Protecting Single-Kidney Allografts from Long-Term Functional Deterioration

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In the past decade, the short-term results of renal transplant surgery have dramatically improved, and 1-yr graft survival for cadaveric kidneys is now more than 80%, approaching 90 to 95% for living related donor transplants (1,2). Better short-term results, however, have not been paralleled by an acceptable improvement in long-term allograft survival (1), so that, after the first year, the number of functioning grafts begins to decline, down to a 5- and 10-yr survival of approximately 70% and less than 50%, respectively, at least for cadaveric grafts (1,3,4). Organ failure rates have remained almost constant in the past 20 yr, with only 40% of new allografts from a cadaver donor expected to survive at 8.5 yr (1,2). Prolonging the survival of transplanted kidneys is therefore a major task of modern nephrology. When death with a functioning graft is excluded as a cause of late renal allograft loss, most grafts fail after a period of renal function deterioration (5,6), which has been attributed to a process of progressive renal structural injury called "chronic rejection" (7,8). However, the pathophysiology of chronic rejection is not clear, and the underlying immunobiology is far from being understood in detail (7).

Pathophysiology of Progressively Deteriorating Renal Function after Transplantation

Renal transplant registry data and single-center studies indicate that immune events can influence long-term graft survival to a major extent (Figure 1). The clinical benefits of HLA matching are appreciable in recipients of renal grafts from living related donors. United Network for Organ Sharing (UNOS) registry data (9) showed estimated half-lives of 26.9 yr for HLA-identical grafts, and, respectively, 12.2 and 10.8 yr for one-haplotype-matched sibling grafts and parental grafts. The advantage of long-term engraftment of HLA-matched kidneys is also documented in cadaveric graft recipients in which estimated half-lives are 17.3 yr for HLA-matched and 7.8 yr for HLA-mismatched renal allografts (10). A threshold level of HLA matching also exists, at least for 3-yr graft survival, which is better in recipients with two or less HLA mismatches than in those with more than two antigen mismatches (11). The UNOS data, however, are not censored for death with a functioning graft. Other researchers (12) have shown that HLA matching has a beneficial effect on long-term outcome only in patients with no rejection episodes, suggesting that the impact of HLA may be dependent mostly on decreased patient mortality in well matched recipients. Moreover, the evidence of unexpectedly higher rates of survival of kidney grafts from spouses and other living unrelated donors obtained in recent years (12-19) has tempered the importance of HLA in living-donor allograft. Although most transplants from living unrelated donors are mismatched for HLA antigens, the survival rates of these grafts are even higher than those of similarly mismatched cadaveric grafts (20). This has been confirmed more recently by the analysis of Terasaki et al. (21) on survival rates of grafts in 368 spouses and 129 living unrelated donors of the UNOS renal transplant registry. In this analysis, the superior survival rate of grafts from unrelated donors could not be attributed to better HLA matching, white race, younger donor age, or shorter cold-ischemia times, but was explained by damage due to shock before removal in 10% of the cadaveric kidneys, which is in keeping with the 10% difference in graft survival rates.

The number of acute rejections is another crucial immune-related risk factor for long-term kidney survival (22-24). In 706 renal transplants with a graft functioning for more than 6 mo and followed for a median of 6.4 yr, the 10-yr survival was 96% in patients who had no acute rejections, 88% in those who had one acute rejection, and 54% in patients with more than one such event (25).

Although acute rejections generally increase the risk of chronic allograft dysfunction, not all episodes have an equal impact on subsequent renal function deterioration, and time and severity of such episodes also play a role. For instance, late forms of acute rejection (i.e., after one or more years from surgery) were associated with a threefold risk of subsequent chronic renal allograft failure (25).

Besides immune events, and possibly even more relevant, factors of a nonimmune nature can also impair long-term graft survival (26,27) (Figure 1), e.g., very young, old, black, or female donors whose kidneys survive less well than grafts from 15- to 55-yr-old white, male donors (28). Posttransplant anuria and recipients' obesity are two other variables consistently associated with reduced long-term graft survival (20). Finally,
arrestal hypertension, which hastens the progression of all forms of renal disease (29), is one of the major issues of concern for long-term transplant outcomes (30).

In this section, we will focus first on immune mechanisms of progressive renal injury and then examine in detail the role of nonimmune events.

