American Journal of Transplantation 2003; 3: 794–803
Blackwell Munksgaard

ISSN 1600-6135

Minireview

Clinical Trials of Transplant Tolerance: Slow But Steady Progress

Jeffrey B. Matthews[a], Eleanor Ramos[a] and Jeffrey A. Bluestone[a,b,*]

[a]*Immune Tolerance Network, and* [b]*Diabetes Center, University of California, San Francisco, CA*
**Corresponding author: Jeffrey A. Bluestone, jbluest@diabetes.ucsf.edu*

The search for tolerance therapies that would thwart the alloimmune response following organ transplantation while preserving a patient's protective immune response has been a formidable goal for clinical immunologists. Over the past few decades, a more detailed understanding of the molecular events associated with T-cell recognition and activation has demonstrated the feasibility of various tolerance approaches, such as costimulation blockade, in numerous animal models of both autoimmunity and transplantation. Yet, only a few promising new therapies have reached the early stages of human clinical development. In contrast, the use of T-cell depleting induction therapy has become widespread, and new trials have been designed with immunosuppressive drug withdrawal in mind. Furthermore, nonmyeloablative mixed chimeric approaches have allowed complete immunosuppressive withdrawal in some limited cases. In the course of these investigations, however, what has become increasingly clear is that the distinctions between immunosuppression and tolerance have been blurred as the success and durability of the therapies rely as much on the state of the organ and organism as they do the mechanism of action of the drug. In this review, we provide a summary of the progress and lessons in promoting clinical transplant tolerance and an overview of promising agents.

Key words: Chimerism, clinical trials, costimulation, T cell depletion, tolerance

Received 23 October 2002, revised 21 February 2003 and accepted for publication 24 February 2003

Introduction

Since the seminal experiments of Medawar, Billingham and Brent in the 1950s, the induction of functional tolerance to allografts has been a major goal in immunology. Although a precise universal definition of 'immunological tolerance' is difficult for most to agree upon, tolerance can be regarded generally as a state of unresponsiveness to self or foreign antigens in the absence of ongoing therapy. Importantly, the tolerogenic state must exist in the context of general immune competence, including normal immune responses to pathogens and cancer risks no different than the general population whenever possible. The benchmark for the establishment of clinical tolerance is the ability to completely and successfully withdraw immunosuppressive drugs. In clinical transplantation, functional tolerance has been achieved both anecdotally and experimentally, as some allograft recipients have been completely withdrawn from chronic immunosuppression. In the last decade, important advances in understanding the mechanisms involved in the induction, maintenance, and loss of T-cell tolerance have translated into new strategies for tolerance induction *in vivo*. The goal therefore is to apply these newer drugs to develop durable and antigen-specific tolerogenic therapies that lead to permanent graft acceptance in the absence of lifelong therapy and deleterious side-effects.

To date, most successes have been limited to permissive mouse models, and it is only recently that clinical strategies aimed at intentionally inducing allograft tolerance in humans have emerged. The reasons for the slow translation of the animal successes to human application are multifold. In most clinical centers, current graft survival numbers are excellent, providing little impetus for evaluation of new methodologies. In addition, the relatively low numbers of patients receiving transplants, the envisioned short-term therapeutic regimens and the likely requirement for combination therapies are not conducive to programmatic development by pharmaceutical and biotechnology companies. Finally, there is a need for convincing surrogate markers or predictive assays of tolerance, which will only be obtained if existing and new clinical trials are accompanied by robust assays of clinical tolerance and its underlying mechanisms. Without such assays, the only measure of success is graft survival, an endpoint that is ethically difficult to justify, as it is hard to withdraw drugs from patients who are doing well on current immunosuppressive regimens unless there is a good reason to believe that drug withdrawal will be successful.

Another important issue in human testing is choice of patient populations and transplant indication. For instance, at first glance, it may seem that islet or kidney transplantation may be the first arenas for drug testing, as a failed

graft is not life-threatening. However, recent data support including liver transplantation as well, not only because the liver can tolerate an acute immunologic insult as a result of its regenerative capacity, but because an episode of acute rejection does not alter the long-term liver graft survival (1,2). These observations are in contrast to the problematic effects of acute rejection in renal or other solid organ transplants (3). It is also notable that liver transplantation has been the most successful setting for immunosuppressive drug withdrawal: liver transplant patients need less immunosuppression than recipients of other solid organ allografts such as hearts or kidneys (4) and early steroid withdrawal is easier to achieve. Many rejection episodes are self-limiting and do not need additional high-dose immunosuppression (5,6).

There is one final issue to consider when moving forward with tolerance studies in humans. Some studies have suggested that calcineurin inhibitors, such as cyclosporinee (CsA), may interfere with the ability of the immune system to attain a tolerant state. For instance, CsA may prevent the induction of tolerance because it inhibits activation-induced cell death (AICD) and thus interferes with clonal deletion (7). However, in certain models of tolerance, such as mixed chimerism, the presence of CsA does not interfere with development of tolerance (8), as clonal deletion occurs by other mechanisms, probably by passive cell death (9). Therefore, the precise immunosuppressive regimen needs to be carefully considered when considering the use of calcineurin inhibitors but they should not be dismissed out of hand.

Clinical trials of pro-tolerogenic therapies

There are four basic processes that promote tolerance: clonal deletion, clonal anergy, immune deviation, and suppression. Each of these mechanisms operate to varying degrees in the induction and maintenance of tolerance and their existence presents a wide range of potential targets for intervention. Indeed, multiple ligands, receptors, and signaling intermediates have been developed into a variety of therapies that have been evaluated in rodent and non-human primate models of transplantation (Figure 1). What has been learned is that development of a durable tolerogenic therapy will rely on exploiting more than one of these regulatory pathways, most likely combining a profound reduction in clonal expansion accompanied by active immune regulation. Clinical exploitation of these therapies will involve an iterative process balancing the duration of therapy, toxicity of the preparative regimen, and long-term role of the transplanted organ itself.

