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REVIEW

# Strategies for minimizing immunosuppression in kidney transplantation

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## Summary

Immunosuppression remains the cause of most morbidity following organ transplantation. However, its use is also responsible for the outstanding graft and patient survival rates commonplace in modern transplantation. Thus, the predominant challenge for transplant clinicians is to provide a level of immunosuppression that prevents graft rejection while preserving immunocompetence against environmental pathogens. This review will outline several strategies for minimizing or tailoring the use of immunosuppressive drugs. The arguments for various strategies will be based on clinical trial data rather than animal studies. A distinction will be made between conventional immunosuppressive drug reduction based on over-immunosuppression, and newer induction methods specifically designed to lessen the need for chronic immunosuppression. Based on the available data we suggest that most patients can be transplanted with less immunosuppression than is currently standard.

## Introduction

Kidney allotransplantation is an increasingly successful therapy for chronic renal failure, and is now generally considered the treatment of choice for most causes of end-stage renal disease [1,2]. To prevent allograft rejection, recipients must have their immune systems altered in some lasting way, currently through generalized immunosuppression. Patients therefore trade their renal disease for a chronic, albeit less lethal, condition – that of relative immuno-incompetence [3–5]. The goal for clinicians has thus remained the same since clinical transplantation began: to balance the considerable benefits of organ replacement, with the immunosuppressant-related risks of infection, malignancy and metabolic morbidity. This challenge is complicated by the lack of a reliable means of predicting patients' immunosuppressive needs, and has been met with empiric pharmacological strategies.

The ultimate goal for transplant researchers has been the development of a strategy sustained immune system adaptation to the graft facilitating allograft survival without any immunosuppression, so called immune tolerance. Indeed, an improved understanding of physiological immunity has produced many promising preclinical tolerance strategies [reviewed in 6,7]. Unfortunately, despite the great potential of these approaches, immunosuppression in some form is still required.

Although tolerance remains clinically elusive, one by-product of early clinical tolerance trials has been the recognition that many patients do exceptionally well for long periods of time on very little immunosuppression [8–14]. These same patients, treated with standard modern immunosuppressive regimens would clearly be over immunosuppressed. Empiric immunosuppression is thus less precise in matching immunosuppressive supply and demand than it perhaps could be, peaking the transplant community's interest in minimal immunosuppression strategies [15].

This review will evaluate many approaches aimed toward minimizing the requirements for maintenance immunosuppression. After an overview of the current state of transplant therapy, two general conceptual approaches will be discussed: the use of current medications to prevent over-immunosuppression and the

application of newer concepts to prospectively reduce the immunosuppressive needs of transplant recipients. The first approach will follow the paradigm that an unimpaired immune system will reject an organ – equating immune recognition with an unfavorable outcome. The later will explore a newer viewpoint that immune recognition is a requisite component of both rejection and acceptance. In this paradigm, the context in which immune recognition occurs is as important in determining the response as the antigen recognized, and immunosuppression can be altered by controlling the conditions of recognition.

## Why are we here? The benefits of poly-pharmacy and the risks of reduction

Modern immunosuppressive maintenance therapies have evolved into multi-drug regimens based on several correct assumptions. Firstly, alloimmunity involves multiple interdependent and redundant pathways and effector mechanisms. These include acquired cellular and humoral responses, innate immune elements, and both memory and naïve responses [16–19]. It is clear that any one of these pathways can, in their most vigorous form, mediate graft loss, and thus, some consideration must be given to each. No single drug uniquely inhibits, at tolerable doses, all mediators of alloimmunity.

This leads to a second supposition, that the immune system is better at recognizing and responding to environmental pathogens, so called authentic ligands, than to alloantigens [20]. There is thus a therapeutic window within which rejection can be prevented but protective immunity can be acceptably preserved. Were this not the case, clinical immuno-incompetence would be far more prevalent in transplant patients than is currently the case.

Finally, it is reasonably safe to assume that chronic drug toxicities are, to some extent, dose dependent [21]. This leads one to suppose benefit not only from drug elimination, but also from dose reduction. Similarly, not all of the side effects of immunosuppression are based on their immune effects. Drugs can be chosen for tolerability without necessarily altering their therapeutic effect.

The assimilation of these concepts has led to the general adoption of combination immunosuppression as the standard, and broadly speaking, no immunosuppressive combination has been shown to be obviously superior [22]. This current standard of care is based on the rational and flexible use of many agents by clinicians who are cognizant of the therapeutic and side effects of the available drugs.

