Calcineurin Inhibitor Nephrotoxicity

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The use of the calcineurin inhibitors cyclosporine and tacrolimus led to major advances in the field of transplantation, with excellent short-term outcome. However, the chronic nephrotoxicity of these drugs is the Achilles' heel of current immunosuppressive regimens. In this review, the authors summarize the clinical features and histologic appearance of both acute and chronic calcineurin inhibitor nephrotoxicity in renal and nonrenal transplantation, together with the pitfalls in its diagnosis. The authors also review the available literature on the physiologic and molecular mechanisms underlying acute and chronic calcineurin inhibitor nephrotoxicity, and demonstrate that its development is related to both reversible alterations and irreversible damage to all compartments of the kidneys, including glomeruli, arterioles, and tubulo-interstitium. The main question—whether nephrotoxicity is secondary to the actions of cyclosporine and tacrolimus on the calcineurin-NFAT pathway—remains largely unanswered. The authors critically review the current evidence relating systemic blood levels of cyclosporine and tacrolimus to calcineurin inhibitor nephrotoxicity, and summarize the data suggesting that local exposure to cyclosporine or tacrolimus could be more important than systemic exposure. Finally, other local susceptibility factors for calcineurin inhibitor nephrotoxicity are reviewed, including variability in P-glycoprotein and CYP3A4/5 expression or activity, older kidney age, salt depletion, the use of nonsteroidal anti-inflammatory drugs, and genetic polymorphisms in genes like *TGF-β* and *ACE*. Better insight into the mechanisms underlying calcineurin inhibitor nephrotoxicity might pave the way toward more targeted therapy or prevention of calcineurin inhibitor nephrotoxicity.

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The introduction of the calcineurin inhibitor (CNI) cyclosporine in human kidney transplantation in the late 1970s revolutionized transplantation medicine, and made transplantation a preferable therapeutic intervention for end-stage renal diseases (1,2). In 1984, the potent immunosuppressive properties of another CNI, tacrolimus, were discovered, and tacrolimus was used successfully in human liver, kidney, and heart allograft recipients (3,4). Currently, 94% of kidney transplant recipients are discharged after transplantation with a CNI-based immunosuppressive regimen (5).

The immunosuppressive properties of cyclosporine and tacrolimus result from inhibition of calcineurin, a calcium- and calmodulin-dependent phosphatase (protein phosphatase 3 [PPP3C], formerly PP2B). Intracellularly, these completely different molecules bind to cyclophylin and FKBP12 for cyclosporine and tacrolimus, respectively (6–9). The competitive binding of cyclosporine-cyclophylin and tacrolimus-FKBP12 complexes to calcineurin inhibits phosphatase activity of calcineurin. This inhibition then suppresses the transcription of IL-2 via inhibition of the dephosphorylation and impaired translocation of the nuclear factor of activated T cells (NFAT) (10–12), which regulates IL-2 transcription and thus T cell activation (13–15). The current insights into the role of calcium, calcineurin and NFAT in the regulation of T cell development and function are summarized in a review by Macian *et al.* (16).

Calcineurin (17–19) and NFAT isoforms (five different isoforms; only NFAT5 is not calcineurin dependent) (20) are, however, not T cell specific, and inhibition of this pathway by cyclosporine and tacrolimus gives rise to toxicity beyond immunosuppression (21). The considerable side effects that accompany treatment with cyclosporine and tacrolimus hamper long-term kidney graft and patient survival, and cause major additional morbidity. This is the case not only for kidney transplantation, in which the direct nephrotoxicity of these agents represents a huge challenge for clinicians, pathologists, and scientists. The nephrotoxic effects of cyclosporine and tacrolimus are also a major concern in nonrenal solid organ transplantation and many other diseases requiring therapy with these drugs (22–25).

In this article, we discuss the clinical and histologic aspects of CNI nephrotoxicity, together with the molecular mechanisms and clinical risk factors associated with acute and chronic CNI nephrotoxicity. We also provide a brief overview on current and potential future approaches to prevent and treat CNI nephrotoxicity.

Pathogenesis, Histology, and Clinical Features of CNI Nephrotoxicity

Because cyclosporine has been used for a much longer time, most data in this field pertain to cyclosporine. The effects of tacrolimus are considered to be similar (see below). The nephrotoxicity of cyclosporine was reported in the first publications on the clinical use of cyclosporine in humans after renal transplanta-

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tion (1,2), whereas prior animal studies had not observed this important side effect (26,27). The first experiences with cyclosporine nephrotoxicity suggested that this phenomenon was attributable to functional changes and was thus reversible (28–30). This reversible, hemodynamically mediated renal dysfunction is now recognized as "acute CNI nephrotoxicity." However, in 1984, Myers *et al.* were the first to demonstrate that the long-term use of cyclosporine in heart transplant recipients was associated not only with a reversible decrease in GFR, but also with irreversible renal functional deterioration as a result of irreversible and progressive tubulo-interstitial injury and glomerulosclerosis (22), called "chronic CNI nephrotoxicity." These chronic effects of long-term cyclosporine use were then confirmed by others, both for cyclosporine (23,31,32) and for tacrolimus (33,34).

Table 1 provides a summary of the histologic lesions typically associated with the use of cyclosporine and tacrolimus. Figure 1 depicts the direct and indirect effects of CNI use on renal physiology, histology and function.

Acute CNI Nephrotoxicity

Vascular Effects: "Acute Arteriolopathy." In 1985, it was suggested that the reversible functional impairment associated

Table 1. Histological lesions associated with calcineurin inhibitor (CNI) use, and the differential diagnosis of CNI nephrotoxicity in post-transplantation biopsies

Lesions associated with calcineurin inhibitor use	Differential diagnosis in post-transplantation biopsies
Acute CNI nephrotoxicity	
Acute arteriolopathy = renal dysfunction without histological alterations	Other causes of altered renal hemodynamics (e.g. drugs interfering with renal vascular resistance and prerenal azotemia)
Tubular vacuolization (isometric)	Osmotic nephrosis due to other agents like mannitol, inulin, glucose, sucrose, dextran, hydroxyethylstarch, urea and radiocontrast agents and also secondary to intravenous immunoglobulins; other causes of tubular ischemia.
Thrombotic microangiopathy (TMA)	Recurrent disease (primary HUS/TTP) and other risk factors for TMA like ischemia-reperfusion endothelial injury, renal infections, vascular rejection, anticardiolipin antibodies, malignancies and various other drugs (*e.g.*, mTOR inhibitors, antiviral agents)
Chronic CNI nephrotoxicity	
Interstitial fibrosis and tubular atrophy (typically striped)	Pre-existing donor injury, aging, ischemia-reperfusion injury, tubulo-interstitial rejection, infection (*e.g.*, UTI, polyomavirus, CMV), chronic ischemia (*e.g.*, renal artery stenosis, size discrepancy in pediatric transplantation), chronic postrenal obstruction, diabetes mellitus
Medial arteriolar hyalinosis	Pre-existing donor injury, aging, diabetes mellitus, hypertension (in these cases more subendothelial deposition)
Glomerular capsular fibrosis	Glomerular ischemia (*e.g.*, renal artery stenosis, chronic arteriolar vasoconstriction, or arteriolar hyalinosis) and other causes of atubular glomeruli (*i.e.*, causes of tubular atrophy)
Global glomerulosclerosis	Pre-existing donor injury, aging, chronic glomerular ischemia (*e.g.*, renal artery stenosis, arteriolar vasoconstriction, or hyalinosis), recurrent primary disease, *de novo* glomerular disease, hypertension secondary to tubular atrophy in a late stage
Focal segmental glomerulosclerosis (FSGS)	Recurrent primary disease; donor–recipient size discrepancy with hyperfiltration injury; FSGS secondary to other causes of glomerulosclerosis
Juxtaglomerular apparatus hyperplasia	Not well established, but likely other causes of hyperreninemia (*e.g.*, transplant renal artery stenosis)
Tubular microcalcifications	Pre-existing donor injury, ischemic tubular injury and acute tubular necrosis, bone and mineral metabolism imbalance, proteinuria

HUS, hemolytic uremic syndrome; TTP, thrombotic thrombocytopenic purpura; TMA, thrombotic microangiopathy; CNI, calcineurin inhibitor; UTI, urinary tract infection; CMV, cytomegalovirus; FSGS, focal segmental glomerulosclerosis.

