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Induction of transplantation tolerance in non-human primate preclinical models

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Short-term outcomes following organ transplantation have improved considerably since the availability of cyclosporine ushered in the modern era of immunosuppression. In spite of this, many of the current limitations to progress in the field are directly related to the existing practice of relatively non-specific immunosuppression. These include increased risks of opportunistic infection and cancer, and toxicity associated with long-term immunosuppressive drug exposure. In addition, long-term graft loss continues to result in part from a failure to adequately control the anti-donor immune response. The development of a safe and reliable means of inducing tolerance would ameliorate these issues and improve the lives of transplant recipients, yet given the improving clinical standard of care, the translation of new therapies has become appropriately more cautious and dependent on increasingly predictive preclinical models. While convenient and easy to use, rodent tolerance models have not to date been reliably capable of predicting a therapy's potential efficacy in humans. Non-human primates possess an immune system that more closely approximates that found in humans, and have served as a more rigorous preclinical testing ground for novel therapies. Prior to clinical adaptation therefore, tolerance regimens should be vetted in non-human primates to ensure that there is sufficient potential for efficacy to justify the risk of its application.

Keywords: cynomolgus; rhesus; tolerance; transplantation; chimerism; costimulation

1. INTRODUCTION

Clinical solid organ transplantation has advanced tremendously over the past two decades, with 1 year graft survival now generally exceeding 90, 80 and 85% for kidney, liver and heart grafts, respectively.[1] These results are largely the consequence of the growing repertoire of potent immunosuppressive agents that have transformed solid organ transplantation from an experimental treatment into the treatment of choice for end stage organ failure. However, in spite of this, modern therapies leave considerable room for improvement. One major limitation is that despite excellent short-term graft survival rates and low rates of acute rejection now attained routinely, improvements in long-term graft survival have not been as impressive (Hariharan *et al*. 2000; Meier-Kriesche *et al*. 2004), as graft survival for kidney, liver and heart grafts falls to 70, 60 and 65%, respectively, at 5 years.[1]

Potential aetiologies for this attrition in graft survival are myriad but adverse side effects of modern immunosuppression figure prominently in the mix. In addition to acute and chronic rejection, graft loss can also result as a consequence of more generalized effects of immunosuppressive drugs on the allograft recipient to include toxicity, hypertension, hyperlipidaemia, diabetes, infection and malignancy. In order to delay graft loss therefore, careful titration between both over- and under-immunosuppression is required: a task made difficult by the fact that no reliable assay for the adequacy of immunosuppression currently exists. Another potential problem with the modern practice of immunosuppression is that it may also be partially counter-productive in that it likely interferes with the development of beneficial immune regulatory activity while it holds rejection at bay (Jones *et al*. 2002; Larsen *et al*. 1996).

Tolerance, as it pertains to organ transplantation, can be defined as an induced state characterized by the absence of a specific, deleterious immunologic reaction against an allograft that can be maintained without the chronic use of immunosuppressant drugs. While self-tolerance is a naturally occurring phenomenon that originates at the embryonic stage of development, and is reinforced peripherally throughout life, induced tolerance to non-self MHC is an aberrant state that is actively opposed by multiple effector arms of the immune system. When it is induced experimentally in animals, it does not appear to be a passive phenomenon, although this had been widely believed to be the case in the past. Instead, tolerance to an allograft is associated with a variety of active immunologic processes such as regulation, deletion and anergy that serve to perpetuate it. Specificity is the key characteristic that differentiates the induction of tolerance from the current practice of generalized immunosuppression using conventional agents. The ability to maintain a normal third party immune response in spite of the absence of a hostile response against the allograft would be expected to reduce the morbidity associated with the

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use of conventional immunosuppression and, perhaps ultimately, increase graft survival.

It has taken decades for potential tolerance induction regimens to make it to the clinical trial phase of development. Since their immune system has been highly characterized, a significant portion of the early discovery and mechanistic work regarding tolerance induction has been carried out in rodents. Unfortunately, these models are hampered by significant limitations in their relevance to clinical practice. Major differences in the patterns of MHC expression combined with a far less complex immune system conspire to make rodent tolerance models considerably more permissive than those of higher animals and man. As a consequence, the transplant literature contains many examples of successful tolerance induction regimens that have been developed in rodents (Jirsch *et al.* 1974; Angelisova *et al.* 1983; Gutstein *et al.* 1986; Chavin *et al.* 1993; Chikaraishi *et al.* 1995; Yang *et al.* 1995; Bashuda *et al.* 1996; Brandt *et al.* 1997; Lehmann *et al.* 1997; Alexander *et al.* 1998; Hayamizu *et al.* 1998; Lindner & Zantl 1998; Gao *et al.* 1999; Zhang *et al.* 2000; Gregori *et al.* 2001; Chiffoleau *et al.* 2002; Adorini *et al.* 2003; Guo *et al.* 2003; Jin *et al.* 2003; Nanji *et al.* 2003) while only a few have been successfully applied to non-human primates (NHPs) or the clinic with any degree of success. Accordingly, prospective protocols developed in rodent models normally require vetting in a large animal model in order to better appreciate their therapeutic potential for use in human trials.

Several large animal species have been used for this purpose including dogs, pigs and NHPs (Binns *et al.* 1970; Watson *et al.* 1993; Brendel *et al.* 1995; Kozlowski *et al.* 1999; Oike *et al.* 2000; Montgomery *et al.* 2001; Kirk 2003). Our experimental work is focused predominantly on the evaluation of tolerance induction protocols in NHPs to determine their potential for clinical adaptation. In our opinion, old world monkeys (Macaca mulatta and Macaca fascicularis) are well-suited for the preclinical evaluation of promising tolerance induction regimens. Strong similarities in MHC organization and function have been noted using a DNA based typing methodology developed for rhesus MHC class II and subsequently class I (Knapp *et al.* 1997*a*,*b*). In particular, the degree of polymorphism at the class II and class I loci was found to be very similar to that observed in humans. Further studies have also confirmed that the function and phenotype of lymphocytes involved in the primate allo-response closely mirror that seen in humans (Nocera *et al.* 1989; Linley *et al.* 1998). These similarities contribute to an alloreactive response that mimics that seen in humans both in respect to intensity and evolution over time (Allegra & Giacomett 1968*a*,*b*; Allegra *et al.* 1968; Schuurman *et al.* 1997*a*,*b*). In addition, while sometimes unpredictable, the cross-reactivity of reagents and drugs developed for humans in NHPs is another factor that can facilitate the adaptation of a treatment regimen between humans and NHPs. Anatomic similarities between humans and NHPs also facilitate the translational aspects of this research.

Another factor that supports the relevance of using out bred adult NHPs for tolerance studies has to do with the roughly comparable antigenic experience accumulated over time. This cumulative exposure to a broad array of antigens in the environment translates into a broad spectrum of heterologous immunity that can influence the alloimmune response (Adams *et al.* 2003*a*,*b*). This heterologous repertoire is much smaller in inbred laboratory rodents due to a highly restricted exposure to environmental antigens and this fact partially accounts for the relative ease with which tolerance induction regimens are developed in rodents. Therefore, a tolerance induction regimen that can overcome this barrier in NHPs likely has a higher potential for efficacy in humans.

