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PRECLINICAL AND CLINICAL STUDIES ON THE INDUCTION OF RENAL ALLOGRAFT TOLERANCE THROUGH TRANSIENT MIXED CHIMERISM

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Abstract

Purpose of review—This review updates the current status of research for induction of tolerance through a mixed chimerism approach in nonhuman primates and humans.

Recent findings—Allograft tolerance has been successfully achieved with a nonmyeloablative conditioning regimen and donor bone marrow transplantation in HLA-matched and mismatched kidney transplantation. In HLA-matched kidney transplantation, persistent mixed chimerism and renal allograft tolerance has been achieved in some patients. In HLA-mismatched combinations, induction of persistent mixed chimerism has not been achieved using a nonmyeloablative preparative regimen. Nevertheless, the transient mixed chimerism that has been achieved has resulted in long-term renal allograft tolerance in the majority of patients. Recent preclinical studies have demonstrated that the presence of heterologous memory T cell responses observed in primates, but not in rodents, may be a major barrier for induction of durable chimerism and tolerance in primates. Strategies to overcome such memory T cell responses may therefore be of great value in the development of reliable protocols for clinical tolerance induction.

Summary—Induction of tolerance in clinical kidney transplantation has been achieved via mixed chimerism approaches. Improvements in the consistency and safety of tolerance induction and extension of successful protocols to other organs and to organs from deceased donors will all be among the next steps in bringing tolerance to a wider range of clinical applications.

Keywords

mixed chimerism; kidney transplantation; tolerance

INTRODUCTION

Despite the remarkable improvement over the past two decades in short-term results following organ transplantation, the long-term results remain disappointing [1], mainly due to chronic rejection and the toxicities of chronically administered immunosuppressive drugs [2]. Therefore, induction of specific immunologic tolerance remains an important goal of organ transplantation. Although many tolerance induction strategies have been developed in rodent models, only a limited number of them have been successfully translated to nonhuman primates (NHP) and even fewer to humans.

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TOLERANCE INDUCTION IN NHP AND CLINICAL TRIALS

Among several tolerance strategies that have been tested in clinical transplantation, induction of mixed chimerism through donor bone marrow transplantation (DBMT) is thus far the only approach that has relatively consistently induced allograft tolerance in clinical transplantation.

The conditioning regimens used for induction of allograft tolerance have generally been nonmyeloablative, following which recipients are able to recover from pancytopenia even without engraftment of donor bone marrow (DBM) cells. The chimerism induced after such nonmyeloablative regimens are generally characterized as “mixed chimerism”, a state in which both donor and recipient lymphohematopoietic elements co-exist in the recipient. This state distinguishes these individuals from those with full (i.e. 100%) chimerism observed after myeloablative conditioning.

Studies of the mixed chimerism approach in NHP

Based on previous rodent studies of mixed chimerism [3,4], we developed a clinically relevant, non-myeloablative preparative regimen that permitted the induction of mixed chimerism and renal allograft tolerance following DBMT in fully MHC-mismatched cynomolgus monkeys [5–7]. Components of the initial preparative regimen for monkeys included total body irradiation (TBI) (150 cGy x 2), plus local thymic irradiation (700 cGy), horse anti-thymocyte globulin (ATG), splenectomy, DBM and a one-month post-transplant course of cyclosporine. Eleven of 13 recipients that received this regimen developed multilineage chimerism and 9 survived long-term with normal renal function, with the longest survival exceeding 16 years [7].

We subsequently developed a modified protocol, in which a short course of anti-CD154 mAb was administered to replace splenectomy. With this modification, all recipients consistently developed mixed chimerism and half of them acquired renal allograft tolerance [8].

The major drawback of our current protocol is its inapplicability to deceased donor transplantation, since the conditioning regimen must be initiated a week before the planned organ transplant. Simple compression into 24 hours of the previously successful 6-day therapeutic protocol not only failed to induce chimerism but also led to unacceptable toxicity. Therefore, we have recently developed a protocol in which recipients first undergo kidney transplantation with conventional immunosuppression, followed by conditioning and donor bone marrow transplantation several months later. Our studies of this “Delayed Tolerance” approach have revealed that additional depletion of CD8 memory T cells (Tmem) over that provided by the previously successful living donor regimen was required to induce mixed chimerism and renal allograft tolerance [9]. More recently, we have shown that timing of attempted tolerance induction is critical. We compared the delayed tolerance protocol at one and four months after kidney transplantation. Although both recipient groups developed comparable levels of mixed chimerism, only recipients who received DBMT at 4 months successfully achieved renal allograft tolerance. Additional studies revealed that significantly higher inflammatory responses were detectable by RT-PCR in the one month group, which likely contribute to the failure of allograft tolerance (manuscript submitted).

