Rethinking Chronic Allograft Nephropathy: The Concept of Accelerated Senescence

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Chronic rejection or chronic allograft nephropathy (CAN) is the major cause of failure of kidney transplants other than patient death, and has been extensively reviewed (1–5). CAN is characterized by functional impairment with nonspecific pathology: tubular atrophy, interstitial fibrosis, and fibrous intimal thickening (FIT) in the arteries, with variable glomerular lesions. The risk of CAN correlates with the input, immune, and load stresses experienced by that kidney. Input refers to the preexisting chronic conditions in the donor (aging, hypertension) plus the acute injury related to the transplant process (brain death, donor maintenance, organ removal, preservation, implantation, reperfusion). Immune stress is due to rejection by antibody or cellular mechanisms, determined by histocompatibility, presensitization, host responsiveness, and the effectiveness of and compliance with immunosuppression. Load reflects hypertension, donor-recipient size disparity, proteinuria, hyperlipidemia, drug toxicity, and infectious agents. Load factors such as donor size and gender probably reflect differences in nephron dose and are relatively weak, suggesting that nephron number does not explain the strong effect of input and immune factors. What distinguishes the transplant from normal tissue is the level of injury. Injury depletes the finite ability of the tissue to repair, and triggers inflammation, which may further stress the parenchyma and vessels, increase immune recognition, and promote fibrosis.

In this article we review the problem of human CAN and propose a model in which the cumulative burden of injury and age exhausts the ability of key cells in epithelium or endothelium to repair and remodel to maintain tissue integrity. We term this exhaustion “senescence” to emphasize the importance of donor aging and the overlap of the pathologic lesions with age-related changes, and to suggest analogy with senescent changes observed in cell culture. When the potential of a tissue to repair is exhausted, the endothelial functions decline and the epithelium atrophies; injury-induced inflammation persists, permitting transforming growth factor-β and other mediators to create fibrosis. Thus, fibrosis may be a default for the failure of normal healing. CAN can be minimized by reducing the burden of injury due to immune and nonimmune mechanisms, but grafts with age- and injury-induced changes may still be useful despite their limitations, and few should be discarded. Recent advances in the cellular basis of senescence in vitro may hold clues to the molecular events that limit the repair of key cells.

Development of the Concept of Chronic Rejection and CAN

The concept of chronic rejection emerged gradually in the 1950s and 1960s. Acute homograft (now allograft) rejection was well known in the late 1950s in the early clinical experience, and few kidneys survived even for months. In 1955 Hume et al. (6) reported a case in which rejection developed within 5½ mo, with obliteration of the arteries. Systematic investigation of late rejection by Porter et al. (7) and Jeannet et al. (8) revealed that arterial intimal fibrosis was frequent and probably represented a reaction to immune injury, perhaps due to alloantibody (7,9). This may explain the belief that chronic rejection is alloantibody-mediated. Transplant glomerulopathy distinct from recurrent glomerulonephritis was recognized by the late 1960s and early 1970s (10–13), and is a variable feature of CAN.

However, these early observations by Hume, Porter, Jeannet, and their colleagues in transplants on minimal immunosuppression bear little resemblance to the type of late graft loss in the 1990s. The syndrome of obliterative arterial disease progressing over months is now rare, and the clinical course of CAN is usually indolent, with graft loss often many years posttransplant. The arterial lesions are often not prominent in biopsies early in the course. However, the old Hume-Porter-Jeannet syndrome remains the basis of the current animal models of chronic rejection, making them of limited relevance to the clinical problem of CAN.

The Problem of Definition

We avoid the term chronic rejection because it implies an ongoing immune response that cannot be proven. We cannot determine the extent of immune involvement in CAN at present, and the risk factors indicate a large nonimmune component. Previous efforts to define chronic rejection as a distinct disease often included an arbitrary rate of progression. Such definitions exclude kidneys with poor but stable function, while including kidneys with better function that have experi-
enced recent deterioration. Moreover, progression is often irregular (14), and progression per se is not a criterion for defining other renal diseases.

We define CAN as a state of impaired renal allograft function at least 3 mo posttransplant, independent of acute rejection, overt drug toxicity, and recurrent or de novo specific disease entities, with typical features on biopsy (see below). One can designate graft loss due to CAN, and can define progressive CAN by an arbitrary change over time. Such definitions avoid the problem of defining chronic rejection so rigorously that one excludes much of the population of interest.

**Magnitude of the Problem of CAN**

The two largest problems in renal transplantation are organ availability and late graft loss. A transplant is beneficial compared to remaining on the waiting list (15) and is the treatment of choice for end-stage renal disease. However, the average cadaver donor transplant fails at about 10 yr. CAN is the main cause of returning to dialysis after a transplant (4,16) and a major cause of end-stage renal disease in the developed world, increasing the number of people on dialysis and using up kidneys for retransplantation. CAN contributes to the prevalence of hypertension and renal insufficiency. For example, currently in the 600 patients with renal transplants followed in Edmonton, 35% have creatinine >150 and 15% >200 mM/L. Improved immunosuppression has reduced acute rejection but has had little effect on CAN and late graft loss. CAN is more common in cadaver donor transplants than living donor transplants but the clinical course is similar in live donor transplants once it is established.

