Late Kidney Allograft Loss: What We Know about It, and What We Can do about It

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Despite dramatic improvements in immunosuppression, late graft loss after kidney transplantation remains a common and difficult problem. Histologic evaluation may reveal changes related to BK polyomavirus infection, hypertension, or calcineurin inhibitor toxicity, which can help to guide therapy. The designation chronic allograft nephropathy should thus be reserved for biopsies with tubular atrophy and interstitial fibrosis without an apparent cause. Although the cause clearly includes both antigen-dependent and antigen-independent events, the approach remains largely to exclude immune mechanisms. Although this review discusses the potential contribution of antibody to chronic injury, it focuses on the basic elements of kidney injury, the role of parenchymal cells in promoting injury, and the proliferative and inflammatory responses that accompany injury. Strategies to manage these recipients include close attention to accompanying hypertension, diabetes, and hyperlipidemia, as well as consideration for altering immunosuppression; however, therapies that limit epithelial-to-mesenchymal transition or directly block fibrosis pathways may reduce chronic allograft fibrosis and may prove to be useful. Understanding the basic pathogenesis sufficiently to allow early intervention may finally benefit patients who are at high risk for tubular atrophy and interstitial fibrosis and promote their long-term graft function.


Chronic allograft injury remains the leading cause of late graft loss after kidney transplantation (1). Characterized by progressive tubular atrophy and interstitial fibrosis (TA/IF) as well as microvascular and glomerular damage in the setting of declining graft function months to years after transplantation, the entity encompassed by the previous descriptive term “chronic allograft nephropathy” (CAN) remains frustrating to manage. In part this is due to the absence of a single or easily modifiable cause resulting in a sense of futility in its management. The incidence of this disorder varies, ranging from 23% at 5 yr after transplantation (2) up to 60% of grafts at 10 yr after transplant (3). Often associated with hypertension and proteinuria, management strategies include aggressive hypertensive and diabetes management and manipulation of immunosuppression via minimization or elimination of the nephrotoxic calcineurin inhibitors (CNI). These are nonspecific strategies, and a more direct approach to limit atrophy and fibrosis may have greater long-term benefit. Investigations into the pathogenesis are critical to identifying early markers of disease as well as potential therapeutic pathways. In this review, we summarize the mechanistic insights as well as current and emerging management strategies. The need for an effective approach is critical because graft loss not only is devastating to our patients but also burdens the already lengthy kidney transplant waiting list.

Role of the Allograft Biopsy

The classification of CAN was added to the Banff criteria of kidney allograft biopsies in 1993 (4). Histologic criteria were based on TA/IF and graded from I (mild) to III (severe). This classification scheme originally included four entities that could not always be distinguished, including chronic rejection, CNI toxicity, hypertensive vascular disease, and chronic infection and/or reflux. At the time, this classification was believed to be sufficient, because it would distinguish immunologic processes and not replace any particular diagnostic category per se. More recent additions to this schema include a severity scale (5) and additional characterizations of histologic features that may be associated with chronic injury, including the detection of “double contours” in glomerular capillary loops as a result of mesangial interpositioning was classified as chronic transplant glomerulopathy (“cg”), mesangial matrix deposition (“mm”), vascular changes (“cv”), and arteriolar hyaline changes (“ah”). Despite publication of these refined criteria, the term CAN was used extensively and, in effect, began to define a disease entity rather than a pathologic notation in which TA/IF was noted in the absence of histology suggestive of immunologic activity. Indeed, retrospective analysis of 600 biopsies during a 10-yr period demonstrated that nonspecific TA/IF was found only 23% of cases (6); however, using protocol kidney biopsies in a
prospective manner in kidney/pancreas transplant recipients, 94% of recipients had TA/IF, albeit mild, within the first posttransplantation year and at 10 yr nearly universal changes consistent with chronic CNI therapy, with 58% of biopsies demonstrating severe TA/IF (3). Such data support “CAN” as a progressive and cumulative lesion and suggest a strong and perhaps inseparable relationship to CNI toxicity.

Considerably greater emphasis has been placed on biopsy and morphologic assessment of the failing graft. For example, BK polyomavirus nephropathy has become a critical contributor to graft loss, the extent of which has not been fully defined outside of case series reported in the literature. Suffice it to say that early detection via molecular methods (7) and the role of biopsy early in the course of disease may abrogate an otherwise inexorable decline in kidney graft function (8). A more detailed discussion of the differential diagnosis and issues in classifying CAN may be found in the 2005 Banff meeting report (9).

