Use of Hematopoietic Cell Transplants to Achieve Tolerance in Patients with Solid Organ Transplants
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Abstract
The goals of tolerance in patients with solid organ transplants are to eliminate the lifelong need for immunosuppressive drugs and to prevent graft loss due to rejection or drug toxicity. Tolerance with complete withdrawal of immunosuppressive drugs has been achieved in recipients of HLA matched and mismatched living donor kidney transplants in 3 medical centers using hematopoietic cell transplants to establish mixed or complete chimerism.

Introduction
The concepts of persistent mixed hematopoietic cell chimerism and immune tolerance of foreign tissues emerged during the middle of the twentieth century with the seminal observations and experiments in cattle and rodents by Ray Owen and Peter Medawar and their colleagues. Based on the latter work and numerous subsequent studies, it was concluded that stable mixed chimerism contributed to tolerance by inducing clonal deletion of alloreactive immune cells in the thymus and peripheral lymphoid tissues. However, clonal deletion alone is not sufficient for induction and maintenance of host immune tolerance to donor organ allografts, and immune regulation is required also. Establishment of stable complete chimerism with replacement of all host with donor hematopoietic and immune cells, has been a key goal for bone marrow transplantation as treatment for hematopoietic malignancies because of the persistent graft anti-tumor activity. There are no host immune cells in the complete chimeras, so there is no need to induce host versus graft immune tolerance. However, there is a considerably greater risk of GVHD in stable complete as compared to stable mixed chimeras. Thus, for purposes of tolerance induction to organs, the achievement of stable mixed chimerism instead of complete chimerism is more desirable, and may be a useful biomarker of the persistence of host versus graft tolerance.

The search for clinically applicable approaches to tolerance to organ grafts that are at least as safe as the standard of care transplant immunosuppressive (IS) drug regimens became the search for the “Holy Grail” of the field. The “Grail” included the elimination of the lifelong need for IS drugs and their cumulative side effects as well as the absence of graft loss due to acute or chronic rejection or drug toxicity. Currently, all kidney transplant recipients must remain on maintenance IS drugs indefinitely to prevent rejection, and even with improvements in potency of the latter drugs, the incidence of gradual graft loss after 3 years has not changed appreciably in the past decade.

A safe preclinical approach to transplantation tolerance was reported in the 1970’s by conditioning of adult MHC mismatched rodents with fractionated lymphoid tissue irradiation (total lymphoid irradiation; TLI), a non-myeloablative conditioning regimen developed for the treatment of Hodgkin’s Disease. Stable mixed chimerism and transplantation tolerance involving deletion and regulation were established after bone marrow transplantation without GVHD. Additional approaches to establish stable mixed chimerism and tolerance in rodents were reported in the 1980’s using conditioning with lethal TBI, and infusion of both donor and recipient marrow cells, or conditioning with sublethal TBI, thymic irradiation, and anti-T cell antibodies before administration of donor marrow cells. The TLI and TBI rodent approaches were adapted for use in non-human primate, canine, and swine models of tolerance induction to organ grafts. Thereafter, the conditioning regimens were modified to achieve tolerance to organ transplants in humans. The first report of clinical tolerance used a combination of TLI and rabbit anti-thymocyte globulin (ATG) to condition recipients of deceased donor HLA mismatched kidney transplants without hematopoietic cell transplantation (HCT) or development of chimerism. However, the incidence of successful tolerization was low, and HCT was added thereafter to try to achieve a higher incidence of success after the establishment of chimerism.

Induction of tolerance in HLA matched patients
The feasibility of combined transplantation of kidney and bone marrow in HLA matched patients with kidney failure and multiple myeloma was reported in 7 recipients using a pretransplant conditioning regimen of cyclophosphamide, thymic radiation, and equine ATG developed by investigators at the Massachusetts General Hospital. Four of these patients with transient (<105 days) mixed chimerism accepted the kidney transplants without the use of maintenance IS drugs for up to 7 years without subsequent evidence of rejection. The results demonstrated the success of the chimerism approach in the induction of tolerance to kidney grafts. However, three of the 7 HLA matched patients who became complete chimeras developed acute or chronic GVHD that was treated with IS drugs.
In further studies, the TLI and ATG conditioning regimen used for HCT in patients with hematologic malignancies was modified to induce tolerance in HLA matched organ transplant patients without malignancies. The latter patients were given kidney transplants combined with HCT at Stanford. The key change for the patients without malignancies was the alteration of the composition of the HCT to induce mixed instead of complete chimerism in order to prevent GVHD. Whereas the patients with malignancies received infusions of unmanipulated “mobilized” blood mononuclear cells containing about 2 to 3x10^8 T cells/kg immediately after the completion of TLI, the kidney transplant patients received column enriched CD34+ cells obtained from the “mobilized” blood mononuclear cells with an add back of 1x10^6 T cells/kg contained in the column effluent.

