidneys are currently procured from elderly deceased donors, donors with pre-existing hypertension, diabetes, cardio-vascular risk factors or disease, and mild renal failure. These grafts are called "extended-criteria donor (ECD) kidneys" (Rosengard et al 2002). The clinical outcome with these kidneys is better than that with dialysis, but some studies have shown a higher risk of late allograft failure compared with allografts procured from non-marginal donors. For the same reasons, kidneys harvested from non heart-beating donors are being transplanted more frequently than in the past, despite the risk of delayed graft

Table 2 Factors involved in the pathogenesis of chronic allograft nephropathy

Immunological factors	Non-immunological factors
Episodes of Acute Rejection	Extended-Criteria Donors
Sub-clinical Acute rejection	Ischemia-reperfusion Injury
Chronic alloimmune response	Delayed Graft Function
	Hypertension
	Hyperlipidemia
Risk factors	Diabetes
HLA sensitization (pregnancy, previous transplantation, blood transfusion), DSA	Infections (Bacterial Pyelonephritis/ Polyomavirus Nephropathy)
Poor HLA Matching	CNI nephrotoxicity
Delayed Graft Function	Surgical complications (Arterial and Urinary Stenosis)
Chronic suboptimal immunosuppression	Recurrent or De Novo Nephropathy
Non-compliance of patients	

Abbreviations: DSA, Donor-Specific Antibodies; CNI, Calcineurin inhibitors.

dysfunction being greater. Both types of kidneys are particularly sensitive to other sources of injury, and particularly to the nephrotoxic effects of calcineurin inhibitors (CNI).

The benefits afforded by CNI in terms of short-term survival, makes their detrimental effects on long-term survival difficult to study. Nankivell et al performed repeated biopsy protocols over 10 years with 120 kidney-pancreas transplant patients receiving cyclosporine or tacrolimus. They showed that histological lesions associated with CNI nephrotoxicity (arteriolar hyalinosis with vascular narrowing, stripped-band fibrosis) increase with time after transplantation and become universal by ten years; these lesions constitute the chief cause of late injury and decline in renal function (Nankivell et al 2003).

Because of the long-term nephrotoxicity of CNI, their metabolic side-effects and the resulting risk of CAN and death of patients, recent immunosuppressive protocols tend to minimize or even avoid the use of CNI. It is now clear that complete withdrawal of CNI from the gold standard triple therapy (with steroids and MMF) results in a significant increase in the rate of late acute rejection. Nevertheless, it seems reasonable, with MMF as anti-proliferative drug, to use lower doses of CNI than previously with azathioprine (Ekberg et al 2007). However, how much long-term toxicity can be reduced by this approach remains to be determined (Kaplan and Budde 2007). An attractive alternative to CNI is costimulation blockade and the possibility for precise T-cell targeting, which could protect patients from many of the side-effects common with classical immunosuppressants.

Immunological basis of the co-stimulation blockade

T-cell activation by three signals

During the alloimmune response, both naïve and memory alloreactive T-cells are engaged by dendritic cells (DCs) of both donor and recipient origin (von Andrian and Mackay 2000). T lymphocyte activation requires three signals (Figure 1). The first involves T-cell receptor triggering by donor antigen on the surface of DCs or other APCs (Bromley et al 2001). The second signal or "costimulation signal" is not antigen-specific. Many pairs of molecules on the surface of T-lymphocytes and APCs can contribute to the costimulation signal (Table 3). However, the B7/CD28 and CD40/CD40L pathways are probably the most important and best characterized in T-cell activation. The costimulation signal is delivered when B7-1/CD80 and B7-2/CD86 on the surface of DCs engage CD28 on T-cells (Bromley et al 2001). These two signals activate three transduction pathways (Halloran 2004) that result in the production of numerous factors, including interleukin-2 (IL-2) and the α-chain of its receptor, CD25, and the CD40 Ligand. CD40 is expressed on all APCs (including B cells) and its ligand (CD40L or CD154) is on activated $CD4^+$ T-cells and on a subset of $CD8^+$ T-cells and 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 amplifies antigen presentation and the first antigen-specific signal (van Kooten and Banchereau 1997).

IL-2 binding to its receptor activates the mTOR ("target of rapamycin") pathway – the third signal – resulting in T-cell clonal proliferation and in the generation of effector T-cells (Halloran 2004).