Chimerism and tolerance

Pre-clinical studies have suggested that one approach to attaining tolerance is the creation of a chimeric state in which large numbers of donor cells are maintained in the recipient (10,11). For instance, donor bone marrow may be introduced into a preconditioned host such that the host immune system (including both the T cells and antigen-presenting cells) is either partially or completely reconstituted with donor cells. The most clinically relevant approaches have been those that use nonmyeloablative host conditioning regimens, as the whole-body irradiation used in other regimens to allow donor bone marrow to become established carries excessive risks of toxicity. Cosimi, Sachs and colleagues have developed a protocol that includes cyclophosphamide, thymic irradiation, and antithymocyte globulin (ATG) with CsA. In a pilot study, two patients with multiple myeloma who received HLA-matched kidney transplants from related donors have been withdrawn from all immunosuppression (10). Other chimerization approaches have been attempted as well with some promising results. Strober and colleagues combined using CD34+ (stem cell)-enriched donor peripheral blood mononuclear cells with total lymphoid irradiation and rabbit antithymocyte globulin treatment on a background of CsA and prednisone in MHC-mismatched living donor renal transplantation. The regimen resulted in the development of mixed chimerism in three of four patients while none developed graft versus host disease (GVHD) (11). Most interestingly, two of four patients were completely weaned from immunosuppression, however, both eventually experienced acute rejection episodes that had to be reversed by high-dose corticosteroid therapy (12). These patients are currently back on immunosuppressive therapies. Ricordi et al. have recently reported preliminary results of six patients receiving islet transplants with donor CD34+-enriched stem cells with daclizumab induction and tacrolimus/sirolimus maintenance, which was gradually weaned 1 year post-transplant (13). Two patients had episodes of acute allograft rejection, while three suffered graft failure following weaning. Together these clinical experiences demonstrate that significant progress has been achieved in this arena. However, they also highlight the challenges that remain. As the trials move forward, special attention will be paid to determining the critical factors essential in promoting 'macrochimerization' as a means of inducing tolerance. However, much of the recent preclinical work has suggested that although donor macrochimerization was essential for tolerance, a prolonged state of macrochimerization may not be essential. Thymic exposure to antigen expressed by the donor cells can result in the development of regulatory T cells within the thymus that migrate to the periphery to modulate T-cell responses (14). In fact, extrathymic deletion of mature T cells has been observed in various situations, including following bone marrow transplantation (15). These data indicate that lasting allograft tolerance may depend on peripheral mechanisms of tolerance to control T-cell alloreactivity. Thus, one of the goals of the recently initiated Immune Tolerance Network (ITN) funded multicenter trial for kidney transplantation in patients with

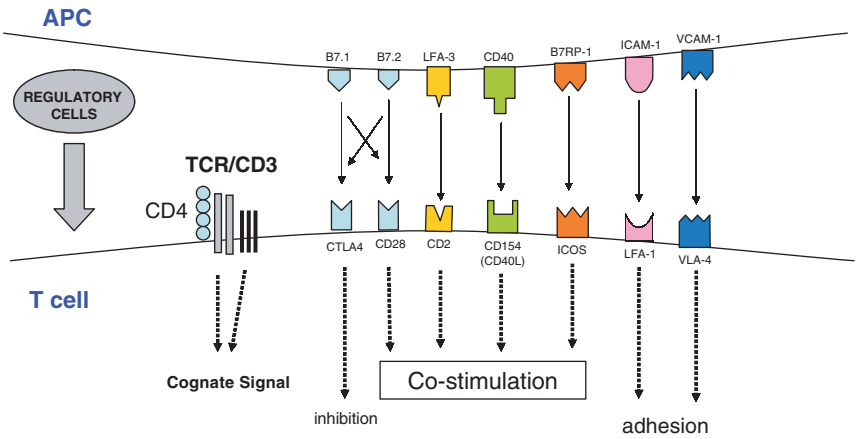

Figure 1: Potential pathways to peripheral tolerance.

multiple myeloma (M. Sykes, principal investigator) is to determine whether durable chimerization is essential for long-term tolerance or whether peripheral immunoregulatory mechanisms take over. The ITN is also supporting a clinical trial investigating the combination of cyclophosphamide, thymic irradiation, humanized anti-CD2 mAb (MEDI-507, Medimmune), CsA, and donor bone marrow infusion in patients undergoing HLA-matched living-related kidney transplants (D. Sachs, principal investigator). In both of these studies, a series of mechanistic studies will be performed to examine the degree of chimerization, the phenotypic and functional consequences of circulating donor antigen, and the relative role of cytokine production to direct vs. indirect antigen presentation.

Targeting cell-surface receptors

T lymphocytes require the engagement of both the TCR and a series of coreceptors, notably costimulatory signals, for complete activation. Blockade of these cell-surface molecules results in incomplete activation and T-cell anergy, presenting an attractive means of promoting tolerance. Indeed, the 'universal' nature of costimulatory pathways renders clinical strategies antigen-independent and it appears that, in general, such agents are relatively nontoxic (Figure 1).

CD28/B7 blockade

The best characterized of the costimulatory pathways involves the CD28 receptor, which binds CD80 and CD86 (B7.1 and B7.2) ligands expressed on antigen-presenting cells (APCs). Engagement of CD28 by CD80/86 costimulates T-cell proliferation, mainly through increasing IL-2 production, while blockade of this interaction inhibits T-cell responses. CTLA-4 (CD152), another CD28 family member, is not expressed on resting T cells but is induced by T-cell activation. As CTLA-4 binds the same CD80/86 molecules but with a 20–50-fold higher avidity, soluble forms of the molecule can compete with CD28 to block costimulatory signals. These observations led to the development of a potent CD28 antagonist, CTLA4Ig, created as a soluble receptor by fusing the extracellular domain of CTLA-4 with an immunoglobulin Fc. CTLA4Ig was shown in the early 1990s to induce tolerance in a number of murine allograft and xenograft settings (16), and early studies in psoriasis showed that the drug is well-tolerated and efficacious (17). This and other preclinical studies suggest that the efficacy of the therapy may depend on a combination of clonal deletion and active immune suppression. Unfortunately, the avidity of the drug, as compared with monoclonal antibodies, required that very large doses, up to 40 mg/kg were necessary to achieve significant efficacy, resulting in a potentially prohibitively high-cost drug. However, a mutant form of CTLA4Ig, LEA29Y, has been developed by Bristol-Myers Squibb (BMS-224818), which has proven to be a strong second generation agent. The single point mutation in the CTLA4Ig molecule leads to at least a 10-fold increase in affinity in LEA29Y, resulting in efficient CD28 blockade with much lower doses of drug. This drug prevents the priming of antidonor T- and B-cell responses and can prolong islet allograft survival indefinitely under cover of sirolimus/tacrolimus in nonhuman primates (18). A 12-site multicenter Phase II/III trial in renal transplantation is currently being conducted using a calcineurin-inhibitor-free regimen with encouraging early results. Future efforts with this important new therapy should include a battery of mechanistic studies to determine the immunologic basis of immune regulation with this therapy.

