Recent advances in immunosuppression and acquired immune tolerance in renal transplants

Federica Casiraghi,1,2 Monica Cortinovis,1,2 Norberto Perico,2 and Giuseppe Remuzzi1,2,3,4

1IRCCS-Istituto di Ricerche Farmacologiche “Mario Negri,” Transplant Research Center “Chiara Cucchi de Alessandri e Gilberto Crespi,” Ranica, Bergamo, Italy; 2IRCCS-Istituto di Ricerche Farmacologiche “Mario Negri,” Clinical Research Center for Rare Diseases “Aldo e Cele Daccò,” Ranica, Bergamo, Italy; 3Unit of Nephrology and Dialysis, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy; and 4Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy

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THE PRIMARY CHALLENGE in organ transplantation continues to be the need to suppress the host immune system long term to ensure prolonged allograft survival (21). In organ transplantation, immunosuppressive agents inhibit one or more steps of the alloimmune response that would otherwise culminate in graft rejection. Unfortunately, the long-term nonspecific immunosuppressive drug use eventually results in systemic and nonspecific inhibition of the graft recipient’s immune system and is associated with an increased risk of serious side effects, such as life-threatening infections and malignancies (30). Therefore, to improve transplantation outcomes, it is critical to continue to develop novel agents to prevent acute rejection and chronic graft dysfunction while limiting the side effects of long-term immunosuppressive therapy. In addition, since the first successful kidney transplant in identical twins in 1954 (43), transplantation immunology has sought to move away from harmful immunosuppressive regimens and toward tolerogenic strategies that promote long-term graft survival, eliminating or limiting the need for medicines. Today, cell-based therapies aim to promote a microenvironment in the host that creates donor-specific tolerance. This technology, while promising, is still a way off.

Novel Immunosuppressive Agents and Regimens

The calcineurin inhibitors (CNI) cyclosporine A (CsA) and tacrolimus (TAC) have significantly improved short-term outcomes after organ transplantation by strikingly minimizing the incidence of acute graft rejection. However, long-term graft and patient survival have not increased markedly (32). The evidence suggests that CNI toxicity (e.g., nephrotoxicity and cardiovascular morbidity) plays an important, albeit not unique, role in the failure to improve long-term outcomes in kidney transplantation (44). Thus, in recent years, researchers have focused on developing novel immunosuppressive strategies that could reduce CNI exposure. A meta-analysis of randomized clinical trials, providing data from >11,000 de novo renal transplant recipients, compared CNI-sparing with standard CNI-based regimens (61). Complete CNI avoidance, achieved by combining a mammalian target of rapamycin inhibitor with mycophenolate mofetil (MMF), resulted in an increased incidence of graft failure and poorer tolerability than the combination of basiliximab and low-dose rabbit anti-thymocyte globulin in kidney transplant recipients given low-dose maintenance immunosuppressive drugs, including the CNI CsA (66).

In the past few years, increasing efforts have also been made to design novel biological agents (i.e., fusion proteins and antibodies) able to selectively target components of the host immune system, so as to avoid or limit the systemic toxic effects of CNI. This approach has led to the development of a second-generation cytotoxic T lymphocyte-associated protein (CTLA)4-Ig, belatacept, a receptor fusion protein consisting of the extracellular binding domain of CTLA4 linked to a modified human IgG1 (IgG1) Fc domain (33). Belatacept selectively inhibits CD28-mediated costimulation of T cells by binding to costimulatory ligands CD80/CD86 on antigen-presenting cells, a critical step for alloreactive T cell activation (20, 72). In 2011, the United States Food and Drug Administration approved belatacept as a first-line immunosuppressive agent based on the results of two pivotal phase III clinical trials that compared two belatacept-based regimens (more-intensive and less-intensive intravenous treatment schedules) with CsA in de novo kidney transplant patients who were also given basilix-
imab, MMF, and steroids (16, 70). In both studies, belatacept-treated groups exhibited similar graft and patient survival rates compared with the CsA arm (16, 70), along with better renal function over a 5-yr posttransplant followup (8, 53). Intriguingly, de novo DSA development was less frequent in patients that received belatacept, who also had better cardiovascular and metabolic risk profiles (69, 71). Despite these benefits, at 12 mo posttransplant, belatacept treatment was associated with higher rates of acute cellular rejection, mostly of high Banff grades, and posttransplant lymphoproliferative disorders (PTLD), especially in patients seronegative for the Epstein-Barr virus (16, 70). This may eventually make it necessary to require a complementary immunosuppressive strategy taming memory T cell subsets.

