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Clinical Operational Tolerance After Renal Transplantation:

Current Status and Future Challenges

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Abstract

In solid organ transplantation, the achievement of an immunosuppression (IS)-free state [also referred to as *clinical operational tolerance* (COT)] represents the ultimate goal. Although COT is feasible and safe in selected cases after liver transplantation, it is an exceptional finding after other types of solid organ transplantation. In the field of renal transplantation (RT), approximately 100 cases of COT have been reported to date, mainly in patients who were not compliant with their immunosuppressive regimens or in individuals who had previously received a bone marrow transplant for hematological disorders. On the basis of promising results obtained in animal models, several tolerogenic protocols have been attempted in humans, but most have failed to achieve robust and stable COT after RT. Molecule-based regimens have been largely ineffective, whereas cell-based regimens have provided some encouraging results. In these latter regimens, apart from standard IS, patients usually receive perioperative infusion of donor bone marrow–derived stem cells, which are able to interact with the immune cells of the host and mitigate their response to engraftment. Unfortunately, most renal transplant patients who developed acute rejection—occurring either during the weaning protocol or after complete withdrawal of IS—eventually lost their grafts. Currently, the immune monitoring necessary for predicting the

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presence and persistence of donor-specific unresponsiveness is not available. Overall, the present review will provide a conceptual framework for COT and conclude that stable and robust COT after RT remains an elusive goal and that the different strategies attempted to date are not yet reproducibly safe or effective.

Solid organ transplantation (SOT) is one of the greatest achievements in the history of modern medicine. However, transplant recipients still have a significantly lower life expectancy and quality of life than the general population.[1,2] Maintenance immunosuppressive therapy administered to prevent allograft rejection is the main culprit, causing known toxicities that can occur in the short, mid, and long term after SOT. Immunosuppressive agents are typically administered empirically at doses that result in adverse effects, which often lead to overimmunosuppression and require subsequent dosage adjustments.[1] Of interest, it has been demonstrated that the number of medications per day and the occurrence of immunosuppression (IS)-related adverse effects have tremendous impact on a patient's quality of life and adherence to treatment.[2] Moreover, dosage adjustments have been associated with acute rejection. Patient noncompliance has become the third leading cause of graft loss in renal transplantation (RT) in some series, after chronic allograft nephropathy and death with a functioning graft.[2] Both chronic allograft nephropathy and death with a functioning graft may also be directly or indirectly related to chronic IS. Therefore, the need for newer and more selective immunosuppressive strategies, which should at the same time be more efficient and tailored to individual patient characteristics, is a pivotal goal in transplantation medicine.

Immunosuppression minimization and withdrawal are 2 possible strategies to adopt.[3] The aim of minimal IS is to target distinct mechanisms of the immune response with different molecules given at lower doses, not only to control allograft rejection and maintain long-term graft function but also to avoid infections and malignancies and prevent drug-specific toxicities. In the field of RT, IS minimization protocols have been implemented in numerous clinical trials but results to date have been unconvincing, inconsistent, and inconclusive.[3]

Theoretically, IS withdrawal is the ultimate treatment to offer a transplant recipient and represents the subject of the present review. The immediate practical goal of this approach is to improve quality of life and overall outcomes in allograft recipients by minimizing exposure to the high costs and side effects of chronic IS such as hypertension, diabetes, hyperlipidemia, target organ damage, and increased susceptibility to malignancies and infections. Technically, the condition in which an SOT recipient exhibits a well-functioning graft and lacks histological signs of rejection after being completely off all IS for at least 1 year is referred to as *clinical operational tolerance* (COT).[4] Importantly, the patient in question is an immuno-competent host capable of responding to other immune challenges, including infections.[4,5] In the review herein, this is the definition of COT that will be adopted. However, it is possible that transplanted organs may exhibit impaired, albeit stable, allograft function for a number of reasons, including the rejection activity that has been previously controlled. Therefore, biologically speaking, *full immuno-logical tolerance*—defined as a state of unresponsiveness to donor antigens—may well exist, even though an

organ has survived events, immunological or otherwise, that have left it with slightly, or more than slightly, impaired graft function.

More than 5 decades of clinical experience in SOT have demonstrated that COT is extremely difficult to achieve and is somewhat organ dependent. Recipients of a liver graft are more capable of developing COT because of the immune-privileged status of the liver.[5] This status is exemplified by a number of interesting observations after liver transplantation, including (1) the relative lack of an effect of either a positive cross-match or blood type incompatibility, the irrelevance of human leukocyte antigen (HLA) matching; (2) the reduced incidence of hyperacute rejection and spontaneous recovery after severe rejection; (3) the fact that acute rejection does not impact adversely on long-term graft and patient-survival outcomes; (4) the ability of liver allografts to protect other extrahepatic allografts from rejection if the latter are derived from the same donor; and (5) a lower overall incidence of chronic rejection that is reversible in up to 30% of cases.[6,7] To date, 168 cases of COT established after liver transplantation have been reported[5,8] out of a sample of 473 individuals in whom a weaning protocol was attempted. Notably, patients who developed acute rejection during the protocols designed to discontinue IS were not exposed to further risks of graft loss, once maintenance IS was resumed. The worldwide experience with COT after liver transplantation has been recently reviewed, and it is now evident that a permanent and stable IS-free state can be safely attempted and sometimes achieved in patients who have received a Liver transplantation (LT) for non–immune-mediated liver diseases.[5]

When other transplantable organs are considered, the scenario changes completely. Clinical operational tolerance has never been reported after intestinal, islet, or whole organ pancreas transplantation, whereas 2 exceptional cases of COT have been described after lung[9] and heart transplantation.[10] When RT is considered, we must remember that the clinical era of transplantation began on December 23, 1954, when the first successful RT between the Herrick twins was performed.[11] Because of genetic identity between the brothers, the recipient was not administered any IS after the operation, thus representing the very first case of COT. In the next 55 years, only sporadic cases of COT have been documented after RT in the absence of genetic identity between the donor and the recipient.

In this review, we will summarize and comment on all cases of COT after RT reported to date. We will demonstrate that the achievement of a permanent and stable IS-free state—namely, COT—after RT is exceptional and that tolerogenic strategies are not yet available for daily clinical practice because they are not effective, practical, or safe. We will also describe the main clinical trials in which presumed tolerogenic regimens administered to RT patients did not provide the expected results despite their success in animal models. We will emphasize the clinical perspective and touch only briefly on possible mechanisms relevant to understanding the pathophysiology of the state of tolerance. Eventually, it will become clear that despite significant progress achieved in the field of clinical transplant immunology, understanding of the immune mechanisms underlying COT remains inadequate.

