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HEMATOPOIETIC STEM CELL INFUSION/TRANSPLANTATION FOR INDUCTION OF ALLOGRAFT TOLERANCE

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Abstract

Purpose of review—This review updates the current status of basic, preclinical, and clinical research on donor hematopoietic stem cell infusion for allograft tolerance induction.

Recent findings—Recent basic studies in mice provide evidence of significant involvement of both central deletional and peripheral regulatory mechanisms in induction and maintenance of allograft tolerance effected through a mixed chimerism approach with donor hematopoietic stem cell infusion. The presence of heterologous memory T cells in primates hampers the induction of persistent chimerism. Durable mixed chimerism, however, now has been recently induced in inbred major histocompatibility complex-mismatched swine, resulting in tolerance of vascularized composite tissue allografts. In clinical transplantation, allograft tolerance has been achieved in human leukocyte antigen-mismatched kidney transplantation after the induction of transient mixed chimerism or persistent full donor chimerism.

Summary—Tolerance induction in clinical kidney transplantation has been achieved by donor hematopoietic stem cell infusion. Improving the consistency and safety of tolerance induction and extending successful protocols to other organs, as well as to organs from deceased donors, are critical next steps to bringing tolerance to a wider range of clinical applications.

Keywords

allograft tolerance; hematopoietic stem cell transplantation (HSCT); donor bone marrow transplantation (DBMT); chimerism; kidney transplantation; vascularized composite allograft

Introduction

With the use of newer immunosuppressive regimens, short-term outcomes, such as one-year patient and allograft survival and rate of acute rejection in the first year after transplantation have steadily improved over the past three decades [1]. Long-term allograft survival, on the other hand, has been less than satisfactory, owing mainly to chronic rejection and the deleterious side effects of immunosuppressive drugs [2]. Cardiovascular disease, infectious

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complications, and malignancies associated with chronic immunosuppression significantly diminish the longevity of transplant recipients [3-5], not to mention their quality of life. A significant number of organ transplant recipients who survive beyond the first year, may also succumb to chronic rejection, which is characterized by perivascular inflammation, fibrosis, and arteriosclerosis [6-8]. Therefore, the development of safe, reliable protocols for allograft tolerance induction remains an important goal in organ transplantation. This article updates the current status of basic, preclinical, and clinical research on donor hematopoietic stem cell (HSC) infusion or transplantation for allograft tolerance induction.

Basic studies in murine models

Mice infused with hematopoietic stem cells (HSC) from allogeneic donors tend to become immunologically tolerant to allogeneic major histocompatibility complex (MHC) antigens and thereby accept allotransplants from the same donors [9-11]. While this principle is well established, the mechanisms by which transplantation tolerance is induced and maintained through hematopoietic chimerism are not completely understood. First, the nature of donor HSC and the level and duration of hematopoietic chimerism required to achieve transplantation tolerance have not been fully elucidated. Second, the relative contributions of thymic deletion vs. peripheral regulation of host alloreactive lymphocytes to the initiation and maintenance of tolerance remain to be determined. Recent studies in mice have shed light on some of these questions.

Stable hematopoietic macrochimerism, defined as the sustained presence of high numbers of donor-derived professional MHC class II⁺ antigen-presenting cells (APCs), such as dendritic cells and B cells, induces continuous depletion of newly developing T and B alloreactive lymphocytes in primary lymphoid organs (i.e., thymus and bone marrow) [12-15]. Prolonged macrochimerism can be achieved regularly in irradiated mice by the infusion of large doses of donor HSC and short-term immunosuppression [9]. Such procedures induce a particularly robust and durable form of tolerance, as evidenced by the ability of chimeric mice to accept, indefinitely, fully MHC disparate skin and solid organ allografts from their HSC donors [16]. The development of non-myeloablative protocols designed to achieve mixed chimerism, in which both host and donor hematopoietic cells coexist in the recipient, has provided an elegant solution to the problem of graft-versus-host disease (GVHD) [17,18].