**Cell-Based Therapy for Immune Tolerance Induction**

To date, transplantation tolerance has been intentionally achieved in a small number of kidney transplant recipients undergoing donor hematopoietic stem cell transplantation under a nonmyeloablative conditioning regimen (Table 1). These encouraging clinical studies are based on early experiments in

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**Table 1. Clinical studies of tolerance induction to kidney transplantation by donor HSC transplantation**

<table>
<thead>
<tr>
<th>Center</th>
<th>Nonmyeloablative Conditioning Regimen and Maintenance immunosuppression (Dose and Days in Respect to Kidney Transplant)</th>
<th>Donor HSCs (HLA Match, Dose, and Day of Cell Transplantation in Respect to Kidney Transplant)</th>
<th>Chimerism</th>
<th>Patient Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stanford University</td>
<td>• Thymoglobulin: 1.5 mg/kg, days 0 to 5</td>
<td>HLA matched</td>
<td>Mixed, stable (at least for the first 6 mo)</td>
<td>Total N = 22</td>
</tr>
<tr>
<td></td>
<td>• Total lymphoid irradiation: 10 doses of 80–120 cGy during the first 14 days posttransplant</td>
<td>Granulocyte colony-stimulating factor mobilized enriched CD3⁺ hematopoietic progenitors (4.3–17.5 × 10⁶ cells/kg) mixed with CD3⁺ T cells (1–10 × 10⁶ total cells/kg) at day +11</td>
<td>Total N = 22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Steroids: days 0 to +10</td>
<td></td>
<td>N = 16 off immunosuppression</td>
<td></td>
</tr>
<tr>
<td>Massachusetts</td>
<td>• Cyclophosphamide: 60 mg/kg, days −2, −1, 0, and +1</td>
<td>HLA haplotype mismatched</td>
<td>Mixed, transient (2–3 wk posttransplant)</td>
<td>Total N = 10</td>
</tr>
<tr>
<td>General Hospital</td>
<td>• Anti-CD2 monoclonal antibody: days −2, −1, 0, and +1</td>
<td>Unprocessed bone marrow cells (2–3 × 10⁶ cells/kg) at day 0</td>
<td>Total N = 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Thymic irradiation: 700 cGy, day −1</td>
<td></td>
<td>N = 5 off immunosuppression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Rituximab: 375 mg/m², days −7, −2 (fourth and fifth patients) and days +5 and +12 (last five patients)</td>
<td></td>
<td>N = 3 graft loss for acute humoral rejection (N = 1, 10 days posttransplant), thrombotic microangiopathy complication (N = 1, 7 mo posttransplant), and acute rejection (N = 1, 3 yr)</td>
<td>N = 2 belatacept for chronic rejection 5 and 7 yr posttransplant</td>
</tr>
<tr>
<td></td>
<td>• Steroids: tapered in 10–20 days</td>
<td></td>
<td>N = 2 belatacept for chronic rejection 5 and 7 yr posttransplant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Maintenance: calcineurin inhibitors, tapered and discontinued in 8–14 mo</td>
<td></td>
<td>N = 1 in taper</td>
<td></td>
</tr>
<tr>
<td>Northwestern</td>
<td>• Cyclophosphamide: 50 mg/kg, days −3 and +3</td>
<td>HLA mismatched</td>
<td>Full, durable</td>
<td>Total N = 19</td>
</tr>
<tr>
<td>University</td>
<td>• Total body irradiation: 200 cGy, day −1</td>
<td>Engineered cellular product enriched for HSC and tolerogenic facilitating cells (2–33 × 10⁶ total cells/kg) at day +1</td>
<td>N = 12 off immunosuppression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Fludarabine: 30 mg/kg, days −4, −3, and −2</td>
<td></td>
<td>N = 5 on immunosuppression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Maintenance: MMF and tacrolimus, tapered and discontinued in 6 mo (MMF) and in 12 mo (tacrolimus)</td>
<td></td>
<td>N = 2 graft loss for viral sepsis (N = 1, 3 mo posttransplant) and for infection (N = 1, 9 mo posttransplant)</td>
<td></td>
</tr>
</tbody>
</table>

