CHIMERISM
2015, VOL. 6, NOS. 1–2, 27–32
http://dx.doi.org/10.1080/19381956.2015.1115584

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REVIEW

Stable mixed chimerism and tolerance to human organ transplants

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ABSTRACT
Tolerance to combined kidney and hematopoietic cell transplant has been achieved in humans after establishment of mixed chimerism allowing for the withdrawal of immunosuppressive drugs. The seminal contributions of Ray Owen provided the scientific basis for the human protocol.

ARTICLE HISTORY
Received 6 August 2015
Revised 21 October 2015
Accepted 27 October 2015

KEYWORDS
human organ transplants; mixed chimerism; tolerance

Introduction

The achievement of immune tolerance in clinical solid organ transplantation has remained a central goal for more than 50 years. Key advantages of tolerance are the elimination of the lifelong need for immunosuppressive drugs and their attendant side effects, and prevention of chronic rejection leading to organ graft failure. The recognition of tolerance started with the seminal contribution of Ray Owen in the middle of the 20th century.

Stable mixed chimerism in utero and tolerance in cattle

The seminal contribution was the observation that dizygotic cattle twins sharing the same placenta developed a lifelong mixing of blood forming cells from their twin.[1] This state of stable mixed chimerism implied that the foreign blood forming cells were not rejected by the recipient immune system during adult life, and instead were "tolerated." The additional observation made by Peter Medawar and his co-workers, that the chimeric cattle did not reject skin grafts from the twin, and did reject skin grafts from non-twin cattle clinched the idea that the chimeric state prevented rejection of foreign tissue transplants by the immune system.[2]

Stable mixed chimerism and tolerance in neonatal mice

The recognition that *in utero* exposure of cattle to foreign blood forming cells resulted in stable mixed chimerism and acceptance of skin grafts throughout the adult life led to the most robust and famous observations in mice by the Medawar group. During the 1950s, the latter investigators showed that intravenous injection of blood forming cells obtained from one mouse strain into neonates of another resulted in stable mixed chimerism and acceptance of skin grafts of the donor strain during the adulthood of the recipients.[3,4] These observations opened the possibility that tolerance to organ transplants could be achieved in humans, thereby obviating the need for immunosuppressive drugs and resulting in the permanent acceptance of the grafts.

However, it became clear that achieving stable chimerism and tolerance in adult rodents was far more difficult than in neonates. Although the immature immune system of the murine neonates had minimal ability to reject the foreign haematopoietic cells, the mature immune system of the adults rapidly rejected these cells and prevented chimerism. An attempt to address this issue was made by treating adult murine recipients with ablative total body irradiation (TBI) that would deplete both blood forming cells and immune cells such that recipient survival was dependent on the injection of foreign bone marrow cells, and the development of chimerism.[5-7] The latter recipients developed complete chimerism with total replacement of the recipient's blood forming and immune cells with that of the donor.[5-7] The latter "radiation chimeras" accepted skin grafts from the

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bone marrow cells donors, and showed the feasibility of this approach.[5-7]

A considerable fraction of the radiation chimeras developed graft versus host disease (GVHD), the most important side effect of allogeneic bone marrow transplantation. Severity depended on the extent of MHC matching, and the strain combinations.[6,7,9] Thus, development of GVHD was a major barrier to the application of the ablative radiation approach to the field of organ transplant tolerance. In contrast, ablative radiation followed by MHC matched allogeneic bone marrow transplantation was a valuable approach taken by E.D. Thomas and his coworkers for the treatment of terminal cancer patients with hematologic malignancies.[8-10] The latter patients all had a uniformly lethal disease, and complete chimerism after bone marrow transplantation was necessary to cure the tumors.[8-10] However, complete chimerism was associated with a high risk of GVHD.[8-10] The risk of GVHD and ablative radiation toxicity was acceptable in this group of patients. Subsequently, non-myeloablative radiation regimens were studied to reduce toxicity and GVHD.