CLASSIFICATION

Tolerogenic strategies in the field of clinical RT have been classically divided in hematopoietic cell transplant– and non– hematopoietic cell transplant–based approaches, depending on the utilization or nonutilization of donor bone marrow cells.[12] Herein, we will categorize all cases of COT into 3 groups described in the literature (Table 1):

1. sporadic cases in which patients discontinued IS for nonadherence to treatment, usually without the knowledge or advice of transplant physicians;
2. planned protocols applied to long-surviving organ recipients who were gradually weaned from IS under close supervision of their physicians after the onset of severe IS toxicity or life-threatening complications;
3. protocols in which COT was the planned objective before the transplant, using both the careful selection of a suitable tolerogenic strategy instituted from the outset and highly individualized patient selection.

All other cases of COT that have occurred in identical twins,[11,13,14] or in whom the causes are unknown,[15] will not be considered. In addition, cases of COT with follow-up shorter than 1 year[16,17] will not be reviewed unless reported in the context of large clinical series or nonanecdotal reports.[18,19]

GROUP A: NONADHERENCE TO IMMUNOSUPPRESSIVE THERAPY

Spontaneous Withdrawal of Immunosuppression

Los Angeles—Owens et al[18] documented 4 cases of COT after RT due to noncompliance. Three patients never developed rejection after 17, 23, and 52 months from IS withdrawal, whereas IS was resumed in the remaining patient who experienced acute rejection 18 months after the withdrawal of IS. It is noteworthy to mention that these patients were taking sporadically 25 mg/d of azathioprine, and therefore they do not fulfill the definition of clinically tolerant patients. In the same paper, they reported on the basis of a survey that they conducted in the United States that of 24 individuals in whom IS was discontinued mainly for nonadherence to the immunosuppressive therapy, only 2 remained IS-free for 9 and 36 months, respectively; all remaining patients resumed IS under the guidance of their physicians after the onset of rejection. However, on the basis of their encouraging personal experience, the authors recommend that once IS has been stopped, consideration should be given to not resuming IS unless signs of acute or chronic rejection appear. Because of limitations in immune monitoring (IM) and the relative insensitivity of serum creatinine as an indicator of renal function, surveillance renal allograft biopsies and donor-specific antibody monitoring are probably warranted in all cases of either planned or unplanned withdrawal of IS. Such unique patients remain of enormous interest; however, as they could potentially provide important clues regarding the immunobiology behind such rare occurrences.

Minneapolis—In the editorial comment to the article by Owens et al,[18] Najarian[20] criticized the above-mentioned position by highlighting the need for the reinstitution of IS whenever an RT recipient has stopped IS. He reported on 6 patients from the University of

Minnesota who discontinued IS for nonadherence. Five of these patients subsequently lost their renal grafts after the development of severe acute rejection, whereas no further data regarding the remaining patient are provided.

Madison—1—In a letter to the editor written in response to the article by Owens et al,[18] Hussey[21] expressed the same concerns as Najarian and concluded that the resumption of IS is strongly indicated but should be done at the lowest possible dose compatible with graft maintenance and patient survival. He also reported his personal experience on 8 nonadherent patients, 7 of whom experienced acute rejection (which was fatal in 2 cases), whereas the remaining patient remained IS-free for more than 40 months with stable graft function.

Madison—2—Uehling et al[22] described a small series of 5 RT recipients who spontaneously stopped all IS for nonadherence to IS. All but 1 individual rejected their grafts within a few months of IS withdrawal. The only patient who did not reject was a 45-year-old woman who had received an HLA-identical graft from her nontwin sister. She intentionally stopped IS 5 years after transplant for personal reasons. She remained IS-free and without signs of rejection for the next 36 months. It is important to note that all of the earlier cases occurred during an era in which maintenance IS consisted solely of prednisone/prednisolone and azathioprine.

Columbus—Madison—In an attempt to define immunological parameters that identify potentially tolerant patients, Burlingham and colleagues[23,24] utilized the human-to-mouse trans-vivo delayed-type hypersensitivity assay. Two of the 3 IS-free patients analyzed in their first study were RT recipients who spontaneously stopped all IS and were IS-free for 3 years without developing any sign of rejection.

By observing that allograft acceptors failed to exhibit donor-reactive delayed type hypersensitivity responses when recipient leukocytes were challenged with donor antigen, although they frequently develop donor-reactive alloantibodies, the authors demonstrated that this pattern of immune response is not due to an absence of allosensitization, but rather to the development of an immune mechanism that actively inhibits anti-donor delayed-type (ie, cell-mediated) immune responses. They emphasized the finding that immune tolerance is a phenomenon based on regulation, rather than suppression of the immune system, in which the different components of the immune system dynamically interact over the time. The same concept will be stressed by the Nantes group later on and substantiated by data from a larger series (see later).

Pregnancy—Fischer et al[25] described 1 case of specific immune tolerance developing during pregnancy in a 24-year-old woman who received a deceased donor RT at the age of 13 for membranoproliferative glomerulonephritis. The patient interrupted the immunosuppressive therapy, consisting of prednisolone and azathioprine, because of fear of fetal abnormalities that could be related to IS. It is noteworthy that no immunological response was observed for 9 years after the withdrawal of IS while the patient was exhibited normal renal function.

It is well known that women may experience considerable amelioration of certain autoimmune diseases during pregnancy.[26] Yet, despite the systemic effect that pregnancy has on the maternal immune system, it seems that pregnancy-induced IS is restricted to responses directed against the fetus and autoimmune targets. In the above case, however, the mechanisms of maternal–fetal tolerance and fetal immune evasion[26] possibly paved the way for the development of inertness of the host immune system toward the renal graft. Deletion and anergy of clones of immune cells reactive against graft antigens, expansion of the number and function of regulatory T cells, maternal–fetal cell exchange, and microchimerism, which have been described in both pregnancy and tolerance models, may have been responsible for the onset of COT in this specific case.[26,27]

Surveys

Boston—Almost 30 years ago, Zoller et al[19] surveyed all US transplant centers to identify any RT patients who were completely off IS. They identified 23 patients who were IS-free for at least 244 days. In particular, 6 patients who had received living-related donor grafts were IS-free for greater than 3 years, 7 additional patients from 1 to 3 years, and 10 recipients for less than 1 year. They demonstrated that at no point after transplantation, it is prudent to stop all IS barring serious drug toxicity. Also, in patients who stop IS surreptitiously and in whom the renal function remains normal, the reinstitution of IS is indicated within 1 year and is advisable up to 3 years after cessation. Conversely, those few patients who do well without IS for greater than 3 years may not need further IS.

Although this conclusion was dictated by common sense, a quarter of century later, researchers at the University of Miami came to the same conclusion that the chances for an organ to be tolerant to the host immune system are higher for long-lasting grafts than for the grafts that have been in place for shorter periods of time.[28] In the context of an international forum on clinical and experimental tolerance published in *Transplantation*, the authors addressed the fundamental topic of when the specificity of an operationally tolerant state occurs. They reviewed a few seminal experimental and clinical studies and concluded that the clinical adage “the longer an organ transplant can stay in place, the harder it is to reject it” is possibly substantiated by the evidence that there are afferent and efferent immune alterations that eventually become established (accommodation) that facilitate a protective state of the graft.[28] However, the currently available data are not strong enough to support the above concept.

