[Frontiers in Bioscience 12, 4248-4253, May 1, 2007]

Induction of allograft tolerance in nonhuman primates and humans

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1. ABSTRACT

Despite remarkable improvement in short-term survival following organ transplantation, long-term results have been less satisfactory, mainly due to chronic rejection or toxicities induced by immunosuppressive drugs. Therefore, induction of specific immunologic tolerance remains an important goal in organ transplantation. Although numerous regimens for the induction of allograft tolerance have been developed in rodents, their application to large animal models has been limited. The mechanisms of action of the approaches that have been successfully applied in monkey models can be divided into three major categories: 1) deletion, 2) co-stimulatory blockade and 3) regulation. Long-term allograft survival has now been achieved in several nonhuman primate models; however, late-onset chronic rejection as well as the toxicity of some of these regimens remain as significant limitations that hamper clinical application.

2. INTRODUCTION

With the use of newly developed immunosuppressive drugs, the incidence of acute rejection following organ transplantation has been significantly decreased. However, these improved short-term results have not greatly improved long-term results, the real "graft half life" for first transplants has only marginally improved (1). Therefore, the induction of specific immunologic tolerance of the transplanted organ has remained an important goal in organ transplantation.

Since Medawar *et al.* reported successful induction of neonatal tolerance in a rodent model in 1953 (2), numerous protocols for induction of tolerance have been developed in rodent models. Nevertheless, only a limited number of protocols have been successfully translated to nonhuman primate models. Those approaches can be largely divided into three major categories: deletion,

costimulatory blockade, and regulation (Fig.1), according to the presumed mechanism of tolerance induction. However, in some protocols, two or all three of these mechanisms may be involved.

In this review, we summarize current protocols applied to nonhuman primate models and clinical transplantation for induction of allograft tolerance.

3. STARATEGIES FOR TOLERANCE INDUCTION IN NON-HUMAN PRIMATES

3.1. Deletion

3.1.1 Peripheral lymphocyte depletion

Anti-CD3 immunotoxin (IT) was initially produced by Neville *et al.* who conjugated the anti-rhesus CD3 mAb, FN18, to the mutant diphtheria toxin protein, CRM9 (3). This immunotoxin effectively depletes rhesus monkey peripheral blood and lymph node T cells to less than 1% of pretreatment levels. Knechtle *et al.* investigated the use of anti-CD3 immunotoxin (IT) for prolongation of renal allograft survival. In their experiments, all monkeys treated with anti-CD3 IT had significantly prolonged allograft survival (4). However, the production of anti-donor alloantibody was not prevented, resulting in alloantibody-mediated glomerular and arterial damage of the kidney allograft (5). Contreras *et al.* also tested anti-CD3 IT, starting at the time of renal transplantation (6). In contrast to the results reported by Knechtle, anti-CD3 IT alone or with methylprednisolone (MP) did not greatly prolong graft survival. Subsequent studies showed that deoxyspergualin (DSG) added to the regimen completely prevented a vascular leak syndrome that had been observed with the original protocol; 87% of monkeys receiving DSG survived long-term (7). With this regimen, IFN-γ levels were depressed at all time points, whereas plasma IL-4 became elevated at 3 weeks after treatment. These investigators concluded that the early control of systemic proinflammatory cytokine levels is essential to subsequent Th2 cytokine deviation and development of tolerance. They suggested that the mechanism of tolerance induction using anti-T cell immunotoxin is based upon mature T cell depletion and cytokine production inhibition with DSG which apparently suppresses activation of nuclear factor-κB (NFκB).

3.1.2. Central Deletion - Mixed chimerism

Based on previous murine studies (8) in which mixed chimerism and skin allograft tolerance were induced by a non-myeloablative conditioning regimen, we successfully developed a similar non-myeloablative protocol for induction of renal allograft tolerance in cynomolgus monkeys (9-11). The previous rodent studies clearly demonstrated thymic deletion of donor-reactive T cells as a mechanism of tolerance in this approach. The initial conditioning regimen used in monkeys was similar to that used in mice with respect to 1) low dose total body irradiation (TBI, 3 Gy), 2) administration of thymic irradiation (TI, 7 Gy), and 3) the use of depleting anti-T cell antibody. However, splenectomy and a one-month course of cyclosporine (CyA) were necessary in the monkey regimen because adequate T-cell depletion proved to be more difficult to achieve in large animals. With this regimen, 12/15 recipients developed chimerism and 8/15 survived long-term. The longest survival currently exceeds 13 years with normal kidney allograft function. Although the induction of permanent chimerism was required for induction of skin allograft tolerance in mice, chimerism induced in the monkeys was only transient, yet capable of inducing renal allograft tolerance. Early thymic chimerism has been detected in these nonhuman primates, suggesting that partial central deletion of donor reactive cells may be operative in the early phase of allograft tolerance induction via this approach (12). However, continued survival of the kidney allograft despite the loss of chimerism suggests that peripheral mechanisms must also be operative. Subsequent studies have shown that splenectomy can be omitted if a short course of CD154 blockade is added (12). With additional CD154 blockade, mixed chimerism was significantly enhanced; all 8 recipients developed chimerism and half of them acquired renal allograft tolerance, with the longest survival exceeding 5 years. Further modifications are currently being tested to improve the consistency of tolerance induction.