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, and CTLA-4 inhibits

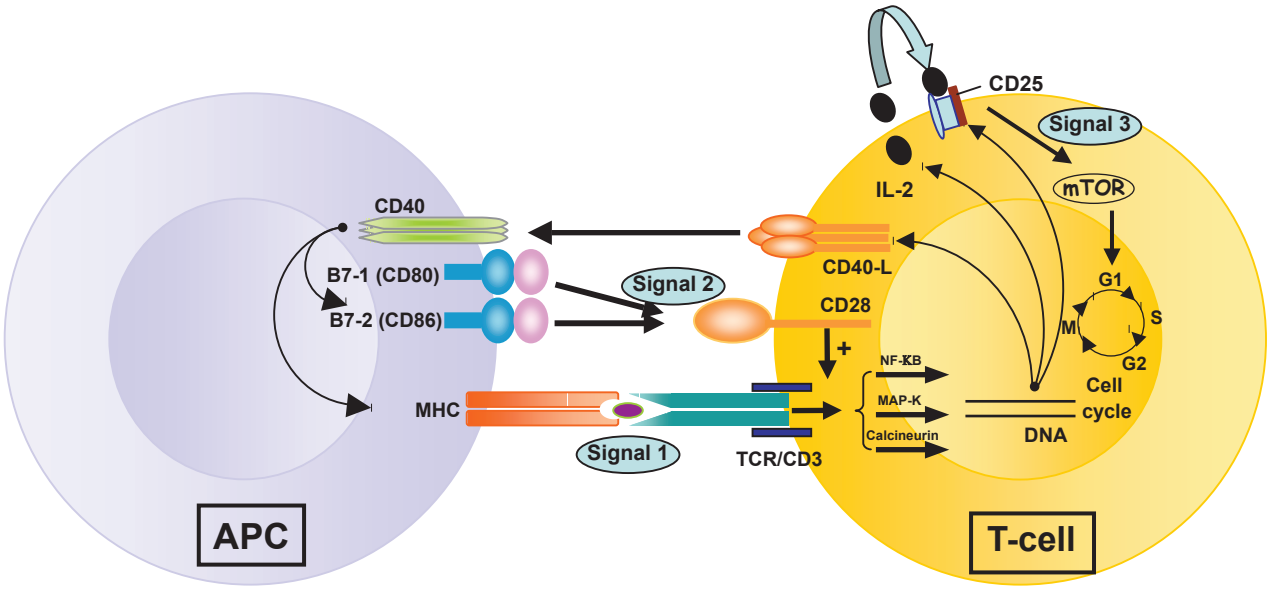

Figure 1 T-cell activation by three signals. The first involves T-cell receptor (TCR) triggering by donor antigen on APCs (antigen presenting cells). The second signal or "costimulation signal" is delivered when B7-1/CD80 and B7-2/CD86 on the surface of APCs engage CD28 on T-cells. These two signals activate three transduction pathways (the Nuclear Factor-κB or Nf-κB pathway, the mitogen-activated protein (MAP) kinase pathway and the calcium-calcineurin pathway) that result in the production of numerous factors, including interleukin-2 (IL-2), the α–chain of its receptor, CD25, and the CD40 Ligand. CD40 is expressed on all APCs (including B cells) and its ligand (CD40L or CD154) is on activated $CD4^+$ T-cells and on a subset of $CD8^+$ T-cells and NK cells. Stimulation of CD40 on APC by CD40L triggers important signals for antibody production by B cells and strongly induces B7 and Major Histocompatibility Complex (MHC) expression on APCs. IL-2 binding to its receptor activates the mTOR ("target of rapamycin") pathway – the third signal – resulting in T-cell clonal proliferation.

T-cell responses (Table 3) (Greenwald et al 2005). CD28 is constitutively expressed on T-cells, whereas CTLA-4 expression is rapidly upregulated following T-cell activation. CTLA-4 has a higher receptor affinity for both B7-1 and B7-2 than CD28.

Recently, a new regulatory mechanism was demonstrated for DCs, through B7/CTLA-4 reverse signaling (meaning from T-cell to DC) (Finger and Bluestone 2002; Grohmann et al 2002). CTLA-4, when binding to B7, may activate the immunosuppressive pathway of tryptophan catabolism in DCs (Grohmann et al 2002). This pathway consists of the production by DCs of Interferon-γ, which stimulates the indoleamine 2,3-dioxygenase (IDO) to degrade tryptophan. The resulting degradation products and tryptophan deprivation inhibit T-cells proliferation and promote their apoptosis. These two-way B7/CTLA-4 interactions may be involved in the inhibition of T-cell responses. Work with a model of murine islet allografts shows that the long-term survival induced by CTLA4-Ig – a fusion protein of CTLA-4 with an immunoglobulin – depends on effective tryptophan catabolism and that this effect disappears in the presence of 1-methyltryptophan, a pharmacological inhibitor of IDO (Grohmann et al 2002). However, Pree et al found divergent results regarding the role of IDO *in vivo* (Pree et al 2007). They tested costimulation blockade as a component of murine bone marrow transplantation protocol for the induction of mixed chimerism and donor-specific skin graft tolerance. They demonstrated that induction of high-level chimerism and permanent skin engraftment obtained with CTLA4-Ig occurred independently of IDO. Indeed, chimerism and graft survival were not affected by implantation of pellets releasing 1-methyltryptophan. The use in this study of the human construct of CTLA4-Ig abatacept, which may have different binding properties than the murine fusion protein, may explain this divergence.

B7/CTLA-4 interactions and regulatory T-cells

A subgroup of $CD4^+CD25^+$ T-lymphocytes, termed regulatory or suppressor T-lymphocytes (T-reg), are central to the establishment of transplantation tolerance in several experimental models (Graca et al 2002). Their clinical significance in human transplantation is currently being investigated. Muthukumar et al (2005) found that urinary levels of *FOXP3* mRNA, a functional factor specific to T-regs, were inversely correlated with serum creatinine at the time of acute rejection. Moreover, levels of FOXP3 mRNA were significantly higher in the group with successful reversal of acute rejection than in the group without reversal. The patients in higher third of *FOXP3* mRNA level had the lowest rate of graft failure

Table 3 Expression and effects of B7/CD28 and TNF/TNF-R family members, and their targeting in transplantation models