Nantes—Soulillou and coworkers[29–37] have extensively studied IS-free RT recipients, for the most part in patients with IS cessation due to noncompliance. In a seminal descriptive single-cohort study on 10 tolerant RT patients [7 of whom spontaneously stopped IS for nonadherence, whereas 2 were withdrawn for posttransplant lymphoproliferative disorder (PTLD) and 1 for recurrent infections and skin cancer], they provided striking clinical evidence that COT may occur in the presence of antidonor class II antibodies, as well as in patients who have experienced previous acute rejection,[29] which is consistent with previously reported findings from the University of Wisconsin.[23,24] In addition, the authors showed that tolerant patients are likely to have received a graft from a young donor (<30 years of age), possibly because the good quality of the graft or its potential reserve may

facilitate the establishment of a sustained COT. In contrast, grafts from older donors are more susceptible to ischemia-reperfusion injury and delayed graft function, which may result in higher antigenicity due to endothelial injury. Of interest, tolerant patients may be low responders to blood transfusion as demonstrated by the low levels of panel-reactive antibodies present in this group of patients when compared with nontolerant patients. However, tolerant patients may develop graft dysfunction at any time, even in the absence of lesions specific for acute rejection. Interestingly, the incidence of infectious diseases in tolerant patients is comparable to normal individuals, suggesting that COT is heterogeneous, with some patients exhibiting a global immunodeficiency and others exhibiting an adapted response to vaccination.[30]

The same patients have been the subject of subsequent studies, in which the same group investigated biomarkers of COT and long-term outcomes after RT.[31–37]

GROUP B: WEANING OF THE IMMUNOSUPPRESSION DRIVEN BY TRANSPLANT PHYSICIANS

Aarhus

There is an anecdotal report of a 21-year-old man who had received 2 haploidentical RTs, the first at age 11 from his mother and the second at age 15 from his father.[38] Three years after the second RT, he developed PTLD. Immunosuppression was promptly interrupted and the PTLD subsequently resolved. At the time of publication, IS had been withdrawn for 3 years without any episodes of rejection.

Nantes

Two cases of COT, developing after intentional withdrawal of IS due to the development of PTLD, have been described, mainly within a larger series.[29] In first, PTLD developed 7 years after the transplant, while the postoperative course had been uneventful until then. Seven years after withdrawal of IS, the onset of proteinuria revealed biopsy-proven chronic rejection. Interestingly, the patient was noted to have antidonor antibodies since the time of IS withdrawal. The second individual developed acute rejection in the immediate post-RT course, was taken off IS 8 years later for PTLD, and has been IS-free for 6 years in absence of any antidonor antibodies.

A third patient from the same series was weaned off IS for recurrent bacterial infections and skin cancer. After experiencing 2 episodes of acute rejection 1 and 7 months after the transplant, graft function progressively deteriorated, and hemodialysis was started. Surprisingly, months later her renal function improved, and she became dialysis-independent again. Overall, she has remained off IS for 11 years and retains acceptable renal function. These 3 cases, in addition to several others, have been studied by the same group in further investigations.[30–37]

GROUP C: IMPLEMENTATION OF PROTOCOLS IN WHICH CLINICAL OPERATIONAL TOLERANCE OF TRANSPLANTED ORGANS WAS THE PLANNED OBJECTIVE BEFORE THE TRANSPLANT

This group will be divided further into 3 subgroups, namely molecule-based, cell-based, and total lymphoid irradiation (TLI) protocols. The molecule-based group will include all cases in which the induction of COT was attempted through administration of presumed tolerogenic drugs. In the cell-based group, RT patients received heavy conditioning regimens in association with the perioperative infusion of immunomodulatory cells, such as hematopoietic stem cells (HSC) or transplant-acceptance inducing cells (TAIC); afterward, maintenance IS was given for a few months until complete withdrawal, when possible. Patients who received RT after bone marrow transplantation (BMT) from the same donor will also be included in this group. Finally, we will discuss 3 cases of COT that developed after TLI.

Molecule-Based Tolerogenic Protocols

In 2003, the University of Pittsburgh published the results of a seminal trial in which the investigators administered ab initio an immunosuppressive regimen deemed to be tolerogenic to 82 adult kidney, liver, pancreas, and intestinal transplant recipients.[39] Their working hypothesis was that the need for continuous high-dose IS can be avoided in most cases with the use of a strong lymphocyte-depleting regimen before engraftment, followed by the administration of low-dose tacrolimus monotherapy. The goal of the induction treatment was the nonspecific removal of clones of immune cells responsible for rejection before contact with foreign donor antigens occurs. Once the donor antigens are in place after implantation of the new organ, repletion of immune cells occurs, favored by the homeostatic expansion triggered by leukocyte depletion. In addition, minimization of maintenance IS was implemented to further reduce the antidonor response with just enough treatment to prevent irreversible immune damage to the graft, but not with such heavy treatment that the donor-specific clonal exhaustion-deletion process is precluded.

After a mean follow-up of 18 months, overall 1-year patient and graft survival rates were 95% and 82%, respectively, IS-related morbidity was virtually eliminated, and 48 of 72 surviving patients were receiving spaced doses of tacrolimus monotherapy. These results were described as groundbreaking, as 25/39 (64%) renal, 12/17 (70%) liver, 5/12 (42%) pancreas, and 6/11 (54%) intestinal transplant recipients were on spaced doses at the time of publication. Even if the finding that no patient could be weaned completely off IS represents a matter of concern and questions the working hypothesis, the striking reduction in the daily doses of IS should be regarded in and of itself as an outstanding achievement for 2 reasons; first, it was obtained after transplantation of organs (viz kidney, pancreas, and intestine) considered highly immunogenic, and second, it led to a significant reduction in overall IS-related morbidity.

It is important to note that other protocols based on a similar strategy—that is, leukocyte depletion followed by the administration of low-dose single-drug IS—have been implemented not only after RT[40–50] but also after liver transplantation,[51] which is more

capable than any other organ to develop COT. However, none of these protocols have achieved COT, nor have they shown convincingly any impact on overall outcomes.

One lesson to learn from this singular experience is that the working hypothesis may be erroneous or incomplete. In fact, several central and peripheral mechanisms other than clonal exhaustion or deletion might be involved in the induction of tolerance, including intrathymic clonal deletion of precursor T cells expressing T-cell receptors, dendritic cells, peripheral clonal deletion of allogeneic T cells, anergy of allogeneic T cells, cytokine deviation, and cellular regulation of T cells and other cell subsets. In most situations, it seems that leukocyte depletion is not accompanied by a permanent and complete deletion of alloaggressive donor-reactive cells, and the establishment of a regulatory network is required to maintain tolerance. Moreover, a number of laboratory studies analyzing samples from recipients treated with leukocyte-depleting agents have shown that antigen experienced or memory T cells are less susceptible to depletion and may be resistant to suppression by some immunosuppressants.[52,53] Conse quently, in some patients, residual memory T cells may abrogate the potential benefits of induction with a leukocyte depleting agent.