HSCs, hematopoietic stem cells; MMF, mychophenolate mofetil; CsA, cyclosporine A.
mice (24) and later studies in patients with hematological malignancies (63, 64) showing that inducing mixed chimerism (coexistence of hematopoietic cells of both recipient and donor origin) through transplantation of donor bone marrow in recipients undergoing myeloablative conditioning achieved long-term tolerance toward a subsequent kidney transplant from the same bone marrow donor. Since then, similar tolerogenic protocols have been applied in clinics with nonallogenical transplant recipients of living donor kidneys. These protocols have been adapted to design less toxic conditioning regimens capable of ablating the recipient bone marrow, eventually allowing various levels of chimerism to occur. Three investigator groups have recently reported the initial results of small clinical trials for transplant tolerance induction using donor hematopoietic stem cells in HLA-matched and mismatched kidney transplantation (Table 1).

The Stanford University study included 22 kidney transplant patients that received peripheral blood CD34+ stem cell infusions and kidney transplants from HLA-matched donors (57–59). Weaning maintenance immunosuppression (CsA/MMF) was based on the presence of durable mixed chimerism. Nineteen of twenty-two patients successfully developed persistent mixed chimerism after the peculiar nonmyeloablative conditioning regimen of the Stanford protocol (Table 1), and sixteen patients were successfully weaned off immunosuppressive drugs. Kidney graft tolerance was achieved independently of whether mixed chimerism, obtained in the first 6 mo posttransplantation, persisted or vanished during or after CsA discontinuation (58, 60). This finding questions about the role of persistent mixed chimerism in promoting graft tolerance, at least in the setting of the Stanford protocol. When applied to kidney transplant patients with HLA-mismatched donors (n = 6), this protocol failed to induce persistent mixed chimerism. Two of the six patients met the criteria for immunosuppressive drug withdrawal [transient chimerism, absence of clinical rejection episodes, and graft-versus-host disease (GVHD) and donor reactivity in a mixed lymphocyte reaction] and were withdrawn from immunosuppression at 12 mo after kidney transplantation. However, both patients developed rejection episodes 3.5 and 5.5 mo after drug discontinuation (40, 60). A more recent attempt in HLA haplotype-matched (3/6 HLA mismatches) recipients (n = 10) applied a dose escalation approach of CD3+ T cells (from 3–50 million) to the same total lymphoid irradiation and antithymocyte globulin conditioning regimen (60). Chimerism lasting at least 12 mo was observed in the two patients given the highest number of CD34+ cells (15–22 × 10⁶ cells/kg) and in the three patients given the highest CD3+ T cell dose (50 × 10⁶ cells/kg). In the two patients given the highest CD34+ cell dose, MMF was successfully discontinued and TAC tapering was in progress (60). This revised regimen is currently being tested in a larger cohort of patients to assess its efficacy in inducing persistent chimerism and immunosuppression drug withdrawal in HLA-mismatched patients (60).

At Massachusetts General Hospital, Kawai and coworkers treated 10 patients with combined kidney and bone marrow transplantation from HLA-haploidentical (3/6 mismatches) related donors under nonmyeloablative conditioning (26, 28). Over time, the protocol was implemented with multiple rituximab doses to prevent the development of DSA, as observed in the initial few patients enrolled (28). Although donor mixed chimerism was completely lost in all patients within the first 21 days after combined cell and kidney transplantation, graft tolerance was achieved in 7 of 10 subjects, 5 of whom remained off immunosuppression long term (27, 28). However, all patients developed engraftment syndrome, characterized by severe but transient renal dysfunction during the first week after cell transplantation (19). Therefore, a modified version of the conditioning protocol with total body irradiation replacing cyclophosphamide has begun in an effort to prevent the engraftment syndrome (27).