The benefits of our current immunosuppressive capabilities must be balanced against several knowledge deficits that leave immune management to empiricism. First, while we recognize that many facets of immunity can mediate rejection, we cannot predict the dominant arm of immunity in a given individual. Although preexisting alloantibodies make humoral rejection likely, we have little, aside from demographics, to predict the likelihood of cellular responses, and essentially nothing to measure potential alloresponsiveness late after transplantation when immunosuppressive weaning typically occurs. As such, we cannot ascertain with any accuracy the appropriate pathway to spare from inhibition.

Similarly, there is no assay for over- or under-immunosuppression that is not dependent on some poor clinical outcome. Also, as alloimmunity is typically heterologous or cross-reactive in origin, a patient's past immune history can impart allospecific memory without actual prior alloantigen exposure [23]. Thus, even genetically identical individuals with identical alloantigen mismatches can have markedly different responses based on different immune histories. Compounding these imperfections is our lack of biologically based measures of drug exposure. Steroids and purine antagonists are not measured in clinical practice, and while we do have levels to follow for calcineurin and mammalian target of rapamycin (mTOR) inhibitors, biologically relevant exposure varies widely between individuals based on intracellular drug concentration, variable rates of metabolism and differences in fat soluble drug distribution for patients of markedly different size [24,25].

The inability to predict immune behavior, and actual drug exposure, has necessitated drug regimen design based on generalizations created to sufficiently inhibit some aspect of many pathways. The use of multiple drugs with proven synergy permits dose reduction and a presumed commensurate reduction in the side effects of each drug. Poly-pharmacy also imparts a modest impunity to noncompliance and probably limits the development of drug resistance. Resistance is not typically considered in this way, but it is clear that mammalian cells can develop resistance to immunosuppressants [26].

The field of drug minimization should thus be entered with the clear understanding that benefits will be offset to some extent by rejections. Clinicians should recognize the need for close patient monitoring and avoid blind reliance on a particular strategy. Furthermore, one should recognize that drug elimination may have unexpected consequences, like changes in drug sensitivity [27] and unmasked noncompliance.

## Why reduce? What to reduce? The principal morbidities of immunosuppression

Most common maintenance regimens consist of a calcineurin inhibitor (CNI), either cyclosporine A (CyA) or

tacrolimus, an anti-proliferative agent, mycophenolate acid, azathioprine, or sirolimus, and corticosteroids [22,28]. Many centers augment peritransplant immunosuppression with anti-lymphocyte antibody induction. These regimens consistently produce excellent short-term survival rates and low acute rejection rates that have translated into improved graft half-lives [2,29]. Which component deserves the most attention for elimination, and which adverse event weighs heaviest in the risk benefit equation? It is apparent that risk-benefit minimization is difficult to individually quantify. Just as the dominant immunosuppressant or arm of the immune system cannot be predicted, neither can risk be ascribed to any single agent. The benefit of drug withdrawal varies with both patient and drug.

Although early acute rejection is easy to measure, it is likely that a single, quickly diagnosed and well-treated acute rejection does not necessarily adversely affect allograft survival or mandate a return to high dose immunosuppression [30]. Instead, late allograft rejection is a more significant problem precipitating chronic allograft nephropathy (CAN) [29–31]. Rejection rates should not be viewed as the sole metric for drug withdrawal success. Rather an aggregate endpoint that considers overall patient outcome is more relevant because the rate of acute rejection will be increased in any reduction scheme.

There are many side effects to consider in combined endpoint analysis (Table 1). The dominant side effect to target varies by patient and the risk of drug withdrawal is similarly variable. Only recently have monotherapy regimens allowed for individual side effects to be analyzed outside the context of a multi-drug regimen. Thus, no single drug should be assumed as the best one to eliminate. Nevertheless, increasing emphasis has been directed toward cardiovascular disease as the cause of most chronic transplant patient mortality. The single most common cause of graft loss in the United States is death with a functioning graft, accounting for 43% of graft losses, of which 36% are attributed to cardiovascular disease and 27% are either infectious or malignant deaths [32]. Kidney recipients clearly have an

**Table 1.** Side effects of approved immunosuppressive agents. A subjective assessment of the toxicities and immunosuppressive effects associated with common immunosuppressants.