Factors of Immune Origin

Animal studies indicate that T cell recognition of alloantigens plays a central role in initiating mechanisms critical for chronic rejection, including activation of CD4+ T lymphocytes and macrophages, CD8+ T cells, and alloantibody-producing B cells. Two pathways of allore cognition have been described thus far (31,32): in the first, termed “direct” pathway, T cells recognize intact allo-MHC molecules on the surface of donor antigen-presenting cells (APC); in the second, termed “indirect” pathway, T cells recognize processed alloantigens presented as allopeptides by self-MHC on APC. The available evidence seems to indicate that early acute allograft rejection is mediated primarily by the direct pathway, whereas the indirect pathway of allore cognition predominates in the chronic rejection process (33). Findings of activation of the indirect pathway shortly after transplant (34,35) are not corroborated so far by enough data on whether there is a persistent recruitment of this pathway that may actually promote chronic graft dysfunction. Independent of the pathways of allore cognition, alloactivated T cells produce distinct sets of cytokines that identify two types of CD4+ T helper cells (36). Type 1 helper cells (Th1 cells) produce interleukin-2 (IL-2) and interferon-γ, which promote macrophage activation and delayed-type hypersensitivity responses. Type 2 helper cells (Th2 cells) secrete IL-4, IL-6, IL-10, and IL-13, which differentiate B cells into antibody-producing cells. However, the relative contribution of Th1- versus Th2-type lymphocytes to the development of chronic rejection remains unknown. Th2 cells may suppress Th1 responses, thus protecting the graft from acute rejection, as shown in transplant models of tolerance induction associated with a state of “immune deviation” toward predominantly Th2 cells (37). On the other hand, Th2 cells, by facilitating the production of alloantibodies by B cells, can play a more relevant role in directly damaging graft vascular endothelia (37). Thus, immunocytochemistry studies of rat renal allografts with chronic rejection found evidence of monocytes/macrophages and CD4 T helper cells infiltrating the kidneys, as well as of immunoglobulin deposition on microvascular endothelia (38,39). Moreover, IgG antibodies against yet unidentified donor tubular basement membrane antigens have been found in sera of rats with chronic renal allograft rejection (40). Thus, it is possible that in chronic rejection Th2-type response dominates over Th1. Cytokines secreted by activated T cells and macrophages in the graft in turn activate endothelial cells to express cell surface adhesion molecules and class I and II MHC molecules, which mediate further recruitment and activation of lymphocytes (41). The precise role of this bidirectional lymphocyte–endothelial cell activation on chronic rejection is unclear. Of interest, cultured endothelial cells effectively activate Th2, not Th1, cells (42), which may eventually enhance B cell-mediated alloantibody production.

T cells, macrophages, and activated graft endothelial cells all secrete growth factors that by promoting smooth muscle cell proliferation can cause vascular intimal occlusion, interstitial fibrosis, and matrix deposition leading to renal scarring.

Noncompliance with immunosuppressive therapy is increasingly recognized as an important cause of allograft loss due to

Figure 1. Immunologic and nonimmunologic factors that play a role in the progressive deterioration of renal function after transplantation.
chronic rejection (43–45). That “suboptimal” immunosuppression is an additional risk factor for chronic rejection has been suggested by some studies (46–48). In the past few years, lower cyclosporin A (CsA) maintenance doses have been used to minimize nephrotoxicity. However, there is some evidence (46–48) that dose reduction does favor chronic rejection. In a recent multivariate analysis, change in CsA pharmacokinetics was correlated with chronic rejection in 204 renal transplant recipients on dual immunosuppression with CsA and prednisone (49). Daily CsA dose, dose frequency, and coefficient of variation of peak CsA concentration values did not correlate with chronic rejection. At variance, a highly significant correlation was found with a greater than 20% coefficient of variation of dose-corrected average CsA blood concentration (49), suggesting that variable bioavailability of CsA is indeed a risk factor for chronic rejection. That more than half of the renal transplant recipients had a greater than 20% coefficient of variation of average blood CsA concentration during 5 yr posttransplant would indicate that CsA absorption remains highly variable even in the long-term (50).

In the pre-CsA era, the use of blood transfusion improved long-term cadaveric graft survival by 10 to 20% compared with nontransfused recipients (51–53), providing further support to the concept that immunologic factors are important in late graft failure. In the past two decades, however, the role of blood transfusion has been questioned (54,55). The Collaborative Transplant Study data (56) actually found less benefit of transfusion in patients on CsA, and presently risks of infection and presensitization appear to outweigh any benefit on graft survival.