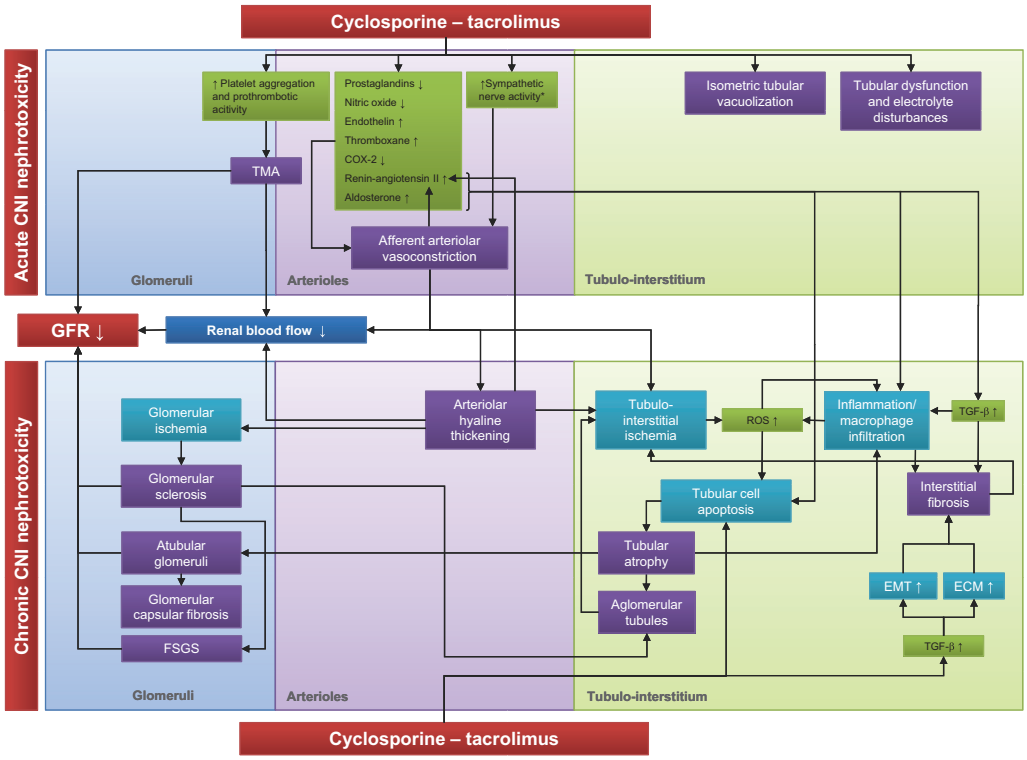

Figure 1. Schematic representation of the etiology of calcineurin inhibitor nephrotoxicity. CNI, calcineurin inhibitor; TMA, thrombotic microangiopathy; EMT, epithelial mesenchymal transition; ECM, extracellular matrix; GFR, glomerular filtration rate; FSFS, focal segmental glomerulosclerosis; ROS, reactive oxygen species. *Only in native kidneys.

with the use of cyclosporine is due to vasoconstriction of the afferent arterioles (35), which was first demonstrated by Murray *et al.* (36). The importance of renal vascular resistance and blood flow in the afferent and efferent arterioles for the acute functional impairment associated with the use of cyclosporine was confirmed by other groups, which unequivocally showed the profound alterations in vascular flow (37–39) and even reduced diameter of the afferent arterioles (40) with cyclosporine treatment.

Several studies indicate that vascular dysfunction results from an increase in vasoconstrictor factors that include endothelin and thromboxane and activation of the renin-angiotensin system (RAS), as well as a reduction of vasodilator factors like prostacyclin, prostaglandin E2, and nitric oxide (NO) (reviewed in 25,41,42). In addition, free radical formation plays a role in acute CNI nephrotoxicity, as well as sympathetic nerve activation in native kidneys. Finally, reversible tubular dysfunction is also recognized as a feature of acute CNI nephrotoxicity. It is currently not known whether these effects are related to calcineurin/NFAT-dependent mechanisms, but the similarities between cyclosporine and tacrolimus, which have a completely different molecular structure and intracellular binding site but a similar toxicity profile, suggest that this may be the case.

Endothelin is a potent vasoconstrictor widely released in the kidney and vascular beds (43). Endothelin was linked to acute CNI nephrotoxicity when it was found that cyclosporine stimulates endothelin release from cultured renal epithelial cells (44). This was later confirmed in animal studies (45) and in humans (46). The pivotal role of endothelin in CNI-associated renal dysfunction is further illustrated by the finding that anti-endothelin antibodies block the functional renal impairment of acute CNI nephrotoxicity, at least in rats (47). The mechanism underlying CNI-associated increase in endothelin production is, however, not yet clear.

Cyclosporine leads to activation of the renin–angiotensin system (RAS), by both direct effects of cyclosporine on juxtaglomerular cells (48) and indirect effects from the renal vasculature hemodynamic changes (arteriolar vasoconstriction) secondary to decreased vasodilator factors and increased endothelin (49). The effects of cyclosporine on the RAS have been reviewed in detail previously (50,51). Because RAS activation reduces renal blood flow through the action of angiotensin II (49), this mechanism represents a vicious circle, which further enlarges the renal hemodynamic changes associated with the use of CNIs. In addition, recruitment of renin-containing cells in the afferent arterioles has been observed in association with cyclosporine use, both in rats (52) and in humans (53). The increased renin secretion associated with calcineurin inhibition is sometimes seen as hyperplasia of the juxtaglomerular apparatus, which is typically observed in states of chronic renin stimulation (54). Moreover, cyclosporine not only leads to activation of the RAS, but also augments the vasoconstrictory effects of angiotensin II in smooth muscle cells by influences on intracellular calcium stores, smooth muscle cell phenotypic maintenance, and contractility (51). The molecular mechanisms by which cyclosporine stimulates renin synthesis in the juxta-

glomerular cells, induces recruitment of renin-containing cells in the afferent arterioles, and leads to alterations in the vascular smooth muscle cells are currently not known.

In addition, cyclosporine induces imbalances in the vasodilator/vasoconstrictor ratio of arachidonic acid metabolites (eicosanoids), which ultimately promotes renal vasoconstriction. An explanation could be found in the association of NFAT and cyclooxygenase-2 (COX-2). The *COX-2* gene promoter contains binding sites for NFAT, and NFAT has been reported to be of major importance for the regulation of *COX-2* gene expression *in vitro* (55). In this light, it could be expected that inhibition of calcineurin activity also attenuates COX-2 expression. This was shown by Höcherl *et al.* (56,57), who demonstrated the selective suppression of renal COX-2 expression by both cyclosporine and tacrolimus in rats. The inhibition of NFAT-mediated COX-2 expression has also been reported in nonrenal cells (58). Furthermore, the renal effects of CNIs and selective COX-2 inhibitors have many similarities, including vasoconstriction of afferent arterioles, reduction in GFR, potassium retention, and sodium retention (59–61). Taken together, although this was not studied directly, COX-2 inhibition and associated decreased formation of prostaglandin E2 by calcineurin/NFAT inhibition could well be a major mechanism by which CNIs lead to renal vasoconstriction and decreased GFR. In addition, there is convincing evidence that COX-derived prostanoids are involved in the regulation of renin synthesis and secretion in the juxtaglomerular apparatus, as well as in tubular salt and water handling (see below) (62,63). Therefore, the effects of CNIs on COX-2 inhibition could be very important for the development of CNI nephrotoxicity.