Stable mixed chimerism and tolerance in adult mice and rats

Although ablative TBI followed by allogeneic bone marrow transplantation resulted in complete chimerism and rapid death due to GVHD in strains with major histocompatibility (MHC) gene differences, the use of total lymphoid irradiation (TLI) as a conditioning regimen instead of TBI prevented the development of GVHD even after MHC mismatched bone marrow transplantation.[11-15] The TLI procedure was developed as a curative treatment of early stage Hodgkin's disease by Henry Kaplan and his co-workers, and risks and benefits had been studies in thousands of patients.[16,17] TLI differed from TBI in that only the lymphoid tissues including the lymph nodes, spleen and thymus were included in the radiation fields, and the remaining tissue were shielded with lead to reduce toxicity to organs such as the lungs, intestines and central nervous system.[16,17] The precise tissue targeting procedure was made feasible by the use of linear accelerators for radiation administration.[16,17] In addition, the radiation was delivered in multiple small doses that markedly reduce normal tissue injury.[16,17] Since at least 50% of the marrow volume was outside the radiation fields, the conditioning was non-ablative, and spontaneous recovery of blood elements occurred without severe neutropenia or thrombopenia.[16,17] The TLI conditioning regimen was adapted from the clinical protocols for use as a murine conditioning regimen for bone marrow transplantation.[11-15]

Initially the TLI regimen was used as a pre-transplant conditioning regimen in mice to achieve chimerism without GVHD after the transplantation of MHC mismatched bone marrow cells, and subsequently used in MHC mismatched mice and rats as a pre-transplant regimen to induce stable mixed chimerism and tolerance to combined bone marrow and organ transplants as reported first in 1976.[12] All chimeric recipients accepted skin grafts from the donor strain and rejected grafts from third party strains.[11-14] The success in achieving tolerance to the donor skin grafts was expected in these chimeras based on the work of Medawar and Owen and their coworkers as discussed above. Alternative non-myeloablative pre-transplant conditioning regimen to achieve mixed chimerism and tolerance avoiding GVHD in MHC mismatched mice was reported by David Sachs and his co-workers in 1989[18] by conditioning recipients with sublethal TBI, irradiation of the thymus and administration of anti-T cell antibodies. The recipients of the combined marrow and organ transplants became stable mixed chimeras.[18,19] This regimen was extended to mini-pigs and to non-human primates.[20,21] In the case of non-human primates the chimerism was transient, and yet the majority of recipients accepted kidney grafts.[22]

In order to make the TLI tolerance induction regimen developed at Stanford applicable to deceased donor organ transplantation in which the timing of organ availability is uncertain, the conditioning regimen was changed to a completely post-transplant procedure in which the organ was transplanted on day 0, and the conditioning regimen was started the same day. Success was achieved in this endeavor by adding rabbit anti-thymocyte serum (ATS) or globulin (ATG) to the TLI regimen such that the first of 10 doses of TLI was combined with 5 doses of ATS or ATG.[23-25] The bone marrow cell injection was delayed until just after the completion of the TLI.[23-25] Almost all MHC mismatched mice and rats given the combined bone marrow and skin or kidney transplants using the

latter regimen developed stable mixed chimerism and tolerance to the organ graft.[25-28]

Cellular and molecular basis of tolerance after TLI

Studies of the cellular and molecular basis of tolerance and chimerism in mice after the TLI and ATS conditioning regimen showed that the conditioning changed the balance of immune cells in the lymphoid tissues for 2 to 3 weeks to favor suppressive cells including natural killer (NKT) cells, Treg cells and myeloid derived suppressor cells (MDSCs) over conventional CD4 and CD8 T cells.[29-33] Each of the suppressor cells was required for chimerism and tolerance induction, since depletion of NKT cells in CD1d$^{-/-}$ or Jalpha 18$^{-/-}$ mice, or depletion of Tregs with anti-CD25 mAb, or depletion of MDSCs with anti-Gr-1 mAb abrogated chimerism and tolerance.[29-33] In each case, chimerism and tolerance were restored by the infusion of the purified suppressive cells from the wild type untreated recipient strain mice.[29-33] In addition to the altered balance of suppressive cells, there was a marked increase in the expression of the negative costimulatory surface receptors, PD-1 and/or Tim-3 on either conventional T cells or Treg cells.[29,30] The latter changes were induced by an IL-4 dependent interaction between the NKT cells and other suppressive cells or conventional T cells.[29,30]