An alternative means of interfering with CD28 signaling is through direct blockade of CD80/86 molecules using monoclonal antibodies (19). Wyeth has developed two humanized mAbs, h1F1 and h3D1, directed against CD80 and CD86, respectively. The therapeutic use of these mAbs has yielded promising results in nonhuman primates (19,20). Preliminary studies in humans suggested that treatment of renal transplant recipients with a combination of the monoclonal antibodies in combination with

CsA, mycophenolate motefil (MMF), and corticosteroids is safe and effective (21). In a similar vein, IDEC Pharmaceuticals is developing a primate anti-CD80 antibody (IDEC-114) for the treatment of autoimmune diseases (psoriasis and rheumatoid arthritis). The demonstration of efficacy in these immune disease settings might provide an impetus for application in the transplant arena (22).

While results using CTLA4Ig and anti-CD80/86 mAbs are ongoing, it is worth noting that these soluble receptors and antibodies also block the interactions of CTLA-4 with its various ligands. This may result in confounding effects, as CTLA-4 is a negative regulator of T-cell responses. Thus, directly targeting the CD28 molecule represents an alternative means of blocking this pathway. Non-stimulating antibodies directed specifically to CD28 would not interfere with natural CTLA-4 engagement by CD80/86, thus permitting the negative regulatory signal of this receptor. A humanized anti-CD28 mAb is currently in development by Diabetogen and Abgenix for use in type 1 diabetes (23) and could potentially be used in the transplant setting.

ICOS/B7RP-1

Another CD28-like molecule, ICOS (inducible costimulator), has been described that binds B7RP-1 (B7 h), a third distantly related member of the B7 family. ICOS is up-regulated after T-cell activation and is effective in several preclinical transplant models wherein anti-ICOS antibody or an ICOSIg fusion protein can suppress intragraft T-cell activation and cytokine expression and prolong allograft survival in rodents either alone or in combination with calcineurin inhibitors (24–26). Millennium Pharmaceuticals currently has an anti-ICOS mAb in preclinical development (24). The ability of ICOS blockade to coexist with traditional immunosuppressants and to synergize with other costimulatory blockers may therefore make it an attractive clinical candidate. However, it should be pointed out that ICOS engagement is particularly effective in costimulating IL-10 and IL-4 secretion, two regulatory cytokines that may be useful in promoting long-term graft acceptance. Thus, like many of these new receptor antagonists, there will be a balance of their pro-tolerogenic and 'antitolerogenic' biologic activities.

CD40/CD40L

CD40 and its ligand CD154 (CD40L) have been shown to play a critical role in regulating both humoral and cell-mediated immunity. Compelling preclinical (nonhuman primate) data showing successful renal allotransplantation (27) prompted clinical testing of Biogen's humanized anti-CD154 mAb (hu5C8/Antova) in renal transplantation and several autoimmune indications. However, these trials were discontinued because of multiple thromboembolic events, and the failure to prevent rejection in five of seven patients receiving renal transplants. IDEC Pharmaceuticals is also developing an anti-CD154 mAb (IDEC 131) with a current focus on autoimmune diseases, but recent thromboembolic events have placed clinical trials on hold until thorough review of the clinical and additional preclinical data indicate that it is safe to proceed (28). While the precise mechanisms behind the thromboembolic events observed following anti-CD40L treatment are not yet known, it is nonetheless interesting to note that CD154 is expressed upon activated platelets, indicating a possible link with anti-CD154 toxicity (29). CD40 itself is expressed on APCs, B cells and to some degree on epithelial cells during inflammation. Non-stimulating antibodies directed at CD40, such as those currently in development for other indications by Tanox Pharmaceuticals, appear to have minimal binding to epithelium-expressed CD40 (30) and may provide an alternate means of blocking CD40/CD154 interactions without the risk of platelet activation.

CD11a/LFA-1

Efalizumab (Xanelim, MHM24) is a humanized mAb targeting the CD11a chain of LFA-1, preventing the LFA1–ICAM interaction. Blockade of LFA-1 has been shown to block T-cell activation, trafficking, and adhesion in rat models and, in combination with anti-ICAM-1, may induce tolerance (31), in part through a shift from Th1 to Th2 cytokine expression (32). In addition, it has recently been reported that combination therapy with CTLA4Ig and LFA-1 can prolong murine cardiac allograft survival indefinitely (33). In humans, efalizumab has been shown to be effective in psoriasis patients in Phase III clinical trials conducted by Xoma Pharmaceuticals (34) and has completed early development in renal transplantation (35). A renal transplant trial using efalizumab in combination with either half dose-CsA/sirolimus/prednisone or full dose-CsA/MMF/prednisone has demonstrated a low rejection rate with this therapy. Unfortunately, high doses of efalizumab may be associated with development of post-transplant lymphoproliferative disease, limiting its clinical development (36). Like all current potentially tolerogenic therapies, the ability of efalizumab to promote lasting, drug-free, graft acceptance remains to be proven but the preclinical data are encouraging (37). A randomized Phase III trial of Sangstat's anti-LFA-1 mAb odulimomab (Antilfa) recently showed little efficacy in the prevention of delayed graft function in high-risk kidney transplant patients (38).

VLA-4/VCAM-1

Very late antigen 4 (VLA-4) and its ligands (VCAM-1, MadCAM-1, and ICAM-4) have an important role in recruiting leukocytes to sites of inflammation, stabilizing the interaction between T cells and APCs, and providing costimulatory signals to T cells. Up-regulation of VCAM-1 expression is observed in renal allografts with acute cellular rejection, and correlates with areas of leukocyte infiltration and vascular inflammation. Animal studies have demonstrated that combined blockade of VLA-4 and LFA-1 can attenuate cardiac (39) and corneal (40) transplant rejection in mice and significantly prolong rat islet allografts with a short treatment course (41). Among the many

inhibitors of VLA-4 currently being evaluated as therapeutic agents is Natalizumab (Antegren), a humanized mAb that is under development by Elan Corp. and Biogen. Natalizumab has completed a Phase II trial multiple sclerosis (42) and a pilot study in Crohn's disease (43), where it was well tolerated and showed significant clinical responses. Phase III trials in MS and Crohn's disease are now proceeding (44).