A number of centers are reporting results of protocol or surveillance biopsies to detect abnormalities early in the posttransplantation course, as potential surrogate markers of future graft survival (10). The presence of ongoing interstitial inflammation (“i score”) without associated tubulitis, even in areas of atrophy and fibrosis, is a negative predictor of graft outcome (11). Moreover, inflammation and tubulitis detected “subclinically,” that is, in the absence of a clinically significant change in renal function, are associated with progressive graft failure (12–14). For example, biopsy at 1-yr after transplantation in the presence of CNI therapy commonly demonstrates some degree of fibrosis, and the combination of interstitial fibrosis and inflammation is strongly associated with graft loss or a 50% decline in GFR within the first 5 yr of transplantation (12). This significant relationship remained regardless of whether the donor was living or deceased and regardless of the extent of “i” score. Thus, biopsy histology, independent of renal function, may define graft prognosis and may identify a population at high risk for progression.

Serial biopsies of living kidney allografts have also demonstrated the presence of fibrosis, not unlike that seen in deceased-donor grafts, and the severity increases over time (15). Moreover, graft survival and renal function at 2 yr were strongly correlated to the extent of allograft fibrosis. These data suggest that ischemic injury and preservation injury do not entirely explain the extent of fibrosis in deceased-donor kidneys and suggest that other variables are important in living graft loss; however, the role of surveillance biopsy in managing this patient population remains unclear. The cost, safety, and practical application with third-party compensation remain uncertain. As demonstrated, early histologic diagnosis can occur before detectable changes in renal function. Further investigation is needed to understand the type(s) of intervention to ameliorate ongoing and early immune responses. Importantly, in the absence of clear treatment options and unequivocal linkage to outcomes, the management of immunosuppression in the context of these infiltrates is not established.

When assessing allograft dysfunction, several new modalities that may supplement histology as a guide to diagnosis and treatment have been identified. These methods include gene and protein expression profiles in the peripheral blood, the urine, and the graft itself, all compartments relevant to the alloimmune response (reviewed in reference [16]). The recent sequencing of the human genome also provides a unique opportunity to develop genomic (17) and proteomic approaches (18) to increase our understanding of events that lead to TA/IF. For example, high-density array analysis of protocol kidney biopsies demonstrated upregulation of genes associated with inflammation and matrix and tissue remodeling, which correlated with histologic evidence of TA/IF (19). Fibrosis-associated gene sets have also been identified in protocol kidney biopsies with subclinical TA/IF and are linked to inflammation and immune markers (20). Previous studies also implicated growth factors such as TGF-β (2,21) and, more recently, connective tissue growth factor (22), the downstream effector of TGF-β, as important molecules in the development of fibrosis in kidney allografts that may be useful biomarkers of disease. The future challenge will be the validation for many of these promising tests in larger patient populations to facilitate the adoption of these tests for disease detection and early management.

What Causes Graft Fibrosis and Tubular Atrophy?

Both immune (antigen dependent) and nonimmune (antigen independent) events may promote graft injury as summarized in Table 1. Regardless of the cause of the initial insult, the result is inflammation, which in the case of renal allografts may never completely resolve. In this regard, allogeneic differences between donor and host lead to a persistence of graft-infiltrating cells, including T cells, B cells, and macrophages, accompanied by a proliferative response, mediated by chemokines, cytokines, and growth factors (reviewed in reference [23]). In particular, TGF-β has been implicated in the fibrotic response after injury in animal models of transplantation (24–26) and also in human (27,28). Moreover, connective tissue growth factor, a downstream effector of TGF-β, has been associated with chronic allograft failure in heart (24) and kidney transplant models (22), with specific ability to induce transformation of kidney tubule epithelial cells into fibroblasts (22). Other factors of potential importance in this response to injury include endothelin-1 (29), PDGF-BB, EGF, and basic fibroblast growth factor (reviewed in reference [30]), which augments TGF-β signaling, and bone morphogenic protein-7 (BMP-7) and hepatocyte growth factor (HGF), which counteract TGF-β signaling and are discussed in “Epithelial-to-Mesenchymal Transition: A Role in Allograft Fibrosis?”. Ultimately, the long-term potential of allograft function may depend on somatic cell death. The combination of ischemic injury and other posttransplantation stresses may accelerate senescence, as indicated by recent studies by Melk et al. (31), in which expression of the cell-cycle inhibitor P16ink4a increased over time after transplant and was further affected by underlying graft age. Thus, a finite period of function that is perturbed by the transplantation procedure and its consequent management may exist.
Tubular Cell Injury: The Starting Point of Allograft Failure?