3.2. Co-stimulatory signal blockade

For full activation of T cells, both TCR-mediated and simultaneously delivered costimulatory signals are necessary. Antigen-specific signals through TCR in the absence of costimulatory signals lead to antigen-specific unresponsiveness in T cells. Among the costimulatory signals that have been identified, blockade of the CD28 or CTLA4 (CD152) /CD80,CD86 and the CD40/CD40 ligand (CD154) pathways have been tested in nonhuman primate models. Although blockade of CD80 and CD86 has not appreciably prolonged graft survival in monkeys (13), blockade of the CD40 / CD154 pathway has proved to be highly effective (14, 15). Unfortunately, these recipients also developed donor specific alloantibody and eventually chronic rejection after discontinuation of immunosuppressive treatment. Kirk also tested a combination of CD154 blockade, sirolimus and donor specific transfusion in rhesus monkeys and achieved long-term renal allograft survival in 3/5 recipients with specific acceptance of skin allograft in two (16). More recently Haanstra reported long-term stable renal allograft tolerance in two of four Rhesus monkeys treated with a combination of anti-CD40 and anti-CD86 mAbs, followed by a 12-week course of CyA (17).

CTLA4Ig has also been tested in nonhuman primates but provided only moderate prolongation of allograft survival (18, 19). The reason for this might has been the inability of this agent to sufficiently block CD28/CD80,CD86, especially CD28/CD86 interactions. A modified version of CTLA4Ig, LEA29Y in which two amino acids were substituted to provide slower dissociation rates for both CD80 and CD86 has recently been tested in monkeys. Initial reports indicate prolongation of renal allograft survival has been limited with only two of five recipients surviving over 200 days even with addition of mycophenolate mofetil and steroids (20).

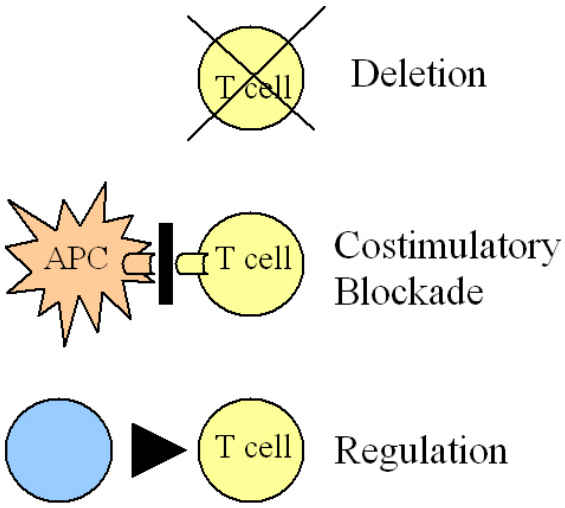

Figure 1. Strategies for tolerance induction tested in non-human primates. Three major approaches for tolerance induction include; central or peripheral deletion, costimulatory blockade and regulation (suppression) of T cells.

3.3. Regulation

3.3.1. Induction of peripheral tolerance by infusion of donor bone marrow cells

Based on the murine studies by Monaco *et al.* (21), Thomas *et al.* developed a regimen which consisted of rabbit anti-thymocyte antibody and donor bone marrow (DBM) cell infusion in rhesus monkeys (22). In these approaches, engraftment of donor bone marrow cells was not a prerequisite to the establishment of immunologic tolerance, and chimerism was typically not detectable by flow cytometric assays. In this protocol, temporary functional activity of specific BM components has been cited as a possible mechanism leading to the prolonged allograft survival achieved in some recipients (23). With this regimen, graft survival exceeded one year in approximately 50% of monkeys, and specific suppression of anti-donor CTL responses was demonstrated in long-term survivors. However, the production of anti-donor alloantibody could not be prevented and most recipients eventually succumbed to chronic rejection (23, 24).

3.3.2. Adoptive transfer of regulatory T cells

Since Sakaguchi defined CD4+CD25+ cells as an important T regulatory cell population to control autoimmunity (25), accumulating evidence has supported that these regulatory T cells are also important in induction and maintenance of allograft tolerance (26). Bashuda *et al.* recently reported successful induction of renal allograft tolerance in 3/6 monkey recipients who received adoptive transfer of anergic T cells induced by coculture with donor alloantigen in the presence of anti-CD80/CD86 antibody (27). Although a large dose of cyclophosphamide (30mg/kg x 8-10 days) was required to condition the recipients, this study was the first demonstration of renal allograft tolerance induction by adoptive transfer of regulatory cells in nonhuman primates.