Factors of Nonimmune Origin

Findings that even in isografted rat kidneys proteinuria got worse with time and that progressive interstitial and glomerular injury became evident (57) raise the question of whether antigen-independent factors also contribute to the chronic deterioration in renal function after transplantation.

Nonimmune mechanisms that may cause structural and functional damage include prolonged graft ischemia, surgical manipulation, and reperfusion injury (58). Long-term nephrotoxicity of immunosuppressants such as CsA or FK506 may add further injury to the primary graft ischemia (59). In addition, single renal allografts may often supply the recipient with an inadequate number of functioning nephrons (28,60). In experimental animals, a too small number of nephrons due to a small renal mass for acquired or innate reasons, triggers a self-perpetuating cycle of events, the hallmark of which is excessive urinary protein excretion followed by interstitial and glomerular inflammation and scarring (61). Hemodynamic determinants of subsequent renal injury in this setting are enhanced intraglomerular pressure and flow, closely involved in the development of renal structural damage (62,63). Glomerular hypertension enhances filtration of macromolecules across the capillary barrier, which are then largely reabsorbed by proximal tubuli (64). This tubular cell activation upregulates genes for inflammatory and vasoactive proteins that, in the long run, contribute additionally to renal scarring (65). All of these mechanisms could logically operate in a kidney graft whose nephron dose may be largely inadequate to meet metabolic demand, contributing significantly to progressive renal function deterioration. Bilaterally nephrectomized Lewis rat recipients of a single Fisher 344 kidney allograft developed proteinuria, hyperlipidemia, and glomerulosclerosis 16 to 24 wk posttransplant, and renal injury with time (38); the damage was greatly attenuated when the Lewis recipient retained one of its native kidneys (66). Putting two Fisher 344 kidneys into bilaterally nephrectomized Lewis recipients limited the late allograft injury despite the excess of antigen load (67). Moreover, cadaver kidneys from older (>50 yr) and younger (<10 yr) donors are associated with decreased graft survival (1,68,69), which could be due to reduction of nephron mass in the former, and to the effect of a rapid increase in growth of initially small kidneys placed under the metabolic demand of the adult recipients in the latter donor category.

Therapeutic interventions, including diet and angiotensinconverting enzyme (ACE) inhibitors, which attenuate the hemodynamic response to a reduced number of nephrons, slow subsequent injury. Thus, dietary protein restriction retarded proteinuria and renal lesions in rats with renal mass ablation (70) and in many other models of progressive nephropathies (71–73), while normalizing glomerular hypertension and hyperfiltration (63,65,72). Agents that reduce serum lipids also slow renal disease progression in various animal models (74,75), with mechanisms independent of changes in glomerular hemodynamics; however, these mechanisms are not yet well defined. ACE inhibitors reduce proteinuria and renal damage in a number of models of rat progressive renal injury due to a lower than normal number of nephrons (76–78). The most plausible explanation of the favorable effect of this class of compounds in experimental renal disease is that ACE inhibitors, due to their peculiar effect of preferentially dilating efferent arterioles, reduce glomerular hypertension and improve glomerular size-selective function, ultimately resulting in less urinary protein and less renal damage (65,76).

The possibility of a common pathogenic mechanism accounting for progressive renal disease in animals and humans with a lower than normal number of nephrons suggests that maneuvers that retard renal dysfunction in progressive diseases other than transplantation might be effective in posttransplant chronic renal injury. There are no data on the effect of dietary protein restriction or lipid-lowering agents in animals with chronic graft dysfunction, but recent studies have found that ACE inhibitors or angiotensin II (AngII) receptor blockers prolonged renal graft survival in rats (79,80). In a recent study, we found that daily administration of losartan to kidney-transplanted Fisher 344→Lewis rats prevented the development of proteinuria, preserved renal structure, and improved graft survival (80). These findings are in accordance with a recent report that in the same model, the ACE inhibitor cilazapril or the AngII receptor blocker L-158,809 reduced urinary protein excretion and limited renal injury (79).

Collectively, these findings confirm that reduced nephron number is a leading cause of chronic renal function deterioration in rat transplantation. Its synergistic interaction with al-
loantigen-specific injury processes poses new challenges to renal transplant physicians and provides the rationale for non-immune strategies in the context of better immunosuppression.

Can We Prevent Chronic Allograft Dysfunction?
Approaches to protect allografted kidney in the long-term again include immune and nonimmune interventions.