Tubular epithelial cells (TEC) comprise more than 75% of renal parenchymal cells, and their susceptibility to injury directs long-term graft function, because severe tubular injury can be a primary cause for nephron loss (32). TEC death after ischemia and inflammation occurs by apoptosis or programmed cell death and necrosis (reviewed in reference [33–35]), which have considerable overlap in etiologies and pathways (34,35). Apoptosis is required for kidney remodeling and repair, but when cell death exceeds the regenerative capacity of the kidney, the result is a loss of function and premature graft failure. Thus, TEC preservation might be a reasonable target to prevent late graft loss.

Fas (CD95) expression on TEC and interaction with Fas ligand (FasL) on self or infiltrating cells has been implicated in apoptotic injury in allografts (36,37). It has been difficult, however, to establish unequivocally a role for this receptor in allograft injury because Fas expression may vary nonspecifically in the graft (38), and TEC death via perforin/Granzyme B–based cytotoxicity is more common in acute renal allograft rejection (38,39). Nonetheless, renal Fas—FasL interactions have been implicated in chronic renal injury (37,40), and Fas polymorphisms (TNFRSF6) of the donor kidney can affect graft survival (41). Moreover, disruption of TEC—TEC Fas—FasL interactions or inhibition of Fas signaling through caspase-8 in vivo has proved successful in preclinical rodent kidney transplant models be it by RNA silencing (42), caspase inhibition by synthetic peptides such as zIETD and zVAD, or genetic manipulation to silence caspase function (42,43) or the proapoptotic enzymes produced by TEC (44). Finally, the regulation of Fas—FasL expression in TEC is of considerable importance such that TEC might be protected in vivo from self-injury during inflammation. This process includes endogenous inhibitors of caspase-8 activation (c-FLIP), which may be downregulated in vivo by cytokines such as IL-2 (45) and other factors including growth factors and endogenous antiapoptotic proteins such as bcl-2 and inhibitor of apoptosis (46) to promote resistance of TEC to death. The complexity of these intrarenal responses to inflammation demonstrates the importance of mechanistic insights to develop strategies to modify late graft injury (37,42).

Epithelial-to-Mesenchymal Transition: A Role in Allograft Fibrosis?

Recently, it has been recognized that TEC injury is associated with the development of fibroblasts with myocyte-like properties within the interstitium of the kidney. Although these cells may be derived from a number of origins, including resident interstitial fibroblasts or circulating mesenchymal cells, it is likely that TEC in response to injury can undergo a phenotypic change through the process of epithelial-to-mesenchymal transition (EMT). Although EMT is a complex process, it has been described in vitro and in vivo in other chronic kidney diseases associated with fibrosis (47) as well as epithelial cells in other organs. Interconversion of mesenchymal and epithelial cells during embryonic development via EMT and mesenchymal to epithelial transition are well established mechanisms in organ development, and renal EMT may be part of a regenerative, albeit dysregulated, response in transplanted kidneys (48).

During kidney allograft fibrosis, conversion of renal TEC into myofibroblasts/fibroblasts would promote late graft loss through the disruption of polarized renal TEC and an increase in fibrotic scar formation. Although mesenchymal cells that infiltrate the vasculature and interstitium of grafts with chronic

| Tubular Cell Injury: The Starting Point of Allograft Failure? |
| Organ viability |
| donor senescence |
| donor age |
| prolonged cold ischemic time |
| delayed graft function/acute tubular necrosis |
| living versus deceased |
| reduced renal mass |
| donor brain injury |
| Treatment |
| drug toxicity |
| Recipient factors |
| lipid disorders |
| diabetes |
| recurrent disease |
| compliance |
| hypertension |
| obstruction |

| Epithelial-to-Mesenchymal Transition: A Role in Allograft Fibrosis? |
| Cellular immunity |
| direct versus indirect allorecognition |
| donor–host mismatch |
| subclinical inflammation |
| co-stimulatory signaling |
| inadequacy of immunosuppression |
| Humoral immunity |
| antibody-mediated rejection |
| previous sensitization |
| Infection |
| CMV |
| BK polyomavirus |

