

The Beginning of Clinical Tolerance in Solid Organ Allografts

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Development of effective multidrug immunosuppressive regimens and improvements in the management of chronically immunosuppressed patients have produced extraordinary patient and allograft survival in clinical organ transplantation. Unfortunately, significant problems of morbidity and mortality related to chronic immunosuppression remain. Thus, there is an enormous motivation and interest in inducing specific unresponsiveness (tolerance) to clinical solid organ allografts. Operational clinical tolerance may be defined as stable, normal graft function in the total absence of a requirement for maintenance immunosuppression. Alternatively, the concept of employing tolerogenic strategies to permit graft acceptance with dramatically reduced immunosuppression requirements is referred to as 'prope' or minimal immunosuppression tolerance. There have been isolated examples of clinical tolerance, usually in the context of spontaneous or induced donor chimerism, excellent HLA matching, and/or drug weaning or patient noncompliance.

The various attempts that are currently being employed to induce some type of clinical tolerance are reviewed in this manuscript. Strategies in which all immunosuppression was to be withdrawn from the recipient (donor-specific unresponsiveness) are first discussed. These include strategies that utilize initial immunoablation with varying doses of irradiation and/or lymphocytic antibodies with or without donor-specific bone marrow infusion and short-term standard

immunosuppressive therapy. Strategies to induce 'prope' or minimal immunosuppression tolerance that utilize induction lymphoablation with polyclonal or monoclonal antilymphocyte antibodies, with or without donor bone marrow infusion, followed by limited low-dose immunosuppressive therapy are also discussed.

The ethical considerations in testing clinical tolerance strategies and protocols are discussed in detail. The limited number of clinical tolerance studies already available affirms that carefully supervised weaning of immunosuppressive drugs in controlled tolerance trials is not unreasonable, especially when monitored by protocol allograft biopsies. Initial results suggest that aggressively treated low-grade steroid-responsive rejection reactions in the absence of immune-mediated tissue destruction does not necessarily require resumption of high-dose immunosuppression. Finally, the role of donor bone marrow infusions in facilitating tolerance/hyporesponsiveness induction needs to be studied and expanded.

Keywords: *Tolerance, Immunosuppression, Nonresponsiveness, and Hyporesponsiveness*

Progress in the development of effective multidrug immunosuppressive regimens and in the management of chronically immunosuppressed recipients has produced dramatic improvement in patient and graft survival after kidney and other organ allografts. One-year patient and graft survival after renal allografting are now regularly in excess of 90% [1]. Unfortunately, significant problems of morbidity and mortality related to chronic immunosuppression remain. Failure to control rejection (acute and chronic rejection), associated drug toxicity and side effects (diabetes, osteonecrosis, cataracts etc.), susceptibility to

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opportunistic infection, and significant occurrence of spontaneous neoplasms [2] continue to plague patients. In addition, reliance on our most effective drugs, calcineurin-inhibitors, is associated with direct nephrotoxicity and hypertension leading to chronic allograft dysfunction and late loss requiring need for repeat transplantation. Also, the excessive cost and need for daily polypharmacy can contribute to patient noncompliance and eventual graft loss. Thus, there is an enormous motivation and interest in inducing specific unresponsiveness to clinical solid organ allografts.

Definition(s) of Clinical Tolerance

Donor Specific Unresponsiveness (Tolerance): Specific unresponsiveness (tolerance) is easily defined in experimental transplantation. Experimentally, tolerance is the specific survival of an organ allograft in a recipient in the absence of a requirement for continuing nonspecific immunosuppression, while the capacity to reject third-party allografts and to accept a second donor-specific allograft is maintained. Implicit in this definition is the fact that the tolerated graft maintains normal histologic characteristics and function. Medawar and colleagues [3] induced actively acquired tolerance to skin allografts by the injection of replicating lymphoid cells into the naturally immunosuppressed neonatal mouse. Tolerance to donor strain skin grafts was associated with persistent donor strain lymphoid cell chimerism; tolerance was thought to be due to an absence of immune activity (central deletion) and was maintained by the persistence of lymphoid cell chimerism. Later detailed studies by Streilein and colleagues [4] demonstrated in adult animals rendered tolerant by neonatal lymphoid cell injection that recipient anti-donor activity could be present by various in vitro assays and skin allografts were still tolerated. Other studies, usually in rodent species have demonstrated that in mice rendered tolerant as adults, several different tolerance-facilitating mechanisms could operate, frequently sequentially during the tolerant state; these mechanisms include besides chimerism, deletion, anergy, clonal reduction, cytokine pattern (TH1-TH2 paradigm) alterations, and the presence of immunoregulatory (suppressor) cells [5].

