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 accompanying 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.

Clin J Am Soc Nephrol 3: S56–S67, 2008. doi: 10.2215/CJN.03040707

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.

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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

ISSN: 1555-9041/302–0056

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 $P16^{ink4a}$ 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.

Table 1.
Causes of allograft injury[a]

Immunologic (Antigen Dependent)	Nonimmunologic (Antigen Independent)
Cellular immunity	Organ viability
direct *versus* indirect allorecognition	donor senescence
donor–host mismatch	donor age
subclinical inflammation	prolonged cold ischemic time
co-stimulatory signaling	delayed graft function/acute tubular necrosis
inadequacy of immunosuppression	living *versus* deceased
Humoral immunity	reduced renal mass
antibody-mediated rejection	donor brain injury
previous sensitization	Treatment
Infection	drug toxicity
CMV	Recipient factors
BK polyomavirus	lipid disorders
	diabetes
	recurrent disease
	compliance
	hypertension
	obstruction

[a]CMV, cytomegalovirus.

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

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.

Although the focus of injury has been on the T lymphocyte and its contribution to alloimmunity, recent investigations by a number of groups have proposed other novel immune mediators of injury. For example, tertiary lymphoid tissues, ectopic accumulations of lymphoid cells formed in states of chronic inflammation through a process of lymphoid neogenesis, have been associated with autoimmunity and infection (61). In human kidney allografts with TA/IF, tertiary lymphoid tissues have been identified in a number of patients with graft loss (62) and also associated with chronic rejection and alloantibody production (63). However, these nodules have not been seen uniformly in all cases; neither is their role understood in this process of graft loss. Similarly, mast cell infiltration of kidney allografts, noted in acute cellular rejection, is associated with the extent of interstitial fibrosis (64), and the extent of infiltration has been associated with long-term decline as well (65,66). These studies highlight the relatively nonconventional hypotheses about TA/IF development. Although these are interesting associations, the mechanism and functional role of mast cells remain under investigation.

Antibody and Allograft Failure

The predictive value of antidonor alloantibody production on graft outcome has long been emphasized by some groups (reviewed in reference [67]). Early studies demonstrated that preexisting anti-HLA antibodies were associated with worse graft survival (68). Although the relative contribution of class I *versus* class II antibodies is not certain, the combination of both antibodies seems to worsen graft survival significantly in kidney transplant recipients of HLA-mismatched grafts (69). The subsequent development of anti-HLA antibodies after transplantation also denotes significant negative impact on graft outcome (70). This alloantibody has been strongly associated with acute rejection (71), but the independent contribution to chronic graft injury is not entirely known. Despite the increased risk for rejection and graft failure associated with alloantibody (72), the detection of donor-specific antibody (DSA) in individuals has not been consistently demonstrated. Some studies showed that $<$20% of individuals with chronic graft injury had detectable antidonor HLA class II antibody detected by ELISA (73), whereas others noted a strong correlation with anti-HLA antibodies and chronic injury, although antibody may not be donor specific (74), and that the development of alloantibody is also correlated with a strong level of antidonor cellular immunity (75). However, the threshold effect and the need for concomitant cellular response in chronic injury remain unknown.

The existence of alloantibody in the graft is established by detecting C4d, a complement pathway product, within peritubular capillaries of kidney allografts (76,77). Criteria have evolved to identify antibody-mediated rejection and include Cd4 deposition in peritubular capillaries, along with several different histologic phenotypes, combined with the detection of circulating DSA (78). The prevalence of C4d deposition may reflect whether the biopsy is performed for cause or in protocol settings where C4d positivity is seen relatively infrequently (79); however, early detection in the first 6 mo after transplantation is associated with a 50% reduction in long-term graft survival from an average of 8 to 4 yr (80). This finding is specific and not related to other causes of late graft failure such as CNI toxicity or nonspecific IF. The concomitant finding of C4d staining and DSA in some studies suggests a role of antibody in chronic allograft injury (6).

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 heparin sulfate antibodies (88) and anti-perlecan 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 TGF-β 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 $\geq$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 fenofibrates 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.

Altering 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 α-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 regimens. 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 waiting 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 antiapoptotic 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.

Acknowledgments

This research was supported by the National Institute of Allergy, Immunology, and Infectious Diseases (A.M.J.), the Canadian Institutes of Health Research (A.M.J.), and the National Institute of Diabetes and Digestive and Kidney Diseases intramural research program (R.B.M.).

We thank Dr. Monique Cho for critical review of this manuscript.

Disclosures

None.

