

Chronic Allograft Nephropathy

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Summary: Chronic allograft nephropathy (CAN) remains the Achilles heel of renal transplantation. In spite of the significant strides achieved in one-year renal allograft survival with newer immunosuppressant strategies, the fate of long-term renal allograft survival remains unchanged. The number of renal transplant recipients returning to dialysis has doubled in the past decade. This is especially important since these patients pose a significantly increased likelihood of dying while on the waiting list for retransplantation, due to increasing disparity between donor organ availability versus demand and longer waiting time secondary to heightened immunologic sensitization from their prior transplants. In this review we analyze the latest literature in detail and discuss the definition, natural history, pathophysiology, alloantigen dependent and independent factors that play a crucial role in CAN and the potential newer therapeutic targets on the horizon. This article highlights the importance of early identification and careful management of all the potential contributing factors with particular emphasis on prevention rather than cure of CAN as the core management strategy. *Semin Nephrol* 27:414-429 © 2007 Elsevier Inc. All rights reserved.

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Chronic allograft nephropathy (CAN) remains the single most prevalent cause of late transplant kidney failure. Although the 1-year graft survival rate has improved steadily to more than 90% over the past few decades with better immunosuppressant strategies, this has not been translated successfully into long-term kidney allograft survival.^{1,2} The 5-year overall adjusted graft survival of expanded criteria donor (ECD), non-ECD, and living donor kidney recipients remains at 54%, 69%, and 80% respectively (Fig. 1).³ Hence, the number of kidney transplant recipients returning to dialysis has nearly doubled in the past decade, adding to the already overburdened list of patients awaiting kidney transplantation. The median time to (re)transplantation for these patients is nearly twice that of patients awaiting a

first transplant, likely reflecting an increased degree of immunologic sensitization. In addition, as the death rate on the waiting list increases with age, these patients have a significantly higher chance of dying while waiting for a kidney transplant.

Chronic allograft dysfunction in kidney transplant recipients is a clinically defined condition characterized by declining renal function (as evidenced by a slow, progressive decrease in glomerular filtration rate), associated with de novo or aggravated hypertension and worsening proteinuria. Reliance on serum creatinine alone as a marker of renal function can underestimate the severity and rate of functional decline,⁴ particularly when the glomerular filtration rate is between 30 and 70 mL/min (which encompasses the majority of kidney transplant recipients). New-onset proteinuria of greater than 0.5 g/24 h or worsening proteinuria in a transplant patient should raise the suspicion of chronic allograft dysfunction, once other causes such as recurrence of primary disease or new-onset de novo disease are excluded.

Histologically CAN is characterized by interstitial fibrosis, tubular atrophy, and variably is

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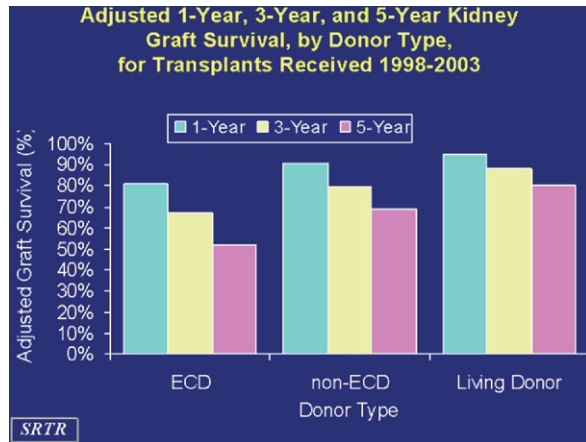


Figure 1. SRTR data showing decreased graft survival by 5 years in ECD, non-ECD, and living donor kidneys. Reprinted with permission from the Scientific Registry of Transplant Recipients (SRTR).

associated with fibrointimal thickening of the arteries within the transplanted kidney, as was originally agreed on by the Banff classification of 1997.^{5,6} The term *CAN* largely has superseded the original term *chronic rejection* and emphasizes that a variety of conditions, in addition to an immunologic insult, could contribute to development of the clinicopathologic syndrome of CAN. The latest meeting of the Banff consensus group (2005) worked towards clearly delineating specific diagnostic entities such as chronic active antibody-mediated rejection, chronic active cell-mediated rejection, calcineurin inhibitor (CNI) toxicity, polyoma (BK) viral nephropathy, and so forth, clearly from a nonspecific fibrotic subtype of CAN. The most widely used classification system applied to chronic lesions is still based on the Banff 1997 system, which semiquantitatively grades the degree of allograft injury according to the severity of interstitial fibrosis and tubular injury, from grades I to III, correlating with mild to severe injury. Kidney allografts with ongoing chronic rejection frequently also show transplant vasculopathy or glomerulopathy in the background of CAN.⁷ The glomerulopathy and arteriolar hyalinosis also can be graded from I to III, but are not necessary for a diagnosis of CAN to be made. Transplant vasculopathy is characterized by fibrointimal thickening of arteries, breaks in the elastic layer, and vessel wall infiltration

with inflammatory cells. Small peritubular capillaries can show basement membrane layering as a marker of transplant capillaropathy.

NATURAL HISTORY OF CAN

Serial protocol biopsy studies have shown that CAN is an almost universal, progressive, time-dependent finding.³ It is commonly a composite end point resulting from multiple insults of both donor and recipient origins such as ischemia-reperfusion injury, hyperfiltration injury secondary to inadequate renal mass, acute/chronic rejection, recurrent or de novo glomerulonephritis, hypertension and metabolic injury such as diabetes and hypercholesterolemia, immunosuppression-related side effects including CNI toxicity, and secondary viral and bacterial infections.