Expression	Molecule on APC	Molecule on T-cell	Expression	Signal	Experimental targeting	Reference
			B7/CD28 Superfamily			
APC (M, DC, B), T-cell	**B7-1 (CD80)**	**CD28**	Constitutive on T-cells	Activation	See Text	
APC (M, DC, B), T-cell	**B7-2 (CD86)**	**CTLA-4**	Induced on T-cells	Inhibition	See Text	
Induced on APC, fibroblasts, endothelial and epithelial cells	**B7-h**	**ICOS**	Induced on activated T-cells NK-cells	Activation	Anti-ICOS blocking mAb + CTLA4-Ig	Ozkaynac 2001 Subudhi
On resting APC, endothelial cells, T and B cells, parenchymal cells up-regulated on activation	**PD-L1**	**PD-1**	Induced on activated T-cells NK-cells, B-cells, monocytes	Inhibition	PD-L1-Ig fusion protein + CsA PD-L1-Ig fusion protein + anti-CD40L	Ozkaynac 2002 Gao
Induced on APC (M, DC)	**PD-L2**					
Induced on APC (M, DC) B-cells, T and NK-cells	**B7-H3**	**Unknown**	Induced on activated T-cells	Activation	Prolonged allograft survival in B7-H3 –/– mice + CsA or RPM	Wang
			TNF/TNF-R Superfamily			
APC (M, DC, B), fibroblasts, endothelial cells	**CD40**	**CD40-L**	Activated T-cells, NK-cells	B7 and MHC expression on APC Ab production	See Text	
APC (M, DC), T-cells	**CD70**	**CD27**	Constitutive on naïve T-cell Induced after antigen recognition	Activation	Anti-CD70 blocking mAb (Prevention of CD28-independent T-cell rejection)	Yamada
APC (M, DC, B), endothelial cells	**OX40-L**	**OX40**	Induced on T-cells	Activation	OX40-Ig Fusion protein Anti-OX40L	Curry Demirci
APC (M, DC, B)	**4-1BB-L**	**4-1BB**	Induced on T-cells	Activation	Anti-4-1-BB blocking mAb	Cho
T-cells, B-cells, DC, myeloid cells, inducible in somatic tissues	**HVEM**	**BTLA**	T-cells, B-cells, DC, myeloid cells	Inhibition	Acceleration of partially MHC mismatched allografts with Anti-BTLA mAb	Tao

Abbreviations: APC, antigen presenting cells; M, monocytes; DC, dendritic cells; CsA, cyclosporine A; RPM, rapamycin; MHC, major histocompatibility complex; mAb, monoclonal antibody.

following an acute rejection episode. In sum, this study suggests that *FOXP3*-expressing T-regs play a role in the limitation of alloimmune response and that the lack of suppression exerted by these cells during an episode of acute allograft rejection increases the risk of incomplete resolution and graft loss. (Muthukumar et al 2005). Moreover, this finding has potential for the identification of patients at risk of graft failure after an episode of rejection.

The lack of expression of B7-1 and B7-2 on the surface of effector cells results in lower susceptibility to suppression exerted by $CD4^+CD25^+$ T-cells. Indeed, direct contact between suppressor and effector cells is required in vitro, through the binding of CTLA-4 on T-reg and B7-1/B7-2 on effector cells. (von Boehmer 2005). *In vivo* suppression also requires B7 expression on effector cells. In a skin transplant model, blockade of the CTLA-4 pathway abolishes immunoregulation by $CD4^+CD25^+$ T-cells, suggesting that CTLA-4 is required for tolerance (Kingsley et al 2002).

Experimental models of "classical" costimulation blockade

The B7/CD28 pathway

One of the first tools used to target the B7/CD28 pathway was the CTLA4-Ig molecule, a fusion protein consisting of the extracellular domain of CTLA-4 and the Fc domain of IgG. CTLA4-Ig may act by directly disrupting B7/CD28 cross-talk and/or by activating the immunosuppressive pathway of tryptophan catabolism in DCs (see above) (Grohmann et al 2002). CTLA4-Ig efficiently prevents acute heart or kidney rejection in many mouse and rat models (Turka et al 1992; Glysing-Jensen et al 1997; Sayegh et al 1995, 1997). In some animal models, it inhibits the T-cell-dependent antibody response and prevents the progression of chronic rejection (Azuma et al 1996; Judge et al 1996). However, it induces long-lasting tolerance in only a few models of heart or kidney transplantation (Lenschow et al 1992; Sayegh et al 1995; Yamada et al 2001; Ito et al 2005), and not in more stringent models, such as mouse skin and islet transplantation (Trambley et al 1999; Rothstein et al 2001; Sho et al 2005). Transplantation tolerance is used to indicate that a single administration of an immunosuppressant at the time of transplantation is sufficient to ensure an indefinite donor-specific graft survival. The inability of CTLA4-Ig to generate such tolerance may be the consequence of various factors:

1. Costimulation blockade is less effective in memory T-cells and effector $CD8^+$ T-cells than in naïve T-cells (Onodera et al 1997; Trambley et al 1999; Valujskikh et al 2002).
2. CTLA4 plays a key role in downregulating T-cell responses, independently of CD28/B7 blockade. CTLA-4 signaling is essential for initial engraftment (Judge et al 1999; Sho et al 2002). Moreover, in the absence of CD28-mediated costimulation (CD28-deficient animals), interaction of CTLA-4 with B7 results in down-regulation of alloimmune response and prolongation of allograft survival. The blockade of CTLA-4/B7 interactions with CTLA-4-Ig or blocking anti-CTLA-4 antibodies abolishes the prolongation allograft survival observed in these animals (Yamada et al 2001).
3. Suppression of the alloimmune response requires a functional CTLA-4/B7 pathway between T-reg and effector cells (see above) (Kingsley et al 2002; Paust et al 2004).

The CD40/CD40L pathway

Similar to CD28/B7, the blockade of the CD40/CD40L pathway with an anti-CD40L mAb rarely results in durable tolerance when used alone (Parker et al 1995; Hancock et al 1996; Larsen et al 1996, 2005). The effects of anti-CD40L mAb are reversible, so prolonged administration is often required (Kirk et al 1999). However, simultaneous CD40/CD40L and B7/CD28 blockade may promote indefinite graft survival, even in stringent models such as mouse skin transplantation and xenotransplantation (Larsen et al 1996; Elwood et al 1998; Li et al 1999).