Much evidence points to endothelial dysfunction as an essential factor in the pathogenesis of acute CNI nephrotoxicity. Cyclosporine and tacrolimus inhibit NO synthesis (64) and endothelium-dependent NO-mediated renal vasodilatation. Cyclosporine is known to increase free radical formation and superoxide production, likely through vasoconstriction-associated hypoxia (see below). By forming peroxynitrite, superoxides decrease NO bioavailability (65–67). In addition, cyclosporine decreases eNOS-mediated NO production through various mechanisms (68–70). This decreased NO bioavailability and production could then lead to decreased vasodilation and unopposed vasoconstriction, which is a main mechanism of cyclosporine-induced hypertension and decreased GFR (71).

Finally, in native kidneys, stimulating effects of cyclosporine on sympathetic nerve activity could play a role in the acute effects of cyclosporine by increased renal vascular resistance and secondarily decreased GFR (72–74). In transplanted kidneys, which lack innervation, the sympathetic effects of cyclosporine were not observed (75). The effects of cyclosporine on sympathetic nerve activity could be explained by the fact that calcineurin is ubiquitously expressed in neural tissue (76,77). Recently, it was suggested that calcineurin inhibition stimulates excitatory renal afferents through effects on synapsins. Synapsins are components of presynaptic vesicles and microvesicles of sensory nerve endings, including renal sensory nerve endings (78,79). Whether these mechanisms contribute to the neurotoxic effects of the CNIs has not been studied to date.

Tubular Effects: "Toxic Tubulopathy." Histologically, acute CNI nephrotoxicity has been associated with isometric vacuolization of the tubular cytoplasm, as a result of enlargement of the endoplasmic reticulum and increased lysosomes (80–84). However, it should be noted that acute nephrotoxicity of CNIs with renal (graft) dysfunction is often present in the absence of morphologic lesions (pure functional/vascular effects). In contrast, isometric tubular vacuolization associated with the use of cyclosporine and tacrolimus can be found in the absence of renal dysfunction, and a recent study has demonstrated that isometric tubular vacuolization is not associated with progression to chronic CNI nephrotoxicity (85).

Notwithstanding the observation that isometric tubular epithelial cell vacuolization is commonly seen in the context of acute CNI nephrotoxicity after kidney transplantation, this vacuolization is not a specific finding and is also seen in allograft biopsies of patients maintained only on steroids and azathioprine, as well as in other clinical settings such as renal ischemia or tubular epithelial injury caused by intravenous administration of hyperosmotic fluids (*e.g.*, including solutions of mannitol, inulin, glucose, sucrose, dextran, hydroxyethylstarch, urea, and radiocontrast agents) (34,86–89). This tubular vacuolization is sometimes called "osmotic nephrosis" and can be distinguished from cyclosporine-induced acute tubular toxicity by the varying size of the vacuoles in osmotic nephrosis, in contrast to cyclosporine-induced vacuolization, which is typically isometric. Finally, intravenous immunoglobulins can also cause vacuolization of tubular epithelial cells, with perhaps a rather macrovacuolar appearance (90–92).

The processes by which CNIs lead to isometric tubular vacuolization are currently not known. Isometric tubular vacuolization could be the consequence of relative ischemia caused by afferent arteriolar vasoconstriction, but the possibility that direct effects of calcineurin inhibition in tubular epithelial cells cause alterations in endoplasmic reticulum structure and functioning cannot be excluded (93).

Next to tubular vacuolization, inclusion bodies are also sometimes noted in the tubular cytoplasm in association with cyclosporine use. Ultrastructurally, these inclusion bodies represent giant mitochondria and autolysomes (81,82). However, giant mitochondria are also nonspecific and occur in a variety of conditions, including ischemic injury, and are often found in preimplantation donor biopsies, which limits their diagnostic value (94). It is currently not known what triggers the formation of these giant mitochondria, but it is clear that cyclosporine has important effects on mitochondrial functioning (95,96).

Finally, experimental and clinical evidence points to more subtle tubular injury. Not only are there significant effects of calcineurin inhibition on tubular electrolyte handling (see below), but other signs of sublethal tubular toxicity are also observed. This toxicity has been related to inhibition of the cell cycle through cyclosporine-induced accumulation of p53 (97,98). In addition, CNI-induced tubular toxicity is also suggested by the finding that urinary retinol binding protein concentration is associated with an increased risk of developing progressive renal failure in heart transplant recipients treated with cyclosporine (99,100). Retinol binding protein is normally

reabsorbed in the proximal convoluted tubules and can be used as a marker for tubular dysfunction associated with CNI use. Other markers of tubular dysfunction like urinary N-acetyl β-D-glucosaminidase, which is a lysosomal enzyme that originates from proximal tubular microsomes, have also been associated with reduced GFR induced by cyclosporine A (101,102). Finally, it was shown that sublethal cyclosporine exposure induces heat shock protein expression (103–105), decreased NO production in cultured tubular epithelial cells (106), and alterations in calcium influx and free cytosolic calcium concentration (107,108), further illustrating the direct toxic effects of calcineurin inhibition on tubular function.

Thrombotic Microangiopathy. Finally, although *de novo* thrombotic microangiopathy (TMA)/hemolytic uremic syndrome after renal transplantation can occur as a result of many factors, including ischemia-reperfusion endothelial injury, renal infections, rejection, anticardiolipin antibodies, malignancies, and various drugs, the use of the CNIs cyclosporine and tacrolimus is clearly an important risk factor for post-transplant TMA (109–113). This is attributed to the endothelial injury secondary to vasoconstriction-associated ischemia, and in addition, it has been suggested that cyclosporine and tacrolimus can increase platelet aggregation and activate prothrombotic factors. The mechanisms underlying TMA after renal transplantation were recently reviewed by Ponticelli (113).

Chronic CNI Nephrotoxicity

Chronic CNI nephrotoxicity is the Achilles' heel of current immunosuppressive regimens (114). Myers *et al.* were the first to demonstrate that cyclosporine not only induces reversible alterations in renal vascular resistance, but is associated with irreversible damage of the renal architecture (22). In this study in native kidney biopsies from heart transplant recipients, interstitial fibrosis and tubular atrophy were described, accompanied by focal glomerular sclerosis. Later, more detailed histologic analyses demonstrated that all three compartments of the kidneys can be irreversibly affected by cyclosporine and tacrolimus treatment: vessels (arteriolar hyalinosis), tubulo-interstitium (tubular atrophy and interstitial fibrosis), and glomeruli (thickening and fibrosis of Bowman's capsule and focal segmental or global glomerular sclerosis) (Table 1) (34,83,84). In protocol biopsy studies, it was shown that lesions suggestive of chronic CNI nephrotoxicity progress with time after transplantation. By 10 yr after transplantation, lesions suggestive of chronic CNI nephrotoxicity were seen in virtually all cases (115). However, this study did not contain a control arm, and the relative contribution of chronic CNI use *versus* other injury processes like rejection, infections, other nephrotoxic drugs, hypertension, diabetes, hyperlipidemia, and aging processes is therefore not known.

The etiology of chronic CNI nephrotoxicity has been studied extensively. A combination of cyclosporine-induced hemodynamic changes and direct toxic effects of cyclosporine on tubular epithelial cells is thought to play a role (Figure 1).

Vascular Effects. Nodular hyaline deposits in the media of afferent arterioles (arteriolar hyalinosis) is regarded as a hallmark of CNI nephrotoxicity, and is characterized by the replacement of necrotic smooth muscle cells by focal or circular lumpy protein (hyaline) deposits at the periphery of the wall of afferent arterioles. Eventually, these nodular hyaline deposits become sufficiently large to cause narrowing of the vascular lumen (83). Arteriolar hyalinosis is commonly regarded as irreversible, although it has been reported that complete regression of severe cyclosporine-associated arteriolopathy and remodeling of arterioles can occur after stopping or reducing the dose of cyclosporine(116,117).

