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Immune Tolerance in Recipients of Combined Haploidentical Bone Marrow and Kidney Transplantation

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Abstract

The success of allogeneic HCT has been limited by transplant-associated toxicities related to the conditioning regimens used and to graft-vs-host disease (GVHD). The frequency and severity of GVHD observed when extensive HLA barriers are transgressed has greatly impeded the routine use of extensively HLA-mismatched HCT. Allogeneic HCT also has potential as an approach to organ allograft tolerance induction, but this potential has not been previously realized because of the toxicity associated with traditional conditioning. This paper reviews an approach to HCT involving reduced intensity conditioning that demonstrated sufficient safety in patients with hematologic malignancies, even in the HLA-mismatched transplant setting, to be applied for the induction of kidney allograft tolerance in humans with no other indication for HCT. These studies provided the first successful example of intentional organ allograft tolerance induction across HLA barriers in humans. Current data and hypotheses on the mechanisms of tolerance in these patients are reviewed.

Keywords

Tolerance; Chimerism; Kidney; T cell receptor

INTRODUCTION

Immune tolerance denotes a state in which the immune system accepts donor organs or tissues but is capable of responding normally to foreign antigens. While recent improvements in immunosuppressive drugs have greatly augmented early organ allograft survival rates, these improvements have not significantly reduced late graft loss due to chronic rejection. High rates of malignancies and opportunistic infections as well as drug-specific metabolic and end-organ toxicities limit the tolerability of long-term chronic immunosuppressive therapy. Induction of donor-specific immune tolerance would avoid these complications while also preventing chronic rejection. However, attempts at tolerance induction in humans require a radical departure from the standard of care, as immunosuppressive therapy is withdrawn, exposing the patient to the risk of rejection. Thus, safety and efficacy of tolerance approaches should be demonstrated in pre-clinical animal models before they are evaluated in clinical trials.

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TOLERANCE INDUCTION BY ALLOGENEIC HCT

Mixed hematopoietic chimerism was first shown to be associated with tolerance in fraternal twin cattle sharing a placental circulation almost 70 years ago¹. However, achievement of this state in adult recipients with preestablished immune systems has presented a major challenge. These barriers largely consist of recipient T cells^{2, 3}, which must be either eliminated or tolerized. Since newly developing T- and B-lymphocytes are tolerized by antigens presented to them, especially by hematopoietic antigen-presenting cells, during lymphocyte maturation, HCT resulting in mixed chimerism can educate these T and B cells to be tolerant of both donor and recipient⁴⁻⁹.

Suitable protocols that achieve engraftment across HLA barriers will be required before HCT can be routinely used for the induction of organ allograft tolerance. Such regimens must be non-myeloablative so that recipient hematopoiesis can protect the recipient from marrow failure if the donor graft is rejected, yet must overcome the T cell barrier to HLA-mismatched grafts. NK cell barriers are easier to overcome with higher HSC numbers¹⁰, but may pose a more serious barrier when T cell suppression is incomplete¹¹, when the number of surviving donor stem cells is reduced. Engraftment of HSCs is promoted by making 'space' in the hematopoietic system using myelosuppressive treatments such as a low dose of total body irradiation (TBI) or busulfan. While this requirement can be overcome with enormous doses of HSCs¹²⁻¹⁴, such doses are not yet clinically obtainable. Following the demonstration that "megadose marrow" enhances allogeneic engraftment in sublethally irradiated rodents¹⁵, an increase in stem cell dose has been used to improve engraftment in human haploidentical transplants¹⁶. Donor hematopoietic progenitor cells can mediate a veto effect that deletes donor-reactive CTLs¹⁷. Very high doses of allogeneic marrow can engraft in mice receiving costimulatory blockade without myelosuppressive conditioning¹⁸ and this marrow dose can be lowered to clinically attainable doses with improved immunosuppression in non-cytoreduced rodents¹⁹.