Kurz et al. have reported that maintenance of transplantation tolerance induced via mixed chimerism following the infusion of allogeneic HSC and anti-CD40L antibodies in conditioned mice resulted in rapid deletion of pre-existing donor-reactive CD4⁺ T cells, obviating the need for regulatory T cells (Tregs) to suppress CD4 cell-mediated alloreactivity [19-23]. However, conflicting findings have been obtained in two studies, implying that deletion of recipient T cells specific for allogeneic MHC molecules on donor APCs (i.e., direct allorecognition) may not be sufficient to maintain allograft tolerance in macrochimeric mice. Uehara et al. reported that skin and heart allografts accepted by mice displaying stable mixed chimerism later developed cardiac allograft vasculopathy (chronic rejection) dependent on the presence of host CD4⁺ T cells [24]. More recently, Shinoda et al. found that depleting Foxp3⁺ T cells abrogated tolerance of skin and heart allografts in

stable mixed chimeras without an associated loss of mixed chimerism [25]. Of note, the authors here found that host T cells collected from the rejecting mice were unable to mount a mixed lymphocyte reaction against donor cells. The latter finding indicates that rejection in these animals was not mediated by T cells directed against intact allogeneic MHC molecules on donor APCs. In other words, it did not involve direct allorecognition [25]. Instead, it is likely that the T cells causing rejection were directed toward cryptic determinants on donor MHC, minor antigens, or graft tissue-specific antigens presented in an indirect fashion, as previously described [26-29]. These allo- and auto-reactive T cells may not undergo clonal deletion. Rather they may be suppressed by T regulatory cells (Tregs) and/or other peripheral regulatory cells and factors. In support of this view, a recent study by Pilat et al. showed that treatment with Tregs prevented chronic rejection of cardiac allografts in a murine mixed chimerism model [30]. Collectively, these reports reinforce the notion that two distinct mechanisms of tolerance, namely, deletion in the thymus and peripheral regulation, may be required in the same recipient to account for the different types of antigens involved in acute and chronic rejection, respectively.

Stable hematopoietic microchimerism, defined as the presence of low numbers of donor leukocytes (< 1% of total leukocytes) in the recipient, is sufficient to induce allograft tolerance under appropriate circumstances [31-34]. It is not clear, however, whether microchimerism results in the deletion of T cell clones that demonstrate medium-to-high affinity for donor MHC antigens, such as those studied in T cell receptor (TCR) transgenic mice [20,35]. Contrary evidence exists implying that induction and maintenance of tolerance in microchimeric mice is dependent on the presence of Tregs in peripheral tissues [34,36,37]. These studies suggest that the tolerance induced by microchimerism may be less robust than that induced by macrochimerism. In support of this view, we note that microchimeric mice usually accept vascularized solid organ transplants (heart, kidney), but rarely more immunogenic skin allografts from the same donor.

Hematopoietic microchimerism also could result from trafficking of leukocytes within the organ transplant, itself. For instance, allogeneic kidney grafts are spontaneously accepted in certain donor-recipient mouse combinations through a dual process involving Tregs and, presumably, microchimerism induced by donor kidney leukocytes [37-43]. Microchimerism is induced regularly during pregnancy through the passage of maternal leukocytes to the fetus [37,42,44-46]. Studies from Burlingham's laboratory showed that maternal microchimerism, consisting of mature leukocytes and HSC, persists in adult offspring [32,47-49]. The presence of these cells ensures some level of immune tolerance to non-inherited maternal antigens (NIMA) in the offspring [32]. Our laboratory at MGH has shown that the presence of NIMA MHC class I K^b alloantigen in the fetal thymus of NIMA-K^b TCR transgenic mice activates anti-K^b T cells but does not lead to deletion [50]. In this model, anti-K^b TCR transgenic offspring, which had been exposed to NIMA during fetal life, accepted K^b+ cardiac allotransplants through a process involving Tregs [50].

It is also unclear whether macro- or microchimerism must necessarily be permanent to maintain tolerance of vascularized solid organ allografts. Some studies show that loss of donor macrochimerism in mice infused with recipient type lymphocytes abrogates tolerance to donor alloantigens. These findings suggest that, in a model relying essentially on

deletional mechanisms, maintenance of tolerance requires sustained macrochimerism. Whether the same rule applies to regulatory tolerance induced via microchimerism is unknown. While tolerance induction requires microchimerism provided by the transplantation of BMCs, it is possible that once accepted some organ transplants may enjoy a degree of immune privilege. Alternatively, microchimerism could be maintained by a few donor leukocytes present in the graft itself or in graft lymphoid-associated structures (i.e., tertiary lymphoid structures or lymph nodes transplanted along with the organ transplant) [51,52]. Further studies are needed to answer these questions. It is now firmly established that allospecific memory T cells induced in mice after infection or allosensitization prevent the establishment of durable donor hematopoietic chimerism and subsequent development of tolerance [53]. These memory T cells are usually resistant to calcineurin inhibitors and costimulatory blockade [54]. On the other hand, the generation and maintenance of anamnestic responses rely on the expression of anti-apoptotic factors of T cells [54]. A recent series of studies from Fehr's laboratory indicates that a Bcl-2/Bcl-XL inhibitor, ABT-737, can be used to induce apoptosis of memory T cells, thereby prolonging skin graft survival in sensitized mice [55]. Furthermore, the same strategy achieved stable mixed chimerism in mice in the absence of myelosuppressive conditioning [56]. Therefore, utilization of pro-apoptotic factors represents a promising strategy for inducing tolerance in sensitized mice and primates that display pre-existing alloreactive memory T cells. It remains to be determined whether excessive depletion of memory T cells will cause increased susceptibility to infections and tumorigenesis.