The high prevalence of CAN at 2 years as reported by the FK 506 Kidney transplant study group,⁸ which compared treatment with cyclosporine or tacrolimus, showed that 72.3% and 62% of biopsy specimens, respectively, showed CAN. Apart from CNI toxicity, older donor age and acute rejection episodes also were associated with the development of CAN. Ten-year follow-up data on recipients of simultaneous kidney-pancreas transplants who had yearly renal protocol biopsy examinations,³ yielded an elegant picture of the longitudinal histologic evolution of CAN in CNI-treated patients. Two thirds of the fibrosis present by 10 years already had appeared by 1 year, during which time interstitial fibrosis exceeded the development of tubular atrophy. Between 1 and 10 years after transplantation, tubular atrophy and interstitial fibrosis progressed simultaneously, with dominant features of chronic CNI toxicity. Classic features of CNI toxicity such as striped interstitial fibrosis and arteriolar hyalinosis with or without calcification developed almost universally by 10 years.

Arteriolar hyalinosis appeared between 3 and 12 months after transplantation and was associated very strongly with CNI dose. By 10 years, 75% of the patients had arteriolar hyalinosis, and in most patients hyalinosis preceded the onset of hypertension. Glomerulosclerosis, which represents the final and irreversible destruction of nephrons, was seen in 2 phases. Early glomerulo-

sclerosis resulted from interstitial fibrosis with development of periglomerular fibrosis and atubular glomeruli. The later phase of glomerular destruction was secondary to high-grade arteriolar hyalinosis, resulting in ischemic glomeruli. Thus, functional failure of the transplanted kidney results from the cumulative loss of individual nephrons, combined with additional disruption of its internal structural integrity. However, the precise natural history of this sequence of events likely is influenced by a variety of factors including donor age, type (live versus deceased donor type), degree of mismatch, prior sensitization, type of immunosuppressant used, and so forth.

PATHOPHYSIOLOGY OF ALLOGRAFT DAMAGE

In the earlier days of transplantation, chronic allograft dysfunction was thought to be purely a result of ongoing immunologic injury, however, the high failure of transplants between identical twins⁹ suggested that factors other than a purely immunologic response were at play. Similarly, animal models of kidney transplantation revealed that long-term renal isografts develop functional and morphologic changes that mimic chronic allograft nephropathy,¹⁰ again suggesting that in addition to alloantigen-dependent factors, alloantigen-independent factors also influenced the development of chronic rejection. Although these factors usually co-exist and are mutually additive in enhancing the damage to the allograft, for the purpose of this review we discuss these mechanisms separately.

Alloantigen Dependent

The role of alloantigen-dependent factors in the development of chronic rejection is undisputable. This fact is supported strongly by the fact that chronic rejection is associated with the degree of histoincompatibility between the recipient and donor, sensitization (pretransplant or posttransplant), acute rejection episodes, and inadequacy or noncompliance with immunosuppression. The immunologic mechanisms that underlie renal allograft rejection are heterogeneous and involve both the cellular and humoral limbs of the adaptive immune response.

ANTIGEN PRESENTATION AND CO-STIMULATION

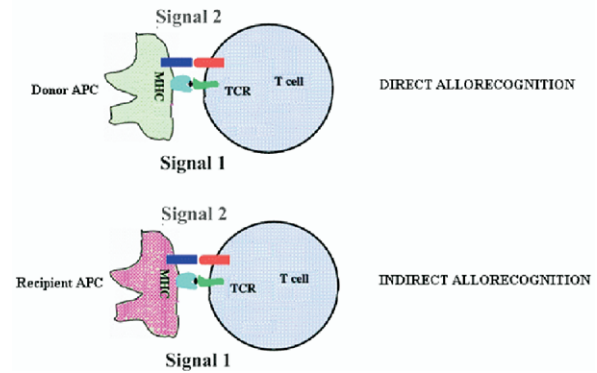


Figure 2. Two different mechanisms of allorecognition between T cells and APCs. Direct allorecognition involves presentation of foreign HLA by donor APCs to the recipient T cells. In contrast, indirect allorecognition involves presentation of foreign HLA sequence by recipient APCs to their T cells. Antigen delivers signal 1 to alloantigen-specific T cells through the T-cell antigen receptor. The costimulatory signal 2 is dependent on the interaction of cell surface receptors with their ligands, typically on APCs (dendritic cells, B cells, monocytes, and macrophages) and is not antigen specific.

Until recently, most studies on the mechanism of renal allograft rejection have focused predominantly on the central role of T-cell-mediated mechanisms that lead to allograft injury and destruction. In recent years, however, with the identification of the complement fragment complement 4d (C4d) and the high correlation of anti-human leukocyte antigen (HLA) donor-specific antibodies in the serum of patients with failing transplant kidney, there has been a renewed interest in the role of humoral response in the development of CAN.

Cellular Immune Response

A key factor driving the underlying pathophysiology of chronic rejection in organ transplants is a persistent T-cell-mediated alloimmune response. T-cell recognition of alloantigen in the presence of appropriate costimulatory signal is of central importance in initiating a directed alloimmune response. Two major modes of alloantigen recognition have been described. In the direct pathway (Fig. 2), recipient T lymphocytes recognize foreign major histocompatibility molecules presented by donor antigen presenting (APC) cells. Although in the indirect pathway, allorecognition occurs when donor

histocompatibility molecules are internalized, processed, and presented as peptides by self-APCs.¹¹ It has been shown that the direct pathway is a predominant player during acute rejection and initiation of the anti-allograft response, whereas the indirect pathway is thought to play a greater role in later forms of alloresponse that lead to chronic rejection.¹² Also, a shift in T-cell response toward different allopeptides of the donor graft over time, a process termed *epitope spreading* or *shifting*, can occur in renal transplant recipients undergoing chronic rejection.^{12,13}

