HHS Public Access

Author manuscript

Bone Marrow Transplant. Author manuscript; available in PMC 2021 June 08.

Published in final edited form as:

Bone Marrow Transplant. 2019 August ; 54(Suppl 2): 793–797. doi:10.1038/s41409-019-0603-4.

Combined kidney and hematopoeitic cell transplantation to induce mixed chimerism and tolerance

Robert Lowsky[1], **Samuel Strober**[1]

[1]Divisions of Blood and Marrow Transplantation and Immunology and Rheumatology, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

Abstract

Based on preclinical studies, combined kidney and hematopoietic cell transplantation was performed on fully HLA matched and haplotype matched patients at the Stanford University Medical Center. The object of the studies was to induce mixed chimerism, immune tolerance, and complete immunosuppressive drug withdrawal. Tolerance, persistent mixed chimerism, and complete withdrawal was achieved in the majority of fully matched patients. Persistent mixed chimerism and partial withdrawal has been achieved in the haplotype matched patients at present.

Benefit of tolerance in kidney transplant patients

Currently, there are about 90,000 patients with renal failure undergoing dialysis in the US. The treatment of choice for these patients is kidney transplantation, since transplantation allows for a return to a normal life style and marked extension of life expectancy [1, 2]. However, recipients of kidney transplants require lifelong treatment with maintenance immunosuppressive (IS) drugs usually with a purine metabolism inhibitor, a calcineurin inhibitor, and steroids in order to prevent rejection of the graft. These drugs are associated with cumulative side effects that increase the risk of cardiovascular disease, diabetes, hypertension, infection, cancer, and nephrotoxicity [3, 4]. In addition, the current IS drugs have not appreciably altered the long-term loss of grafts due to chronic rejection, and about 50% of deceased and living donor HLA mismatched grafts are lost in ~12—15 years respectively [5, 6]. In the best circumstance of fully HLA matched kidney transplants, ~50% of grafts are lost in 22–25 years, but the latter patients also require lifelong IS drugs and are subject to their attendant side effects to prevent rejection. The induction of tolerance in kidney transplant patients can transform the field of organ transplantation by providing significant improvements in the health outcome of these patients by eliminating the lifelong need for IS drugs, and by improving graft survival by eliminating chronic rejection.

Samuel Strober sstrober@stanford.edu.

Compliance with ethical standards

Conflict of interest SS and RL receive consulting fees and owns equity in Medeor Therapeutics Inc., and receive royalties payments on patents held by their employer.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Mixed chimerism and tolerance in mice and rats after conditioning with total lymphoid irradiation

Murine recipients of bone marrow transplants in strains differing at the MHC genes developed rapid death due to GVHD when conditioning was myeloablative total body irradiation [7–11]. This problem was overcome when the conditioning was changed to non-myeloablative total lymphoid irradiation (TLI), a radiation regimen developed in humans for the treatment of Hodgkin's disease by H.S. Kaplan and his co-workers [12, 13]. In contrast to the inclusion of all organs in the radiation fields during the administration of TBI, TLI is confined to the lymph nodes, spleen, and thymus [12, 13]. In clinical bone marrow transplantation, the entire intestines are included in the radiation fields used with TBI, and there is considerable gut injury and release of endotoxin that predisposes to GVHD. In contrast, TLI shields most of the intestines in patients given this regimen. Severe neutropenia and thrombopenia are avoided by shielding of the majority of the bone marrow.

As reported in 1976, the TLI regimen was used as a pretransplant conditioning regimen in adult mice to achieve mixed chimerism without GVHD after the transplantation of MHC mismatched bone marrow cells. This approach was adapted to organ transplantation in MHC mismatched adult mice and rats as a pre-transplant regimen to induce stable mixed chimerism and tolerance after combined bone marrow and organ transplantation [7]. Almost all recipients developed mixed chimerism, accepted skin grafts from the donor strain and rejected grafts from third party strains [7–11]. Tolerance to the donor skin grafts was expected in these chimeras based on the work of Medawar and Owen and their co-workers [14, 15], since in utero exposure to donor cells or neonatal injection of donor cells resulted in persistent chimerism and tolerance in adults.