In summary, preliminary findings using costimulatory and other cell-surface receptor antagonists are encouraging. However, the results to date illustrate the profound difficulties in translating animal model success to the clinical arena. The reasons for these difficulties remain unclear but likely relate to the redundancy of the immune system in compensating for the blockade of one specific pathway, the challenges posed by a more dynamic immune system in the outbred, environmentally exposed human being and delicate balance of pro-tolerogenic and antitolerogenic biologic activities of these drugs. In fact, these complexities have led to the obvious speculation that tolerance may only be achieved through combining targets that maximize complementary pathways of immune activation. Not unlike cancer therapies, a multidimensional approach may be essential to tolerize the different arms of the immune system including CD4 and CD8 T cells, and memory and naïve T cells, etc. In this regard, it is interesting to note that a number of studies have demonstrated a synergistic effect of combining CD28 and CD154 blockade, either alone or in combination with donor cell infusions (45). Similarly, success in some mouse models of mixed chimerism is achieved by using a combination of bone marrow and CTLA4Ig and anti-CD154 (46) or anti-CD154 alone (47). Importantly, in this setting the need for cytoreductive radiation therapy is dramatically reduced. Thus, the challenge will be to develop partnerships between pharmaceutical companies and the academic community to develop robust combination therapies when the individual pro-tolerogenic drugs do not provide sufficient efficacy for independent licensure.

T-cell receptor targeting

CD2

CD2 was one of the first molecules known to enhance the TCR recognition signal, and antibodies specific for CD2 have been found to inhibit the T-cell response to antigen. Anti-CD2 antibody therapy can delay allograft rejection to various extents in rodents and can induce a tolerant state when combined with anti-CD3 (48) or CTLA4Ig (49). In humans, LO-CD2a (BTI-322), a rat IgG2b antihuman CD2, which likely works via ADCC (antibody-dependent cellular cytotoxicity), has been shown to deplete activated T cells and inhibit activation, as well as significantly decrease the incidence of first rejection episodes when used in combination with CsA (50). More recently, a Phase II study of MEDI-507 (humanized BTI-322) for the treatment of steroid-refractory acute graft-vs.-host disease (GVHD) in allogeneic marrow or blood stem cell transplant recipients was proven effective with little toxicity (51). This agent will be tested in an ITN supported trial exploring mixed chimerism in renal transplant recipients (see Chimerism and Tolerance section earlier). Recently, another rat anti-CD2 mAb able to strongly inhibit both mitogenic and allogeneic responses has been found to prolong renal allograft survival in a nonhuman primate study (52). Clinical trials with anti-CD2 mAbs may prove to be tolerogenic when combined with other immunotherapies such as anti-CD3 strategies, as described in preclinical models (48).

Alefacept (Amevive, Biogen), a soluble LFA-3-Ig fusion protein that binds to CD2 and prevents its interaction with CD58 expressed on APCs, has completed the Phase III clinical evaluation for psoriasis. In Phase II trials Alefacept treatment was well tolerated and resulted in significant clinical responses (53). Alefacept has the ability to bind both CD2 and Fc receptors, thereby preventing T-cell activation and proliferation and promoting selective T-cell apoptosis. LFA-3Ig fusion proteins have shown efficacy in prolonging allograft survival in both rodent and nonhuman primate models (54,55), and thus there may be an incentive for examining this therapy in the transplant arena.

Anti-CD4 mAbs

Short courses of anti-CD4 antibodies have been shown to induce tolerance to allogeneic heart, islet, skin and bone marrow models, in some cases over major MHC mismatch barriers (56). Several murine anti-CD4 mAbs have entered the clinic for the treatment of autoimmune diseases or transplant rejection. In the NIAID Collaborative Trials in Kidney Transplantation, for example, the murine anti-CD4 monoclonal antibody OKT4A in combination with a standard regimen of cyclosporinee, azathioprine, and prednisone was associated with a 26% rejection rate and human antimurine antibody was observed. In studies of rheumatoid arthritis, the murine-human chimeric mAb (cM-T412) caused severe and prolonged depletion of CD4 cells, even following a single dose (57). Thus, limited effectiveness, as well as concerns of toxicity observed as a consequence has limited the clinical utility of anti-CD4 mAbs.

Recent observations, however, have provided evidence that T-cell depletion is not necessary for tolerance induction with anti-CD4 mAbs. The development of CD4+ regulatory T cells may be responsible for tolerance and is reflected in findings of linked suppression and infectious tolerance. These results in animal models seem to indicate a renewed interest in transplantation for these well-characterized human Abs (58–61). Thus, although not targeted specifically for transplant indications, several non-depleting anti-CD4 antibodies are currently under clinical investigation in autoimmune indications. Clenoliximab

(IDEC 151), a primatized anti-CD4 mAb (62) has been shown safe and effective in a Phase I trial of rheumatoid arthritis and is currently in Phase II trials. Of concern, however, is the finding that Genmab's Humax-CD4, a fully humanized anti-CD4 mAb, failed in Phase II efficacy trials of RA (63). However, a Phase IIb trial in psoriasis is proceeding since the drug was well tolerated (64). In non-human primate studies, TolerRx's TRX1, an anti-CD4 mAb with a mutated Fc portion has been shown to induce antigen-specific tolerance while maintaining immuno-competence and the ability to respond to newly presented antigens (65).

Anti-CD3 mAb

OKT3 was the first FDA approved monoclonal antibody for use in kidney transplantation. Although it has been highly efficacious, the side-effects, often severe as a result of the mitogenic activity and subsequent elicitation of a so-called cytokine storm, have limited the use of the mAb outside the organ transplant setting for the reversal of rejection. To address this problem, several humanized Fc receptor-nonbinding anti-CD3 antibodies that, unlike their murine counterpart (OKT3), do not elicit a very toxic cytokine syndrome have entered the clinic in both autoimmune disease and transplantation settings. Initial human clinical studies in the context of renal allograft rejection demonstrated that an FcR non-binding form of humanized OKT3 [hOKT3γ1 (Ala-Ala)] was efficacious in reversing allograft rejection (66). Preliminary studies by Hering et al. in islet transplantation with this antibody have shown similar efficacy in promoting islet allograft acceptance when combined with low-dose tacrolimus and sirolimus (67). The ITN is supporting a clinical trial of combination hOKT3γ1 (Ala-Ala) and sirolimus maintenance immunosuppression in islet transplantation with planned sirolimus withdrawal at a defined time point after transplantation (B. Hering, principal investigator). Protein Design Labs' HuM291 is a humanized anti-CD3 monoclonal antibody engineered to reduce binding to Fcγ receptors and complement fixation. In Phase I dose escalation studies in kidney transplantation, it reversed allograft rejection while exhibiting only minor adverse effects (68). Phase II trials in kidney transplantation were terminated to focus this drug on the treatment of acute GvHD, as well as autoimmunity (69). Campath 3 (ChAglyCD3), another FcR nonbinding anti-CD3mAb, has been tested in an open pilot trial in which acute renal allograft rejection was reversed in seven of nine patients (70). The drug was well tolerated in this study. The ITN is currently supporting a Phase II clinical trial of Campath 3 in combination with sirolimus and MMF in renal transplantation (H. Kreis, principal investigator). Although complete immunosuppressive withdrawal will not be attempted in this study, it is anticipated that mechanistic data obtained through the ITN assay program in the trial may provide important clues into the mechanisms of allograft survival.