If the above experimental definition is applied clinically, it would require that a tolerant recipient be off all immunosuppressive drugs with normal

graft function and histology while maintaining essentially all other immune responses. However, recent studies in patients subjected to various tolerance strategies suggest that clinically tolerant patients may show antidonor immune activity in vitro [6]. Also cellular infiltration of normally functioning allografts with cells of a immunoregulatory phenotype [7] may be protolerogenic. Currently, the definition of tolerance requiring normal graft function and histology off all immunosuppression is an incomplete one and presents a difficult clinical conundrum. All established transplant groups have had the experience with one or more transplanted patients who have taken themselves off immunosuppression and who have maintained normal graft function for several to many years before graft function deteriorated from chronic rejection. If an excellent transplant recipient is slowly tapered off all immunosuppression (or if a noncompliant patient takes himself or herself off immunosuppression) but still maintains normal function and histology for several years before beginning rejection, was the patient tolerant during this drug-free period? Thus, added to the definition of tolerance must somehow be the parameter of time, i.e. if "tolerance" is established, there is no guarantee it will be permanent (see below). Indeed, the decision to pronounce a patient off immunosuppressive drugs "tolerant" must be considered a qualified diagnosis closely monitored over time.

Donor-Specific Hyporesponsiveness (Prope' Tolerance, Minimal Immunosuppression-MIST-Tolerance): It is well established in experimental transplantation systems involving multiple histocompatibility antigens that induction of unresponsiveness to some of the antigens produces prolonged or attenuated survival without immunosuppression or indefinite survival with reduced immunosuppression, i.e. a partial tolerance. It is likely that any protocol that attempts to induce clinical tolerance may be only partially effective, so that allograft responses between recipient and donor may be only partially abrogated, leading to modified (prolonged) but not indefinite survival. In such circumstances, one or more tolerance mechanisms (partial clonal deletion or reduction, suppressor cells, cytokine alteration) may be transiently operative [8]. This might be demonstrated clinically by better survival and function for the same dose of immunosuppression in the putatively tolerized recipients. Obviously, such improvements in

survival with less immunosuppression would be (intuitively) accomplished with better preservation of immune responses to bacterial, viral, and other antigens, which, in turn, would be reflected by reduced morbidity and mortality. The concept of employing tolerogenic strategies to permit graft acceptance with dramatically reduced immunosuppression was first formally introduced by Calne et al [9] who coined the application proper (Latin almost) tolerance. Monaco [8] has referred to it as minimal immunosuppression (MIST) tolerance; he noted that in tolerance associated with immunoregulatory cell mechanisms, a minimum dose of chronic immunosuppression is frequently required to achieve and maintain clonal reduction for maximum suppressor cell effect.

Historical Examples of Clinical Tolerance to Solid Organ Allografts

Although effective chronic maintenance immunosuppression has been an almost absolute requirement for persistent, long-term solid organ allograft survival, there have been isolated examples of clinical tolerance—usually in the context of spontaneous or induced donor chimerism, excellent HLA matching, and/or drug weaning or patient noncompliance.

It is now recognized that patients who are haematopoietic chimeras after myeloablative therapy and bone marrow transplantation for treatment of haematopoietic malignancy will accept renal transplants and other tissue transplants from their specific bone marrow donor without requirement for immunosuppressive therapy [10]. Unfortunately, the infectious and graft-versus-host disease (GVHD) morbidity and mortality associated with stringent myeloablative therapy required for bone marrow transplantation has up to now recently precluded routine application of this approach for induction of tolerance in recipients of solid organ allografts (see below).

Essentially, every established transplant program has experience with one or two patients with long-term, excellent functioning organ allografts off all immunosuppression. These patients usually (but not always) occur in HLA identical matches and, for the most part, have been weaned from initial maintenance immunosuppression, frequently by noncompliant patients. The incidence of this phenomenon is not known; a long-term registry of such patients would be desirable. The

natural outcome of the dogma for required maintenance immunosuppression is that when such a drug-free patient is encountered, they are invariably restarted on immunosuppression. This might not be necessary. There have been few formal analyses of such patients, but two recent papers are relevant. Knechtle and Burlingham [7] studied two interesting cases of human renal allograft tolerance. The first case involved an HLA identical kidney recipient transplanted in 1967 who had become noncompliant [11] and has been off all immunosuppression for 30 years with a current normal creatinine (1.2 mg). They used their unique trans-vivo DTH assay [12] to show that there were present in the recipient peripheral blood CD8⁺ CTL effector cells held in check by CD8⁺ regulatory cells specific for the same antigen but with lower apparent affinity. They also suggested that suppression by CD8⁺ T regulatory cells was mediated by TGF- β and IL-10. A second case involved a recipient of a maternal haploidentical renal allograft who discontinued immunosuppression after 2 years and was followed for more than 9 years. The recipient peripheral blood mononuclear cells and skin were microchimeric; one in 10,000 PBMC were of maternal origin; when these donor cells were isolated and added back to a secondary MLR culture, a specific suppression of the CTL response to the donor, but not to a third party, was identified. Nevertheless, after 6 years of perfect function, acute and chronic rejection ensued and the graft was lost and dialysis necessitated. These authors maintain that tolerance is a metastable state that develops over time in a donor-specific manner and is mediated in part by donor-specific regulatory T cells. The role of regulatory T cells in experimental and clinical tolerance has recently been the subject of extensive attention [13].