*CMV, cytomegalovirus.
injury can be of both recipient and donor origin (49), the primary form of tubule regeneration seems to occur through the survival of dedifferentiated epithelial cells that proliferate and
re-differentiate into mature functional epithelial cells (50) or via bone marrow-derived cells (51); therefore, halting the process of EMT may be of considerable benefit in preventing TA/IF and late graft loss. Emerging data suggest that adult renal fibroblasts might retain parts of their original embryonic imprint and plasticity, which can be re-engaged by systemic administration of BMP-7 to mediate repair of tubular injury even with established renal fibrosis (48,52). Treatment with BMP-7 reduced fibrosis and improved renal function in a number of rodent models and attenuated disease via antagonism of TGF-β-mediated EMT (53). BMP-7 signaling is enhanced by kielin/ chordin-like protein, and in its absence, mouse kidneys seem more susceptible to tubular injury and fibrosis (54). Similarly, HGF seems to block TGF-β-mediated EMT in vitro and attenuated fibrosis in a variety of rodent models (reviewed in reference [55]). This effect is mediated by HGF modulation of TGF-β expression through transcriptional repressors.

Within allografts, expression of S100A4, a calcium-binding protein and a marker of EMT, is associated with infiltrating CD8+ T cells in allografts with TA/IF and suggest that TGF-β produced by these cells may directly induce epithelial cells to transform and migrate into the interstitium (56). Cyclosporin A induces EMT in cultured proximal TEC and is associated with a profibrotic transcriptional signature (57,58). The presence of EMT has been confirmed in human allograft biopsies from recipients with a decline in renal function and the hallmark histology of tubular atrophy and interstitial fibrosis (59). Moreover, protocol biopsies of kidney allografts at 3 mo have demonstrated that approximately 40% expressed markers of EMT, and these grafts were more often associated with rejection and longer cold ischemic times (60). Thus, a greater understanding of the potential of TEC within the graft or other mesenchymal cells to contribute to TA/IF is critical because it may provide us with new direction in both early diagnosis of progressive fibrosis and an early interventional target to prevent subsequent fibrosis. Disruption of the process of EMT may be clinically feasible in the near future, but the impact on graft regeneration as well as the ongoing immune response and allograft failure must be considered.

Another entity that is receiving more attention in late allograft loss is chronic transplant glomerulopathy. This lesion is characterized by widespread involvement of all glomeruli, with enlargement and duplication of the glomerular basement membrane (GBM), and endothelial cell activation. By electron microscopy, there is subendothelial accumulation of electron lucent material, reduplication of the GBM, and interposition of mesangial cells into the capillary wall (81,82). Associated risk factors for development include the presence of anti-HLA antibodies at the time of transplantation and late acute rejection
(83,84). Glomerular C4d staining may also be seen in this lesion, indicating antibody-mediated immune responses (83). Endothelial C4d deposition is associated with chronic transplant glomerulopathy, and its detection in peritubular capillaries in otherwise morphologically normal biopsies has been associated with subsequent development of this lesion (85). The generation of antidonor antibody may be a response to structural proteins in the kidney, rather than allogeneic responses leading to anti-HLA antibodies. Alternatively, anti-GBM antibodies, specifically to heparan sulfate proteoglycan agrin, may play a role (86). That this lesion is a result of immune activation is further supported by the observation that intraglomerular and periglomerular leukocytes express CXCR3 and ICOS as markers of T cell activation, compared with their absence in biopsies with CAN lesions alone (87). These studies support the need for further investigation into the prospective development of this lesion and the contributions of both cellular and antibody-mediated components.

The case for non-HLA antibodies in chronic graft injury is also growing. These include anti-GBM heparan sulfate antibodies (88) and anti-perlecain and anti-α1(VI)/α5(IV) collagen antibodies (89) in rat transplant models. In human kidney transplant recipients, antibodies directed against vascular endothelial cells have been associated with biopsy-proven chronic rejection and worsened graft outcome (90). Anti-angiotensin II type A receptor antibody has been detected in a subset of kidney transplant recipients with refractory vascular rejection episodes and malignant hypertension (91). Finally, detection of antibody toward MHC class I-associated proteins is relatively uncommon after kidney transplantation but is associated with worse graft outcome (92) and more frequent acute rejection episodes (93). Thus, chronic antibody production, both HLA and non-HLA directed, has reemerged as an important potential cause in immune-mediated late graft loss; however, the management of this disorder remains poorly defined. Although there are anecdotal reports of management with plasmapheresis and anti-B cell antibody therapy such as rituximab, the management of this recipient remains under study and potential intervention has considerable practical issues.