Recent comparisons of outcomes of kidney transplant patients who achieved tolerance either by the Stanford (60) or Massachusetts General Hospital (27) protocols demonstrated that tolerant patients had improved graft survival versus patients on standard immunosuppression (60) and reduced incidence of immunosuppression-related complications such as hypertension, hyperlipidemia, de novo diabetes, malignancies, and infections (27).

Northwestern University has also reported successful induction of allograft tolerance in HLA-mismatched kidney transplant recipients by almost completely replacing the recipient hematopoietic cells with donor cells (full chimerism) (34, 35). The tolerogenic protocol includes transplanting the cellular product enriched with hematopoietic stem cells and “tolerogenic facilitating” cells. “Facilitating” cells are a CD8+ T cell receptor (TCR)–bone marrow-derived mixed cell population that favor the engraftment of bone marrow without promoting GVHD (75). The mechanism through which these facilitating cells promote bone marrow engraftment appears to involve the development of regulatory T cells (Tregs) as well as IL-10-producing type 1 Tregs (75). Twelve of nineteen patients achieved durable chimerism and are off immunosuppressive drugs. Five other patients did not develop stable donor cell engraftment and are continuing with immunosuppressive drug therapy, and two subjects experienced allograft loss. The investigators reported that no GVHD has occurred for up to 5 yr despite very high levels of donor chimerism (35). Evaluation of immune reconstitution and immunocompetence in chimeric subjects revealed that most of these individuals retained memory for hepatitis B, measles, mumps, rubella, and varicella, suggesting that mixed, rather than full chimerism, was present in these transplant patients. In addition, chimeric recipients developed protective immunity in response to pneumococcal vaccination, providing evidence for immunocompetence (35).

Collectively, these studies documented the feasibility and efficacy of cell-based therapy to induce tolerance in organ transplant recipients. However, challenges related to the procedure and clarification of the mechanism(s) involved in the protolerogenic process must still be addressed before these tolerogenic cell-based protocols will be part of current kidney transplantation management. Indeed, cell-based therapies with bone marrow or hematopoietic stem cells, with or without facilitating cells, require peritransplant conditioning regimens to promote cell engraftment and chimerism (Table 1). These conditioning protocols have been modified to improve the clinical outcomes, but this has not eliminated their inherent dangers and the likelihood of infections and GVHD. This shortcoming must be balanced with the short- and long-term risks/benefits of current pharmacological immunosuppressive therapies in kidney transplantation. Furthermore, whether inducing chimerism is the cornerstone for achieving transplantation tolerance or an epiphenomenon remains unclear. According to the above studies, graft tolerance can be achieved
after inducing either mixed (60) or almost full (35) chimerism or even with very low and transient (37) chimerism, yet it is not clear why some chimera become tolerant to the donor kidney and some do not. In mouse models of mixed chimerism, the lifelong contribution of donor antigen-presenting cells that mediate the central deletion of donor-reactive T cells in the thymus is the major mechanism of long-term tolerance of the transplanted organs (55). The transient nature of chimera in human patients (2, 37) and the rapid expansion of peripheral T cells after bone marrow or hematopoietic stem cell transplantation (35, 57, 58) would, however, suggest that peripheral rather than intrathymic mechanisms play a role in immune tolerance induction, at least immediately after organ transplantation. It has recently been proposed that in the long term, extrathymic deletion of donor-reactive CD4+ and CD8+ T cell clones plays a role in maintaining tolerance (42).

Although hematopoietic stem cell-based therapies hold promise for kidney transplantation, alternative cell-based treatments that do not require peritransplant conditioning regimens are being investigated for potential use in immunotherapy in solid organ transplantation.