Stable mixed chimerism and tolerance in adult humans

The extensive rodent studies of tolerance after conditioning with TLI provided a basis for application of the regimen to large outbred laboratory animals and to humans. The TLI regimen was initially applied to clinical kidney transplantation in the early 1980s based on the use of the regimen in dogs and nonhuman primates.[34-38] The initial clinical trials studied recipients of deceased donor kidney grafts by extending the duration of administration of pretransplant TLI treatments until the kidney graft became available.[34,35,37] A course of rabbit ATG was given posttransplant followed by maintenance low dose prednisone, and patients received a total dose of TLI of at least 1,800 cGy.[34,35,37] Three of 28 patients using this protocol without haematopoietic cell transplantation were withdrawn from immunosuppressive drugs without subsequent rejection episodes. The latter patients had donor specific unresponsiveness in the MLR, and met the criteria for tolerance in humans.[37] However, there was a high incidence of rejection episodes during the first year after transplantation in patients who could not be withdrawn from immunosuppressive drugs.

In view of the low success rate of immunosuppressive drug withdrawal in the patients conditioned with pretransplant TLI, and the development of a posttransplant TLI and ATS regimen in laboratory animals that was successful when combined with bone marrow transplantation, clinical trials with posttransplantation TLI and ATG conditioning were begun in 2000 using combined kidney and haematopoietic cell transplantation with HLA matched and mismatched living donor grafts.[39-42] The goals of the latter trials were to achieve stable mixed chimerism and tolerance to the kidney transplant such that the lifelong need for maintenance immunosuppressive drugs was eliminated. In parallel, a clinical trial using the same TLI and ATG conditioning regimen with HLA matched haematopoietic cell transplantation was started as treatment for leukemia and lymphoma using fully HLA matched living donors.[43] The goals of the cancer treatment trial were to establish complete chimerism with tumor eradication while preventing GVHD in patients who were not eligible for ablative radiation protocols due to advanced age or medical comorbidities.[43,44]

The combined kidney and haematopoietic cell transplantation trials showed that about 75% of recipients of fully HLA matched recipients developed persistent mixed chimerism for at least 1 year, and immunosuppressive drugs could be completely withdrawn without subsequent rejection episodes.[42] These recipients were given donor cell infusions containing at least 4×10^6 purified CD34$^+$ cells/kg and 1×10^6 T cells/kg obtained from G-CSF "mobilized" donor blood.[42] The recipients who were successfully withdrawn showed specific unresponsiveness to donor cells in the MLR and potent responses to microbial recall antigens in vitro.[40-42] Thus, tolerance was achieved in the majority of the HLA matched recipients using this protocol with observations for as long as 10 years.[42]

In 2010, the same protocol was adapted for use with living related HLA haplotype matched donor grafts.[42] A donor cell dose escalation study showed that the donor T and/or CD34$^+$ cell requirement to achieve

persistent mixed chimerism in HLA mismatched recipients was considerably greater than in the matched patients. However, it has not yet been determined whether the latter chimeras have developed tolerance, and can be completely withdrawn from immunosuppressive drugs.[42] Investigators at Northwestern University have reported achieving tolerance associated with complete chimerism after combined kidney and haematopoietic cell transplantation in HLA mismatched patients.[45,46] In contrast, investigators at the Massachusetts General Hospital have achieved tolerance associated with transient mixed chimerism after combined kidney and haematopoietic cell transplantation in HLA mismatched patients[47,48] based on their studies in non-human primates and mini-pigs.[8,23]

Conclusion

The discovery that immune tolerance to allogeneic tissues is linked to chimerism established *in utero* or neonatally was made about 70 years ago. Since then, the field of organ transplantation has provided life saving treatments for patients with organ failure such that patients can return to normal activities. During that 70 year period, laboratory research on tolerance and chimerism has led to the first demonstrations that clinical tolerance can be achieved in association with chimerism in recipients of kidney transplants.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

Research on clinical tolerance at Stanford was supported by a grant (5 PO1 HL075462) from the National Heart, Lung and Blood Institute.

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