Regulatory T Cells in Kidney Transplant Recipients: Active Players but to What Extent?

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CD4⁺CD25⁺ T cells are now well established as naturally occurring immune regulatory T cells. Resurrected from the suppressor T cell adversity, Sakaguchi et al. (1) showed in mid-1990 that a subset of CD4⁺ T cells expressing IL-2 receptor α chain in naïve animals had the ability to control autoreactive T cells in vivo. These T cells were not only hyporesponsive to antigenic stimulation but also suppressive to other otherwise responsive T cells via cell-cell contact mechanism. Although originally identified in the mouse system, it is now clear that a T cell population with the same phenotypic and functional properties does exist in humans (2). These T cells are crucial in preventing various forms of autoimmune diseases, which makes them a key regulatory element in maintaining self-tolerance. This raises an obvious question regarding the role regulatory T cells may play in allograft tolerance. Although still an elusive goal in clinical transplantation, immunologic tolerance against MHC-incompatible organ allografts is now readily inducible in a number of murine and some primate transplant models (3,4). In addition, few human studies have reported rejection-free states in the absence of immunosuppression (5–7). Importantly, immunologic tolerance does not mean complete unresponsiveness toward the graft, but rather a lack of destructive immune response against it in the face of generalized immune competence (8). Multiple tolerogenic mechanisms, including depletion, anergy, and immune regulation operate in different forms or combinations depending on the animal model and immunosuppression. The role of immune regulation is of particular interest and under intensive study nowadays. Different from the case of autoimmune diseases, regulatory T cells involved in transplantation tolerance are not limited to the CD4⁺CD25⁺ subset. With varied efficacies, CD4⁺CD25⁺, CD8⁺CD28⁻, NK, or DN T cells have been shown to exert regulatory functions in allografted hosts (9). Two distinctive and dynamic stages can be envisioned during the acquisition of tolerance in adult transplant recipients: the induction and maintenance phases. The immune regulation is the only active mechanism capable to control coexisting alloreactive T cells (i.e., those that escaped deletion or are continuously produced in the thymus) in immunocompetent recipients. The role of distinct types of regulatory T cells, or CD4⁺CD25⁺ T cells may well be different in those two stages. Adoptive transfer experiments, so often used to address the role of CD25⁺ T cells in murine models, do not mimic the real physiologic situation in reference to the immunologic environment (e.g., ratios of effector:regulatory T cells), and their results primarily reflect the function of regulatory T cells in the induction phase. Indeed, no reliable models to probe the role of regulatory T cells in the maintenance of tolerance have been established.

The articles by Salama et al. (10) and Game et al. (11) in this issue of JASN pioneer the clinical effort to study the role and the relevance of well-established regulatory CD4⁺CD25⁺ T cell subset in the regulation of alloimmune responses during the maintenance of renal transplants. Unlike in other human studies in which cells from healthy volunteers were employed, they sampled blood lymphocytes from transplant recipients with defined clinical diagnosis of graft function, and focused on allore cognition pathways relevant for regulatory T cells. This is the first clinical report on the putative role of CD4⁺CD25⁺ T cells in maintaining hyporesponsiveness of recipients’ own T cell repertoire toward the donor-type alloantigen, and on the mechanism of their activation in long-term and stable transplant patients. Both groups used similar ex vivo assays to contrast alloreactivity before and after depletion of the CD4⁺CD25⁺ subset. Complementary to each other, they measured either direct or indirect alloresponses. Their conclusions imply the important role of indirect rather than direct pathway in alloreactive regulatory T cell function.