**The Pathology of CAN**

The pathology of CAN is nonspecific and requires exclusion of specific entities. The CAN triad—tubular atrophy, interstitial fibrosis, and FIT—is shown in Figure 1, and is the basis for the Banff classification of CAN (17,18). (The updated diagnostic categories for CAN are listed at http://tpis.upmc.edu/tpis/schema/index.html.) All CAN lesions including the vascular lesions often considered the hallmark of CAN overlap the lesions in the aging kidney (19). Thus, many kidneys now being transplanted fulfill the criteria for CAN at the time of transplant. The prevalence of CAN lesions in cadaver transplants is about 60 to 70% by 2 yr (20), but it is not clear in most studies how much was present at the time of transplant. The key feature may be epithelial atrophy, associated with a reduction in renal mass. There is an impressive increase in relative interstitial area and alpha smooth muscle actin in sections, but it is difficult to assess the absolute increase in collagen and the prominence of myoblasts in the interstitium because the kidney has decreased in size (21). A new lesion, splitting of the peritubular capillary basement membrane detected by electron microscopy, may be more specific for CAN and is under evaluation (22,23).

Chronic rejection has been considered synonymous with transplant vascular sclerosis, based on the assumption that vascular sclerosis causes the epithelial atrophy and interstitial fibrosis. This may not be correct, and vascular sclerosis is not a synonym for CAN or chronic rejection. Compared with focal, eccentric, and proximal lesions of conventional atherosclerosis, the arterial lesions in CAN have been described as generalized, concentric, and distal, similar to the lesions in chronic rejection of coronary arteries in heart transplantation. However, such generalizations are suspect. By light microscopy, no lesions are present in CAN that are not also present in some donor kidneys pretransplant (24), and one of the strong associations of CAN lesions is with donor age (25). Indeed, the majority of graft dysfunction at 6 mo posttransplant is determined by the donor (25,26). Similarly, in heart transplants examined by intravascular ultrasound, the transplant coronary artery disease actually is proximal and eccentric like conventional atherosclerosis (27), not the distal, concentric process originally described. There are differences in the extracellular matrix composition between conventional atherosclerosis and transplant coronary artery disease, but these may reflect the age of the lesion or the speed of development. Thus, the arterial changes in kidney transplants and coronary vasculopathy in heart transplants may represent acceleration of the conventional arterial disease associated with aging.

FIT in arteries involves smooth muscle cell proliferation and increased lipid- and glycosaminoglycan-rich matrix in the intima, narrowing the lumen. But part of the loss of lumen in diseased vessels is due to failure of the vessel wall to dilate in response to decreased flow (28), and represents exhaustion of the normal remodeling process, possibly due to decreased endothelial function. According to Schwartz (29), “atherosclerosis results from growth of plaque and, ultimately, from the failure of the rest of the vessel to dilate as it should in response to reduced blood flow.” Thus, the focus on the intimal proliferative changes should be replaced by the concept of a more global disorder of arterial remodeling.

In CAN, both renal function (30) and histology (31) correlate with the ultimate prognosis of the graft. For example, the chronic allograft dysfunction index at 2 yr correlates with transplant function at 6 yr, and with eventual graft failure. However, it is doubtful whether pathology, with its inherent sampling error, predicts progression more accurately than does the serum creatinine or GFR, which lack the elegance of pathology but are more precise, reproducible, and quantitative.

**Clinical Factors: Input, Immunity, Load**

Our understanding of CAN has emerged from the human renal transplant experience. Because most late graft loss is due

![Figure 1. The injury triangle.](image-url)
to CAN, the risk factors for late allograft failure can be taken as a surrogate for CAN. Factors that increased the probability of graft failure in various databases are summarized as quality of the transplanted tissue (input), the rejection events, and the posttransplant load or stress on the organ (Table 1) (3,26,30,32–50).

**Input: The Quality of the Transplanted Tissue**

There are two aspects of input quality: chronic changes from stresses and injuries in the donor, and the acute peritransplant injuries that arise in donation and transplantation. The use of older and “marginal” donors or expanded criteria for donor acceptability are increasing the importance of acute and chronic input effects (51). In the past, the donors were usually young male trauma victims, and now are increasingly likely to be older, with strokes, and female. The result is that nonspecific input injury is rivaling rejection as the major process causing CAN and graft failure.

**Chronic Pretransplant Injuries**

Donor age is the strongest predictor of poor long-term graft survival (52–55), and CAN lesions strongly correlate with older donor age (25). Kidneys from older donors show an increased frequency of later adverse features: delayed graft function and elevated baseline serum creatinine. The aging kidney develops increasing functional impairment, particularly in men (56), due to age and age-related diseases such as hypertension and vascular disease (57). Age-related loss of renal mass is primarily cortical with sparing of the medulla. The number of glomeruli decreases due to global sclerosis, and the remaining glomeruli enlarge. Focal glomerular sclerosis is not a major finding in age-related changes (or for that matter in CAN). The percentage of sclerotic glomeruli increases from 5% in the fourth decade to 10 to 30% in the eighth decade (57). Lesions similar to the CAN triad are prominent in the aging kidney. The FIT lesions correlate with diminished renal weight, but it is not clear that FIT causes nephron loss or glomerulosclerosis. Infiltrates of inflammatory cells in the interstitium occur probably as a response to injury.

Effects of donor age explain about 30% of the variance in kidney transplant outcomes beyond 1 yr (55). The effects of donor aging have been considered to be due to reduced nephron dose. However, the effects of donor age and other input parameters are considerably stronger than the measures of nephron dose, such as donor size and gender, which contribute only 1 to 2% of the variance (55). Thus, intrinsic changes in the aging kidney probably both contribute more to the reduced survival than nephron dose per se. As we argue below, this may be a result in inherent “clocks” in the cells, rather than overwork due to nephron dose. In other words, the age of the nephrons and the number of nephrons may both affect graft survival.

