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New Considerations for Chronic Kidney Allograft Injury

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Abstract

There are a number of new observations in the literature about chronic allograft injury that merit consideration. Not only is the Banff '05 report important as a new pathological classification schema, but our understanding of factors that may drive chronic allograft injury is changing with observations about basic clinical circumstances, molecular mechanisms of injury and fibrosis, and a greater recognition of humoral responses that do not dissipate, but linger and lead to a decline in kidney transplant function over time. **(Trends in Transplant. 2007;1:95-103)**

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Key words

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Introduction

The term chronic allograft nephropathy was introduced in 1991 as a generic alternative to the then popular term "chronic rejection"[1]. This nonspecific term has been used to denote fibrotic changes in the allograft. It is preferable to labeling all fibrotic changes as "chronic rejection", as occurred in the past, since rejection by definition implies injury due to inflammatory processes targeting alloantigens. Acceptance of the terminology "chronic allograft nephropathy" succeeded in reversing the misconception that all late scarring of the graft was due to alloimmune injury or rejection. However, the term is a tacit admission that specific features defining pathogenesis are often not present or recognized.

Many publications during the last decade have fostered the idea that chronic allograft nephropathy is a specific disease rather than just a term noting nonspecific parenchymal scarring. The Banff '05 Report authors argued that this idea inhibited the accurate diagnosis and appropriate therapy for the different causes of chronic kidney allograft dysfunction[2].

This review outlines several factors that merit reconsideration or new consideration as stimuli for chronic allograft injury. That noted, it is important to recognize some fundamental and accepted features about chronic allograft injury. Chronic allograft injury drives progressive chronic allograft dysfunction through different mechanisms (Table 1). The most rele-

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Table 1. Causes of chronic allograft injury and morphological correlates

Etiology	Typical Morphological Changes
Nonimmune injury	
Interstitial fibrosis and tubular atrophy due to calcineurin inhibitor nephrotoxicity	Arteriolar hyalinosis with peripheral hyaline nodules and/or progressive increase in the absence of arterial hypertension or diabetes. Tubular cell injury with isometric vacuolization
Interstitial fibrosis and tubular atrophy due to arterial hypertension	Fibrointimal thickening with elastica reduplication, usually with small artery hyaline changes
Chronic urinary tract obstruction	Marked tubular dilation. Large Tamm-Horsfall protein casts with extravasation into interstitium, and/or lymphatics
Viral nephropathy (especially BK virus nephropathy)	Viral inclusions on histology and immunohistology and/or electron microscopy, several grades of tubulointerstitial inflammation and chronic nephritis
Bacterial pyelonephritis	Intratubular and peritubular neutrophils, lymphoid follicle formation
Immune injury	
Chronic alloantibody-mediated rejection	C4d deposition in peritubular capillaries (PTC) with combinations of PTC basement membrane multilayering, glomerular basement membrane splitting and duplication (transplant glomerulopathy) or fibrous intimal thickening in arteries without duplication of the internal elastica Other findings: mononuclear inflammatory cells in PTC, transplant glomerulitis, interstitial plasma cell infiltrate
Chronic T-cell-mediated rejection	Arterial intimal fibrosis with mononuclear cell infiltration in fibrosis and formation of neo-intima

vant nonimmune causes of allograft injury are calcineurin inhibitor toxicity and arterial hypertension. Chronic calcineurin inhibitor nephrotoxicity can be found in protocol biopsies as early as one month after kidney transplantation. It produces hyaline arteriolar changes, tubular atrophy and interstitial fibrosis (TA/IF) either in "striped" ischemic or diffuse patterns[3,4]. Arterial hypertension, if undertreated, promotes pathological changes recognizable in the allograft, including arterial fibro-intimal thickening with duplication of internal elastica (fibroelastosis), small artery hyalinosis, glomerulosclerosis, and of course TA/IF. Chronic urinary tract obstruction, viral nephritis, especially due to bradykinin (BK) virus[5], and bacterial pyelonephritis, are other causes of chronic allograft injury, relevant in the differential diagnosis.