In the most recent report of 22 HLA matched patients enrolled in the TLI and ATG tolerance protocol, 18 who had at least 12 months of mixed chimerism were completely withdrawn from IS drugs. Withdrawal occurred after 1 month of mycophenolate mofetil, and 6 to 12 months of cyclosporine treatment. Patients were observed for up to 7 years after IS drug withdrawal (median 29 months), and none had subsequent rejection episodes or GVHD. All 22 had good kidney graft function at the last observation point. None of the 22 patients were hospitalized for bacterial, fungal, or viral infections, and one had severe (<500 cells/mm³) neutropenia.

Another trial of combined HLA matched hematopoietic and kidney transplantation was reported by investigators at Northwestern University in which unmanipulated bone marrow or G-CSF “mobilized” blood mononuclear cells were injected into recipients along with standard of care IS drugs. Eight of 10 recipients given transplants were completely withdrawn from IS drugs during the second year posttransplant, and 3 of the 8 patients subsequently developed rejection episodes, and were returned to IS drugs. The remaining 5 patients had no evidence of rejection during a follow up period of up to 32 months off IS drugs.

**Induction of Tolerance in HLA Haplotype Matched Patients**

Investigators at the Massachusetts General Hospital enrolled 10 HLA haplotype matched patients in a tolerance protocol of combined kidney and unmanipulated bone marrow transplantation. Conditioning included pretransplant cyclophosphamide, thymic irradiation, and anti-CD2 monoclonal antibodies with or without rituximab, and all patients developed mixed chimerism that was lost after a few weeks. Seven of the patients were completely withdrawn from IS drugs, and 4 of the latter patients maintained good graft function without subsequent rejection episodes with 4 to 11 years of observation off IS drugs. Three of the 7 patients were returned to IS drug therapy after 6 to 8 years of discontinuation due to the development of chronic rejection (2 patients) or to relapse of the underlying kidney disease (one patient). Three of 10 patients had graft loss within 18 months after transplantation due to either rejection (2 patients) or to thrombotic microangiopathy (1 patient).

In contrast to the above study of haplotype matched kidney transplant patients with transient mixed chimerism, investigators at Northwestern University performed a tolerance induction trial designed to achieve complete chimerism based on a conditioning regimen developed at Johns Hopkins University for haplotype matched patients with hematologic malignancies. The conditioning included pre and posttransplant cyclophosphamide with pretransplant fludarabine and TBI (200 cGy). Donor cells were G-CSF “mobilized” blood mononuclear cells that were manipulated by Regenerex LLC to enrich for hematopoietic progenitors and “facilitator cells” while reducing the T cell content to about 4x10^5 T cells/kg. Mycophenolate mofetil and tacrolimus were withdrawn by the end of one year. Of 19 patients who were enrolled in the Northwestern University protocol and followed for at least 18 months after transplantation, 12 patients who achieved durable chimerism were completely withdrawn from IS drugs for 8 to 48 months without subsequent rejection episodes. Eleven of 12 patients had stable complete chimerism, and one had stable mixed chimerism. Two of 19 patients had graft loss, and the remainder had good graft function. One patient developed GVHD. Almost all patients had initial severe neutropenia (ANC < 100 cells/mm³), and 11 patients developed bacterial or fungal infections that were effectively treated.

Investigators at Stanford performed a trial of combined kidney and HCT transplantation in haplotype matched patients using the same conditioning regimen as with fully HLA matched patients. The composition of the injected donor cells was changed in order to achieve persistent mixed chimerism in a T cell dose escalation study reported in 10 patients. The results of the study showed that achievement of high levels of chimerism (>40% donor cells among granulocytes) that persisted at least 70 days required high levels of CD34+ cells (≥ 10x10^6 cells/kg), and high levels of T cells (50x10^6 cells/kg) when as few as 10x10^6 CD34+ cells/kg were infused. Although 4 of 10 haplotype matched patients achieved persistent mixed chimerism in the latter study, the success of withdrawal of IS drugs was not determined in these patients due to the short term nature
of the study, and further follow up is required.\textsuperscript{41}

Table 1 summarizes the achievement of tolerance after combined kidney and HCT transplantation in the three medical centers. A total of 41 HLA matched and mismatched patients were completely withdrawn from IS drugs without subsequent rejection episodes or return to IS drugs at the last observation point. The results clearly show that HCT is a feasible approach to allow the complete withdrawal of IS drugs from kidney transplant patients with observation periods of up to 10 years (median 20 to 62 months).