In cadaveric renal transplantation, the degree of glomerulosclerosis (as a percentage of the total glomeruli seen) has been used as the basis for excluding certain donors. This may not be valid, due to sampling errors, particularly in superficial wedge biopsies, and because sclerosis may not be the best predictor of subsequent graft performance. The extent of FIT may better predict renal tissue quality (H. Wang, K. Solez, S. Cockfield, manuscript in preparation; B. Kasiske, personal communication).

In multivariate analysis, the gender and size of the donor affects the outcome significantly but weakly (55). Gender and size differences probably reflect nephron dose (58), and are discussed below as load factors.

**Acute Peritransplant Injuries**

Delayed graft function (DGF) is a strong correlate of reduced graft survival, reflecting acute injury related to the donation and transplant process, although it is likely that pre-existing chronic changes such as FIT also increase DGF. This reflects the increased susceptibility of the kidney with chronic stress to acute injury, as seen in acute renal failure of other causes (59). DGF has been attributed to ischemia-reperfusion injury, but is more complex than this. One key factor may be renal injury due to brain death itself, which can now be studied in rats (N. Tilney, personal communication), and is both profound and poorly understood in many tissues in the hours following brain death. DGF reflects the total of many injuries: brain death, donor maintenance, warm ischemia, cold flush and preservation, re-warming during the anastomosis, reperfusion, and intrarenal arterial spasm in the hours after the anastomosis. DGF is increased in patients with high panel-reactive antibod-

### Table 1. Risk factors for renal graft loss

<table>
<thead>
<tr>
<th>Input</th>
<th>Chronic</th>
<th>Acute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Brain death</td>
<td>Type of brain death</td>
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<tr>
<td>Hypertension</td>
<td>Type of brain death</td>
<td>Type of brain death</td>
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<tr>
<td>Vascular disease</td>
<td>ICU measures</td>
<td>Preservation</td>
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<tr>
<td>Genetic factors</td>
<td>Harvesting</td>
<td>Acute rejection</td>
</tr>
<tr>
<td></td>
<td>Implantation</td>
<td>Drug compliance</td>
</tr>
<tr>
<td>Load</td>
<td>Immune Injury/Stress</td>
<td>Posttransplant Stress</td>
</tr>
<tr>
<td>PRA</td>
<td>Size mismatch</td>
<td></td>
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<tr>
<td>HLA match</td>
<td>Hypertension</td>
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<tr>
<td>Immunosuppression</td>
<td>Drug toxicity</td>
<td></td>
</tr>
<tr>
<td>Acute rejection</td>
<td>Recurrent disease</td>
<td></td>
</tr>
<tr>
<td>Drug compliance</td>
<td>Lipid disorders</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Donor CMV positive</td>
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*ICU, intensive care unit; PRA, panel-reactive antibody; CMV, cytomegalovirus.
ies (60), perhaps because undetected alloantibody-mediated rejection can present as acute tubular necrosis.

Despite HLA mismatching, grafts from living spousal donors show enhanced survival compared with cadaveric transplants (38), emphasizing the long-term consequences of the peritransplant stresses of cadaveric transplantation. Increasing cold ischemic time and other preservation/harvesting times (warm ischemic time, anastomosis time) represent risk factors for immediate function and long-term survival, suggesting that current conditions for cadaveric donation and preservation are not optimal. The concepts of renal preservation date to the 1960s and need to be reevaluated (61). Even the idea of cold flush and cold preservation should be reconsidered, since cold stress could be counterproductive (62). Moreover, the practices in organ donation have been adjusted to improve the function of the lungs, liver, heart, pancreas, etc., sometimes at the expense of added renal stress. These stresses may interact with the chronic input stresses in the kidneys with age-related changes.

Kidneys from donors who die of brain damage by physical trauma (e.g., motor vehicle trauma) have better survival than those from donors dying from cerebrovascular events (55). Other features of the donor after the brain insult, such as disseminated intravascular coagulation (63), may also influence the future of the graft.

Nonrandom associations among factors such as causes of death, gender, age, and age-related disease complicate the interpretation of these relationships. Young male donors with cerebral trauma, large renal mass, no hypertension, and little vascular disease are compared to older donors who are more likely to be female, and to have intravascular accidents, hypertension, and vascular disease. We must continue to refine the multivariate statistical models for these complex, nonrandom, and incompletely understood associations.

Immunity

Immunologic parameters (histocompatibility differences, acute rejection episodes, presensitization) have a major effect on graft survival. Acute rejection episodes, especially if severe, recurrent, late, or not responding well to treatment, strongly predict the early development of CAN. In some patients, progression of CAN is associated with laboratory evidence of an ongoing donor-specific immune response, e.g., proliferative responses to allopeptides (64) and circulating anti-donor antibodies (65,66). Such findings are neither universal nor proven to be causal, and the prevalence and pathogenic role of alloantibodies and T cells in CAN remains unresolved some four decades after the issue was first raised.