In addition to nonimmune causes of TA/IF, chronic allograft injury can be mediated by alloantibodies or by T-cells[1,2,6]. In both types of injury, mixed components may be present, either a cellular infiltrate concomitantly infiltrating an allograft during antibody-mediated rejection (AMR) or evidence of allo-immunity concurrent with predominantly T-cell-mediated damage. The development of C4d as a specific marker of alloantibody deposition in the capillary endothelium and the use of specific techniques to detect alloantibodies have increased the awareness for chronic AMR, its possible diagnosis and intervention[6,7]. Typical features are C4d deposition in peritubular capillaries (PTC), in conjunction with a variety of chronic histologic changes, detailed in table 1. Chronic active T-cell-mediated rejection is recognized in the biopsy by arterial intimal fibrosis with mononuclear cell infiltration in fibrosis and formation of neointima[2]. The presence in protocol biopsies of histologic findings suggestive of acute T-cell-mediated rejection, without apparent deterioration of

Table 2. Chronic injury categories in the new Banff schema for kidney allograft pathology

Category*	Morphological Diagnostic Criteria
Category 2 (Antibody-mediated rejection) – Subcategory 2 (Chronic active antibody-mediated rejection)	Deposition of C4d in peritubular capillaries (PTC) with at least one of the following: – PTC basement membrane multilayering, – glomerular basement membrane splitting and reduplication (transplant glomerulopathy), – fibrous intimal thickening in arteries without duplication of the internal elastica, – simple interstitial fibrosis and tubular atrophy
Category 4 (T-cell mediated rejection) – Subcategory 2 (Chronic active T cell-mediated rejection)	Chronic allograft arteriopathy: arterial intimal fibrosis with mononuclear cell infiltration in fibrosis and formation of neo-intima
Category 5 (Tubular atrophy and interstitial fibrosis [TA/IF], no evidence of any specific etiology)	
Grade I	Mild TA/IF (< 25% of cortical area)
Grade II	Moderate TA/IF (26-50% of cortical area)
Grade III	Severe TA/IF (> 50% of cortical area) (may include nonspecific vascular and glomerular sclerosis, but severity graded by tubulointerstitial features)
Category 6 (Changes unrelated to acute or chronic rejection [all nonimmune-related changes in table 1])	See table 1

*Category 1 is "normal" and category 3 is "borderline changes".

kidney function, does not fit any category in the new Banff schema. However, recent reports suggest this subclinical rejection is associated with chronic allograft injury, fibrosis and atrophy[8]. The significance of subclinical C4d PTC deposition is unknown.

According to the new Banff '05 schema, a special category (Category 5), includes TA/IF cases in which no specific etiologies can be defined (Table 2). Quantitation of these changes is based on the percentage of cortex involved by TA/IF. Histologic damage is commonly observed without significant clinical impact in protocol biopsies[9,10]. However, the progressive decline in kidney function manifested by an increasing serum creatinine, or the development of proteinuria often alerts the clinician to the presence of this form of chronic kidney allograft injury. The decline in kidney function is a sign of late disease, usually implying irreversible histologic damage with fibrosis and glomerulosclerosis[11-13]. Changes in serum creatinine are widely used in clinical practice to detect chronic allograft injury; however, these changes occur after mechanisms of progression have usually set in and, therefore, may be too late to allow successful changes in therapy. Monitoring changes in kidney function over time using estimating equations and measurements of proteinuria remains essential for early detection of chronic allograft injury.

Proteinuria

Proteinuria is a prognostic marker of progression of kidney disease, of patient survival, and a marker of kidney allograft survival[14-16]. It also reflects the severity of the underlying glomerular and tubulointerstitial injury. In the transplant setting, proteinuria presumably contributes to transplant dysfunction and fibrosis through putative mechanisms involving aberrant proximal tubule protein uptake and

tubular cell toxicity[17,18]. It is remarkable that despite evidence demonstrating the significance of proteinuria in native kidney mediated disease, only recently has proteinuria been examined more closely in kidney transplantation[15,16,19,20]. Persistent proteinuria is present in almost one third of kidney transplant patients one year after transplantation[15,21] and proteinuria > 1 g/day is a predictor of graft loss[22].

Abnormally filtered proteins damage kidney tubular cells and activate multiple pathways of interstitial inflammation and fibrosis[18]. As these proteins are transported across the brush border and into the tubular cell, inflammatory mediators such as monocyte chemoattractant protein 1 (MCP-1), regulated on activation, normal T-cell expressed and secreted (RANTES), fractalkine and transforming growth factor-β are upregulated in the interstitium[17,18]. This promotes fibrogenesis.