The study on the etiology of arteriolar hyalinosis associated with the use of CNIs is complicated by the fact that it has been difficult to reproduce this process in animal studies. In salt-depleted rats, however, which appeared to be a valid model for chronic CNI nephrotoxicity (118), it was shown that arteriolar hyalinosis lesions begin with granular eosinophilic transformation of smooth muscle cells in afferent arterioles, followed by vacuolization of smooth muscle cells and discrete hyaline deposits in vessel walls (119). The underlying processes and molecular mechanisms leading to these smooth muscle cell changes are currently not well elucidated, but may be related to the important function of calcineurin-NFAT in smooth muscle cells (120–122). Furthermore, it could be hypothesized that there is an association between prolonged arteriolar vasoconstriction induced by imbalances in the release of vasoactive substances and cyclosporine and tacrolimus treatment (see above), but this has not been shown directly.

Tubular-Interstitial Effects. In the context of chronic cyclosporine and tacrolimus use, it is likely that the associated arteriolopathy and narrowing of the arteriolar lumen is a major contributor to the development of (typically striped) interstitial fibrosis and tubular atrophy, as well as glomerular sclerosis. Local hypoxia or ischemia of the tubulo-interstitial compartment, resulting from renal vasoconstriction induced by cyclosporine or tacrolimus, lead to the formation of free radicals or reactive oxygen species (66,123,124). The importance of free radical formation in CNI nephrotoxicity was first suggested by the beneficial effects of vitamin E on lipid peroxidation and cyclosporine-induced renal damage (125,126). Like is the case with hypoxia in other organs, renal vasoconstriction can lead to renal hypoperfusion and hypoxia-reoxygenation injury and subsequently to the formation of reactive oxygen species or free radicals, which then causes cellular injury and promotes cellular death by apoptosis (66,127). This is further illustrated by the finding that catalase, an enzyme that specifically breaks down the reactive H_2O_2 into H_2O and O_2 and acts as a scavenger of reactive oxygen species, reduces cyclosporine-associated renal tubular epithelial cell senescence (128). This finding, however, does not exclude possible direct effects of cyclosporine in the generation of reactive oxygen species. This second hypothesis is supported by the finding that rat proximal tubular epithelial cells exposed to cyclosporine accumulate intracellular reactive oxygen species and lipid peroxidation products, along with an altered glutathione redox state (129).

In addition, upregulation of TGF-β is considered an important etiologic factor in chronic CNI nephrotoxicity. TGF-β expression of tubular epithelial cells is directly upregulated by cyclosporine and has also been observed after treatment with

tacrolimus (130–135), independent of the hemodynamic effects of calcineurin inhibition (136). TGF-β promotes interstitial fibrosis by decreasing the degradation and increasing the production of extracellular matrix proteins (137,138). In addition, TGF-β induces epithelial mesenchymal transition (EMT). EMT is a multistep process in which renal tubular epithelial cells lose their epithelial phenotype and acquire new characteristic features of mesenchyme, which includes disruption of polarized tubular epithelial cell morphology, *de novo* α-smooth muscle actin expression and actin reorganization, loss of cell–cell adhesions through downregulation of E-cadherin, destruction of basement membrane, and increased cell migration and invasion. EMT is recognized as a major mechanism contributing to renal interstitial fibrosis (139–143) and is related to chronic cyclosporine use (144).

Angiotensin II not only is important for its hemodynamic actions and acute CNI nephrotoxicity (see above), but also has pleiotropic effects including aldosterone release, stimulation of tubular transport, proinflammatory effects, and profibrogenic and growth stimulatory actions, which are mainly mediated by AT_1 receptors and induction of TGF-β (49,138). Activation of the RAS, therefore, not only is important for its hemodynamic contribution to CNI nephrotoxicity, but also directly promotes renal interstitial fibrosis and chronic CNI nephrotoxicity, as has been shown in rats (145,146). The observation that salt depletion (leading to RAS activation) is essential for achievement of chronic CNI nephrotoxicity in animal models supports the important role of RAS activation and angiotensin II generation (118,119,147). However, it should be noted that the mechanisms of CNI nephrotoxicity observed in salt-depleted rats do not fully overlap with the pathophysiology of chronic CNI nephrotoxicity in humans. An in-depth analysis of these differences is provided by Lee *et al.* (50). Increased aldosterone secondary to RAS activation could also in itself aggravate interstitial fibrosis through production of growth factors (like TGF-β) and reactive oxygen species, as well as inhibition of extracellular matrix degradation (see below), as was recently reviewed by Remuzzi *et al.* (148).

Calcineurin inhibition may also directly activate apoptosis genes and increase apoptosis in tubular and interstitial cells, thereby inducing tubular atrophy (96,127,149–151). In addition, inflammation with macrophage infiltration has been associated with chronic CNI nephrotoxicity (42,136,152,153). Finally, cyclosporine competitively inhibits P-glycoprotein (multidrug resistance protein 1 [MDR1] or ATP-binding cassette subfamily B, member 1 [ABCB1]) activity, which is a drug transporter expressed at the apical membrane of tubular cells. From this, it is possible that decreased excretion of endogenous toxic substances accumulate locally in the kidney when cyclosporine therapy is used (see below) (154).

Glomerular Effects. The chronic use of CNIs can induce many different types of glomerular injury. Most commonly, global glomerulosclerosis results from severe CNI-associated arteriolar hyalinosis and arteriolopathy and secondary glomerular ischemia (115,155,156), as was also seen in diabetes mellitus (157). Furthermore, tubular damage (see above) leads to the development of atubular glomeruli, which are perfused glomeruli that are disconnected from their proximal tubule. Atubular glomeruli appear smaller and often have periglomerular fibrosis (capsular fibrosis), or may become severely contracted within an enlarged glomerular cyst (155,158,159). In addition, calcineurin inhibition may cause focal segmental glomerulosclerosis lesions, possibly caused by hyperfiltration injury associated with either arteriolar hyalinosis or global glomerulosclerosis (84,155,160–163).

Electrolyte Disturbances

In addition to the impact of CNI use on renal hemodynamics and structure (see above), cyclosporine and tacrolimus also lead to tubular functional alterations and ion homeostasis disturbances like hyperkalemia, hypomagnesemia and magnesium wasting, hyperchloremic metabolic acidosis (distal tubular acidosis), and hyperuricemia (164–168).

Some of the effects of cyclosporine and tacrolimus on tubular function can be explained by reduced expression of the Na^+-K^+-$2Cl^-$-cotransporter (NKCC2) at the apical membrane of tubular epithelial cells (169,170). As was shown in patients with type I Bartter's syndrome (NKCC2 mutations) and in patients treated with NKCC2 inhibitors (*e.g.*, furosemide), decreased expression of NKCC2 would lead to polyuria, nephrocalcinosis, magnesium wasting, hyperreninemic hyperaldosteronism, and juxtaglomerular apparatus hyperplasia (171), which are all features of CNI use. The finding that cyclosporine attenuates the natriuretic effects of loop diuretics, likely by the inhibition of COX-2–mediated renal prostanoid formation, fits well with this model (57). However, the only factors that do not fit with the Bartter-like syndrome are hyperkalemia and metabolic acidosis (172). The hyperkalemia seen with calcineurin inhibition is likely multifactorial and relates to inhibitory effects on Na^+-K^+-ATPase in collecting ducts(169,170,173,174) and possibly to distal tubular acidosis. In addition, there is evidence that decreased numbers of mineralocorticoid receptors, which are detected in 75% of patients who are treated with cyclosporine, lead to hyperkalemia and metabolic acidosis as a result of aldosterone resistance (175). Recently, it was demonstrated that cyclosporine reduces paracellin-1 expression in thick ascending limb cells (176). The resulting decrease in magnesium transport likely contributes to the magnesium wasting and hypomagnesemia induced by cyclosporine (176), which is in itself associated with chronic interstitial fibrosis, a faster rate of decline of kidney function, and increased rates of graft loss in renal transplant recipients with CNI nephrotoxicity (177,178). Finally, it was shown that cyclosporine indirectly opens ATP-sensitive K^+ channels by inhibition of calcineurin, which could contribute to the CNI-associated hyperkalemia (179). The effects of cyclosporine on hyperuricemia are reviewed by Clive (167). These findings, however, do not explain the whole picture, and the effects of calcineurin inhibition on tubular transport are likely much more complex and vary between the different nephron segments (180), as well as between cyclosporine and tacrolimus (181–183). The molecular mechanisms underlying the inhibitory effects of CNIs on tubular transport function are currently not known, and further research is needed in this field.