Starzl and colleagues [14] noted that long-term hepatic and renal allograft recipients requiring minimal or no maintenance immunosuppressive therapy frequently have persistent donor cells in peripheral tissue, i.e. were microchimeras. Recently, Starzl et al [15] concluded that long-term, drug-free allograft survivors occurred more frequently after immunosuppressive protocols that did not emphasize heavy, multidrug posttransplant immunosuppression. Thus, they noted that 9 of 46 (19.6%) of live-related renal allografts performed in 1962-63 had continuous graft function for most of the succeeding 40 years; 7 of 9 of these patients are currently immunosuppressive drug-free for 3 to 38

years. Although all were related donors, only 2 were HLA-identical. These authors attributed this clustering of clinically tolerant patients to use of the prevailing drug protocols which minimized post-transplant immunosuppression until rejection occurred. A similar group of drug-free tolerant liver allograft recipients was generated in the early 1970s when immunosuppression consisted of azathioprine (or cyclophosphamide) combined with a short course of prednisone [16]. Twelve of 42 patients in this group had been off immunosuppression for 1 to 17 years and many of the remaining 30 have since been weaned from drugs under supervision [17]. With the introduction of calcineurin-inhibitor drugs in multidrug immunosuppressive protocols, the high mortality and rejection rates associated with liver transplantation declined, but the frequency of drug-free recipients did not increase. With adoption of multidrug, high-dose prophylactic immunosuppression, complete drug withdrawal has become rare. Only when liver recipients received cyclosporine or tacrolimus monotherapy with high-dose prednisone reserved for rejection, has complete drug withdrawal become more achievable. A theoretical basis for this observation has been proposed by Starzl and Zinkernagel [18].

Attempts to Induce Clinical Tolerance to Solid Organ Allografts

In this section, the various attempts to induce some type of clinical tolerance to solid organ allografts are reviewed. Strategies in which all immunosuppression was to be withdrawn from the recipient (donor-specific unresponsiveness) are discussed initially; minimal immunosuppression strategies (Prope' or MIST tolerance strategies) are also reviewed.

Donor-Specific Unresponsiveness: Based on a long series of studies in rodents and canines [19,20] using pretransplantation lymphoablation employing total lymphoid irradiation to induce tolerance, Strober and colleagues [21] reported 28 cadaver kidney recipients given TLI (2000 cGy) pretransplant followed by 6 injections of antithymocyte globulin (ATG) posttransplant; maintenance immunosuppression was prednisone 10 mg/day. No donor-specific lymphoid/hematopoietic cell infusions were performed. Eleven of 28 patients had no rejections during the first year, and 9 of 11 showed donor-specific unresponsiveness in MLR [22]. Subsequently Strober et al [23] reported 3 patients

(two from this series and one from South Africa) who had been completely withdrawn from immunosuppressive drugs; all 3 were tolerant in that they had normal function without drugs and exhibited specific unresponsiveness to donor cells in MLR and CML in vitro assays. One of these patients had normal graft function off drugs with no evidence for microchimerism 12 years later [24]. Because pretransplant conditioning with TLI was difficult to apply in relation to predictability of cadaver organ availability, the Stanford group developed a posttransplant conditioning regimen using TLI [25] and antithymocyte globulin and infusion of donor bone marrow derived cells. Milan et al [26] subsequently applied this protocol to 4 recipients of living donor renal allografts. Patients received kidney transplants and posttransplant conditioning with TLI of 80 cGy in 10 doses (total 800 cGy) from day 0 to day 10 and ATG (6 doses of 1.5 mg/kg from day 0 to day 14); subsequently patients were infused with cytokine-mobilized donor-specific bone marrow-derived cells on day 11 as a purified inoculum of 1×10^5 CD3⁺ cells/kg and 3 to 5×10^6 CD34⁺ cells/kg. Prednisone and cyclosporine were added as maintenance immunosuppression. Steroids were to be withdrawn in 6 to 9 months and cyclosporine at 12 months if all of the following criteria were met: macrochimerism was demonstrable during the first 3 months post transplantation, no clinical or protocol biopsy evidence of rejection occurred, and donor specific unresponsiveness in MLR assay was achieved. Three of 4 patients were chimeric but only 2 of 4 patients fulfilled all criteria and were taken off drugs. Both of these patients after being drug-free for nearly a year had a slight rejection episode at 17 months posttransplant and were restarted on low-dose immunosuppression with excellent function. These studies show that rejection crises after tolerance strategies can occur after a drug-free period; they also demonstrate that such rejections can be easily managed and that negative in vitro assays do not necessarily reflect a true state of clinical tolerance [27].