| | Pred | Aza | MMF | Siro | CyA | Tacro | OKT3 | RATG | Anti-IL2 |
|---|---|---|---|---|---|---|---|---|---|
| Infectious | | | | | | | | | |
| Viral | + | + | ++ | + | + | ++ | +++ | ++ | + |
| Fungal | ++ | + | + | + | + | + | + | + | + |
| Bacterial | ++ | + | + | + | + | + | + | + | + |
| Malignant | | | | | | | | | |
| Lymphoma/EBV | + | + | ++ | + | ++ | ++ | +++ | ++ | + |
| Skin/HPV | + | ++ | + | + | ++ | + | + | + | + |
| Toxic | | | | | | | | | |
| Cardiovascular | ++ | – | – | + | ++ | ++ | – | – | – |
| Gut/hepatic | ++ | ++ | +++ | + | + | + | – | – | – |
| Neurologic | + | – | – | + | + | ++ | – | – | – |
| Hematologic | | | | | | | | | |
| Anemia | – | + | + | ++ | – | – | – | + | – |
| Leukopenia | – | ++ | ++ | ++ | – | – | + | ++ | – |
| Thrombocytopenia | – | + | + | + | – | – | – | ++ | – |
| Renal | – | – | – | + | +++ | +++ | – | – | – |
| Metabolic | | | | | | | | | |
| Hypertensive | ++ | – | – | + | +++ | + | – | – | – |
| Diabetic | +++ | – | – | – | + | +++ | – | – | – |
| Osteoporotic | +++ | – | – | – | – | – | – | – | – |
| Hyperlipidemic | ++ | – | – | +++ | ++ | + | – | – | – |
| Cosmetic | | | | | | | | | |
| Fat distribution | +++ | – | – | – | – | – | – | – | – |
| Hirsutism | ++ | – | – | – | +++ | – | – | – | – |
| Acne | +++ | – | – | – | ++ | – | – | – | – |
| Cytokine release | – | – | – | – | – | – | +++ | ++ | – |
| Anti-rejection efficacy | $+^{lo}/+++^{hi}$ | + | + | ++ | ++ | ++ | +++ | +++ | + |

The scale is based on the experience of the authors with conventional and monotherapy immunosuppressive protocols. Toxicities are categorized as negligible (–), atypical/not dose limiting (+), typical/dose limiting (++), or typical and requiring active prophylaxis (+++). Immunosuppressive potency is categorized as requiring additional therapy (+), sufficient as a single maintenance agent for appropriately selected/induced patients (++), or reversal agents/stand alone agents limited by immunosuppressive toxicity for chronic use (+++).

increased risk of ischaemic heart disease as predicted by Framingham Heart Study criteria [33] because both their primary disease and subsequent immunosuppression are associated with hypertension, hyperlipidemia, and diabetes mellitus. While it is difficult at times to attribute hypertension solely to immunosuppression, it is clearly associated with decreased graft and patient survival and an increased incidence of CAN [34,35]. In contrast, post-transplant diabetes mellitus (PTDM) is an important complication that is clearly a reversible effect of steroids and CNI. The incidence of PTDM in the US is 24% at 36 months (higher in African and Native Americans), and has been clearly associated with reduced graft and patient survival [36–39].

The two primary immunologic complications of immunosuppression are malignancy and opportunistic infections, accounting for 27% of deaths with a functioning graft. The risk of malignancy in immunosuppressed patients is increased three to five times the normal population predominantly because of skin cancers and post-transplant lymphoproliferative disease (PTLD)/lymphoma [40]. The oncogenic viruses, Epstein–Barr virus (EBV) and human papillomavirus are the primary causes of transplant malignancy [40,41] and thus, malignancy in this setting can also be considered an opportunistic infection of sorts. Aggressive immunosuppression in any combination increases the risk of PTLD [42,43], almost certainly by limiting EBV-specific T-cell function and immune surveillance of EBV-infected cells [44]. Just as allograft rejection can be prevented in many ways, so too can viral rejection.

Despite significant advances in prophylaxis, opportunistic infections remain an important risk [4]. The high prevalence of seropositivity for cytomegalovirus (CMV) and herpes simplex places patients at risk of both primary infection from the donor, and reactivation of latent disease. This is particularly true in the induction phase of immunosuppression [4,45], although viral clearance is impeded by maintenance therapy as well [46]. The most concerning viral infection in recent years has been polyoma virus that occurs in up to 45% of kidney recipients associated with graft loss in as many as 50% of infected patients [47–57]. Just as PTLD is the cause for immunosuppression reduction, we now view polyoma viremia as a biological indicator of over-immunosuppression.