If the above conditions are met, then donor HSC engraftment can lead to multilineage mixed hematopoietic chimerism, which is associated with lifelong central, deletional T cell tolerance⁴⁻⁸ allowing acceptance of any donor allograft without immunosuppression²⁰⁻²². However, if used solely to achieve organ allograft tolerance, mixed chimerism must be achieved without GVHD, which is an unacceptable risk in this setting. This represents a major challenge, as HLA barriers are routinely transgressed in organ transplantation.

EXPERIMENTAL APPROACHES TO ACHIEVING MIXED CHIMERISM

A number of experimental non-myeloablative conditioning regimens have achieved mixed chimerism without GVHD in animals, including TLI²³, sublethal TBI²⁴, administration of cyclophosphamide following sensitization with allogeneic donor antigens²⁵, and the use of mAbs against host T-cells along with other modalities^{26, 27}. When global T cell ablation and non-myeloablative TBI are combined, a purely central (intrathymic) deletional mechanism achieves and maintains tolerance, with no significant role for suppressive mechanisms⁷. Less T cell ablative conditioning regimens have utilized costimulatory blockade^{27, 18, 28}. The ability to replace recipient T cell depletion with costimulatory blockade is important, as it has been difficult to achieve T cell depletion with antibodies in large animals and humans

that is as exhaustive as that in the above rodent models. Moreover, if truly exhaustive T cell depletion could be achieved in humans, T cell recovery from the thymus is likely to be dangerously slow in older patients with diminished thymic function²⁹. After pre-existing T cells are tolerized, long-term tolerance is maintained by intrathymic deletion in mixed chimeras prepared with costimulatory blockade^{18, 27, 30}. However, regulatory CD4 cells play a role in the initial tolerance induction³¹ but not in long-term tolerance in such models in which tolerized T cells are gradually deleted^{32, 33, 18, 27, 34}. Other BMT models using costimulatory blockade that are associated with less complete deletion of pre-existing donor-reactive T cells involve long-term regulatory mechanisms^{35, 36}.

A non-human primate model for mixed chimerism and renal allograft tolerance induction across MHC barriers utilizes 3 Gy TBI and 7 Gy thymic irradiation, equine ATG and a short (28 day) course of cyclosporine to suppress residual T cells not depleted by ATG. A high percentage of cynomolgus monkeys receiving class I- and II-mismatched marrow with this protocol develop transient mixed chimerism and donor kidney allograft acceptance³⁷.

ROLE FOR MIXED CHIMERISM IN THE TREATMENT OF HEMATOLOGIC MALIGNANCIES

Many non-myeloablative clinical HCT protocols have been developed in the last few decades. Most of these aim to achieve full donor chimerism, as mixed chimerism is associated with leukemic relapse. However, GVHD remains a major complication of these regimens and may occur either spontaneously or following donor leukocyte infusion (DLI). In contrast to HCT for the induction of organ allograft tolerance, some GVHD is considered acceptable in the setting of hematologic malignancies because of the associated anti-tumor effects³⁸. However, the frequency and severity of GVHD observed when extensive HLA barriers are transgressed has hindered the use of extensively HLA-mismatched HCT. Based on observations in animal models, we have attempted to overcome this limitation to HLA-mismatched HCT in a series of clinical protocols using non-myeloablative conditioning that includes recipient and donor graft T cell depletion and aims to achieve initial mixed chimerism without any GVH response from the initial transplant. The key observation leading to these trials is that conditioning-induced tissue inflammation plays an important role in promoting GVHD³⁹. MHC-directed alloreactivity can be confined to the lymphohematopoietic system when non-tolerant donor T cells are given in DLI to mixed chimeras after host recovery from the initial conditioning regimen has occurred. The unopposed GVH response of DLI converts these animals to full donor chimerism and achieves strong graft-vs-leukemia/lymphoma (GVL) effects^{40, 41}. However, this GVH alloresponse against lymphohematopoietic cells is not associated with GVHD, even though the T cell numbers given cause rapidly lethal GVHD in freshly conditioned recipients^{40, 42}. Nevertheless, GVH-reactive T cells in DLI become activated and proliferate in established mixed chimeras receiving DLI^{39, 43}. The presence of recipient APCs expressing both class I and class II MHC play a critical role in inducing this anti-host reactivity and maximal GVL^{41, 43}. Despite converting to the “effector/memory” phenotype following activation in established mixed chimeras, DLI-derived T cells do not migrate to the epithelial GVHD target tissues, due to the absence of inflammatory signals in those tissues³⁹. Such inflammatory signals, including chemokines and probably adhesion molecules, are induced