Costimulation blockade in non-human primates

Non-human primate (NHP) models must be used to make the jump from small animal models to clinical protocols. In adolescent rhesus monkeys, kidney graft survival increases from 8 days to 30 days when CTLA4-Ig is used alone and to up to six months if CTLA4-Ig plus humanized anti-CD40L antibody (hu-anti-CD40L) are used (Kirk et al 1997). Survival was similar with hu-anti-CD40L antibody alone, suggesting that CD40/CD40L pathway blockade is critical (Elster et al 2001). Treatment did not prevent chronic rejection as routine biopsies on day 28 showed, in all animals, donor-specific antibodies and focal perivascular infiltrate (Kirk et al 1999).

Delivery of the first signal without costimulation signal drives T-cells to a state of anergy (Schwartz 1990; Lombardi et al 1994). New protocols, based upon the suppressor properties of these anergic T-cells, can improve the results of costimulation blockade in NHPs. In human bone marrow transplantation, the treatment of donor bone marrow ex vivo with CTLA4-Ig and its coculture with irradiated

recipient-cells to induce donor-specific anergic T-cells before its re-injection to the recipient resulted in a low risk of graft-versus-host-disease (Guinan et al 1999). Similarly, in NHP models, when recipient splenic T-cells are cocultured with irradiated donor splenocytes in the presence of both anti-human CD80 and CD86 mAbs and re-injected into recipient, graft survival was indefinite in 50% of cases (Bashuda et al 2005). The simplicity, safety and efficacy of this protocol make it suitable for application to human transplantation.

Development of LEA29Y (belatacept) and first clinical trial in renal transplantation

It can be difficult to transpose experimental findings in NHPs to human clinical trials, because primates lack "heterologous immunity". Indeed, the animals used are raised in captivity and have limited exposure to non-self antigens; consequently they may not have an extensive repertoire of effector/memory cells (Elster et al 2004).

Preliminary clinical results with CD40 pathway targeting

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 unanticipated thromboembolic events (Kawai et al 2000). This complication reflects the importance of costimulatory molecule expression by non-lymphoid cells. Indeed, CD40L has been detected on the surface of activated platelets and has been shown to activate endothelium. Moreover, the absence of CD40L affects the stability of arterial thrombi and delays arterial occlusion *in vivo*, in a CD40-independent manner (Andre et al 2002). This antibody was not able to prevent acute rejection in 5 of 7 seven kidney transplant patients in a phase I trial (Vincenti 2002). Thus, targeting CD40 rather than its ligand was considered as an alternative, so as to avoid these thromboembolic complications. A chimeric anti-human CD40 antibody, Chi220, has recently been developed to circumvent these adverse events. It has proved particularly effective in combination with CTLA4-Ig for islet transplantation in NHPs, with the property of transiently depleting B cells (Adams et al 2005).

Development of LEA29Y

Similar to the CD40/CD40L pathway, the two components of the B7/CD28 pair have been targeted with biological immunosuppressive agents. h1F1 and h3D1 are two humanized antibodies directed against CD80 and CD86, respectively, which have been shown to inhibit CD28-dependent T-cell proliferation in vitro. In vivo, provided that both mAbs are used together, they prolong kidney allograft survival in NHPs (Hausen et al 2001). At phase I clinical trial stage, in combination with steroids, cyclosporine and mycophenolate mofetil, they proved to be safe and effective. However, they were subsequently withdrawn from further development, despite the good safety profile, for financial reasons (Vincenti 2002).

CTLA4-Ig (abatacept) is a fusion protein consisting of the extracellular domain of CTLA-4 and the Fc domain of IgG. The affinity of CTLA4-Ig for CD80 is 200 times higher than that for CD86 and it is 100 times more potent for the blockade of CD80-dependent costimulation than for that of CD86-dependent costimulation (Linsley et al 1994). Insufficient blockade of CD28/B7 interaction may partly account for the limited results obtained in NHP models. However, treatment with CTLA4-Ig is associated with a significant clinical improvement of T-cell-mediated autoimmune conditions, including psoriasis and rheumatoid arthritis (Abrams et al 1999; Kremer et al 2003).

A mutagenesis and screening strategy using various CTLA4-Ig variants has been used to identify high-avidity mutants with slower dissociation rates (Larsen et al 2005). Two amino-acid substitutions in the binding domain (L104E and A29Y) were identified as potentially useful, leading to the development of a new molecule, LEA29Y or belatacept. Its binding avidity to CD80 and CD86 was twofold and fourfold higher, respectively, and inhibition of T-cell activation was tenfold more powerful than those of CTLA4-Ig. NHPs studies showed a prolongation of kidney allograft survival and an inhibition of anti-donor humoral response when belatacept was used alone or in combination with steroids and MMF (Larsen et al 2005).

First clinical trial with belatacept in renal transplantation

A large-scale 12-month study has been conducted to assess the efficacy of a strategy based upon costimulation blockade with belatacept in renal transplantation (Vincenti et al 2005). This phase 2 multicenter study included 218 adult kidney recipients at low immunological risk, randomly assigned to receive an intensive regimen of belatacept, a less-intensive regimen, or cyclosporine A (CsA). Belatacept was administered as peripheral intravenous injections each lasting

30 minutes. Both belatacept regimens included an early phase of 10 mg/kg body weight injections every two weeks, and a late phase of 5 mg/kg injections (at four-week or eight-week intervals). For the intensive regimen, the early phase involved 11 injections over 6 months and the less intense regimen involved 5 injections over 3 months. Patients of all three groups also received basiliximab induction therapy, mycophenolate mofetil and steroids.