In summary, studies of hematopoietic micro- and macrochimerism in mice have provided essential information regarding the mechanisms by which bone marrow and stem cell transplantation can achieve immune tolerance of allogeneic transplants. Overreliance on results obtained in transgenic mice should be avoided, however, since these strains have a limited alloreactive TCR repertoire confined to single clones displaying high affinity for alloantigens. More important, it is increasingly apparent that inbred laboratory mice raised in sterile environments display limited gene polymorphism and are essentially devoid of bona fide allospecific memory T and B cells, which represents a major barrier to chimerism and induction of tolerance [53,57-59]. Hence, protocols designed to achieve transplantation tolerance via hematopoietic chimerism in laboratory mice need to be further validated in a "non-laboratory" milieu, i.e., wild-caught mice or primates, prior to clinical application.

Preclinical studies in large animal models

Unlike murine studies, in which persistent mixed chimerism can be readily achieved [60,61], thus far induction of stable mixed chimerism has been extremely difficult to achieve in primates [62-64]. Investigators at Emory University attempted to induce stable mixed chimerism by adding CTLA4Ig and anti-CD154 mAb to a Busulfan-based nonmyeloablative conditioning regimen [65,66]. Their results confirmed previous findings wherein increasing MHC matching resulted in significant prolongation of chimerism. Nevertheless, donor BMC engraftment was eventually lost after recovery of rigorous T-mediated alloreactivity, even in an MHC-matched cohort [66]. The same group subsequently found a synergistic effect on chimerism induction after combined treatment with belatacept and anti-CD40 mAb, but persistent chimerism still was not achieved [67]. Kidney transplantation also was attempted

in this study, but kidney allografts were rejected despite ongoing whole blood chimerism [68].

In contrast to the Emory experience, successful induction of renal allograft tolerance has been achieved after induction of transient chimerism with a nonmyeloablative regimen developed at the Massachusetts General Hospital (MGH) [62,64,69,70]. The MGH regimen consisted of low dose total body and local thymic irradiation, horse anti-thymocyte globulin (ATG), a cosimulatory blockade (either by anti-CD154 mAb or belatacept), and a weak course of cyclosporine (CyA). Although the reason for the disparate outcomes between the Emory and the MGH studies remains to be clarified [71], absence of lymphoid chimerism in recipients of the Emory regimen may be a key factor in the failure of renal allograft tolerance, since previous studies at MGH showed that induction of lymphoid chimerism was correlated with long-term allograft survival [63].

To extend the MGH regimen to deceased donor transplantation, an alternative approach, termed “delayed tolerance,” was perfected. In this approach, kidney transplantation was performed first with conventional immunosuppression followed by conditioning and donor bone marrow transplantation (DBMT) at a later time. Since the kidney allograft could potentially activate memory T cells prior to transplantation of donor bone marrow, anti-CD8 mAb [72] or Alefacept (LFA3 Ig) [73] was needed to delete or suppress CD8 memory T cells and to ensure a state of mixed chimerism and renal allograft tolerance using this approach.

Since successful allograft tolerance after induction of transient chimerism appears so far to be restricted to the kidney [74], more robust chimerism induction protocols may be necessary to induce tolerance in non-renal allografts. Although induction of persistent mixed chimerism has been difficult to accomplish in outbred nonhuman primates (NHP) and humans, induction of stable mixed chimerism has been achieved in inbred canine and swine models [75]. Mathes et al. reported successful induction of stable mixed chimerism and tolerance of vascularized composite tissue allografts (VCA) in dog-leukocyte antigen–matched, minor antigen mismatched canine [76]. Using a nonmyeloablative conditioning regimen, consisting of 1 Gy total body irradiation (TBI) 1 Gy, anti-CD3 immunotoxin, HSCs, and a 30-day course of CyA, Leonard et al. successfully induced persistent mixed chimerism using MHC haplotype identical swine donors. In this study, all six recipients that developed stable mixed chimerism accepted VCA without histological evidence of rejection. This study provided proof of concept that long-term VCA tolerance can be achieved if persistent mixed chimerism is induced.