Previous Bone Marrow Transplant for Hematologic Disorders

Bone marrow transplantation, when successful, generally results in the total replacement of the recipient's bone marrow with the donor's bone marrow hematopoietic cells, a condition referred to as *full chimerism*.[54] Full chimerism can be obtained rapidly through the ablation of the recipient's marrow and immune system with high-dose radiation and/or chemotherapy; also, it can be induced more slowly by nonablative conditioning regimens, followed by the infusion of donor's marrow to colonize the recipient completely. This phenomenon paves the way for the onset of tolerance in the case of a subsequent SOT from the same donor (Table 2).

Sayegh et al[55] described 2 cases of BMT followed by living-related donor RT, in which the same individuals donated both the bone marrow and the renal graft. The 2 recipients did not require any maintenance IS, apart from low-dose steroids given for BMT-related chronic lung disease, and therefore these individuals do not fulfill the strict criteria of tolerance adopted in the present article. However, these cases are considered the first report of COT after metachronous bone marrow and RT. Subsequently, more cases of combined BMT and RT[56–61]—or solid organ transplants in general[62]—have been described. It is noteworthy to highlight that in all cases, the use of BMT with its potentially fatal complications such as graft-versus-host disease was justified on the basis of the need for treatment of hematological malignancies. In other words, in these cases, RT was performed only years after BMT were done successfully to replace the patients' bone marrow but never when BMT was performed simultaneously with RT (Table 2). In such cases, the hematolymphoid system of the recipient, by the time he or she receives the RT from the same donor, has already been completely replaced by the donor bone marrow cells and thus will not mount immune responses against a non-HLA-identical solid organ from the same donor. These cases are very different from simultaneous BMT/SOT cases in which a new solid organ is being transplanted at the same time that new bone marrow cells are being introduced because stable full or partial chimerism has not yet been achieved.

Perioperative Infusion of Hematopoietic Stem Cells

Background—Based on the groundbreaking discovery by Billingham et al[63] that the inoculation of fetal mice with lymphoid cells from an allogeneic adult donor mouse of a different strain led to later acceptance of skin grafts from the same original donors, Monaco and Wood[64,65] demonstrated that the addition of donor bone marrow to a strong lymphocyte-depleting regimen resulted in the long-lasting survival of skin allografts in mice, without the need for maintenance IS. On the basis of these findings, Monaco and Wood attempted to translate this strategy into humans in order to eliminate the need for IS and to avoid IS-related toxicity. Their rationale was to convert the state of nonspecific suppression of the immune system, similar to that induced by standard IS, to a specific recipient tailored state of nonreactivity to donor antigens by early exposure to donor antigens.[66] Therefore, they tried to implement the same strategy in 1 patient who received antilymphocyte serum (days 0–14) as a preconditioning regimen, followed by the infusion of donor BM cells on post-RT day 25, along with conventional doses of prednisone and azathioprine. Immunosuppression was progressively tapered and renal function remained stable for 8 months, until the patient died of a perforated sigmoid diverticulitis. At the time of death, the patient was receiving only 2.5 mg of prednisone per week.

Subsequently, much experimental data have confirmed that the infusion of donor-derived bone marrow cells can prolong allograft survival by still incompletely understood mechanisms.[67] However, the translation of this model from animals to humans has remained a very challenging task. In particular, an IS-free state has been achieved only sporadically after living-related donor RT, whereas similar findings have never been documented after deceased donor RT.[54,68–72] In some studies, the perioperative infusion of donor bone marrow seems to reduce the incidence of acute and chronic rejection,[54,69,70] and to improve graft function when infused not only systemically but also intrathymically.[71,72]

The Massachusetts General Hospital Experience

The group at Massachusetts General Hospital adopted a similar strategy in 6 patients with renal failure due to multiple myeloma, who received simultaneous kidney and BMT from HLA-identical sibling donors after a nonmyeloablative conditioning regimen consisting of cyclophosphamide, antithymocyte globulin, and thymic irradiation[72–75] (Table 3). In this context, the myeloma represented not only the cause of their end-stage renal disease but also the primary indication for BMT. Maintenance IS consisted of cyclosporine for 2 months. Donor lymphocytes were also infused at different times in 4 cases, to enhance the graft-versus-host myeloma effect and convert chimerism to full-donor hematopoiesis. Patients were followed for a mean of 4.2 (range, 2–7.3) years and the last mean serum creatinine level was 2.2 (0.9–5.6) mg/dL. One patient had an episode of rejection on day 104 and could not be weaned off IS until 18 months after transplant. Two more patients were completely weaned off IS on days 73 and 76, but the latter patient was displaying a rising serum creatinine level (2.0 mg/dL) at the time of publication. The remaining 3 patients continued to receive IS. Interestingly, 3 patients lost detectable chimerism, showing that a combined kidney/BMT with nonmyeloablative conditioning regimen can achieve renal allograft tolerance and excellent myeloma responses, even in the presence of donor marrow rejection. However, only 3 patients could be completely weaned off IS.

In 2008, the same group reported on a series of 5 renal failure patients, whose grafts and bone marrow were harvested from HLA-mismatched (haploidentical) parent or sibling donors.[76] The 5 individuals received a standard nonmyeloablative conditioning regimen consisting of cyclophosphamide, anti-CD2 monoclonal antibody, and thymic irradiation; in 2 cases, rituximab and prednisone were also given. Cyclosporine was the only immunosuppressant for maintenance therapy. The authors were able to withdraw IS in 4 of 5 patients, who retained their grafts and maintained stable, renal function for 1.2 to 4.6 years after the complete withdrawal of IS. Serum creatinine levels ranged from 1.2 to 1.8 mg/dL in these 4 patients. The remaining patient experienced graft loss after the onset of severe acute rejection (Table 3). Cyclosporine was withdrawn after a mean time of 294 (range, 240–272) days. However, it should be emphasized that the mean creatinine clearance (67 mL/min; range, 60–75) and serum creatinine (1.5 mg/dL; range, 1.2–1.8) levels were slightly abnormal. The investigators report that no signs of rejection were present on graft biopsies performed for cause to date. Long-term monitoring of these 4 patients is clearly required to ensure that any level of donor-specific immunological unresponsiveness induced is stable and maintained.