Optimizing Chronic Immunosuppressive Strategies
Current anti-rejection regimens, very effective in controlling acute rejection, appear of limited value in chronic rejection. There is no doubt that CsA has contributed to the dramatic improvement of 1-yr graft outcome, but its impact on long-term graft survival is questionable (2). In 570 kidney transplant patients on CsA and low-dose steroids, there was a 23-yr graft half-life that compared well to 16.6 yr in 171 grafts in patients given azathioprine, high-dose steroids, and antilymphocyte globulins (81). However, results from large series did not find any better long-term outcome after CsA was introduced in routine protocols (1,2). Experimental and clinical evidence has suggested that some of the current immunosuppressants may participate in the development of chronic allograft dysfunction. This is well documented for CsA, and is attributed to its significant nephrotoxic potential in routine clinical usage, related to renal vasoconstriction and its ability to promote the synthesis of the fibrogenic cytokine transforming growth factor-β (TGF-β) (59).

Data collected from 304 centers in 45 countries have also shown that different combination regimens of the three most used immunosuppressive agents may have different effects on the long-term outcome of a kidney transplant (82). Analysis of these data from the first to the fifth year after surgery showed that renal transplant recipients maintained with CsA monotherapy had better long-term graft survival than those on steroids and CsA, or azathioprine and steroids but no CsA (82). However, a recent randomized study has shown that monotherapy with CsA did not provide a better 4-yr graft survival compared to double therapy with CsA and steroid (83). Recent follow-up data of a prospective conversion study from CsA to azathioprine (beginning 3 mo after transplantation) showed an 8-yr graft survival rate of 64% in the CsA group versus 76.6% in the azathioprine group (84), whereas in another recent article (85) 10-yr graft survival was 56% in patients randomized to CsA and 35% in those randomized to azathioprine, both given with steroids.

The variability of oral CsA absorption with the traditional Sandimmune formulation, one of the risk factors for chronic rejection (49), has been limited by the new microemulsion formulation (86), which allows a consistent drug absorption and reduces intraindividual variability of the area under the time-CsA concentration curve (AUC) during a 6-mo follow-up period (87,88). If the new formulation reduced variability on CsA absorption in the long run, it would possibly overcome one of the obstacles to long-term transplant success.

The search for more active and specific immunosuppressants to control chronic allograft dysfunction has continued in the past decade, but information on the efficacy of these new drugs is scant. Data reported to the UNOS Kidney Transplant Registry appear encouraging with regard to the potential benefit of tacrolimus on long-term kidney graft survival (89). Thus, by actuarial analysis, a 10-yr graft survival rate of 67% can be projected from a 3-yr kidney graft survival for the first cadaveric renal transplant patients maintained with tacrolimus compared with the projected 48% rate for kidney recipients currently on CsA. This indicates that recipients given tacrolimus might have a previously unseen long-term gain in graft survival compared with CsA regimens. Caution, however, is warranted when drawing causal inferences from this study, because tacrolimus data were relatively scarce, and important data regarding dosage levels and other concomitant anti-rejection therapies were not analyzed.

A possible explanation of tacrolimus' effect lies in the recent demonstration that the drug and the growth factor TGF-β share the same binding site, the immunophilin FKBP12 (90). Thus, tacrolimus could interfere with TGF-β signaling through competitive binding, antagonizing the fibrosis-promoting effect of this growth factor.

It is also possible that the new selective immunosuppressant mycophenolate mofetil (MMF) (91), developed for the prevention and treatment of acute graft rejection, may in fact reduce the risk of chronic allograft dysfunction. This is based on experimental evidence that MMF reduced the incidence of graft coronary disease in the rat model of cardiac allograft (92) and in a primate cardiac xenograft model (cynomolgus monkey to baboon), and prevented functional and structural injury in chronic renal allograft rejection in rats (93).

Because MMF specifically inhibits the proliferative response of both T and B lymphocytes and blocks humoral responses in vitro and in vivo (94), it would affect processes thought to be involved in chronic graft rejection. Angiography and intravascular ultrasound data from a recent MMF clinical trial in cardiac transplant recipients show a significant reduction in the degree of graft arteriosclerosis 1 yr after surgery (95). However, analysis of the three collaborative multicenter MMF trials in the United States, Europe, Australia, and Canada found no significant difference in 3-yr kidney graft survival compared with the azathioprine-based immunosuppressive regimen (96,97). Moreover, analysis of renal biopsies at 1-yr posttransplant did not show a difference between MMF and control groups. These studies, however, were not originally designed to demonstrate any effect of MMF on chronic allograft dysfunction. Therefore, they might not have the statistical power to document a clear graft survival advantage of MMF at this relatively short time after transplantation.