References

1. Pascual M, Theruvath T, Kawai T, Tolkoff-Rubin N, Cosimi AB: Strategies to improve long-term outcomes after renal transplantation. *N Engl J Med* 346: 580–590, 2002
2. Harris S, Coupes BM, Roberts SA, Roberts IS, Short CD, Brenchley PE: TGF-beta1 in chronic allograft nephropathy following renal transplantation. *J Nephrol* 20: 177–185, 2007
3. Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Allen RD, Chapman JR: The natural history of chronic allograft nephropathy. *N Engl J Med* 349: 2326–2333, 2003
4. Solez K, Axelsen RA, Benediktsson H, Burdick JF, Cohen AH, Colvin RB, Croker BP, Droz D, Dunnill MS, Halloran PF: International standardization of criteria for the histologic diagnosis of renal allograft rejection: The Banff working classification of kidney transplant pathology. *Kidney Int* 44: 411–422, 1993
5. Racusen LC, Solez K, Colvin RB, Bonsib SM, Castro MC, Cavallo T, Croker BP, Demetris AJ, Drachenberg CB, Fogo AB, Furness P, Gaber LW, Gibson IW, Glotz D, Goldberg JC, Grande J, Halloran PF, Hansen HE, Hartley B, Hayry PJ, Hill CM, Hoffman EO, Hunsicker LG, Lindblad AS, Yamaguchi Y: The Banff 97 working classification of renal allograft pathology. *Kidney Int* 55: 713–723, 1999
6. Mauiyyedi S, Pelle PD, Saidman S, Collins AB, Pascual M, Tolkoff-Rubin NE, Williams WW, Cosimi AA, Schneeberger EE, Colvin RB: Chronic humoral rejection: Identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 12: 574–582, 2001
7. Brennan DC, Agha I, Bohl DL, Schnitzler MA, Hardinger KL, Lockwood M, Torrence S, Schuessler R, Roby T, Gaudreault-Keener M, Storch GA: Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. *Am J Transplant* 5: 582–594, 2005
8. Hirsch HH, Brennan DC, Drachenberg CB, Ginevri F, Gordon J, Limaye AP, Mihatsch MJ, Nickeleit V, Ramos E, Randhawa P, Shapiro R, Steiger J, Suthanthiran M, Trofe J: Polyomavirus-associated nephropathy in renal transplantation: Interdisciplinary analyses and recommendations. *Transplantation* 79: 1277–1286, 2005
9. Solez K, Colvin RB, Racusen LC, Sis B, Halloran PF, Birk PE, Campbell PM, Cascalho M, Collins AB, Demetris AJ, Drachenberg CB, Gibson IW, Grimm PC, Haas M, Lerut E, Liapis H, Mannon RB, Marcus PB, Mengel M, Mihatsch MJ, Nankivell BJ, Nickeleit V, Papadimitriou JC, Platt JL, Randhawa P, Roberts I, Salinas-Madriga L, Salomon DR, Seron D, Sheaff M, Weening JJ: Banff '05 Meeting Report: Differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am J Transplant* 7: 518–526, 2007
10. Mengel M, Chapman JR, Cosio FG, Cavaille-Coll MW, Haller H, Halloran PF, Kirk AD, Mihatsch MJ, Nankivell BJ, Racusen LC, Roberts IS, Rush DN, Schwarz A, Seron D, Stegall MD, Colvin RB: Protocol biopsies in renal transplantation: Insights into patient management and pathogenesis. *Am J Transplant* 7: 512–517, 2007
11. Mengel M, Gwinner W, Schwarz A, Bajeski R, Franz I, Brocker V, Becker T, Neipp M, Klempnauer J, Haller H, Kreipe H: Infiltrates in protocol biopsies from renal allografts. *Am J Transplant* 7: 356–365, 2007
12. Cosio FG, Grande JP, Wadei H, Larson TS, Griffin MD, Stegall MD: Predicting subsequent decline in kidney allograft function from early surveillance biopsies. *Am J Transplant* 5: 2464–2472, 2005
13. Moreso F, Ibernon M, Goma M, Carrera M, Fulladosa X, Hueso M, Gil-Vernet S, Cruzado JM, Torras J, Grinyo JM, Seron D: Subclinical rejection associated with chronic allograft nephropathy in protocol biopsies as a risk factor for late graft loss. *Am J Transplant* 6: 747–752, 2006
14. Shishido S, Asanuma H, Nakai H, Mori Y, Satoh H, Kamimaki I, Hataya H, Ikeda M, Honda M, Hasegawa A: The impact of repeated subclinical acute rejection on the progression of chronic allograft nephropathy. *J Am Soc Nephrol* 14: 1046–1052, 2003
15. Cosio FG, Grande JP, Larson TS, Gloor JM, Velosa JA, Textor SC, Griffin MD, Stegall MD: Kidney allograft fibrosis and atrophy early after living donor transplantation. *Am J Transplant* 5: 1130–1136, 2005
16. Mannon RB, Kirk AD: Beyond histology: Novel tools to diagnose allograft dysfunction. *Clin J Am Soc Nephrol* 1: 358–366, 2006
17. Sarwal MM: Chipping into the human genome: Novel