The incidence of proteinuria in kidney transplant patients ranges between 10-31%[15,23] while nephrotic-range proteinuria occurs in up to 13% of all kidney transplant patients[22]. Transplant-associated proteinuria has been noted more frequently at three months posttransplantation in those who had one or more episodes of acute rejection, especially multiple rejection episodes at three and six months[18,24]. This suggests that injury related to rejection may contribute to proteinuria. Yet, Halimi, et al. found that donor age older than 60, prolonged warm and cold ischemia time, and cardiovascular death were also determinants of early, low-grade (< 1 g) proteinuria[24]. Such data implicate kidney quality as another cause for proteinuria. Residual kidney function also contributes early to posttransplant proteinuria, dissipating within one to ten weeks posttransplantation[19]. Interestingly, no matter the cause, proteinuria seems to confer a poor prognosis. Rosenkrantz and Meyer found a close correlation between tubulointerstitial inflammation, atrophy, and the degree of proteinuria in kidney allografts[16].

Most patients with TA/IF present with some degree of proteinuria. Artz, et al. noted that the median protein excretion in patients with chronic allograft injury was 3.3 g at biopsy, and this tended to influence graft survival[25]. Nankivell, et al. found calcineurin inhibitor nephrotoxicity was the main cause of late histologic injury and decline in kidney function, with nodular hyaline arteriolar changes and proteinuria to some degree almost uniformly present as well[12]. Transplant glomerulopathy also has significant proteinuria with classical pathology, including double contouring in the capillary loops, an increase in mesangial matrix, mesangiolysis and glomerulosclerosis[1]. Recurrent glomerulonephritis is the other well-recognized common cause of proteinuria after transplantation. It occurs in 6-19% of kidney transplant patients[26,27].

Proteinuria is an independent risk factor impacting graft survival and risk of patient death, from all causes, but especially death from cardiovascular causes. Halimi, et al. noted that a 0.1 g/24 hour increase in proteinuria led to a 25% increased risk of graft loss in those with low-grade proteinuria and 15% graft loss in the entire cohort[24]. The half-life of the kidney allograft in those with persistent proteinuria is 5.6 years compared to 16.5 years in those without persistent proteinuria[21].

Oxidative stress and chronic allograft injury

Oxidative stress is a term that recognizes damage to cells, tissues and organs caused by reactive oxygen species (ROS) including superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot}$) and peroxynitrite ($ONOO^{\cdot}$). Peroxynitrite is a potent oxidizing agent generated when sub-micromolar concentrations of nitric oxide ($NO^{\cdot}$) compete for $O_2^{\cdot -}$ with endogenous superoxide dismutase (SOD) enzymes[28,29]. The principal intracellular sources of ROS include the mito-

chondrial electron transport system, peroxisomes, cytochrome p450 and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzymes[28,29], whereas commonly described exogenous factors involved in the generation of ROS are represented by inflammatory cytokines, chemotherapeutic drugs and toxins[28,29]. Copper-zinc and manganese superoxide dismutase (CuZnSOD and MnSOD), catalase and glutathione peroxidase are key antioxidant enzymes that reduce $O_2^{\cdot-}$ to H_2O_2 and water and glutathione, vitamins A, C, and E constitute the major nonenzymatic antioxidant molecules[28,29]. The balance between ROS production and antioxidant defenses defines oxidative stress in a given tissue. A prooxidant milieu can alter and denature nucleic acids, carbohydrates, lipids and proteins, resulting in cell toxicity.

Oxidative stress is involved in the pathogenesis of tissue injury in experimental models of hypertensive, diabetic, and obstructive kidney disease and systemic biomarkers of oxidative stress are increased in kidney transplant recipients[30-32]. Oxidative stress is increased in allografts with chronic tubulointerstitial fibrosis. Hydrogen peroxide-positive cells were increased in the interstitium of human kidney allografts with chronic TA/IF[33]. Similarly, $O_2^{\cdot-}$ levels were increased in graft-infiltrating and tubular cells of rat and rhesus allografts with chronic TA/IF[32,34]. MacMillan-Crow, et al. demonstrated that allograft tubular MnSOD was nitrated and inactivated in human kidneys with chronic TA/IF[35]. Nitration of MnSOD and cytochrome c occurred prior to the onset of kidney allograft dysfunction, suggesting that protein nitration and inactivation of antioxidant enzymes were early events in the pathogenesis of chronic tubulointerstitial injury[36]. These observations, confirmed by other groups, demonstrate protein and lipid nitration with peroxynitrite formation in tubular and graft-infiltrating cells in rat, rhesus, and human allografts with chronic TA/IF[32,33,37]. Interstitial and tubular levels of inducible nitric oxide synthase enzyme (iNOS) were also increased in chronic allograft TA/IF[32,33,38].