T-cell depleting therapies

For T-cell depleting agents, the working hypothesis is that a transient but profound T-cell depletion can reset the immune system to a tolerized state in the presence of alloantigen presentation. Pre-clinical studies have shown that anti-CD3 immunotoxin, combined with immunosuppressants such as 15-deoxyspergualin (DSG), is an effective tolerogenic therapy. The approach of following the depleting protocol with a therapy that alters T-cell function during the reconstitution phase may be most efficacious (71). An antihuman anti-CD3ε single-chain immunotoxin based on truncated diphtheria toxin has been described by Thomson et al. and may be suitable for human trials in transplantation (72). However, there are already a number of promising agents moving into clinical trials, which will yield some interesting results in the near future.

Campath 1H (Alemtuzumab)

Initial success using a T-cell depleting induction therapy to induce long-term allograft unresponsiveness was demonstrated by Calne et al. using Campath 1H (anti-CD52 mAb) with CsA maintenance therapy (73). Based on these initial studies a humanized version of Campath 1 was developed. Campath-1H has been shown to rapidly deplete peripheral blood B cells and T cells without affecting stem cells and was approved for the treatment of B-cell chronic lymphocytic leukemia. Preliminary data in renal transplant recipients who received Campath-1H (20 mg $\times$ two doses) with a short course of corticosteroids in combination with sirolimus showed unacceptably high incidence of acute rejection (74), as did data from a separate trial of Campath 1H with infliximab and sirolimus (75). These results indicate that modification of the regimen is needed to safely navigate the early post-transplant immunosuppression period before attempting drug withdrawal at a later time point. The ITN will be supporting a trial with Campath-1H, sirolimus and low-dose tacrolimus in renal transplantation (S. Knechtle, principal investigator) and Campath-1H plus sirolimus in islet transplantation (J. Shapiro, principal investigator). In both trials, the objective is to test the ability to completely withdraw immunosuppression.

Thymoglobulin

Thymoglobulin is a polyclonal rabbit antihuman thymocyte globulin (SangStat) that is approved for the treatment of acute renal transplant rejection and is a powerful lymphocyte depleting agent, possibly acting through the down-regulation of fas and bcl-2, negative regulators of apoptosis (76). Thymoglobulin has been tested in combination with sirolimus monotherapy (without calcineurin inhibitor or corticosteroids) in renal transplantation, where preliminary data suggest that it is safe and effective (77). Unexpectedly, a number of very recent studies (many from the

Pittsburgh transplant group) in kidney, pancreas, kidney/pancreas, and intestinal transplantations have revealed that thymoglobulin may be more than 'just' an immunosuppressive drug. Their studies suggest that thymoglobulin induction therapy results in a requirement for significantly less maintenance immunosuppression. More significantly, full drug withdrawal, as evidenced by a number of initial study reports using thymoglobulin, seems possible in some cases, suggesting that the therapy may potentially induce donor-specific tolerance (78–82). Ongoing studies have been designed to complete the withdrawal of immunosuppressives following careful weaning protocols.

Anti-CD45RB

While antilymphocyte antibodies (ALGs) such as thymoglobulin are powerful depleting therapies that have found widespread application, they are polyclonal, targeting multiple surface molecules including CD2, CD3, CD4, C5, CD8, CD11a/18, CD25, and CD74 (83,84). Significantly, a significant proportion of the antibodies found in ALGs bind to the leukocyte common antigen CD45 (85), a transmembrane protein tyrosine phosphatase involved in the coupling of signals from the T-cell receptor to the proximal signaling apparatus. Anti-CD45RB antibodies have been shown to induce long-term survival and tolerance in various experimental models of solid organ and islet transplantation and xenotransplantation, and have been found to be efficacious in models of autoimmune diseases (86). Combined blockade with mAbs to both CD45RB and CD40L has been found to result in a synergistic effect on allograft survival (87). Non-human primate models have shown that short-term therapy with a mouse anti-CD45RB mAb can establish permanent engraftment and reverse acute rejection episodes. Thus, it is clear that anti-CD45RB antibodies should be tested in the clinical transplant setting. In this regard, a humanized version of the antibody is currently in development for clinical use (86).

Where do we go from here?

Nearly 50 years have passed since the concept of inducing transplant tolerance was first proposed. In that time, there has been remarkable progress in elucidating the mechanisms and means to achieving a durable long-term graft acceptance without ongoing immunotherapy. The fundamental processes involved in the immune response have been characterized to a large degree; new drug targets and avenues of intervention have been identified; numerous regimens tested in various animal models have provided proof-of-concept and the impetus for human clinical development; and, as the various studies described in this review illustrate, initial human trials have finally begun.

When judging the progress made during these early trials, it is important to remember that these studies are, in fact, our initial foray into a complex adventure. To this point, most of our knowledge of the mechanisms of transplant tolerance induction have been conceptual or in the context of animal models. These initial studies are our first glimpse at tolerance induction in humans. While there have been some disappointments along the way, there have already been some clinical successes. More importantly, both the failures and successes inform our understanding of the mechanisms of tolerance induction in the human transplant setting and help define the problems that remain.

Another important lesson from these early trials is that there are many opportunities for progress that may be missed if safety and efficacy are the only endpoints of interest. Each trial, in fact, presents the opportunity to answer pressing questions related to the mechanisms of induction, loss, and maintenance of tolerance to allografts in humans. Assays for gene expression, polymorphisms, cytokine expression, etc., that complement the clinical trials will help guide future clinical trials. Such studies may also evolve into specific assays for surrogate or predictive markers of tolerance that could help target therapies to responsive individuals.