Unlike the previous rodent studies, mixed chimerism observed in NHP has been transient [6,7,10] and continued survival of the kidney allograft despite the loss of chimerism suggests that peripheral mechanisms are also operative. In vitro assays to elucidate the mechanisms of tolerance after transient chimerism have suggested involvement of T regulatory cells. For example, donor specific MLR hyporesponsiveness in tolerant recipients

can be restored by removing recipient CD25+ cells from the MLR (Andreola G. et.al Am J Transpl 2011, in press). We also applied CSFE-MLR in tolerant recipients, which revealed donor specific expansion of Foxp3+ cells (manuscript in preparation).

Another interesting observation has been the organ specific nature of tolerance induction via the mixed chimerism approach. Although successful induction of renal allograft tolerance has been achieved in the majority of recipients after transient mixed chimerism, heart allograft tolerance has not been achieved with the same protocol [11]. This has led us to pursue approaches to inducing more persistent chimerism. Studies in both mice [12] and miniature swine [13] from our center have demonstrated that the induction of persistent mixed chimerism and allograft tolerance was possible even with less intense recipient conditioning, if very high doses of bone marrow cells were transplanted. We thus established a protocol to mobilize and collect peripheral blood stem cells in cynomolgus monkeys, which enabled us to infuse an approximately tenfold increased number of CD34+ cells than that provided by regular BMT. These peripheral blood stem cells (PBSC) were infused to the recipients after administration of a nonmyeloablative regimen. Although the levels of chimerism were significantly higher and persisted longer, recipients consistently lost chimerism by day 50 [14*]. This study did demonstrate, however, that renal allograft tolerance can be induced with PBSC as well as DBM. Larsen et.al recently studied the impact of MHC matching on the stability of mixed chimerism after nonmyeloablative conditioning in rhesus monkeys [15**]. Using a busulfan-based pre-transplant preparative regimen, followed by maintenance immunosuppression with sirolimus and CD28 plus CD154 blockade, all recipients achieved donor engraftment. The results confirmed that increasing MHC matching resulted in significant prolongation in engraftment. Nevertheless, donor engraftment was eventually lost after immunosuppression withdrawal, even in the two haplotype MHC-matched cohort. Chimerism induced after conditioning was predominantly myeloid, with very low levels of T cell chimerism. Since the majority of T cells after stem cell transplantation were recipient T cells, the authors speculated that a surge of donor-reactive memory T cells was responsible for rejection of donor bone marrow-derived cells after discontinuation of immunosuppression.

These obstacles to inducing chimerism/tolerance may be attributed to a broad spectrum of memory T cell (Tmem) responses that are present in primates but not in laboratory rodents. Humans and monkeys are exposed to the numerous environmental pathogens, vaccinations, transfusions and transplantation, all of which lead to significant memory Tmem development. It has been demonstrated that these T mem cross-react with unrelated pathogens or alloantigens, a phenomenon known as heterologous immunity [16–18]. These heterologous Tmem responses have been demonstrated to cause resistance to tolerance induction by costimulatory blockade [19] and by the mixed chimerism approach [20]. Homeostatic proliferation of Tmem observed following T cell depletion has also been demonstrated to generate allospecific Tmem and induce similar resistance to tolerance induction by costimulatory blockade [21]. Since most clinical tolerance induction trials include severe T cell deletion [22–24], homeostatic proliferation following T cell depletion could block the process of chimerism/tolerance induction.

We also tested memory T cell responses to alloantigens by ELISPOT in over 100 cynomolgus monkeys. Memory alloreactivity mediated via direct but not indirect allorecognition was detected in all animals but the frequency of allospecific memory T cells varied dramatically depending upon the nature of the responder/stimulator monkey combinations tested. Although MHC gene matching was generally associated with a low memory alloreactivity, this was not consistent. Thus, favorable chimerism/tolerance induction may be achievable by selecting donor/recipient combinations displaying a low memory alloresponsiveness even without favorable MHC matching [25**]. Other modalities

for specific suppression of Tmem have been reported in NHP, which may be critically important to achieve more sustained chimerism [26] [27].