The best drug reduction or elimination strategy is thus predicated on the dominant side effect anticipated in the recipient. CNIs may be best avoided in individuals with a tendency toward type 2 diabetes, steroids in persons with prior steroid psychosis or growing children, purine analogues in persons with chronic marrow suppression, prior myelodysplastic disease or viral skin lesions, and mTOR inhibitors in individuals with difficulty in controlling hyperlipidemia. Economic considerations are also relevant to battle problems of noncompliance.

## Immunosuppression reduction/elimination based on clinical over-immunosuppression

By definition, immunosuppression should be reduced in patients that are over-immunosuppressed. The immune system has evolved to reject environmental pathogens, not organs, and pathogens are, as empirically surmised, more easily rejected than organs. Patients who cannot reject pathogens also cannot, generally speaking, reject their organs. Accordingly, viral replication may be considered a biological surrogate for over-immunosuppression.

With the use of more intense immunosuppressive regimens the potential for opportunistic infection has grown [4]. Prophylactic anti-infective regimens have successfully been employed to counter this risk resulting in reduced CMV, PCP (Pneumocystis carinii pneumonia), and urinary tract infections. In the early post-transplant period, induction therapy of is to some degree necessary and not indicative of inappropriate immunosuppression. Late post-transplant however, the presence of infection is indicative of excessive immunosuppression.

A recent example over the past decade is BK polyomavirus nephropathy, which has emerged as a serious pathogen [47–56] and can be used as an illustrative example of immunosuppressive reduction for cause. Infecting between 10 and 45% of kidney recipients [48,49], BK virus may result in nephropathy in approximately 6% [49]. While it has been speculated that improved disease recognition has contributed to the increased incidence, immunosuppressive potency is the most likely cause [50]. Polyomavirus has a predilection for urogenital epithelium, and the presence of actively replicating virus may be detected simply by urine cytology demonstrating virally infected uroepithelial cells (decoy cells) in the urine. This technique is very sensitive for identifying viral replication in the urinary tract [47]. However, the positive predictive value of this qualitative technique for identifying nephropathy is quite low (<20%) [51]. Several centers have therefore utilized quantitative urine and plasma assays. Viremia is strongly associated with nephropathy, and frequently precedes its development [49,52,53]. In clinical reports using plasma quantitation, reductions in immunosuppression frequently result in decline in the viral copy load [49,52]. This ability to monitor viral load noninvasively offers substantial advantage to the clinical management of infected patients in providing a noninvasive method for determining the appropriate duration of anti-viral treatment and/or guiding immunosuppressive reduction.

We, and others, have adopted management algorithms based on the presence of BK DNA in plasma and urine with specific intent on using BK positivity as an indication for immunosuppressive reduction [54–56]. Emphasis is placed

on prospective assessment of BK infection using PCR based assays. We incrementally reduce immunosuppression, either an anti-metabolite or CNI, if plasma levels become positive, and to a lesser extent based on the intensity of the urine level. Using such a strategy, recipients can be classified in one of three groups: intermittent viruria lasting <3 weeks, sustained viruria without viremia, and progression to viremia [54]. Sustained high level viruria ($\geq 3.0 \times 10^7$ copies/ml) is associated with progression to viremia and viremia rarely exists in the absence of viruria. While long-term follow-up of patients in BK surveillance studies are pending, active surveillance and preemptive actions appear effective in dealing acutely with BK infection and prevention, and in doing so, lead patients to lower immunosuppressive burdens without apparent additional rejection risk. Prospective surveillance in our center has demonstrated detectable viruria in the immediate postoperative period that is managed with immunosuppressant reduction and clearance of viruria, with absence of nephropathy on subsequent protocol biopsies [56]. Thus, preemptive viral monitoring allows the clinician to achieve a more risk neutral immunosuppressive balance.

Similar strategies can be adopted with CMV and PTLD and indeed, immunosuppressive reduction in the face of PTLD has resulted in patients being successfully withdrawn from immunosuppression completely [57]. We currently advocate routine screening for BK, EBV and CMV during the first year post-transplant. Whether tolerant or over immunosuppressed, patients with active viral disease need less immunosuppression and comprise a category of individuals in whom reduction should be considered as a matter of standard therapy.

## Immunosuppression reduction/elimination in the face of clinical stability

The rationale for immunosuppressive reduction in patients who are clinically stable may seem less intuitive than in patients with active viral infection, but the justification for considering this is equally valid. First, even stable patients suffer serious consequences from chronic immunosuppression. Thus, there should be an implied benefit providing graft survival is not worsened. Secondly, it is unlikely that any prescribed regimen is precisely the right amount of immunosuppression for every patient. There is room for error that can perhaps be minimized by judicious drug withdrawal built into every regimen. All agents commonly used in triple drug regimens have been reduced in prospective trials with reasonable degrees of success. However, it should be reiterated that all reduction regimens will be associated with increased acute rejection, and should therefore be linked to heightened clinical monitoring.