The primary non-inferiority objective was reached, with the following incidences of acute rejection at six months: 6% (less-intensive belatacept), 7% (intensive belatacept) and 8% (CsA). Subclinical rejection at month-12 routine biopsy was more common with less-intensive belatacept (20 %) than with intensive belatacept (9 %) or cyclosporine (11%). The glomerular filtration rate measured with iohexol clearance at 12 months was significantly higher in patients receiving belatacept than in those treated with CsA (66.3 and 62.1 versus 53.5 ml/min/1.73 m^2). By month 12, the incidence of chronic allograft nephropathy as assessed from protocol biopsy was lower in patients receiving belatacept: 29% (less intensive) and 20% (intensive) versus 44% in the cyclosporine arm. Four patients in the cyclosporine arm died (two from cardiovascular disease) and one in the less intensive belatacept arm (from infection).

The side effects were also noted for the three groups. The frequency of infections was similar (around 75%) in all three groups. 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 further patients treated with the intensive regimen 2 and 13 months after the replacement of belatacept with tacrolimus at the end of study. 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 one year of treatment and no additional case of PTLD occurred after up to four years of follow-up. For metabolic cardiovascular and markers, total, LDL and HDL cholesterol levels were similar in the three groups but significantly more patients in the cyclosporine arm were receiving lipid-lowering drugs. Similarly, the percentage of patients treated for hypertension was higher under cyclosporine than under belatacept (92% versus 88 and 83%). The incidence of post-transplantation diabetes mellitus was 8% under cyclosporine and 1% in each belatacept group. The rate of discontinuation was similar in the three groups (22%, 25% and 27%).

In summary, belatacept belongs to a new class of immunosuppressants and is the first maintenance drug to have an immune specificity. Contrary to many biological agents used for induction or treatment of acute rejection (murumonab, thymoglobulin), belatacept does not appear to act by depleting T-cells or to affect number and function of regulatory T-cells (Moreland et al 2002; Hirose et al 2004; Chavez et al 2007). Although its long-term safety remains to be confirmed, its administration is well tolerated and seems to protect patients against adverse renal, cardiovascular and metabolic effects encountered with calcineurin inhibitors, whilst providing equally effective immunosuppression. Furthermore, this totally calcineurin inhibitor-free regimen is the first to be associated with a very low risk of acute rejection. The more disappointing aspect is the need to combine belatacept with standard immunosuppressants (basiliximab, steroids and mycophenolate mofetil). This may explain the similar rates of infection in the three groups and possibly the unanticipated incidence of PTLD in the belatacept arm. Indeed, the absence of infectious and neoplasic complications observed in rheumatoid arthritis with abatacept and belatacept (in studies against placebo) suggests that the side effects reported in renal transplant patients are due to drugs associated with belatacept (Moreland et al 2002).

Targeting 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 (London et al 2000; Valujskikh et al 2002) and $CD8^+$ effector T-cells (Ensminger et al 2000), 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-Receptor (TNF-R) superfamilies deliver positive or negative costimulatory signals, early or late after encountering antigen (Table 3). These signals are not limited to T-cell/APC interactions, but also involve interplay between T-cells and other T-cells, B-cells or parenchymal cells. Several of these molecules have been targeted in animal models of transplantation.

In the B7/CD28 superfamily, the Induced Costimulatory Molecule (ICOS) and Programmed Death-1 (PD-1) share homology with CD28. However, unlike CD28 these two molecules are not expressed on naïve T-cells but are induced on T-cells after activation. Thus, ICOS signaling is required for the activation and function of effector and memory T-cells, whereas CD28 primes naïve T-cells

(Coyle et al 2000). PD-1 expression is not restricted to T-cells like other members of the CD28 family (Greenwald et al 2005). Its two ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC) are also constitutively expressed on a large panel of non-lymphoid organs, suggesting a peripheral regulation of self-reactive T- or B-cells. PD-1 ligation transmits a potent inhibitory signal in the early stages of T-cell activation. B7-H3 is a B7 homolog inducible in APCs, which binds a putative receptor on T-cells, resulting in increased T-cell proliferation.

Simplistically, TNF-R-family members are expressed by T-cells and their ligands are expressed by APCs (Table 3) (Croft 2003). TNF-R/TNF interactions are critical for the clonal expansion/effector phases of immune responses, and involve DC/T-cell and B/T-cell interactions. TNF-Rs may be either constitutively expressed by naïve T-cells, as is the case for CD27, or induced after antigen recognition, as are OX40, 4-1BB, and CD27. Their ligands OX40L, 4-1BBL, and CD70 are not constitutively expressed by APCs but are induced simultaneously with their receptors on T-cells, one to several days after activation (Croft 2003). Despite variability in expression, binding to TNF-Rs increases cytokine secretion and the proliferation of T-cells receiving the TCR signal (Croft 2003).

The pair of costimulatory molecules B- and T-lympocyte attenuator (BTLA)/herpervirus-entry mediator (HVEM) is unique in the fact that their interaction bridges the two superfamilies of receptors, CD28 for BTLA and TNF-R for HVEM. This crosstalk is unusual because BTLA is known to inhibit T-cell responses whereas HVEM is an activator receptor (Murphy et al 2006). Their widespread expression (Table 3) suggests a regulatory role for many cell types. Few and contradictory data are available about the role of BTLA in allogeneic response. Tao et al have shown that targeting of BTLA with a blocking antibody prompted rapid rejection of partially MHC-mismatched murine heart allografts whereas it prolonged survival of fully MHC-mismatched allografts (Tao et al 2005). The role of BTLA and its ligand has to be precised by further experimental models.

The use of single agents blocking costimulation has proved to be insufficient in stringent models of transplantation. In particular, the results obtained with LEA29Y in NHP models of kidney transplantation led investigators to combine it with conventional immunosuppressive reagents for clinical applications so as to minimize the risk of acute rejection (Larsen et al 2005; Vincenti et al 2005). Given the diversity, redundancy and complementary nature of all the various costimulation molecules described above, strategies for human transplantation in the future may involve the simultaneous blockade of several selected pathways or the concomitant use of currently approved conventional immunosuppressants.