To date, only two fundamental approaches to tolerance induction have enjoyed some measure of success in primate models. One approach involves the implementation of various lymphocyte depletion manoeuvres (i.e. radiation, antibody) prior to exposure to donor antigen in order that reconstitution of the lymphocyte populations occurs in the presence of the allograft. The second approach modifies the immune response of the host through the selective alteration of costimulatory pathways necessary for the initiation of a competent alloimmune response. Both approaches have been combined with the administration of donor haematopoietic cells in an attempt to facilitate tolerance through the induction of immunoregulatory pathways or a transient state of mixed chimerism. This manuscript will review, in roughly chronologic fashion, the progress that has been observed over the past several decades by a select group of investigators who have chosen to pursue this challenge.

2. DEPLETIONAL APPROACHES TO TOLERANCE

(a) *Lymphoid irradiation*

Total lymphoid irradiation (TLI) continues to be one of the most potent immunosuppressive therapies available. In the early era of clinical and experimental immunosuppression, prior to the widespread availability of depletional antibodies and calcineurin inhibitors, it was a key component of conditioning regimens developed for the purpose of inducing tolerance. At Stanford University in 1979, Bieber *et al.* conducted a series experiments exploring the effects of TLI combined with rabbit antithymocyte globulin (RATG) (Bieber *et al.* 1979). In this series 18 abdominal heterotopic cardiac allografts were performed on rhesus macaques. The animals were divided into four treatment groups. A control group received no therapy. Group two received polyclonal antilymphocyte globulin (ALG) alone. Group three received TLI (600 rad) alone. Group four received TLI and ALG. The investigators found that TLI and ALG acted synergistically to lower peripheral lymphocyte counts. The survival for group four was significantly longer than for the other three groups, with the mean being 169 ± 15 days. Persistent cellular infiltration and narrowing of the coronary arteries was seen in all rejecting grafts. These findings have since been recognized as the hallmarks of cardiac allograft

rejection. The authors concluded that even though TLI and ALG increased allograft survival time, it was likely that additional immunosuppressive agents would have to be added to this regimen to make it clinically applicable.

TLI in orthotopic cardiac transplantation was subsequently studied by the Stanford group (Pennock *et al*. 1981). Because the heart is exposed to the load of pumping blood, orthotopic cardiac transplantation more accurately mimics clinical conditions. In this series, 31 cynomolgus monkeys were placed into six different treatment groups. A control group received no treatment. The second group received only postoperative cyclosporine A (CyA). The third group received 600 rad preoperative TLI, eight doses of postoperative antilymphocyte globulin (ATG), and azathioprine. The fourth group received the same treatment as the third, with the TLI dose increased to 1800 rad. The fifth group received 600 rad preoperatively and CyA postoperatively. The sixth group received the same treatment as the fifth, with the 1800 rad TLI dose. The survival associated with the use of CyA alone was 59 ± 30 days. Although all treatment groups showed significantly increased survival when compared to the untreated controls, no group significantly exceeded the survival of the group treated with CyA alone. In the sixth group (1800 rad + Cy A) two of the animals died of widely metastatic lymphoma. Not surprisingly, there were numerous incidents of early animal demise due to infection in most treatment groups.

Successful induction of tolerance using a combination of donor specific bone marrow (BM) and fractionated TLI in mouse, rat, and dog models led to experiments studying the efficacy of these treatments in NHPs. The Johannesburg group studied the effects of BM and TLI in baboon liver and renal transplantation (Myburgh *et al*. 1980*a*,*b*). In these experiments the authors sought to establish the optimal timing and dosage of radiation, BM, and allograft transplantation. They found that fractionated irradiation to the entire torso with a total of 1600 rad over 8 doses, followed by immediate BM injection, and then delayed liver grafting (3–4 weeks), demonstrated a survival of between 63 and 106 days. Low levels of transient chimerism were found in two of four animals in this group. These experiments were repeated with a renal transplantation model; similar results were obtained.

Subsequent experiments were performed to facilitate the translation of this approach to a relevant clinical scenario. In 1981 Myburgh *et al*. investigated various regimens of TLI in the baboon that were aimed at lengthening the period between the original dose of TLI and eventual transplantation (Myburgh *et al*. 1981). In this series of experiments they found that a priming dose of 2200 rad over three months, with additional 200 rad doses every one to three months thereafter was shown to be as effective as the original 200 rad eight dose regimen. These experiments also demonstrated that BM was not necessarily required to achieve long-term graft survival. It was suggested that this extended period of irradiation would suit patients awaiting transplantation while on dialysis. The TLI could begin before a graft was actually procured and 'touch up' doses of radiation could keep the recipient primed for transplantation.

In a follow-up study the Johannesburg group concluded that the optimal dose of radiation in the baboon was 8 fractions of 1 Gy each over four weeks (Myburgh *et al*. 1984). In this same series, attention was turned to the lymphocyte populations affected by TLI. By using monoclonal antibodies it was determined that after TLI, the CD4 T cell population increased while the cytotoxic/suppressor T cell population decreased. Interestingly, after transplantation the opposite occurred. These findings led to the theory that after TLI and transplantation, a subset of specific suppressor T cells was responsible for maintaining graft survival. These studies also reiterated that donor specific BM transfusion did not increase efficacy of TLI.

In 1994 Stark *et al*. reported that an inhibitory factor was found in the serum of baboons after TLI and renal transplantation (Stark *et al*. 1994). Serum obtained from baboons rendered tolerant to renal allografts suppressed mixed lymphocyte culture (MLC) reactivity against donor cell stimulators. This activity was not found in serum obtained from baboons that did not achieve durable tolerance. This inhibition activity was also transient. It could be demonstrated 3–4 months post transplant but not thereafter. Further characterization of the serum demonstrated the factor to be a low affinity IgG antibody. The authors concluded that this antibody could act as a mask, blocking APC presentation to alloreactive T cells. This effect could be overcome by addition of rIL-2.

(b) *Polyclonal antilymphocyte antibody*

Polyclonal antilymphocyte antibody is generated by the repeated immunization of an animal (generally horse or rabbit) with lymphocytes obtained from either the thymus, lymph node or spleen of the animal species against which it will by used. For purposes of tolerance induction, it has frequently been paired with the infusion of donor haematopoietic cells, usually in the form of donor BM.

In 1971 Myburgh and Smit reported that a combination of ALG and donor specific BM transfusion resulted in graft survivals that equaled or exceeded those associated with immunosuppressant protocols existing at that time (Myburgh *et al*. 1971). It should be noted that standard anti-rejection medication at this time consisted of prednisone and azathioprine. In this liver allograft study, baboon ALG was manufactured by injecting horses with baboon lymph nodes and lymphocytes. Seven different groups received various combinations of ALG, donor liver antigen, donor BM, and/or prednisone and azathioprine. Survival was found to be enhanced in animals receiving ALG for 6 days postoperatively followed by a one-time dose of 25×10^6 donor specific BM cells. The survival of these animals equaled or slightly bettered the group receiving continuous prednisone and azathioprine therapy. MLC on the baboons in this group were negative at 7–10 days after transplantation and remained negative for 2–4 weeks. Subsequently, these animals rejected and died 43–66 days after transplantation. The results of these MLCs indicated that a transient period of anti-donor

hyporesponsiveness had been achieved in these animals.