The principal considerations about the role of acute rejection in CAN have become clearer in recent years:

1. Freedom from acute rejection correlates strongly with protection from CAN and from late graft loss.
2. The rejection episodes that correlate with late graft loss include those followed by impaired function (32,41); late, severe, or recurrent rejection; and rejection affecting arteries (43,48,67,68).
3. Perfect HLA matching decreases acute rejection rates both early and late and increases graft survival (G. Opelz, personal communication).
4. Decreasing rejection by immunosuppression has had less effect than expected on CAN and late graft loss to date (43,69). The new immunosuppressive agents such as cyclosporin A (CsA), mycophenolate mofetil, and tacrolimus have failed to alter histology or renal function or graft survival despite reducing the rate of acute rejection. Some data favor tacrolimus over CsA for reducing CAN (70), but these may reflect aggressive dosing of tacrolimus compared with CsA in the early tacrolimus studies and have not been confirmed in recent analyses in the Collaborative Transplant Study (G. Opelz, personal communication).
5. Nonimmune parameters such as DGF, donor age, and brain death due to stroke have a powerful influence on graft survival in cadaver transplant databases (37,71), at least as strong as HLA mismatch or any other immune parameter. The role of brain death per se is emphasized by the observation that unmatched spousal transplants survive as well as HLA one haplotype-matched live related donor transplants and better than fully matched cadaver transplants (38).
6. Because injured kidneys reject more, rejection may in part be a marker for input injury and vice versa (60), and rejection may also account for some of the effect of DGF. DGF reduces graft survival even when no acute rejection episodes are recorded, indicating that the effects of DGF are only partially immune-mediated (72).
7. Noncompliance with medications can precipitate acute rejection even very late after transplantation, and may contribute to the failure of immunosuppressive agents to alter transplant half-life. This may explain why HLA matching effects tend to have more effect on transplant half-life than immunosuppressive drugs. (Noncompliance is also a factor in the treatment of hypertension and hyperlipidemia.)

Input Injury Interacts with Immune Recognition

Kidneys with DGF have more acute rejection (60), and grafts lost without recovering function usually show severe rejection (73). The combination of DGF and rejection gives particularly poor survival (44). Possible reasons for immune problems in kidneys with acute input injury include difficulties in the diagnosis of rejection in DGF, but there may be a true increase in immune recognition in injured tissues. A variety of types of injury elicit a cascade of inflammatory events that contribute to a general stereotyped response to tissue injury and not just to ischemia-reperfusion, i.e., the injury response (74). The immune system is governed by geographic rules, such that antigen expressed in normal tissue tends to be ignored and antigen expressed in injured tissue (which is automatically inflamed via the injury response) is likely to provoke and activate an immune response (75), perhaps in part due to proinflammatory cytokines (76). Injury may act as an adjuvant, increasing expression of MHC antigens in epithelium and endothelium as well as recruiting and activating antigen-presenting cells (77). Injury and inflammation could prevent favorable immune adaptations in the host, perpetuating the im-
Acute input injury could thus favor rejection, and rejection injury could induce inflammation and new immune activation—the injury triangle (Figure 2). The importance of this sequence in clinical transplantation remains unknown.

Whereas acute input injuries (as manifest by DGF) increase rejection, the chronic stresses such as age-related changes may not. It is conceivable that donor atherosclerosis might render vessels and parenchyma more immunogenic. Atherosclerosis has features of chronic inflammation, with increased T cells (CD8-positive more than CD4-positive T cells) and monocytes in the intima of atherosclerotic vessels, MHC class II expression, and adhesion molecules ELAM-1, VCAM-1, and ICAM-1 expressed in endothelial cells, and increased expression of many cytokines (78). The FIT and atherosclerotic lesions could thus theoretically facilitate immune recognition. However, in human transplants the deleterious effects of advancing donor age and vascular disease are clear but their association with rejection is not.

**Gender of the Recipient**

Graft survival is slightly better in female recipients of male kidneys, an effect usually ascribed to nephron dose (see below), although factors relating to patient survival, underlying diseases, sensitization, and other factors complicate such analyses. Females have more active immune responses, and pregnancy could affect female responsiveness to alloantigens through sensitization, tolerance, or persistent microchimerism.

**Nephrone Dose and the Hyperfiltration Hypothesis**

Low nephron mass or “nephron endowment” has been proposed as a risk for progression of primary renal disease (58) and CAN. Very large recipient size and recipient male gender significantly reduce graft survival in multivariate analyses (55), although it is not clear that these risks reflect increased CAN in the transplant. Excessive donor size could induce hyperfiltration (79,80) and subsequent glomerular sclerosis, as documented in rats (81,82). However, the effects of gender mismatch and recipient size are small compared with the effects of donor age and DGF, and there is little evidence that focal sclerosis is a principal mechanism of progression in human CAN, or even that CAN primarily affects glomeruli.

A large mass of transplanted tissue relative to the recipient mass may dampen the immune response. In experimental animals, more tissue generally correlates with more stability, buffering the immune attack and promoting stability, and thus conferring an immunologic advantage (83). Tissue mass may contribute to the tolerogenic properties of liver transplants (84). Very low nephron mass may also evoke inflammation, as has been shown in rat models (85).

**CMV Infection**

Kidneys from CMV-positive donors are associated with small reductions in graft survival in United Network of Organ Sharing (86), U.S. Renal Data System (35), and the Collaborative Transplant Study (G. Opelz, personal communication). It is not clear whether this reflects an increased risk of CAN. CMV may be associated with chronic rejection of liver transplants (87) and heart transplants (88,89). Analysis of symptomatic CMV illness has been correlated with the development of chronic rejection, but such observations are colored by the association of clinical CMV manifestations with heavy antirejection therapy and thus with severe rejection. Thus, the case that CMV causes human CAN remains unproven. If CMV plays some role in CAN, improvements in CMV prophylaxis and treatment may reduce CAN.

