Trends in Transplantation 2007;1:56-60

Impact of Subclinical Rejection on Transplantation

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Abstract

The long-term survival of renal transplants has not improved significantly in the modern era despite a major decrease in the incidence of acute rejection episodes. Most graft failures are due to chronic alloimmune or autoimmune injury and are therefore the result of insufficient immunosuppression. Excessive immunosuppression, however, can also be injurious, either directly through drug-induced nephrotoxicity, or indirectly by promoting the growth of nephropathogenic viruses.

Early diagnosis of insufficient or excessive immunosuppression offers the best opportunity to potentially affect late outcomes. The use of protocol biopsies in selected cases may be beneficial in this regard. However, in the future, longitudinal noninvasive monitoring with tests such as urine proteomics may offer the best chance of improving long-term outcomes in renal transplant patients. **(Trends in Transplant 2007;1:56-60)**

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Key words

Subclinical rejection. Proteomics. Protocol biopsy.

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Introduction

The idea that subclinical renal transplant rejection can cause interstitial fibrosis, tubular atrophy (IF/TA) and late deterioration of renal function has been controversial and the procurement of protocol biopsies has therefore not been universally accepted[1-4].

There are several arguments that can be put forward by the opponents of protocol biopsies. First, as by definition subclinical rejection can only be diagnosed with a protocol biopsy, there has been reluctance to perform this procedure, despite increasing evidence of its safety[5,6] in the recipient of a well-functioning graft. Furthermore, it is clear that modern immunosuppression is decreasing the prevalence of subclinical rejection, at least in some patient populations, and the benefit of performing biopsies in all patients may therefore be questionable[7]. Another concern is cost. Finally, there is the potential that some of the graft infiltrates may be immunoregulatory and therefore beneficial[8], although, even in clinically rejecting patients, mononuclear cells with immunoregulatory phenotypes have been reported in the graft[9].

In this brief paper, the concept of subclinical rejection in renal transplantation will be discussed, both with and without reference to the protocol biopsy, and current indications for protocol biopsies will be suggested.

The problem of late graft failure

Despite the reduction in clinical rejection episodes in the modern immunosuppressive era, long-term renal transplant survival has failed to improve[10], and renal transplant failure has become an increasingly more frequent cause of patients requiring dialysis[11]. The most common cause of renal transplant failure is inadequate immunosuppression that results in chronic alloimmune or autoimmune forms of injury (e.g. chronic humoral rejection, recurrent glomerulonephritis) and accounts for approximately 50% of cases[12]. On the other hand, excessive immunosuppression can be equally deleterious, resulting in drug nephrotoxicity[12], and more recently BK virus nephropathy[13]. The combined incidence of all the above pathologies[12,13] may explain about 65% of the findings in renal biopsies performed in failing renal transplants, 35% of which will have IF/TA of unknown cause[12]. In this latter group however, as well as in non-biopsied patients with deteriorating function, evidence of both anti-donor cellular and humoral immunity has been reported to be increased when compared to patients with stable grafts[14], suggesting that at least a proportion of patients with IF/TA of unknown cause has previous or persistent alloimmune injury to the graft.

The early diagnosis of the above pathologies would offer, at least theoretically, the best opportunity for improved management. However, there is no clinical test that is able to determine the adequacy of immunosuppression in a patient with a normally functioning graft in whom the consequences of under- or over-immunosuppression may already be present.

On the other hand, the procurement of renal biopsies at set times posttransplantation, irrespective of graft function (per protocol) has shown that acute tubulo-interstitial inflammation or IF/TA of an extent sufficient to satisfy Banff criteria for acute rejection and chronic allograft nephropathy (CAN)[15], respectively, are present in a proportion of grafts with normal function.

Subclinical acute and chronic renal transplant pathology

Two eras can be defined in the procurement of protocol biopsies in relation to the

immunosuppressive regimen(s) that were most commonly used at the time. In the first era, when protocol biopsies were first introduced (early 1990-2000), most immunosuppressive regimens consisted of the original formulation of cyclosporine (CsA), azathioprine, and prednisone. Our group in Winnipeg[16-18] and others[19,20] reported a prevalence of ~ 30% of Type I A rejection (ai2at2) in protocol biopsies obtained in well-functioning grafts in the first three post-transplant months. Treatment of subclinical rejection resulted in a less IF/TA[18] whereas failure to treat resulted in an increase[19,20] in IF/TA in subsequent protocol biopsies. A clear "compartment-specific"[20] relation was suggested by the correlation between acute interstitial and tubular inflammation and the subsequent development of IF/TA that could be prevented with corticosteroids. The link between acute tubulo-interstitial inflammation/injury and the development of IF/TA may be the result of epithelial-mesenchymal transition (EMT). The term EMT refers to the process during which epithelial cells acquire the phenotypic and functional characteristics of mesenchymal cells that is central to many normal events (e.g. embryogenesis), but also to pathologic processes such as fibrosis and cancer. A general review of EMT is provided by Thiery, et al.[21] and a discussion of EMT and its potential role in IF/TA in renal allografts is provided by Nankivell, et al.[22].