Late graft losses have been reported in other settings. For example, Burlingham reported on a late graft rejection in a patient who received an RT 9.5 years earlier from his mother after donor-specific blood transfusion and who had been IS-free for 7 years; a gradual rise in serum creatinine level to 2.0 mg/dL prompted a biopsy that did not show any rejection, but 10 months later the serum creatinine level rose to 3.4 mg/dL, and a second biopsy revealed severe cellular rejection.[77]

The Stanford Experience

Strober and colleagues[78,79] applied a similar tolerogenic protocol in 2 distinct series of 4 and 6 patients. All patients received a renal graft followed by the perioperative infusion of HSC from the same HLA-mismatched and HLA-matched donors, respectively. The conditioning regimen consisted of TLI and rabbit antithymocyte globulin, followed by cyclosporine and prednisone as maintenance therapy. Steroids were discontinued on day 10 and mycophenolate mofetil was administered for 1 month after the intravenous injection of HSC, which was performed during the third postoperative week.

In the first trial, only one patient achieved an IS-free state. However, the last documented serum creatinine level was slightly elevated at 1.4 mg/dL. In the second trial, one individual could be weaned off IS but renal function was not shown, whereas 2 individuals developed acute rejection and the remaining 3 were still under the weaning protocol at the time of publication. It was claimed that such findings demonstrate that it is possible to achieve persistent mixed chimerism and COT without the development of graft-versus-host disease. However, these studies need to be verified by others as the risk of development of GVHD, which can be potentially fatal, is only warranted in situations in which BMT is used for treatment of otherwise fatal malignancies. Furthermore, report on the long-term follow-up of these patients, regarding persistence of chimerism and lack of the need for IS, is needed before these methods can be adapted to a wider range of patients.

Perioperative Infusion of Transplant-Acceptance Inducing Cells

Transplant-acceptance inducing cells were originally identified as the principal derivative of a rat embryonic stem cell line that is able to induce tolerance to allogeneic heart grafts[80,81] (Table 4). Because TAIC are able to influence recipient antidonor reactivity through unknown mechanisms, they have been used in 2 safety trials (labeled as TAIC I and TAIC II studies, the latter being a subproject of the Reprogramming the Immune-System for the Establishment of Tolerance consortium). The 2 trials differ in several respects including methods for TAIC preparation, numbers of cells infused, induction IS, and timing of infusions. In the TAIC I trial, the tolerance-inducing cells were given perioperatively to 12 individuals receiving renal grafts from deceased donors. After a mean follow-up of 36 months, results were difficult to interpret. No patient achieved a permanent and robust IS-free state, 2 dropped out of the study for nonimmunological causes, and 8 patients experienced rejection despite preservation of graft function after appropriate treatment and resumption of IS. The remaining 2 individuals did well and were under tacrolimus monotherapy at the end of the study. In the TAIC II study, 5 patients were enrolled. TAICs were administered 5 days before a living-related RT. Immunosuppression could be completely withdrawn in only 1 individual, who finally rejected the graft 34 weeks later (see Table 4).

Overall, although these trials demonstrated that the infusion of TAIC is feasible, major concerns remain regarding the efficacy and safety of such an approach. Whether this approach confers any benefit in the establishment of minimal IS in RT patients when compared with the protocols currently adopted is unclear. Lastly, the optimal dose and timing of cell infusions, and the most appropriate concomitant IS regimen, remains to be determined.

Mesenchymal Stem Cells in SOT: A Potential Immunomodulatory Tool Under Investigation

Mesenchymal stem cells (MSCs) were originally isolated from bone marrow but now can be isolated from almost any tissue in the human body and possess fascinating tissue repair and immunoregulatory properties.[82–85] Interestingly, despite many unknowns about their precise immunobiology, MSCs are currently being evaluated for a wide variety of clinical applications including the treatment of disorders characterized by a dysfunction of immune regulation, such as graft-versus-host disease after bone marrow transplantation and rejection after cell or organ transplantation.[86] Organ transplantation represents another potential field of application as MSCs seem to be able to promote engraftment of allogeneic cells/tissues/organs and to prevent and/or treat rejection in preclinical models. Although there are no published reports to date on their potential in the setting of clinical SOT, it is expected that in the near future we will see numerous reports using MSCs as immune modulators after SOT.[87]

TOTAL LYMPHOID IRRADIATION

Strober et al[88–90] documented COT obtained by discontinuance of treatment soon after RT in 3 cadaveric donor RT recipients whose IS consisted of TLI, a perioperative course of antithymocyte globulin, and maintenance prednisone that was gradually weaned and

eventually stopped. Donor-specific nonreactivity of lymphocytes from their drug-free patients was demonstrated with mixed leukocyte reaction and cell-mediated lympholysis assays. Two patients remained IS-free for 12 years and 69 months, respectively, whereas the remaining patient developed severe urinary tract obstruction 47 months after transplant and 10 months after withdrawal of IS, for which he was eventually retransplanted. It is noteworthy to report that 25 other RT recipients were administered the same protocol without developing COT, and chimerism was not detected.

Total lymphoid irradiation was originally developed as a nonmyeloablative treatment for Hodgkin disease[10] In SOT, this treatment modality was first used about 40 years ago to induce prolonged renal allograft survival. However, TLI has significant short- and long-term effects on lymphocyte subpopulations by suppression of activated T cells and the interleukin-2 pathway. Importantly, as the doses of radiation required for TLI to be effective are high, its clinical application is limited by the toxicity that occurs with such high doses. With the advent of more effective immunosuppressive drugs and cytolytic therapy with antithymocyte globulin and monoclonal antibodies, the use of TLI has declined considerably and is mainly applied—as shown earlier[73–79]—as a nonmyeloablative preparative regimen of TLI in combination with the infusion of donor-derived cells to induce a state of lymphohematopoietic chimerism.

IMMUNE MONITORING

A major concern raised by the implementation of tolerogenic strategies after SOT is the risk for graft loss once acute rejection has occurred. Ideally, we should be able to identify those recipients who may be good candidates for complete withdrawal of IS, to abrogate the subsequent risk for acute rejection and graft loss. Unfortunately, the lack of predictable assays to measure the net state of IS beyond pharmacological monitoring and methods for effective monitoring of the patient response to the withdrawal of IS represent major challenges in the development of tolerogenic strategies. In fact, methods currently used for therapeutic drug monitoring of IS do not provide any assessment of the overall status of the immune response. Moreover, graft dysfunction is usually detected after significant immunological damage has occurred and findings from allograft biopsies may not be predictive of clinical events (see the case reported earlier by Burlingham).[36,77]