Future directions for costimulation blockade

In conclusion, a short course of a costimulation-blocking agent to achieve drug-free antigen-specific tolerance in transplantation patients does not appear to be a realistic goal in the immediate future. However, costimulation blockade with belatacept has proved to be a powerful alternative to calcineurin inhibitors, and the non-immune side effects and consequences on graft and patient survival can therefore be avoided. This molecule is currently being evaluated in phase III trials versus cyclosporine in recipients of extended-criteria donor kidneys.

Nevertheless, there are issues that require careful consideration at this stage of development:

1. The short half-life of classical oral immunosuppressive drugs implies a relative reversibility of their effects, and this is a major point in cases of malignant or infectious complications. Future trials need to determine whether the long half-life of biological agents precludes their safety. In the belatacept trial, the three PTLD affecting patients receiving belatacept were not expected, and indicate that this new agent does not provide specific immunosuppression and may block immune surveillance of tumors. These patients should be carefully followed-up because the long-term safety of belatacept is not known.
2. More practically, although some patients are happy to take fewer tablets each day, the intravenous route of administration makes the ambulatory follow-up of transplant patients more complicated. The development of subcutaneous delivery systems is an essential step if the use of these agents is to become more generalized. In our experience, patients found intravenous administration of belatacept every one or two months acceptable, because they have to come to the hospital for medical visits anyway, and this meant that they had fewer tablets to take. Indeed, the list of drugs that a transplant patient has to take to treat the side-effects of immunosuppressive molecules is long and may result in non-adherence with treatment; this is an established risk factor for late allograft failure. (Humar et al 1999)
3. The best use of costimulation blocking agents in the long term needs to be determined, and it is not known such long-term use has an effect, positive or negative, on the process of accommodation, in other words whether it is possible to give lower doses over time as is the case for drugs currently in use.

4. Monitoring tools adapted to these costimulation blocking agents are required, as with other immunosuppressants. For example, monitoring of belatacept concentrations in blood is possible and studies are necessary to find out whether there is a correlation with the incidence of acute rejection.

It is clear that single therapy cannot currently be used, probably because of the redundancy of the various costimulation pathways. Consequently, costimulation blocking agents are actually envisioned as components of combination therapy. The need to use additional drugs may be responsible for a rate of infectious and neoplasic complications that is similar to that observed in classical protocols. Consequently, combined targeting of several costimulation pathways of both B7/CD28 and TNF/TNF-R families could result in a reinforced immunosuppression whilst retaining a degree of immune specificity. Experimental transplantation models using agonists or blocking reagents have already been used to investigate most of these pathways, and several 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.

References

Abrams JR, Lebwohl MG, Guzzo CA, et al. 1999. CTLA4Ig-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris. *J Clin Invest*, 103:1243–52.

Adams AB, Shirasugi N, Jones TR, et al. 2005. Development of a chimeric anti-CD40 monoclonal antibody that synergizes with LEA29Y to prolong islet allograft survival. *J Immunol*, 174:542–50.

Andre P, Prasad KS, Denis CV, et al. 2002. CD40L stabilizes arterial thrombi by a beta3 integrin–dependent mechanism. *Nat Med*, 8:247–52.

Azuma H, Chandraker A, Nadeau K, et al. 1996. Blockade of T-cell costimulation prevents development of experimental chronic renal allograft rejection. *Proc Natl Acad Sci USA*, 93:12439–44.

Bashuda H, Kimikawa M, Seino K, et al. 2005. Renal allograft rejection is prevented by adoptive transfer of anergic T-cells in nonhuman primates. *J Clin Invest*, 115:1896–902.

Bromley SK, Iaboni A, Davis SJ, et al. 2001. The immunological synapse and CD28-CD80 interactions. *Nat Immunol*, 2:1159–66.

Chavez H, Beaudreuil S, Abbed K, et al. 2007. Absence of CD4-CD25 regulatory T-cell expansion in renal transplanted patients treated in vivo with belatacept mediated CD28-CD80/86 blockade. *Transpl Immunol*, 17:243–8.

Cohen DJ, St Martin L, Christensen LL, et al. 2006. Kidney and pancreas transplantation in the United States, 1995-2004. *Am J Transplant*, 6:1153–69.

Coyle AJ, Lehar S, Lloyd C, et al. 2000. The CD28-related molecule ICOS is required for effective T-cell-dependent immune responses. *Immunity*, 13:95–105.

Croft M. 2003. Co-stimulatory members of the TNFR family: keys to effective t-cell immunity? *Nat Rev Immunol*, 3:609–20.

Denton MD, Magee CC, Sayegh MH. 1999. Immunosuppressive strategies in transplantation. *Lancet*, 353:1083–91.

Ekberg H, Grinyo J, Nashan B, et al. 2007. Cyclosporine sparing with mycophenolate mofetil, daclizumab and corticosteroids in renal allograft recipients: the CAESAR Study. *Am J Transplant*, 7:560–70.

Elster EA, Xu H, Tadaki DK, et al. 2001. Treatment with the humanized CD154-specific monoclonal antibody, hu5C8, prevents acute rejection of primary skin allografts in nonhuman primates. *Transplantation*, 72:1473–8.

Elster EA, Hale DA, Mannon RB, et al. 2004. The road to tolerance: renal transplant tolerance induction in nonhuman primate studies and clinical trials. *Transpl Immunol*, 13:87–99.

Elwood ET, Larsen CP, Cho HR, et al. 1998. Prolonged acceptance of concordant and discordant xenografts with combined CD40 and CD28 pathway blockade. *Transplantation*, 65:1422–8.