4. CLINICAL TRIALS

The first clinical attempt for renal allograft tolerance was reported by Murray *et al.* in 1960 (28). In this clinical trial, high dose TBI was administered with or without DBM transplantation to induce fully allogeneic chimerism. However, only one recipient out of six patients survived long-term, while others died due to either infectious or hemorrhagic complications. After this report, although "spontaneous tolerance" was reported especially in liver transplant recipients, formal clinical trials for operational tolerance were not pursued for the subsequent three decades.

In 1991, Barber *et al.* reported a clinical trial using DBM combined with ALG. Although significantly better allograft survival was observed in recipients treated with DBM, immunosuppression was not completely discontinued in any of these recipients and clear evidence of tolerance was not demonstrated in these studies (29).

In 1992, Starzl *et al.* reported seminal observations of 30 recipients of livers or kidneys with long-term stable graft function. With sensitive cytostaining and PCR techniques, small numbers of donor leukocytes (microchimerism) were identified in one or more peripheral recipient locations in all 30 patients (30, 31). It was hypothesized that responses of coexisting donor and recipient cells can result in reciprocal clonal exhaustion, followed by peripheral clonal deletion (32). To enhance such "microchimerism" state after organ transplantation, groups in Pittsburgh and Miami evaluated DBM infusion with conventional immunosuppression in various organ transplants. In these studies, although superior graft survival with less incidence of acute or chronic rejection was reported, clear evidence of tolerance induction has not been demonstrated (33, 34).

Tolerance induction through mixed chimerism has been attempted in HLA-matched or mismatched kidney transplants. In HLA matched kidney transplants, six renal failure patients with refractory multiple myeloma underwent combined kidney and bone marrow transplantation (CKBMT) after a cyclophosphamide based non-myeloablative conditioning regimen (35, 36). Three patients developed transient mixed chimerism and their immunosuppressants were successfully discontinued, with the longest kidney graft survival exceeding 6 years. In the other three patients, mixed chimerism was converted to full chimerism for treatment of myeloma, after which minimum amount of immunosuppression was required to control chronic GVHD (37). In HLA haplotype mismatched kidney transplant, five patients with end stage renal disease without malignancies underwent CKBMT after a nonmyeloablative conditioning. All five recipients developed transient mixed chimerism and immunosuppression was successfully discontinued in four of five patients with the longest immunosuppression free survival exceeding 3 years (Kawai *et al.* manuscript submitted).

Clinical trials of anti-CD154 mAb in kidney transplantation were suspended because of thromboembolic

complications observed in the initially tested patients. The precise mechanisms of thrombophilia associated with anti-CD154 monoclonal antibody (mAb) treatment have not been clarified. However, in our monkey studies, we found that Ketorolac, a non-steroidal anti-inflammatory drug, is markedly effective for preventing thromboembolic complications. This indicates involvement of platelet activation as a cause of thrombosis (38).

Several groups have also attempted tolerance induction by profound lymphocyte depletion. Calne el al. first reported a condition described as "Prope (almost) tolerance", in which low rejection rates of renal allografts were achieved with low dose cyclosporine monotherapy after peri-transplant alemtuzumab (Campath-1H) (39). Alemtuzumab is a humanized monoclonal antibody that binds CD52, which is expressed on various hematopoietic cells including T cells, B cells, monocytes, natural killer (NK) cells and dendritic cells. Rapid and profound lymphocyte depletion was achieved with only two doses of this antibody. Starzl *et al.* reported 90 clinical kidney transplant recipients who were treated with alezutumab or rabbit thymoglobulin (40). In these studies, spaced weaning of immunosuppressant was achieved in selected patients. However, there was no improvement in patient and graft survival compared with historic controls, and chronic allograft nephropathy (CAN) progressed at the same rate (41). Kirk *et al.* added deoxyspergualine (DSG) to alemtuzumab to test the strategy developed by Thomas (7). However, despite profound T-cell depletion and therapeutic DSG dosing, all alemtuzumab/DSG patients developed reversible rejection that was similar to that seen in patients treated with alemtuzumab alone (42). Knechtle *et al.* has been testing rapamycin monothrapy followed by profound lymphocyte depletion by alemtuzumab (43).

5. SUMMARY AND PERSPECTIVE

With the use of non-myeloablative conditioning regimens that allow DBMC engraftment or with the administration of recently developed agents such as anti-CD3 IT or anti-CD40L mAb, long-term allograft survival has now been achieved in several nonhuman primate models. Some of these animals have developed *in vitro* donor-specific unresponsiveness and have even accepted donor-specific skin grafts. However, late-onset chronic rejection, as well as the toxicity of some of these regimens, remain as significant limitations that hamper clinical application.

6. ACKNOWLEDGEMENT

Supported by NIH grant 2RO1 AI37692006A1 and 5PO1 HL 1864625. The authors thank Ms. Crystal Augusta for editorial assistance.

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Key Words: Transplantation, Immune Tolerance, Chimerism, Costimulatory Blockade, Non-Human Primates, Review

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