Many immune cells with regulatory properties, such as tolerogenic dendritic cells, regulatory macrophages, Tregs, and mesenchymal stromal cells (MSCs) have been proposed for cell therapy (Table 2) (73). Among them, Foxp3+ Tregs, either naturally occurring or peripherally induced (25), and MSCs (7) have been particularly in the spotlight.

Tregs can exert dominant tolerance to alloantigens in vivo by inducing regulatory properties in alloreactive T cells in animal models (11) and may establish a tolerant state that could obviate the need for immunosuppressive drugs (65). The clinical use of regulatory cells in organ transplantation is currently being explored in several ongoing trials (Table 2) (67). Since Tregs are small in number in the peripheral circulation, they required ex vivo expansion before infusion into patients. Either expanded naturally occurring Tregs or induced Tregs from naïve CD4+ T cells stimulated by donor alloantigens are being proposed as cellular therapy to control the unwanted alloim-

### Table 2. Regulatory cell-based therapy in kidney or liver transplant patients

<table>
<thead>
<tr>
<th>Study Identifier</th>
<th>Center</th>
<th>Regulatory Cells</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02091232</td>
<td>Massachusetts General Hospital, Boston, MA</td>
<td>Tregs</td>
<td>Living donor kidney transplant</td>
</tr>
<tr>
<td>NCT02129881</td>
<td>Guy’s Hospital, London and Oxford Transplant Centre, Oxford, United Kingdom</td>
<td>Polyclonal natural Tregs</td>
<td>Living donor kidney transplant</td>
</tr>
<tr>
<td>NCT02371434</td>
<td>Charité University Medicine, Berlin, Germany</td>
<td>Donor alloantigen reactive Tregs</td>
<td>Living donor kidney transplant</td>
</tr>
<tr>
<td>NCT02234801</td>
<td>University of California, San Francisco, CA</td>
<td>Donor alloantigen reactive Tregs</td>
<td>Living donor kidney transplant</td>
</tr>
<tr>
<td>NCT02145325</td>
<td>Northwestern University, Chicago, IL</td>
<td>Polyclonal Tregs</td>
<td>Kidney transplantation</td>
</tr>
<tr>
<td>NCT02088931</td>
<td>University of California, San Francisco, CA, Planned</td>
<td>Antigen-specific type 1 Tregs (T10 cells)</td>
<td>Living donor kidney transplant</td>
</tr>
<tr>
<td>NCT02188719</td>
<td>University of California, San Francisco, CA, and Mayo Clinic, Rochester, MN</td>
<td>Donor alloantigen reactive Tregs</td>
<td>Living donor liver transplant</td>
</tr>
<tr>
<td>NCT2474199</td>
<td>University of California, San Francisco, CA, and Mayo Clinic, Rochester, MN</td>
<td>Donor alloantigen reactive Tregs</td>
<td>Living donor liver transplant</td>
</tr>
<tr>
<td>NCT02166177</td>
<td>Guy’s and St Thomas’ National Health Service Foundation Trust, London, United Kingdom</td>
<td>Autologous Treg product TR002</td>
<td>Liver transplant</td>
</tr>
<tr>
<td>NCT01624077</td>
<td>Nanjing Medical University, Jiangsu, China</td>
<td>Donor alloantigen-specific Tregs</td>
<td>Liver transplant</td>
</tr>
<tr>
<td>NCT02252055</td>
<td>Nantes University Hospital, Nantes, France</td>
<td>Autologous tolerogenic dendritic cells</td>
<td>Living donor kidney transplant</td>
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<tr>
<td>NCT02085629</td>
<td>University of Regensburg, Regensburg, Germany</td>
<td>Donor-derived regulatory macrophages</td>
<td>Living donor kidney transplant</td>
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<tr>
<td>NCT02012153</td>
<td>Mario Negri Institute, Bergamo, Italy</td>
<td>Autologous MSCs</td>
<td>Living donor kidney transplant</td>
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<tr>
<td>NCT02409940</td>
<td>Institute of Medical Education and Research, Chandigarh, India</td>
<td>Autologous or donor MSCs</td>
<td>Living donor kidney transplant</td>
</tr>
<tr>
<td>NCT02490020</td>
<td>Zhirui Hospital, Guangdong, China</td>
<td>MSCs</td>
<td>Kidney transplant</td>
</tr>
<tr>
<td>NCT01429038</td>
<td>University Hospital of Liege, Liege, Belgium</td>
<td>Third-party MSCs</td>
<td>Kidney and liver transplant</td>
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<tr>
<td>NCT02492490</td>
<td>Fuzhou General Hospital, Fujian, China</td>
<td>Autologous stromal-vascular fraction-derived MSCs</td>
<td>Kidney transplant</td>
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<td>NCT02387151</td>
<td>Leiden University Medical Center, Leiden, The Netherlands</td>
<td>Third-party MSCs</td>
<td>Kidney transplant</td>
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<td>Fuzhou General Hospital, Fujian, China</td>
<td>Autologous stromal-vascular fraction-derived MSCs</td>
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<td>Third-party MSCs</td>
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<td>Third-party MSCs</td>
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<tr>
<td>NCT01841632</td>
<td>University Hospital Regensburg and Athersys Incorporated, Regensburg, Germany</td>
<td>Third-party multipotent progenitor cells</td>
<td>Liver transplant</td>
</tr>
</tbody>
</table>