Development of mixed chimerism and tolerance avoiding GVHD in MHC mismatched mice was reported by David Sachs and his co-workers in 1989 [16] by conditioning recipients with sublethal TBI. This regimen required additional high dose irradiation of the thymus and administration of anti-T cell antibodies. The recipients of the combined marrow and organ transplants became tolerant to organ grafts and remained stable mixed chimeras [16, 17]. This regimen was extended to mini-pigs and to non-human primates [15, 18]. In the case of non-human primates the chimerism was lost within the first 90 days, and yet the majority of recipients accepted kidney grafts [19].

The TLI tolerance induction regimen developed at Stanford was made applicable to deceased donor organ transplantation in which the timing of organ availability is uncertain, by changing the conditioning regimen to a completely posttransplant procedure. The organ was transplanted on day 0, and the conditioning regimen was started the same day by adding rabbit anti-thymocyte serum (ATS) or globulin (ATG) to the TLI regimen. Ten doses of TLI were combined with 5 doses of ATS or ATG that was started immediately after organ transplantation [20–22]. The bone marrow cell injection was delayed until just after the completion of the TLI [20–22]. Almost all MHC mismatched mice and rats given this posttransplant conditioning regimen and combined bone marrow and skin or kidney transplants developed stable mixed chimerism and tolerance to the organ graft [20, 23–25].

The TLI and ATS conditioning regimen changed the balance of immune cells in the lymphoid tissues for 2–3 weeks to favor immune suppressive regulatory cells including natural killer (NKT) cells, Treg cells and myeloid derived suppressor cells (MDSCs) over conventional CD4 and CD8 T cells [26–30]. Each of the regulatory cells was required for chimerism and tolerance induction. Loss of NKT cells in genetically engineered CD1d$^{-/-}$ or Jalpha 18$^{-/-}$ mice, or depletion of Tregs after administration of anti-CD25 mAb, or depletion of MDSCs after administration of anti-Gr-1 mAb abrogated chimerism and tolerance [26–30]. The infusion of purified regulatory suppressive cells restored tolerance and chimerism, when the infused cells were obtained from the wild-type untreated recipient strain mice [26–30]. The altered balance of suppressive cells was accompanied by a marked increase in the expression of the negative co-stimulatory surface receptors, PD-1 and/or Tim-3 on either conventional T cells or Treg cells [26, 27]. IL-4-dependent interaction between the NKT cells and other suppressive cells or conventional T cells was required for the changes in the surface receptor expression. The expression of PD-1 On Tregs was associated with the increased secretion of IL-10 [27, 30].

Mixed chimerism, IS drug withdrawal and tolerance after HLA matched kidney and hematopoietic cell transplantation

At present, we have enrolled 29 recipients into an HLA-matched tolerance protocol, and all recipients were given 10 doses of TLI of 120 cGy each and 5 doses of ATG after kidney transplantation. All recipients were given an infusion of column enriched CD34^{+} cells (4.3–17.5 × 10^{6} cells/kg) immediately after the last dose of TLI. In addition to the CD34 cells, 28 patients were given a defined dose of 1 × 10^{6} CD3^{+} T cells. One patient with active lupus was given 10 × 10^{6} CD3^{+} T cells/kg to further promote engraftment of CD34^{+} cells and prevent rejection of the donor cells. IS drug withdrawal criteria was (1) persistent chimerism for at least 6 months, (2) lack of clinical rejection episodes, (3) lack of GVHD, and (4) lack of rejection on a protocol biopsy obtained within 2 weeks before the discontinuation of IS drugs.

Postrransplant prednisone was discontinued after 10 days. Cyclosporine starting at day 0 was tapered such that discontinuation occurred after at least 6 months of chimerism. Standard doses of MMF were administered for 30 days after the infusion of donor cells as reported previously [31]. Twenty-four patients were successfully withdrawn from IS drugs at 6–14 months posttransplant with an observation period of up to 11 years (median 5 years). Three patients developed recurrence of kidney disease, and were returned to maintenance IS drugs in order to treat the relapsed disease.