insights for transplantation. *Immunol Rev* 210: 138–155, 2006
18. Schaub S, Wilkins JA, Rush D, Nickerson P: Developing a tool for noninvasive monitoring of renal allografts. *Expert Rev Proteomics* 3: 497–509, 2006
19. Flechner SM, Goldfarb D, Modlin C, Feng J, Krishnamurthi V, Mastroianni B, Savas K, Cook DJ, Novick AC: Kidney transplantation without calcineurin inhibitor drugs: A prospective, randomized trial of sirolimus versus cyclosporine. *Transplantation* 74: 1070–1076, 2002
20. Park W, Griffin M, Grande JP, Cosio F, Stegall MD: Molecular evidence of injury and inflammation in normal and fibrotic renal allografts one year posttransplant. *Transplantation* 83: 1466–1476, 2007
21. Saurina A, Campistol JM, Lario S, Oppenheimer F, Diekmann F: Conversion from calcineurin inhibitors to sirolimus in kidney transplant patients reduces the urinary transforming growth factor-beta1 concentration. *Transplant Proc* 39: 2138–2141, 2007
22. Cheng O, Thuillier R, Sampson E, Schultz G, Ruiz P, Zhang X, Yuen PS, Mannon RB.: Connective tissue growth factor is a biomarker and mediator of kidney allograft fibrosis. *Am J Transplant* 6: 2292–2306, 2006
23. Thuillier R, Mannon RB: The immunology of chronic allograft injury. N.l Ahsan. *Eurekah Bioscience Database* Chronic Allograft Failure: Natural History, Pathogenesis, Diagnosis and Management, Landes Biosciences, 2007, http://www.eurekah.com/chapter/3237
24. Csencsits K, Wood SC, Lu G, Faust SM, Brigstock D, Eichwald EJ, Orosz CG, Bishop DK: Transforming growth factor beta-induced connective tissue growth factor and chronic allograft rejection. *Am J Transplant* 6: 959–966, 2006
25. Mannon RB, Kopp JB, Ruiz P, Griffiths R, Bustos M, Platt JL, Klotman PE, Coffman TM: Chronic rejection of mouse kidney allografts. *Kidney Int* 55: 1935–1944, 1999
26. Mannon RB, Doyle C, Griffiths R, Bustos M, Platt JL, Coffman TM: Altered intragraft immune responses and improved renal function in MHC class II-deficient mouse kidney allografts. *Transplantation* 69: 2137–2143, 2000
27. Coupes BM, Newstead CG, Short CD, Brenchley PE: Transforming growth factor beta 1 in renal allograft recipients. *Transplantation* 57: 1727–1731, 1994
28. Gaciong Z, Koziak K, Religa P, Lisiecka A, Morzycka-Michalik M, Rell K, Kozlowska-Boszko B, Lao M: Increased expression of growth factors during chronic rejection of human kidney allograft. *Transplant Proc* 27: 928–929, 1995
29. Tikkanen JM, Koskinen PK, Lemstrom KB: Role of endogenous endothelin-1 in transplant obliterative airway disease in the rat. *Am J Transplant* 4: 713–720, 2004
30. Wahab NA, Mason RM: A critical look at growth factors and epithelial-to-mesenchymal transition in the adult kidney: Interrelationships between growth factors that regulate EMT in the adult kidney. *Nephron Exp Nephrol* 104: e129–e134, 2006
31. Melk A, Schmidt BM, Vongwiwatana A, Rayner DC, Halloran PF: Increased expression of senescence-associated cell cycle inhibitor p16INK4a in deteriorating renal transplants and diseased native kidney. *Am J Transplant* 5: 1375–1382, 2005
32. Pagtalunan ME, Olson JL, Tilney NL, Meyer TW: Late consequences of acute ischemic injury to a solitary kidney. *J Am Soc Nephrol* 10: 366–373, 1999
33. Bonegio R, Lieberthal W: Role of apoptosis in the pathogenesis of acute renal failure. *Curr Opin Nephrol Hypertens* 11: 301–308, 2002
34. Ortiz A, Lorz C, Catalan MP, Danoff TM, Yamasaki Y, Egido J, Neilson EG: Expression of apoptosis regulatory proteins in tubular epithelium stressed in culture or following acute renal failure. *Kidney Int* 57: 969–981, 2000
35. Ortiz A: Nephrology forum: Apoptotic regulatory proteins in renal injury. *Kidney Int* 58: 467–485, 2000
36. Du C, Guan Q, Yin Z, Masterson M, Zhong R, Jevnikar AM: Renal tubular epithelial cell apoptosis by Fas-FasL-dependent self-injury can augment renal allograft injury. *Transplant Proc* 35: 2481–2482, 2003
37. Du C, Jiang J, Guan Q, Yin Z, Masterson M, Parbtani A, Zhong R, Jevnikar AM: Renal tubular epithelial cell self-injury through Fas/Fas ligand interaction promotes renal allograft injury. *Am J Transplant* 4: 1583–1594, 2004
38. Boonstra JG, van der Woude FJ, Wever PC, Laterveer JC, Daha MR, Van Kooten C: Expression and function of Fas (CD95) on human renal tubular epithelial cells. *J Am Soc Nephrol* 8: 1517–1524, 1997
39. Wever PC, Boonstra JG, Laterveer JC, Hack CE, van der Woude FJ, Daha MR, ten Berge IJ: Mechanisms of lymphocyte-mediated cytotoxicity in acute renal allograft rejection. *Transplantation* 66: 259–264, 1998
40. Schelling JR, Cleveland RP: Involvement of Fas-dependent apoptosis in renal tubular epithelial cell deletion in chronic renal failure. *Kidney Int* 56: 1313–1316, 1999
41. Cappellesso S, Valentin JF, Giraudeau B, Boulanger MD, Al Najjar A, Buchler M, Halimi JM, Nivet H, Bardos P, Lebranchu Y, Watier H: Association of donor TNFRSF6 (FAS) gene polymorphism with acute rejection in renal transplant patients: A case-control study. *Nephrol Dial Transplant* 19: 439–443, 2004
42. Du C, Wang S, Diao H, Guan Q, Zhong R, Jevnikar AM: Increasing resistance of tubular epithelial cells to apoptosis by shRNA therapy ameliorates renal ischemia-reperfusion injury. *Am J Transplant* 6: 2256–2267, 2006
43. Zheng X, Zhang X, Sun H, Feng B, Li M, Chen G, Vladau C, Chen D, Suzuki M, Min L, Liu W, Zhong R, Garcia B, Jevnikar A, Min WP: Protection of renal ischemia injury using combination gene silencing of complement 3 and caspase 3 genes. *Transplantation* 82: 1781–1786, 2006
44. Mohib K, Guan Q, Diao H, Du C, Jevnikar AM: Pro-apoptotic activity of indoleamine 2,3-dioxygenase (IDO) expressed in renal tubular epithelial cells. *Am J Physiol Renal Physiol* 293: F801–F812, 2007
45. Du C, Guan Q, Diao H, Yin Z, Jevnikar AM: Nitric oxide induces apoptosis in renal tubular epithelial cells through activation of caspase-8. *Am J Physiol Renal Physiol* 290: F1044–F1054, 2006
46. Deveraux QL, Roy N, Stennicke HR, Van Arsdale T, Zhou Q, Srinivasula SM, Alnemri ES, Salvesen GS, Reed JC: IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. *EMBO J* 17: 2215–2223, 1998
47. Kalluri R, Neilson EG: Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest* 112: 1776–1784, 2003
48. Zeisberg M, Shah AA, Kalluri R: Bone morphogenic protein-7 induces mesenchymal to epithelial transition in