T-cell costimulation plays a critical role in deciding the fate of a T-cell response to antigen. During this interaction the T cell receives signal 1, provided by T-cell receptor engagement of the antigen presented on the major histocompatibility complex on the APC, this provides the specificity of the immune response. Costimulatory signals are not antigen specific and are dependent on the interaction of T-cell surface receptors with their ligands on APCs. Both positive and negative costimulatory pathways have been described. Delivery of positive co-

stimulatory stimulus to the T cell triggers cytokine production, alloantigen-specific clonal expansion, and acquisition of a memory/effector phenotype capable of mediating a sustained immune response.¹⁴ In contrast, negative costimulatory signals can lead to inhibition of T-cell proliferation and cytokine production, thereby causing anergy, apoptosis, or induction of regulatory cells.¹⁵

Interruption of several positive and enhancement of many negative costimulatory pathways (Fig. 3) have been shown to prevent chronic rejection in rodent models of transplantation, with blockade of the CD28/CTLA4:B7 and CD40:CD40L pathways showing the most potency in nonhuman primate kidney transplant studies. CTLA4Ig is a recombinant fusion protein that blocks the ligation of CD28 and B7 interaction, a crucial costimulatory signal for T-cell activation. LEA29Y (belatacept), a high-affinity variant of CTLA4Ig, has shown great promise as a combination agent with anti-CD40 in nonhuman primate studies.^{16,17} Importantly, this agent has now entered stage II human clinical trials as a calcineurin-sparing agent in human renal transplantation.¹⁸ One-year posttransplantation studies show that belatacept did not appear to be inferior to cyclosporine in preventing acute rejection, but may preserve the glomerular filtration rate and reduce the rate of chronic allograft nephropathy.¹⁹ Studies looking at the ability of belatacept as a long-term substitute for CNIs in kidney transplant recipients with biopsy evidence of CAN as outcome are being planned.

Humoral Immune Response

Association of preformed antibodies and hyperacute rejection of the allograft has been well recognized since the earliest days of transplantation and is the reason that recipients' serum is tested against donor cells. Experimental evidence that the humoral response contributes to the development of chronic immunologic injury also has been reported. Russell et al²⁰ showed that cardiac allografts placed in immunodeficient severe combined immunodeficiency (SCID) mice develop the vascular lesions of chronic rejection with repeated doses of donor-specific antibodies. In the clinical setting, C4d deposition (as a marker of anti-HLA

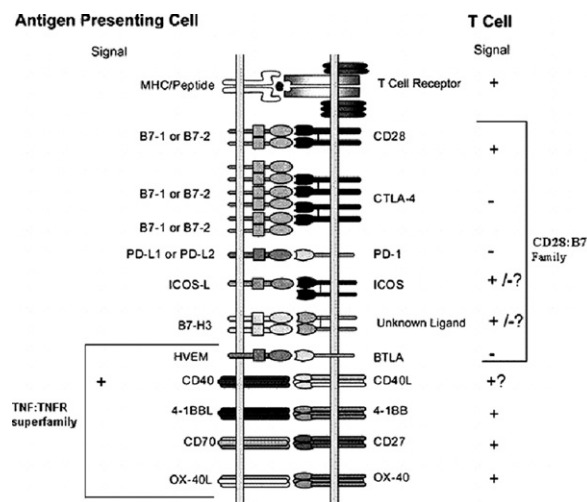


Figure 3. Costimulatory molecules are grouped broadly into CD28:B7 and the tumor necrosis factor: tumor necrosis factor receptor (TNF:TNFR) families based on their structural characteristics. Positive costimulation in concert with alloantigen-specific T-cell receptor engagement results in T-cell proliferation, differentiation, activation, and cytokine reduction. In contrast, negative costimulatory signals during T-cell receptor alloantigen-specific T-cell engagement leads to T-cell anergy/apoptosis.

antibodies) in the peritubular capillaries of the kidney have been correlated with both acute rejection and with slowly failing kidney allografts. C4d is one of the split products generated during complement activation of the classic pathway, triggered by antidonor antibodies. It binds rapidly to the target structures and remains bound for several days or weeks. C4d staining may be a more sensitive and specific marker than the presence of circulating antibodies because antibodies may be removed from the circulation through adsorption to the graft.²¹ Mauiyyedi et al²² observed that 61% of patients with classic features of chronic rejection had positive peritubular capillary C4d stain and, of those, 88% (in those with a serum sample obtained at the time of biopsy) had circulating donor-specific antibodies. This is in contrast to C4d positivity in only 1 of 21 biopsy specimens that showed CNI toxicity. Acute rejection with positive C4d staining is more likely to result from (32%-44%) and also predict the subsequent onset of transplant glomerulopathy and chronic rejection compared with C4d-negative acute rejection (8%-14%).²³

Both HLA and the less well defined non-HLA (major histocompatibility complex class I-related chain A [MICA], antiendothelial, and so forth) antibodies have been shown to be implicated strongly in both acute and chronic rejection. In contrast to pre-existing (anamnestic) alloantibodies, which are present in large titers and cause rapid graft destruction, the development of antibodies against the allograft posttransplant is thought to cause injury through repetitive waves of gradual injury and repair steps contributing to an atypical morphologic picture of duplication of glomerular basement membrane and peritubular capillary membrane multilayering.²⁴ In a prospective study more than 2,000 kidney transplant recipients were tested for the presence of anti-HLA antibodies. Two years after the testing 15.1% of grafts failed in those patients who had developed antibodies posttransplant compared with a 6.8% graft failure rate in those without antibodies.²⁵ The use of newer, more sensitive assays such as flow cytometry and Luminex (Austin, TX) has led to an increased ability to define highly sensitized patients both pretransplant and posttransplant

and identify even low titers of donor-specific antibodies in patients with antibody-mediated rejection. However, currently there is no commercially available method available for the reliable and reproducible detection of non-HLA antibodies. Panel reactive antibody (PRA) testing by the cytotoxicity assay still may have the greatest predictive value of long-term graft function because it detects antibodies against both HLA and non-HLA antigens.