Mixed chimerism approach in HLA-identical kidney transplantation

Using TLI and DBMT, successful induction of stable mixed chimerism and renal allograft tolerance has been reported in HLA identical kidney transplantation [28]. The protocol consisted of TLI (80 cGy X 10, days 1–14), anti-rabbit thymocyte globulin (1.5 mg/kg X 5, days 0–4), and HLA-matched peripheral blood stem cells on day 14. The first patient received triple immunosuppressive therapy (mycophenolate mofetil, prednisone and cyclosporine) after transplantation, but all immunosuppressive medications were slowly tapered over 6 months. The patient continued to do well, with immunosuppression-free normal kidney function and stable mixed chimerism for more than 28 months. This protocol was subsequently tested in 12 additional HLA-matched kidney transplant recipients (Strober et.al Abstract #O43.05 in XXIII International Congress of The Transplantation Society, 2010). All 12 patients developed persistent mixed chimerism with excellent graft function for 4–53 months of observation. At the time of report, six had been completely withdrawn from immunosuppressive medications, one for 3 months and 5 for 1–3 years. A potential advantage of this approach might be applicability to deceased donor transplantation, since all treatments in the conditioning regimen are initiated after organ transplantation. However, extension of this protocol to HLA-mismatched kidney transplantation would be required and attempts to do so have been less promising (see below).

At Massachusetts General Hospital (MGH), ten patients with renal failure secondary to multiple myeloma have received HLA identical combined kidney and bone marrow transplantation (CKBMT) after conditioning with a non-myeloablative regimen. Long-term clinical outcome of seven of these patients has recently been summarized [29*]. Our preparative regimen consisted of cyclophosphamide (60mg/kg X2), local thymic irradiation 700 cGy, horse ATG and a 60-day course of post-transplant cyclosporine administration [30–32]. All seven patients developed mixed chimerism, which became undetectable by day 100 after CKBMT in four of them. Remission of myeloma was observed in five of the seven patients but one died 7 years later due to recurrence of myeloma. In two of the patients with early myeloma recurrence, one lost renal function due to myeloma recurrence and eventually died four years later. The other patient underwent second stem cell transplantation one year later and achieved remission of the myeloma. Except for one patient with early myeloma recurrence, all kidney allografts were accepted with no evidence of rejection. Four patients successfully discontinued all immunosuppression, but two patients with stable chimerism have been on low dose immunosuppression to control minor symptoms of chronic GVHD. Three additional patients with refractory myeloma and renal failure have subsequently undergone CKBMT using the same protocol, and chimerism in two of these cases has been stable, with normal kidney function at the time of this writing.

These observations demonstrate that CKBMT with a non-myeloablative regimen from an HLA-matched donor can be an excellent option for renal failure secondary to myeloma, a clinical situation in which no other effective treatment option has been previously available.

Mixed chimerism in HLA-mismatched kidney transplantation

As described above, a successful HLA-matched CKBMT for renal failure secondary to refractory myeloma was reported in 1999. Shortly thereafter, the Stanford group attempted DBM with a TLI-based regimen for induction of renal allograft tolerance in HLA mismatched kidney transplantation [33]. Their protocol consisted of kidney transplantation on day 0, immediately followed by rabbit ATG (five doses), TLI (a cumulative dose of 1220–2000 cGy), and CD34+ donor stem cell transplantation immediately after the last dose

of TLI (day 11), which was based on their previously reported studies in both rodents [34,35] and patients [36,37]. In this clinical trial, three of four recipients developed transient multilineage chimerism but rejection developed when immunosuppression withdrawal was attempted [33]. These investigators concluded that ,despite its successful application to recipients of HLA identical kidneys, this protocol did not induce allograft tolerance in HLA mismatched recipients [38].

At MGH, we applied a non-myeloablative conditioning regimen to one-haplotype HLA-mismatched kidney transplantation. Unlike the situation for myeloma patients, GVHD at any level would be unacceptable for renal transplant recipients without malignant disease. Fortunately, ongoing studies at MGH using one-haplotype mismatched BMT for the treatment of hematologic malignancies, had been attempting to replace ATG with an anti-CD2 mAb (MEDI-507) in a non-myeloablative protocol [39], and one of the regimens tested for HLA mismatched BMT appeared to be exactly what was needed for our CKBMT protocol. This particular preparative regimen was not ideal for treatment of hematologic malignancies because the chimerism induced was transient, but unlike the ATG regimen, no GVHD was observed. In 2002, using this regimen, we initiated the first of two ITN/NIAID-sponsored tolerance induction studies via the mixed chimerism approach in HLA-mismatched transplant recipients.

