Editorial

Measuring T Cell Alloreactivity to Predict Kidney Transplant Outcomes: Are We There Yet?

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Assessment of pretransplant immunological risk is a critical part of improving renal transplant outcome. By carefully determining pretransplant immunological risk assessment, it may be possible to optimize individual therapy decisions related to immunosuppression regimens, thereby improving transplant outcomes. Improving transplant outcomes would lessen the need for retransplantation, which in turn would have a positive impact on the shortage of donor organs. Furthermore, pretransplant immunological risk assessment could potentially help the transplant community make more informed decisions related to organ allocation, and that may also improve overall outcomes and alleviate organ shortages. Currently, pretransplant tests to assess transplant rejection and graft loss risk include HLA matching between donor and recipient and routine screening for panel reactive antibodies (PRA) in the recipient. At the time of transplant, a final cross-match is preformed to assess antidonor antibodies and to make a final decision on acceptance of the donor; a positive cross-match being a contraindication to transplantation between the recipient and donor pair.

It is well accepted that HLA matching has a significant benefit in predicting transplant outcomes (1,2), but besides sharing of six antigen-matched kidneys, HLA matching is not currently used to make decisions on organ allocation because of the notion that current immunosuppression obviates the benefits of matching (3). Furthermore, it has been known for some time that the presence of a high PRA predicts worse outcome (4–6). However, PRA testing focuses on humoral immunity. T cell–mediated antidonor immunity is not routinely assessed pretransplantation. T cells are central mediators of transplant rejection (7,8). T cells have the ability to directly cause graft damage through a variety of mechanisms and play an important role in providing help to B cells and the generation of the humoral immune response to donor antigens. An important issue is whether pre-existing alloreactive antibodies in the host can predict cellular alloreactivity and *vice versa*. Given the central role played by T cells in transplant rejection, it may be

critical to develop methods to assess T cell alloreactivity pretransplantation to improve accuracy in assessing risk pretransplantation.

How does one measure T cell alloreactivity *in vitro*? For pretransplantation risk assessment, assays of T cell reactivity must be reliable, rapid, reproducible, quantitative, and able to be performed using limited patient material. Classically, T cell alloreactivity has been measured *in vitro* using ^{3}H-thymidine incorporation to assess T cell proliferation in the mixed lymphocyte reaction (MLR). T cell alloreactivity has also been measured by analyzing the ability of T cells to kill allogenic targets in cytotoxic T lymphocyte (CTL) assays. MLR assays are of limited usefulness because they primarily measure CD4 T cell proliferation in the case of HLA class II disparities between host and donor, and are highly variable. In the MLR, naive CD4 T cell responses mediated *via* the direct pathway (recognition of HLA antigens on allogenic antigen–presenting cells) are most pronounced. CTL assays typically assess CD8 T cell–mediated killing, also predominantly through the direct pathway. Measurement of indirect responses to donor HLA peptides presented by responder or recipient antigen–presenting cells typically requires prior sensitization *in vivo*. Therefore, neither assay (MLR or CTL) give a comprehensive picture of alloreactivity (both direct and indirect). Furthermore, neither assay can measure the frequency of alloreactive T cells unless performed in limiting dilution, which often requires restimulation *in vitro*, which can further skew actual *in vivo* frequencies. More recently, enzyme-linked immunosorbent spot (ELISPOT) assays have been developed for use in assessing alloreactivity based on cytokine production (9). ELISPOT assays are able to assess the frequency of cytokine-producing T cells directly *ex vivo*, can measure alloreactivity mediated through the direct and indirect pathways, and can define the phenotype of the responding T cells (Th1/Th2) (10–12). Most recently, the use of multiplexing assays based on technology such as Luminex (Luminex Corp., Austin, TX) have made it possible to simultaneously detect production of up to 100 cytokines using a small number of T cells in culture (13).