Salama et al. (10) report on twenty-three renal transplant patients, grouped into two cohorts with or without the history of acute rejection. These patients were chosen on the basis of their low reactivity to the mismatched donor-derived HLA-DR antigen. By employing ELISPOT assay, the authors were able to detect significant increase in the frequency of IFN-γ-producing cells stimulated by donor-derived mismatched HLA-DR peptides, after depletion of the CD25⁺ subset. This increase was alloantigen-specific, as the response to recall mumps antigen was unaffected by CD25 depletion. Significantly, this frequency increase was associated with the history of graft rejection, and the initial status of alloresponses toward the mismatched alloantigen in vitro. In contrary to this “positive” finding, Game et al. (11) in their CD25 depletion experiments failed to detect any changes in the direct alloreactivity specific to donor-type alloantigens. By screening twelve stable
renal transplant patients, the authors measured the effects of CD4+CD25+ T cell depletion on alloresponses in the direct pathway (i.e., MLR) by LDA, as well as by ELISPOT for IFN-γ. The patients shared one HLA-DR with organ donors. The third-party controls were chosen on the basis of the sharing of one DR allele with the recipient but expressing mismatched allele different from that of donor. In 11 of 12 cases, no significant increases were detected in the frequency of donor-specific T cells after depletion of the CD25+ subset. In one case, the increase occurred in both donor- and third-party-reactive T cells. Thus, they concluded that CD4+CD25+ T cells are not the major regulators responsible for donor-specific direct T cell hyporesponsiveness. This conclusion is supportive of their previous experimental results showing anergy as one of the mechanisms of hyporesponsiveness of anti-donor T cells in the direct pathway (12).

Collectively, these results suggest that CD4+CD25+ T cells do regulate anti-donor T cell alloreactivity in the indirect pathway. However, to fully appreciate these experimental data, we need to consider the key factors working selectively in the ex vivo setting. To detect cell-cell regulation, both cell populations need to be activated. Indeed, it is plausible that they may recognize different antigens or the same antigen but in different forms. Second, since quantitative changes were measured, and the relative quantities of these two interacting cell populations are critical for the outcome of potential regulation. These two factors are most difficult to control and to properly assess results from any ex vivo experiment designed to replicate the in vivo scenario. Another issue that needs to be considered while using peripheral blood lymphocytes, is a possible accumulation of putative regulatory T cells at the graft site, as demonstrated in a number of murine studies, including our own (13,14). As to the antigen-specificity, this may be even more confounded ex vivo, considering the well-described phenomenon of regulatory T cells linked suppression (15,16).

Regardless of the conclusions from these two important articles, we may consider what will be necessary for regulatory T cells to achieve clinical tolerance. One consensus assay for specific tolerance induction in animal models is the acceptance of a new donor-type but rejection of a third-party test graft. As shown by different groups, regulatory T cells can indeed act as the major regulators in preventing the rejection of secondary donor-type grafts (17–19). Obviously, alloreactive T cells in the direct pathway, which may not be depleted completely in tolerant recipients, are subject to such a regulation for tolerance maintenance. It is impossible to predict how the criterion of a secondary “test graft” could translate with regard to longevity of human transplants. The issue of allore cognition during immune regulation consists of two aspects. The first question relates to the pathway for regulatory T cells to be activated, and the second focuses on the pathway for the effector T cells to be regulated. Both articles actually address the latter aspect. A more defined assay distinctive of the two aspects, possibly first developed in experimental animals, is required to fully address this complex issue.

There is no doubt that ever-increasing shortage of donor organs means that every transplant should provide life-long function replacement. Hence, achieving a “true” tolerance, i.e., long-term, drug-free graft survival with normal function is of utmost importance. Accurate clinical assays to examine immune regulation and to assess the acquisition or eventual breakdown of the tolerant state over time are urgently needed. This is another reason why the reports of Salama et al. and Game et al. are of such an interest. In addition to measuring direct and indirect T cell alloreactivity, new promising candidates for a clinical tolerance assay to examine immune regulation are emerging (20). These include peripheral and/or intragraft profiling of lymphocyte activation markers, screening of humoral immune responses, as well as gene chip microarrays and proteomics defining tolerance genes/proteins. However, a widespread clinical validation of these assays is still lacking. It remains to be seen as to whether or not these assays will be used to detect “tolerant” patients, and thus allow prospectively withdrawing or minimizing immunosuppression under aggressive immune monitoring, and thus guiding us in designing future tolerance studies.

References