To date, a total of 10 subjects have been enrolled into these two studies. The results of the first trial have been published [40]. The conditioning protocol was modified following the observations made in the first 3 subjects of the first trial, with the addition of more intense anti-B cell depletion (Rituximab), in an effort to better control humoral responses, plus a brief course of steroids, to ameliorate the symptoms associated with the “engraftment syndrome” (see below). All recipients developed transient mixed chimerism, lasting up to 21 days. Withdrawal of immunosuppressive therapy was accomplished according to protocol design in 8 of these 10 subjects. Stable renal allograft function without maintenance immunosuppression, has been maintained in 7 of these 8 subjects for follow-up periods to date of 14 to >90 months. Only one of these 8 recipients developed an episode of acute cellular rejection, which occurred seven weeks after immunosuppressive therapy had been withdrawn. Six months following re-institution of immunosuppression, his renal allograft function has improved, but has remained compromised (Kawai et al., manuscript in preparation). Another of these recipients has developed anti-donor class II antibody and evidence of C4d deposition on protocol biopsies, although his allograft function has remained normal for over five years, with no albuminuria. All 7 recipients show donor-specific unresponsiveness by in vitro assays. Although the mechanisms of long-term kidney tolerance remain incompletely defined, our in vitro observations suggest that suppressive T cell populations play a role in tolerance induction, after which there appears to be deletion of donor-reactive T cells (Andreola G. et.al, Am J Transpl 2011, in press).

The clinical courses of the two recipients who were not able to be weaned from immunosuppression as planned were complex. The first developed irreversible humoral rejection during the second post-transplant week, as reported [40]. The other developed progressive thrombotic microangiopathy (TMA), thought to be the consequence of tacrolimus toxicity, although some element of cellular rejection could not be ruled out. Humoral rejection was ruled out by the absence of C4d staining and failure to detect serum donor-specific alloantibodies. Calcineurin inhibitor immunosuppression was discontinued, but the allograft eventually failed and she was returned to dialysis, six months after transplantation (Kawai et al, manuscript in preparation).

In summary, the outcomes for 7 of 10 subjects enrolled into these trials have fulfilled the current operational definition of tolerance as successful withdrawal of all

immunosuppressive therapy for greater than one year [41,42]. Nevertheless, in order to increase the applicability of this mixed chimerism approach, additional modifications of the protocol are being considered to decrease morbidity of the procedure. In particular, the most significant adverse event experienced by 9 of the 10 recipients has been a constellation of symptoms and signs including fever, fluid retention and renal dysfunction, referred to as "engraftment sydndrome" ([40] and manuscript submitted). This syndrome, which has been described in recipients of both allogeneic and autologous bone marrow transplants [43], is of most concern in patients undergoing this protocol because of its effects on renal function, which is undoubtedly already compromised in a newly transplanted kidney in a patient being treated with calcineurin inhibitors, which are known to be nephrotoxic [44]. We are therefore planning further investigations of the mechanism of this syndrome as well as considering use of a less nephrotoxic T cell suppressant during the second post-transplant week, when the syndrome is observed.

CONCLUSIONS

The long-term, full hematopoietic chimerism which results from myeloablative conditioning and reconstitution with HLA-matched bone marrow, usually for the treatment of hematologic malignancies, has been known for many years to induce tolerance of other organs from the same donor [45]. However, as described above, it is now clear that tolerance of renal allografts can also be achieved in primates and in the clinic by protocols leading only to chimerism that is transient rather than long-term and mixed rather than full. In addition, such protocols can be effective even across HLA disparities [40]. To date, however, these protocols have only been applicable to transplants from living donors and have been effective for kidney but not heart transplants. These limitations, as well as the morbidity of the preparative regimens utilized to date, make it clear that more work needs to be done in order to achieve more wide-spread applicability of tolerance regimens to the treatment of organ failure by transplantation. Nevertheless, for the patients in whom renal allograft tolerance has been achieved, this treatment has restored them to a much more normal life than can be achieved for patients on chronic immunosuppression. In addition, these studies have demonstrated that induction of transplantation tolerance is feasible not only in animal models but also in humans. As such, these results represent a start for application of tolerance in the clinic, and will hopefully lead to additional trials capable of extending the benefits of drug-free transplant survival to much broader donor and recipient populations.

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KEY POINTS

- Induction of allograft tolerance has been achieved via the mixed chimerism approach in HLA matched or mismatched clinical kidney transplantation.
- Induction of transient mixed chimerism has been sufficient to induce renal allograft tolerance in nonhuman primates and humans.
- In vitro studies have shown involvement of T regulatory cells in the maintenance of tolerance after transient chimerism.