So where do we stand? There are currently immunosuppressive protocols that are calcineurin inhibitor-free that result in long-term graft acceptance. Bone marrow transplant (BMT) protocols have been evaluated that in some, (albeit limited) cases patients have been removed from immunosuppression altogether. Indeed, it is now evident that bringing new tolerogenic agents into clinical testing in transplantation is difficult even in the case of single agent studies. Much of the clinical work undertaken, particularly by biotechnology or pharmaceutical companies, has been concentrated on autoimmune disease, with less development taking place in clinical transplantation. The lack of pursuit of the transplant indication is likely to be multifactorial, including a hesitancy to place patients at risk of graft loss. However, pursuing a less risky population such as recipients of islet transplants, where the consequences of graft failure are relatively less severe, or liver transplantation, where regeneration is possible, might offer a more suitable starting point for full clinical evaluation of these agents. Nonetheless, clinical trials in autoimmune diseases harbor important lessons for transplantation because of the common mechanistic bases of tolerance between the two indications.

However, there are a number of trials underway in which planned immunosuppressive withdrawal is built-in to the clinical protocols. Perhaps surprisingly, we have learned that induction therapy, with agents such as thymoglobulin, may indeed fit into the category of 'tolerance therapies.' In addition, much of what these early trials have taught us is what we need to do differently and what should be done next. For instance, given the available data, it appears likely that multiple mechanisms will be operational in patients who ultimately develop specific tolerance to the

allograft, yet maintain normal immunity to pathogens. In such patients, for example, induction of tolerance may be promoted by deletional (such as mixed chimerism, use of depleting antibodies, etc.) or anergy-promoting regimens, but the tolerant state may be maintained by other mechanisms such as the development of suppressor cells that produce regulatory cytokines and continuously control the alloreactive response.

Thus, one key goal is for organizations, such as the ITN, to promote the use of these new classes of potentially tolerogenic drugs, alone and in combination, in the transplant setting. Efforts to work with industry to remove the barriers to multidrug studies must be continued. The hope is that as a community we can capitalize on knowledge gained in other indications by providing an interdisciplinary environment and by including complementary mechanistic studies in every clinical trial in order to further the field of transplant immunotherapy and achieve the long sought after goal of tolerance.

References

1. Neuberger J, Adams DH. What is the significance of acute liver allograft rejection. J Hepatol 1998; 29: 143–150.
2. Calne RY. An opportunity in organ transplantation. Nat Med 1995; 1: 20–22.
3. Ojo AO, Meier-Kriesche H-U, Hanson JA et al. Mycophenolate mofetil reduces late renal allograft loss independent of acute rejection. Transplantation 2000; 69: 2405–2409.
4. Ramos HC, Reyes J, Abuelmagd K et al. Weaning of immunosuppression in long-term liver-transplant recipients. Transplantation 1995; 59: 212–217.
5. Dousset B, Hubscher SG, Padbury RT et al. Acute liver allograft rejection is treatment always necessary? Transplantation 1993; 55: 529–534.
6. Padbury RT, Gunson BK, Dousset B et al. Steroid withdrawal from long-term immunosuppression in liver allograft recipients. Transplantation 1993; 55: 789–794.
7. Li Y, Li XC, Zheng XX, Wells AD, Turka LA, Strom TB. Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance. Nat Med 1999; 5: 1298–1302,.
8. Lan F, Hayamizu K, Strober S. Cyclosporine facilitates chimeric and inhibits nonchimeric tolerance after posttransplant total lymphoid irradiation. Transplantation 2000; 69: 649–655.
9. Wekerle T, Kurtz J, Bigenzahn S, Takeuchi Y, Sykes M. Mechanisms of transplant tolerance induction using costimulatory blockade. Curr Opin Immunol 2002; 14: 592.
10. Cosimi A. Calcineurin inhibitors are not antagonistic to tolerance induction. Transplant Proc 2002; 34: 1376–1377.
11. Millan MT, Shizuru JA, Hoffmann P et al. Mixed chimerism and immunosuppressive drug withdrawal after HLA-mismatched kidney and hematopoietic progenitor transplantation. Transplantation 2002; 73: 1386–1391.
12. Millan MT, Shizuru JA, Hoffmann P et al. Mixed chimerism and donor-specific unresponsiveness without graft-versus-host disease after MHC-mismatched hematopoietic stem cell infusion and kidney transplantation [abstract 59]. Transplantation 2002; 74: 37.
13. Ferreira JV, Froud T, Caulfield A et al. Can enriched bone marrow infusion induce donor tolerance in solitary islet cell transplantation? [abstract 290] Transplantation 2002, 74.
14. Jordan MS, Riley MP, von Boehmer H, Caton AJ. Anergy and suppression regulate CD4 (+) T cell responses to a self peptide. Eur J Immunol 2000; 30: 136–144.
15. Wekerle T, Sayegh MH, Chandraker A, Swenson KG, Zhao Y, Sykes M. Role of peripheral clonal deletion in tolerance induction with bone marrow transplantation and costimulatory blockade. Transplant Proc 1999; 31: 680.
16. Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 System of T cell costimulation. Ann Rev Immunol 1996; 14: 233–258.
17. Moreland LW, Alten R, Van den Bosch F et al. Costimulatory blockade in patients with rheumatoid arthritis: a pilot, dose-finding, double-blind, placebo-controlled clinical trial evaluating CTLA-4Ig and LEA29Y eighty-five days after the first infusion. Arthritis Rheum 2002; 46: 1470–1479.
18. Adams AB, Shirasugi N, Durham MM et al. Calcineurin inhibitor-free CD28 blockade-based protocol protects allogeneic islets in nonhuman primates. Diabetes 2002; 51: 265–270.
19. 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: 377–384.
20. 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: 1128–1137.
21. Vincenti F. What's in the pipeline: new immunosuppressive drugs in transplantation. Am J Transplant 2002; 2: 898–903.
22. Schopf RE. IDEC-114 (IDEC). Curr Opin Invest Drugs 2001; 2: 635–638.
23. Diabetogen press release, March 26, 2001.
24. Özkaynak E, Gao W, Shemmeri N et al. Importance of ICOS–B7RP:-1 costimulation in acute and chronic allograft rejection. Nat Immunol 2001; 2: 591–596.
25. Guo L, Li XK, Funeshima N et al. Prolonged survival in rat liver transplantation with mouse monoclonal antibody against an inducible costimulator (ICOS). Transplantation 2002; 73: 1027–1032.
26. Nakamura Y, Yasunami Y, Hirakawa E et al. Acceptance of islet allografts in the liver of mice by blockade of an inducible costimulatory [abstract 462]. Transplantation 2002; 74: 160.
27. Kirk AD, Burkly LC, Batty DS et al. Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. Nat Med 1999; 5: 686–693.
28. IDEC press release, June 10, 2002.
29. Henn V, Steinbach S, Buchner K, Presek P, Kroczek RA. The inflammatory action of CD40 ligand (CD154) expressed on activated human platelets is temporally limited by coexpressed CD40. Blood 2001; 98: 1047–1054.
30. Boon L, Laman JD, Ortiz-Buijsse A et al. Preclinical assessment of anti-CD40 Mab 5D12 in cynomolgus monkeys. Toxicology 2002; 174: 53–65.
31. Isobe M, Yagita H, Okumura K, Ihara A. Specific acceptance of cardiac allograft after treatment with antibodies to ICAM-1 and LFA-1. Science 1992; 255: 1125–1127.
32. Isobe M, Suzuki J, Yamazaki S et al. Regulation by differential development of Thl and Th2 cells in peripheral tolerance to cardiac allograft induced by blocking ICAM-1/LFA-1 adhesion. Circulation 1997; 96: 2247–2253.