Whether CMV can cause arterial disease in humans is unknown. A link between CMV infection and vasculopathy has been proposed for coronary restenosis (90). However, studies of kidney allografts with FIT by *in situ* hybridization, immu-
nohistochemistry, and PCR failed to show CMV protein or DNA in the arteries (91).

**Proteinuria**

In grafts with heavy proteinuria, the filtered proteins may be toxic to the tubules. Although this cannot initiate renal injury, it may contribute to progression in some cases (92).

**Hypertension**

Hypertension in the recipient is significantly associated with CAN and late graft failure (39,93), either as a cause or an effect. The prevalence in posttransplant hypertension defined by antihypertensive treatment is approximately 75% (94). Pretransplant hypertension in the recipient, the presence of the native kidneys, history of hypertension in the donor, recurrent rejection episodes, impaired graft function, and immunosuppressive drugs such as CsA and steroids correlate with posttransplant hypertension (53,95–99). There is a need to establish the extent to which rigorous control of hypertension can prevent CAN in controlled prospective trials; this would establish that hypertension contributes to CAN.

**Hyperlipidemia**

Hypercholesterolemia and hypertriglyceridemia are risk factors for the development of atherosclerosis and are common in transplant patients and presumably contribute to patient mortality from cardiac disease (100,101). The role of hyperlipidemia in the incidence of CAN is separate from the risks of generalized recipient atherosclerosis. Hypertriglyceridemia correlates with CAN (101), but whether lipid abnormalities cause CAN remains unproven. Increased pretransplant cholesterol levels were associated with higher graft damage scores in renal biopsies in one prospective study (102), but other studies have not found a clear association (103,104). The combination of increased triglycerides and VLDL proteins may correlate with future graft failure in kidney transplants (101,103) and in heart transplant recipients (105,106). Thus, lipid abnormalities, especially hypertriglyceridemia, enjoy guilt by association with CAN. At present, lipid lowering with HMG-CoA reductase inhibitor is not indicated for all allograft recipients but should be used if other cardiovascular risk factors are present (107). There are provocative data about immunosuppressive effects of HMG-CoA inhibitors in small studies (108,109). Lipid issues will intensify with the increasing use of rapamycin.

One problem for those who advocate that hypertension or lipid abnormalities cause CAN is why such abnormalities would be worse in the transplant versus the host vessels. Perhaps input and immune stress in the transplant blood vessel predisposes them to damage from blood pressure and lipids.

**Nephrotoxicity of CsA and Tacrolimus**

The contribution of nephrotoxicity from the calcineurin inhibitors (CsA and tacrolimus) to CAN is difficult to estimate. By definition, CAN excludes obvious cases of drug toxicity. The typical lesion of renal toxicity from calcineurin inhibitors is in afferent arterioles (Figure 3), which develop nodular hyaline thickening with protein deposits, sometimes associated with myocyte changes, and necrosis of individual smooth muscle cells on electron microscopy (110–113). CsA and tacrolimus produce sustained constriction of the afferent arteriole with upregulation of endothelin receptors (114), causing ischemia and glomerular collapse. GFR may initially appear stable due to adaptive growth and hypertrophy in intact glomeruli. Some analyses suggest that CsA or tacrolimus can increase CAN especially if there are early episodes of toxicity, but higher CsA or tacrolimus levels may also protect against the powerful effects of immune injury (24,25,115). The studies of the administration versus withdrawal of calcineurin inhibitors reflect this balance between beneficial and toxic activities (43,116–118).

Whether dosing and monitoring of calcineurin inhibitors affect CAN is not clear. Variable oral bioavailability of CsA correlates with chronic rejection in one study (119), suggesting that variability is deleterious. This observation should be confirmed in other data sets. Low CsA levels in one population study correlated with increased graft loss (47), but recently it has been difficult to prove that the doses and concentrations of these drugs affect graft survival. Thus, it remains unknown whether in properly managed renal transplants CsA or tacrolimus nephrotoxicity contribute to the problem of progressive loss of function due to CAN, or whether chronic administration of CsA or tacrolimus (or any other immunosuppressive drug) provides protection against CAN.

**Interpreting the Risk Factors in the Human Databases**

In interpreting these observations, there are two extreme views: immune and nonimmune. The immune view is that all negative influences on long-term graft function are due to immune mechanisms. Thus, nonspecific tissue injury evokes the inflammatory response, which increases the probability of rejection. The immunologic process could be recurrent clinical or subclinical acute rejection episodes, or a continuous low-level immune process such as alloantibody production. The best argument for the immune view is the excellent long-term survival of kidneys with perfect HLA matches in the United States.

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**Figure 3.** Histopathology of CsA nephrotoxicity, showing the characteristic lesion of hyalinization of an arteriole.
States (55) and Europe (54). Thus, posttransplant load factors can be borne successfully by the solitary kidney when immune injury is minimal. HLA mismatches increase acute rejection episodes even very late after transplantation, and the benefit of HLA matching tends to increase with time. The strong benefit of being rejection-free in cadaver transplantation also argues for the immune view.