To address these issues, the concept of IM has been introduced. *Immune monitoring* is defined as a method of measuring functional and molecular correlates of immune reactivity to provide clinically useful information for therapeutic decision making.[1] The group in Nantes has concentrated much effort in the identification of specific biologic signatures of COT aiming to identify new perspectives for targeted rather than empiric weaning of IS.[35–37] Briefly, they have identified a small biomarker panel, using gene-expression profiling of peripheral blood from spontaneously tolerant RT recipients.[35] This analysis, performed across 91 adults including normal adults and 5 cohorts of renal transplant recipients in different clinical contexts (among whom, 17 were IS-free), identified a minimal set of 49 genes and differentially expressed gene transcripts in drug-free tolerant patients when compared with other patients, with tolerance class prediction scores of more than 90%. Quantitative real time–polymerase chain reaction across a subset of 33 of these 49 genes can

accurately confirm tolerance in an independent validation group of tolerant patients with a specificity of 99%. In other words, they were able to define patients who might be eligible for a progressive decrease in their immunosuppressive medications and, more important, identify patients who need to stay on their current IS dose. The same group has also used the potential of high throughput microarray technology to study peripheral blood-specific gene expression profiles and corresponding molecular pathways associated with operational tolerance in a cohort of 8 human kidney graft recipients.[36,37] In comparison with patients with chronic rejection, tolerant patients displayed a set of 343 differentially expressed genes, mainly immune and defense genes, in their peripheral blood mononuclear cells, of which 223 were also different from healthy volunteers. Using the expression pattern of these 343 genes, they were able to classify correctly more than 80% of patients in a cross-validation analysis and classified correctly all of the samples over time. Collectively, this study identified a unique peripheral blood mononuclear cell gene signature associated with human operational tolerance in kidney transplantation by a classical statistical microarray analysis and, in the second part, by a nonstatistical analysis.

Further investigations have been conducted in parallel in Europe and in the United States by the European Union Indices of Tolerance Network and the National Institute of Health's Immune Tolerance Network, respectively. The European consortium showed that IS-free RT patients present a distinctive expansion of peripheral blood B lymphocytes and natural killer cells and differential expression of several immune-relevant genes in the absence of donor-specific antibodies.[91,92] Similar population expansion of B immune cells and selective expression of B cell–related genes in samples obtained from tolerant individuals were noted by the American consortium.[93] Overall, the European investigators claimed that the combination of identified biomarkers and bioassays are able to identify tolerant patients with a specificity of 0.964 and a sensitivity of 0.933.[92] It is noteworthy to mention that studies from Stanford and Emory Universities have also contributed significantly to progress in this field.[94–97]

Given the critical utility of IM, the next theoretical step should be to validate such findings in large clinical trials. However, we believe that this is not yet practical because the present review shows that the impact of acute rejection on the outcome of an RT is detrimental and typically leads to graft loss. In other words, in the field of RT where the heavier burden on investigators is the demonstration of safety rather than efficacy, the presumed accuracy and efficacy of IM are not yet counterbalanced by the safety of the weaning procedure itself. These considerations render the routine clinical application of tolerogenic protocols unacceptable, no matter if driven by any IM or not, and suggest that such protocols should be implemented only in experienced centers willing to put forth the exceptional effort required to safely perform these extremely complex and high-risk trials.

WHAT IS THE MECHANISM OF COT?

Our efforts to understand the mechanisms underlying the phenomenon of COT and the ways to achieve it have been mostly in vain to date. In addition to the frustrating failure of all molecule-based strategies, we have learned that stem cells exert a powerful modulatory effect on the immune system but we do not yet understand why COT occurs and when the

opportunities for COT to develop are greatest. Cases of COT described late after successful BMT,[55–60] where the immune system of the host has been completely replaced by the donor's bone marrow, demonstrate that the depletion of the host immune cell compartment followed by repletion of this latter with donor cells is important in the onset of COT. Yet, several cases of COT have been documented in the absence of any chimerism or in the presence of only transient chimerism detectable in the RT recipient.[73–76]

WHAT IS THE “GOLD STANDARD” TO INDUCE COT AFTER RT, IF ANY?

This review demonstrates that strategies that have been investigated to date with the objective of achieving a permanent IS-free state have been numerous and heterogeneous in terms of concept, immunological background and rationale, patient age, endpoints, deceased-versus-living donor RT, length of the weaning period and follow-up, presence or absence of donor chimerism, full or partial chimerism, and timing. Most cases of COT have developed in individuals who spontaneously stopped IS. However, when COT is the planned objective from the time of transplantation, it seems that it is essential to combine standard IS with lymphocyte-depleting regimens, followed by the infusion of donor-derived immune modulating cells.

CONCLUSIONS

The worldwide experience reported in the English literature to date could be summarized as follows: (1) After RT, COT is an exceptional finding; (2) patients who do not become tolerant and develop rejection are exposed to an unacceptable risk for graft loss; and (3) whereas all molecule-based strategies have failed, the cell-based approach seems promising, but its efficacy and safety remain a matter of major concern. Most cases of COT are attributable mainly to patient nonadherence to IS, whereas some additional cases of COT may develop after a previous BMT or—very rarely—through the implementation of cell-based tolerogenic strategies. It is evident that molecule-based strategies, despite success in animal models, fail when they are translated into the clinic.

Overall, the withdrawal of IS after RT as attempted with the currently available technologies cannot be encouraged yet, because it is neither effective nor safe and still remains in an experimental phase. No reliable in vitro assays or predictors of tolerance are currently available. Efforts to identify a peripheral blood transcriptional biomarker panel associated with COT after RT are laudable but, as long as the safety of the withdrawal of IS will not be guaranteed, any clinical implementation of such an endeavor should proceed with great caution because—as correctly formulated by Kirk—“regimens that stray from accepted standards require more explicit proof of safety than those carrying accepted practice to a subsequent level”[98] (p 947); and so far, the lack of efficacy of tolerogenic protocols represents their major weakness.

Yet, failures in these experimental settings should not be overblown. Instead, they should be clearly noted and incorporated, as the potential benefit deriving to SOT recipients from the successful implementation of tolerance-inducing strategies remains extraordinary. In terms of risk-to-benefit relationship, as transplant physicians should be aware of their

responsibilities to patients through risk minimization, both the risk of failure to induce tolerance and the risk of failure to offer tolerance should be considered.[99] Many questions still need to be answered, but answers will not be provided unless hard work continues in this arena. Therefore, clinical research in the field not only remains appropriate but also should be strongly encouraged in experienced centers.

FUTURE PERSPECTIVES

The failure of non–cell-based protocols means that such strategies may be suboptimal. The pathways of the immune response triggered by the engraftment of an allogeneic organ may be too numerous to be controlled by just 1 or a few compounds, so cell-based modulation of the immune response after transplantation may be the method of choice to pursue, given our current knowledge and technology. This has been shown in anecdotal reports after both liver[5,100–103] and RT (see earlier). However, there is still no reliable cell-based therapeutic protocol allowing for the induction of COT after allogeneic RT in a safe, practical, and reproducible manner.