### Steroids reduction/elimination

Glucocorticosteroids have been proved to be adjuvant immunosuppressants since the early 1960s [58] and their usefulness in preventing allograft rejection has stood the test of time. Their broad mechanism of action leads to myriad side effects and contributes to morbidity and mortality. However, most attempts to simply eliminate steroids without some compensatory therapy have lead to unacceptable rates of rejection exceeding 30% [59,60]. As immunosuppressive agents with more defined spectra have become available, compensated steroid avoidance has become more easily achieved in even high-risk individuals. Recently reported steroid withdrawal or avoidance using tacrolimus and sirolimus or extended daclizumab are examples of compensatory steroid withdrawal [61–63].

It is important to recognize, however, that many of the chronic effects attributed to steroids, including osteoporosis, are likely better ascribed to chronic immunosuppression in general. There are no clear data demonstrating that graft or patient mortality are improved when steroids are removed but replaced with equally potent maintenance immunosuppression. It is our current contention that most of the beneficial effects of steroids are related to inhibition of antigen presentation. Thus, their avoidance may not mandate continuous replacement, but rather compensation at the time of initial allograft recognition. We thus favor induction strategies over steroid replacement strategies to avoid steroid usage, and view chronic compensatory replacement to be excessive.

### Calcineurin inhibitor avoidance/elimination

The CNI CyA and tacrolimus have dramatically improved renal transplantation. Despite a common mechanism of action and shared relative T-cell specificity, they have unique side-effect profiles (Table 1). Recently reported prospective studies comparing the two agents have revealed comparable graft and patient survival [64–67]. Tacrolimus is a more potent agent and cyclosporine thus has more flexibility for incremental dosing. Tacrolimus has a higher incidence of PTDM and worse neurotoxicity and less cosmetic side effects. Substitution of one CNI for another is an acceptable option for minimizing a dominant side effect in selected recipients [68], but should not be considered immunosuppression reduction *per se*. Both acute and chronic nephrotoxicity are well described with even judicious CNI use, and are the major dose-limiting effects of these drugs [69,70]. Chronic CNI arteriolar vasoconstriction is additive to other factors leading to CAN and has provided the major impetus to reduce or eliminate CNI [71–74].

Several centers have evaluated cyclosporine withdrawal with varied results. A meta-analysis of CNI withdrawal showed an 11% increase in rejection in cyclosporine withdrawal studies but no change in overall survival [75]. CNI withdrawal remote from the time of transplantation is generally well tolerated although the rate of rejection is approximately fivefold higher than persons remaining on triple immunosuppressive therapy. Randomized, controlled studies evaluating CyA withdrawal versus standard therapy have revealed an acceptable rejection rate of approximately 10% versus 2% in nonwithdrawn arms [74,76]. The majority of rejections have been mild and reversible and a significant improvement in renal function has been consistently seen. However, reports of higher rejection rates approaching 25% and more severe rejection scores continue to be reported when early withdrawal is attempted without some compensatory induction [77,78]. Mycophenolate substitution for azathioprine may facilitate CyA withdrawal [79]. Thus, late after transplantation it is possible that as many as 90% of patients are over-immunosuppressed, when considering short-term outcome. The ultimate fate of CNI reduction will rely on long-term follow-up. Even with equivalent outcome, the economic benefit may be considerable.

### The role of sirolimus in immunosuppressive reduction

The approval of sirolimus as a maintenance immunosuppressant has provided another potential option for CNI avoidance – again, compensated drug elimination. Sirolimus binds to FK-binding protein remote from the tacrolimus-binding site inhibiting the mTOR and the signal transduction associated with interleukin-2. This accentuates the tendency of activated T cells to undergo apoptosis [80], and might be beneficial in promoting antigen-specific activation-induced apoptosis, a requirement for peripheral tolerance [81]. This potential effect is likely only applicable in the absence of CNI. The fact that many studies have been performed showing that sirolimus can be combined with CNI to give acceptable results, however is no evidence that combination use is less or more specific immunosuppression [63,82–84]. Based on the studies of cyclosporine withdrawal with mycophenolate, at least two groups have initiated similar protocols with sirolimus. Two multicenter, randomized, controlled studies demonstrated the safety of cyclosporine withdrawal at 3 months followed by concentration-controlled rapamycin levels and also found significantly improved renal function and control of hypertension with worsening hyperlipidemia [85,86]. Sirolimus is specifically approved in the US for achieving CNI withdrawal.