in GVHD target tissues by conditioning treatment and subside over time in the absence of GVH reactivity⁴⁴.

In clinical trials of this approach, proof of principle has been obtained that GVH responses can be confined to the lymphohematopoietic system and thereby fail to induce GVHD in patients receiving non-myeloablative HCT with an initially T cell-depleted product, followed by delayed DLI, even across extensive HLA barriers⁴⁵. However, some patients who are mixed chimeras and show no evidence for GVHD prior to receiving DLI do develop GVHD after receiving the DLI. One major difference between these patients and the mouse model is that T cell recovery in patients is generally poor at the time when DLI are given⁴⁶, resulting in bacterial, viral and fungal infections. In contrast, mice have excellent T cell recovery due to robust thymopoiesis by the time DLI are given. It appears that these T cells protect from DLI-induced GVHD largely by preventing inflammatory stimuli induced by infection⁴⁷. Even in “quiescent” established mixed chimeras, activation of toll-like receptors (TLRs), as occurs in infection, promotes the trafficking of DLI-derived T cells to the GVHD target tissues^{39, 47}. Systemic TLR stimuli (mimicking a systemic infection) result in systemic GVHD. In contrast, a local TLR stimulus, applied to the skin, promotes DLI-induced GVHD only in the treated area of skin³⁹. Therefore, regulatory cells, which are present in mixed chimeras by the time of DLI administration, are insufficient to prevent the development of GVHD when an inflammatory stimulus is provided by TLR activation. These results also show that local inflammatory stimuli are critical in promoting the trafficking of GVH-reactive T cells into the skin and hence the induction of GVHD. This “inflammatory checkpoint” confines GVHD to the inflamed tissue. Improved immune recovery, which would lead to better control of post-transplant infections and prevent TLR-dependent immune activation, would likely improve the ability to separate GVHD and GVL in patients who receive delayed DLI.

TRANSLATIONAL STUDIES OF MIXED CHIMERISM INDUCTION FOR TREATMENT OF HEMATOLOGIC MALIGNANCIES TO TOLERANCE INDUCTION

The successful achievement of renal allograft tolerance in a non-human primate model using non-myeloablative conditioning for mixed chimerism induction³⁷, combined with the above clinical studies aimed at achieving GVL without GVHD by inducing mixed chimerism and later giving DLI^{48, 49}, allowed the first successful trials of organ allograft tolerance induction to be carried out in humans, initially in patients with multiple myeloma and consequent renal failure. These patients have accepted their kidney graft without any immunosuppression for follow-ups as long as 16 years, even though chimerism in a significant fraction of patients was only transient^{50, 51}. The myeloma remissions achieved in this group raised the possibility that transient chimerism followed by marrow rejection, as was evident in sensitized anti-donor T cell responses⁵⁰, could lead to anti-tumor responses. This hypothesis was subsequently verified in a mouse model^{52–55}. The renal allograft tolerance that was nevertheless achieved suggested that the kidney graft itself may participate in tolerance. Indeed, the patients demonstrated unresponsiveness to donor renal tubular epithelial cells⁵⁰, suggesting that tolerance was specific for minor histocompatibility antigens expressed on the kidney graft itself. In the primate model described above,

chimerism is also transient, but both BMT and early renal transplantation are required for achievement of tolerance³⁷.