Ensminger SM, Witzke O, Spriewald BM, et al. 2000. $CD8^+$ T-cells contribute to the development of transplant arteriosclerosis despite CD154 blockade. *Transplantation*, 69:2609–12.

Finger EB, Bluestone JA. 2002. When ligand becomes receptor–tolerance via B7 signaling on DCs. *Nat Immunol*, 3:1056–7.

Glysing-Jensen T, Raisanen-Sokolowski A, Sayegh MH, et al. 1997. Chronic blockade of CD28-B7-mediated T-cell costimulation by CTLA-4-Ig reduces intimal thickening in MHC class I and II incompatible mouse heart allografts. *Transplantation*, 64:1641–5.

Graca L, Thompson S, Lin CY, et al. 2002. Both CD4(+)CD25(+) and CD4(+)CD25(−) regulatory cells mediate dominant transplantation tolerance. *J Immunol*, 168:5558–65.

Greenwald RJ, Freeman GJ, Sharpe AH. 2005. The B7 family revisited. *Annu Rev Immunol*, 23:515–48.

Grohmann U, Orabona C, Fallarino F, et al. 2002. CTLA-4-Ig regulates tryptophan catabolism in vivo. *Nat Immunol*, 3:1097–101.

Guinan EC, Boussiotis VA, Neuberg D, et al. 1999. Transplantation of anergic histoincompatible bone marrow allografts. *N Engl J Med*, 340:1704–14.

Halloran PF. 2004. Immunosuppressive drugs for kidney transplantation. *N Engl J Med*, 351:2715–29.

Hancock WW, Sayegh MH, Zheng XG, et al. 1996. Costimulatory function and expression of CD40 ligand, CD80, and CD86 in vascularized murine cardiac allograft rejection. *Proc Natl Acad Sci USA*, 93:13967–72.

Hariharan S, Johnson CP, Bresnahan BA, et al. 2000. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med*, 342:605–12.

Hausen B, Klupp J, Christians U, et al. 2001. 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*, 72:1128–37.

Hirose K, Posselt AM, Stock PG, et al. 2004. Treatment of kidney transplant patients with the novel co-stimulatory blocker LEA29Y and anti-IL2 receptor does not impede the development of regulatory T-cells. *Am J Transplant*, 4(Suppl 8):442.

Humar A, Kerr S, Gillingham KJ, et al. 1999. Features of acute rejection that increase risk for chronic rejection. *Transplantation*, 68:1200–3.

Ito T, Ueno T, Clarkson MR, et al. 2005. Analysis of the role of negative T- cell costimulatory pathways in CD4 and CD8 T-cell-mediated alloimmune responses in vivo. *J Immunol*, 174:6648–56.

Judge TA, Tang A, Turka LA. 1996. Immunosuppression through blockade of CD28:B7-mediated costimulatory signals. *Immunol Res*, 15:38–49.

Judge TA, Wu Z, Zheng X-G, et al. 1999. The Role of CD80, CD86, and CTLA-4 in alloimmune responses and the induction of long-term allograft survival. *J Immunol*, 162:1947–51.

Kaplan B, Budde K. 2007. Lessons from the CAESAR Study: calcineurin inhibitors–can't live with them and can't live without them. *Am J Transplant*, 7:495–6.

Kauffman HM, Cherikh WS, Cheng Y, et al. 2005. Maintenance immunosuppression with target-of-rapamycin inhibitors is associated with a reduced incidence of de novo malignancies. *Transplantation*, 80:883–9.

Kawai T, Andrews D, Colvin RB, et al. 2000. Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. *Nat Med*, 6:114.

Kingsley CI, Karim M, Bushell AR, et al. 2002. $CD25^+CD4^+$ regulatory T-cells prevent graft rejection: CTLA-4- and IL-10-dependent immunoregulation of alloresponses. *J Immunol*, 168:1080–6.

Kirk AD, Burkly LC, Batty DS, et al. 1999. Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. *Nat Med*, 5:686–93.

Kirk AD, Harlan DM, Armstrong NN, et al. 1997. CTLA-4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc Natl Acad Sci USA*, 94:8789–94.

Kremer JM, Westhovens R, Leon M, et al. 2003. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA-4-Ig. *N Engl J Med*, 349:1907–15.

Larsen CP, Elwood ET, Alexander DZ, et al. 1996. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature*, 381:434–8.

Larsen CP, Pearson TC, Adams AB, et al. 2005. Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA-4-Ig with potent immunosuppressive properties. *Am J Transplant*, 5:443–53.

Lee PC, Terasaki PI, Takemoto SK, et al. 2002. All chronic rejection failures of kidney transplants were preceded by the development of HLA antibodies. *Transplantation*, 74:1192–4.

Lenschow DJ, Zeng Y, Thistlethwaite JR, et al. 1992. Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA-4-lg. *Science*, 257:789–92.

Li Y, Li XC, Zheng XX, et al. 1999. Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T-cells and induction of peripheral allograft tolerance. *Nat Med*, 5:1298–302.

Linsley PS, Greene JL, Brady W, et al. 1994. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. *Immunity*, 1:793–801.

Lombardi G, Sidhu S, Batchelor R, et al. 1994. Anergic T-cells as suppressor cells in vitro. *Science*, 264:1587–9.

London CA, Lodge MP, Abbas AK. 2000. Functional responses and costimulator dependence of memory $CD4^+$ T-cells. *J Immunol*, 164:265–72.

Meier-Kriesche HU, Schold JD, Kaplan B. 2004. Long-term renal allograft survival: have we made significant progress or is it time to rethink our analytic and therapeutic strategies? *Am J Transplant*, 4:1289–95.