One patient returned to IS drugs after year 4 due to a rejection episode. The episode was completely reversed without continued graft dysfunction. Twenty three of 24 showed no evidence of rejection after IS drug withdrawal. Five patients did not meet drug withdrawal criteria due to clinical rejection episodes during early IS drug taper, microscopic rejection on a protocol biopsy, or lack of chimerism for more than 6 months. Among the 24 patients who developed chimerism for at least 6 months and had IS drugs successfully discontinued, 10 had stable chimerism during and after IS drug withdrawal. These patients had drug

independent mixed chimerism. In contrast to preclinical observations, 15 patients developed IS drug dependent chimerism beyond the first year posttransplant. The loss of chimerism occurred without subsequent organ graft rejection [31–33].

The mechanisms of tolerance in the patients who lost chimerism in the blood is unclear, and may include persistence of chimerism in the solid lymphoid tissues and maintenance of tolerance by antigen specific regulatory T cells. Since we were successful in inducing persistent chimerism and tolerance in HLA matched patients, we attempted to apply the success to HLA haplotype matched patients. Accordingly, we initiated a new study of T cell and CD34 cell dose escalation in haplotype matched patients to find doses to achieve chimerism and tolerance.

Mixed chimerism and IS drug withdrawal after HLA haplotype matched kidney and hematopoietic cell transplantation

The patients enrolled in this study received the same TLI (10 doses of 120 cGy each) and ATG (5 daily doses) regimen given to the HLA matched patients. However, the content of the donor cell infusion was changed to develop a dose finding study of $CD3^+$ T cells that would promote persistent mixed chimerism for at least 12 months. Donor T cells have been shown to promote the engraftment of hematopoietic progenitor cells, but the risks of GVHD increased if complete instead of mixed chimerism is established [34, 35]. The range of infused $CD34^+$ cells/kg was from 8 to 22 × 10^6 cells/kg in the haplotype matched patients [31–33].

The dose of $CD3^+$ T cells added to the $CD34^+$ cells started at 3 × 10^6 cells/kg, and was increased to 10 × 10^6 cells/kg in the next 4 patients, 20 × 10^6 cells/kg in the next two, 50 × 10^6 cells/kg in 6 patients, 70–75 × 10^6 cells/kg in 2 patients, and 100 × 10^6 cells/kg in 6 patients. Based on the data from the fully HLA matched patients, our goal was to find a dose of T cells and CD34 + cells in the mismatched patients that would induce a peak of whole blood chimerism of at least 30% donor type cells observed at 60 days posttransplant, since that profile was correlated with stable mixed chimerism for at least 1 year in HLA matched recipients. We were able to achieve the goal of stable mixed chimerism for at least 1 year in 9 of the 21 haplotype matched recipients enrolled in the protocol. There was a correlation between persistence of chimerism and the number of CD34 donor cells infused. In order to achieve persistent chimerism the CD34 cell dose had to be at least 10 × 10^6 cells/kg [31–33]. Chimeric patients were tapered from three IS drugs to tacrolimus monotherapy at the end of the 1st year. Protocol biopsies were performed in 6 out of 9 patients at the end of the first year, and 6 out of 6 biopsies showed no microscopic evidence of rejection [31–33].

During the 2nd year posttransplant 6 of the 9 patients with persistent chimerism were tapered off the tacrolimus monotherapy, and chimerism was lost shortly after the blood levels of tacrolimus became subtherapeutic. Three of these patients had rejection episodes that were easily reversed with IS drugs, and the patients were returned to maintenance IS drug therapy. The remaining three continued on therapeutic doses of tacrolimus with persistence of chimerism during the 2nd year. In conclusion, the haplotype matched patients could be tapered to tacrolimus monotherapy with persistence of chimerism, but could not be

withdrawn from IS drugs completely due to loss of chimerism and associated rejection episodes [31–33].