Use of sirolimus from the time of transplantation as a base therapy to replace CNIs is attractive both in terms of reduced nephrotoxicity and potential pro-tolerant adaptation. Improved renal function and blood pressure have been demonstrated in a multicenter European trial replacing CNI with sirolimus [87]. The rejection rate noted in this study may be improved by substituting mycophenolate for azathioprine [88]. A more recent study performed at Cleveland Clinic randomized sirolimus versus cyclosporine with mycophenolate and steroids after basiliximab induction and found excellent graft survival with improved renal function [89]. Thus, sirolimus is a reasonable compensatory strategy for CNI elimination for patients in whom CNI toxicity is burdensome. However, there is no evidence that immunosuppression with sirolimus is better than immunosuppression with CNI when all cause morbidity or mortality is considered.

### Common themes for compensatory immunosuppressant elimination

Several common themes are identifiable in the literature cited above and in the transplant literature in general. The first is that patients clearly need less immunosuppression late after transplantation than they do at the time of transplantation. Although there is no objective way to assess this need, there is general agreement that grafts do indeed 'heal in' over time. Empirically, this seems to take approximately 6 months, as immunosuppressive reduction that takes place prior to this is fraught with higher rates of rejection. Another observation is that no trial has been shown to actually improve patient outcome. Trials that reduce one drug often improve the effects of that drug, but not graft and patient survival. Thus, most trials exploring immunosuppressive reduction by altering maintenance medications have merely taken advantage of the natural reduction in immunosuppressive need that occurs following transplantation, or have provided another means of supplying immunosuppression. Indeed, it is increasingly clear that most patients on triple immunosuppression do not need this for life, and an argument can be made that the general resistance to acknowledge this has been as much economically driven as it has been driven by biology. Furthermore, most trials have not reduced immunosuppression so much as they have eliminated over-immunosuppression – still a laudable and important goal, but not one that is based on altering the biology of alloantigen recognition. Thus, the fundamental causes of allograft rejection should be considered to plot a new course on the road toward true immunosuppressive minimization.

## Considering the context of rejection

If one is to truly reduce immunosuppressive requirements below that which is routinely required, one must

reconsider the fundamental causes of allograft rejection. It is beyond the scope of this review to comprehensively discuss this topic, and indeed this subject and the introduction to 'context-based therapy' has recently been reviewed in depth [90]. However, several general points should be reinforced to construct a conceptual framework around new strategies for maintenance minimization.

Clearly, alloantigens are targeted by the immune system for elimination, and the pathways associated with alloantigen recognition, namely the T-cell receptor and T-cell transcriptional pathways, are ripe targets for inhibiting alloimmunity. However autoantigens can also serve as targets for counter adaptive immunity (as demonstrated by patients with lupus or diabetes) but typically do not. Thus, many factors other than the antigen itself shape the immune posture toward an allograft, and this suggests that pathways other than those associated with T-cell activation should be taken into consideration when determining how much immunosuppression is required. Indeed, the context in which an antigen is recognized and the shear number of cells participating in the event must have considerable influence on the direction of an immune response apart from whether a T-cell can recognize the antigen in question. As such, considering these contextual aspects of immunity may illuminate many methods for reducing immunosuppression.

In this light, we should consider that a human vascularized allograft rejects not only because it is antigenic, but also because it is transplanted into a recipient with a nonphysiologically high antigen specific T-cell frequency – a context of over-crowding [91,92]. Thus, physiological mechanisms for controlling autoimmunity caused by low-prevalence autoreactive cells are perhaps unable to control high-prevalence alloreactive cells. Transplants are also performed in a manner, surgically with ischemic injury, that accentuates chemotaxis to the organ and the activation of tissue-based donor antigen presenting cells (APC) [93,94]. This also increases the prevalence of cells at the site by increasing the efficiency of allospecific T-cell activation and their homing to the allograft, again, likely overwhelming local mechanisms for regulation that have evolved to handle low prevalence cells with auto-reactivity or specificity for a single viral antigen. Similarly, immune responsiveness to the graft strongly skews the immediate specificity of the acquired immune repertoire toward the alloantigen. This is not likely a posture that is maintained with time. As the immune system must subsequently respond to other antigens and do so while maintaining an immune system with a stable size, time and apoptosis eventually reduces the cells available to respond to the graft [reviewed in 95].