Diagnosis, Differential Diagnosis, and Nonspecificity of the Histologic Lesions

In kidney transplantation, the differential diagnosis between cyclosporine/tacrolimus-related nephrotoxicity and other injury phenomena remains very difficult. Opposite phenomena share the same clinical picture of gradual decrease in renal graft function and lead to nonspecific interstitial fibrosis, tubular atrophy, and finally complete scarring of the renal allograft (32). Lesions considered more specific for CNI nephrotoxicity, such as tubular vacuolization and new onset or progressive arteriolar hyalinosis, are also seen secondary to other processes. Table 1 provides an overview of the histologic lesions associated with the use of CNIs, together with the differential diagnosis for each lesion. A combination of several histologic features associated with the use of CNIs and exclusion of other processes that lead to similar histologic findings would be mandatory for making a positive diagnosis of CNI nephrotoxicity. The CNI nephrotoxicity score recently proposed by Kambham *et al.* represents a first step in the standardization of the composite histologic damage induced by CNIs (184), but further validation studies are necessary.

After nonrenal organ transplantation, the picture may be clearer, and the study of CNI nephrotoxicity in native kidneys may be regarded as less troublesome (22–24,185), although the underlying disease can be associated with or lead to renal problems. Also the frequent use of concomitant nephrotoxic agents may mask the true effects of the chronic use of CNIs on native kidneys. In addition, it should be kept in mind that an essential difference between native kidneys and transplanted kidneys is the absence of innervation of transplanted kidneys; this innervation plays a prominent role in the regulation of renal vascular resistance (see above). Therefore, the extrapolation of findings on CNI nephrotoxicity in native kidneys to transplanted kidneys should be interpreted cautiously.

Another concern for the histologic diagnosis of CNI nephrotoxicity is the sometimes poor reproducibility of the histologic grading of the lesions (186–191). It is important to take into account the inter- and intraobserver variability of the histologic scoring according to the evolving Banff classification of renal allograft pathology (192–196), especially when the histologic data are used for scientific purposes and for comparison between different centers. But also for routine clinical practice, the moderate to even poor reproducibility of histologic scoring of renal allograft biopsies is of concern. This can be partly overcome by intensive and continuous training of involved and dedicated pathologists, and by rescoring biopsies for studies in a blinded way by a "central" pathologist, which obviously avoids interobserver variability. In addition, the reproducibility of interstitial fibrosis can be improved by using computerized quantitative scoring methods (197,198), and the scoring of other lesions like arteriolar hyalinosis could be refined for improvement of reproducibility (191).

Comparison Between Cyclosporine and Tacrolimus

Although cyclosporine and tacrolimus are not structurally related (and despite initial optimism), it has been demonstrated that tacrolimus has nephrotoxic properties similar to those of cyclosporine and induces similar histologic injury (83,199,200). In addition, in a study with 61 cyclosporine analogs, it was shown that the ability to induce nephrotoxicity *in vivo* correlates with the immunosuppression activity of these agents (201). This study very strongly suggested that the mechanisms of immunosuppression are the same as those of toxicity, that is, calcineurin inhibition (201). The association between the tissue-specific degree of calcineurin inhibition (greatest in kidney) and the tissue-specific side effects of cyclosporine further supported the direct role of calcineurin inhibition in the side effects of these drugs (18,19). Finally, for many aspects of CNI nephrotoxicity, there is now also increasing molecular evidence that the calcineurin-NFAT pathway could be pivotal in its pathogenesis (see above). Intriguingly however, a recently developed CNI with increased immunosuppressive potential (ISA247; voclosporin) induced renal dysfunction in only a very low number of patients treated for plaque psoriasis (202). In renal transplantation, preliminary results demonstrate that voclosporin is not inferior to tacrolimus with regard to biopsy-proven acute rejection, with a similar or slightly better safety profile: lower triglyceride and higher magnesium levels and less post-transplant diabetes mellitus. However, interim results did not show a benefit of voclosporin over tacrolimus with regard to kidney function (203,204). More detailed analyses of these data and longer term results are awaited.

There is substantial evidence that tacrolimus has a lower nephrotoxicity potential than cyclosporine. Animal studies have demonstrated that the vasoconstrictive effect of tacrolimus is weaker than of cyclosporine (205–207), and this was also confirmed in humans (208,209). Moreover, the fibrogenic potential of tacrolimus also appears to be lower (210), although these results could not be confirmed in a more recent study (135). In nonrenal solid organ transplantation, there are single- and multicenter studies as well as registry analyses demonstrating the benefit of tacrolimus over cyclosporine with regard to renal function (24,211–214), although other studies did not observe this benefit of tacrolimus over cyclosporine (215).

In renal transplantation, this analysis is much more difficult to perform, because of the interference with renal allograft rejection phenomena. However, when tacrolimus and cyclosporine efficacy were at similar level in renal transplantation (similar incidence of acute rejection), tacrolimus therapy was associated with significantly lower serum creatinine levels or higher GFR compared with cyclosporine (216–218), and even better graft survival (219). A change from cyclosporine to tacrolimus for renal allograft dysfunction was also associated with significant improvement in renal function (220,221). The SYMPHONY study, a recent large randomized multicenter trial comparing different immunosuppressive regimens, demonstrated the benefit of low-dose tacrolimus over cyclosporine in terms of both graft function and graft survival (222).

In addition, there are also marked differences in the nonrenal toxicity profile of cyclosporine *versus* tacrolimus. In a recent meta-analysis and a randomized trial in kidney transplantation, tacrolimus was associated more with diabetes mellitus, tremor, headache, dyspepsia, vomiting, diarrhea, and hypomag-

nesemia than was cyclosporine, which was associated more with hirsutism, gingival hyperplasia, higher LDL cholesterol, and higher triglyceride levels (218,219). The reasons for these differences between cyclosporine and tacrolimus are currently not known.

Risk Factors for CNI Nephrotoxicity

A brief summary of the clinical risk factors for CNI nephrotoxicity is provided in Table 2.

Systemic Levels of Cyclosporine and Tacrolimus

Concentration-Effect and Concentration-Toxicity Relationship. The main issue after renal transplantation is to maintain a reasonable balance between efficacy (avoid rejection) and toxicity (especially CNI nephrotoxicity) of the immunosuppressive agents used. In the first years of clinical use of cyclosporine, immunosuppressants were administered in fixed doses based on body weight. Dose adjustments were made only for apparent side effects, and a dose–effect relationship was already suggested by Calne in 1979 (2). In 1981, Keown *et al.* were the first to establish an association between serum concentrations of cyclosporine and the immunosuppressive capacity in renal allograft recipients (223), which was later confirmed (18). Klintmalm *et al.* were the first to demonstrate a relationship between cyclosporine doses, plasma levels, and renal allograft interstitial fibrosis (31,224). This association between cyclosporine nephrotoxicity and high cyclosporine doses or levels was then confirmed by others (225).

Table 2. Clinical risk factors for the development of calcineurin inhibitor nephrotoxicity

Risk Factors for Calcineurin Inhibitor Nephrotoxicity
Systemic overexposure to cyclosporine and tacrolimus
Local exposure to cyclosporine and tacrolimus
interactions with drugs interfering with ABCB1-mediated transport in the tubular epithelial cells (*e.g., m*TOR inhibitors)
ABCB1 genotype of the kidney
ABCB1 expression in renal tubular epithelial cells
Exposure to metabolites of cyclosporine and tacrolimus
CYP3A4/5 genotype of the patient
CYP3A5 expression in renal tubular epithelial cells
interactions with other drugs which lead to altered exposure to calcineurin inhibitor metabolites (e.g. ketoconazole)
Older kidney age
Use of nonsteroidal anti-inflammatory drugs
Salt depletion and diuretic use
Genetic polymorphisms of other genes (*e.g., TGF-β, ACE*)

ABCB1, ATP-binding cassette subfamily B, member 1; TGF-β, transforming growth factor β; ACE, angiotensin converting enzyme.