Graft survival in HLA matched and mismatched transplant patients

We analyzed kidney transplant graft survival of HLA matched patients using Kaplan–Meier statistical methods, and compared the first 22 HLA matched patients enrolled in the tolerance induction protocol with control HLA matched patients given conventional immunosuppressive therapy at the Stanford Medical Center during the same period of time. The patients enrolled in the tolerance induction protocol had 100% actuarial graft survival over an observation period of over 11 years from 2005 to 2016. The control group of HLA-matched patients were observed for over 12 years from 2001 to 2013. The group had an actuarial graft survival of about 86%, and the graft survival difference between tolerance protocol and control patients was not statistically significantly different (Greenwood formula) due to the small number of protocol patients. We performed another comparison of the graft survival of the combined group of the first 20 HLA matched and the first 10 HLA mismatched patients (total 30) enrolled in the tolerance protocols using as controls conventionally treated living donor kidney transplant patients with similar pretransplant characteristics and similar initial IS drug therapy as recorded in the US national registry maintained by the Organ Procurement Transplant Network/Scientific Registry of Transplant Recipients (OPTN/SRTR). Co-variate parameters of the protocol patients including donor and recipient age, gender and BMI, number of HLA mismatches, race of donor and recipient, and anti-rejection drugs were matched with those of registry patients. A nomogram was previously constructed that accurately predicts 5-year graft survival based on the co-variates [36]. The observed 5-year graft survival of protocol patients (100%) was compared with the predicted graft survival of all patients in the registry with matched characteristics based on the nomogram. The registry patients were predicted to have ~83% 5-year graft survival, and the latter survival was significantly lower than the observed.

Summary and future directions

None of the 50 HLA matched and mismatched patients enrolled in the tolerance protocol had graft loss or chronic rejection during the observation period from 2005 through 2017. About 80% of the fully HLA matched were successfully withdrawn from IS drugs. In contrast, IS drugs in the haplotype matched recipients could be reduced to tacrolimus monotherapy, but could not be completely withdrawn. The T cell dose escalation study is continuing in order to determine whether there is a threshold donor T cell dose that will increase the levels of mixed chimerism such that during tapering of tacrolimus monotherapy during the 2nd year will allow for persistence of mixed chimerism after the complete withdrawal of IS drugs, thereby, preventing rejection of the kidney transplants.

Methods

This is a minireview of the experience of a single center in which preclinical and clinical studies of combined hematopoietic cell and kidney transplantation were performed at

Stanford University to induce immune tolerance. Search criteria were articles related to the above subject. Thirty six articles were identified, and 36 were included.

Funding

The research was supported by grants from their National Institutes of Health (P01HL075462 and R01 AI 1085024), and a grant from the California Institute of Regenerative Medicine (CLIN2–09439). Publication of this supplement was sponsored by Gilead Sciences Europe Ltd, Cell Source, Inc., The Chorafas Institute for Scientific Exchange of the Weizmann Institute of Science, Kiadis Pharma, Miltenyi Biotec, Celgene, Centro Servizi Congressuali, Almog Diagnostic.

The research was supported by grants from their National Institutes of Health (P01HL075462 and R01 AI 1085024), and a grant from the California Institute of Regenerative Medicine (CLIN2–09439). Publication of this supplement was sponsored by Gilead Sciences Europe Ltd, Cell Source, Inc., The Chorafas Institute for Scientific Exchange of the Weizmann Institute of Science, Kiadis Pharma, Miltenyi Biotec, Celgene, Centro Servizi Congressuali, Almog Diagnostic.