adult renal fibroblasts and facilitates regeneration of injured kidney. *J Biol Chem* 280: 8094–8100, 2005
49. Grimm PC, Nickerson P, Jeffery J, Savani RC, Gough J, McKenna RM, Stern E, Rush DN: Neointimal and tubulointerstitial infiltration by recipient mesenchymal cells in chronic renal-allograft rejection. *N Engl J Med* 345: 93–97, 2001
50. Duffield JS, Park KM, Hsiao LL, Kelley VR, Scadden DT, Ichimura T, Bonventre JV: Restoration of tubular epithelial cells during repair of the postischemic kidney occurs independently of bone marrow-derived stem cells. *J Clin Invest* 115: 1743–1755, 2005
51. Duffield JS, Bonventre JV: Kidney tubular epithelium is restored without replacement with bone marrow-derived cells during repair after ischemic injury. *Kidney Int* 68: 1956–1961, 2005
52. Eddy AA: Ramping up endogenous defences against chronic kidney disease. *Nephrol Dial Transplant* 21: 1174–1177, 2006
53. Zeisberg M, Hanai J, Sugimoto H, Mammoto T, Charytan D, Strutz F, Kalluri R: BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nat Med* 9: 964–968, 2003
54. Lin J, Patel SR, Cheng X, Cho EA, Levitan I, Ullenbruch M, Phan SH, Park JM, Dressler GR: Kielin/chordin-like protein, a novel enhancer of BMP signaling, attenuates renal fibrotic disease. *Nat Med* 11: 387–393, 2005
55. Liu Y: Epithelial to mesenchymal transition in renal fibrogenesis: Pathologic significance, molecular mechanism, and therapeutic intervention. *J Am Soc Nephrol* 15: 1–12, 2004
56. Robertson H, Ali S, McDonnell BJ, Burt AD, Kirby JA: Chronic renal allograft dysfunction: The role of T cell-mediated tubular epithelial to mesenchymal cell transition. *J Am Soc Nephrol* 15: 390–397, 2004
57. McMorrow T, Gaffney MM, Slattery C, Campbell E, Ryan MP: Cyclosporine A induced epithelial-mesenchymal transition in human renal proximal tubular epithelial cells. *Nephrol Dial Transplant* 20: 2215–2225, 2005
58. Slattery C, Campbell E, McMorrow T, Ryan MP: Cyclosporine A-induced renal fibrosis: A role for epithelial-mesenchymal transition. *Am J Pathol* 167: 395–407, 2005
59. Vongwiwatana A, Tasanarong A, Rayner DC, Melk A, Halloran PF: Epithelial to mesenchymal transition during late deterioration of human kidney transplants: The role of tubular cells in fibrogenesis. *Am J Transplant* 5: 1367–1374, 2005
60. Hertig A, Verine J, Mougenot B, Jouanneau C, Ouali N, Sebe P, Glotz D, Ancel PY, Rondeau E, Xu-Dubois Y: Risk factors for early epithelial to mesenchymal transition in renal grafts. *Am J Transplant* 6: 2937–2946, 2006
61. Drayton DL, Liao S, Mounzer RH, Ruddle NH: Lymphoid organ development: From ontogeny to neogenesis. *Nat Immunol* 7: 344–353, 2006
62. Kerjaschki D, Regele HM, Moosberger I, Nagy-Bojarski K, Watschinger B, Soleiman A, Birner P, Krieger S, Hovorka A, Silberhumer G, Laakkonen P, Petrova T, Langer B, Raab I: Lymphatic neoangiogenesis in human kidney transplants is associated with immunologically active lymphocytic infiltrates. *J Am Soc Nephrol* 15: 603–612, 2004
63. Thaunat O, Field AC, Dai J, Louedec L, Patey N, Bloch MF, Mandet C, Belair MF, Bruneval P, Meilhac O, Bellon B, Joly E, Michel JB, Nicoletti A: Lymphoid neogenesis in chronic rejection: Evidence for a local humoral alloimmune response. *Proc Natl Acad Sci U S A* 102: 14723–14728, 2005
