Use of hematopoietic cell transplants to achieve tolerance in patients with solid organ transplants

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The goals of tolerance in patients with solid organ transplants are to eliminate the lifelong need for immunosuppressive (IS) drugs and to prevent graft loss due to rejection or drug toxicity. Tolerance with complete withdrawal of IS 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. (Blood. 2016;127(12):1539-1543)

Introduction

The concepts of persistent mixed hematopoietic cell chimerism and immune tolerance of foreign tissues emerged during the middle of the 20th century with the seminal observations and experiments in cattle and rodents by Owen, Anderson et al, and Billingham et al. 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 also required. The establishment of stable complete chimerism with replacement of all hosts with donor hematopoietic and immune cells, has been a key goal for bone marrow transplantation (BMT) as treatment of 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 vs graft immune tolerance. However, there is a considerably greater risk of graft-versus-host disease (GVHD) in stable complete as compared with 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 vs 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 1970s by conditioning of adult major histocompatibility complex mismatched rodents with fractionated lymphoid tissue irradiation (total lymphoid irradiation; TLI), a non-myeloablative conditioning regimen developed for the treatment of Hodgkin disease. Stable mixed chimerism and transplantation tolerance involving deletion and regulation were established after BMT without GVHD. Additional approaches to establish stable mixed chimerism and tolerance in rodents were reported in the 1980s using conditioning with lethal total body irradiation (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 nonhuman primates, 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 tolerance 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 BM 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 Harvard University (MGH). 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, 3 of the 7 HLA-matched patients who became complete chimeras developed acute or chronic GVHD and were 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 University Medical Center (SU). 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 3 × 10⁶ T cells/kg immediately after the completion of TLI. In the study of haplotype-matched kidney transplant patients, investigators at the MGH enrolled 10 HLA haplotype-matched patients.42 The kidney transplant patients received column enriched CD34⁺ cells obtained from the “mobilized” blood mononuclear cells with an add-back of 1 × 10⁶ T cells/kg contained in the column effluent.43-44

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 cyclosorine treatment.41 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 patients had good kidney graft function at the last observation point.41 None of the 22 patients were hospitalized for bacterial, fungal, or viral infections, and 1 had severe (<500 cells/mm⁳) neutropenia.41

Another trial of combined HLA-matched hematopoietic and kidney transplantation was reported by investigators at Northwestern University Medical Center (NW), in which unmanipulated BM or granulocyte colony-stimulating factor “mobilized” blood mononuclear cells were injected into recipients along with standard of care IS drugs.44 Eight of the 10 recipients were completely withdrawn from IS drugs during year 2 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.44

### Induction of tolerance in HLA haplotype-matched patients

Investigators at the MGH enrolled 10 HLA haplotype-matched patients in a tolerance protocol of combined kidney and unmanipulated BMT.45-49 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.49 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.49 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 (1 patient).49 Three of 10 patients had graft loss within 18 months after transplantation due to either rejection (2 patients) or to thrombotic microangiopathy (1 patient).49

In contrast to the above study of haplotype-matched kidney transplant patients with transient mixed chimerism, investigators at NW 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.50 The conditioning included pre- and posttransplant cyclophosphamide with pretransplant fludarabine and TBI (200 cGy).51-53 Donor cells were granulocyte colony-stimulating factor “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 4 × 10⁶ T cells/kg.51-53 Mycophenolate mofetil and tacrolimus were withdrawn by the end of 1 year.51-53 Of 19 patients who were enrolled in the NW 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.53 Eleven of 12 patients had stable complete chimerism, and 1 had stable mixed chimerism.53 Two of 19 patients had graft loss, and the remainder had good graft function. One patient developed GVHD.54 Almost all patients had initial severe neutropenia (absolute neutrophil count <100 cells/mm³), and 11 patients developed bacterial or fungal infections that were effectively treated.53

Investigators at SU performed a trial of combined kidney and HCT transplantation in haplotype-matched patients using the same conditioning regimen as with fully HLA-matched patients.52 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.41 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 (≥10 × 10⁶ cells/kg), and high levels of T cells (50 × 10⁶ cells/kg) when as few as 10 × 10⁶ CD34⁺ cells/kg were infused.41 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.41

### Table 1. Summary of achievement of tolerance to kidney transplants combined with HCT

<table>
<thead>
<tr>
<th>Number of patients off IS drugs at last observation (N = 41)</th>
<th>Medical center</th>
<th>HLA-matched or mismatched</th>
<th>Duration off IS drugs at last observation range (median mo)</th>
<th>Chimerism status (# of patients)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3*†</td>
<td>MGH</td>
<td>Matched</td>
<td>3-85 (62)</td>
<td>Transient mixed (3)</td>
<td>35</td>
</tr>
<tr>
<td>17</td>
<td>SU</td>
<td>Matched</td>
<td>2-66 (29)</td>
<td>Transient mixed (10)</td>
<td>38-41</td>
</tr>
<tr>
<td>3</td>
<td>NW</td>
<td>Matched</td>
<td>15-32 (20)</td>
<td>Transient mixed (3†)</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>MGH</td>
<td>Mismatched</td>
<td>45-127 (51)</td>
<td>Transient mixed (4)</td>
<td>45-47</td>
</tr>
<tr>
<td>12</td>
<td>NW</td>
<td>Mismatched</td>
<td>8-48 (29)</td>
<td>Stable complete (11)</td>
<td>51-54</td>
</tr>
</tbody>
</table>

*Patients all had myeloma.
†Chimerism <3% peak in blood.

### 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. Specific unresponsiveness to donor alloantigens was observed in tolerant patients as judged by the mixed leukocyte reaction in some studies, and by measurement of reduced frequencies of alloreactive clones as judged by high throughput sequencing in others. As in the preclinical studies, contributors to tolerance included immune regulation and clonal deletion. 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.

Mechanisms after conditioning that promote persistent chimerism and prevent GVHD

The conditioning regimen at NW and SU 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. 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 natural killer T (NKT) cells, regulatory T cells (Tregs), facilitator cells, myeloid-derived suppressor cells (MDSCs), and dendritic cells (DCs) contributed to tolerance.

An example of the complex interactions of regulatory cells after conditioning of mice with TLI is shown in Figure 1. Due to the high sensitivity of conventional T cells to radiation-induced cell death, the more radio-resistant CD8+ DCs, NKT cells, Tregs, and MDSCs markedly increased in percentage among residual cells. There was a sequential activation of the host regulatory cells starting with the CD8+ DCs, then the NKT cells, and followed by the MDSCs and Tregs as judged by upregulation of surface receptors such as programmed death 1 (PD-1) and PD ligand 1, and the production of secreted regulatory proteins such as indoleamine 2, 3-dioxygenase from DCs, arginase-1 from MDSCs, interleukin (IL)-4 from NKT cells, or IL-10 from Tregs. Activation of the host Tregs and MDSCs 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 Tregs that suppressed the allosecreativity of donor conventional T cells to prevent GVHD.

Conclusion

In conclusion, the feasibility of inducing tolerance to living donor kidney transplants for up to 10 years has been demonstrated using HCT. Multicenter 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.

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Authorship

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

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