In the stem cell era, the field of SOT has just started to address interest toward new types of stem cells, for example, MSCs, which possess impressive immunomodulatory properties. As MSCs have already been used to treat conditions characterized by immunologic dysregulation such as Crohn disease and graft-versus-host disease after allogeneic HSC transplantation, we may speculate that the same immunomodulatory properties might be potentially useful for the prevention or treatment of SOT rejection and for the induction of COT. Notably, as MSCs are capable of promoting tissue repair, harnessing both the immunomodulatory capabilities of such cells and their potential for tissue repair provides an exciting opportunity for further research in the field of SOT.[82–85,87]

Also, other immune cells are currently being explored. Regulatory T cells hold much promise as therapeutic agents for SOT.[104] This class of immune regulatory cells is known to play an unequivocal role in modulating the host immune response to the engraftment. In fact, a large body of experimental data has demonstrated that immunoregulatory mechanisms dependent on donor-specific regulatory T cells are critical in the induction and maintenance of the tolerant state. Consequently, strategies exploiting antigen-specific regulatory T cells for the induction of COT are currently under investigation.[105] In addition, the identification and characterization of regulatory T cells that control immune responses to self-antigens and non–self-antigens have become the focus of many studies.[106] Finally, dendritic cells have been shown to be a major component in the regulation of T-cell responsiveness. In particular, immature dendritic cells are able to induce donor-specific anergy, to favor the generation of T-cells with regulatory properties in vitro, and, by continuously acquiring antigens from the engrafted organ in vivo, promote a state of donor-specific tolerance.[105]

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TABLE 1

Synoptic View of All Successful and Unsuccessful Cases of COT After RT*

Classification	Subtype	Center	Type of Study	Claimed Cases of COT	Length of Follow-up From the Time of the Withdrawal of the IS	Histocompatibility
Nonadherence to IS	Spontaneous withdrawal	Los Angeles[18]	Case report	4, but eventually 1 rejected after 18 mo	17, 23, 52 mo. However, they were taking azathioprine sporadically	In 2 cases, histocompatibility was not matched. First 2A, the second available 4A
		Minneapolis[20]	Case report	6, but 5 rejected after few months	Not available	Not available
		Madison[21]	Case report	8, but 7 rejected after few months	40	Not available
		Madison[22]	Case report	5, but 4 rejected after few months	5 y	Not available in 1 case, unclear in 3. HLA identical in the remaining case
		Columbus-Madison[23,24]	Case report	2	5 and 27 y	Patient no. 1: HLA A1, 2 B44, 62 DR4, 13 donor; A1, 2 B37, 60 DR4, 13 recipient Patient no. 2: HLA identical
	Pregnancy	Erlangen-Munster[25]	Case report, pregnancy	1	9 y	Only HLA class I tissue typing was available. Donor: A2, A28, B8, B40. Recipient: A1, A28, B8, B40
	Survey	Boston[19]	Descriptive, observational	13	>1 y	Unclear
		Los Angeles[18]	Descriptive, observational	24, but 22 rejected after few mo	9, 36 mo	Not available
		Nantes[29]	Descriptive, investigative	7, one of whom rejected after being IS-free for 13 y	9.7 (range, 1–20) y	One patient was HLA-identical to the donor. One, 2, and 3 patients had 1, 3, or 4 mismatches for HLA, respectively. For HLA DR (not available in 2 cases), 3 patients presented 1

Classification	Subtype	Center	Type of Study	Claimed Cases of COT	Length of Follow-up From the Time of the Withdrawal of the IS	Histocompatibility
						mismatch, whereas 1 presented 2 mismatch mismatch, whereas 1 presented 2 mismatch mismatch, whereas 1 presented 2 mismatch
		Nantes[30]	Descriptive, investigative	4	10.3 (5–20) y	Not available
		Nantes[31]	Descriptive, investigative	5	8.4 (3–17) y	One patient was HLA-identical to the donor. Two more patients had 4 and 2 mismatches, respectively. Typing was not available in 2 patients
		Nantes[32]	Descriptive, investigative	4	8 (2–12) y	Not available
		Nantes[33]	Descriptive, investigative	2	10 and 12 y	Not available
		Nantes[34]	Descriptive, investigative	6	2–13 y, mean values not being available	Not available
		Nantes[35]	Descriptive, investigative	17. The reasons for IS withdrawal was not specified per single patients	>2 y	COT patients are divided in 2 groups. One group of 5 patients presents a mean HLA incompatibility score of 3.2 (range, 3–4). The other group of 12 individuals shows a score of 3 (range, 2–4)
		Nantes[36,37]	Descriptive, investigative	6	6.4 (17.3) y	One patient was HLA-identical. One patient showed 3 HLA mismatches. Four patients presented either 1 (2 cases) or 4 (2 cases) mismatches
		European Consortium for tolerance[91,92]	Descriptive, investigative	11. The reasons for IS withdrawal was not specified per single patients	>1 y	Not available
		American network for immune tolerance[93]	Descriptive, investigative	25. The reasons for IS withdrawal was	>1 y	Not available

Classification	Subtype	Center	Type of Study	Claimed Cases of COT	Length of Follow-up From the Time of the Withdrawal of the IS	Histocompatibility
				not specified per single patients		
				not specified per single patients		
Physician-driven weaning of IS	PTLD	Aarhus[38]	Case report	1	>3 y	HLA identical, living related
	Survey	Nantes[29]	Descriptive, investigative	3, of whom 2 for PTLD and 1 for recurrent infections and basal cellular carcinoma; one rejected after being IS-free for 7 y	8.6 y (mean time, range, 6–11)	Patient no. 1: HLA/HLA DR incompatibility number 3 and 2, respectively Patient no. 2: 3, 1 Patient no. 3: 3, 0
		Nantes[30]	Descriptive, investigative	1	6 y	Not available
		Nantes[31]	Descriptive, investigative	2, of whom 1 for PTLD and 1 for CNI toxicity	3 and 8 y	Both presented 3 HLA A-B-DR mismatches
		Nantes[32]	Descriptive, investigative	2	8 (2–12) y	Not available
		Nantes[33]	Descriptive, investigative	3, of whom 2 for PTLD and 1 for recurrent infections and basal cellular carcinoma; one rejected after being IS-free for 7 years	6.3 (3–8)	Not available
		Nantes[34]	Descriptive, investigative	2, of whom 1 for PTLD and 1 for CNI toxicity	2–13 y, mean values not being available	Not available
		Nantes[35]	Descriptive, investigative	17. The reasons for IS withdrawal were not specified per single patient	>2 y	COT patients are divided in 2 groups. One group of 5 patients presents a mean HLA incompatibility score of 3.2 (range, 3–4). The other group of 12 individuals shows a score of 3 (range, 2–4)