Cosimi and Sachs and the MGH group have studied the use of the so-called mixed chimerism model of tolerance in rodents [28], miniature swine [29], and nonhuman primates [30]. In the latter species, they utilized a tolerance protocol involving pretransplant and peritransplant low-dose total body irradiation, thymic irradiation, and antithy-

mocyte globulin treatment combined with donor-specific bone marrow infusion at time of kidney transplant and splenectomy. Cyclosporine was administered for 1 month posttransplant and then terminated. Recipients of this regimen generally developed transient multilineage chimerism for the first several weeks and 70% survived long-term. There was a remarkable lack of toxicity and GVHD was not observed. This protocol was subsequently adapted for combined HLA-matched bone marrow and kidney transplantation in patients with multiple myeloma and renal failure [6]. The protocol was modified so that TBI was replaced by cyclophosphamide (60 mg/kg IV x 2 pretransplant) and splenectomy was not done; cyclosporine was discontinued after 2 or 3 months. Five patients have been studied since 1998. With the exception of one patient requiring low-dose cyclosporine for mild GVHD, immunosuppressive drugs have been withdrawn for all patients; there has been no evidence of chronic rejection, with the longest patient surviving over 5 years. Of interest was the finding that in these patients peripheral chimerism was transient, usually becoming undetectable after approximately 3 months. Also, *in vitro* assays showed some recipient anti-donor reactivity. Encouraged by these results, the authors extended this tolerance strategy to patients with renal failure without malignancy and without an HLA-matched donor [31]. In this trial, ATG (ATGam) was replaced with an experimental humanized anti-CD2 monoclonal antibody because of its protective effect against GVHD. Thus far, 3 patients have been entered into this trial with encouraging results. The first patient was a recipient of a second renal allograft, having lost the first transplant because of necessity to withdraw chronic immunosuppression due to multiple skin neoplasms. This patient has been off immunosuppression for 16 months without evidence of rejection, suggesting tolerance has been established. The second patient sustained a mild humoral rejection and has required continuation of low-dose tacrolimus and prednisone. A third patient sustained humoral rejection early after transplantation and currently has compromised graft function on maintenance immunosuppression. The authors concluded that allograft tolerance via mixed chimerism appears achievable in recipients of HLA mismatched kidneys but may require intensified suppression of humoral responses.

Donor-Specific Hyporesponsiveness (Prope' or Minimal Immunosuppression (MIST) Tolerance)

In this section, the various formal clinical attempts involving application of a "tolerance" fostering protocol aimed to deliberately reduce maintenance immunosuppression are considered. This concept was first clearly and formally championed by Calne [9]. He noted that there may be different mechanisms by which tolerance is achieved, but from the patient's viewpoint operational tolerance is the goal whereby, after a brief induction procedure, the patient will maintain good allograft function indefinitely without maintenance immunosuppression. Such a goal may be hard to achieve with a tolerance-inducing strategy because of variation between donors and recipients relative to tissue matching, innate immune activity, response to tolerogenic drugs, and susceptibility to disturbances to the tolerant state by infections or allergic reactions. Therefore, he makes the case that it may be in the patient's best interest to maintain graft acceptance by a low, nontoxic dosage of maintenance immunosuppression that may or may not be required indefinitely, *i.e.* *prope'* tolerance. Monaco has suggested that in certain forms of tolerance based on immunoregulatory cells, reduction of target clone size by minimal immunosuppression enhances the efficacy of suppressor cells; thus minimal immunosuppression tolerance (MIST) may be a true variant of the tolerant state [8]. Calne [32,33] has reported on 31 patients treated with a powerful lymphocyte depleting monoclonal antibody Campath 1H (alemtuzumab) followed by low-dose maintenance cyclosporine monotherapy to produce trough levels of 75 to 125 mg/mL. Twenty-nine of 31 patients have sustained function in their grafts for over 5 years posttransplant. Only 3 patients are receiving prednisone (10 mg/day) and azathioprine (100 mg/day) after rejection episodes. A theoretical basis for possible induction of donor-specific unresponsiveness (hyporesponsiveness) by this strategy has been described [9]. If regularly reproducible in large numbers of patients, this strategy would offer significant advantages. It is simple and relatively inexpensive, steroid free, and as a monotherapy, less susceptible to patient noncompliance.

Kirk et al [34] performed a study to determine if profound lymphocyte depletion with Campath 1H (alemtuzumab) would induce tolerance in human renal allografts; the study also permitted evaluation of the immune response in the setting of profound

T-cell depletion. Seven unsensitized living donor kidney recipients were treated perioperatively with alemtuzumab (0.3 mg/kg for 3 or 4 doses). SoluMedrol to control cytokine release syndrome was given with the first 3 doses. Thereafter, no immunosuppression was given. Profound lymphocyte depletion was achieved in both the blood (periphery) and secondary lymphoid tissues. All patients developed reversible rejection episodes within the first month that were characterized by predominantly monocyte (not lymphocyte) infiltrates with only rare T-cells in the blood or allograft. These episodes were responsive to steroids or Rapamycin (sirolimus) or both. After such therapy, patients remained rejection free on reduced immunosuppression, usually sirolimus monotherapy, despite recovery of lymphocytes to normal levels. They concluded that profound T-cell depletion does not induce tolerance in humans. Also they noted a predominant role for responding monocytes in human allograft rejection in the setting of lymphocyte depletion.