Successful induction of stable mixed chimerism by injection of donor HSC into swine fetuses also has been reported. Although the clinical application of this approach is limited in organ transplantation, two studies on intra-uterine HSC transplantation (HSCT) have been published. Mathes et al. infused MHC- mismatched BMCs into swine fetuses between days 50-to-55 of gestation [77]. After birth, the recipients were found to have multilineage donor chimerism ranging from 1.8 – 90% throughout life. All chimeric animals showed in vitro evidence of donor-specific hyporesponsiveness and accepted VCAs for over 130 days.

Investigators at Children's Hospital of Philadelphia also reported stable long-term mixed chimerism by in utero HSCT in an MHC-mismatched canine model [78]. They identified 40 days' gestation, when thymic selection begins, as the optimal time point for intra-uterine HSCT. They also found that intracardiac injection of HSC resulted in higher levels of chimerism compared with intraperitoneal injection. The animals were chimeric for up to 2 years after birth, and donor-specific tolerance was shown by successful kidney transplantation from the donor of the HSC. Although clinical application of this approach in organ transplantation is limited, it proves that neonatal tolerance, first shown by Billingham et al. in 1953 [79], can be achieved in large animals.

Clinical studies

Three major centers, Stanford, MGH, and Northwestern, have been actively pursuing allograft tolerance induction through HSCT in human leukocyte antigen (HLA)-matched and mismatched kidney transplantation.

Stanford approach—The Stanford group initiated clinical trials for renal allograft tolerance induction in the 1980s, using a total lymphocyte irradiation (TLI)-based regimen with or without donor bone marrow infusion. Although successful discontinuation of immunosuppression was initially reported [80], the results failed to extend graft survival over conventional immunosuppression [81] [82].

Subsequently, the Stanford group revised their TLI-based regimen by adding CD34+ and CD3+ cells in an attempt to induce stable mixed chimerism. Their current protocol consists of TLI (80-120 cGy, 10 doses total on days 0-9), rabbit ATG (1.5 mg/kg, 5 days total on days 0-4), followed by HLA-matched peripheral blood CD34+ stem cell, and CD3+ cell infusion on day 11. Mycophenolate mofetil (MMF) and cyclosporine are initiated on day 0 and tapered off over the ensuing 6 months. In HLA-matched kidney transplantation, the majority of patients (19/22) receiving the Stanford protocol successfully developed persistent mixed chimerism, and 16 patients were successfully weaned off immunosuppression. However, in HLA-mismatched kidney transplantation, the initial attempt to induce stable mixed chimerism and renal allograft tolerance failed [83,84]. A revised regimen with increased CD34+ cell and CD3+ cell doses is currently being tested to improve engraftment of HLA-mismatched donor HSC.

MGH approach—Based on decades-long basic and preclinical studies, a nonmyeloablative regimen was developed at MGH in collaboration with the Immune Tolerance Network (ITN) to induce mixed chimerism and renal allograft tolerance in HLA-mismatched kidney transplantation [85-88]. The MGH protocol consisted of cyclophosphamide (60mg/kg X2), local thymic irradiation (7Gy), anti-CD2 mAb, and combined kidney and bone marrow transplantation, followed by an 8-14 month course of calcineurin inhibitor. All 10 recipients who received this protocol developed transient mixed chimerism up to day 21, and seven of them successfully discontinued immunosuppression by 14 months. Among these seven recipients, four remained immunosuppression free for 5-12 years, while three resumed immunosuppression at 5, 7, and 8 years after kidney transplantation as a result of recurrence of the original disease or chronic rejection [88,89]. These results were compared with 21

living donor kidney transplant recipients, of comparable age and pathology, who were maintained with conventional immunosuppression. During the 10-year observation period in recipients of conventional immunosuppression, four lost their kidney grafts as a result of rejection, and many of them suffered significant post-transplant morbidity, including hypertension requiring medical management (85%), hyperlipidemia (65%), new onset insulin dependent diabetes (35%), and serious infectious complications (25%). In addition, two patients developed malignant skin lesions. In contrast, less than half of the tolerant recipients are currently on anti-hypertensive medications and none has developed hyperlipidemia, de novo diabetes mellitus, serious infection, or malignancy [89], which clearly indicates that induction of tolerance is ideal for sustaining health and longevity among transplant recipients.