Although there can be some debate as to the importance of each of these mechanisms relative to the source of the donor organ, it is clear that all of these processes augment immune responsiveness to some extent. As the discussion is not of tolerance, but rather of immunosuppressive reduction, the absolute contribution is not as important as the fact that each contributes. Thus, attention to these processes can incrementally lessen the magnitude of an alloimmune response, and in doing so, reduce the immunosuppressive requirements. It is important to point out, however, that alloimmune responsiveness is not static. Immunity versus environmental pathogens begets alloimmune cross-reactivity such that states of quiescence can be broken by viral infection and subsequent heterologous immunity [23,96].

The common feature of all of these factors is that they are present at the time of transplantation, and wane with time. Thus, we currently favor aggressive attention to minimizing the pro-inflammatory aspects of the transplant and reducing the initial magnitude of the immune response, rather than relying on sustained immunosuppression. The technique that best addresses this purpose is lymphocyte depletion. This single maneuver reduces the absolute number of cells responding to the graft, prevents antigen recognition and interaction with APC in the secondary lymphoid tissue, and delays the opportunity for T-cell activation. This permits one to immediately use immunosuppression that would typically be inadequate until late after transplantation, making low dose immunosuppression the default.

## Maintenance reduction/elimination strategies using context-based concepts

The natural tendency to require less immunosuppression relates either to a need for time to pass from the transplant procedure or for the injury of the procedure to abate. Induction with depletion antibodies offers the opportunity to satisfy these requirements. Yet, depletion induction has been used for decades without dramatic impact on graft or patient survival [97]. It thus seems that conventional induction does not serve the purposes sought.

For depletion to segregate alloantigen from the context of transplantation it must attenuate not just the peripheral lymphocyte burden (which we view as of minor importance), but also the lymphocytes in the secondary lymphoid tissues [reviewed in 98]. Indeed, it is clear that depletion methods that leave detectible levels of T cells in the periphery do little to alter the nodal architecture. Studies have suggested that depletion can be dosed by following the peripheral T-cell count [99,100], and when used solely as an immunosuppressant along with triple therapy, this is prudent to avoid over-immunosuppression. However, when specifically used to foster

maintenance minimization, the peripheral count should be reduced as low as possible, as in our experience only trace T-cell presence correlates with a reasonable impact on the secondary lymphoid tissues [13].

Many animal studies have clearly shown that depletion is, in and of itself, adequate to induce tolerance [101,102]. However, the use of depleting agents in humans has been limited by concerns for infectious and malignant morbidity. When used clinically, depleting antibody preparations are typically used sparingly and generally do not achieve prolonged peripheral or nodal depletion [97,103]. As such, aggressive T-cell depletion has not until recently been studied in humans [8,10,12–14]. It is now clear, that when used aggressively, maintenance therapy with single agents, either cyclosporine, sirolimus or tacrolimus, is possible in most nonsensitized patients.

A growing number of investigators have demonstrated that lymphocyte depletion with either alemtuzumab or rabbit anti-thymocyte globulin at the time of transplantation greatly reduces the need for maintenance immunosuppression and appears to be relatively well tolerated. This is at first glance inconsistent with the literature showing that depletion induction increases immunosuppressive morbidity [97,104]. Indeed, the use of aggressive T-cell depletion in concert with full maintenance immunosuppression will increase infectious morbidity. However, when employing depletion specifically to lessen the need for immunosuppression, it appears to be acutely safe, and effective to apply these temporary agents aggressively.

We have recently investigated the nature of the postdepletion T-cell repertoire and found that not all T-cell types are equivalently depleted [105]. Indeed, T cells with certain memory phenotypes are relatively resistant to antibody-mediated depletion. This provides one explanation for why depletion in the absence of aggressive maintenance immunosuppression is well tolerated. Memory responses are to some extent preserved, and we have found no evidence that antibody or T-cell responses to environmental pathogens are eliminated by depletion. This also suggests that depletion and maintenance minimization may not be a rational strategy for presensitized individuals, as it is unlikely to erase allospecific memory. In those patients with high panel reactive antibody in whom we have applied alemtuzumab induction, we have not seen a subsequent reduction in the degree of sensitization over time. Indeed, we have seen serious (but reversible) vascular rejection in sensitized patients treated with RATG (rabbit anti-thymocyte globulin) and sirolimus. Here it is important to re-state that testing for alloantibody does not predict cellular sensitization. As alloreactive T cells are, in general, responding through cross-reactivity with environmental pathogens, some patients will have alloreactive memory as a result of prior viral exposure that will not be sufficiently controlled with depletion. This is also likely the cause of rejection in the minority of patients who are not adequately maintained on sirolimus following alemtuzumab induction [12]. In these instances, we find that CNI control memory activation better than mTOR inhibitors and we currently view the combination of depletion and CNI to be the optimal initial combination.