In contrast to cyclosporine, for which no dose-finding studies were performed before its introduction into clinical practice, extensive studies were performed before the general introduction of tacrolimus. Similar to cyclosporine, significant concentration–rejection and concentration–toxicity correlations were found (226–228). It should be noted that the ranges used in these dose-finding studies for tacrolimus were very broad (predose trough level range, 5 to 25 ng/ml). After it became clear that cyclosporine and tacrolimus are drugs with a narrow therapeutic window, great care was given to keep dosage within preset target ranges (228,229).

Pharmacokinetics–Pharmacogenetics. Maintaining the concentrations of the CNIs cyclosporine and tacrolimus within preset target ranges turned out to be complicated by their high inter- and intraindividual pharmacokinetic variability, resulting in very low dose–concentrations correlations(230,231). This unpredictable dose–concentration relationship results from a high variability in absorption, distribution, metabolism, and elimination of these compounds. The clinical pharmacokinetics of cyclosporine and tacrolimus are reviewed by Fahr (230) and Staatz and Tett (231). Intestinal absorption of both cyclosporine and tacrolimus is low and variable, and is influenced by concomitant ingestion of food, diabetes, uremia, ethnicity, gastrointestinal problems, and diarrhea. This can be largely attributed to variability in expression and function of the metabolizing cytochrome P450 3A isozymes (mainly CYP3A4 and CYP3A5) and of the multidrug efflux transporter P-glycoprotein (from permeability-glycoprotein; also MDR1 and ABCB1). In blood, cyclosporine and tacrolimus are extensively distributed in erythrocytes. In plasma, more than 90% of cyclosporine and tacrolimus is bound to plasma proteins like α1-acid glycoprotein, albumin, globulines, and lipoproteins. Metabolism of cyclosporine and tacrolimus occurs mainly in the liver and the gastrointestinal tract through the hydroxylating and demethylating actions of CYP3A4 and CYP3A5 isozymes. Metabolism of these agents is virtually complete, with less than 1% of the parent drug appearing in urine or feces. After metabolization, cyclosporine and tacrolimus metabolites are eliminated in the bile; less than 5% is excreted in the urine.

Significant interindividual and intraindividual variability in the expression and function of CYP3A5, CYP3A4, and P-glycoprotein contribute to the highly variable pharmacokinetics of both CNIs. This can be partly attributed to interactions with other drugs, which cause either inhibition or stimulation of expression or activity of these enzymes and transporter (*e.g.*, corticosteroids, antibiotics, antihypertensive or antiarrhythmic agents, antimycotics, antiepileptics). In addition, interfering disease conditions like diarrhea likely affect expression or activity of these enzymes or transporters, as has been suggested for tacrolimus (232,233). The long-term maturation of tacrolimus pharmacokinetics, in both adult and pediatric patients (234,235), is likely also mediated through alterations in CYP3A4/5 activity or expression (234). Finally, in recent years, there has been extensive evidence that CNI pharmacokinetics can be influenced by genetic polymorphisms in *CYP3A5* (236–

241); the impact of single nucleotide polymorphisms in *ABCB1* is less clear. The largest cohort studies did not demonstrate an association of *ABCB1* polymorphisms with cyclosporine pharmacokinetics(238,242–244), and the effects of *ABCB1* polymorphisms on tacrolimus pharmacokinetics are not consistent (238–240,245,246). This demonstrates that *ABCB1* variants, at maximum, explain tacrolimus pharmacokinetics to a limited degree. The effects of *CYP3A4*, *CYP3A5*, and *ABCB1* single nucleotide polymorphisms on cyclosporine and tacrolimus pharmacokinetics have been reviewed in detail by Hesselink *et al.* (247) and more recently by Anglicheau *et al.* (248).

Therapeutic Drug Monitoring. The low therapeutic index of cyclosporine and tacrolimus, the high variability in the pharmacokinetics, and the concentration–effect and concentration–toxicity associations suggest that therapeutic drug monitoring of these agents is useful. Routine monitoring of cyclosporine and tacrolimus levels is now common practice. In most centers, CNI dose is adjusted according to the predose trough (C_0) concentrations(249,250). However, especially for cyclosporine, there is only a poor correlation between C_0 levels and total exposure (measured as area under the time-concentration curve; $AUC_{0\ to\ 12}$). Monitoring of cyclosporine levels 2 h after administration (C_2) appeared to be the single time point that correlates best with total exposure (251,252), and could offer additional benefit over C_0 (253). Application of C_2 monitoring is, however, more complicated than C_0 monitoring, and there are no conclusive direct studies showing a significant benefit of C_2 over C_0 monitoring, which is a main reason most centers still rely on C_0 monitoring for cyclosporine (249,254). For tacrolimus, the correlation between C_0 and total $AUC_{0\ to\ 12}$ is much better, and although it is obvious that measurement of abbreviated pharmacokinetic profiles or the use of Bayesian forecasting models would increase accuracy importantly (250,255,256), C_0 level monitoring is generally regarded as sufficient (249). This better correlation of tacrolimus C_0 with total $AUC_{0\ to\ 12}$ could be one of the reasons tacrolimus could be less nephrotoxic than cyclosporine.

Therapeutic Drug Monitoring to Avoid CNI Nephrotoxicity. Despite these major efforts in establishing accurate therapeutic strategies for CNI monitoring, the application of such monitoring in routine clinical practice has not prevented kidney transplant recipients from developing acute rejection, nor has it avoided CNI nephrotoxicity, which is virtually universal by 10 yr after transplantation (115). However, the important interindividual variability in the rate at which chronic CNI nephrotoxicity develops is yet unexplained.

Acute CNI nephrotoxicity has been associated with relatively higher systemic exposure to cyclosporine (156) and tacrolimus (85,91). A recent study could not confirm this association, although there was a clear trend for tacrolimus, without reaching statistical significance (257). The lack of an association between tacrolimus exposure and acute CNI nephrotoxicity in this last study could be due to low numbers of patients treated with tacrolimus.

Whether the rate of development of lesions suggestive of chronic CNI nephrotoxicity is associated with the interindividual, relatively small variation in systemic exposure to cyclosporine and tacrolimus, if kept within preset target ranges, is still a matter of debate. For cyclosporine, although some studies suggested an association of chronic CNI nephrotoxicity lesions with higher cyclosporine exposure (156,258), other studies have found that relatively low levels are associated with higher chronic histologic damage (259,260). A similar association of lower levels of tacrolimus with higher increase in chronic tubulo-interstitial damage was reported recently (261). In addition, this association was seen only when no additional induction therapy with IL-2 receptor blockers was given (261). Taken together, these last studies suggest that the observed progression of chronic damage is more the result of subclinical alloimmune phenomena than of overexposure to CNIs. This seems counterintuitive and provocative in the light of recent trials demonstrating short-term benefit of CNI-minimization protocols, with even shorter time to biopsy-proven acute rejection in the standard-dose group compared with the low-dose group, and equal prevalences of adverse events and similar graft function at 12 mos after transplantation between low- and high-dose groups (222,262). However, these differences in rejection timing are possibly more related to differences in mycophenolic acid (MPA) exposure between these groups, or in the use of daclizumab in the low-dose group. Regarding CNI nephrotoxicity, in these two randomized trials (222,262), the high-exposure group did not have significantly lower graft function, but because no protocol biopsies were performed in these studies and no long-term results are available yet, it is not known whether there could be differences in the prevalence of subclinical CNI toxicity between patients treated with high-dose *versus* low-dose cyclosporine. From this, it can be concluded that to date, there is no hard evidence that systemic exposure to cyclosporine and tacrolimus represents the major determinant of the risk for chronic CNI nephrotoxicity. Whether lower CNI systemic exposure in *de novo* renal transplantation leads to lower incidence or slower progression of chronic CNI nephrotoxicity needs to be tested in prospective clinical trials, which should include subclinical histologic appearance of the allografts as a surrogate end-point. Finally, it should be noted that several studies have related the risk for "chronic rejection" and renal transplant outcome with a high intraindividual variability in cyclosporine exposure, but the underlying reasons remain unknown (263,264).