References

1. Collins AJ, Foley RN, Chavers B, Gilbertson D, Herzog C, Ishani A, et al. US Renal Data System 2013 Annual Data Report. Am J Kidney Dis. 2014;63(Suppl 1):A7. [PubMed: 24360288]
2. Suthanthiran M, Strom TB. Renal transplantation. New Engl J Med. 1994;331:365–76. [PubMed: 7832839]
3. Marcen R Immunosuppressive drugs in kidney transplantation: impact on patient survival, and incidence of cardiovascular disease, malignancy and infection. Drugs. 2009;69:2227–43. [PubMed: 19852526]
4. Srinivas TR, Schold JD, Guerra G, Eagan A, Bucci CM, Meier-Kriesche HU. Mycophenolate mofetil/sirolimus compared to other common immunosuppressive regimens in kidney transplantation. Am J Transplant. 2007;7:586–94. [PubMed: 17229066]
5. Cecka JM. The UNOS Scientific Renal Transplant Registry–ten years of kidney transplants. Clin Transplant, 1997;1–14. [PubMed: 9067686]
6. Yang H Maintenance immunosuppression regimens: conversion, minimization, withdrawal, and avoidance. Am J Kidney Dis. 2006;47:S37–51. [PubMed: 16567240]
7. Slavin S, Strober S, Fuks Z, Kaplan HS. Long-term survival of skin allografts in mice treated with fractionated total lymphoid irradiation. Science. 1976;193:1252–4. [PubMed: 785599]
8. Strober S, Slavin S, Gottlieb M, Zan-Bar I, King DP, Hoppe RT, et al. Allograft tolerance after total lymphoid irradiation (TLI). Immunol Rev. 1979;46:87–112. [PubMed: 39027]
9. Liu YP, Li Z, Nador RG, Strober S. Simultaneous protection against allograft rejection and graft-versus-host disease after total lymphoid irradiation: role of natural killer T cells. Transplantation. 2008;85:607–14. [PubMed: 18347541]
10. Slavin S, Reitz B, Bieber CP, Kaplan HS, Strober S. Transplantation tolerance in adult rats using total lymphoid irradiation: permanent survival of skin, heart, and marrow allografts. J Exp Med. 1978;147:700–7. [PubMed: 147301]
11. Slavin S, Strober S. Induction of allograft tolerance after total lymphoid irradiation (TLI): development of suppressor cells of the mixed leukocyte reaction (MLR). J Immunol. 1979;123:942–6. [PubMed: 156766]
12. Coleman C, Burke J, Varghese A, Rosenberg S, Kaplan H. Malignant lymphomas: etiology, immunology, pathology, treatment (ed H'S Kids Rosenberg 5). Academic Press; New York City, 1982. p. 259–76.
13. Kaplan H Hodgkin's disease. Harvard University Press; Cambridge, MA, 1980.
14. Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. Nature. 1953;172:603–6. [PubMed: 13099277]
15. Owen RD. Immunogenetic consequences of vascular anastomoses between bovine twins. Science. 1945;102:400–1. [PubMed: 17755278]