Subsequent experiments focused on modulation of the recipient immune system, in order to prolong graft survival, with varied levels of success. One such strategy consisted of administering postoperative polyspecific cytotoxic serum (PSS) (Myburgh & Smit 1972). This was a fractionated allospecific serum created by multiple immunizations of 10 baboons with lymphoid tissue from 50 unrelated baboons. A combination of PSS and donor specific BM demonstrated a modest increase in survival. Other strategies included radioactive antigen 'suicide' (Myburgh & Smit 1973) and immunization of recipients with donor specific T cell lymphoblasts (Myburgh & Smit 1979).

In the late 1970s, Judith Thomas initiated a series of NHP tolerance studies predicated on the use of anti-thymocyte globulin (ATG) in conjunction with donor lymphoid cell infusion, a technique that is still being investigated by multiple groups to this day. The use of ATG treatment was a derivative of the work done by Lance and Medawar using heterologous anti-lymphocyte serum for tolerance induction during the early 1970s (Lance & Medawar 1970*a*,*b*). During the same period of time, Wood and Monaco *et al*. paired allogeneic BM with anti-lymphocyte serum for tolerance induction (Wood *et al*. 1971). The first primate model in which Thomas chose to test the efficacy of this regimen was a rhesus skin transplantation model (Thomas *et al* 1979). She demonstrated prolonged allograft survival from 7.7 days in untreated animals to 32.5 days in animals that received a 5 day course of rabbit anti-human thymocyte globulin. With the addition of donor lymphoid infusion—spleen and BM—on day 10 after transplant, survival was improved to 42.8 days. Armed with encouraging data using the secondarily vascularized model of skin allografts, Dr Thomas moved on to vascularized renal allografts (Thomas *et al*. 1983*a*,*b*). Similar to the induction protocol that was optimized in the skin allografts, rhesus monkeys received renal allografts and a short 5 day course of rabbit anti-thymocyte globulin (RATG). The timing of the donor unfractionated BM infusion was shifted to day 12 after transplantation. Thomas *et al*. demonstrated significantly prolonged allograft survival from 9.2 days without treatment to 35.8 days with RATG treatment alone. In four animals, greater than 150 days survival was noted with the addition of BM (Thomas *et al*. 1983*a*). While the mean survival time using this protocol was ultimately determined to be less than 100 days (Thomas *et al*. 1989), these results were truly remarkable at the time considering they were obtained without the use of chronic immunosuppression.

With further investigation into the mechanisms behind prolonged renal allograft survival using RATG and BM, a role for veto cells in allowing the development of immunoregulation was proposed (Thomas *et al*. 1991, 1994). Through fractionation of the BM inoculum, Thomas *et al*. determined that recipients given BM depleted of HLA-DR+ cells or HLA-DR+ and CD3+ cells had significantly prolonged allograft survival. In fact, 40–50% of these animals went on to long-term survival of greater than 150 days. These *in vivo* data were consistent with concurrent *in vitro* data revealing that a small population of the BM (CD2+, CD8+, CD16+, HLA-DR−, CD3−, CD38−) suppressed cytotoxic T-lymphocyte assays. Furthermore, veto activity was abolished if the BM inoculum was irradiated, or treated with mitomycin C. Regardless of the type of manipulation performed on the BM, consistent long-term survival was not achieved. In an attempt to improve the rates of graft survival, Thomas *et al*. investigated the effects of adjunctive immunosuppression (Thomas *et al*. 1989). The addition of cyclosporine and low-dose prednisone increased mean survival by 50% but resulted in chronic rejection and calcineurin toxicity. Therefore, the need to alter the approach to tolerance induction was realized.

Finally, based on work by other groups (Posselt *et al*. 1990; Goss *et al*. 1992), Knechtle *et al*. attempted to induce tolerance in a rhesus kidney allograft model by combining intra-thymic injection of donor antigen with peripheral lymphocyte depletion using ATG. Due to a limiting amount of the reagent, only four experimental renal allografts were attempted. In the three animals treated with intrathymic injection of donor lymphocytes and ATG, survival times of 24, 25, and 1227 days were noted. One animal treated with ATG alone survived 30 days (Knechtle 2001). The difficulty associated with the production of ATG prompted investigators to seek another avenue of aggressive lymphocyte depletion.

(c) *Monoclonal antibody*

An initial attempt at the induction of tolerance using a purely depletional monoclonal antibody was conducted at the Massachusetts General Hospital in 1990 (Conti & Cosimi 1990; Cosimi *et al*. 1990). The model consisted of a heterotopic kidney transplant performed in cynomolgus monkeys. The recipients were treated with either intact monoclonal antibody targeting CD4 or the corresponding F (ab′) two fragments for 12 days following the kidney transplant. No other immunosuppression was administered. The mean survival in the recipients receiving a low dose of monoclonal antibody was 25 days. The group receiving high dose monoclonal antibody therapy demonstrated a mean graft survival of 39 days. Of note, serial flow cytometry demonstrated that CD4 cells were not depleted, but were transiently coated by the monoclonal antibody. All animals treated with monoclonal antibodies developed antibodies against mouse antibody.

In an attempt to improve the therapeutic efficacy and limit the anti-murine responses to the original OKT3 for monoclonal antibody, a humanized form employing grafted murine complementarity determining regions were developed (Powelson *et al*. 1994). In contrast to the murine antibody, the humanized form effected significant CD4 positive T cell depletion. Nonetheless, administration of this antibody resulted in only modest increases in renal allograft survival.

The limitations of this approach to immunosuppression in a NHP model was emphasized in a subsequent publication focusing on the effect of CD4 positive T cell coating or depletion on the kinetics of the

alloantibody response generated in the recipient (Wee *et al.* 1994). Control animals generated significant levels of anti-donor antibody within 10 days of kidney transplantation. Coating of CD4 positive T cells with a murine monoclonal antibody delayed production of antibody to approximately 20 days. Depletion of CD4 positive T cells using the humanized form of the monoclonal antibody delayed production of alloantibody for an additional 10 days.

The importance of antibody isotype in determining the biologic function of humanized monoclonal antibodies targeting CD4 was emphasized in a later study (Mourad *et al.* 1998). This study demonstrated that humanized antibodies based upon an IgG4 isotype coated but did not deplete CD4 positive T cells. In contrast, use of humanized antibodies based upon an IgG1 isotype resulted in effective depletion of CD4 positive T cells. Moreover, these effects were seen in the lymph nodes. While both preparations were capable of prolonging kidney allograft survival compared to untreated controls, the depletional antibody was clearly superior.

The search for a more potent depleting agent with efficacy in primates led to the development of FN18-CRM9 immunotoxin, an anti-CD3 monoclonal antibody conjugated to a mutated diphtheria toxin, by Neville *et al* (Neville *et al.* 1992). Immunotoxin is capable of a 2–3 log reduction of T cell numbers in the periphery and 1–2 log reduction in secondary lymphoid organs with minimal toxicity. The ability to deplete lymphocytes in lymph nodes differentiates it from the other anti-T cell monoclonal antibodies available at the time. In their initial set of experiments, they used a 3 day course of immunotoxin 0.2 mg kg^{-1} on days -7 to -5. One group of animals received an intrathymic injection of donor lymphocytes. Amazingly, 50% of animals treated with immunotoxin had prolonged renal allograft survival of greater than 200 days (Knechtle *et al.* 1997). In an appropriate test of tolerance, 5–6 months after renal transplantation a secondary immunologic challenge was performed with donor and third party skin allografts. Five of six long-term surviving animals accepted donor skin grafts and promptly rejected third party grafts.