In nonrenal transplantation, the scene should be clearer and not complicated by rejection phenomena. However, because no routine kidney biopsies are performed in nonrenal transplant recipients, not much data are available on the evolution of histologic CNI nephrotoxicity. In a study in cardiac transplant recipients, patients treated with low doses of cyclosporine had slightly lower mean serum creatinine concentrations, but similar pathologic changes were seen on the renal biopsies when compared with biopsies from patients treated with higher cyclosporine doses (265). This again suggests that low *versus* high dosage of cyclosporine confers only marginal protection against CNI-mediated injury. With regard to the impact of systemic cyclosporine exposure on renal dysfunction after cardiac transplantation, two large studies demonstrated no relationship between cyclosporine concentration and the decline in

renal function measured as the slope of serum creatinine as a function of time or by serial GFR measurements (266,267), and a case-control study (268) showed that cyclosporine doses and trough levels in the 24 heart transplant patients who developed end-stage renal disease were not different from those of patients who maintained stable renal function. Likewise, in liver transplantation, no differences were found in renal function or the incidence of nephrotoxicity between high- and low-dose cyclosporine groups, at least in the short term (269). Furthermore, in the first randomized trial on CNI minimization in liver transplantation, delayed introduction of lower-dose tacrolimus has been associated with better renal function in the first 6 mo, but this effect disappeared by 1 yr after transplantation (270).

Taken together, these studies in both renal and nonrenal solid organ transplantation suggest that interindividual susceptibility to chronic CNI nephrotoxicity is not directly related to interindividual variability in systemic exposure, if CNI levels are kept within relatively narrow target ranges using therapeutic drug monitoring.

Local Renal Exposure to Cyclosporine and Tacrolimus

A main reason close monitoring of systemic levels of cyclosporine and tacrolimus has not prevented development of chronic CNI nephrotoxicity could be that local levels in the kidney allograft are not directly related to the systemic levels, and that other factors could determine the local levels of these drugs in kidney tissue. Both for cyclosporine and tacrolimus, it was demonstrated that levels in renal tissue are much higher than in blood (18,271), but the degree of interindividual variability and the determinants of local cyclosporine or tacrolimus levels are currently not known.

There is, however, accumulating indication that the interindividual variability in local renal cyclosporine or tacrolimus exposure in kidney tissue contributes significantly to chronic CNI nephrotoxicity. First, there is the observation that both sirolimus and everolimus increase chronic nephrotoxicity of CNIs, both in rats (272) and in humans (273–275). This is explained by robust evidence that these mTOR inhibitors interact with cyclosporine excretion in tubular epithelial cells through competition for P-glycoprotein (276,277), and hence lead to accumulation of cyclosporine in these cells. Local renal accumulation of cyclosporine was demonstrated *in vitro* by Anglicheau *et al.* (277) and *in vivo* by Napoli *et al.* (278) and Podder *et al.* (279). These latter studies measured increased local renal cyclosporine concentrations in kidney tissue after concomitant treatment with sirolimus in rats. In addition, the study by Podder *et al.* (279) demonstrated that higher local renal concentrations of cyclosporine correlated significantly with decreased renal function and increased histologic damage (279). Although the first report on the combination of tacrolimus with sirolimus was encouraging (280), later studies also observed increased nephrotoxic potential of this combination of tacrolimus with sirolimus (281,282). It should, however, be noted that the synergistic effects of tacrolimus/sirolimus appears to be less important compared with the combination cyclosporine/sirolimus, both in animal studies and in a clinical setting (282,283).

Further evidence for the importance of local renal CNI concentrations is found in studies associating P-glycoprotein expression or function with CNI nephrotoxicity. P-glycoprotein is expressed at the apical side of tubular epithelial cells and is the main protein responsible for the excretion of the CNIs tacrolimus and cyclosporine (284,285), although the renal excretion of these CNIs is only limited (see above). In 1997, del Moral *et al.* suggested that variability in local P-glycoprotein expression is important for chronic CNI nephrotoxicity by using immunohistochemistry and Western blotting in a rat model of chronic CNI nephrotoxicity (286). Another study in rats showed that exposure of cultured renal tubular cells to cyclosporine induces P-glycoprotein overexpression (287). In addition, del Moral *et al.* demonstrated that the up-regulation of P-glycoprotein was inversely related to the incidence of arteriolar hyalinosis, interstitial fibrosis, and periglomerular fibrosis (286). Human studies confirmed this upregulation of tubular epithelial P-glycoprotein expression with cyclosporine treatment, as well as lower expression of P-glycoprotein in renal allograft biopsies with CNI nephrotoxicity (288,289). These human studies confirm the animal data and suggest that interindividual variability in local renal P-glycoprotein expression contributes to the local susceptibility to CNI nephrotoxicity. However, these case-control studies were not prospective and need to be validated in larger and, more importantly, prospective studies, as the association found does not prove any causal role of local P-glycoprotein expression variability. Whether a similar upregulation of P-glycoprotein expression is also present with tacrolimus treatment is currently not known.

In addition to this suggestion that the variability in tubular P-glycoprotein expression contributes to the susceptibility to CNI nephrotoxicity, a recent study has also demonstrated that variability in P-glycoprotein function could be important in the development of CNI nephrotoxicity. A single nucleotide polymorphism in *ABCB1,* the *TT* genotype at position *3435* of *ABCB1* of the donor was associated with chronic CNI nephrotoxicity (290). This frequent polymorphism has been associated with altered conformation and function of P-glycoprotein (291–293). However, another case-control study in liver transplant recipients found the opposite effect: an association of the *TT* genotype at position *2677* (which is in linkage disequilibrium with the *TT* genotype at position *3435*) with lower risk for kidney dysfunction (294). The reason for this discrepancy is not clear but likely relates to differences in the effects on CNI pharmacokinetics, which was not assessed in these studies but could have played a role (see above). In addition, it should be emphasized that the definition of CNI nephrotoxicity was not very stringent in these studies and was not based on histologic criteria.

The most plausible hypothesis to explain these studies associating P-glycoprotein expression or activity with CNI nephrotoxicity is local accumulation of cyclosporine when apical P-glycoprotein expression or function are lower. This was shown for the interaction between cyclosporine and sirolimus (see above). In humans, no studies directly associating renal P-glycoprotein with local renal CNI concentrations are available. However, a study in mice found no differences in local renal

cyclosporine levels between MDR1 knock-out and wild-type mice, but a strong effect on cyclosporine concentrations in brain (295). This needs to be validated in humans, but if the absence of influence of P-glycoprotein expression on local renal exposure to CNIs is also true in humans, alternative hypotheses need to be formulated. It has been shown previously that decreased expression or altered function of P-glycoprotein plays a role in the apoptotic response to nephrotoxic agents and protects proximal tubular cells against apoptotic stress (296). Because cyclosporine use is associated with apoptotic mechanisms (see above), defective P-glycoprotein activity or decreased expression could increase the direct nephrotoxic effects of cyclosporine, which would explain the association of *ABCB1* genotype or expression with CNI nephrotoxicity in previous human studies. This hypothesis would also be an explanation for the apparent discrepancy between the minor importance of the kidney in cyclosporine and tacrolimus pharmacokinetics, and for the suggested contribution of local renal P-glycoprotein to CNI nephrotoxicity.

From this, it is clear that well-controlled prospective studies including pharmacokinetic data and robust histologic analyses are necessary to elucidate the exact role of renal P-glycoprotein expression and function and *ABCB1* polymorphisms on local CNI concentrations, apoptosis, and the development of CNI nephrotoxicity.

