Immunosuppression and Regulation: Cast in New Light?

David M. Rothstein

Department of Internal Medicine, Section of Nephrology, Yale University, New Haven, Connecticut


Research and clinical trials aimed at prolonging allograft survival have historically been viewed as promoting either immunosuppression or tolerance. However, new studies reveal that some immunosuppressive agents may also promote tolerance through enhanced generation of regulatory T cells (Treg). These findings lead to new ways of thinking about these agents and how they might be combined to promote tolerance while suppressing the immune response. This requires a new view of tolerance and immunosuppression as a continuum rather than a dichotomy.

The outcome of kidney transplantation continues to improve through judicious use of potent drugs that prevent rejection by “nonspecifically” suppressing the immune system while trying to avoid deleterious consequences of overimmunosuppression. In contrast, research, including pilot clinical trials, continues to focus on achieving immunologic tolerance, where the immune system is rendered specifically unresponsive to the allograft in the absence of ongoing therapy. Tolerance promises to reduce rejection and improve long-term outcome, while avoiding the dangers of chronic immunosuppression. Conventional wisdom holds that tolerance has only been achieved in rodents and that standard immunosuppressive agents may actually hinder its development. However, a truth lies between these extremes. First, tolerance may be viewed as a balance rather than “all or none” and intermediate levels are still clinically useful. Whatever the mechanisms, most allograft recipients require much less immunosuppression after six months than they did initially. Patients whose immune systems lean toward tolerance may suffer fewer rejections and require less maintenance immunosuppression. Second, some patients, albeit a minority, on standard therapy do actually develop tolerance. If we could only identify these patients, immunosuppression could be stopped or tapered with anticipation of a favorable outcome rather than by trial and error. Indeed, development of assays to identify tolerant recipients are the subject of a review on immunological monitoring by Najafian et al. in this issue of JASN (1). Also in this issue, Lopez et al., and other reports noted below, suggest that certain standard immunosuppressive agents may directly promote tolerance through generation of regulatory T cells (Tregs) (2). This challenges previous thinking about immunosuppression and tolerance, and provides new insight into Treg biology.

Tolerance capitalizes on the immune system’s endogenous regulatory mechanisms. Key among these are CD4+ Treg cells. Five to ten percent of CD4 cells emerging from the thymus constitutively express CD25 (IL-2R α-chain) and other activation markers (3). Depletion of these cells, dubbed natural Treg cells (nTregs), precipitates systemic autoimmunity in mice. Unlike CD25 and other markers, the Foxp3 transcription factor is not expressed by activated murine effector T cells, and is specific for nTregs. Indeed, humans and mice deficient in Foxp3, lack nTregs and develop severe systemic autoimmunity. nTregs normally respond to antigenic challenge in vivo and dampen the effector response. While other regulatory populations (both expressing and lacking Foxp3) may be induced in the periphery under certain conditions, the details of this induction and the role of induced versus nTregs remains unclear (Figure 1). In experimental models, Tregs can prevent autoimmunity, prevent graft-versus-host disease (GVHD), and play an important role in both the induction and maintenance of allograft tolerance (3–5). Given their important role in immune homeostasis, there is great interest in trying to manipulate Tregs for therapeutic advantage.

While nTregs are normally present even in transplant recipients on conventional immunosuppressives, there is hope that boosting levels of such cells may promote tolerance. In humans, CD25+ Tregs have been shown to be increased in transplant patients with stable graft function (6,7). Moreover, a recent study notes that although Foxp3 mRNA increases in urine of patients undergoing acute renal allograft rejection, patients with the highest increases were most responsive to treatment (8). Based on such findings, several groups are attempting ex vivo expansion of pre-existent nTregs followed by reinfusion, for clinical trials in prevention/treatment of GVHD and type 1 diabetes (5). However, cell culture approaches present a significant challenge and it is important to identify agents that can boost the number of endogenous Tregs and/or induce conversion of nonregulatory T cells into Tregs in vivo. Conversion is particularly attractive because it targets 90 to 95% (rather than 5 to 10%) of the CD4 population (Figure 1).

Enter the study of Lopez et al., which shows that rabbit antithymocyte globulin (ATG), widely believed to immunosuppress through T cell depletion, can induce a five-fold increase in CD4 cells expressing CD25 and Foxp3 after just 24 h in vitro. Although the number of CD25–CD4 cells decreased after ATG, this was accompanied by a similar increase in the number...
of CD25+ CD4 cells, suggesting conversion rather than selective depletion of CD25− cells. In agreement, the multi-fold increase cannot be explained by proliferation of pre-existing CD25 cells given the short time-frame, and indeed 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE) labeling revealed only 16% of CD25+ cells had proliferated. Finally, incubation of CD25− Foxp3− cells with ATG still results in a five-fold increase in CD25+ and Foxp3+ cells, which in 24 h can only be explained by phenotypic conversion.