There is likely a role for mTOR inhibitors as they are thought to foster activation-induced cell death and may in fact permit pro-tolerant adaptation over time [81]. We have seen a reduction in direct alloreactivity in patients maintained on monotherapy sirolimus after several years. Thus, a regimen that uses CNI early and switches to mTOR inhibition after 6 months may be a suitable use of both classes of agent. Further investigation along these lines is warranted.

### Altering central immune repertoire development

Depletion induction seems adequate for eliminating the possibility of naïve responses. However, it is likely that, in the absence of central repertoire change, the requirement for immunosuppression will continue. The use of donor antigen, particularly donor bone marrow, has been suggested as a means of skewing the T-cell repertoire toward tolerance. Clinical studies conducted and reported over the past 15 years evaluating the effect of donor bone marrow infusion in recipients of solid organ transplants give cause for guarded optimism.

Early studies concentrated on the administration of donor bone marrow as an adjuvant to conventional immunosuppression. The first major controlled trial demonstrating a modest benefit in cadaveric kidney graft survival and *in vitro* donor-specific hyporesponsiveness was conducted by Barber [106]. Unfortunately, the poorer than expected graft survival observed in the control arm of this study tempered enthusiasm for this approach. Subsequently, investigators at the University of Pittsburgh infused donor bone marrow as an adjunct to a conventional tacrolimus-based regimen [107]. No induction with depleting antibodies was performed. The regimen was applied to recipients of kidney, liver, lung and multiple organ allografts. A significant reduction in the incidence of acute rejection at 6 months was noted. The regimen was also associated with a higher incidence of *in vitro* donor-specific hyporesponsiveness and increased levels of chimerism. In a separate study from the same group, the effect of donor bone marrow infusion on pancreas allograft survival was evaluated [108]. Again these authors documented a reduced incidence of acute rejection

episodes in the first year post-transplant and increased donor chimerism by PCR analysis. Investigators at the University of Miami have also reported on the beneficial effects of the administration of donor bone marrow in conjunction with conventional immunosuppression in recipients of liver and kidney allografts. One-year graft survival was improved and the rate of acute rejection was reduced significantly in liver allograft recipients that received donor bone marrow [109]. Kidney transplant recipients that were infused with donor bone marrow demonstrated a reduced incidence of CAN and improved long-term graft survival [110]. Low but progressively increasing levels of multilineage donor chimerism were demonstrated by a combined *in situ* PCR flow cytometric approach. Interestingly, these chimeric cells demonstrated the ability to suppress anti-donor T-cell proliferation *in vitro* [111].

More recently, emphasis has shifted toward using progressively more aggressive forms of recipient conditioning prior to the infusion of donor bone marrow (or peripherally mobilized stem cells) in order to effect a greater therapeutic response. A report from Stanford described their experience in kidney recipients using total lymphoid irradiation, donor stem cell infusion, lymphocyte depletion, and conventional immunosuppression that was weaned approximately 1 year post-transplant [11]. While a transient chimeric state was achieved and the majority of patients were weaned off of immunosuppression, subsequent rejection was common. Perhaps most promising of all, the group at The Massachusetts General Hospital has successfully adapted their long established protocol for induction of a transient state of mixed chimerism and tolerance into the clinic [112] . They administered human leukocyte antigen identical donor bone marrow to kidney transplant recipients with multiple myeloma and renal failure. The patients were conditioned with a combination of cyclophosphamide, thymic irradiation, lymphocyte depletion, donor lymphoid infusion and a tapered course of immunosuppression. These patients developed transient donor chimerism, resolution of their multiple myeloma and long-term kidney survival without the need for maintenance immunosuppression.

## Conclusion

Immunosuppression remains a necessary therapy for allotransplantation. However, most regimens that have been adopted to optimize first year rejection rates appear overly immunosuppressive late after transplantation. Accordingly, judicious reduction in immunosuppression over time, guided by indications of immunosuppressive toxicity, monitored to detect burgeoning alloimmunity, and cognizant of the needs of the individual patient, is becoming the accepted standard. Depletion-induction protocols may allow for reduced immunosuppressive regimens to be employed from the time of transplantation, but will require longer follow-up to determine their ultimate role in clinical practice.

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