Mechanistic work performed on these animals revealed a suppression of donor specific cytotoxic T cell (CTL) precursor frequency (Fechner *et al.* 1997*a*,*b*). Of note, the initial decline of CTL precursors was non-specific during the first six months after depletion. Subsequently, when the T cell count had normalized (approximately six months after renal transplant) the donor CTL precursor frequency remained suppressed but third party CTL number rebounded. Again, this was evident in the prompt rejection of third party skin grafts. In these experiments, Knechtle *et al.* found that *in vitro* anti-donor MLR reactivity was not significantly reduced. Additionally, alloantibody formation was not suppressed. Therefore, they coined the term 'split tolerance' to indicate the selective inhibition of the cytotoxic T cell compartment.

Interestingly, Knechtle *et al.* noted significant interstitial infiltrate in the surviving animals without evidence of acute rejection or tubular injury (Knechtle *et al.* 1997). Many of these animals went on to reject their allografts 3–5 months after transplant in the setting of alloantibody mediated glomerular and arterial injury. Knechtle postulated that immunotoxin effectively ablated T cell mediated acute cellular rejection but did not prevent all T cell allosensitization thereby promoting later T-dependent alloantibody mediated graft loss (Armstrong *et al.* 1998). This group has gone on to show that immunotoxin treated animals are a model for chronic graft rejection (Torrealba *et al.* 2003). These animals develop severe interstitial fibrosis, tubular atrophy, chronic transplant glomerulopathy and chronic vascular sclerosis. They found a correlation between the degree of CD68+ macrophage infiltration, anaemia and chronic rejection. More recently, they have been able to characterize a state of metastable tolerance that develops in the long-term surviving animals (Knechtle & Burlingham 2004; Torrealba *et al.* 2004). This state is associated with the eventual recruitment of regulatory T cells to the allograft interstitium.

In the meantime, Knechtle's group was testing other approaches to tolerance in their well-developed NHP renal allograft model. For example, they paired immunotoxin with conventional immunosuppressants and found that mycophenolate and steroids given in the perioperative period could enhance allograft survival (Fechner *et al.* 2001). They also attempted to pair costimulation blockade in the form of CTLA4-Ig and anti-CD40L with aggressive lymphocyte depletion and found encouraging results (Knechtle *et al.* 1999).

At the same time that Knechtle's group at the University of Wisconsin was having great success using pretransplant lymphocyte depletion with anti-CD3 immunotoxin, Thomas also adopted its use in the hope that it might prove to be more efficacious than RATG. Work in Thomas' lab focused on the peritransplant administration of immunotoxin—for a 2 or 3 day treatment—to more closely model the cadaveric transplant scenario. The fact that immunotoxin did not require an ancillary immune effector mechanism for depletion of both circulating and lymph node-resident T cells made it a very attractive alternative to RATG. In contrast to the striking results achieved with pretransplant aggressive T cell depletion (Knechtle *et al.* 1997), only two of six recipients that received immunotoxin in combination with donor BM infusion developed tolerance with survival times of 4 and more than 6 years (Thomas *et al.* 1997, 2001). Also, unlike the improvement in graft survival seen when post-transplant TLI was added to RATG and donor BM (Kawai *et al.* 1995), TLI did not improve immunotoxin treated graft survival. Therefore, the lack of consistent tolerance induction did not result from inconsistent or insufficient T cell depletion.

Thomas sought an immunologic explanation for the failure of peritransplant immunotoxin for tolerance induction as compared to its use 7 days prior to transplant. Evaluation of plasma cytokine levels revealed that kidney transplant recipients treated with immunotoxin had a significant proinflammatory cytokine response within hours of administration (Contreras *et al.* 1998). Thomas reasoned that when immunotoxin was given 7 days prior to transplant,

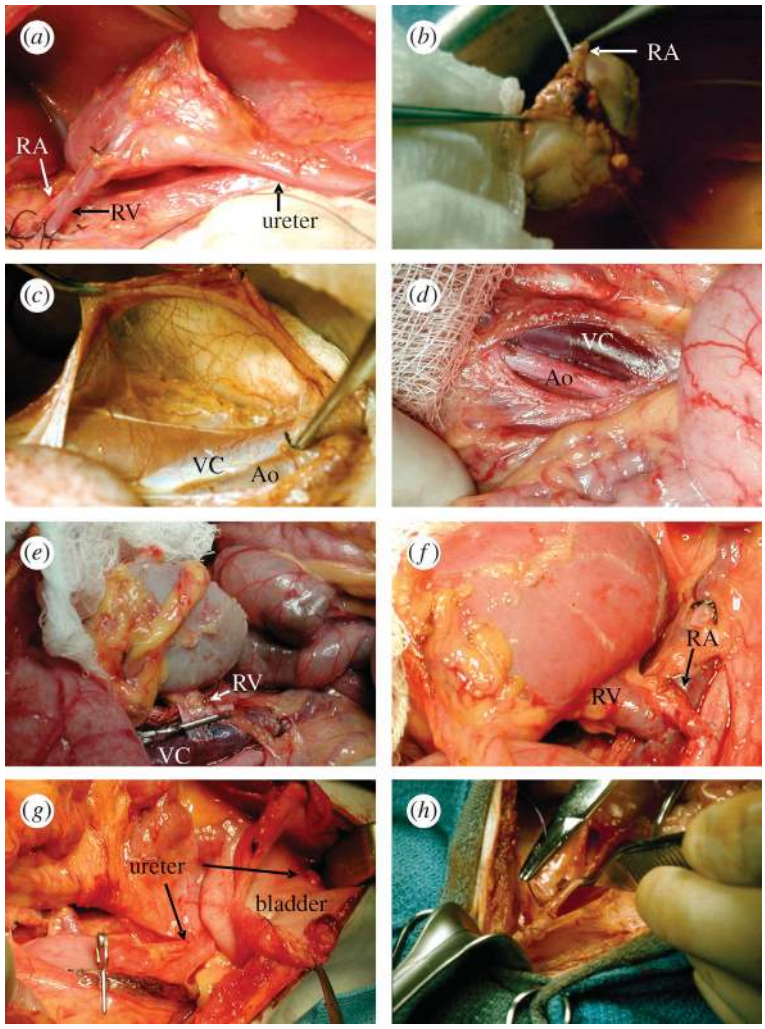

Figure 1. Kidney transplant method. (*a*) The donor kidney is isolated on a pedicle consisting of the renal vein (RV), renal artery (RA) and ureter. (*b*) The donor kidney is excised and flushed with UW preservation solution. (*c*) A retroperitoneal pocket lateral to the vena cava (VC) is raised. (*d*) The recipient's VC and aorta (Ao) are prepared for anastomosis. (*e*) The venous anastomosis has been completed and the donor RV clamped to permit restoration of flow through the VC. (*f*) The completed vascular anastomoses consisting of end to side donor RV to VC and donor RA to aorta. (*g*) The bladder has been incised and the donor ureter passed through the retroperitoneal pocket and through the posterior wall of the bladder. (*h*) The bladder is closed in two layers.