There is also experimental evidence that rapamycin, a new immunosuppressant macrolide, prevents arteriosclerosis after allogeneic aorta transplantation in the rat (98), but its role in long-term prevention of chronic rejection needs to be confirmed in humans.

The goal of transplant medicine, however, remains specific acceptance of the allograft without immunosuppression, i.e., the induction of tolerance (99). Clinical strategies of tolerance
have been investigated with the use of blood transfusion (100) and donor bone marrow infusion (101), but their impact on long-term graft outcome remains to be established.

**Approaches Aimed at Nonimmune Events**

Interventions to control nonimmunologic determinants of chronic allograft dysfunction have included protein restriction, lipid-lowering agents, and antihypertensive medications.

**Low Protein Diet.** Feehally et al. (102) reported a lessening of the decline in the inverse of serum creatinine over time with protein restriction in a small group of long-term transplant patients, but there were no control subjects. Kootte and Paul (103) compared the effects of dietary protein restriction and control diet in patients who had clinical or biopsy diagnosis of chronic rejection. There was no difference in the numbers of patients who returned to dialysis. However, the patients on a low protein diet tended to have better preserved renal function. In 14 patients with chronic kidney rejection randomly assigned in a crossover design to an 11-d period of low- or high-protein diet, protein restriction was associated with an improvement in glomerular permeselectivity to macromolecules, with no changes in blood pressure and renal function (104). These findings indicate a potential beneficial effect of dietary protein restriction in kidney transplant recipients. The level of protein intake reached with a low protein diet regimen used in chronic renal failure might be insufficient for transplant patients and result in negative nitrogen balance, ultimately exposing patients to risk of infections (105). Actually, nitrogen balance can be maintained even on a low protein intake (0.6 g/kg per d) in stable renal transplant recipients provided an adequate caloric intake (>25 kcal/kg per d) (106). However, these findings should be considered with caution because they are derived from a short-term study of a very small number of patients. Additional trials are clearly needed to establish the safe level of dietary protein restriction and its long-term effects on the progression of chronic allograft dysfunction before a low-protein diet can be recommended as a treatment option for transplant patients.

**Lipid-Lowering Agents.** Lipid-lowering agents have been used because 75% of kidney transplant recipients have hyperlipidemia (107) and a higher incidence of acute and chronic rejection (108,109). 3-Hydroxy-3-methyl coenzyme A reductase inhibitors safely and effectively control posttransplant hyperlipidemia (110–112), but there are no data on patient and graft outcome. In a prospective randomized trial of kidney transplant recipients, pravastatin, a 3-hydroxy-3-methyl coenzyme A reductase inhibitor, reduced the incidence of acute rejection episodes to 25% compared with 58% in control transplanted patients (113). Indeed, there is growing evidence that these drugs have immunosuppressive properties (113–115). However, whether the lipid reduction will affect long-term graft survival by preventing progressive renal function deterioration is unclear and merits further investigation.

**Antihypertensive Medications.** There is no question that chronic renal injury to native kidneys is exacerbated by high systemic blood pressure (116). Hypertension after transplantation is also associated with a poorer outcome, i.e., faster decline in creatinine clearance and a greater likelihood of return to dialysis or death (26,117,118). Moreover, in patients with chronic rejection, a significant negative correlation was found between renal allograft survival and the degree of hypertension (119,120). Unfortunately, few studies have addressed the question of whether adequate blood pressure control limited time-dependent deterioration of graft function.

The prevalence of posttransplant hypertension has increased from no more than 40 to 50% in the pre-CsA era to nearly 80% since the drug was introduced into clinical practice (121–123). CsA-induced hypertension is apparently multifactorial: It was considered primarily a consequence of sodium retention and therefore volume-dependent (124), but it appears to have an additional role for enhanced peripheral vascular resistance linked to the effect of the drug in constricting the renal vascular bed.