64. Goto E, Honjo S, Yamashita H, Shomori K, Adachi H, Ito H: Mast cells in human allografted kidney: Correlation with interstitial fibrosis. *Clin Transplant* 16[Suppl 8]: 7–11, 2002
65. Ishida T, Hyodo Y, Ishimura T, Takeda M, Hara I, Fujisawa M: Mast cell numbers and protease expression patterns in biopsy specimens following renal transplantation from living-related donors predict long-term graft function. *Clin Transplant* 19: 817–824, 2005
66. Roberts IS, Brenchley PE: Mast cells: The forgotten cells of renal fibrosis. *J Clin Pathol* 53: 858–862, 2000
67. McKenna RM, Takemoto SK, Terasaki PI: Anti-HLA antibodies after solid organ transplantation. *Transplantation* 69: 319–326, 2000
68. Terasaki PI, Kreisler M, Mickey RM: Presensitization and kidney transplant failures. *Postgrad Med J* 47: 89–100, 1971
69. Susal C, Opelz G: Kidney graft failure and presensitization against HLA class I and class II antigens. *Transplantation* 73: 1269–1273, 2002
70. Terasaki PI, Ozawa M: Predicting kidney graft failure by HLA antibodies: A prospective trial. *Am J Transplant* 4: 438–443, 2004
71. Piazza A, Poggi E, Borrelli L, Servetti S, Monaco PI, Buonomo O, Valeri M, Torlone N, Adorno D, Casciani CU: Impact of donor-specific antibodies on chronic rejection occurrence and graft loss in renal transplantation: Post-transplant analysis using flow cytometric techniques. *Transplantation* 71: 1106–1112, 2001
72. Worthington JE, Martin S, Al Husseini DM, Dyer PA, Johnson RW: Posttransplantation production of donor HLA-specific antibodies as a predictor of renal transplant outcome. *Transplantation* 75: 1034–1040, 2003
73. Supon P, Constantino D, Hao P, Cagle L, Hahn A, Conti DJ, Freed BM: Prevalence of donor-specific anti-HLA antibodies during episodes of renal allograft rejection. *Transplantation* 71: 577–580, 2001
74. Lee PC, Terasaki PI, Takemoto SK, Lee PH, Hung CJ, Chen YL, Tsai A, Lei HY: All chronic rejection failures of kidney transplants were preceded by the development of HLA antibodies. *Transplantation* 74: 1192–1194, 2002
75. Poggio ED, Clemente M, Riley J, Roddy M, Greenspan NS, Dejelo C, Najafian N, Sayegh MH, Hricik DE, Heeger PS: Alloreactivity in renal transplant recipients with and without chronic allograft nephropathy. *J Am Soc Nephrol* 15: 1952–1960, 2004
76. Feucht HE, Felber E, Gokel MJ, Hillebrand G, Nattermann U, Brockmeyer C, Held E, Riethmuller G, Land W, Albert E.: Vascular deposition of complement-split products in kidney allografts with cell-mediated rejection. *Clin Exp Immunol* 86: 464–470, 1991
77. Feucht HE, Schneeberger H, Hillebrand G, Burkhardt K, Weiss M, Riethmuller G, Land W, Albert E: Capillary deposition of C4d complement fragment and early renal graft loss. *Kidney Int* 43: 1333–1338, 1993
78. Racusen LC, Colvin RB, Solez K, Mihatsch MJ, Halloran PF, Campbell PM, Cecka MJ, Cosyns JP, Demetris AJ, Fishbein MC, Fogo A, Furness P, Gibson IW, Glotz D, Hayry P, Hunsickern L, Kashgarian M, Kerman R, Magil AJ, Montgomery R, Morozumi K, Nickeleit V, Randhawa