33. Corbascio M, Ekstrand H, Osterholm C et al. CTLA4Ig combined with anti-LFA-1 prolongs cardiac allograft survival indefinitely. Transpl Immunol 2002; 10: 55–61.
34. Papp K, Bissonnette R, Krueger JG et al. The treatment of moderate to severe psoriasis with a new anti-CD11a monoclonal antibody. J Am Acad Dermatol 2001; 45: 665–674.
35. Dedrick RL, Walicke P, Garovoy M. Anti-adhesion antibodies efalizumab, a humanized anti-CD11a monoclonal antibody. Transpl Immunol 2002; 9: 181–186.
36. Vincenti F, Mendez R, Rajagopalan PR et al. A phase I/II trial of anti-CD11a monoclonal antibody in renal transplantation [abstract #562]. Am J Transplant 2001; 1 (Suppl. 1): 276.
37. Seville F, Vanhove B, Soullilou J-P. Mechanisms of tolerance induction: blockade of costimulation. Phil Trans R Soc London B 2001; 356: 649–657.
38. Antilfa Study Group. Prospective study of anti-cd11a antibody in prevention of delayed graft function (dgf) in recipients of high-risk renal cadaver grafts [abstract 471]. Am J Transplant 2001; 1 (Suppl. 1): 253.
39. Suzuki J, Isobe M, Izawa A et al. Differential Th1 and Th2 cell regulation of murine cardiac allograft acceptance by blocking cell adhesion of ICAM-1/LFA-1 and VCAM-1/VLA-4. Transpl Immunol 1999; 7: 65–72.
40. Hori J, Isobe M, Yamagami S, Tsuru T. Acceptance of second corneal allograft by combination of anti-VLA-4 and anti-LFA-1 monoclonal antibodies in mice. Transplant Proc 1998; 30: 200–201.
41. Yang H, Issekutz TB, Wright JR Jr. Prolongation of rat islet allograft survival by treatment with monoclonal antibodies against VLA-4 and LFA-1. Transplantation 1995; 60: 71–76.
42. Tubridy N, Behan PO, Capildeo R et al. The effect of anti-VLA4 integrin antibody on brain lesions activity in MS. The UK Antegren Study Group. Neurology 1999; 53: 466–472.
43. Gordon FH, Hamilton MI, Donoghue S et al. A pilot study of treatment of active ulcerative colitis with natalizumab, a humanized monoclonal antibody to alpha-4 integrin. Aliment Pharmacol Ther 2002; 16: 699–705.
44. Ghosh S, Goldin E, Gordon FH et al. Natalizumab for Active Crohn's Disease. N Engl J Med 2003; 348: 24–32.
45. Wekerle T, Blaha P, Langer F, Schmid M, Muehlbacher F. Tolerance through bone marrow transplantation with costimulation blockade. Transpl Immunol 2002; 9: 125–133.
46. Wekerle T, Kurtz J, Ito H et al. Allogeneic bone marrow transplantation with co-stimulatory blockade induces macrochimerism and tolerance without cytoreductive host treatment. Nat Med 2000; 6: 464–469.
47. Durham MM, Bingaman AW, Adams AB et al. Administration of anti-CD40 ligand and donor bone marrow leads to hematopoietic chimerism and donor-specific tolerance without cytoreductive conditioning. J Immunol 2000; 165: 1–4.
48. Chavin KD, Qin L, Lin J, Yagita H, Bromberg JS. Combined anti-CD2 and anti-CD3 receptor monoclonal antibodies induce donor-specific tolerance in a cardiac transplant model. J Immunol 1993; 151: 7249–7259.
49. Woodward JE, Qin L, Chavin KD et al. Blockade of multiple costimulatory receptors induces hyporesponsiveness: inhibition of CD2 plus CD28 pathways. Transplantation 1996; 62: 1011–1018.
50. Besse T, Malaise J, Mourad M et al. Prevention of rejection with BTI-322 after renal transplantation (results at 9 months). Transplant Proc 1997; 29: 2425–2426.
51. Przepiorka D, Phillips GL, Ratanatharathorn V et al. A phase II study of BTI-322, a monoclonal anti-CD2 antibody, for treatment of steroid-resistant acute graft-versus-host disease. Blood 1998; 92: 4066–4071.
52. Dehoux JP, Talpe S, Dewolf N et al. Effects on human and nonhuman primate immune response of a new rat anti-CD2 monoclonal antibody. Transplantation 2000; 69: 2622–2633.
53. Ellis N, Krueger GG. Treatment of chronic plaque psoriasis by selective targeting of memory effector T lymphocytes. N Engl J Med 2001; 345: 248–255.
54. Sultan P, Schechner JS, McNiff JM et al. Blockade of CD2–LFA−3 interactions protects human skin allografts in immunodeficient mouse/human chimeras. Nat Biotechnol 1998; 15: 759–762.
55. Kaplon RJ, Hochman PS, Michler RE et al. Short course single agent therapy with an LFA-3-IgG1 fusion protein prolongs primate cardiac allograft survival. Transplantation 1996; 61: 356–363.
56. Waldman H, Cobbold S. How do monoclonal antibodies induce tolerance? A role for infectious tolerance? A Review. Immunol 1998; 16: 619–644.
57. Van der Lubbe PA, Reiter C, Breedveld FC et al. Chimeric CD4 monoclonal antibody cM-T412 as a therapeutic approach to rheumatoid arthritis. Arthritis Rheumatism 1993; 36: 1375.
58. Honey K, Cobbold SP, Waldmann H. Dominant tolerance and linked suppression induced by therapeutic antibodies do not depend on Fas–FasL interactions. Transplantation 2001; 69: 1683–1689.
59. Kohlhaw K, Sack U, Lehmann I et al. The monoclonal anti-CD4 antibody RIB5/2 induces donor-specific tolerance in the high-responder liver transplant model in the rat. Transplant Proc 2001; 33: 2371–2373.
60. Guo Z, Wu T, Kirchhof N et al. Immunotherapy with nondepleting anti-CD4 monoclonal antibodies but not CD28 antagonists protects islet graft in spontaneously diabetic nod mice from autoimmune destruction and allogeneic and xenogeneic graft rejection. Transplantation 2001; 71: 1656–1665.
61. Thiel MA, Takano T, Hawksworth N, Coster DJ, Williams KA. Low-dose, short-term treatment with anti-CD4 monoclonal antibody prolongs corneal allograft survival. Transplant Proc 2001; 33: 635–636.
62. Newman R, Hariharan K, Reff M et al. Modification of the Fc region of a primatized IgG antibody to human CD4 retains its ability to modulate CD4 receptors but does not deplete CD4 (+) T cells in chimpanzees. Clin Immunol 2001; 98: 164–174.
63. Genmab press release, September 24, 2002.
64. Genmab press release, September 19, 2002.
65. Winsor-Hines D, Cobbold SP, Merrill CJ et al. Induction of durable, antigen specific tolerance in primates with a short therapeutic course of non-depleting anti-cd4 antibody [abstract 254]. 2nd Annual Meeting of the Federation of Clinical Immunology Societies, San Francisco, CA.
66. Woodle ES, Xu D, Zivin RA et al. Phase I trial of a humanized, Fc receptor nonbinding OKT3 antibody, huOKT3gamma1 (Ala-Ala) in the treatment of acute renal allograft rejection. Transplantation 1999; 68: 608–616.
67. Hering BJ, Kandaswamy R, Harmon JV et al. Insulin independence after single-donor islet transplantation in type 1 diabetes with hOKT3gamma1 (Ala-Ala), sirolimus, and tacrolimus therapy. Am J Transplant 2001; 1 (Suppl. 1): 180A.
68. Norman DJ, Vincenti F, de Mattos AM et al. Phase I trial of HuM291, a humanized anti-CD3 antibody, in patients receiving renal allografts from living donors. Transplantation 2000; 70: 1707–1712.
69. Trajkovic V. Nuvion protein design laboratories. Curr Opin Invest Drugs 2002; 3: 411–414.
70. Friend PJ, Hale G, Chatenoud L et al. Phase I study of an engineered aglycosylated humanized CD3 antibody in renal transplant rejection. Transplantation 1999; 68: 1632–1637.