**Endothelial Cell Injury: A Parallel Pathway to Graft Loss?**

The presence of arterial intimal proliferation with fibrointimal inflammation is a recognized feature of chronic rejection (5). Although the focus of this review has been on epithelial cell injury and interstitial fibrosis, it should be noted that endothelial cell injury may interplay to lead to late graft dysfunction. Vasculopathy (“cv”) may be manifest not only in arteries but also in arterioles and the glomerular tuft and peritubular capillaries. Apoptosis of endothelium leads to a series of events that culminate, including mononuclear leukocyte recruitment, vascular smooth muscle cell proliferation, neointima formation, and abnormal vascular remodeling (reviewed in reference [94]). Monocyte-derived TGFB is enhanced, promoting a fibroproliferative response, with myofibroblast accumulation within the neointima of arteries and capillaries and concomitant interstitial fibrosis (95). Understanding the contribution of endothelial cell injury and intimal proliferation in chronic graft injury, particularly the cross-talk with the interstitial compartment, may define a new therapeutic avenue to salvage the failing allograft.

Injury to the microvasculature of the kidney may also be underrecognized as a contributor to late graft loss with resultant hypoxia and ischemic injury. In end-stage grafts with TA/IF, reduced microvascular density has been associated with increased endothelial proliferation of remaining cells (96). Interstitial lymphatics that contain macrophages were also prevalent in these failed grafts, associated with enhanced expression of inducible nitric oxide synthase and vascular endothelial growth factor-C. These observations suggest another pathway with potential therapeutic implications in disrupting the decline in renal function and anatomy.

**Key Factors in Managing Chronic Graft Injury**

In general, the development of late graft failure is a variant of chronic kidney disease; as such, standard management principals for reduced GFR apply. These include addressing hypertension, adequate control of blood sugar in diabetes, attention to hyperlipidemia, and anemia support based on the Kidney Disease Outcomes Quality Initiatives (K/DOQI) guidelines for patients after kidney transplantation (97). Moreover, both hypertension and diabetes strongly contribute to the risk for coronary artery disease, and such risk factors need appropriate management to limit the death with a functioning graft (98); however, it should be clearly noted that there have been limited studies into specific interventions in this patient population, and, clearly, further investigation is warranted to identify relevant strategies to improve graft outcome.

Hypertension is extremely common in kidney transplant recipients, ranging from 60 to 80% depending on the center. In a report from the Collaborative Transplant Study of 262 European transplant centers, 76% of patients had hypertension, defined as a BP ≥130 systolic (99). Elevated BP also has a significant negative effect on long-term kidney graft outcome, and increased levels of systolic and diastolic BP after transplantation are associated with a graded increase of subsequent graft failure (99). Thus, K/DOQI guidelines recommend control with BP <130/80 mmHg. A variety of agents have been studied in posttransplantation recipients. These include the use of dihydropyridine calcium channel blockers, which are not only effective in BP management (100,101) but also are associated with arteriolar vasodilation and may ameliorate the vasoconstriction associated with calcineurin therapy and improved GFR (101,102). Randomized comparison between nifedipine and lisinopril demonstrated that although BP control was similar, GFR was significantly better at 2 yr after transplantation in the nifedipine group (103), although this has not been a consistent finding (104). Angiotensin-converting enzyme inhibitors (ACEI) may provide some specific benefits, including reduction in left ventricular hypertrophy compared with placebo (105) and a reduction in proteinuria compared with β blocker therapy (106). Although one report suggested that ACEI may improve patient survival (107), a retrospective analysis of 17,209
kidney transplant recipients in the Collaborative Transplant Study with functioning grafts and ACEI use at 1 yr demonstrated no significant differences in either patient or graft survival (108). Thus, the available evidence is not sufficient to recommend one class of agent over the other, and the utility of ACE inhibition might be best suited to those with proteinuria.