P, Regele H, Seron D, Seshan S, Sund S, Trpkov K: Antibody-mediated rejection criteria: An addition to the Banff 97 classification of renal allograft rejection. *Am J Transplant* 3: 708–714, 2003
79. Mengel M, Bogers J, Bosmans JL, Seron D, Moreso F, Carrera M, Gwinner 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, Land W, Feucht HE: Impact of humoral alloreactivity early after transplantation on the long-term survival of renal allografts. *Kidney Int* 59: 334–341, 2001
81. Habib R, Zurowska A, Hinglais N, Gubler MC, Antignac C, Niaudet P, Broyer M, Gagnadoux MF: A specific glomerular lesion of the graft: Allograft glomerulopathy. *Kidney Int Suppl* 42: S104–S111, 1993
82. Maryniak RK, First MR, Weiss MA: Transplant glomerulopathy: Evolution of morphologically distinct changes. *Kidney Int* 27: 799–806, 1985
83. Sijpkens YW, Joosten SA, Wong MC, Dekker FW, Benediktsson H, Bajema IM, Bruijn JA, Paul LC: Immunologic risk factors and glomerular C4d deposits in chronic transplant glomerulopathy. *Kidney Int* 65: 2409–2418, 2004
84. Gloor JM, Sethi S, Stegall MD, Park WD, Moore SB, Degoey S, Griffin MD, Larson TS, Cosio FG: Transplant glomerulopathy: Subclinical incidence and association with alloantibody. *Am J Transplant* 7: 2124–2132, 2007
85. Regele H, Bohmig GA, Habicht A, Gollowitzer D, Schillinger M, Rockenschaub S, Watschinger B, Kerjaschki D, Exner M: Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: A contribution of humoral immunity to chronic allograft rejection. *J Am Soc Nephrol* 13: 2371–2380, 2002
86. Joosten SA, Sijpkens YW, van Ham V, Trouw LA, van der Vlag J, van den Heuvel B, van Kooten C, Paul LC: Antibody response against the glomerular basement membrane protein agrin in patients with transplant glomerulopathy. *Am J Transplant* 5: 383–393, 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 glomerulopathy. *Am J Transplant* 3: 1116–1120, 2003
88. van den BJ, van den Heuvel LP, Bakker MA, Veerkamp JH, Assmann KJ, Berden JH: A monoclonal antibody against GBM heparan sulfate induces an acute selective proteinuria in rats. *Kidney Int* 41: 115–123, 1992
89. JJoosten 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 renal allograft rejection in the rat. *Am J Pathol* 160: 1301–1310, 2002
90. Ball B, Mousson C, Ratignier C, Guignier F, Glotz D, Rifle G: Antibodies to vascular endothelial cells in chronic rejection of renal allografts. *Transplant Proc* 32: 353–354, 2000
91. Dragun D, Muller DN, Brasen JH, Fritsche L, Nieminen-Kelha M, Dechend R, Kintscher U, Rudolph B, Hoebeke J, Eckert D, Mazak I, Plehm R, Schonemann C, Unger T, Budde K, Neumayer HH, Luft FC, Wallukat G: Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. *N Engl J Med* 352: 558–569, 2005
92. Zou Y, Stastny P, Susal C, Dohler B, Opelz G: Antibodies against MICA antigens and kidney-transplant rejection. *N Engl J Med* 357: 1293–1300, 2007
93. Panigrahi A, Gupta N, Siddiqui JA, Margoob A, Bhowmik 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* 68: 362–367, 2007
94. Cailhier JF, Laplante P, Hebert MJ: Endothelial apoptosis and chronic transplant vasculopathy: Recent results, novel mechanisms. *Am J Transplant* 6: 247–253, 2006
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, Robertson F, Hughes J, Marson LP: Peritubular capillary rarefaction 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
99. Opelz G, Wujciak T, Ritz E: Association of chronic kidney graft failure with recipient blood pressure. Collaborative Transplant Study. *Kidney Int* 53: 217–222, 1998
100. Rahn KH, Barenbrock M, Fritschka E, Heinecke A, Lippert J, Schroeder K, Hauser I, Wagner K, Neumayer H: Effect of nitrendipine on renal function in renal-transplant patients treated with cyclosporin: A randomised trial. *Lancet* 354: 1415–1420, 1999
101. van Riemsdijk IC, Mulder PG, de Fijter JW, Bruijn JA, van Hooff JP, Hoitsma AJ, Tegzess AM, Weimar W: Addition of isradipine (Lomir) results in a better renal function after kidney transplantation: A double-blind, randomized, placebo-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 Transplant* 13: 2327–2334, 1998
103. Midtvedt K, Hartmann A, Foss A, Fauchald P, Nordal KP, Rootwelt K, Holdaas H: Sustained improvement of renal graft function for two years in hypertensive renal transplant recipients treated with nifedipine as compared to lisinopril. *Transplantation* 72: 1787–1792, 2001
104. Mourad G, Ribstein J, Mimran A: Converting-enzyme inhibitor versus calcium antagonist in cyclosporine-treated renal transplants. *Kidney Int* 43: 419–425, 1993
105. Hernandez D, Lacalzada 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 ventricular hypertrophy by lisinopril after renal transplantation: Role of ACE gene polymorphism. *Kidney Int* 58: 889–897, 2000
106. Hausberg M, Barenbrock M, Hohage H, Muller S, Heidenreich S, Rahn KH: ACE inhibitor versus beta-blocker for