T cell depletion had already reached its nadir and, therefore, cytokine production was inhibited. Therefore, Thomas proposed the addition of a short-course of the NF-κB inhibitor 15-deoxyspergualin (DSG) to immunotoxin treatment in order to limit the early inflammatory response. An unexpected number of long-term graft survivals and tolerant animals resulted from this combination (Thomas *et al.* 1999). Overall, 12 of 16 animals were found to be tolerant with follow up of more than 2 years (Thomas *et al.* 2000, 2001). These animals were found to have no evidence of chronic rejection and/or alloantibody production. In addition, the same protocol produced similar results in streptozocin-induced diabetes treated with islet allografts. Most notably, these results were not dependent on donor BM infusion or chronic immunosuppression.

The powerful synergy between immunotoxin and DSG led Thomas to devise the STEALTH paradigm of primate allograft tolerance (Thomas *et al.* 2001). She proposed that the powerful tolerogenic potential of this treatment stemmed from the juxtaposition of three factors in the immediate post-transplant period: (i) more than 99% depletion of mature T and memory T cells in lymph nodes thereby preventing interactions with antigen presenting cells; (ii) marked increase in Th2 cytokines such as IL-4, IL-10 and TGFβ1; (iii) maturation arrest of dendritic cells (DC) during the period of graft healing which persists throughout the life of the DC. To date, the success that Thomas *et al.* achieved using immunotoxin and DSG has yet to be reproduced using other tolerogenic strategies. A limited scale trial using the CD52 specific T cell depleting monoclonal antibody alemtuzumab as a surrogate for immunotoxin in combination with DSG in humans has unfortunately not yielded the same encouraging results (Kirk *et al.* 2005). With further determination of the unique set of circumstances surrounding the tolerance induction by immunotoxin and DSG, perhaps a reliable and consistent mode of prolonged graft survival can be induced.

This initial work done using immunotoxin in NHPs by Knechtle and Thomas provided the basis for a landmark clinical study conducted by Calne *et al.* (1998). He combined an induction course of

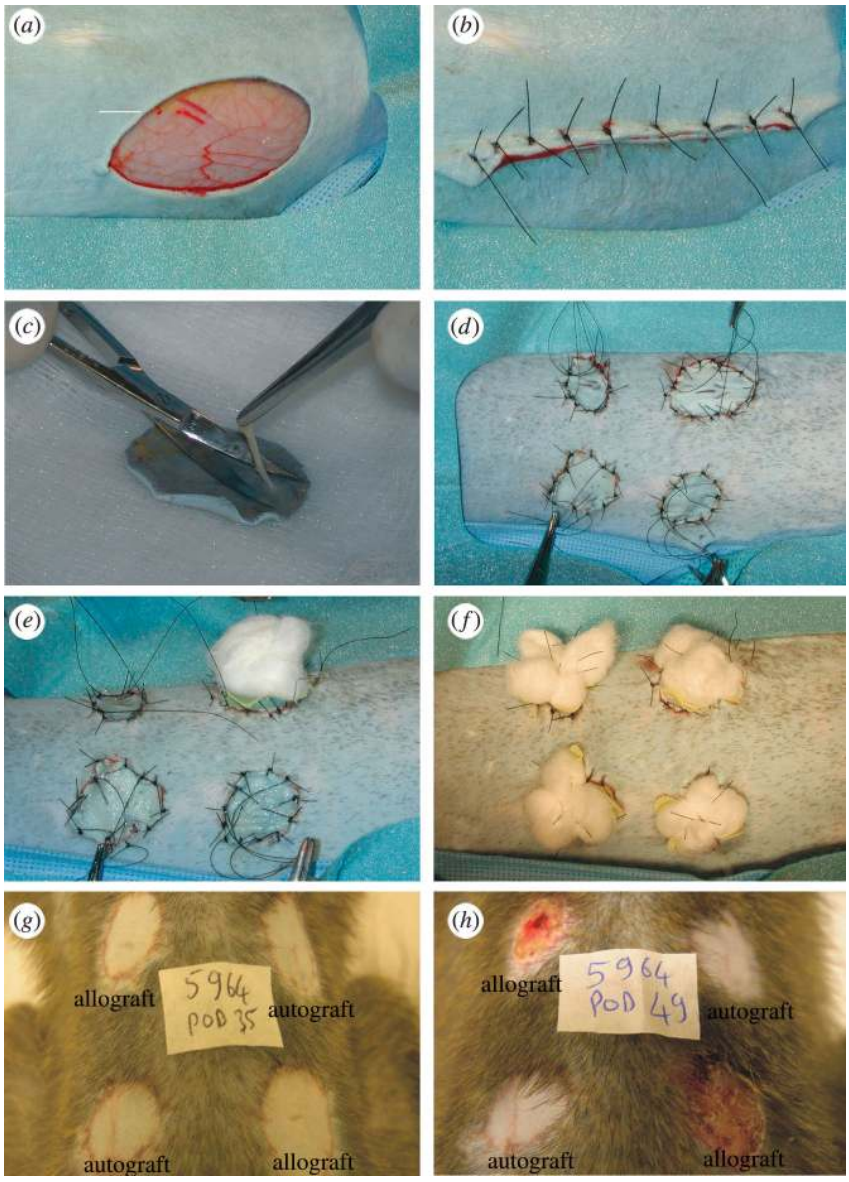

Figure 2. Skin graft method. (*a*) A full thickness ellipse of hairless donor skin is excised from the abdominal wall. (*b*) The skin graft donor site is repaired. (*c*) The skin graft is defatted. (*d*) Skin grafts are perforated and sewn into skin defects made on the back of the recipient. (*e*) Pressure is maintained on the grafts through the use of a cotton bolster placed over a petroleum gauze. (*f*) Completed skin graft procedure. (*g*) Appearance of allografts and control autografts prior to rejection. (*h*) Appearance of rejecting allografts.

alemtuzumab, as a surrogate for immunotoxin, with low dose cyclosporine monotherapy maintenance immunosuppression. Insofar as mid term graft survival and rates of rejection were equivalent to those obtained using conventional three-drug maintenance immunosuppression, Calne coined the immunologic state achieved in these patients as *prope* (almost) tolerance. Both Knechtle and Kirk subsequently confirmed these results substituting the mTOR inhibitor sirolimus for cyclosporine (Kirk *et al.* 2003; Knechtle *et al.* 2003). This is an example of a clinical approach that was explicitly predicated on the preclinical results in NHPs, in effect validating the model.

3. COSTIMULATION BLOCKADE

Prevailing theory suggests that T cells require supporting signals through costimulation pathways, in addition to specific recognition via the T cell receptor/antigen/MHC interaction, for optimal activation and proliferation (June *et al.* 1994). It was not long after these pathways were elucidated that it was discovered that blockade of these signals at the time of T cell receptor/antigen/MHC engagement resulted in T cell anergy (Gimmi *et al.* 1993; Johnson & Jenkins 1993). Consequently, this information was exploited to demonstrate that interruption of costimulation interactions had therapeutic potential in rodent transplantation models (Lenschow *et al.* 1992; Lenschow *et al.* 1995; Larsen *et al.* 1996). Translation to a NHP model was soon to follow.