The second era is that of the combined use of tacrolimus (TAC), mycophenolate mofetil (MMF), and prednisone as baseline immunosuppression (2001 to the present). In general, the replacement of CsA by TAC has resulted in a decrease in the prevalence of Type I A subclinical rejection of up to 25%[23-25]; however, in a recent study in which protocol biopsies were performed at one year in such patients, the prevalence of IF/TA between TAC/MMF vs. CsA/MMF was the same[25].

There are at least two possible explanations for this apparent paradox. First, it is possible that the similar degree of IF/TA between TAC- and CsA-treated treated patients reported by Rowshani, et al.[25] was the result of inadequate control of antidonor alloreactivity in both patient groups. Thus, the reported prevalence of subclinical rejection in the six-month protocol biopsy (defined as either Type 1 A or "borderline" inflammation) was 39% in CsA-treated and 15.2% in TAC-treated patients. It is possible, therefore, that this extent of subclinical inflammation was sufficient to cause the similar degree of IF/TA observed in the protocol biopsy procured at one year in both groups of patients (see below). Second, however, it should be also be recognized that subclinical alloimmunity is not the only cause of IF/TA in the renal allograft, and that the more efficient suppression of subclinical inflammation by TAC/MMF observed in the study of Rowshani, et al. may have resulted in other causes of IF/TA becoming more prevalent such as drug nephrotoxicity, perhaps favored by the smaller doses of anti-fibrogenic corticosteroids that was used.

It is important to note, however, that there is evidence that tubulo-interstitial inflammation below the ai2at2 Banff threshold for rejection, if persistent, may result in decreased renal function after one and two years[26]. Similarly, protocol biopsies that show a combination of any degree inflammation and fibrosis at either six or 12 months posttransplantation identify grafts that have increased rates of progressive dysfunction and failure in the longer term[27,28]. The implications of these findings are most important, as they suggest that potentially any amount of inflammation in the graft whether driven by alloimmune, autoimmune, viral disease, or other forms of injury may be associated with the development of IF/TA, and eventual graft dysfunction and loss. Elucidation of the mechanisms behind the development of IF/TA is required before rational treatment can be offered, and these findings caution against the routine reduction

or withdrawal of immunosuppressive medications in all patients.

A final point to be made regarding the apparent pathogenicity of less extensive inflammation in the modern era of renal transplantation is that older donors, "expanded-criteria donors", and donors after cardiac death are providing an increasing number of the kidneys that are transplanted, and the ability of these kidneys to withstand repeated injury may be limited[29].

The search for noninvasive biomarkers: urine proteomics

Diffuse mononuclear cell infiltrates are less often present in protocol biopsies than in those done for graft dysfunction[26] so that monitoring of graft infiltrates by protocol biopsies carries an increased risk of sampling error that would underestimate the extent of inflammation in the graft. Indeed, the proof that subclinical rejection contributes to adverse long-term outcomes in renal transplant patients will likely require the development of novel diagnostic tests. Such tests would ideally be non-invasive, thus allowing for frequent sampling of the graft with low risk. A current focus of our group for this purpose is the study of the urine proteome.

Our initial study showed that a unique urine proteome pattern consisting of three protein peaks of distinct molecular mass was found in patients with graft dysfunction due to acute rejection as compared to several control groups that included normal controls and controls with urinary tract infection, and various renal transplant populations such as histologically and functionally normal grafts, non-oliguric ATN, and recurrent glomerular disease[30]. In a subsequent study all three protein peaks were found to arise from the proteolytic cleavage of the same protein, namely β_2-microglobulin[31].

Although in these initial "discovery phase" studies urinary cleaved fragments of β_2-microglobulin appeared to be restricted to the clinically rejecting patients, in more recent "validation-phase" studies we have observed that in a proportion of histologically normal renal grafts, the urine levels of cleaved β_2-microglobulin are increased as compared to those of normal controls; furthermore, the same was true for other low molecular weight proteins studied, the levels of which correlated in general with those of cleaved β_2-microglobulin (unpublished). Increased excretion of these lower molecular weight proteins likely represents nonspecific renal tubular injury, but whether the dissociation between normal tubular histology and abnormal tubular function represents sub-histologic tubular injury or is simply the result of sampling error is not known. In any case, the persistent, increased excretion of these urinary proteins may herald the subsequent development of IF/TA irrespective of the etiologic agent involved.

More fundamental insights regarding the pathogenesis of injury, however, will arise from the full characterization of all the proteins and their patterns, and their correlation with individual pathologic diagnoses and long-term outcomes. Our group has begun looking at the urine proteome in several well-characterized clinical and histologic patient cohorts with this goal in mind; however this is an onerous task that will require time.

Current indications for protocol biopsies

Despite its limitations, and until noninvasive biomarkers of renal transplant pathology are available, it is the opinion of this author that the protocol biopsy remains an indispensable tool for the monitoring of renal transplant patients. Patients that are most likely to benefit from such biopsies include those at high

immunologic risk (e.g. patients that are sensitized to their donor), and those participating in studies of drug avoidance, minimization, or withdrawal, where protocol biopsies should ideally be done at baseline and at later time points to ensure trial safety. Similarly, protocol biopsies should be performed in patients in whom new immunosuppressive regimens are being tested and in controlled trials where there is prolonged exposure to potentially nephrotoxic agents. Some of these points have been recently summarized by Racusen[32].

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