16. Sharabi Y, Sachs DH. Mixed chimerism and permanent specific transplantation tolerance induced by a nonlethal preparative regimen. J Exp Med. 1989;169:493–502. [PubMed: 2562984]
17. Tomita Y, Khan A, Sykes M. Role of intrathymic clonal deletion and peripheral anergy in transplantation tolerance induced by bone marrow transplantation in mice conditioned with a non-myeloablative regimen. J Immunol. 1994;153:1087–98. [PubMed: 8027542]
18. Sykes M Mixed chimerism and transplant tolerance. Immunity. 2001;14:417–24. [PubMed: 11336687]
19. Kawai T, Cosimi AB, Colvin RB, Powelson J, Eason J, Kozlowski T, et al. Mixed allogeneic chimerism and renal allograft tolerance in cynomolgus monkeys. Transplantation. 1995;59:256–62. [PubMed: 7839449]
20. Hayamizu K, Lan F, Huie P, Sibley RK, Strober S. Comparison of chimeric acid and non-chimeric tolerance using posttransplant total lymphoid irradiation: cytokine expression and chronic rejection. Transplantation. 1999;68:1036–44. [PubMed: 10532547]
21. Woodley SL, Gurley KE, Hoffmann SL, Nicolls MR, Hagberg R, Clayberger C, et al. Induction of tolerance to heart allografts in rats using posttransplant total lymphoid irradiation and anti-T cell antibodies. Transplantation. 1993;56:1443–7. [PubMed: 8279017]
22. Zeng D, Ready A, Huie P, Hayamizu K, Holm B, Yin D, et al. Mechanisms of tolerance to rat heart allografts using posttransplant TLI. Changes in cytokine expression. Transplantation. 1996;62:510–7. [PubMed: 8781618]
23. Higuchi M, Zeng D, Shizuru J, Gworek J, Dejbakhsh-Jones S, Taniguchi M, et al. Immune tolerance to combined organ and bone marrow transplants after fractionated lymphoid irradiation involves regulatory NK T cells and clonal deletion. J Immunol. 2002;169:5564–70. [PubMed: 12421933]
24. Lan F, Hayamizu K, Strober S. Cyclosporine facilitates chimeric and inhibits nonchimeric tolerance after posttransplant total lymphoid irradiation. Transplantation. 2000;69:649–55. [PubMed: 10708124]
25. Nador RG, Hongo D, Baker J, Yao Z, Strober S. The changed balance of regulatory and naive T cells promotes tolerance after TLI and anti-T-cell antibody conditioning. Am J Transplant. 2010;10:262–72. [PubMed: 20041865]
26. Hongo D, Tang X, Baker J, Engleman EG, Strober S. Requirement for interactions of natural killer T cells and myeloid-derived suppressor cells for transplantation tolerance. Am J Transplant. 2014;14:2467–77. [PubMed: 25311657]
27. Hongo D, Tang X, Dutt S, Nador RG, Strober S. Interactions between NKT cells and Tregs are required for tolerance to combined bone marrow and organ transplants. Blood. 2012;119:1581–9. [PubMed: 22174155]
28. Lan F, Zeng D, Higuchi M, Higgins JP, Strober S. Host conditioning with total lymphoid irradiation and antithymocyte globulin prevents graft-versus-host disease: the role of CD1-reactive natural killer T cells. Biol Blood Marrow Transplant. 2003;9:355–63. [PubMed: 12813443]
29. Lan F, Zeng D, Higuchi M, Huie P, Higgins JP, Strober S. Predominance of NK1.1 + TCR alpha beta + or DX5 + TCR alpha beta + T cells in mice conditioned with fractionated lymphoid irradiation protects against graft-versus-host disease: “natural suppressor” cells. J Immunol. 2001;167:2087–96. [PubMed: 11489992]
30. Pillai AB, George TI, Dutt S, Strober S. Host natural killer T cells induce an interleukin-4-dependent expansion of donor CD4 + CD25 + Foxp3 + T regulatory cells that protects against graft-versus-host disease. Blood. 2009;113:4458–67. 10.1182/blood-2008-06-165506. [PubMed: 19221040]
31. Scandling JD, Busque S, Shizuru JA, Lowsky R, Hoppe R, Dejbakhsh-Jones S, et al. Chimerism, graft survival, and withdrawal of immunosuppressive drugs in HLA matched and mismatched patients after living donor kidney and hematopoietic cell transplantation. Am J Transplant. 2015;15:695–704. [PubMed: 25693475]
32. Kawai T, Leventhal J, Wood K, Strober S. Summary of the third international workshop on clinical tolerance. Am J Transplant. 2018. 10.1111/ajt.15086.
33. Scandling JD, Busque S, Lowsky R, Shizuru J, Shori A, Engleman E, et al. Macrochimerism and clinical transplant tolerance. Hum Immunol. 2018;79:266–71. [PubMed: 29330112]

34. Cao TM, Shizuru JA, Wong RM, Sheehan K, Laport GG, Stockerl-Goldstein KE, et al. Engraftment and survival following reduced-intensity allogeneic peripheral blood hematopoietic cell transplantation is affected by CD8 + T-cell dose. Blood. 2005;105:2300–6. [PubMed: 15572597]

35. Urbano-Ispizua A, Rozman C, Pimentel P, Solano C, de la Rubia J, Brunet S, et al. The number of donor CD3(+) cells is the most important factor for graft failure after allogeneic transplantation of CD34(+) selected cells from peripheral blood from HLA-identical siblings. Blood. 2001;97:383–7. [PubMed: 11154212]

36. Tiong HY, Goldfarb DA, Kattan MW, Alster JM, Thuita L, Yu C, et al. Nomograms for predicting graft function and survival in living donor kidney transplantation based on the UNOS Registry. J Urol. 2009;181:1248–55. [PubMed: 19167732]