Early studies regarding the efficacy of costimulation blockade were carried out in a rhesus macaque kidney transplant model by Kirk and colleagues (Kirk *et al.* 1997). The surgical model employed was adapted from Knechtle and is illustrated in figure 1. Early studies involved the co-administration of the fusion protein CTLA4-Ig and hu5C8 (a humanized monoclonal antibody directed against CD154). These

experiments were unique in that they targeted two major costimulatory pathways, the B7/CD28 pathway and the CD40/CD154 pathway alone and in combination. Animals receiving CTLA4-Ig alone had modestly prolonged survivals of 20–30 days. Those treated with a two week course of anti-CD154 alone were rejection free for 95–100 days, with these rejections being responsive to repeat therapy with anti-CD154 antibody. Animals treated with a combination of CTLA4-Ig and anti-CD154 antibody had similar rejection free survivals to those treated with anti-CD154 antibody monotherapy. After rescue with repeat doses of anti-CD154 antibody, the animals developed slow rejection over the course of a year, with the longest survivor being 370 days.

Since it was noticed that anti-CD154 antibody seemed as efficacious as the combination of CTLA4-Ig and anti-CD154, a series of renal transplant experiments focusing on humanized anti-CD154 (hu5C8) alone were performed (Kirk *et al.* 1999; Xu *et al.* 2001). The antibody was tested in doses ranging from 5 to 20 mg kg^{-1} on days 0, 3, 7, 10, 18, and 28, followed by a monthly dose for 1–12 months. Untreated controls rapidly rejected at 7 days. Animals receiving only 5 mg kg^{-1} rejected during therapy (Xu *et al.* 2001). One of two animals receiving 10 mg kg^{-1} for six months achieved long-term survival. The 20 mg kg^{-1} dose was most effective, with no animals rejecting during therapy. One month of therapy produced a single long-term survivor. Treatment for six months produced reliable long-term rejection free survival that was not significantly improved by prolongation of therapy to 12 months. Those animals treated for at least six months had a mean survival time of 750 ± 400 days, with two animals still alive at more than 913 and more than 1529 days after transplant. This experiment demonstrated that anti-CD154 given at a dose of 20 mg kg^{-1} for several months could reliably induce long-term survival without the addition of CTLA4-Ig in NHPs receiving renal allografts. They also highlighted the importance of intervention early in the postoperative course with high doses of antibody for optimal effect.

Since clinical adaptation of costimulatory agents would almost certainly require their use in conjunction with conventional agents, the effects of established immunosuppressants on treatment with anti-CD154 antibody were subsequently investigated (Kirk *et al.* 1999; Cho *et al.* 2001; Montgomery *et al.* 2002*a*; Xu *et al.* 2003*a*). A six month course of antibody was combined with conventional drugs in one of four arms and compared to animals treated with hu5C8 alone. One group received twice daily tacrolimus for a nine month course. The second group received steroids for nine months and mycophenolate mofetil (MMF) for 12 months. Another group received only 5 days of steroids, followed by 12 months of MMF. The last group received the anti-CD25 monoclonal antibody daclizumab. None of the animals treated with hu5C8 alone or hu5C8 with a short course of steroids and MMF experienced acute rejection during the course of antibody therapy. There was a trend towards a detrimental effect on rejection free survival for the groups receiving tacrolimus and those receiving chronic steroids. Three of five animals treated with hu5C8, MMF, and chronic steroids suffered acute rejection while still receiving hu5C8, as did one of three animals treated with tacrolimus. A second tacrolimus animal died of a viral infection with worsening renal function and histological evidence of rejection. Of the animals ($n=4$) that received daclizumab, one rejected during the six months of hu5C8 therapy. The few animals that completed their course of steroids or tacrolimus without rejection then demonstrated survival comparable to that of animals treated with hu5C8 alone.

Since hu5C8 was so effective in preventing renal allograft rejection, it was subsequently tested in a more rigorous skin allograft model (Elster *et al.* 2001*a*,*b*). The technical aspects of this model are illustrated in figure 2. Two untreated controls rejected rapidly at 5 and 7 days. Five animals were treated with hu5C8, using the 20 mg kg^{-1} six month course described above. In spite of a transient period of erythema between 7 and 21 days, these grafts survived for 66, 137, 312, more than 345, and more than 365 days. This demonstrated the robustness of costimulation-based therapy in the NHP, where acceptance of skin allografts has been otherwise difficult to achieve.

Given the success of manipulation of the CD154 pathway, attention was directed to evaluating other costimulation-based antibodies. The efficacy of monoclonal antibodies against CD80 and CD86 was investigated in the NHP renal allograft model (Kirk *et al.* 2001*b*). Monotherapy with either of these antibodies produced only modest increases in time to rejection. Survival was increased compared to untreated controls, but the longest survivor was only 40 days. Combination therapy with the clones 1f1 (anti-CD80) and 3d1 (anti-CD86) or their humanized counterparts was then investigated. The murine versions of these antibodies produced survival times of 25, 42, 77, and 227 days when used in combination and therapy was started on the day of transplantation. Delaying the initiation of therapy until postoperative day 2 produced survival times of 7 and 23 days. Four animals were treated with a combination of humanized 1f1 and 3d1 for two months following surgery. These animals survived for 47, 67, 227, and more than 906 days. One of these animals remains alive and well with no further therapy. None of these treatments prevented the eventual development of donor specific alloantibody.

Since the combination of these two antibodies against CD80 and CD86 was successful in prolonging survival but did not induce long-term tolerance, a regimen consisting of monoclonal antibodies against CD80, CD86, and CD154 was tested (Montgomery *et al.* 2002*b*). Two animals were treated with hu5C8 and murine 1F1 and 3D1 and two received hu5C8 in combination with the humanized anti-B7 antibodies. Their survival times were 668 and 911 days for the recipients of murine antibodies and 309 and 375 for the humanized anti-B7 antibodies. This was better than those treated with the anti-B7 antibodies alone, but it did not represent an improvement in survival over that achieved with hu5C8 alone. One interesting difference noted when the combined anti-B7/anti-CD154 therapy

Table 1. Renal and skin allograft rejection-free survival and treatment of all transplanted monkeys.

treatment group	renal allograft rejection-free survival	skin allograft survival
no treatment	7, 7, 6, 5, 2	8, 7, 7
sirolimus alone	23, 21, 10	11, 10
sirolimus, DST	10, 6, 5	9, 8
IDEC-131 alone	352, 44, 21, 7, 3	16, 16, 15, 13, 13, 13, 10, 10
IDEC-131, DST	42, 35, 7	10, 9, 7
IDEC-131, sirolimus	178, 108, 42, 9, 9	246, 84, 45, 35,15, 14
IDEC-131, sirolimus, DST	>1000, >1000, >750, 269, 168	246, 202, 76, 76, 33, 12

was compared to hu5C8 monotherapy was that animals receiving the combination therapy had a marked delay in the development of alloantibody. Animals treated with hu5C8 alone developed alloantibody during the first three months of therapy. Monkeys treated with combination therapy did not develop alloantibody until over 1 year had passed since kidney transplantation.

No opportunistic infections, neoplastic diseases, or wound complications were noted during therapy with any of the antibodies directed against costimulatory molecules alone. One of the animals treated with tacrolimus and hu5C8 did develop infectious diarrhoea, but other animals remained healthy until the time of rejection.