Hyperlipidemia, defined as a total cholesterol >240 mg/dl (approximately 6 mmol/L), LDL >130 mg/dl (approximately 3.5 mmol/L), HDL <35 mg/dl (approximately 0.9 mmol/L), and triglycerides >200 mg/dl (approximately 5 mmol/L), is seen in approximately 60% of recipients. The pathogenesis is multifactorial and includes the presence of preexisting hyperlipidemia, posttransplantation weight gain, immunosuppression (particularly sirolimus and steroids), and the presence of the coexistent diabetes or hypothyroidism. Agents for therapy include statins as well as fenofibrate to control LDL and triglycerides. Moreover, statins have been implicated as beneficial agents beyond their lipid-lowering ability because of their potential to regulate fibrogenic mechanisms, as well as their impact on endothelial dysfunction. In a landmark study of >2000 renal transplant recipients, the daily use of fluvastatin was found to be safe and effective in lowering total cholesterol and LDL cholesterol (109). In addition, fluvastatin therapy resulted in a 32% reduction of myocardial infarction and a 38% reduction in cardiac death but did not reduce interventions and other cardiac morbidity; however, there was no impact on graft function or outcome. Thus, the primary treatment goal at present should be aimed at improving overall patient health and survival and avoiding adverse effects of drugs while the benefit on graft function has not completely been established.

Posttransplantation diabetes is increasingly recognized as a significant issue. Because of a significant negative impact on patient and graft survival (110), treatment of this disease should be included in the management strategy of late graft failure. Identification of diabetes after transplantation, management goals and strategies, and therapeutic interventions will be discussed in another section of this supplement.

Alterating Immunosuppression: What Can Help?
The long-term use of CNI, even when monitored, has been implicated as a substantial contributor to the development of TA/IF (3). Lower rates of “CAN” have been associated with the use of mycophenolate mofetil (111). Such studies suggest that antimetabolites may uniquely affect fibrogenesis or that these agents can facilitate reduction of CNI, which may be of greater impact. Consequently, limiting the long-term use of CNI or avoiding them altogether has been under intense investigation in the past 5 yr. Although initial attempts to withdraw CNI demonstrated limited efficacy (112), further investigations have been facilitated by the frequent adoption of induction immunosuppression that includes nondepleting antibodies that block CD25 (19) and co-stimulatory blockade (113) or via depletional strategies using rabbit anti-thymocyte globulin (114) or alemtuzumab (115). The addition of sirolimus to the list of approved drugs to prevent kidney graft rejection has further facilitated trials in CNI withdrawal (116–118) or avoidance (114,119–122), and such strategies have been met with mixed results. The use of sirolimus in and of itself could provide some benefit as a result of its recognized antiproliferative effects on fibroblast proliferation as demonstrated recently by the stabilization of TA/IF grade and a reduction in a-smooth muscle actin expression after treatment in patients with moderate graft dysfunction (123) as well as the reduction in arteriolar hyalinosis and tubular degeneration in another study (124). Further studies should be considered and reviewed with the recognition that approaches for long-term maintenance of stable graft function may differ from those for the already failing transplanted kidney. Ultimately, identifying a strategy for optimal immunosuppressive manipulation for late graft injury will depend on a strategy that is not only non-nephrotoxic but also as equally efficacious as standard calcineurin-containing regimes. The reader is referred to the more detailed discussion of the role of specific strategies and their implications in graft function and outcome elsewhere in this supplement.

Abrogating Matrix Deposition: Novel Approaches
As already discussed, management of recipients with late graft failure includes attention to modifying comorbidities to slow the rate of decline and also to modify the contribution to coronary artery disease, but these strategies are only indirect. In the kidney with interstitial fibrosis, matrix synthesis is no longer in balance with matrix degradation as a result of increased synthesis, decreased degradation, or a combination of both. Blocking matrix formation in and of itself may be powerful in clinical transplant settings, because it obviates knowing precisely which insult is the cause.