Moreland LW, Alten R, Van den, Bosch F, et al. 2002. Costimulatory blockade in patients with rheumatoid arthritis: a pilot, dose-finding, double-blind, placebo-controlled clinical trial evaluating CTLA-4-Ig and LEA29Y eighty-five days after the first infusion. *Arthritis Rheum*, 46:1470–9.

Murphy KM, Nelson CA, Sedy JR. 2006. Balancing co-stimulation and inhibition with BTLA and HVEM. *Nat Rev Immunol*, 6:671–81.

Muthukumar T, Dadhania D, Ding R, et al. 2005. Messenger RNA for FOXP3 in the urine of renal-allograft recipients. *N Engl J Med*, 353:2342–51.

Nankivell BJ, Borrows RJ, Fung CL, et al. 2003. The natural history of chronic allograft nephropathy. *N Engl J Med*, 349:2326–33.

Onodera K, Chandraker A, Schaub M, et al. 1997. CD28-B7 T-cell costimulatory blockade by CTLA-4-Ig in sensitized rat recipients: induction of transplantation tolerance in association with depressed cell-mediated and humoral immune responses. *J Immunol*, 159:1711–17.

Parker DC, Greiner DL, Phillips NE, et al. 1995. Survival of mouse pancreatic islet allografts in recipients treated with allogeneic small lymphocytes and antibody to CD40 ligand. *Proc Natl Acad Sci USA*, 92:9560–4.

Pascual M, Theruvath T, Kawai T, et al. 2002. Strategies to improve long-term outcomes after renal transplantation. *N Engl J Med*, 346:580–90.

Paust S, Lu L, McCarty N, et al. 2004. Engagement of B7 on effector T-cells by regulatory T-cells prevents autoimmune disease. *Proc Natl Acad Sci USA*, 101:10398–403.

Pree I, Bigenzahn S, Fuchs D, et al. 2007. CTLA4Ig promotes the induction of hematopoietic chimerism and tolerance independently of Indoleamine-2,3-dioxygenase. Transplantation, 83:663–7.

Rosengard BR, Feng S, Alfrey EJ, et al. 2002. Report of the Crystal City meeting to maximize the use of organs recovered from the cadaver donor. *Am J Transplant*, 2:701–11.

Rothstein DM, Livak MF, Kishimoto K, et al. 2001. Targeting signal 1 through CD45RB synergizes with CD40 ligand blockade and promotes long term engraftment and tolerance in stringent transplant models. *J Immunol*, 166:322–9.

Sayegh MH, Akalin E, Hancock WW, et al. 1995. CD28-B7 blockade after alloantigenic challenge in vivo inhibits Th1 cytokines but spares Th2. *J Exp Med*, 181:1869–74.

Sayegh MH, Zheng XG, Magee C, et al. 1997. Donor antigen is necessary for the prevention of chronic rejection in CTLA4Ig-treated murine cardiac allograft recipients. *Transplantation*, 64:1646–50.

Schwartz RH. 1990. A cell culture model for T-lymphocyte clonal anergy. *Science*, 248:1349–56.

Sho M, Kishimoto K, Harada H, et al. 2005. Requirements for induction and maintenance of peripheral tolerance in stringent allograft models. *Proc Natl Acad Sci USA*, 102:13230–5.

Sho M, Yamada A, Najafian N, et al. 2002. Physiological mechanisms of regulating alloimmunity: cytokines CTLA-4, $CD25^+$ Cells, and the alloreactive T-cell clone size. *J Immunol*, 169:3744–51.

Snanoudj R, Beaudreuil S, Arzouk N, et al. 2005. Immunological strategies targeting B cells in organ grafting. *Transplantation*, 79:S33–6.

Tao R, Wang L, Han R, et al. 2005. Differential effects of B and T-lymphocyte attenuator and programmed death-1 on acceptance of partially versus fully MHC-mismatched cardiac allografts. *J Immunol*, 175:5774–82.

Terasaki PI, Ozawa M. 2004. Predicting kidney graft failure by HLA antibodies: a prospective trial. *Am J Transplant*, 4:438–43.

Trambley J, Bingaman AW, Lin A, et al. 1999. Asialo GM1(+) CD8(+) T-cells play a critical role in costimulation blockade-resistant allograft rejection. *J Clin Invest*, 104:1715–22.

Turka LA, Linsley PS, Lin H, et al. 1992. T-cell activation by the CD28 ligand B7 is required for cardiac allograft rejection in vivo. *Proc Natl Acad Sci USA*, 89:11102–5.

Valujskikh A, Pantenburg B, Heeger PS. 2002. Primed allospecific T-cells prevent the effects of costimulatory blockade on prolonged cardiac allograft survival in mice. *Am J Transplant*, 2:501–9.

van Kooten C, Banchereau J. 1997. Functions of CD40 on B cells, dendritic cells and other cells. *Curr Opin Immunol*, 9:330–7.

Vincenti F. 2002. What's in the pipeline? New immunosuppressive drugs in transplantation. *Am J Transplant*, 2:898–903.

Vincenti F, Larsen C, Durrbach A, et al. 2005. Costimulation blockade with belatacept in renal transplantation. *N Engl J Med*, 353:770–81.

von Andrian UH, Mackay CR. 2000. T-cell function and migration. Two sides of the same coin. *N Engl J Med*, 343:1020–34.

von Boehmer H. 2005. Mechanisms of suppression by suppressor T-cells. *Nat Immunol*, 6:338–44.

West M, Sutherland DE, Matas AJ. 1996. Kidney transplant recipients who die with functioning grafts: serum creatinine level and cause of death. *Transplantation*, 62:1029–30.

Yamada A, Kishimoto K, Dong VM, et al. 2001. CD28-independent costimulation of T-cells in alloimmune responses. *J Immunol*, 167:140–6.