Potential sources for ROS in kidney allografts with TA/IF are inflammation, immunosuppressive drugs, comorbid clinical conditions, hypoxia, and interstitial myofibroblasts. Inflammation has long been considered a contributing factor to chronic allograft injury[1,13,39]. Graft-infiltrating monocyte/macrophages produced iNOS and proinflammatory cytokines including MCP-1 and IL-6 in the Fisher to Lewis model of chronic allograft injury[38,40,41]. We recently examined NADPH oxidase enzymes and graft-infiltrating cells in human and nonhuman primate kidney allografts undergoing chronic TA/IF, and demonstrated that $CD68^+$ cells (macrophages) and not $CD3^+$ cells (T lymphocytes), were an important source of NADPH oxidase based on greater intracytoplasmic levels of Gp91[32].

Immunosuppressive drugs represent another potential source of ROS in chronic allograft TA/IF. Cyclosporine-treated rats had greater lipid peroxidation and decreased antioxidant (glutathione) levels in kidney tissue[42]. Similarly, rat proximal tubular epithelial cells exposed to cyclosporine accumulated intracellular ROS and lipid peroxidation products, along with altered glutathione redox state[43]. Cyclosporine also increased isoprostane production in thoracic aortic segments[44].

Tissue hypoxia could also contribute to ROS in kidney allografts. We evaluated intrarenal oxygenation in human kidney allografts with chronic TA/IF using blood oxygen level-dependent magnetic resonance imaging. Medullary and cortical R_2^* levels (corresponding to deoxyhemoglobin concentrations) were significantly decreased in allografts with chronic TA/IF[45]. Deoxyhemoglobin levels correlate with tissue oxygenation when capillaries are intact as oxygen can diffuse freely towards the tissue. Because chronic allograft

TA/IF is associated with peritubular capillary rarefaction[46], it is possible that interstitial fibrosis and poor blood supply limit tissue oxygen extraction and lower deoxyhemoglobin levels[45]. Interestingly, serum H_2O_2 and HSP27 levels were significantly increased, while urine total antioxidant potential and NO levels were decreased in patients with chronic allograft TA/IF[45]. There was also a significant correlation between medullary and cortical oxygenation (R_2* levels) and serum/urine biomarkers of oxidative stress, suggesting that abnormal intrarenal oxygenation may aid in generating ROS[45].

How could oxidative stress result in allograft injury? Evidence addressing this question is limited, but points towards a potential profibrotic, proapoptotic and proinflammatory role. Interstitial fibroblasts are the principal source of kidney fibrosis[47,48]. Up to a third of all disease-related fibroblasts can originate from tubular epithelia at the site of injury through epithelial-to-mesenchymal transition (EMT)[48]. This EMT can contribute to native[49,50] and transplant kidney injury, including chronic allograft TA/IF[34,51-53]. We demonstrated that oxidative stress was associated with EMT in experimental allograft TA/IF[34]. Moreover, myofibroblasts had significantly greater intracytoplasmic gp91 expression compared to fibroblasts, suggesting that these activated fibroblasts may be a source of oxidative stress in chronic tubulointerstitial fibrosis[32]. Oxidative stress can also contribute to tubular atrophy through apoptosis[41,54]. We observed increased oxidative stress and apoptosis, together with upregulation of FasL, Bax and HSP27 in areas of tubular injury in kidney allografts with chronic TA/IF[34,41]. Furthermore, oxidative stress can activate proinflammatory pathways, including c-Jun N-terminal kinase, p38-MAPK[55,56], nuclear factor kappa B[57,58] and activator protein-1[59]. Yet, association does not imply causation and the extent of oxidative stress-mediated allograft injury will require further mechanistic investigation.

Alloimmune insults

In acute T-cell-mediated rejection, hyaluronan production leads to edema and congestion. Cytokine and adhesion molecules activation stimulates adjacent fibroblasts through molecules such as platelet derived growth factor, tumor necrosis factor alpha, interferon gamma and interleukin-2 with resulting tubular injury. Ultimately, this injury leads to a transformation in the kidney milieu with fibrosis replacing functional tissue. This model describes chronic fibrosis with an episode of T-cell mediated rejection. However, this can be initiated by a number of different processes.

Recent advances in transplant have shed light on another distinct form of rejection – antibody mediated rejection (AMR). While acute AMR has long been recognized as an infrequent yet devastating event in kidney transplantation, chronic AMR had not been recognized as an important cause of graft loss. The term chronic AMR was initially defined in a consensus meeting at the National Institutes of Health (NIH) on the basis of a handful of reports[7,60], and its histologic characteristics have been recently revised[2]. Chronic AMR is now an established entity, and its impact on allograft and patient outcomes is gradually being demonstrated.