Currently available effective therapeutic measures against antibody-mediated allograft injury include switching to tacrolimus and mycophenolate combination treatment, high-dose intravenous immunoglobulins (IVIg), rituximab (anti-CD20 monoclonal antibody directed against B cells), plasmapheresis, and immunoadsorption. However the treatment strategy for grafts with C4d stain positivity without accompanying acute rejection is currently not clear. Grafts in patients with higher antibody titers seem to have a survival advantage with tacrolimus and mycophenolate mofetil (MMF) treatment compared with other combinations. The benefits of the use of rituximab in this population currently is being studied in trials such as the National Institutes of Health-sponsored Clinical Trials in Organs Transplantation study.

Acute Rejection Episodes

Acute rejection (AR) is the single most important risk factor for long-term allograft outcome and a major determinant of CAN. However, not all AR episodes lead to chronic rejection. Type, severity, frequency, time of occurrence, and response to antirejection treatment determine outcome. Acute vascular rejection has a poorer longer-term outcome when compared with tubulointerstitial rejection.^{26,27} Multiple AR episodes also are associated with a greater risk of developing CAN.^{28,29} CAN has been shown to be significantly more common in patients who had more than 1 AR episode compared with those with only 1 episode (34.8% versus 8.9%, respectively). Likewise, a single late AR episode (occurring >3 months after transplantation) carries a much higher risk of graft failure than early (<3 months) acute rejections (relative risk of graft failure, 5.27 versus 3.07).³⁰

Meier-Kriesche et al³¹ showed that among patients with AR episodes who recovered their

baseline renal function to more than 95% at 1 year posttransplantation had significantly higher 6-year graft survival compared with their counterparts who had less than 75% baseline recovery (72.7% versus 38%, respectively). Matas et al³² studied the half-life of the renal allografts that survived the first year. The half-life of the renal allograft in patients with no AR episodes was 46 years, compared with 25 years for those who had 1 episode during the first year. However, the recipients with more than 1 AR episode had a marked and statistically significant decrease in their half-life of 5 years.

Although there are no definitive prospective studies that show a correlation between decreased acute rejection episodes and better long-term graft survival, acute rejection is recognized to be deleterious to the graft. Measures to prevent and aggressively treat AR episodes should be an important clinical objective.³³

Histocompatibility Match

Major histocompatibility complex (MHC) molecules are the principal targets of the immune response posttransplantation. HLA matching is one of the most important predictors for survival of deceased donor renal allografts.^{34,35} HLA-matched grafts have an estimated half-life of 12.4 years, as compared with 8.6 years for mismatched grafts.³⁶ HLA-DR matching has been shown to have the earliest and most beneficial effect on graft outcome,³⁷ although HLA-A and HLA-B matching also positively impact graft survival.³⁸ Each HLA subtype has a large number of alleles. This large number of HLA alleles can be grouped into a small number of closely related groups that share common HLA-derived antigenic targets; these groups are known as cross-reactive groups (CREGs). CREG matching is associated with a reduced frequency of late acute rejection episodes and better long-term graft survival.^{39,40} According to the United Network for Organ Sharing database, CREG-mismatched patients have a 62% higher risk of developing chronic rejection than HLA- and CREG-matched recipients.⁴¹ However, the use of HLA matching in allocation policies does not provide a complete answer because it may increase waiting times on dialysis⁴² for potential recipients and it dispro-

tionably disadvantages minorities' access to a life-saving transplant.⁴³⁻⁴⁵

PRA

Patients with higher, preformed, non-donor specific anti-HLA antibodies (PRA or highly sensitized) experience an increased number of rejection episodes posttransplant and have a poorer graft survival rate. In transplants among HLA-identical siblings, those who had more than 50% PRA had a 55% 10-year graft survival rate compared with a 63% survival rate in similar identical sibling transplants with a 1% to 50% PRA and 72% survival rate in nonsensitized HLA-identical siblings. Thus, a higher PRA indicates a state of heightened responsiveness to alloantigens or potentially could be an indicator of reactions against non-HLA antigens present on the donor cell panel.^{46,47} In presensitized patients with a higher PRA, desensitization protocols involving high-dose IVIg or low-dose IVIg and plasmapheresis have been shown to significantly increase the chances of being transplanted and improved posttransplant graft outcome.⁴⁸ Some drugs with anti-B-cell activity such as rituximab, MMF, and sirolimus have shown some promise in improving longer-term outcomes in presensitized patients, however, large, well-controlled, multicenter trials are needed to clarify their benefits.

Immunosuppression Adequacy

Adequacy of immunosuppression is the key goal in achieving long-term allograft survival. Inadequate immunosuppression either through patient noncompliance or attempts to reduce existing immunosuppression have been shown to be a strong risk factor for chronic allograft dysfunction.^{49,50} However, on the other hand, overimmunosuppression also limits allograft longevity through drug toxicity (such as CNI toxicity) and by increasing the risk of BK viral nephropathy, cytomegalovirus (CMV) disease, pyelonephritis, and so forth. This is complicated further by the lack of a reliable assay to assess the therapeutic adequacy of overall immunosuppression.

Alloantigen-Independent Factors

Non-alloantigen-dependent factors play an equally important role in the pathogenesis of

CAN. These could be subdivided into donor-related, transplant-associated, and recipient factors. The successful outcome of a transplant could be improved by limiting the number, duration, and severity of these insults to the allograft.

Donor-related factors include donor age, state of donor at the time of harvest (living versus brain dead versus non-heart beating donors), cause of death, their premorbid illnesses (such as hypertension, diabetes mellitus, and so forth), prior renal dysfunction, renal integrity (vasculopathy, glomerulosclerosis, interstitial fibrosis, tubular atrophy, and so forth), and the dose of nephron mass relative to the recipient's requirement.