Knechtle et al [35] modified this approach for a pilot study by combining a similar dose of Compath-IH (alemtuzumab) induction followed by low-dose Rapamycin. They found that only 30% of patients experienced acute rejection, usually within the first 1 to 3 weeks. They responded for the most part to steroids and other immunosuppressive treatment. Approximately one half of these rejections were aggressive humoral responses. Approximately 60% of the study patients have been maintained on sirolimus monotherapy for up to 3 years in some cases. Interestingly, only 1 of 13 patients over 45 years of age developed rejection, in contrast to 7 of 16 patients younger than 45 years. These encouraging results suggest that some of these patients could be tolerant, but no formal attempt at immunosuppressive withdrawal was performed. In vitro analysis suggested that 70% of the patients displayed some level of CD4 and/or CD8 T-cell hyporesponsiveness to donor antigens by MLR in vitro assay and cytokine production.

Miller and colleagues [36,37] have reported 6-year follow-up results in kidney transplant recipients infused with donor-specific bone marrow after transient antilymphocyte antibody lymphoablation with subsequent standard cyclosporine or tacrolimus-based triple immunosuppression. They noted that at 3 years and later, significantly decreased chronic rejection and higher graft survival in the BM-infused recipients versus noninfused

controls. They noted persistent chimerism in the bone marrow of infused recipients; notably the chimeric cells increased in iliac crest bone marrow but not in the peripheral blood. The Miami group has also shown that in certain bone marrow recipients with residual antidonor MLR responses 1 to 2 years posttransplant, purified posttransplantation iliac crest chimeric cells of either donor or recipient phenotype inhibited recipient immune responses in MLR to the donor more strongly than freshly obtained (nonchimeric) cells from the same donor in the same reactions. This indicated an ongoing increased regulatory role for chimeric cells of both the donor and recipient origin with a degree of donor specificity in BM-infused recipients [38]. Interestingly, Maki and colleagues [39] observed the same phenomenon in mice with tolerant skin grafts after antilymphocyte serum treatment and donor BM-infusion. Unfortunately, the BM-infused patients of the Miami group have not been subjected to formal, controlled studies of immunosuppressive drug withdrawal.

Starzl and colleagues [40] have utilized the protocol of peritransplant lymphocyte depletion with high-dose thymoglobulin (SangStat) followed by reduced tacrolimus monotherapy to achieve recipient lymphocyte depletion and reduce the anti-graft response to a readily deletable range. This high-dose antibody-induction therapy is combined with reduced posttransplant maintenance immunosuppression (tacrolimus) so that antigen-specific immune activation can occur in a graded or limited manner to facilitate exhaustion-deletion of reactive host cells [15]. These authors applied these concepts to 82 patients with kidney and other organ transplants. Patients received preoperative thymoglobulin (5 mg/kg, single infusion) followed by tacrolimus monotherapy twice daily. After 4 months, tacrolimus monotherapy was weaned to varying levels from once daily to once per week. Immunosuppression morbidity was virtually eliminated. Patient survival was 95%, and graft survival was 88% at 13 to 18 months with 43 of 72 patients on spaced monotherapy. There was a high incidence of biopsy-proven rejections in weaned kidney patients that responded relatively easily to steroid boluses and/or adjusted tacrolimus dosages. The high incidence of steroid-responsive rejections was reminiscent of the frequent, low-grade, easily reversible rejections observed in successful recipients of donor-specific blood transfusions [41]. Patients who

responded to rejection could resume weaning. This important and unique study showed that excellent short-term patient and graft survival could be obtained with relatively modest tacrolimus monotherapy after lymphocyte-depletive induction therapy. The high incidence of rejection reactions (called immune activations by the authors) may presage late organ injury or loss. Only time will tell. It is incumbent on the authors to provide long-term follow-up on these important patients.

Ethical Considerations in Clinical Tolerance

Kirk [42] has provided a thought-provoking analysis of the ethical considerations in the performance of clinical tolerance trials; everyone involved in clinical organ transplantation should review this work. He examined the internationally accepted guidelines for human experimentation as they relate to organ transplantation trials. These guidelines are based on the single premise: "First, do no harm." Investigators in clinical transplantation must therefore demonstrate safety over efficacy, since the results of current transplantation practice are very satisfactory alternative treatments for specific organ failure. Clinical tolerance is a laudable and valuable goal that is an appropriate subject for research, but it is not an end in itself; as immunosuppressive drugs become less toxic, more effective, and more devoid of late, long-term complications, the need and appropriateness of specific tolerance trials must be regularly reevaluated.