In this regard, a number of agents have been investigated and are awaiting additional preclinical testing and/or have been tested in nontransplantation situations and could represent novel therapeutic strategies for our patients (reviewed in reference [125]). For example, blockade of prolyl-4-hydroxylase, a rate-limiting step in collagen biosynthesis (126,127), resulted not only in a reduction of fibrosis and graft inflammation but also in improved graft function compared with vehicle-treated controls, with no evidence of gross toxicity in the mouse (128). Inhibition of matrix metalloproteinase enzymes, regulatory enzymes in matrix degradation, ameliorate proteinuria and histology in rat kidney allografts, depending on the time course of dosing (129). Retinoids, recognized for their anti-inflammatory capacity (reviewed in reference [130]), not only reduce acute rejection severity in the rat (131) but also ameliorate fibrosis in chronic nephropathy (132). Finally, disrupting EMT may be another therapeutic route. HGF treatment improved graft survival and renal function in rat kidney allografts (133) and also ameliorated the fibrosis associated with cyclosporine nephrotoxicity (134). These effects not only are mediated by reduction in gene transcripts for TGF-β and matrix molecules (133,134) but also are associated with a reduction in macrophage infiltration (133) and protection of tubular injury, perhaps by anti-apoptotic effects. Thus, there are a number of approaches, but the issue to consider includes whether such class of agent may ameliorate established matrix and show clinical utility in trans-
plant surgical settings where wound and graft anastomotic healing are a necessity.

Conclusions
Late allograft loss by TA/IF is a consistently identified and progressive condition for which no current single approach has entirely predictable results. That chronic graft loss is unchanged despite remarkable reductions in acute rejection rates suggests that immune mechanisms are not primarily responsible; however caution is required in this interpretation because the pattern of response to diverse forms of injury in the kidney is TA/IF, and as we alter therapy to try to prevent this form of graft loss, we may be unaware of interchange between immune and nonimmune mechanisms. The role of tubular injury and mechanisms of graft fibrosis have been discussed. In a practical sense, limiting nephrotoxic immunosuppressive agents while still providing adequate antirejection coverage remains a reasonable goal but needs to consider immune process more insidious than cellular infiltrate can occur, including antibody and complement. Management of comorbidities that are known to contribute to GFR decline in nontransplant chronic kidney dysfunction should also be addressed. Emerging concepts of the mechanisms of injury and fibrosis should lead to early biomarkers as well as therapeutic agents so that we will finally be able to tackle specifically this considerable clinical problem.

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None.