Classification	Subtype	Center		Type of Study	Claimed Cases of COT	Length of Follow-up From the Time of the Withdrawal of the IS	Histocompatibility
		Nantes[36,37]		Descriptive, investigative	2, of whom 1 for PTLD and 1 for CNI toxicity	3 and 10 y	Both patients were presenting 3 HLA DR A-B-DR mismatches
		European Consortium for tolerance[91,92]		Descriptive, investigative	11. The reasons for IS withdrawal were not specified per single patient	>1 y	Not available
		American network for immune tolerance[93]		Descriptive, investigative	25. The reasons for IS withdrawal were not specified per single patient	>1 y	Not available
Implementation of tolerogenic strategies ab initio	Molecule based	Pittsburgh[39]		Prospective, noncomparative	0/39	—	Not available
		Cambridge[41–45]		Prospective, comparative, nonrandomized	0/33	—	Not available
		Oxford[40]		Prospective, noncomparative	0/13	—	Not available
		Bethesda[47]		Prospective, noncomparative	0/7	—	Not available
		Bethesda[48]		Prospective, noncomparative	0/5	—	Not available
	Cell based	Previous BMT	Boston BWH[55]	Case report	2*	7 and 3 y	HLA identical
			Geneva[56]	Case report	1	2 y	HLA identical
			Copenhagen[57]	Case report	1	1 y	Donor: HLA A24, B7, DR5/A1, B8,DR2, blood group 0 Recipient: A24, B8, DR3/A1,B8, DR2, blood group A
			San Francisco[58]	Case report	1	2 y	HLA mismatched, haploidentical (the donor being the patient's father)
			Milwaukee[59]	Case report	2	15 and 30 mo	All HLA identical
			Birmingham, AL[60]	Case report	1	7	HLA identical
		HSC	Boston MGH[73–75]	Prospective, noncomparative	3/6	7.3, 5.3, 2 y	HLA identical
			Boston MGH[76]	Prospective, noncomparative	4/5	4.6, 3.4, 2.2 and 1.2 y	HLA mismatched

Classification	Subtype	Center	Type of Study	Claimed Cases of COT	Length of Follow-up From the Time of the Withdrawal of the IS	Histocompatibility
		Stanford[78]	Prospective, noncomparative	1/4	14 y	HLA mismatched. Patient no. 1: A1, 31, B44, 60, DR7, 9 donor; A2, X, B44, 60, DR1, 4 recipient. Patient no. 2: A11, 34, B13, B* 1535, DR4, 8 donor; A34, X, B* 1521, B*1535, DR4, 15 recipient Patient no. 3: A1, 3 B8, 27, DR1, 17 donor; A3, 31, B27, 35, DR1, X recipient Patient no. 4: A3, 11, B60, X, DR4, 4 donor; A2, 29, B7, 44, DR1, 8.
		Stanford[79]	Prospective, noncomparative	1/3	28 m	HLA identical
		Gujarat[71]	Prospective, nonrandomized	0/24	—	Not available
		Gujarat[72]	Prospective, randomized	0/12	—	Not available
		Miami[70]	Prospective, nonrandomized, comparative	0/63	—	Not available
	ESC	Gujarat[16]	Prospective, randomized	1/1	3 mo	HLA identical
	TAIC	Kiel[80]	Prospective, noncomparative	0/12	—	All degrees of matches (not better specified)
		Kiel[81]	Prospective, noncomparative	0/5	—	One HLA identical, the other being mismatched
	Total body irradiation	Stanford[88,89]	Prospective	3/28, but 1 rejected after 10 mo for nonimmunological reason	144 and 69 mo	Patient no. 1: A28/30, B14/17, DR2/8 donor; A2/11, Bw44/y, DR4/5 Patient no. 2: A2/x, B7/18, DR3/- donor;

Classification	Subtype	Center	Type of Study	Claimed Cases of COT	Length of Follow-up From the Time of the Withdrawal of the IS	Histocompatibility
						A2/11, Bw44/y, DR1/7 A2/11, Bw44/y, DR1/7 Patient no. 3: A3/10, B7/35 donor; A10/ w26, B7/w40

[*] Overall, 240 RT patients have been reported to be or to have been IS-free for at least 1 year. Yet, the actual number of tolerant patients cannot be accurately calculated because some series have been the object of numerous investigations, which lead to many papers.

TABLE 2

Synoptic View of the 6 papers Reporting on the Cases of COT Developed After Sequential BMT and RT*

First Author	Center	Journal, Year	Number of Patients	HLA	Time Elapsed Between BMT and RT, y)	Length of Follow-up From IS Withdrawal, mo
Sayegh[55]	Brigham and Women Hospital, Boston, MA	Ann Int Med, 1991	2†	Identical	7, 3	12, 24
Helg[56]	Geneva, CH	Transplantation, 1994	1	Identical	2‡	24
Jacobsen[57]	Copenhagen, DK	Lancet, 1994	1	Nonidentical	1	17
Sorof[58]	San Francisco, CA	Transplantation, 1995	1	Nonidentical	2	15
Butcher[59]	Milwaukee, WI	Clin Transplant 1999	3	Identical	3, 11, 18	15, 30
Sellers[60]	Birmingham, AL	Transplantation 2001	1	Identical	7	72

*It should be emphasized that all patients received both transplants from the same donor. This is a critical issue as demonstrated by the Milwaukee[59] series, which actually included 3 additional patients who received bone marrow and kidney graft from 2 different donors; they all experienced acute rejection and needed maintenance IS. The interpretation of such finding is that complete donor chimerism produced by the previous BMT facilitates acceptance of the renal graft derived from the same donor.

†Yet, as these 2 patients received prednisone at small doses to treat the idiopathic pneumonia complicating BMT, they cannot be labeled as completely tolerant, according to the definition herein adopted.

‡Both BMT and RT were planned at the baseline and the patient's mother consented to donate both the bone marrow and the kidney. Therefore, this strategy somehow anticipates the protocol that will be described by Sachs' group a few years later.

TABLE 3

Summary of the Experience Reported by the Transplant Biology Research Croup and the Massachusetts General Hospital

Patient	Follow-up From RT	Rejection	IS	Last Creatinine, mg/dL
Myeloma HLA-matched series[73–75]				
1	7.3 y	None	Off by day 73	0.9
2	5.3 y	None	Off by day 76	2
3	4.3 y	None	Yes	1.5
4	3.5 y	None	Yes	1.5
5	2.8 y	None	Yes	5.6
6	2y	Day 104	Off by 1.8 y	1.7
HLA-mismatched series[76]				
1	5.3 y	None	Off by day 240	1.2
2	4.7 y	None	Off by day 422	1.5
3	0.45 y	Yes, with graft loss	Yes after second RT	—
4	2.9 y	None	Off by day 244	1.5
5	1.9 y	None	Off by day 272	1.8

TABLE 4

Synoptic View of the Results of TAIC II Trial[81]

Patient No.	Steroid Withdrawal	Tacrolimus Withdrawal (wk)	Acute Rejection
1	Yes, by the eighth wk	Yes, by the 43rd wk	Yes
2	Yes, by the 10th wk	Yes, spontaneously withdrawn by the patient	Yes
3	Yes, by the eighth wk	No	Yes, steroid-resistant
4	No, as the patient was suffering from rheumatoid arthritis	No	No
5	Yes, by the eighth wk	No	No