Immunotolerance, which is nonimmunologic reactivity to specific tissue, was demonstrated in animals in the 1950s. However, despite assiduous efforts, it has not been reproduced in human solid-organ transplant to date. Fortuitously, clinical operational tolerance, which is stable graft function for > 1 year with no immunosuppression, has been demonstrated, primarily in a few nonadherent recipients of kidney and liver transplant. Vigorous efforts to identify a biomarker to distinguish recipients with clinical operational tolerance from nontolerant recipients have so far not been successful. However, weaning of immunosuppression in stable pediatric and adult liver transplant recipients has been successful in 60% and 20% of recipients. In kidney transplant recipients, clinical operational tolerance has been induced by combined kidney and hematopoietic cell transplant to induce chimerism and subsequent weaning of immunosuppression. Recently, the ex vivo expansion of autologous regulatory T cells with subsequent infusion has facilitated weaning of immunosuppression in liver transplant recipients. Protocols have been initiated to expand the use of regulatory T cells to kidney transplant recipients. These new methodologies have the potential to induce clinical operational tolerance in all recipients of a solid-organ transplant in the future, thus avoiding the long-term consequences of continued dependence on immunosuppressive medications for stable graft function.

Key words: Avoidance, Immunosuppression, Rejection, Withdrawal

What Is the Definition of Tolerance?
Immunologic tolerance is specific immunologic unresponsiveness to specific donor tissue. Operational tolerance is long-term (> 1 y) allograft acceptance with clinically stable graft function without the requirement for continuous immunosuppressive therapy. Prope tolerance is almost tolerance with stable allograft function being maintained with low doses, presumably nontoxic, of immunosuppressive agents, which is required indefinitely.

The ability to produce immunologic tolerance was first demonstrated experimentally by Billingham and associates when they showed that inoculation of fetal mice or chick embryos with donor tissue resulted in permanent acceptance of donor skin allografts after birth or hatching. Third-party allografts were rejected. Sir Peter Medawar subsequently received the Nobel Prize for this discovery.

Unfortunately, for more than 60 years since the first successful solid-organ transplant between identical twins (isograft) in 1954 by Murray and colleagues in Boston, despite intense investigation directed toward developing the methodology to produce immunologic tolerance in human solid-organ transplant, immunologic tolerance has not been clinically successful to date. Joseph Murray also received the Nobel Prize for this effort.

In the absence of the ability to achieve immunologic tolerance, clinical advances in human solid-organ transplant have progressed significantly during the past > 60 years by targeting suppression of the immune system with a myriad of immunosuppressive agents.

Why Is It Important to Achieve Some Form of Tolerance in Human Solid-Organ Transplant?
Despite dramatic improvements in short-term renal allograft function and survival with current immunosuppressive agents, the long-term allograft survival
rates, especially with regard to the kidney, have improved minimally. In addition, adverse effects from immunosuppressive agents produce morbidity and adversely affect the quality of life of recipients and curtail long-term graft and patient survival rates. These adverse effects include an increased incidence of malignancy, especially involving the skin, an increased incidence of cardiovascular disease, nephrotoxicity, not only involving the kidney graft but affecting native kidneys of other solid-organ recipients, cosmetic adverse effects, and, in pediatric recipients, growth retardation and nonadherence.

What Is the Current Status of Clinical Operation Tolerance and Which Recipients of Solid-Organ Transplant Have Exhibited Clinical Operation Tolerance?

Clinical operational tolerance (COT) is so named as a result of the following circumstances: (1) recipients who, despite nonadherence with immunosuppressive medications, exhibit long-term graft survival with good graft function; (2) recipients who are weaned off or have their immunosuppressive medications discontinued by their transplant physician because of severe toxicity or life-threatening complications (posttransplant lymphoproliferative disease, infection); (3) clinically stable long-term survivors (liver transplant recipients) who follow protocols for planned weaning of immunosuppressive medications with eventual discontinuation of immunosuppressive medications; (4) patients who follow protocols combining hematopoietic cell and kidney transplant from the same donor with nonmyeloablative conditioning to establish temporary or persistent mixed chimerism with subsequent rapid discontinuation of immunosuppressive therapy.