Peritransplant factors include cold and warm ischemic time and reperfusion-associated injury. Marginal kidneys such as those obtained from extended criteria donors and non-heart beating donors are more susceptible to insult. Although several trials have concluded that kidneys preserved by machine perfusion show better early function than preservation by cold storage in this group,^{51,52} few studies have looked at long-term outcome. A 6-year, single-institution review showed better early and long-term renal function in kidneys preserved with machine perfusion.⁵³

Recipient factors including age, race, cause of native kidney disease, body mass index, smoking, hypertension, hyperlipidemia, diabetes, and compliance with medication among others have been shown to influence graft outcome to a lesser or greater degree. Because it is beyond the scope of this review to discuss all of these in detail, we concentrate on some of the most important factors.

Quality of the Renal Allograft

Although the renal transplant waiting list is steadily growing, the number of standard criteria deceased donors has almost reached a static state in most industrialized countries. This has led to the increasing use of kidneys obtained from ECD and non-heart beating donors. The main common denominator of these grafts may be summarized as an insufficient functional nephron mass for the recipient's requirement. Although these kidneys show a somewhat decreased graft survival, the patient mortality lev-

els are lower than for those patients remaining on dialysis. Lower functional nephron mass, particularly those from pediatric donors, female donors to male recipients, and donors older than age 60 are of particular concern because these kidneys, in theory, have a relatively decreased functional reserve. Experimental and clinical studies have confirmed this concept. In rat experiments, functional mass reduced renal allografts and isografts show accelerated chronic allograft nephropathy as evidenced by functional and morphologic measurements.^{54,55} Similarly, in clinical studies the graft/recipient mass ratio and the effect of sex have been shown to impact graft survival significantly. Kidneys from elderly female donors transplanted to male recipients and those from elderly deceased donors who died of vascular accidents, as compared with relatively younger donors who died from road traffic accidents, are associated with poorer longer-term outcomes.^{56,57} Hyperfiltration injury and reduced compensatory mechanisms that protect against insults lead to this accelerated senescence of the graft.

High glomerulosclerosis, interstitial fibrosis, vasculopathy, or tubular atrophy scores on pretransplant donor kidney biopsy are associated with poorer long-term graft survival. The extent of acceptability or delineation of cut-off values for chronic changes in the donor kidney is not well defined. Although most of these measurements may suffer from sampling bias, there is a positive correlation of CAN with more than 20% glomerulosclerosis, higher interstitial fibrosis scores, and associated myointimal elastosis of large arterial branches or hyaline arteriosclerosis of the arterioles. Finally, enlarged glomerular size also correlates with worse prognosis, suggesting that such hypertrophied glomeruli might have limited adaptation reserves.⁵⁸

Impact of Death on Organ Quality

Data from Scientific Registry of Transplant Recipients (SRTR) and various transplant registries around the world clearly show that both short- and long-term function of organs from living donors, regardless of the relationship to the recipient, are superior to those of deceased donor origin. In the deceased donor pool, organs procured from brain-dead donors have

been shown to have better survival than those of non-heart beating donor origin.

Brain death has been shown to initiate a cascade of events resulting in rapid swings in blood pressure, pulse rate, temperature dysregulation, coagulopathy, and electrolyte abnormalities.⁵⁹ In animal models, rapid hemodynamic swings, related to this autonomic storm, have been shown to result in reduced organ perfusion and increased structural damage of organs. Human studies have shown that brain death leads to a heightened inflammatory state within the kidneys, as evidenced by increased expression of cytokines such as E-selectin, intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and interstitial leukocyte accumulation and MHC class II expression even before organ retrieval.⁶⁰ The cytokine up-regulation appears to increase the allogenicity of the transplanted organ. In a rat model of brain death, peripheral organs harvested after brain death showed up-regulation of MHC class I and II antigens, the T-cell costimulatory molecule B7, as well as increased mRNA expression of lymphocyte- and macrophage-associated products.⁶¹ Experimentally, when these kidneys are transplanted they are rejected in an accelerated manner^{62,63} compared with controls, which may explain the clinical observation of increased rejection associated with deceased donor kidneys.

In non-heart beating donors, prolonged warm ischemic time may add to organ injury. A recent single-center study from Spain⁶⁴ noted significantly higher primary nonfunction and delayed graft function rates in the non-heart beating donors group when compared with heart-beating-deceased donors. However, in the grafts that survived early injury, 1-, 5-, and 10-year graft survival was no different when compared with heart-beating-deceased donors. Therefore, limiting early insults to the graft by short cold ischemic time, limited ischemic injury through better perfusion techniques, and avoidance of calcineurin inhibitors may help improve the graft survival in this group.

Ischemia-Reperfusion Injury

Ischemia-reperfusion injury plays a major role in both short-term renal function and long-term allograft changes consistent with CAN. The du-

ration and degree of ischemic insult to the kidney, which is composed of a preretrieval injury, cold storage ischemia, ischemic re-warming (warm ischemia), and reperfusion, influences the overall injury. Acute ischemia makes endothelial cells lose their antiadhesive properties and develop a thrombogenic and adhesive surface. Thus, endothelial permeability of the graft along with expression of other adhesion molecules and inflammatory genes is up-regulated significantly. On reperfusion, ischemia-primed endothelial cells are prone to leukocyte and platelet adhesion. The adherent leukocytes release reactive oxygen species and a variety of cytokines. The resulting acute inflammatory response leads to graft dysfunction, as manifested by delayed graft function. ECD kidneys in particular are vulnerable to this effect, as manifested by the increased need of dialysis post transplantation.

