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Review

Biomarkers of operational tolerance following kidney transplantation – The immune tolerance network studies of spontaneously tolerant kidney transplant recipients

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ABSTRACT

Studies of kidney transplant recipients who have developed spontaneous and sustained tolerance have revealed an association with B cells. Unexpectedly tolerant individuals are characterized by increased numbers and frequencies of B cells in the blood and increased expression of genes associated with B cells in the blood and urine. Comparisons of the B cell repertoires of tolerant individuals and those receiving immunosuppression reveal that not only are the B cells more numerous but developmental differences result in a repertoire comprised of more naïve and transitional B cells in the tolerant cohort. B cells isolated from tolerant individuals also display functional differences compared to those from individuals receiving immunosuppression. Many of these differences may serve to suppress alloimmunity. Lastly a significant number of transplant recipients receiving standard immunosuppression display B cell-biased patterns of gene expression predictive of tolerance or a pro-tolerogenic state. Interestingly, this pattern is associated with improved renal allograft function. While recent studies have raised the concern that immunosuppressive drugs heavily influence B cell-based "signatures of tolerance", a substantial body of work suggests that differences in B cells may be a useful tool for identifying tolerant kidney transplant recipients or guiding their immunosuppressive management.

1. Introduction

Spontaneous tolerance following kidney transplantation in humans, as opposed to tolerance intentionally induced by a specific treatment regimen, is not a newly observed phenomenon. As early as 1975 a small series of patients who had stopped immunosuppression and not acutely rejected was reported [1]. Although two of the six patients ultimately experienced acute rejection, the authors concluded that once immunosuppression was stopped, unless rejection occurred it was not necessary to resume immunosuppressive therapy. However, a subsequent report of a larger number kidney transplant recipients displaying spontaneous tolerance emphasized the high frequency of acute rejection and subsequent graft loss and urged resumption of immunosuppression with the possible exception of those who had maintained stable function for greater than three years after stopping all immunosuppression [2]. Concerns about the wisdom of pursuing tolerance to transplanted kidneys are far from resolved. Even recently authorities in the field of kidney transplantation have voiced new concerns about the safety and long-term outcomes of complete immunosuppressive drug withdrawal in kidney transplant recipients [3]. These reports highlight the fact that tolerance in the clinical setting as opposed to the laboratory is considered to be operational. Operational tolerance is defined as the persistence of normal function in the absence of immunosuppression. The study of tolerance is hampered by the absence of validated assays or biomarkers capable of confirming the existence of robust donor-specific unresponsiveness. Furthermore, there are currently no biomarkers capable of determining how robust or long lasting a state of operational tolerance may be. This absence of validated biomarkers of tolerance is a significant barrier to the study of tolerance in the clinic, the immunosuppressive management of patients receiving little or no immunosuppression, and the weaning of immunosuppression. Two recent reports describing studies attempting to wean calcineurin inhibitors from patients predicted to be at a low risk of rejection on the basis of clinical characteristics (absence of DSA, stable graft function, biopsies without evidence of inflammation) demonstrate the challenges of immunosuppressive drug minimization in stable kidney transplant recipients. Both studies were stopped prematurely due to high rates of rejection and/or the formation of DSA following attempted drug withdrawal [4,5].

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2. Goals, design, and limitations of the ITN studies of spontaneously tolerant kidney transplant recipients

Unlike studies of tolerance following liver transplantation where the rates of operational tolerance are significantly higher than kidney [6] and the long-term consequences of rejection following immunosuppressive drug reduction or withdrawal limited with the prompt diagnosis and reintroduction of more intensive immunosuppression [7], it is generally thought that spontaneous tolerance following kidney transplantation is a rare event and that episodes of rejection associated with drug withdrawal likely to compromise long-term graft function and survival. Thus in the absence of validated biomarkers of operational tolerance most in the field believe it is unsafe to intentionally withdraw immunosuppression unless prompted by a clinical indication. Realizing that there were rare patients who had ceased all immunosuppression and continued to display stable, good function of the transplanted kidney and had thus already assumed the risk of drug withdrawal of their own volition we chose a study design that sought to identify kidney transplant recipients who had previously stopped all immunosuppression. Identified patients who agreed to participate provided demographic and clinical data as well as biological samples for mechanistic assays. When feasible, almost exclusively in the setting of living donor kidney transplantation, efforts were made to also obtain donor cells for additional mechanistic assays. Following enrollment subjects underwent testing to assess renal function (serum creatinine and calculation of eGFR), allograft injury (proteinuria and allograft biopsy), alloimmunity (cellular assays of immunity and screening for DSA), and more general studies to determine the phenotype of peripheral blood cells by flow cytometry as well as gene expression profiles of peripheral blood cells (gene array and QT-PCR) and shed urinary epithelial cells (QT-PCR). Data and biological samples were obtained from several additional cohorts for the purpose of comparison.