The observation that therapy directed at altering T cell costimulation leads to prolonged allograft acceptance but not tolerance in a primate allograft model is supported by additional work by several groups investigating kidney, heart and pancreatic islet transplantation (Levisetti *et al.* 1997; Kenyon *et al.* 1999*a*,*b*; Pierson *et al.* 1999; Hausen *et al.* 2001; Birsan *et al.* 2003).

Unfortunately, despite the exceptional promise of hu5c8 in NHPs, when this drug was used in early human trials, it was associated with thrombotic side effects that precluded completion of the trials. It also failed to prevent rejection in the few patients studied (Kirk *et al.* 2001*a*). As a result, a great deal of interest was thus focused on pursuing other anti-CD154 monoclonal antibodies, and identifying adjuvant immunosuppressants to limit the dose and duration of anti-CD154 therapy.

IDEC-131, a different anti-CD154 monoclonal antibody, had been used previously in clinical trials for systemic lupus erythematosus (Davis *et al.* 2001), and had been used by Pierson and colleagues with limited success as a monotherapy in a NHP cardiac transplant model (Pfeiffer *et al.* 2001; Pierson *et al.* 2001). In recognition of the fact that rodent studies had demonstrated a synergistic relationship between costimulation blockade and either donor specific transfusion or sirolimus (Parker *et al.* 1995; Rossini *et al.* 1996; Markees *et al.* 1998; Wells *et al.* 1999; Iwakoshi *et al.* 2000), this approach was subsequently adapted to the NHP using IDEC-131. Xu *et al.* in Kirk's lab undertook a trial to assess the efficacy of IDEC-131 in conjunction with donor-specific transfusion and sirolimus in a rhesus skin allotransplantation model (Xu *et al.* 2003*b*). They were able to demonstrate a clear prolongation of allograft survival using IDEC-131 in combination with sirolimus (table 1). Although the data suggested a beneficial effect of DST, the small numbers in the study precluded a definitive conclusion.

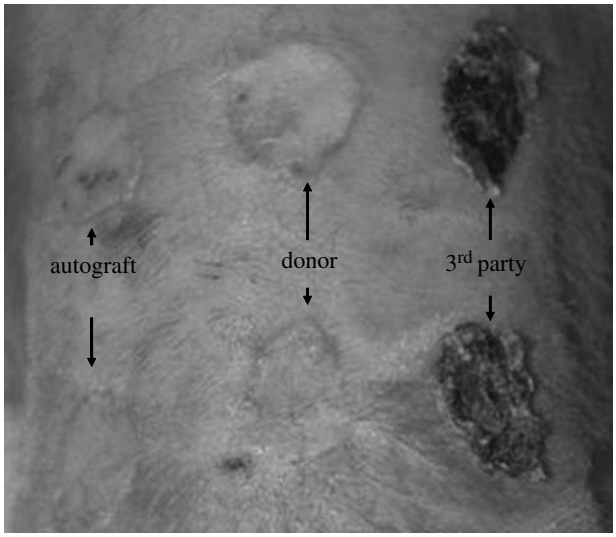

Figure 3. Appearance of second skin grafts performed 1 year after kidney transplant. Autografts and donor grafts are viable while third party grafts rejected in normal fashion.

This approach was extended to the NHP renal allograft model. Preston *et al.* were able to show marked improvement of rejection-free allograft survival using the triple therapy of IDEC-131 (for two months rather than six months), DST (a single perioperative 7 ml kg^{-1} blood transfusion)and sirolimus (dosed for three months) (Preston *et al.* 2005). All five animals that were given the three agents remained rejection-free during the period of therapy (90 days after transplant). Three of five animals have had indefinite rejection-free survival of greater than 750 days (table 1). In addition, two animals were tested for tolerance through a secondary immunologic challenge with donor and third party skin grafts at 1 year after renal transplant (see figure 3). These animals promptly rejected the third party grafts and accepted the donor skin while maintaining stable renal function. Interestingly, alloantibody formation was delayed until the time of rejection in animals that received IDEC-131. This is in contrast to other preclinical trials using anti-CD154 therapy where alloantibody developed prior to rejection (Elster *et al.* 2001*b*; Montgomery *et al.* 2002*b*; Ringers *et al.* 2002). As has been noted in all anti-CD154 approaches, stable renal allograft function was noted in the face of an interstitial T cell infiltrate on protocol biopsies. In

those animals that experienced rejection, graft dysfunction was coincident with monocyte/macrophage infiltration accompanied by T cell infiltrate, while isolated T cell infiltrates were not associated with allograft dysfunction. Perhaps most importantly, using a clinically applicable prophylaxis regimen of perioperative heparin and aspirin, there was evidence of pulmonary or cerebral thromboembolism. At the present time, neither hu5c8 nor IDEC-131 is available for clinical investigation. Other groups have evaluated the efficacy of costimulation blockade in a NHP allograft model. In 2002 Pearson *et al.* examined multiple antibodies aimed at costimulation blockade in rhesus monkeys (Pearson *et al.* 2002). Animals underwent renal transplantation and were treated with Chi220—a monoclonal antibody against CD40, CTLA4-Ig—a blocker of the B7/CD28 pathway, and H106—against CD40L. Although H106 targets the same pathway as Chi220, a recent study had shown that it also activated T cells (Blair *et al.* 2000). Median survival in the anti-CD40 group was 48.5 days. Median survival in the CTLA4-Ig group was 8 days. CD40 and CD40L were also combined with CTLA4-Ig. No synergistic increase in survival was seen with either combination. When CD40 was used alone, B-cell depletion occurred. This however did not prevent alloantibody formation. The addition of CTLA4-Ig to CD40 did prevent the formation of alloantibody. The study also found that costimulation blockade apparently increased susceptibility to overwhelming CMV infection by CMV+ recipients. This led to the recommendation that clinical trials involving costimulation blockade be done in seronegative recipients.

Costimulation blockade was also examined in islet cell transplantation. In this study Adams *et al.* examined LEA29Y, a mutant CTLA4-Ig analogue with increased binding activity (Adams *et al.* 2002). Rhesus monkeys underwent pancreatectomy and subsequently had islet cell transplantation. The monkeys were assigned to two treatment groups. The control group received rapamycin and anti IL-2R. The experimental group received LEA29Y, IL-2R, and rapamycin. Rapamycin was tapered and discontinued by day 121. LEA29Y was discontinued at around day 150. All treated animals' grafts functioned significantly longer than the control group but rejected after discontinuation of the LEA29Y. Throughout the experiment recipient peripheral blood lymphocytes were reacted to donor antigen and resulting INF-gamma was measured. In the control group, production of IFN-gamma was shown during rejection. No IFN-gamma production was seen in the experimental group during treatment. In four out of five experimental monkeys, alloantibody was not detectable. The authors concluded that this regimen, both steroid and calcineurin inhibitor free, was a viable strategy for clinical trials.

Thus, multiple strategies targeting costimulatory pathways have been proven safe and effective in NHPs. However, the early adverse experience with hu5c8 has tempered the enthusiasm for this approach. At present, LEA-29Y (Belatacept) is being tested as a calcineurin inhibitor sparing immunosuppressive agent in human kidney transplantation. No other costimulation blocking agents are actively being used in humans.