Feasibility or pilot studies involving small numbers of patients addressed to answering a specific question without contemporaneous controls are reasonable and appropriate in so far as they provide a basis for subsequent investigation that should address the concept of benefit with appropriate comparative groups involving adequate numbers, controlled variables, and careful patient monitoring. Furthermore, failure in a tolerance trial should not acutely equate with mortality. No clinical tolerance trials should proceed without Institutional Review Board (IRB) approval. Transplantation trials require considerable resources for rigorous follow-up. Investigators should be obligated for long-term (minimal 5 years) follow-up—a relative rarity in transplantation studies. Since rejection in tolerance studies can occur late after immunosuppression withdrawal, comprehensive follow-up with regular protocol biopsies are essentially an absolute requirement. It is generally accepted that investigator-

designed tolerance trials should be based on preclinical studies involving evidence obtained progressively in rodents and large animal models. Nevertheless, drugs or therapies used in humans for nontransplantation circumstances could appropriately be incorporated in transplantation tolerance protocols without additional testing in large animals. Finally, a strong case is made for the concept that addition of any known effective immunosuppressive drug to a standard regimen should be accompanied by a suitable reduction in another effective drug.

Kirk also makes the very strong point that there are a multiplicity of effective immunosuppressive drug protocols and regimens in widespread use. Thus, the standard of care is the result, not a particular drug regimen. Therefore, pilot tolerance trials should be compared with contemporaneously reported results for comparable patients in the UNOS database. In comparative studies, the control group must still reach a level comparable to the national standard. In assessing safety, the protocol must be compared with the best available therapy, yet when determining the merit of the trial, the distance between the results of the accepted therapy and the health of a normal individual must be considered. In terms of value, a tolerance regimen of moderate risk that can be applied to only a small number of patients, if successful, has less potential value than a therapy that can be applied to all, despite similar risks.

Conclusions

The studies to induce tolerance presented above already have generated important information that suggests the basis for further investigations.

1. The fact that isolated examples of spontaneous tolerance generated by drug noncompliance or toxicity-necessitated drug withdrawal exist may provide an important research resource. A tolerance registry of such patients should be established, perhaps under the aegis of UNOS or some other national transplant organization. A registry of such patients would provide opportunity to determine the phenotype of tolerance patients. Theoretically, blood, urine, and other tissue samples could be made available for suitable analysis and testing.
2. Successful organ transplantation is currently

based on almost dogmatic use of maintenance immunosuppression. A persistent incidence of opportunistic infection and spontaneous neoplasms suggests intuitively that most patients—especially those given some type of anti-T-cell induction antibody—are probably significantly over immunosuppressed utilizing currently accepted protocols for maintenance multidrug immunosuppression. Attempts involving drug minimalization or withdrawal have shown varying degrees of success, and they have done little harm over the short-term; unfortunately, they have been frequently poorly controlled and long-term follow-up has been lacking. We need to establish whether currently used maintenance programs in individual patients may be safely reduced over time to levels consistent with the concepts of 'prope' or MIST tolerance.

3. The above tolerance studies affirm that cautious, carefully supervised weaning of immunosuppressive drugs in controlled trials is not unreasonable, especially when monitored by protocol biopsies. They have shown that aggressively treated low-grade steroid-responsive rejection reactions in the absence of immune-mediated tissue destruction does not necessarily require resumption of high-dose immunosuppression—a clear conceptual change from previously held dogma. These observations alone should permit elaboration of controlled tolerance drug minimalization/complete withdrawal studies without excessive fear that significant, irreversible harm would be done to rejecting recipients. At the same time, these studies have shown that in drug minimized or completely withdrawn individuals, although T-cell-mediated rejection may not occur, antibody or other non-T-cell-mediated rejection reactions can occur. Strategies to avoid this problem of non-T-cell-mediated rejection must be elaborated.
4. Thus far, all clinical drug minimalization/tolerance strategies utilize some type of recipient transient lymphoablation/depletion; it is likely that a number of such techniques will be effective to varying degrees. Subsequent clinical immunosuppression is maintained for a finite target time and then abruptly withdrawn. Alternatively, it may be slowly weaned to low levels but not completely withdrawn. Currently, monitoring of recipient immune status is essentially limited to

protocol graft biopsies. Development of molecular diagnostic assays for rejection/tolerance will greatly simplify and facilitate monitoring efforts.