After initial organ recovery and a period of quiescence signs of CAN such as glomerulosclerosis, interstitial fibrosis, arterial obliteration, and tubular atrophy with accompanying proteinuria become apparent. Experimental rat models of ischemia reperfusion (IR) injury clearly have shown this temporal relationship in both isografts and allografts.^{65,66} Our group, using a rat uninephrectomized ischemic single-kidney model, has shown that in addition to T-cell and macrophage infiltration, up-regulation of the costimulatory molecule B7 occurs within 24 hours of injury and peaks at 3 days. Blockade of T-cell CD28-B7 costimulation with CTLA4Ig resulted in significant inhibition of leukocyte infiltration and activation, leading to significantly better long-term protection of the kidney.⁶⁷ Superoxide dismutase has been shown to improve graft survival in deceased donor kidney recipients, possibly by preventing reperfusion injury through its antioxidant properties.⁶⁸ The chronic effects of IR injury on CAN depend on the individual response of the kidney to the insult, most importantly the balance between damaging genes such as oxidases and proteases against protective genes such as heme-oxygenase-1 (HO-1) and vascular endothelial growth factor.⁶⁹ In a rat transplant model, HO-1 induction by a single treatment of cobalt protoporphyrin in brain-dead donors

lead to enhanced allograft survival.⁷⁰ Newer clinical trials are on the way to study this effect in human beings by up-regulating these protective gene expressions by measures such as carbon monoxide.

Impact of Hypertension

Death with a functioning graft and CAN are the 2 major causes of chronic graft loss. The role of hypertension as a contributing factor to both CAN and cardiac death have been well documented. The etiology of hypertension in this population is multifactorial, resulting from the pressor response of CNIs, graft renal artery stenosis, chronic allograft nephropathy, de novo glomerulonephritis, high renin output from native kidneys, and polycythemia. Opelz et al⁷¹ in their Collaborative Transplant Study, a large retrospective study involving 29,751 patients, first showed an association between posttransplant blood pressure (BP) and renal allograft failure. Subsequent studies have shown that this finding was independent of acute rejection and baseline renal function, suggesting that progressive renal dysfunction was the result of, rather than the cause of, increased blood pressure.^{72,73} Further analysis of the Collaborative Transplant Study data⁷⁴ indicated an association of systolic BP with cardiovascular morbidity in the renal transplant population, suggesting a linear relationship between higher death rate in patients with high (>140 mm Hg) systolic BP at 1 and 3 years than in patients with consistently low (<140 mm Hg) systolic BP (relative risk, 2.14; $P < .001$). This study suggested that the window of opportunity to derive benefit from lowering BP is wide and that BP lowering undertaken even several years after transplantation can still confer significant benefit for graft survival. It should be noted that even though this was a large study, it was a retrospective, nonrandomized trial in which only a small fraction of the patients analyzed were non-Caucasian.

The National Kidney Foundation Task Force on Cardiovascular Disease established targets for the transplant individuals as less than 130/85 mm Hg (without proteinuria) and less than 125/75 mm Hg (with proteinuria).⁷⁵ There is no agreement among the transplant community concerning the optimal antihypertensive therapy in renal transplant recipients. Properties of angiotensin-con-

verting enzyme inhibitors and angiotensin II receptor blockers such as their clear renoprotection effect (reduction of intraglomerular pressure reduction and thereby proteinuria in patients with native renal disease), cardioprotective effects, posttransplant erythrocytosis limitation, and inhibition of transforming growth factor (TGF)- β 1 (a potent cytokine responsible for CAN) have led to their increasing use in the population. Lin et al,⁷⁶ in their retrospective study involving 63 patients with biopsy-proven chronic allograft nephropathy, showed a trend toward slowing of the renal insufficiency as well as a significant survival benefit in the combined end point of allograft failure or death in patients treated with renin-angiotensin system (RAS) inhibitors. On the other hand, a large recently published retrospective study found no patient or graft survival benefit associated with the use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (ARBs).⁷⁷ There is a well-documented association with the development of anemia in transplant patients with the use of these agents, suggesting an altered physiology in transplanted kidneys.

The rationale for the use of calcium channel blockers (CCBs) stems from their potential to counteract afferent arterial vasoconstriction caused by calcineurin inhibitors. However, because of their dilatation of preglomerular vessels, strict control of BP must be ensured when CCBs are used. Although β -blockers may aggravate the metabolic disturbances associated with immunosuppression, they do have proven benefits in the risk reduction of cardiovascular events, which is a definite plus in this high-risk population. Because posttransplant hypertension often needs multiple medications, α -blockers with their beneficial effect on insulin sensitivity and lipid profile and diuretics in patients with edema or hyperkalemia may be a rationale choice in some transplant patients.

Dyslipidemia

Posttransplantation hypercholesterolemia is an independent risk for graft loss⁷⁸ and renal transplant recipients have up to a 16-fold higher risk of developing ischemic heart disease compared with age- and risk-matched controls.⁷⁹ Risk factors include genetic predisposition, patient sex and age, body weight, renal dysfunction, proteinuria, and use of other agents, such as

β -blockers and diuretics. Among drug-related causes of dyslipidemia, cyclosporine, sirolimus, and prednisone mainly are implicated and the lipid profile differs between individual agents. At least 60% of adult renal transplant recipients develop dyslipidemia, which can occur as early as 1 month after the initiation of immunosuppressive therapy.⁸⁰ Cyclosporine has an increased propensity to cause dyslipidemia through its effect on total cholesterol (TC), low-density lipoproteins, and triglyceride (TG) levels when compared with tacrolimus. Currently, there are no data to support that the use of MMF or azathioprine is associated with dyslipidemia.⁸¹ Sirolimus can have an early, profound, and dose-dependent effect on TC (3%-77% increase) and TG (13%-97% increase) versus baseline.^{82,83} However, there is some evidence from human and rodent studies that predicted that the risk of sirolimus inducing atherosclerosis may not be as high because of its associated antiproliferative effect.^{83,84}