4. MIXED CHIMERISM

Donor BM infusion has been employed in experimental animals and in humans as a means of improving the efficacy of immunosuppressive regimens or as a component of tolerance induction regimens since the 1950s. The application of a conditioning regimen that leads to the engraftment of donor stem cells and a state of transient or permanent mixed chimerism is what differentiates this line of investigation from those previously discussed. At the NHP level, the demonstration of a transient state of macrochimerism has been shown to be sufficient for the induction of tolerance. To date, the most consistently successful and clinically applicable approaches for achieving mixed chimerism and tolerance in NHPs have involved the administration of conditioning regimens that may be associated with excessive morbidity to justify their use in solid organ transplantation.

After nearly a decade of studying the prospects of employing the mixed chimerism approach to tolerance in a rodent model (Sykes & Sachs 1988, 1990), the group at The Massachusetts General Hospital then took the painstakingly developed regimen and applied it to a cynomolgus monkey renal allograft model (Kawai *et al.* 1995; Kimikawa *et al.* 1997*a*). This study evaluated the effects of various manipulations of the ATG/irradiation preparative regimen on allograft survival and the induction of chimerism. The basic regimen consisted of horse anti-human ATG, two fractions of 150 rad each of total body irradiation, 700 rad of local thymic irradiation, followed by kidney transplantation and splenectomy. Donor specific BM cells at a dose of approximately 1×10^{8}cells kg^{-1} were administered on the day of transplant. This study was notable for the finding that monkeys treated with this regimen developed transient multilineage chimerism and accepted renal allografts long-term with no further immunosuppression. Conversely, if total body irradiation was administered in a single dose of 300 rad or a single dose of 150 rad, chimerism could not be demonstrated and there was no long-term graft survival even with the addition of cyclosporine to the regimen. Similarly, monkeys treated with the basic regimen minus the infusion of donor specific BM rejected the kidney allografts within 52 days. This study suggests that in this model, at least transient engraftment of donor BM appears to be essential for the induction of donor specific tolerance.

The importance of the depletional agent utilized in this regimen was emphasized in a subsequent study comparing the efficacy of horse ATG, rabbit ATG, and a monoclonal antibody (Medi-502) specific for CD2 (Kawai *et al.* 2000; Sogawa *et al.* 2001). Interestingly, the level of T cell depletion observed did not correlate with the induction of chimerism. While the horse ATG was the least effective T cell depleting agent, it was the most consistently successful regimen for producing chimerism. This could possibly be related to the fact that the non-depletional effects of horse ATG were effective in suppressing in T cell function.

This group conducted further studies to clarify the importance and toxicity of the various components of their mixed chimerism tolerance induction regimen (Kimikawa *et al.* 1997*b*). They did this by fractionating or reducing the amount of whole body irradiation administered, adding DSG, or individually omitting the various components. The studies clearly demonstrated that regimens omitting donor BM, cyclosporine or irradiation failed to facilitate the development of chimerism and were less effective in producing long-term graft survival. While six of six monkeys given 300 cGy of whole body irradiation became chimeric and demonstrated long-term graft survival, only two of four monkeys given 250 cGy did the same. This loss of efficacy could be partially offset by the administration of a two week course of DSG. Overall, the development of significant albeit transient lymphocyte chimerism (greater than 1.5%) was the most important predictor for tolerance.

To study the role of splenectomy and of the kidney allograft itself in producing a state of tolerance, this same group then manipulated the timing of kidney transplant and/or splenectomy on long-term kidney allograft survival (Kawai *et al.* 1999). These studies found that the immediate transplantation of the kidney at the time of BM infusion was not required for the induction of tolerance. In contrast, the ultimate development of anti-donor antibody and graft rejection were seen if splenectomy was not performed at the time of initial conditioning. These studies suggest a crucial role for splenectomy in the induction of B cell tolerance.

Subsequent extension of this tolerance induction protocol to non-kidney allografts has yielded interesting results. Nearly 3 years following the induction of a transient state of chimerism and tolerance to a kidney allograft, these investigators administered islets from the original kidney donor after having rendered the tolerant recipient diabetic through an injection of streptozocin (Kawai *et al.* 2001). This monkey received nearly 20 000 islet equivalents per kg overall. Approximately 11 000 islet equivalents per kg were infused into the portal venous system. In addition, 3000 islet equivalents per kg were placed under the kidney capsule. After six months, another infusion of 6000 islet equivalents per kg was made into the portal venous system. No immunosuppression was administered. Initial control of blood glucose levels was followed by the reestablishment of diabetes 300 days following islet transplantation. The animal was subsequently sacrificed and the autopsy demonstrated viable islets with strong insulin staining both in the liver and under the kidney capsule. There was no histologic evidence of rejection. It should be noted that renal allograft function remained normal throughout this period and there was no evidence of acute or chronic rejection on histopathological examination of the kidney allograft.

This classical model for the induction of tolerance through mixed haematopoietic chimerism was also applied to a heterotopic cardiac allograft model in cynomolgus monkeys (Kawai *et al.* 2002). In contrast to kidney allograft recipients, long-term heart allograft recipients eventually developed humoral and cellular immunity against the donor and rejected the grafts. Only three of five monkeys developed detectable multilineage chimerism and at least initially demonstrated *in vitro* evidence of donor specific hyporesponsiveness. Graft survival in these monkeys was 138, 428 and 509 days.

Subsequent refinements to the Massachusetts General Hospital model involved the addition of costimulation blockade to the conditioning regimen through the administration of anti-CD154 monoclonal antibodies. This agent was added to the primate model following the demonstration of efficacy of this approach in rodent models (Wekerle *et al.* 1998; Wekerle & Sykes 1999; Wekerle *et al.* 1999*a*,*b*). The primate experiments demonstrated that the addition of a short course of anti-CD154 monoclonal antibody to the standard regimen induced mixed chimerism more consistently and significantly enhanced the level and duration of chimerism observed (Kawai *et al.* 2004). Perhaps more importantly, the addition of anti-CD154 monoclonal antibody to the standard regimen precluded the need for splenectomy. Unfortunately, late chronic rejection was observed in three of eight recipients that had received anti-CD154 monoclonal antibody in lieu of splenectomy. These observations are important insofar as eliminating the requirement for splenectomy would greatly increase the appeal of this approach in human trials. Of note, in this study the authors reported that they were able to avoid the problematic prothrombotic complications associated with the use of anti-CD154 monoclonal antibodies through the concomitant administration of the anti-inflammatory agent ketorolac (Koyama *et al.* 2004).

The mixed chimerism approach has been reduced to clinical practice in an initial trial involving patients with both multiple myeloma and renal failure. Recipients of HLA identical BM and a renal transplant from the same donor in combination with non-myeloablative conditioning have been successfully rendered myeloma free and weaned from all immunosuppressive drugs (Buhler *et al.* 2002). This approach is now being investigated in the more generalizable setting of non-HLA identical donation in patients without a haematogenous malignancy.

5. SUMMARY

Many methods for greatly prolonging allograft survival with markedly reduced chronic immunosuppressive requirements have been tested and proven effective in NHPs. Although this rigorous model has not been completely predictive of success in the clinic, it has directly facilitated translational and proof-of-concept work in humans that would not otherwise have been possible. Given the marked similarities between NHPs and humans, it is likely that the reproducible success achieved in NHPs will eventually be realized in humans. A fluid transition back and forth between the NHP model and early phase clinical work will smooth the progress toward transplantation without the chronic requirement for immunosuppression.

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ENDNOTE

[1]http://www.optn.org/data/.

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