5. The role of donor bone marrow infusions in facilitating tolerance/hyporesponsiveness induction needs to be studied and expanded. Donor bone marrow infusions have induced specifically augmented survival/tolerance to every tissue or graft tested in every species studied since the first demonstration of bone-marrow-induced tolerance in antilymphocyte serum-treated mice [43]. The salutary effect of bone marrow in two studies [31,37] confirms this principle in man. More clinical studies are needed to validate the general use of donor bone marrow as a biological reagent to facilitate tolerance.
6. The guidelines for appropriate clinical tolerance research have been nicely elaborated [42]. Although much is to be gained by studies in experimental animals, a strong case is now made that thoughtful tolerance strategies can be studied in man in most transplantation programs. The clinical transplant community should be inspired to pursue such efforts.

References

1. Cecka JM. The UNOS Renal Transplant Registry pg 1-20 in *Clinical Transplants 2002*. JM Cecka and P Terasaki eds. UCLA Immunogenetics Center, Los Angeles, CA.
2. Kyllonen L, Pukkala E, Eklund B. Cancer incidence in a kidney transplant population. *Transplant Int* 1994; 7 (Suppl 1) 5350
3. Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. *Nature* 1953; 172: 603-606
4. Streilein JW. Neonatal tolerance of H-2 alloantigens: Procuring graft acceptance the old-fashioned way. *Transplantation* 1991; 52:1
5. Hale DA, Gottschalk R, Umemura A, Maki T, Monaco AP. Immunological mechanisms in tolerance produced in mice with non-radiation based lymphoablation and donor specific bone marrow. *Transplantation* 2002; 74:477-484
6. Spitzer TR, Delmonico F, Tolkooff-Rubin N, McAfee S, Sackstein R, Saidman S et al. Combined histocompatibility leukocyte antigen-matched donor bone marrow and renal transplantation for multiple myeloma with end stage renal disease: The induction of allograft tolerance through mixed lymphohematopoietic chimerism. *Transplantation* 1999; 68: 480-484
7. Knechtle SJ and Burlingham WJ. Metastable tolerance in non-human primates and humans. *Transplantation* 2004; 77: 936-939
8. Monaco AP. Antilymphocyte serum, donor bone marrow and tolerance to allografts: The journey is the reward. *Trans Proc* 1999; 31:67-71
9. Calne R, Friend P, Moffatt S, Bradley A, Hale G, Firth J et al. Prope tolerance, perioperative campath 1H, and low-dose cyclosporin monotherapy in renal allograft recipients. *Lancet* 1998; 351:1701-1702
10. Dey B, Sykes M, Spitzer T. Outcomes of recipients of both bone marrow and solid organ transplants: A review. *Medicine* 1998; 77:355-369.
11. Uehling DT, Hussey JL, Weinstein AB, Wank R, Bach FH. Cessa-