Therapeutic lifestyle changes such as a reduced intake of saturated fats and cholesterol, an increased intake of dietary fiber, weight reduction, and increased physical activity should be advocated. Limitation of alcohol consumption and strict control of hyperglycemia should be encouraged. The National Kidney Foundation guidelines recommend statins as first-line therapy unless the TG levels equal or exceed 500 mg/d.⁸⁵ Statins primarily reduce TC and low-density lipoprotein levels with variable effects on TG levels and also may have other favorable pleiotropic effects on endothelial function, coagulation, and plaque stability, and on systemic inflammation. In the Assessment of Lescol in Renal Transplantation (ALERT) trial, the first large-scale renal transplant lipid intervention trial with long-term follow-up evaluation, fluvastatin was shown to significantly reduce the combined end point of cardiac death and nonfatal myocardial infarction by 35% ($P = .005$), but appeared to have no effect on noncardiovascular death, graft loss, or renal function.⁸⁶ Fibrates are recommended after failure of dietary therapy to reduce TG levels when they are greater than 500 mg/dL or when maximum statin doses fail to improve the lipid profile. Other strategies, including modification of

the immunosuppressive regimen and the addition of other lipid-modifying agents, also have yielded positive results.

Abnormal Body Mass Index

With increasing levels of obesity, the role of body mass index in transplant outcome has to be addressed. An analysis of more than 50,000 renal transplant recipients in the United States Renal Data System showed adverse graft survival among underweight and obese recipients independent of other risk factors predictive of graft outcome.⁸⁷ Gore et al⁸⁸ also showed that compared with normal weight patients, morbid obesity was associated independently with an increased risk of delayed graft function, prolonged hospitalization, acute rejection, and decreased overall graft survival ($P = .001$). This has been attributed to associated comorbidities such as hypertension, dyslipidemia, subclinical hyperinsulinemia (causing diabetic nephropathy-like changes even in the absence of diabetes), glomerular hyperfiltration injury, underachievement of optimal immunosuppression, and possible adipocytokine-mediated injury. Donor obesity has not been shown to have any long-term graft-adverse outcome. Factors limiting graft survival in underweight recipients may include malnutrition and inappropriate immunosuppression, leading to CAN. Patients should be counseled frequently to adhere to therapeutic lifestyle changes both pretransplant and posttransplant and in overweight patients steroid minimization or avoidance protocols may be particularly beneficial.

BK Viral Nephropathy

Over the past decade, BK viral nephropathy (BKVN) has emerged as a major cause of allograft dysfunction. BK virus is an endemic polyoma virus with a high prevalence rate and has a low morbidity and long latency in immunocompetent individuals. Its prevalence and contribution to graft loss has been correlated strongly with the increasing use of more potent immunosuppressant combinations. The use of anti-lymphocyte preparations as induction therapy was not associated significantly with BK viremia, viremia, or BKVN,^{89,90} however, use of these agents as rescue therapy to treat steroid-resistant rejections has been shown to activate BK

viral replication in patients receiving triple immunosuppressive therapy.⁹¹ With a few exceptions, BKVN has been diagnosed in patients receiving all commonly used maintenance immunosuppressive therapeutic agents such as calcineurin inhibitors, antimetabolites, mammalian target of rapamycin (mTOR) inhibitors, and corticosteroids. Avoidance or early cessation of steroids in calcineurin inhibitor-based regimens may reduce the incidence of polyoma viral associated nephropathy (PVAN).^{92,93} Prior rejection episodes and antirejection treatment with steroid pulses both were associated with an increased risk of viruria, viremia, or BKVN.^{89,94}

Screening techniques for early prediction of BK virus reactivation include judicious combination of urine cytology for the detection of decoy cells and plasma and urine assays for the detection of BK virus DNA by polymerase chain reaction. If BK virus reactivation is suspected, a transplant kidney biopsy to identify intranuclear polyomavirus inclusion bodies in tubular epithelial and/or glomerular parietal cells is crucial. PVAN may be very focal, affecting only scattered nephrons, and can be associated with varying degrees of inflammatory cell infiltrates, tubular atrophy, and fibrosis.⁹⁵ The treatment of BKVN consists of cautious reduction in immunosuppressive therapy; few small published studies have shown the effectiveness of antiviral therapy with cidofovir or leflunomide or a combination of both. Other agents such as quinolones have been tried with some success. Until now, approximately 30% to 60% of subjects with BKVN have experienced irreversible graft failure. The combination of high suspicion, early screening, prompt diagnosis, and appropriate reduction in immunosuppressive therapy is the key to prevent a poor outcome.

CMV Infection

Apart from its damaging effects early posttransplantation, there is growing evidence that CMV contributes to the pathogenesis of chronic allograft injury. CMV infection has been particularly linked to accelerated coronary atherosclerosis in cardiac allograft recipients. In a rat renal allograft model, CMV infection resulted in significantly higher chronic allograft damage in

dex (CADI) scores as early as 6 to 7 days post-transplant, when compared with uninfected controls. CMV enhanced the expression of endothelial TGF- β 1 and platelet-derived growth factor proteins in renal allografts causing transplant vasculopathy and by up-regulating connective tissue growth factor-induced interstitial fibrosis.⁹⁶ As with BK viral infection, overimmunosuppression is a major risk factor and appropriate prophylaxis with oral (val)ganciclovir, early diagnosis in the event of infection, and optimal treatment will help minimize allograft injury.