There is ample direct and indirect evidence from both retrospective and prospective studies linking anti-class I and class II antibodies to chronic AMR[7,61,62]. Non human leukocyte antigen (HLA) humoral immunity has been demonstrated to have a significant impact in the fate of the kidney allograft. Opelz and the Collaborative Transplant Study have reported an association between plasma renin activity and long-term graft loss in HLA-identical sibling kidney transplantation[63]. The vascular endothelial cell system is a minor histocompatibility system genetically linked to the major histocompatibility complex, yet less polymorphic[64]. These antigens are expressed

in both endothelial cells and monocytes, which has allowed for the development of a monocyte crossmatch used for the detection of these antibodies. Although, in one retrospective study, rejection occurred in 80% of patients with a positive monocyte crossmatch *vis-à-vis* 9% in patients with a negative crossmatch[65], other studies have not found an association between anti-endothelial cell antibodies, acute rejection, or poor allograft outcomes[66,67]. In spite of the ability of anti-endothelial cell antibodies to induce the apoptosis of endothelial cells *in vitro* – a potential mechanism in the pathogenesis of accelerated graft arteriosclerosis – the association of these antibodies and chronic AMR remains loose at best[66,67].

The MIC system is a minor histocompatibility system of HLA-class I-like molecules closely linked to the HLA-B locus, consisting of more than 55 alleles, induced by stress and expressed on kidney microvascular endothelial cells and tubular epithelial cells[64,68]. As far as chronic AMR is concerned, Terasaki, et al.[69] have provided the strongest evidence of a deleterious effect of MICA antibodies on graft survival. In a prospective study of patients included in the 14th workshop[62], one-year graft survival in recipients of a deceased donor transplant without MICA antibodies was 96.8% compared to 82.7% for patients with MICA antibodies alone ($p = 0.0005$).

In both acute AMR and chronic AMR the target of injury is the endothelium of the graft microvasculature. In chronic AMR, MAC-induced injury of the endothelium is sublytic, resulting in smoldering endothelial cell damage and activation[2,70]. The mechanisms behind the sublytic nature of complement activation in chronic AMR are not well understood, yet "partial accommodation" resulting from upregulation of complement inhibitory proteins and antiapoptotic pathways (i.e. Bcl-2 and Bcl-x) have been proposed as potential mechanisms[2,70].

Sublytic MAC-induced injury results in the production of ROS, cytokines and growth factors. The production of profibrotic cytokines such as basic fibroblast growth factor, platelet derived growth factor and thrombospondin-1 (a known activator of latent transforming growth factor-β-1 via PI3-k/Akt) further links sublytic MAC-activation to the fibrogenesis and vasculopathy typical of CR[71,72].

The interactions between antibodies and cells through the binding of antibodies to the Fc-γ-receptor (Fc-γR) expressed on B-cells and natural killer-cells, and macrophages/monocytes result in antibody-dependent cell cytotoxicity, activation of macrophages and release of proinflammatory cytokines, and enhanced leukocyte adhesion to the activated endothelial cells[73]. Apoptotic death of endothelial cells and smooth muscle cells of the arterial media has been reported as an antibody-induced mechanism of injury[74,75]. The proapoptotic pathways activated by anti-HLA and non-HLA antibodies remain to be determined.

These smoldering complement-dependent and independent mechanisms of antibody injury generate a feedback loop that leads to endothelial cell lysis, activation, inflammation and the chronic cycle of injury and repair that lie behind the histologic triad of chronic AMR.

Summary

Our therapeutic approach to chronic allograft injury remains somewhat empirical and caught in a tug-of-war between balancing the right amount of immunosuppression and its untoward effects. Yet, we now have a classification schema that provides structure to our observations and a unique and dynamic tension in chronic allograft injury that arises nowhere else in the setting of chronic kidney disease, the juxtaposition of alloimmune and

nonimmune stimuli that ultimately affect the parenchyma, limiting its ability to function.

Our knowledge of the factors that can potentiate chronic allograft injury has expanded significantly in the last several years. We now have a greater ability to recognize multiple factors that can shorten the life and decrease the function of an allograft. Notably, while our pace of identification has increased, our understanding of how these potential stimuli ultimately affect allograft function remains limited and in its infancy. The nomogram of injury, e.g. what factors are affecting the allograft at what point in time, remains elusive but we are moving closer to it. With that, we will be able to translate observations into more directed therapy and hopefully extend the functional life of the allograft for the benefit of the patients.

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