What does this finding mean from a biologic and clinical perspective? First, it shows that agents widely used to prevent transplant rejection may not merely immunosuppress, but may actively promote engraftment through the induction of Tregs. In this regard, rapamycin appears to preferentially inhibit growth of T effector cells stimulated in vitro, favoring the outgrowth of Foxp3+ Tregs (9). This property is already being utilized to enhance the purity of Tregs grown ex vivo in the studies noted above. Moreover, rapamycin can also promote growth of endogenous Tregs over T effectors in vivo, limiting autoimmune diabetes (10). However, this is not a property shared by all immunosuppressive agents, as calcineurin inhibitors (CNI) inhibit Treg expansion and regulatory activity (11). Moreover, liver transplant patients with stable graft function who were withdrawn from immunosuppression exhibited an increased percentage of Tregs (7). This is not to say that patients on CNI do not have Tregs (6), only that the balance may be shifted because there are fewer of them and they are less active.

What distinguishes ATG from other agents (e.g., IL-10 or TGF-β) is its ability to rapidly convert Foxp3− cells into Foxp3+ cells in the absence of T cell proliferation. It should be noted that unlike mice, where Foxp3 expression is highly restricted, Foxp3 may be induced in human Foxp3− cells after normal activation. Nonetheless, such cells also appear to exhibit Treg function (12). Thus, although Foxp3 expression is less specific for Tregs per se, in humans, it still identifies cells with Treg activity. This is supported by the Lopez study, where suppressor function was seen in treated but not untreated cells, although the actual phenotype of cells with Treg activity was not defined. In this regard, Foxp3 and CD25 expression after ATG increased proportionally, and most data shows that only 20 to 25% of CD25+ cells also express Foxp3. Unfortunately, Foxp3 expression per se cannot be monitored in live human T cells. However, Liu et al. recently showed that IL-7 receptor (CD127) and Foxp3 expression are inversely correlated on human Tregs (13). Thus, ATG might be used to directly expand Tregs ex vivo. While Foxp3+ Tregs still comprise a minority of the CD25+ population, they might be enriched in the CD127− subpopulation. Alternatively, incubation with ATG might be used to significantly augment the precursor frequency of CD25+ CD127− Tregs before further ex vivo expansion through T cell receptor stimulation, as noted above.

What implications does the article by Lopez et al. have for use of ATG in vivo? Optimal concentrations of ATG for in vitro generation of Treg appear to occur at significantly lower levels than those achieved in serum after treatment in vivo. However, antibody levels in secondary lymphoid tissues may be significantly lower, so Treg generation might occur in these relevant locations. Moreover, the relative resistance of Tregs to apoptosis may promote tolerance through preferential depletion of T effectors. Indeed this strategy has been used by others to gen-

Figure 1. Regulatory CD4 cell populations. Natural regulatory T cells (NTreg) develop in the thymus and specifically express Foxp3 in addition to T cell activation markers, such as CD25, CTLA-4, and GITR. NTreg can expand by proliferating in the periphery. Although Foxp3− CD4 cells primarily give rise to peripheral effector cells, under certain circumstances (e.g., activation under the influence of IL-10 or TGF-β) they can develop into cells with Treg activity. Some of these (referred to here as induced NTregs) express Foxp3, while others (referred to here as adaptive Tregs) remain Foxp3-negative. The distinctions between these three Treg populations are currently under investigation. Rabbit antithymocyte globulin (ATG) appears to augment proliferation of CD25+ Foxp3+ NTregs as well as generate induced NTregs.
erate tolerance and appears operative in mice receiving antilymphocyte globulin (14,15). Clearly, in vivo studies optimizing doses for Treg generation are needed. Moreover, the above studies suggest therapeutic enhancement of Tregs might best be achieved by combining ATG with rapamycin while avoiding CNI. Whether this will be sufficient to keep the T effector response at bay after withdrawal of ATG is a serious concern. Regardless, Lopez et al. and the other studies noted above indicate that immunosuppressive drugs can have tolerogenic roles mediated by generation of Tregs, and we still have much to learn about using these drugs in this new context.

In summary, the findings of Lopez et al. suggest that ATG will soon be examined as adjunctive therapy for tolerance by enhancing expansion of Tregs ex vivo and in vivo. Moreover, identification of the surface molecules targeted by polyclonal ATG preparations that actually mediate conversion of nonregulatory into regulatory T cells will provide new insight into Treg biology and allow development of therapeutic agents that are more potent and specific in generating Tregs. Such agents would have important impact in transplantation as well as in the treatment of autoimmune disease and glomerulonephritis (5,16). Ultimately, new drugs that effectively control T effectors will be combined with those that efficiently enhance Tregs to promote tolerance, and the differences between tolerogenic and immunosuppressive agents will be viewed more as a spectrum rather than as black or white.

References