the treatment of hypertension in renal allograft recipients. *Hypertension* 33: 862–868, 1999
107. Heinze G, Mitterbauer C, Regele H, Kramar R, Winkelmayer WC, Curhan GC, Oberbauer R: Angiotensin-converting enzyme inhibitor or angiotensin II type 1 receptor antagonist therapy is associated with prolonged patient and graft survival after renal transplantation. *J Am Soc Nephrol* 17: 889–899, 2006
108. Opelz G, Zeier M, Laux G, Morath C, Dohler B: No improvement of patient or graft survival in transplant recipients treated with angiotensin-converting enzyme inhibitors or angiotensin II type 1 receptor blockers: A collaborative transplant study report. *J Am Soc Nephrol* 17: 3257–3262, 2006
109. Holdaas H, Fellstrom B, Jardine AG, Holme I, Nyberg G, Fauchald P, Gronhagen-Riska C, Madsen S, Neumayer HH, Cole E, Maes B, Ambuhl P, Olsson AG, Hartmann A, Solbu DO, Pedersen TR: Effect of fluvastatin on cardiac outcomes in renal transplant recipients: A multicentre, randomised, placebo-controlled trial. *Lancet* 361: 2024–2031, 2003
110. Kasiske BL, Snyder JJ, Gilbertson D, Matas AJ: Diabetes mellitus after kidney transplantation in the United States. *Am J Transplant* 3: 178–185, 2003
111. Ojo AO, Meier-Kriesche HU, Hanson JA, Leichtman AB, Cibrik D, Magee JC, Wolfe RA, Agodoa LY, Kaplan B: Mycophenolate mofetil reduces late renal allograft loss independent of acute rejection. *Transplantation* 69: 2405–2409, 2000
112. Kasiske BL, Chakkera HA, Louis TA, Ma JZ: A meta-analysis of immunosuppression withdrawal trials in renal transplantation. *J Am Soc Nephrol* 11: 1910–1917, 2000
113. Vincenti F, Larsen C, Durrbach A, Wekerle T, Nashan B, Blancho G, Lang P, Grinyo J, Halloran PF, Solez K, Hagerty D, Levy E, Zhou W, Natarajan K, Charpentier B: Costimulation blockade with belatacept in renal transplantation. *N Engl J Med* 353: 770–781, 2005
114. Larson TS, Dean PG, Stegall MD, Griffin MD, Textor SC, Schwab TR, Gloor JM, Cosio FG, Lund WJ, Kremers WK, Nyberg SL, Ishitani MB, Prieto M, Velosa JA: Complete avoidance of calcineurin inhibitors in renal transplantation: A randomized trial comparing sirolimus and tacrolimus. *Am J Transplant* 6: 514–522, 2006
115. Kirk AD, Hale DA, Mannon RB, Kleiner DE, Hoffmann SC, Kampen RL, Cendales LK, Tadaki DK, Harlan DM, Swanson SJ: Results from a human renal allograft tolerance trial evaluating the humanized CD52-specific monoclonal antibody alemtuzumab (CAMPATH-1H). *Transplantation* 76: 120–129, 2003
116. Kaplan B, Schold J, Srinivas T, Womer K, Foley DP, Patton P, Howard R, Meier-Kriesche HU: Effect of sirolimus withdrawal in patients with deteriorating renal function. *Am J Transplant* 4: 1709–1712, 2004
117. Oberbauer R, Segoloni G, Campistol JM, Kreis H, Mota A, Lawen J, Russ G, Grinyo JM, Stallone G, Hartmann A, Pinto JR, Chapman J, Burke JT, Brault Y, Neylan JF: Early cyclosporine withdrawal from a sirolimus-based regimen results in better renal allograft survival and renal function at 48 months after transplantation. *Transpl Int* 18: 22–28, 2005
118. Stallone G, Di Paolo S, Schena A, Infante B, Grandaliano G, Battaglia M, Gesualdo L, Schena FP: Early withdrawal of cyclosporine A improves 1-year kidney graft structure and function in sirolimus-treated patients. *Transplantation* 75: 998–1003, 2003
119. Flechner SM, Kurian SM, Solez K, Cook DJ, Burke JT, Rollin H, Hammond JA, Whisenant T, Lanigan CM, Head SR, Salomon DR: De novo kidney transplantation without use of calcineurin inhibitors preserves renal structure and function at two years. *Am J Transplant* 4: 1776–1785, 2004