- tion of immunosuppression after renal transplantation. *Surgery* 1976; 79:278-282
12. Cai J, Lee J, Jankowska-Gan E et al. Minor H antigen HA-1 specific regulator and effector CD8⁺ T cells and microchimerism in allotolerance. *J Exp Med* 2004 (in press)
 13. Morris PJ and Monaco AP. Editorial Comment: Regulatory T cells finally get the attention they deserve. *Transplantation* 2004; 77 (1 Suppl) S2-3
 14. Starzl TE, Demetris AJ, Murase N, Ildstad S, Ricordi C, Trucco M. Cell migration chimerism, and graft acceptance. *Lancet* 1992; 339:1579-1582.
 15. Starzl TE, Murase N, Demetris AJ, Trucco M, Abu-Elmagd K, Gray EA et al. Lessons of organ-induced tolerance learned from historical clinical experience. *Transplantation* 2004; 77: 926-929.
 16. Starzl TE, Demetris AJ, Trucco M, Murase N, Ricordi C, Ildstad S et al. Cell migration and chimerism after whole organ transplantation: The basis of graft acceptance. *Hepatology* 1993; 17:1127-1152
 17. Starzl TE, Demetris AJ, Murase N, Trucco M, Thomson AW, Rao AS. The lost chord: Microchimerism. *Immunology Today* 1996; 17:577-584
 18. Starzl TE, Zinkernagel R. Antigen localization and migration in immunity and tolerance. *N Engl J Medicine* 1998; 339:1905-1911
 19. Strober S, Slavin S, Gottlieb M, Zan-Bar I, King DP, Hoppe RT et al. Allograft tolerance after total lymphoid irradiation (TLI). *Immunol Rev* 1979; 46:87-112
 20. Modry DL, Strober S, Hoppe R et al. Total lymphoid irradiation models and clinical application in organ transplantation. *Heart Transplantation* 1983; 2:122
 21. Saper V, Chow D, Engleman ED, Hoppe RT, Levin B, Collins G et al. Clinical and immunological studies of cadaveric renal transplant recipients given total irradiation and maintained on low dose prednisone. *Transplantation* 1988; 45: 540-546
 22. Chow D, Saper V, Strober S. Renal transplant patients treated with total lymphoid irradiation show specific unresponsiveness to donor antigens in the mixed lymphocyte reaction (MLR). *J Immunology* 1987; 138: 3746-3750
 23. Strober S, Dhillon M, Schubert M, Holm B, Engleman E, Benike C et al. Acquired immune tolerance to cadaveric renal allografts: A study of three patients treated with total lymphoid irradiation. *N Engl J Med* 1989; 321: 28-33
 24. Strober S, Benike C, Krishnaswamy S, Engleman EG, Grumet FC. Clinical transplantation tolerance twelve years after prospective withdrawal of immunosuppressive drugs: Studies of chimerism and anti-donor reactivity. *Transplantation* 2000; 69:1549-1554
 25. Hayamizu K, Zeng D, Huie P, Garcia-Ojeda ME, Bloch DA, Fong L et al. Donor blood monocytes but not T or B cells facilitate long-term allograft survival after total lymphoid irradiation. *Transplantation* 1998; 66: 585-593
 26. Millan MT, Shizuru JA, Hoffmann P, Dejbakhsh-Jones S, Scandling JD, Grumet FC et al. Mixed chimerism and immunosuppressive drug withdrawal after HLA-mismatched kidney and hematopoietic progenitor transplantation. *Transplantation* 2002; 73(9):1386-1391
 27. Strober S, Lowsky RJ, Shizuru JA, Scandling JD, Millan MT. Approaches to transplantation tolerance in humans. *Transplantation* 2004; 77:932-936
 28. Ildstad ST, Sachs DH. Reconstitution with syngeneic plus allogeneic or xenogeneic bone marrow lead to specific acceptance of allografts or xenografts. *Nature* 1984; 307:168-170
 29. Guzzetta PC, Sundt TM, Suzuki T, Mixon A, Rosengard BR, Sachs DH. Induction of kidney transplant tolerance across MHC barrier by bone marrow transplantation in miniature swine. *Transplantation* 1991; 51:862-866
 30. Kawai T, Cosimi AB, Colvin RB, Powelson J, Eason J, Kozlowski T et al. Mixed allogeneic chimerism and renal allograft tolerance in cynomolgus monkeys. *Transplantation* 1995; 59:256-262
 31. Cosimi AB, Sachs DA. Mixed chimerism and transplantation tolerance. *Transplantation* 2004; 77:943-946
 32. Calne R, Moffatt SD, Friend PJ, Jamieson NV, Bradley JA, Hale G et al. Campath 1H allows low dose cyclosporine monotherapy in 31 cadaveric renal allograft recipients. *Transplantation* 1999; 68:1613-1616
 33. Calne RY. Prope tolerance: The future of organ transplantation—From the laboratory to the clinic. *Transplantation* 2004; 77:930-932
 34. Kirk AD, Hale DA, Mannon RB, Kleiner DE, Hoffmann SC, Kampen RL et al. Results from a human renal allograft tolerance trial evaluating the humanized CD52-specific monoclonal antibody alemtuzumab (Campath 1H). *Transplantation* 2003; 76:120-129
 35. Knechtle SJ, Pirsch JD, H Fechner J Jr, Becker BN, Friedl A, Colvin RB et al. Campath 1H induction plus rapamycin monotherapy for renal transplantation: Results of a pilot study. *Am J Transplant* 2003; 3:722-730
 36. Ciancio G, Miller J, Garcia-Morales RO, Carreno M, Burke GW 3rd, Roth D et al. Six year clinical effect of donor bone marrow infusions in renal transplant patients. *Transplantation* 2001; 71:827-835
 37. Miller J, Mathew JM, Esquenazi V. Toward tolerance to human organ transplants: A few additional corollaries and questions. *Transplantation* 2004; 77:940
 38. Mathew JM, Garcia-Morales R, Fuller L, Rosen A, Ciancio G, Burke GW et al. Donor bone marrow-derived chimeric cells present in renal transplant recipients infused with donor marrow. I. Potent regulators of recipient antidonor immune responses. *Transplantation* 2000; 70: 1675-1682
 39. Maki T, Gottschalk R, Wood ML, Monaco AP. Specific unresponsiveness to skin allografts in ALS-treated, marrow-injected mice. Participation of donor marrow-derived suppressor T cells. *J Immunology* 1981; 127:1433
 40. Starzl TE, Murase N, Abu-Elmagd K, Gray EA, Shapiro R, Eghtesad B et al. Tolerogenic immunosuppression for organ transplantation. *Lancet* 2003; 361: 1502-1510
 41. Monaco AP, Wood ML, Maki T, Madras PN, Sahyoun AI, and Simpson, MA. Attempt to induce unresponsiveness to human renal allografts with antilymphocyte globulin and donor specific bone marrow. *Trans Proc* 1985; 17:1312-1314
 42. Kirk AD. Ethics in the quest for transplant tolerance. *Transplantation* 2004; 77: 947-951
 43. Monaco AP, Wood ML. Studies on heterologous antilymphocyte serum in mice. VII. Optimal cellular antigen for induction of immunologic tolerance with antilymphocyte serum. *Trans Proc* 1970; 2: 489-496