The alternative is the nonimmune view that rejection is simply one of many adverse effects which program a chronic nonimmune process. The fact that preventing acute rejection by immunosuppression has little effect on graft survival supports this view, as are studies which show that early rejection is benign if it is fully reversed (43). Rejection that fails to recover to baseline may be associated with CAN (41) because the failure to recover marks those kidneys nearing exhaustion of their repair capacity, due to the cumulative total of the previous injuries, e.g., aging. Thus, preventing acute rejection in such kidneys will do little to alter the long-term outcome.

Neither view is adequate to explain all of the human data, and a composite of these views is the best option. In the view that combines all of the factors, there is a powerful effect of chronic and acute input stresses, and of immune activity, and some effect of load factors. The effect of both input and immune injury may be to stress the tissue and reduce its life expectancy by accelerating the aging processes, as discussed below.

Animal Models of Chronic Allograft Dysfunction

Problems with Animal Models of Human Chronic Diseases

Animal models of chronic human diseases must elicit pathology resembling a human disease that ordinarily takes years or decades within weeks or months. Once the model has been adjusted to produce the desired lesion, interventions are tested on the assumption that a lesion which resembles the human disease must have a similar pathogenesis. This assumption is flawed, and the value of using animal models to simulate human diseases over a much longer time frame is limited. This point has been made by Schwartz et al. in the context of animal models of atherosclerosis and postangioplasty restenosis (29): The animal models may be fundamentally different diseases from the human diseases. For example, age or age-associated diseases contribute significantly to human CAN but are not well studied in other animals. There are major species differences involved in the response to injury and in the aging process itself. Rat kidneys have a propensity to develop focal segmental glomerulosclerosis after a single insult, which does not happen in humans. For example, ischemic injury in rat kidney transplants triggers a chronic process with prominent focal sclerosis, which is interesting but is not like the human version, where single episodes of injury that recover fully are seldom followed by chronic deterioration (120).

Many risks for human CAN are not usually present in rat and mouse or even primate models: advanced donor age, donor vessel disease, donor hypertension, brain death, prolonged preservation, delayed graft function. Most animal models if anything resemble the best live donor transplants in human: no brain death, young donors, minimal preservation injury, and no chronic vascular disease. Old rat kidneys lack the FIT lesion typical of human renal aging, illustrating the difficulty of extrapolating between species (57,121). Thus, CAN in animal models tends to be driven by immune mechanisms and suppressed by immune interventions, unlike the human counterpart.

In nephrology, several recent failures in clinical trials attest to the difficulty of using animal models to predict the outcome of human trials: the anaritide trial (122,123), the IGF-1 trial (124), and the Modification of Diet in Renal Disease trial of protein restriction (125,126). Conversely, some trials such as the renal protective effect of angiotensin-converting enzyme inhibitors in diabetic nephropathy (127) and proteinuria (128) were predicted by animal models, and animal models of acute rejection have been valuable for screening new immunosuppressive drugs. The lesson is that we must continue to study animal models but remember that they may not be valid models of the human process. More relevant models should be sought that represent the complexity of human CAN. Given the limitations of animal models of CAN, we will touch on them briefly to highlight certain themes.

Immune-Inflammatory Mechanisms in Animal Models of CAN

Lesions in animal models tend to be driven by immune mechanisms such as interferon-γ (129) and macrophages (130). Thus, immune interventions in animal models often abrogate allograft arteriosclerosis or CAN, e.g., anti-CD4 or -CD8 (131), mycophenolate mofetil (132), or CTLA4lg (133,134). (Surprisingly, CTLA4lg prevents ischemia/reperfusion injury in rat kidneys [135,136].) Hyperlipidemia, CMV, and hormones affect vasculopathy in some models. Aortic allografts in rats fed a high fat diet develop intimal thickening (129,137), and rats infected with CMV on immunosuppression showed accelerated CAN (138,139). Estrogen may affect the antigen representation system by altering MHC expression (140), and has a variety of other effects on experimental CAN models (141). Estradiol treatment abolishes some IGF-1 effects (140), and high levels of estrogen favor some immune processes (142).

The rat kidney is the prototype for effects of reduced nephron mass (85,135,143–145), which leads to glomerulosclerosis, proteinuria, and inflammation (81). In rats, decreased renal mass accelerates and intensifies changes resembling CAN in some respects (85) (146), with the expression of growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor-β, adhesion molecules, and endothelin (147). The initial events are not reversible by retransplantation into the donor after a certain “point of no return” (148).

Many molecular mechanisms are being explored as intracellular or extracellular mediators of vascular lesions. For example, the mitogen-activated protein kinase pathway can either stimulate or inhibit growth of arterial smooth muscle cells
depending on the availability of specific downstream enzyme targets (149). PDGF modulation of connective tissue synthesis may thus be critical (150), and the cross-talk between different signal transduction pathways could determine the net effect of PDGF (151). Metalloproteinase disintegrin-like cysteine-rich proteins regulate cell-cell and cell-matrix interactions as well as matrix degradation (152). Smooth muscle cell proliferation may be regulated through collagen via early integrin signaling and upregulation of cdk2 inhibitors (153). These and many other mechanisms would be considered targets for new interventions if they prove to be relevant to the human process of CAN.

**Somatic Cells Have a Finite Life: Theories of Tissue and Cellular Senescence**

Since the pathology of CAN overlaps that of aging and age-related disease, recent work on the cell biology of aging and senescence may be relevant. Somatic cells in culture are usually limited in their number of cell cycles. This finite cycling capacity has been called the Hayflick limit (154). At the end of this cycle number, the cell irreversibly shuts down many processes and usually dies. This condition, termed replicative senescence, is only one aspect of senescent behavior in worn-out cells. Other processes such as energy generation or membrane damage may also be limited. It is as if each cell has clocks that determine their life expectancy.