At the outset it should be emphasized that several elements of the study design created perceived or actual limitations in with respect to the studies' conclusions. The first potential limitation arises from the absence of a true control group. Unlike studies of tolerance performed in the laboratory where it is possible to design a control group that mimics the experimental group in all meaningful variables aside from the therapy used to induce tolerance or the tolerant state itself, this is not feasible in the clinical setting. The importance of the comparison group chosen is illustrated by the findings of Brouard et al. [8]. In this group's seminal study of gene expression profiles in spontaneously tolerant kidney transplant recipients they chose to use subjects with chronic rejection, which they defined as immune-mediated kidney allograft failure with return to dialysis and cessation of immunosuppression as their primary control group. This choice likely contributes to differences between many of the findings in this study and subsequent studies by this or other groups where the primary comparison of tolerant subjects was to those with stable renal allograft function receiving conventional immunosuppression. In designing the ITN study protocol several comparison groups were considered. Indeed numerous cohorts that could be considered as an appropriate comparison for one or more variables were enrolled including subjects with stable function while receiving conventional immunosuppression, subjects receiving conventional immunosuppression who on the basis of clinical features and biopsy findings were determined to have alloimmune-mediated graft injury, patients with stable function while receiving corticosteroid monotherapy, recipients of kidneys from an identical twin donor, and healthy volunteers.

We chose as our primary comparison group kidney transplant recipients receiving conventional immunosuppression who had good and stable graft function. This choice was based on our goal of identifying a signature of tolerance to transplanted kidneys that could be used as a tool to facilitate the safer minimization or complete withdrawal of immunosuppression in the clinical setting. We reasoned that patients already experiencing significant graft dysfunction or those with significant infectious or neoplastic conditions would not likely be candidates of protocol guided management of immunosuppression but would be managed based on other more pressing clinical considerations. However, our choice of comparing tolerant patients to those receiving ongoing immunosuppression raised the very real concern that we may be measuring a signature of the absence of immunosuppression. Consistent with this concern two groups [9,10] as well as our own data to be discussed later demonstrate that the choice of immunosuppressive agents can influence the prevalence of B cells, a factor associated with spontaneous tolerance to transplanted kidneys in numerous studies. While the impact of immunosuppressive drugs on the prevalence of a tolerance signature derived from the comparison of tolerant patients to those receiving any immunosuppression remains a concern, two factors suggest that the described B cell-based tolerance signatures are not solely related to the effects of immunosuppressive agents. Firstly, as discussed later a not inconsequential proportion of patients receiving immunosuppression are consistently predicted to be tolerant based on an increase in the number of B cells or an increased expression of B cell-related genes. Secondly, comparing tolerant liver transplant recipients to those receiving immunosuppression fails to demonstrate the changes in B cells and B cell-related genes that characterize spontaneous tolerance following kidney transplantation [11]. Together these findings suggest that the absence of immunosuppression alone is not responsible for the B cell-related changes that have been associated with spontaneous tolerance to transplanted kidneys.

A second concern directly related to the study design arises from the enrollment of patients who already display the tolerant phenotype rather than enrolling patients prior to the development of tolerance. This becomes a concern if the mechanisms responsible for tolerance evolve and change over time as first proposed by the late Charley Orosz [12]. In this case determining biomarkers in patients with established tolerance may detect biomarkers reflective of mechanisms that maintain the tolerant state but are perhaps distinct from the mechanisms contributing to the initial development of tolerance. This is possibility is supported by the findings that the cell populations associated with the development and maintenance of tolerance following liver transplantation differed in samples obtained prior to and following the weaning of immunosuppression [13]. Similarly, initial reports describing immunologic differences between tolerant and non-tolerant participants in the Massachusetts General Hospital tolerance trials reported that at early time points regulatory T cells were enriched in the blood and allografts of tolerant subjects [14]. At later time points differences in regulatory cell frequency between the groups disappeared at the same time as donor alloantigen specific T cells were deleted from the repertoire [15].

The final design element that influences the interpretation of our studies is the absence of biopsy data. Although the initial protocol included allograft biopsies at the time of the first study visit, the protocol was modified based on an adverse event early in the study in which a protocol biopsy resulted in hematuria, acute kidney injury, and a small arteriovenous fistula that resolved spontaneously. The absence of allograft tissue precludes histologic assessment for factors such as subclinical inflammation or causes of allograft injury distinct from alloimmunity (recurrent disease, drug toxicity, infections, etc.). In a study of operationally tolerant kidney transplant recipients Brouard et al. noted that among the 27 originally tolerant individuals 13 had a functional graft without evidence of sensitization to the donor (DSA), six had a functional allograft with evidence of donor sensitization, and eight experienced graft loss due to a mixture of alloimmune and non-alloimmune causes [16]. Obviously the inability to distinguish between declining function or graft loss caused by alloimmune and non-alloimmune causes would be important when considering how accurately biomarkers of tolerance predict the persistence of tolerance. In addition without allograft tissue it is not possible to interrogate the allograft itself with respect to immunologic processes that may be occurring in the transplanted organ. This is potentially very important as some groups have found that assessment of immune processes occurring