In a 2010 study by the European Consortium for Tolerance, 11 kidney allograft recipients who exhibited COT were identified: 8 as a result of nonadherence, 1 subsequent to malignancy, 1 who received a bone marrow transplant from the same donor, and 1 with unknown cause. Three of these recipients were < 21 years of age. The recipients had been off all immunosuppressive medications for 3 to 21 years at the time of the report.

Simultaneously, in a 2010 study from the American Network for Immune Tolerance, 25 kidney allograft recipients with COT were identified: 20 as a result of nonadherence, 2 resulting from caregiver discontinuation consequent to medical necessity, and 3 with unknown causes. The recipients had been off all immunosuppression for 1 to 32 years at the time of the report.

Clinical operational tolerance in 5 liver transplant recipients was initially described by Starzl and associates in 1993. The recipients were 12.5 to 18.6 years posttransplant and had been off immunosuppression for 5 to 11 years at the time of the report. In a 1997 follow-up report by Mazariegos and associates, 1 recipient had died in a vehicular accident, 1 was retransplanted because of recurrent hepatitis C virus infection 9 years after discontinuation of immunosuppressive medications, and 3 had normal graft function 14 to 17 years after all immunosuppression was stopped.

What Is the Current Status of Planned Weaning of Immunosuppression in Adult Liver Transplant Recipients?

Successful weaning occurs in approximately 20% of adult liver transplant recipients; however, the true incidence of potential spontaneous COT is unknown. The prevalence of successful COT after weaning is increased in adult recipients > 10 years after transplant at the initiation of the weaning process. The risks to liver graft damage during the weaning process are limited because, if clinical rejection occurs, it is mild and very responsive to treatment with increased immunosuppression. One note of caution is that outcomes regarding morbidity and mortality with long-term reductions and establishment of COT are at present unknown.

What Are the Data From Prospective Weaning Protocols in Pediatric Liver Allograft Recipients?

In initial studies from a Kyoto group with pediatric recipients of related living-donor liver transplant, a success rate of approximately 15% was shown. This led to a National Institutes of Health-sponsored trial conducted at 2 centers in the United States involving 20 recipients of related living-donor liver transplants who underwent a 36-month weaning process. The results were more positive, with 12/20 recipients (60%) achieving COT with long-term (> 5 y) excellent graft function after discontinuation of all immunosuppression. This success led to the initiation of an expanded weaning protocol involving 12 centers, which included pediatric recipients who received
living-related donor or deceased-donor liver transplants.

**What Is the Current Status of Inducing Temporary or Persistent Chimerism With the Same Donor for Kidney and Hematopoietic Cell Transplant to Facilitate Rapid Discontinuation of All Immunosuppression in Kidney Transplant Recipients?**

The most recent successful approach to the development of COT in kidney transplant recipients involves the use of a facilitating cell to establish chimerism. The facilitating cell involves 2 phenotype populations that facilitate hematopoietic stem cell engraftment with the development of chimerism. The use of the facilitating cell has resulted in the establishment of high levels of donor chimerism without the development of either graft-versus-host disease or engraftment syndrome after nonmyeloablative conditioning in mismatched related and unrelated kidney graft recipients.

Leventhal and associates published the most extensive series of successful COT utilizing the facilitating cell. In a protocol that included related and unrelated living-donor kidney transplant plus facilitating cell hematopoietic cell transplant on postoperative day 1 with nonmyeloablative pretransplant conditioning, 12 of 19 recipients (63%) were off of all immunosuppressive agents for 8 to 48 months. Serum creatinine levels of these recipients varied between 0.7 and 1.54 mg/dL. In addition, 11 of the 12 recipients who stopped all immunosuppression exhibited persistent 100% chimerism. Two grafts (19%) were lost, 1 from calcineurin inhibitor thrombotic microangiopathy and 1 resulting from sepsis originating in a native polycystic kidney.

These results are promising as a method to establish COT in kidney transplant recipients. This approach has the potential to be extended to deceased-donor kidney transplants because facilitating cell infusion occurs on postoperative day 1.