120. Groth CG, Backman L, Morales JM, Calne R, Kreis H, Lang P, Touraine JL, Claesson K, Campistol JM, Durand D, Wramner L, Brattstrom C, Charpentier B: Sirolimus (rapamycin)-based therapy in human renal transplantation: Similar efficacy and different toxicity compared with cyclosporine. Sirolimus European Renal Transplant Study Group. *Transplantation* 67: 1036–1042, 1999
121. Kreis H, Cisterne JM, Land W, Wramner L, Squifflet JP, Abramowicz D, Campistol JM, Morales JM, Grinyo JM, Mourad G, Berthoux FC, Brattstrom C, Lebranchu Y, Vialtel P: Sirolimus in association with mycophenolate mofetil induction for the prevention of acute graft rejection in renal allograft recipients. *Transplantation* 69: 1252–1260, 2000
122. Morales JM, Wramner L, Kreis H, Durand D, Campistol JM, Andres A, Arenas J, Negre E, Burke JT, Groth CG: Sirolimus does not exhibit nephrotoxicity compared to cyclosporine in renal transplant recipients. *Am J Transplant* 2: 436–442, 2002
123. Stallone G, Infante B, Schena A, Battaglia M, Ditonno P, Loverre A, Gesualdo L, Schena FP, Grandaliano G: Rapamycin for treatment of chronic allograft nephropathy in renal transplant patients. *J Am Soc Nephrol* 16: 3755–3762, 2005
124. Wali RK, Mohanlal V, Ramos E, Blahut S, Drachenberg C, Papadimitriou J, Dinits M, Joshi A, Philosophe B, Foster C, Cangro C, Nogueira J, Cooper M, Bartlett ST, Weir MR: Early withdrawal of calcineurin inhibitors and rescue immunosuppression with sirolimus-based therapy in renal transplant recipients with moderate to severe renal dysfunction. *Am J Transplant* 7: 1572–1583, 2007
125. Mannon RB: Therapeutic targets in the treatment of allograft fibrosis. *Am J Transplant* 6: 867–875, 2006
126. Pihlajaniemi T, Myllyla R, Kivirikko KI: Prolyl 4-hydroxylase and its role in collagen synthesis. *J Hepatol* 13[Suppl 3]: S2–S7, 1991
127. Prockop DJ, Kivirikko KI: Collagens: Molecular biology, diseases, and potentials for therapy. *Annu Rev Biochem* 64: 403–434, 1995
128. Franceschini N, Cheng O, Zhang X, Ruiz P, Mannon RB: Inhibition of prolyl-4-hydroxylase ameliorates chronic rejection of mouse kidney allografts. *Am J Transplant* 3: 396–402, 2003
129. Lutz J, Yao Y, Song E, Antus B, Hamar P, Liu S, Heemann U: Inhibition of matrix metalloproteinases during chronic allograft nephropathy in rats. *Transplantation* 79: 655–661, 2005
130. Kuenzli S, Tran C, Saurat JH: Retinoid receptors in inflammatory responses: A potential target for pharmacology. *Curr Drug Targets Inflamm Allergy* 3: 355–360, 2004
131. Kiss E, Adams J, Grone HJ, Wagner J: Isotretinoin ameliorates renal damage in experimental acute renal allograft rejection. *Transplantation* 76: 480–489, 2003
132. Adams J, Kiss E, Arroyo AB, Bonrouhi M, Sun Q, Li Z, Gretz N, Schnitger A, Zouboulis CC, Wiesel M, Wagner J, Nelson PJ, Grone HJ: 13-cis retinoic acid inhibits develop-

ment and progression of chronic allograft nephropathy. *Am J Pathol* 167: 285–298, 2005

133. Azuma H, Takahara S, Matsumoto K, Ichimaru N, Wang JD, Moriyama T, Waaga AM, Kitamura M, Otsuki Y, Okuyama A, Katsuoka Y, Chandraker A, Sayegh MH, Nakamura T: Hepatocyte growth factor prevents the development of chronic allograft nephropathy in rats. *J Am Soc Nephrol* 12: 1280–1292, 2001
134. Mizui M, Isaka Y, Takabatake Y, Mizuno S, Nakamura T, Ito T, Imai E, Hori M: Electroporation-mediated HGF gene transfer ameliorated cyclosporine nephrotoxicity. *Kidney Int* 65: 2041–2053, 2004