71. Thomas JM, Eckhoff DE, Contreras JL et al. Durable donor-specific T and B cell tolerance in rhesus macaques induced with peritransplantation anti-CD3 immunotoxin and deoxyspergualin: absence of chronic allograft nephropathy. Transplantation 2000; 69: 2497–2503.
72. Thompson J, Stavrou S, Weetall M et al. Improved binding of a bivalent single-chain immunotoxin results in increased efficacy for in vivo T-cell depletion. Protein Eng 2001; 14: 1035–1041.
73. Calne R, Friend P, Moffatt S et al. Prope tolerance, perioperative campath 1H, and low-dose cyclosporin monotherapy in renal allograft recipients. Lancet 1998; 351: 1701–1702.
74. Knechtle SJ, Pirsch JD, Becker BN et al. A pilot study of Campath-1H induction plus rapamycin monotherapy in renal transplantation [abstract 45]. Transplantation 2002; 74: 32.
75. Kirk AD, Hale DA, Hoffmann SC et al. Results from a human tolerance trial using campath-1h with and without infliximab [abstract 47]. Transplantation 2002; 74: 33.
76. Daller A, Woodside KJ, Meng T, Hu M, Gugliuzza KK, Hunter GC. Altered lymphocyte expression of fas and bcl-2 in renal transplant patients receiving induction therapy [abstract 3010]. Transplantation 2002; 74: 594.
77. Swanson SJ, Hale DA, Mannon RB, Harlan DM, Kleiner DE, Kirk AD. Sirolimus monotherapy in kidney transplantation following high dose thymoglobulin induction [abstract 13]. Transplantation 2002; 74: 22.
78. Corry R, Potdar S, Shapiro R et al. Pancreas and kidney-pancreas transplantation under a tolerogenic regimen of preconditioning with thymoglobulin and post-transplant tacrolimus [abstract 85]. Transplantation 2002; 74: 46.
79. Harris C, Bond G, Janson D et al. Thymoglobulin (rATG) single high dose pretreatment for human intestinal transplantation: lymphocyte depletion, drug levels and immune reconstitution [abstract 590]. Transplantation 2002; 74: 199.
80. Shapiro R, Scantlebury VP, Jordan ML et al. Kidney transplantation under a tolerogenic regimen of thymoglobulin preconditioning and post-transplant tacrolimus monotherapy [abstract 3343]. Transplantation 2002; 74: 673.
81. Abu-Elmagd KM, Bond GJ, Murase N et al. Tolerance for human intestinal transplantation [abstract 61]. Transplantation 2002; 74: 38.
82. Abu-Elmagd M, Bond G, Murase N et al. Graft immunomodulation and tolerance enhancing strategy for intestinal transplantation [abstract 640]. Transplantation 2002; 74: 218.
83. Fabre JW, Williams AF. Quantitative serological analysis of a rabbit anti-rat lymphocyte serum and preliminary biochemical characterisation of the major antigen recognised. Transplantation 1977; 23: 349–359.
84. Warr GW, Marchalonis JJ. Glycoproteins of murine thymocyte and splenocyte surface membranes; binding to concanavalin A and recognition by heterologous antilymphocyte serum. Immunochemistry 1976; 13: 753–758.
85. Bonnefoy-Berard N, Vincent C, Revillard JP. Antibodies against functional leukocyte surface molecules in polyclonal antilymphocyte and antithymocyte globulins. Transplantation 1991; 51: 669–673.
86. Luke PPW, O'Brien CA, Jevnikar AM, Zhong R. Anti-CD45RB: Monoclonal antibody-mediated transplantation tolerance. Curr Mol Med 2001; 1: 533–543.
87. Rothstein DM, Livak MF, Kishimoto K et al. Targeting signal 1 through CD45RB synergizes with CD40 ligand blockade and promotes long term engraftment and tolerance in stringent transplant models. J Immunol 2001; 166: 322–329.