Monitoring for Immune Reconstitution, Chimerism, and Immune Tolerance

Profound immunosuppression was induced by the conditioning regimens for at least 6 months followed by immune reconstitution of the T cell repertoire and responses to microbial antigens.\textsuperscript{37-41,47,53} Specific unresponsiveness to donor alloantigens was observed in tolerant patients as judged by the mixed leukocyte reaction (MLR) in some studies, and by measurement of reduced frequencies of alloreactive clones as judged by high throughput sequencing in others.\textsuperscript{37-40,47,53-56} As in the preclinical studies, contributors to tolerance included immune regulation and clonal deletion.\textsuperscript{38,40,47,48,55,56} Chimerism appeared to be required for the induction but not maintenance of tolerance in 2 centers that established mixed but not complete chimerism. However, the development of chronic rejection several years after IS withdrawal in some patients with transient chimerism indicates that tolerance may wane in the absence of stable chimerism.\textsuperscript{49}

Mechanisms after conditioning that promote persistent chimerism and prevent GVHD

The conditioning regimens at Northwestern University and Stanford University protected against GVHD despite persistent chimerism by using posttransplant cyclophosphamide to deplete proliferating alloreactive donor cells in the former case, and TLI to induce host regulatory cells to block donor cell alloreactivity in the latter.\textsuperscript{37,41,51-53} The immune monitoring studies in patients and in associated preclinical models indicated that regulatory, innate and adaptive immune cells from both the donors and hosts including NKT cells\textsuperscript{37,40,42,57-64}, Treg cells\textsuperscript{37,57,58,65,66}, facilitator cells\textsuperscript{67,68}, myeloid derived suppressor cells (MDSCs) and dendritic cells (DCs) contributed to tolerance.\textsuperscript{59,66,69}

An example of the complex interactions of regulatory cells after conditioning of mice with TLI is shown in the diagram in Figure 1. Due to the high sensitivity of conventional T cells to radiation induced cell death, the more radioresistant CD8\textsuperscript{+} dendritic cells (DCs), NKT cells, Treg cells, and MDSCs markedly increased in percentage among residual cells.\textsuperscript{57-59,69-71} There was a sequential activation of the host regulatory cells starting with the CD8\textsuperscript{+} DCs, then the NKT cells, followed by the MDSCs and Treg cells as judged by upregulation of surface receptors such as PD-1 and PDL-1, and production of secreted regulatory proteins such as IDO from DCs, arginase-1 from MDSCs, IL-4 from NKT cells or IL-10 from Treg cells.\textsuperscript{57-62,69} Activation of the host Tregs and MDSCs cells by the host NKT cells was IL-4 dependent. In addition, there was an IL-4 dependent interaction between the host NKT cells and donor Treg cells that suppressed the alloreactivity of donor conventional T cells to prevent GVHD.\textsuperscript{65,66}

Conclusions

In conclusion, the feasibility of inducing tolerance to living donor kidney transplants for up to 10 years has been demonstrated using HCT. Multi-center randomized trials are needed to compare the short and long term safety of the tolerance protocols to regimens with the lowest doses of standard of care IS drugs. The success of tolerance induction to living donor grafts using a completely posttransplant regimen suggests the feasibility of application to deceased donor grafts with at least partial HLA matching.
Figure Legends

Figure 1. The diagram summarizes the cell interactions and molecular products that prevent rejection of combined donor hematopoietic cell and organ transplants by host conventional T cells (Tcon), and prevent GVHD induced by donor conventional T cells after conditioning with TLI and ATG. Cell death in the lymphoid tissues induced by fractionated irradiation is sensed by host CD8+ DCs that become tolerogenic with indoleamine 2, 3-dioxygenase (IDO) production, and then activate host invariant (i)NKT cells to upregulate surface markers such as PD-1 and NKG2D, and polarize their cytokine production toward IL-4. The host NKT cells, in turn, activate host Treg cells to upregulate PD-1 and polarize their cytokine production towards IL-10. The host NKT cells also activate host MDSCs to upregulate PDL-1 and IL-4Rα, and to secrete arginase-1 (Arg-1). These activated innate and adaptive host immune cells suppress rejection by host conventional T cells, and promote chimerism and organ graft acceptance. In parallel, the activated host NKT cells also activate donor Treg cells to expand, and polarize their cytokine production towards IL-10. The donor Treg cells block GVHD.

Acknowledgments

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Authorship

Contribution: S.S. wrote and edited the manuscript.
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References


2002;169(10):5564-5570.
69. Hongo D, Strober S. Tolerogenic interactions between CD8+ myeloid dendritic cells and natural killer T cells are required for acceptance of combined organ and bone marrow transplants. Abstract submitted. 2015.
Table 1.

Summary of Achievement of Tolerance to Kidney Transplants Combined with HCT

<table>
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<th>Number of Patients off IS Drugs at Last Observation (N=41)</th>
<th>Medical Center b</th>
<th>HLA Matched or Mismatched</th>
<th>Duration off IS Drugs at Last Observation range (median) months</th>
<th>Chimerism Status - Number of Patients</th>
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a patients all had myeloma

b MGH - Massachusetts General Hospital Harvard University
SU - Stanford University Medical Center
NW - Northwestern University Medical Center

c chimerism < 3% peak in blood
Figure 1
Use of hematopoietic cell transplants to achieve tolerance in patients with solid organ transplants

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