Initially, CsA-induced hypertension was managed by sodium restriction and diuretics (124), but, by reducing blood volume, this precipitated prerenal azotemia and further enhanced the CsA-dependent reduction of renal perfusion. Nephrologists then started to use calcium channel blockers (CCB), which appeared to be the ideal alternative (125,126). These drugs were very effective in salt-sensitive forms of hypertension (127) and increased renal perfusion in cases of vasoconstriction elicited by specific agonists (128–130), which was taken as a strong basis for using them in CsA-induced hypertension. It is now acknowledged that CCB are natriuretic in experimental models and in humans (131,132), and enhance urinary sodium in a way that can be dissociated from the increase in filtered sodium in that GFR remains unchanged. These effects, however, do not necessarily last forever, and with time sodium excretion may return to pretreatment values, as found with felodipine given for essential hypertension (133).

In the past few years, the idea has developed that calcium antagonists protect against CsA-induced renal vasoconstriction. Although the exact mechanism is far from clear, it is possible that CCB prevent thromboxane A2- and endothelin-induced increase in afferent arteriolar tone in CsA-treated animals and humans (59,131,134).

We found that seven days' treatment with the calcium antagonist lacidipine completely prevented the transient daily reduction in renal plasma flow (RPF) and GFR invariably associated with CsA administration in renal transplant patients with stable renal function (135), an effect that was independent of changes in CsA pharmacokinetics. These findings agree with the recent demonstration that felodipine enhanced RPF and GFR, and reduced systemic blood pressure in CsA-treated renal transplant recipients given the drug in a randomized, placebo-controlled trial (136,137).

Experimental and clinical studies (59) have suggested that the repeated episodes of transient renal vasoconstriction and hypoperfusion after daily CsA dosing may favor structural alterations of glomerular vessels and subsequent glomerular and tubulointerstitial injury, the so-called "chronic CsA nephropathy" (138). This is characterized primarily by focal interstitial fibrosis and tubular atrophy. Renal arteriolar abnormalities consisting of either necrosis of smooth muscle cells
and nodular protein deposits in the wall of afferent glomerular arterioles or arteriolar intimal hyalinosis are also seen. In addition to vascular and tubulointerstitial changes, a series of lesions has been described in the glomeruli, including focal and segmental sclerosis, and thickening and wrinkling of capillary basement membrane. Theoretically, CCB, by antagonizing CsA-induced vasoconstriction of preglomerular vessels, may protect the kidney from structural injury and eventually prevent end-stage renal failure. There are no solid data, however, on whether CCB improve transplant graft survival in the long-term.

In a retrospective study (139), the 1-yr graft survival rate was higher in 17 patients given verapamil or nifedipine (94%) than in 24 control subjects (75%). Another retrospective study, however, showed no differences in graft function and graft survival at 3 and 12 mo in CCB versus conventional antihypertensive groups (140). Dawidson et al. (141) reported a prospective study in 59 patients randomized to receive either verapamil for 14 d or no calcium antagonist, along with conventional four-drug immunosuppression. The verapamil-treated patients had a 93% 1-yr graft survival rate compared with 72% of control subjects. Analyzing the 4-yr follow-up of a prospective randomized open trial in patients given diltiazem from the day of kidney transplant, Neumayer and coworkers (142) found a tendency toward a better survival rate compared with control subjects, but differences were of marginal clinical significance. Moreover, it appeared that most of the beneficial effect of the calcium antagonist was confined to the very early posttransplant period, after which kidney graft survival in diltiazem-treated patients paralleled that of untreated control subjects.

In a Spanish series by Morales and coworkers (143), during a 5-yr follow-up renal transplant patients treated with CsA and given nifedipine to control blood pressure had an improvement of renal function compared with those on β-blockers, but this was not associated with better graft or patient survival at 1, 3, and 5 yr from transplant, and there are a number of other studies that reach substantial similar conclusions (144). Collectively, there appears to be some advantage of CCB over conventional antihypertensive medications in the early posttransplant period, but no consistent effect in the long-term.

Besides the fact that CCB do not provide a significant benefit in prolonging long-term graft survival, one should consider that their use may be associated with side effects such as edema, which may cause discomfort to patients. Moreover, CCB—which stimulate cell proliferation, at least in vitro—should be used with caution in immunosuppressed patients who are per se at high risk of developing neoplasia. The safety of CCB as antihypertensive agents has been questioned further by the recent observation of a higher incidence of myocardial infarction in patients on short-acting dihydropyridines than in those on diuretics and β-blockers (145).

ACE inhibitors have been mentioned as alternatives in posttransplant hypertension. For years nephrologists were reluctant to use them in renal transplant recipients because of a number of alarming reports on the possibility to induce renal insufficiency (146–149). Now, however, using ACE inhibitors for renal transplant recipients is no longer a rarity. In the past few years, it has become apparent that ACE inhibition is an excellent alternative to serial phlebotomy to reduce posttransplant erythrocytosis (150–152).