Role of Calcineurin Inhibitors in CAN

The introduction of CNIs significantly reduced acute rejection rates among kidney transplant recipients. However, the intrinsic nephrotoxicity of these agents and their contribution to CAN has greatly diminished the enthusiasm for their long-term use. The advent of newer potent immunosuppressive medications such as MMF and sirolimus has enabled the minimization, withdrawal, or avoidance of CNIs altogether. Weir et al⁹⁷ studied 118 patients with declining renal function and biopsy-proven CAN, either by minimization or withdrawal of CNI (cyclosporine [CsA] or tacrolimus), with the addition or continuation of MMF and low-dose steroids. After a mean follow-up period of 650 days, the patients were observed to have an improved slope or lack of deterioration in renal function in 72% of patients in the CNI withdrawal group, compared with 54% in the reduced CsA group and 40% in the reduced tacrolimus group. By using a similar strategy, Dudley et al⁹⁸ showed that the combination of CsA withdrawal and the addition of MMF in patients with deteriorating renal function stabilized or significantly improved renal function in 58% of patients compared with 28% of CsA-treated patients. A cyclosporine avoidance study by Flechner et al⁹⁹ using sirolimus or cyclosporine in combination with basiliximab and MMF showed that both combinations efficiently prevented acute rejection episodes, but the SRL-treated patients had a significantly higher creatinine clearance than CsA-treated patients, and 2-year protocol bi-

opsy specimens showed a much lower rate of chronic allograft nephropathy (37% versus 78%).

Most of the earlier studies have compared CNI-sparing therapies with cyclosporine-based combinations and also at a later stage when CAN already is established. They generally showed better short-term renal function or improved slope of renal function deterioration. However, tacrolimus may be less nephrotoxic when compared with CsA, and the tacrolimus/MMF combination is perceived to be the most efficacious, least nephrotoxic, and most commonly used regimen currently. Larson et al,¹⁰⁰ in their randomized prospective trial, evaluated a head-to-head comparison of sirolimus and tacrolimus. Both arms received thymoglobulin induction therapy. Results showed that a CNI-free regimen using sirolimus-MMF-prednisone produced a similar acute rejection rate, graft survival, and renal function 1 to 2 years posttransplantation compared with tacrolimus-MMF-prednisone. In the 1-year protocol biopsy specimens, chronicity as judged by the Banff schema showed no difference in interstitial, tubular, or glomerular changes, but fewer chronic vascular changes were present in the sirolimus group. However, this was a single-center study looking primarily at living donor kidney transplants in contrast to the European multicenter trial of CsA withdrawal in which a high proportion of the candidates were deceased donor recipients. It is possible that these kidneys are less vulnerable to CNI toxicity and this study may be underpowered to pick up subtle differences over the relatively short follow-up period. Longer-term results from this study may answer some of these questions.

Finally, in a multicenter trial, belatacept (a modified, long-acting form of CTLA4Ig), was compared with CsA in conjunction with steroids, MMF, and basiliximab. At 1 year after renal transplant, belatacept did not appear to be inferior to cyclosporine in preventing acute rejection, but it may preserve the glomerular filtration rate and reduce the rate of chronic allograft nephropathy.¹⁹

POTENTIAL THERAPEUTIC TARGETS ON THE HORIZON

Chronic allograft injury is a fibrogenic response secondary to both proliferative and infiltrative responses mediated by chemokines, cytokines, and growth factors. TGF- β has been particularly implicated in the fibrogenic process and epithelial mesenchymal transition (EMT) may be the mediator of that response.¹⁰¹ In a naive mouse cyclosporin nephrotoxicity model, administration of TGF- β -neutralizing antibody has been shown to ameliorate CsA-induced morphologic alterations and preserve renal function.¹⁰² However, it must be translated cautiously to a transplant setting because at high doses it has been shown to abrogate the immunosuppressive effect of CsA, but at low doses it retained its protective effect against CsA nephrotoxicity without interfering with graft survival.¹⁰³ Several other experimental agents such as TGF- β inhibitors (decorin, pirfenidone, and relaxin), endothelin-1 inhibitor (bosentan), and connective tissue growth factor inhibitor (antisense oligonucleotides) have been tried with some success in limiting renal fibrosis in rodent models.¹⁰¹

EMT should be considered as a critical process and may be the end result of both alloantigen-dependent and -independent insults. In human renal biopsy specimens EMT has been shown to be the hallmark of interstitial fibrosis and tubular atrophy. Disruption of EMT may be clinically feasible in the near future with bone morphogenetic protein-7 and hepatocyte growth factor. Although a number of these approaches are promising in renal disease and preclinical models, they may encounter practical limitations in the transplant scenario because TGF- β itself is immunosuppressive and plays a beneficial role in the acute transplant setting.¹⁰¹

Although measures mentioned previously are both preventative and active, the ultimate goal in transplantation is to create a state of donor-specific tolerance to an allograft. Transplant tolerance is defined as a selective lack of an immune response to foreign antigens expressed by an allograft, leading to indefinite survival and acceptance of the graft, without the need for continuous nonspecific immunosuppression.

In rodent studies and in some nonhuman primate studies,¹⁰⁴⁻¹⁰⁶ achieving transplant tolerance through peripheral tolerance by costimulation blockade and other mechanisms such as mixed chimerism with minimal or no chronic rejection has been possible but the induction of tolerance in transplant models does not universally lead to a lack of chronic rejection. Whether these strategies will lead to a true state of transplant tolerance in human beings with prolonged allograft survival alleviating chronic rejection remains to be seen.

CONCLUSIONS

Even patients with the best functioning renal allograft already have compromised renal function. Multiple insults from both alloantigen-dependent and alloantigen-independent mechanisms further contribute to accelerated renal functional decline. It is doubtful that a single measure or treatment will ever be able to universally prevent the development of CAN. Careful management of all the potential contributing factors remains the mainstay of treatment, with emphasis on prevention rather than cure of CAN at the present time.

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