Tregs, regulatory T cells; MSCs, mesenchymal stromal cells.
mune response in clinical settings (Table 2) (17). So far, expanded Tregs have been used as treatment for patients with GVHD (68), as GVHD prophylaxis in patients that received hematopoietic stem cell transplantation (13) and in pediatric patients with new-onset type 1 diabetes (39). Tregs have been also used in liver transplant recipients, but the results of this trial have not yet been published (74). In this study, donor antigen-driven Tregs were infused posttransplant (day 13) in 10 cyclophosphamide-treated patients undergoing living donor liver transplantation. Withdrawal of maintenance immunosuppressive drugs was achieved in six patients.

Bone marrow-derived MSCs are multipotent progenitor cells able to interact with and influence a wide range of cells involved in the immune response. They are capable of suppressing effector T cells (15), including effector/memory T cells (31, 51), and promoting the development of Tregs (1, 18). In animal models of solid organ transplantation, MSCs shift the balance between Tregs and effector/memory T cells toward a more tolerogenic profile, eventually leading to long-term graft tolerance (6). These encouraging experimental studies did suggest that MSCs could potentially be a suitable tolerance-inducing strategy in human transplantation programs. Available studies in transplant recipients of living donor kidneys have provided data of the immunomodulatory ability of autologous MSCs, in particular to control donor-specific effector/memory CD8+ T cell proliferation and long-lasting CD8+ T cell cytotoxic function, an effect current immunosuppressive drugs do not share (49, 50).

Several clinical trials using regulatory cell-based therapies (Tregs and MSCs) to induce tolerance are currently recruiting patients (Table 2) and will soon provide evidence on the safety and efficacy of regulatory cell-based therapies in improving long-term outcomes after solid organ transplantation.

Operational Tolerance to Kidney Transplantation

In addition to induced tolerance (achieved by the approach of hematopoietic stem cell transplantation under nonmyeloablative conditioning in selected kidney transplant recipients) a “spontaneous” state of long-term graft acceptance, referred to as “operational tolerance,” has been observed in a small number of kidney transplant recipients (47). These patients have discontinued immunosuppression, either owing to nonadherence or to physician-led intentional weaning, and, despite weaning off immunosuppression, conserved good graft function (47, 54) and resistance against infection (5). These patients provide a serendipitous proof of principle that immune tolerance may be possible in humans. Therefore, several groups have collected and analyzed samples from spontaneously tolerant kidney transplant recipients with the aim of identifying biomarkers of tolerance (4, 45, 48, 56). These biomarkers would allow the identification of candidate patients for minimization and potential discontinuation of immunosuppression, in addition to provide hypotheses for testing underlying mechanism(s) of tolerance.