In this issue of *JASN*, two groups report the development of a relatively simple method to assess, pretransplant, recipient T cell alloreactivity in a manner analogous to PRA assessment (14,15). The authors in both papers assessed T cell alloreactivity to a panel of allogenic stimulators from healthy volunteers by

analyzing IFN-γ production *in vitro* using cytokine ELISPOT assays to determine the frequency of alloreactive T cells in blood, thereby establishing a panel-reactive T cell (PRT) assay. In each paper, the response of memory or primed T cells was analyzed, which may be an important part of risk assessment because it is generally accepted that such T cells are most difficult to control with immunosuppression or tolerance protocols (16,17). Cytokine ELISPOT assays are ideal for this application because they are sensitive, quantitative, can be preformed with relatively little patient material, and are noninvasive. T cell responses were defined as the percentage of stimulators that stimulated IFN-γ production. Although the number of patients analyzed in these pilot studies was small, the results suggest that assessment of T cell alloreactivity based on PRT may be of significant value for risk stratification in transplant candidates. In both studies, patients exhibited significant variability in PRT-reactivity. Some patients exhibited reactivity to a broad range of stimulators, whereas others showed a relative paucity of reactivity. Based on an analysis of 41 hemodialysis patients and 10 healthy volunteers, Andree *et al.* observed that hemodialysis patients had higher PRT values when compared with controls. Based on analysis of 41 hemodialysis patients, Poggio *et al.* observed that patients who showed relatively high PRT values (PRT-75, defined by a positive response [>25 spots/300,000 peripheral blood leukocyte] to 40 to 75% of the stimulators) tended to be younger and African-American, and that a positive PRA was more prevalent among females but had no correlation with race or age. Perhaps most interesting was the observation in both studies that T cell alloreactivity based on PRT did not routinely predict humoral sensitization based on PRA and *vice versa*. Poggio *et al.* showed that 12% of their cohort was PRT-75$^+$/PRA$^-$, whereas only 5% were PRA$^+$/PRT-75$^+$. Andree *et al.* showed that 5 of 10 PRT$^+$ patients were PRA$^-$ and only 4 of 10 PRA$^+$ patients were PRT$^+$. A preliminary posttransplant outcome correlation performed by Poggio *et al.* in seven patients showed that, of the two patients who were PRT-75$^+$, one showed histologic evidence of acute rejection and remained PRA$^-$. These results imply that humoral and cellular sensitization can be independent of each other. Importantly, the observation that a relatively high proportion of PRA$^-$ patients are PRT$^+$ suggests that PRA assessment alone is not an accurate measure of alloreactivity.

Because of the limited size of the studies preformed and the lack of follow-up study, it is not yet possible to conclude that pretransplant assessment of PRT reactivity will lead to improvements in transplant outcome. However, based on the observation that PRA does not always predict T cell reactivity and *vice versa*, it seems reasonable that the use of PRT to assess pretransplant risk could aid in risk stratification for transplant candidates. Although PRT results, like PRA, do not necessarily reflect specific antidonor responses, it may be important to assess how overall alloreactivity affects outcome, and to address pre-existing T cell alloreactivity by using individualized immunosuppression strategies. The studies presented, though in early stages, appear promising and clearly highlight the importance of developing pretransplant risk assessment assays

that take into account cellular and humoral immunity. By assessing both, it may be possible to tailor immunosuppression regimens for individual transplant recipients, optimize organ allocation, and improve outcomes. Well-designed prospective studies are needed to answer these important questions and related ones on the effect of induction antibody therapy and maintenance immunosuppressive drugs.

There are several related but important questions to the points above. Does PRT reactivity have a place in transplantation other than assessment of pretransplant risk? Can similar assays be developed to monitor a patient after transplantation to preempt organ damage by adjusting immunosuppression? Could similar assays be used to monitor immunosuppression minimization or withdrawal, such as in pilot tolerance trials (18)? Though the answers to these questions are obviously not presently known, the development of sensitive and specific assays that give a broad picture of T cell (both direct and indirect) and humoral alloreactivity before and after transplantation could have a significant impact in transplantation medicine and patient care. The studies by Poggio *et al.* and Andree *et al.* remind us of the current shortcomings in pretransplant risk assessment and underscore the importance of research in the area. The use of cytokine ELISPOT assays to detect T cell alloreactivity pretransplantation based on IFN-γ production is only the beginning. More refinement and development of assays to measure direct and indirect alloreactivity in the CD4 and CD8 T cells subsets, as well as production of a larger array of cytokines could provide an additional layer of risk stratification. Such assays may make it possible to assess the role of the direct and indirect response in the pathogenesis of rejection processes and may help in developing better and earlier management strategies to prevent graft destruction (19). Furthermore, such refinements may make it possible to predict graft rejection and tolerance, individualize therapies, and improve long-term outcome. Though we are not there yet, the exciting journey has started already!

Acknowledgment

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