Acute kidney injury (AKI), observed in most recipients (9 of 10) between 10 and 20 days post-transplant, is a major complication of these clinical trials. It is associated with hematopoietic cell recovery and rapid loss of chimerism. Since AKI was not observed in the NHP studies that TBI rather than cyclophosphamide, a revised conditioning regimen with low-dose TBI but without cyclophosphamide has recently been tested in two recipients. Both recipients have done well and are without AKI. Immunosuppression in the first patient was discontinued one year post-transplant (manuscript in preparation). Further clinical trials are planned using a new regimen including belatacept, which was developed in the NHP study [90].

Northwestern approach—The group at Northwestern University has reported successful induction of allograft tolerance in HLA-mismatched kidney transplant recipients by replacing recipient hematopoietic cells with donor cells (full chimerism). Until recently, bone marrow transplantation from HLA-mismatched donors could not be routinely performed because of the unacceptable risk of GVHD. However, a novel protocol for HLA-mismatched bone marrow transplantation was recently developed at Johns Hopkins Hospital to achieve HSC engraftment without risk of GVHD. This protocol effectively deletes anti-host T cells attributable to GVHD through the administration of cyclophosphamide on days 3 and 4 after HSCT [91]. The group at Northwestern used a novel tolerogenic “facilitating cell” therapy in which a mixture of CD8+/TCR- facilitating cells [92] was added to the Johns Hopkins protocol to further reduce the risk of GVHD. In mice models, these cells have been found to improve engraftment and to prevent GVHD, possibly through the induction of regulatory T cells [93,94].

The full Northwestern protocol for induction of renal allograft tolerance consists of TBI (200 Gy), fludarabine (30 mg/kg x 3) and cyclophosphamide (50 mg/kg on day -3), kidney transplantation on day 0, the administration of donor HSCs combined with facilitating cells on day 1, and finally, another cyclophosphamide (50 mg/kg) treatment on day 3. Mycophenolate mofetil and tacrolimus are started on day 0 and slowly tapered off by one year. A trial using this conditioning regimen has enrolled 15 recipients to date [95]. Nine of these 15 patients have developed full donor chimerism, and immunosuppression has been successfully discontinued in 6 patients. Tolerance was not achieved in the three patients who developed transient chimerism only. Longer term follow-up of the recipients with full donor

chimerism as well as improvement of the consistency of the results will be necessary before widespread adoption of this approach.

The Northwestern group recently reported another strategy to induce renal allograft tolerance for HLA-matched kidney transplant recipients. This immunosuppressive regimen includes alemtuzumab induction, donor HSCs, tacrolimus/mycophenolate immunosuppression converted to sirolimus, and complete drug withdrawal by 24 months post-transplantation. Among 10 recipients, all of whom had at least 36 months of follow-up after transplantation, immunosuppression was successfully withdrawn in 5 for a period of 16-36 months, two had disease recurrence, and three had subclinical rejection in protocol biopsies. In this study, microchimerism disappeared after 1 year. The regulatory function of T or B regulatory cells and/or the immunodeficiency created by DBMC have been postulated as mechanisms of tolerance in this approach [96].

Conclusion

Tolerance induction is now a clinical reality in humans, at least for patients undergoing kidney transplantation. The Transplantation Society recently hosted an international workshop in Boston with approximately 50 physicians and scientists in attendance to establish the current consensus and recommendations for tolerance induction [97]. Chief among the consensus recommendations are the need (1) to establish a registry to record the results of patients enrolled in tolerance trials; (2) to create standardized protocols for sample collection and storage; (3) to define standardized biomarkers and assays; (4) to include children 12 years and older in protocols that have been validated in adults; and (5) to establish a task force to engage third party payers to participate in the costs associated with tolerance trials. Improving the consistency and safety of tolerance induction and extending successful protocols to other organs and to organs from deceased donors will be among the next crucial steps to bringing tolerance to a wider range of clinical applications.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

*of special interest

** of outstanding interest

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Key Points

- Durable macrochimerism in mice is associated with deletion of alloreactive T cell clones displaying medium to high affinity for donor MHC. However, peripheral regulatory mechanisms presumably maintain suppression of T cell clones with low affinity to donor MHC and other minor and auto-antigens.
- Allospecific memory T cells represent a major barrier to establishing hematopoietic chimerism and subsequent transplant tolerance induction and maintenance.
- Durable mixed chimerism and composite tissue allograft tolerance has been achieved across MHC barriers in an inbred swine model.
- Stable renal allograft tolerance in HLA mismatched kidney transplantation has been achieved by induction of transient mixed chimerism.
- Durable full donor chimerism and allograft tolerance have been achieved in HLA mismatched clinical kidney transplantation.