References
17. Sarwal MM: Chipping into the human genome: Novel


48. Zeisberg M, Shah AA, Kalluri R: Bone morphogenic protein-7 induces mesenchymal to epithelial transition in


P, Regele H, Seron D, Seshan S, Sund S, Trpkov K: Antibi-
body-mediated rejection criteria: An addition to the Banff
97 classification of renal allograft rejection. Am J Transplant
79. Mengel M, Bogers J, Bosmans JL, Seron D, Moreso F,
Carrera M, Gwiner W, Schwarz A, De Broe M, Kreipe H,
Haller H: Incidence of C4d stain in protocol biopsies from
renal allografts: Results from a multicenter trial. Am J
Transplant 5: 1050–1056, 2005
80. Lederer SR, Kluth-Pepper B, Schneeberger H, Albert E,
van Veelen PA, Van Kooten C, Paul LC: Antibody
response against perlecan and collagen types IV and VI in
graft rejection.
81. Regele H, Bohmig GA, Habicht A, Gollowitzer D, Schill-
enburg C, Unger T, Kelha M, Dechend R, Kintscher U, Rudolph B, Hoebeke J,
Goldring MICA and anti-HLA antibodies is associated with acute
rejection episodes and renal allograft loss. Hum Immunol
82. Maryniak RK, First MR, Weiss MA: Transplant glomeru-
lopathy: Evolution of morphologically distinct changes. Kid-
ney Int 27: 799–806, 1985
83. Sijpkens YW, Joosten SA, Wong MC, Dekker MC, Bajema
IM, Bruijn JA, Paul LC: Immuno-
logic risk factors and glomerular C4d deposits in chronic
84. Wood J, Aburu F, Stagell MD, Park WD, Moore SB, Degoe-
y S, Griffin MD, Larson TS, Cosio FG: Transplant glomeru-
lopathy: Subclinical incidence and association with alloan-
85. Regele H, Bohmig GA, Habicht A, Gollowitzer D, Schill-
gen M, Rockenschaub S, Watschinger B, Kerjaschki D,
Exner M: Capillary deposition of complement split product
C4d in renal allografts is associated with basement mem-
brane injury in peritubular and glomerular capillaries: A
contribution of humoral immunity to chronic allograft re-
86. Joosten SA, Sijpkens YW, van Ham V, Trouw LA, van der
Vlag J, van den Heuvel B, van Kooten C, Paul LC: Anti-
body response against the glomerular basement membrane
protein agrin in patients with transplant glomerulopathy. Am J
Transplant 3: 583–593, 2005
87. Akalin E, Dikman S, Murphy B, Bromberg JS, Hancock
WW: Glomerular infiltration by CXCR3+ ICOS+ activated
T cells in chronic allograft nephropathy with transplant
88. van den Heuvel BP, Bakker MA, Veerakamp JH,
Assmann KJ, Berden JH: A monoclonal antibody against
GBM heparan sulfate induces an acute selective protein-
89. Joosten SA, van Dixhoorn MG, Borrias MC, Benediktsson
H, van Veelen PA, Van Kooten C, Paul LC: Antibody
response against perlecan and collagen types IV and VI in
chronic allograft rejection in the rat. Am J Pathol 160:
1301–1310, 2002
90. Ball B, Mousson C, Ratiognier C, Guignier F, Glotz D, Rifle
G: Antibodies to vascular endothelial cells in chronic re-
91. Dragun D, Muller DN, Brasen JH, Frischa L, Nieminen-
Kelma H, Dechend R, Kintscher U, Rudolph B, Hoebeke J,
Eller D, Mazak I, Plehm R, Schonemann C, Unger T,
Budde K, Neumayer HH, Luft FC, Wallukat G: Angioten-
sin II type 1 receptor activating antibodies in renal-allo-
D, Guleria S, Mehra NK: Post transplant development of
MICA and anti-HLA antibodies is associated with acute
rejection episodes and renal allograft loss. Hum Immunol
and chronic transplant vasculopathy: Recent results, novel
95. Shimizu A, Yamada K, Sachs DH, Colvin RB: Persistent
rejection of peritubular capillaries and tubules is associated
with progressive interstitial fibrosis. Kidney Int 61: 1867–
1879, 2002
96. Adair A, Mitchell DR, Kipari T, Qi F, Bellamy CO, Robert-
son F, Hughes J, Marson LP: Peritubular capillary raref-
cation and lymphangiogenesis in chronic allograft failure.
Transplantation 83: 1542–1550, 2007
97. K/DOQI clinical practice guidelines for chronic kidney
disease: Evaluation, classification, and stratification. Am J
Kidney Dis 39[Suppl 1]: 1–266, 2005
98. Meier-Kriesche HU, Baliga R, Kaplan B: Decreased renal
function is a strong risk factor for cardiovascular death
after renal transplantation. Transplantation 75: 1291–1295,
2003
graft failure with recipient blood pressure. Collaborative
100. Rahn KH, Barenbrock M, Fritsche E, Heinecke A, Lippert
J, Schroeder K, Hauser I, Wagner K, Neumayer H: Effect of
nitrendipine on renal function in renal-transplant patients
101. van Riemsdijk IC, Mulder PG, de Fijter JW, Bruijn JA, van
of isradipine (Lomir) results in a better renal function after
kidney transplantation: A double-blind, randomized, pla-
cebo-controlled, multi-center study. Transplantation 70:
122–126, 2000
102. Madsen JK, Sorensen SS, Hansen HE, Pedersen EB: The
effect of felodipine on renal function and blood pressure in
cyclosporin-treated renal transplant recipients during the
first three months after transplantation. Nephrol Dial Trans-
103. Midvdett K, Hartmann A, Fossa A, Fuchhalp P, Nordal KP,
Rootwelt K, Holdaas H: Sustained improvement of renal
graft function for two years in hypertensive renal trans-
plant recipients treated with nifedipine as compared to
104. Mourad G, Ribstein J, Mimran A: Converting-enzyme in-
hibitor versus calcium antagonist in cyclosporine-treated
renal transplant recipients with malignant hypertension. Am J
105. Hernandez D, Latalcada J, Salido E, Linares J, Barragan A,
Lorenzo V, Higueras L, Martin B, Rodriguez A, Laynez I,
Gonzalez-Posada JM, Torres A: Regression of left ventric-
ular hypertrophy by lisinopril after renal transplantation:
106. Hausberg M, Barenbrock M, Hohage H, Muller S, Heiden-
reich S, Rahn KH: ACE inhibitor versus beta-blocker for