**What Are Regulatory T Cells?**

Regulatory T cells (Tregs) are a distinct T-cell population that is produced by the thymus and previously known as suppressor T cells that modulate the immune system, retain self-tolerance, and eliminate autoimmunity. The maintenance of self-tolerance is obtained by suppressing aberrant or excessive immune responses.

**What Are the Mechanisms of Regulatory T Cell Immunosuppression?**

Regulatory T cells suppress the activity of antigen-presenting cells and effector T cells by direct contact. The suppression of antigen-presenting cells (dendritic cell) function and maturation occurs by generation of the suppressive cytokines interleukin 10 and transforming growth factor β (TGF-β). Regulatory T cells destroy effector T cells through generation of perforin and granzyme A.

The usual number of Treg cells in humans is shown in Table 1. It should be noted that the number of circulating Treg cells is exceedingly miniscule compared with the number of other circulating lymphocytes.

<table>
<thead>
<tr>
<th>Table 1. Usual Number of Regulatory T Cells in Humans</th>
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<tbody>
<tr>
<td>Cell Type</td>
</tr>
<tr>
<td>Lymphocyte</td>
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<tr>
<td>CD4-positive T cell</td>
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<tr>
<td>Regulatory T cell</td>
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**What Is the Current Status of the Use of Regulatory T Cells in Clinical Solid-Organ Transplant?**

The use of ex vivo expanded autologous Treg cells to both treat chronic graft-versus-host disease and prevent acute graft-versus-host disease in hematopoietic stem cell transplant recipients was initially reported in 2009. Protocols have been proposed to use expanded donor alloantigen reactive Treg cells in adult liver and kidney transplant recipients to induce COT with subsequent discontinuation of all immunosuppression in the early posttransplant period.

The only data demonstrating the ability to achieve COT was shown in liver transplant recipients with expanded donor alloantigen reactive Treg cell-based treatment, which was recently report by Todo and associates. Ten adult related living-donor liver transplant recipients received autologous expanded donor alloantigen reactive Tregs after coculture with irradiated donor cells. In addition to corticosteroids, mycophenolate mofetil, and tacrolimus, the recipients also received cyclophosphamide (40 mg/kg) on day 5 after transplant, with donor alloantigen reactive Tregs infused on day 13 after transplant. Corticosteroids and mycophenolate mofetil were discontinued 1 month after transplant, with tacrolimus discontinuation initiated 6 months after transplant and completed 18 months after transplant.
In the study, 7 of the 10 recipients (70%) were successfully weaned off of all immunosuppression and at the time of the report were off of all immunosuppression for 16 to 33 months. The remaining 3 recipients developed mild rejection and resumed immunosuppressive therapy. The primary liver disease was an autoimmune process in all 3 of these recipients. The authors suggested that an alternative approach was probably required for recipients who have an autoimmune process affecting their native livers.

**What Is the Current Status of Utilizing Biomarkers to Identify Genes by Microarray Transcriptional Profiling, Which Could Be Used as a “Fingerprint” to Accurately Separate Potentially Clinically Operationally Tolerant From Nontolerant Recipients of Liver or Kidney Transplants?**

Extensive investigative efforts by a number of groups have attempted to identify biomarkers by studying COT recipients. This biomarker or fingerprint could be utilized to identify prospectively recipients who are more likely to exhibit COT weaning of immunosuppressive therapy in the early posttransplant period.

To date, natural killer cell transcripts are the most robust markers in liver transplant recipients with COT, and B-cell gene expression is the most robust marker in kidney transplant recipients with COT. In addition, tissue genes involved in iron homeostasis have been recently identified, which are shown before liver transplant in recipients who developed COT.

No prospective studies have been performed to validate the efficiency of utilizing a biomarker to successfully accomplish COT. Such investigational efforts are required in the future to move forward the concept of COT in solid-organ transplant.

**Conclusions**

The ability to induce COT by weaning solid-organ transplant recipients off of all immunosuppressive therapy is becoming a clinical reality. The ability to identify recipients who can become operationally tolerant with biomarkers will greatly enhance the use of the present induction methodologies in the future. At present, the use of ex vivo-expanded autologous Treg cells offers the least toxic method to induce COT.

**References**