Senescence is an organized state that shuts down worn-out cells with potential for mutations to avoid malignant transformation. Indeed, the genes that are activated in senescence are the antitumor genes: p53, p16, p21, and others (155–157). Tumor suppressor gene products mediate cell cycle arrest at checkpoints, which are intrinsic mechanisms to desensitize cells to external growth signals as a programmed response to proliferative age. Recent studies implicate a limited number of other gene products in controlling the life span of simple organisms such as yeast cells or the worm *Caenorhabditis elegans*. Examples include the *Caenorhabditis elegans* gene cik-1, a central regulator of metabolism and life expectancy; the yeast gene RAS2, which controls the response to stress; and PHB1, the yeast homologue of the mammalian prohibitin gene, which regulates growth arrest.

Telomere erosion is one of the cell’s life span clocks. The telomere is the special region of repeat DNA sequences at the ends of each linear chromosome, interacting with specific proteins. It is designed to circumvent the problem that the ends of linear chromosomes cannot be completely replicated and some DNA is lost with each cycle, and to protect the ends of chromosomes from being recognized as DNA breaks. In immortal cells, the problem of telomere erosion is circumvented by a special DNA-replicating enzyme called telomerase, as well as by other mechanisms for maintaining telomere length (158). In somatic cells, telomerase is lacking and telomeres shorten with age. There may be other mechanisms involved in controlling telomere length. Telomere length acts as a clock because when shortening becomes critical the chromosome becomes unstable and the cell is shut down. Telomere shortening has been shown in human lymphocytes (159) and in aging blood vessels in vivo (160), and may explain the Hayflick limit.

Cellular senescence is determined by multiple factors, including the genetic regulation of metabolism, time, number of cell cycles of replication, and the level of injury and stress. The time to senescence in culture is dependent on the donor age at the time of harvest (161) and oxidant stress in tissue culture (162,163). Exposure to cytokines can accelerate the process of senescence (164). Aging mechanisms may be fundamentally different between species; for example, mouse and rat chromosomes have very long telomeres and mouse somatic tissues have active telomerase unlike human tissues, which could affect senescence mechanisms (165).

There is no automatic relationship between senescence in vitro and organ aging in vivo, but it is reasonable to assume that common mechanisms exist. Ultimately, the aging of a tissue must reflect changes in key cells within that tissue, which will also change the extracellular matrix. In an epithelium or an artery, the survival of the tissue is limited by the ability of certain cells to maintain the tissue against wear and tear and to restore it after acute injury. In arteries, the endothelial cells may be limiting: Endothelial cells organize the development of blood vessels in embryogenesis and regulate vasomotion and remodeling of the vessel wall (166). In hypertension or aging, exhaustion of the endothelial capacity for repair and remodeling of the artery may underlie the vascular sclerosis (FIT) lesions. Perhaps vascular age is limited by endothelial senescence, since vascular sclerosis is a feature of the aging kidney and is accelerated by disease states such as hypertension. The endothelial cells subjected to pressure and lipid injury may lose their ability to control remodeling of the arterial wall, resulting in fibrosis. There may be other limiting cells in the vessel wall, e.g., the vascular smooth muscle cells. Similar events may be postulated to occur in the epithelia: Cycles of injury and repair may reach the limit of the capacity of the epithelium to repair, leading to atrophy.

Thus, senescence of key cells would be particularly unfortunate when the tissue received unusual stresses. Injury evokes dedifferentiation, proliferation, expression of antitumor genes such as p53, and inflammation (167,168). If the epithelial cells can heal, the injury resolves and the inflammation resolves with minimal scarring. If the epithelium cannot heal, the inflammation persists and will result in scarring, perhaps because the injury response cannot be terminated by restoration of healthy epithelium. Thus, fibrosis may compensate for exhausted healing potential, putting fibrosis downstream of the primary senescence process.

**A Model for Chronic Allograft Nephropathy**

The salient human observations are as follows. (1) No specific lesions separate human CAN from aging or age-related diseases. (2) The concept of chronic rejection as an exclusively immune disease as described by Hume, Porter, Jeannet, and their colleagues does not fit the current disease of CAN, much of which reflects nonimmune factors. (3) The reduction of acute rejection by immunosuppression has had less effect on
CAN than predicted. (4) Donor age and organ quality strongly predicts CAN. (5) Absence of acute rejection correlates with protection against CAN. (6) Perfect HLA matching protects against CAN. (7) Animal models of chronic rejection do not reflect the complexity of human CAN.

We suggest that aging, nonimmune injury, and rejection drive renal tissue down a common pathway to a state of depletion of finite repair potential that we call renal senescence (Figure 4). This is manifest by progressive epithelial atrophy and endothelial deterioration with secondary fibrosis. Senescence is driven by time (age), age-related diseases before the transplant, peritransplant injury, rejection, and load factors such as hypertension, viruses, lipid abnormalities, and nephrotoxic drugs. This is integrated by the inherent genetic program of the donor cells, and thus may vary among individuals. CAN thus represents not a unique disease but a final common pathway that all kidneys must follow sooner or later, greatly accelerated by the special stresses on the transplant. At some point, the excess workload per nephron may itself drive the process to completion via glomerulosclerosis in some kidneys, but this is more a result of epithelial and endothelial deterioration.