Using combined approaches of gene expression profiling and immune cell phenotyping by flow cytometry, a B cell signature was identified in tolerant kidney transplant patients. Compared with kidney transplant recipients with stable graft function under maintenance immunosuppression, operationally tolerant patients showed an increased number of circulating B cells and overexpression of B cell-associated genes in the peripheral blood and urine (4, 45, 48, 56). In particular, tolerant patients showed specific expansion of transitional, naïve B cells (45, 48) and of B cells that express an inhibitory phenotype, such as FcγRIIB (a receptor transducing inhibitory signals) and B cell scaffold protein with ankyrin repeats-1 (which negatively modulates B cell-CD40-mediated AKT activation) (48, 56). Mechanistically, transitional B cells from operationally tolerant patients secreted high level of IL-10 after in vitro polyclonal stimulation (45) and expressed a higher level of microRNA-142 3p, the forced expression of which in the Raji B cell line upregulates the expression of numerous B cell immune response genes, including those previously identified in operationally tolerant patients (12). Moreover, B cells from tolerant patients did not fully differentiate into plasma cells (10) and suppressed in vitro effector T cell function by a contact- and granzyme B-dependent pathway (9). These findings suggest that B cells may actively regulate the immune response to the transplanted kidney promoting T cell unresponsiveness to donor alloantigens. Indeed, despite a normal phenotype (41), circulating T cells from tolerant patients showed decreased alloreactivity (23), which, however, does not involve an active Treg-mediated immunoregulation (23, 38). Until recently, the role of Tregs in spontaneous tolerance has remained ill defined. Initial studies showed that the number of circulating Tregs and their ex vivo regulatory properties were not significantly modified in operationally tolerant patients compared with those with stable graft function under standard immunosuppression (23, 38). A more recent study extensively characterized circulating Tregs in tolerant patients and found that operational tolerance was characterized by circulating CD4+ T cells with a high level of demethylation of the Fox3 Treg-demethylated region and by expansion of Fox3+memory T cells with stronger suppressive properties compared with patients with stable graft function on immunosuppression or with healthy volunteers (3). Interestingly, this study shed new light on the potential role of memory Tregs in long-term graft survival (3).

Overall, these findings suggest that long-term graft acceptance in operational tolerant patients involves a complex interplay of mechanisms that ultimately lead to emergence of B cells and T cells with suppressive and regulatory functions.

On this line, a recent longitudinal analysis of the B cell tolerance signature in tolerant (spontaneous or induced) patients showed that the increased frequency of transitional and naïve B cells as well as the increased expression of B cell-associated genes persist over time in operational tolerant patients. Of major interest, a similar overexpression of the most predictive B cell-associated genes was found in kidney transplant patients who achieved tolerance using the Massachusetts General Hospital protocol of combined bone marrow transplantation under the nonmyeloablative conditioning regimen (46), suggesting that spontaneous and induced immune tolerance in kidney transplantation may share similar biomarkers or even mechanisms.

Conclusions

In the past few years, novel immunosuppressive agents for preventing graft rejection in organ transplantation, including kidney transplantation, have been developed and approved.
Unfortunately, the initial promise of significant improvements in the management and outcomes of transplant patients has only been partially fulfilled, and major concerns remain for their safety profile and early as well as long-term efficacy. This calls for more collaborative work between basic and clinical academic research institutions and pharmaceutical companies to better dissect the complex cellular and humoral mechanisms underlying the long-term inexorable deterioration of graft function that still occurs in a large percentage of organ transplant recipients. It also means there is a need to develop more targeted and safer immunosuppressive agents. In parallel, at this stage, more small clinical studies are needed so that preclinical discoveries can translate better into tangible benefits for organ transplant recipients. Overall, these considerations may be particularly important for new biological agents, such as cell-based therapies, where despite encouraging initial results, uncertainty about safety and efficacy still exists.

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AUTHOR CONTRIBUTIONS
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54. Scandling JD, Busque S, Debjaksh-Jones S, Benike C, Sarwal M, Millan MT, Shizuru JA, Lowsky R, Engleman EG, Strober S. Toler-


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