Recently, this approach has been extended to patients without malignant disease who have renal failure from other causes, who received HLA-mismatched combined kidney and bone marrow transplantation^{56, 57} (CKBMT). Safety data in a trial involving HLA-mismatched BMT in patients with hematologic malignancies, using the above approach of non-myeloablative mixed chimerism induction followed by delayed DLI, provided the impetus to extend this approach to the HLA-mismatched setting⁴⁵. Only transient chimerism was achieved, and loss of chimerism was associated with robust recipient hematopoiesis, documenting that the regimen was truly non-myeloablative. Importantly, none of these patients developed GVHD. These observations, combined with the non-human primate data above, justified a combined kidney/BMT clinical tolerance trial using haploidentical related donors in recipients without malignant disease. The two HLA-mismatched combined kidney/BMT trials in patients without malignant disease involved 10 patients (5 each), with follow-up now from about 5 to 12 years. Seven patients were successfully taken off their initial immunosuppressive monotherapy. Although 3 of 4 patients in the first trial returned to single-drug immunosuppression 6–7 years after their transplants due to chronic antibody-mediated rejection (2 patients) and recurrent autoimmune kidney disease (1 patient), modifications in the second trial have thus far avoided any requirement for immunosuppression in the 3 patients who were successfully withdrawn⁵⁷.

In vitro analyses of these patients revealed the progressive development of donor-specific unresponsiveness, with robust third party alloresponses, in both MLR and CML assays in the four patients who achieved immunosuppression withdrawal in the first study^{56, 58}, suggesting that systemic donor-specific tolerance developed. The difference between these subjects and the tolerant recipients of HLA-identical transplants, who sometimes showed sensitization to donor hematopoietic antigens in association with loss of chimerism, raises the hypothesis that in both groups tolerance is restricted to antigens expressed by the kidney. Minor histocompatibility antigens expressed by hematopoietic cells may not all be shared by the kidney, resulting in the “split tolerance” observed in recipients of HLA-identical CKBMT with transient chimerism⁵⁰. In recipients of HLA-mismatched CKBMT, in contrast, the pre-existing anti-donor response may disappear following transplant because most allogeneic MHC/peptide complexes responsible for the strong direct alloresponse are shared by both the kidney and the hematopoietic cells. Thus, tolerance to those complexes expressed on the kidney would lead to loss of the bulk MLR and CML response. *In vitro* results in patients with hematologic malignancies who received a similar haploidentical BMT regimen without a kidney transplant did not show donor unresponsiveness⁴⁶, strongly suggesting a role for the kidney in inducing unresponsiveness in the CKBMT recipients. The donor-specific unresponsiveness achieved in the second set of CKBMT recipients was somewhat less complete in the time of follow-up compared to the first set⁵⁹.

It is unlikely that central deletion mediates long-term tolerance in HLA-mismatched CKBMT recipients, given the very transient nature of the chimerism^{56, 60}. Moreover, initial T cell recovery in these patients appears to be mainly from the residual peripheral T cell pool rather than from the thymus^{50, 58}. Ingraft levels of FoxP3 relative to Granzyme B

mRNA were increased in tolerant patients compared to patients on immunosuppression, raising the possibility that regulatory T cells might play a role in tolerance⁵⁶. Regulatory cells are enriched among the circulating T cells initially present in recipients of this regimen with⁵⁸ or without⁴⁶ a kidney transplant, and removal of Tregs revealed anti-donor reactivity *in vitro* during the first year, but not later after transplant in some of the patients⁵⁸. These results led us to hypothesize that initial tolerance involves induction/expansion of donor-specific Tregs, while long-term tolerance may reflect eventual deletion of donor-reactive T cells resulting from repeated encounter with an uninflamed kidney allograft. We recently developed a novel strategy for identifying and tracking donor-reactive TCRs that employs high throughput CDR3 region sequencing. The results of this study are consistent with the interpretation that long-term renal allograft tolerance in these patients is indeed due to a deletional mechanism⁶¹. Further understanding of these mechanisms will lead to further advances in the use of CKBMT for tolerance induction.

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