**Renoprotective Properties of ACE Inhibitors: To What Extent Can Data on Diabetic and Nondiabetic Proteinuric Nephropathies Be Extrapolated to Transplant Nephropathy?**

Blockade of the renin–angiotensin system with ACE inhibitors or AngII receptor antagonists reduces urinary protein excretion and protects against glomerulosclerosis better than conventional therapy in rat models of chronic renal disease due to lower than normal nephron numbers (76–80), and in humans with proteinuric renal diseases (65,153,154).

Thus, in a double-blind controlled trial in patients with insulin-dependent diabetes mellitus, captopril was better than conventional therapy in lowering protein excretion, preserving renal function, decreasing the need for dialysis, and reducing the mortality rate (155). Similarly, in non-insulin dependent diabetic patients with either macro- (156) or microalbuminuria (157), enalapril showed a better antiproteinuric effect and slowed the rate of renal function decline more than conventional antihypertensive treatment.

In the largest trial to date, in 583 patients with chronic renal disease who took part in the Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency Study (158), benazepril halved the overall risk of progressive renal insufficiency (defined as doubling of baseline serum creatinine) over a 3-yr follow-up period compared to placebo, with a greater effect in patients with baseline proteinuria higher than 1 g/24 h. In the above trial, patients on the ACE inhibitor showed a remarkably better control of systemic blood pressure, whereas in the Ramipril Efficacy in Nephropathy (REIN) Study (159), the mean GFR decline and the risk of renal end point in patients with baseline urinary proteins of 3 g/24 h or more were significantly lower in ramipril than in the placebo group at comparable levels of blood pressure control. Collectively, trials in diabetic and nondiabetic progressive renal disease showed that, at a comparable level of blood pressure control, ACE inhibitors slow the rate at which renal function is lost better than other antihypertensive agents (153,154). Of interest, such a renoprotective effect is restricted to proteinuric chronic nephropathies and is consistently associated with a substantial limitation of urinary protein excretion. That reduction in proteinuria precedes and consistently correlates with the renoprotective properties of these drugs in the long-term (155,158,159) is consistent with the possibility suggested by many animal studies that protein traffic has a pathogenic role in renal disease progression (160). Proteins filtered in excessive amounts are reabsorbed by proximal tubular cells, a process associated with perinuclear organelle overload, leading to upregulation of vasoactive and inflammatory genes (214) that contribute to a process of tubular-interstitial injury and progressive renal scarring (160).

Of interest, in transplantation progressive decline in renal
function mostly occurs in patients with measurable proteinuria and correlates with the amount of proteins excreted in the urine (5). Similarities between remnant kidney models and transplant nephropathy, as underscored in the previous sections of this review, represent a promising background for the use of ACE inhibitors to protect renal function in transplantation, but no appropriate controlled studies of this kind are available.

In 10 patients with posttransplant hypertension, on immuno-suppressive therapy with azathioprine and prednisone, who discontinued their previous antihypertensive medications 6 to 72 mo after surgery, fosinopril taken for 12 mo reduced mean arterial pressure, and all patients reached normotension after four months’ treatment (161). Normalization of blood pressure with the ACE inhibitor was followed by a decrease in GFR, which returned to baseline values when the drug was discontinued. The progressive reduction in 24-h urinary protein excretion and the normal response to acute protein intake during ACE inhibition therapy was believed to indicate an effect of limiting glomerular hypertension.

In 22 patients with posttransplant nephrotic syndrome on double or triple immunosuppressive therapy, incremental doses of enalapril for 1 yr resulted in a significant fall in mean daily proteinuria without changes in renal function, measured as predicted creatinine clearance by the Cockroft–Gault equation (162). Analysis of individual data showed that the rate of deterioration of renal function did not increase in 17 of the 22 patients. Again, no serious side effects were observed with enalapril. The short-term efficacy and safety of ACE inhibition in kidney transplant recipients was confirmed in eight patients in whom microalbuminuria improved, with no significant changes in mean blood pressure or GFR after three months’ therapy (163). A significant decline in urinary protein excretion was also reported in 76% of proteinuric transplant patients given enalapril or captopril for an average of 21 mo (164). The favorable effect of ACE inhibition on proteinuria was associated with stabilization of renal function in 62% of cases.