A recent observation supports the concept that unusual stresses of transplantation can accelerate aging. In allogeneic bone marrow transplants, the transplanted cells show about a 16-yr acceleration of telomere shortening (169). It is not unreasonable to postulate that the stress of renal transplantation subjects key renal cells to abnormal acceleration toward their limits.

The infrequency of renal failure in normal aging even with a solitary kidney argues that the aging process under normal load conditions is seldom limiting unless accelerated by acute nonspecific injury or immune injury. This interpretation is compatible with the infrequency of renal failure in patients with one kidney removed for organ donation, the excellent survival of HLA-compatible live donor kidneys, and the relatively weak effect of sex mismatches (female kidneys into male recipients). Thus, the extra workload of a reduced nephron mass (within the limits of these situations) is not a powerful factor compared with input and/or immune stresses. Nevertheless, the high frequency of end-stage renal disease of various types in the elderly would suggest that injury repair mechanisms might be very limited in old kidneys when abnormal stresses emerge due to a primary renal disease.

In this model of CAN and for that matter in normal aging, the interstitial and intimal fibrosis are deemed to be secondary to the exhaustion of the epithelial and endothelial cells, respectively. Postulating that some epithelial atrophy is secondary to ischemia from FIT in small vessels does not fundamentally change the concept—the limiting senescent changes are those in the endothelium and the vessels.

The inflammatory response to injury may be either benign or maladaptive. Renal injury evokes inflammation and resolves with little fibrosis when the epithelium heals. Inflammation and fibrosis are thus controlled when the healing process is healthy. When critical elements required for tissue healing are exhausted (e.g., senescence of epithelial cells), inflammation persists. Inflammation may interact with immune response to facilitate immune surveillance of the injured area, and thus promote rejection, or may itself provide an additional stress on the tissue which accelerates senescence and triggers fibrosis. The possible roles of inflammation include: triggering fibrosis, acting as an additional stress on the tissue, and promoting the specific immune response.

**Advantages and Implications of a Senescence Model of CAN**

The present model explains the prominent effect of donor age and the accelerating effect of nonspecific injury and rejection, and accounts for the extensive overlap between the le-

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**Figure 4.** Three variations of the new model for CAN including possible roles of inflammation and rejection.
sions of CAN and those of aging and of other renal diseases. The model should generate experiments on the cell biology of the epithelial and endothelial cells in CAN and for that matter in other chronic renal diseases.

A kidney transplant is like a used car. The initial quality, mileage, wear and tear, and abnormal damage could predict its useful life and its ability to withstand subsequent stress. The customer who gets such a car would know what to expect, and would accept the limitations of the vehicle (informed consent). In a severe car shortage, we would seldom discard a heavily worn car: We would value it, minimize its stress, and examine ways to extend its useful life. We would seldom give two old cars to one person, without carefully considering the impact on the overall car shortage. Similarly, kidneys with significant wear and tear that may last only a few years need not be discarded but offered to patients with end-stage disease as a finite but useful alternative to dialysis. Rather than discard every suboptimal kidney, we must learn how to use such organs optimally and safely with informed consent.

It may be possible to assess quantitatively the state of senescent change in each donor kidney, like determining the mileage on a used car. The clocks such as telomere length may permit us to tell how much time a kidney has left. For now, we should evaluate the lesions of senescence carefully by biopsy. Special measures to minimize acute input stress to the tissue near exhaustion may be rewarding. Perhaps older kidneys should be allocated to older recipients, because immune and inflammatory mechanisms are attenuated in older recipients. Older patients would thus subject the kidney to less stress. Immunologic stress may be reduced by excellent HLA matching, balanced against detrimental effects of prolonged preservation.

Different immunosuppression may be useful for kidneys with advanced changes, perhaps emphasizing non-nephrotoxic agents. We should minimize hypertension and hyperlipidemias and proteinuria should be aggressively managed with angiotensin-converting enzyme inhibitors (170,171). Would inhibition of fibrosis be beneficial? In our model, fibrosis in CAN is triggered when the critical cells in the tissue—epithelium and endothelium—exhaust their capacity to proliferate, repair, and remodel injured tissue. Nevertheless, fibrosis itself may aggravate the situation, e.g., by further narrowing resistance vessels and adding ischemia to the other stresses. If so, inhibition of fibrosis could be useful.

Transplanting two kidneys to one recipient has been proposed, but this solution leaves many patients with no transplant. The option of transplanting both kidneys from a marginal donor to one host is preferable to discarding both, but may be based on a false premise. If in fact the progression of CAN is due primarily to a program in the kidney itself, rather than to workload, the benefit of two kidneys will be less than anticipated. For now, two kidneys from a marginal donor should only be given to one recipient in the context of a clinical trial in which the performance of transplants can be compared with that of two recipients each given one kidney. Such a trial could succeed if renal survival were more than doubled, which seems unlikely given the number of grafts lost due to patient death. Pending new clinical trials, the best strategy is simply to transplant each kidney into one recipient with informed consent and accept that the kidneys will have reduced survival and will need replacement.

Some of these concepts have obvious implications for understanding progression in chronic renal disease that cannot be fully examined here. For example, the stress of a continued insult (e.g., proteinuria) could exhaust the repair potential of the epithelial cells and lead to atrophy with secondary fibrosis. Perhaps studies of the transplanted kidney could help us develop an understanding of these more general processes.

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