The above studies were not designed to compare the effects of ACE inhibitors and other antihypertensive agents. In a prospective randomized study in renal transplant recipients maintained on CsA, the ACE inhibitor lisinopril, given alone or in association with furosemide, had similar antihypertensive effects when compared to nifedipine, alone or combined with atenolol, during 2.5 yr of follow-up (165). In both the ACE inhibitor and the CCB groups, GFR remained stable, urinary albumin excretion did not change, and trough blood levels of CsA were identical. Interestingly, only in patients given the ACE inhibitor was filtration fraction decreased, suggesting a reduction in glomerular capillary pressure.

In a group of 20 hypertensive CsA-treated renal transplant patients studied in a 4-wk double-blind crossover trial, lisinopril had a significant antihypertensive effect, somewhat less pronounced than that of amlodipine (166). Although CCB treatment was associated with an increase in GFR and RPF, renal hemodynamics did not change with lisinopril. In the 13 patients with proteinuria exceeding 0.5 g/dl, neither drug had any significant effect. These findings contrast with those of Sennesael and coworkers (167), who reported that eight weeks’ treatment with perindopril or amlodipine was equally effective in lowering blood pressure and reducing renal vascular resistance while maintaining GFR and RPF in patients on CsA transplanted more than 6 mo earlier.

Combined treatment with perindopril and nifedipine for 2 mo was more effective than nifedipine alone in the management of posttransplant hypertension in patients with stable renal function (168). Despite the short-term follow-up, a significant reduction of proteinuria was found at the end of the 2-mo treatment with perindopril.

It would therefore appear that ACE inhibitors are effective in controlling posttransplant hypertension, with no major negative effects on renal function. Up to now, however, there has been no information about the impact of ACE inhibition on progressive renal graft dysfunction and ultimately on long-term graft survival.

Conclusions

In the past two decades, long-term results of renal transplantation have not paralleled the progress shown in short-term results, on account of our inability to prevent progressive chronic allograft dysfunction. The pathophysiology of the progressive deterioration of renal graft function is still a matter of debate, and the process has been recently considered to result from a complex interaction of immune and nonimmune events. The immune process may involve Thl-dependent and alloantibody-mediated episodes of tissue injury, not blocked by current immunosuppressants. Nonimmune factors may also be involved, related to the inadequate supply of functioning nephrons with a single kidney allograft, exacerbated by a further reduction in nephron numbers due to graft ischemia, CsA nephrotoxicity, and hypertension early after the transplant. This sequence of events is believed to reproduce the self-perpetuating cycle responsible for renal structural and functional damage in experimental models of reduced nephron number due to a lower than normal renal mass for genetic or acquired reasons.

Therefore, approaches to protect the allograft kidney in the long-term have been designed to control immune and nonimmune events. We are still awaiting better immunosuppressive strategies, including new immunosuppressants. In light of its protective effect in experimental animal studies, MMF is a drug that may be used in the future to inhibit immune factors and affect long-term graft survival, but convincing data in transplant patients are still lacking.

Measures to control nonimmune determinants of chronic allograft dysfunction have included dietary protein restriction and lipid-lowering agents, but the available data on graft outcome are scant. However, there is more information for antihypertensive medications in renal transplantation. Currently, CCB are the most widely used class of drugs to prevent delayed graft function in the immediate postsurgery period, to reduce CsA nephrotoxicity and normalize posttransplant hypertension. It is clear that CCB improve early posttransplant renal function and control systemic blood pressure, but their effects on long-term graft outcome remain to be clarified.

ACE inhibitors are a possible alternative for posttransplant
hypertension, but until recently their use in kidney graft recipients was limited by their potential for inducing acute renal insufficiency, at least in some patients. However, the recent use of ACE inhibitors to reduce posttransplant erythrocytosis has demonstrated their safety, and, as a result, this class of antihypertensive agents is now being reconsidered for chronic graft dysfunction. The potential renoprotective effect of ACE inhibitors in transplantation rests on their documented efficacy in diabetic and non-diabetic proteinuric nephropathies, in which the reduced number of functioning nephrons is the key event in progressive renal injury, as has been postulated for single-kidney transplant. Posttransplant hypertension is well controlled by ACE inhibitors, which also reduce proteinuria. Available thus far are only short-term follow-up data with this class of compounds, which are not enough for predictions on their impact on kidney graft survival in the long run. This issue is under investigation in clinical trials designed ad hoc.

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