Exposure to Metabolites of Cyclosporine and Tacrolimus

Another yet-unanswered question is whether there is any contribution of cyclosporine and tacrolimus metabolites to CNI nephrotoxicity. It has been demonstrated previously that cyclosporine metabolite levels in patient blood often greatly exceed levels of the parent drug (297), and the same could be expected in renal tissue. Although most of these cyclosporine metabolites are considerably less immunosuppressive than the parent molecule (298,299), some were shown to reduce GFR of isolated perfused rat kidneys (297) and to cause tubular vacuolization (300) comparable to that of the parent drug. This could represent a causal explanation for the positive association between cyclosporine metabolite levels in blood and renal dysfunction in humans (301). Although some of these metabolites share immunosuppressive characteristics with cyclosporine, the effects of these metabolites on tubular function, TGF-β expression, and other aspects of CNI nephrotoxicity are currently not known. In contrast to cyclosporine, only the tacrolimus metabolite MII appears to have immunosuppressive capacity; the other metabolites do not (302,303). The nephrotoxic potential of tacrolimus metabolites has not been studied to date.

However, because CNI-metabolite levels can greatly exceed cyclosporine levels in patient blood, especially in patients with abnormal liver function (304), these metabolites maybe represent an important and unrecognized factor in the development of clinical CNI nephrotoxicity. *In vivo* evidence supporting this hypothesis is found in combination therapy of ketoconazole with CNIs. The combination of ketoconazole with cyclosporine and tacrolimus appeared to reduce the levels of the CNI metabolites by inhibition of the cytochrome P450 system (304–306). Coadministration of these agents leads to dramatic increases in total exposure to cyclosporine and tacrolimus and could lead to acute toxicity (307). However, when systemic levels of cyclosporine and tacrolimus were maintained at a similar level compared with the control arms, renal function was significantly better in the ketoconazole groups, both for cyclosporine (308) and for tacrolimus (309). Also, in the tacrolimus-ketoconazole group, there was a significant improvement in graft function over the first years after transplantation, whereas this was not seen in the tacrolimus control group. In heart transplant recipients, however, although the combination of cyclosporine with ketoconazole appeared to be safe, no benefit of combining cyclosporine with ketoconazole on renal function was observed, although it should be noted that in this study only short-term results were available; the effect of ketoconazole coadministration on chronic CNI nephrotoxicity was not reported (305).

Recently, common polymorphisms in *CYP3A4* and *CYP3A5* genes have been associated with the development of chronic CNI nephrotoxicity late after renal transplantation in patients treated with tacrolimus (234). Although no tacrolimus levels were measured in renal tissue, the finding that carriers of the *CYP3A5*1* polymorphism (expressors of CYP3A5; 236) had a higher risk of developing chronic CNI nephrotoxicity compared with nonexpressors (*CYP3A5*3/*3*) (234), supports the hypothesis that tacrolimus metabolites play a role in the development of CNI nephrotoxicity. Because this association was found with recipient's *CYP3A5* genotype (which will affect intestinal and hepatic but not renal allograft CYP3A5), hepatic and intestinal CYP3A5 should be responsible for this and would affect the levels of tacrolimus metabolites in the blood. Indeed, the generation of tacrolimus metabolites was higher in liver microsomes expressing CYP3A5 compared with nonexpressing microsomes, and kidneys contribute only marginally to tacrolimus metabolism (310). The *CYP3A5* genotype of the kidney donors was not assessed in this study (234). Another study in liver transplantation has assessed the impact of the recipient *CYP3A5* genotype on the cumulative incidence of renal dysfunction over the first year after transplantation and reported completely opposite results, with the CYP3A5 expressors (*CYP3A5*1* carriers) having significantly less renal dysfunction compared with nonexpressors (311). The reason for this apparent discrepancy in the impact of the *CYP3A5*1* carrier status on renal (allograft) function remains to be clarified, but likely relates to the fact that renal transplant recipients expressing CYP3A5 in liver (related to the recipients' *CYP3A5* genotype) have higher systemic exposure to tacrolimus and its nephrotoxic metabolites, whereas CYP3A5 expression in kidney tissue (related to the recipients' genotype in liver transplantation) could be protective in the sense that liver transplant recipients expressing CYP3A5 have less local renal tacrolimus accumulation. This last hypothesis was suggested previously by Joy *et al.* in an immunohistochemical case-control study relating decreased local renal CYP3A5 expression with CNI nephrotoxicity (312). It should also be noted that a recent study in heart transplant recipients did not observe any association between *CYP3A5*1* polymorphism and renal function (313).

Taken together, there is some suggestion that cyclosporine

and tacrolimus metabolites could contribute to clinical CNI nephrotoxicity. However, these human studies do not provide robust proof that cyclosporine and tacrolimus metabolites contribute to CNI nephrotoxicity because no CNI metabolites were measured in blood or renal tissue. These studies certainly need to be reproduced in controlled *in vitro* and animal studies and should be validated in larger prospective human studies.

Local Renal Susceptibility Factors for CNI Nephrotoxicity

In addition to the degree of exposure of kidneys to cyclosporine and tacrolimus or their metabolites, there is also evidence that interindividual variability in susceptibility to CNI nephrotoxicity is determined by local renal factors, independent of local cyclosporine or tacrolimus levels.

First, there is some evidence that the age of a kidney is a major determinant of its susceptibility to CNI nephrotoxicity. It is obvious that higher age of a kidney is independently associated with chronic histologic damage, as was described in autopsy studies (314). From this, it is no surprise that higher kidney age is an independent predictor of tubular atrophy, interstitial fibrosis, and functional impairment after renal transplantation, which was shown in protocol biopsy studies in adult and pediatric renal allograft recipients (85,261,315,316). However, these studies not only showed an association with nonspecific chronic histologic damage, but also demonstrated an association of higher donor age with medial arteriolar hyalinosis suggestive of CNI nephrotoxicity (85,261). A similar and independent association of higher age with lesions suggestive of CNI nephrotoxicity was described in patients treated with cyclosporine for other reasons (317). In animal studies, older animals with pre-existing age-related renal dysfunction developed significantly worse nephrotoxicity compared with younger animals (318), although another study described similar increase in collagen mRNA in older *versus* younger animals (319). In a human setting, it is difficult to prove that higher kidney age is associated with increased susceptibility to CNI nephrotoxicity because the relative contribution of age *versus* CNI effect is hard to assess, because no lesion is really specific for any process. Some indirect evidence could be found in the interaction between kidney age and CNI cessation and its impact on the evolution of renal allograft function, with older donor kidneys having significantly less improvement of GFR after cyclosporine cessation compared with younger donor kidneys (320). Although this is no definite proof, this finding suggests that CNIs induce more irreversible damage to the kidneys from older donors compared with those from younger donors, that is, increased susceptibility of older donor kidneys to CNI nephrotoxicity. Whether pre-existing renal disease, hypertension, and diabetes mellitus increase local susceptibility to nephrotoxicity, independently of kidney age, remains to be elucidated.

The reason older kidneys could be more susceptible to chronic CNI nephrotoxicity likely relates to age-related changes in renal autoregulation, with excessive vasoconstriction and anatomical changes of the preglomerular vasculature and subsequent ischemia of the glomeruli (321–323). It could be expected that the CNI-associated alterations in vasodilator and vasoconstrictory responses (see above) have more impact when these anatomical lesions and dysfunction are pre-existing. In addition, senescence of renal tissue is associated with impaired cellular repair mechanisms (324), which is particularly unfortunate for allografts from older donors in the context of increased susceptibility to CNI nephrotoxicity. Finally, there is some suggestion that CNIs accelerate cell senescence, possibly through the formation of reactive oxygen species (see above) (128). If this is true, renal cells from older kidneys exposed to a CNI will reach their cycling limit much sooner than similar kidneys not exposed to CNIs, and develop nonspecific molecular and morphologic changes associated with renal aging at a much faster pace (325–327).

Second, as was mentioned above, local renal P-glycoprotein not only could play a role in renal accumulation of cyclosporine and tacrolimus, but could also be important for tubular epithelial cell detoxification and protection against apoptotic stress.

Third, the use of nonsteroidal anti-inflammatory drugs (NSAIDs) has been shown to increase renal susceptibility to acute CNI nephrotoxicity. In healthy volunteers, indomethacin catalyzes cyclosporine-induced decrease in GFR (328), and in clinical studies, it was shown that NSAIDs and cyclosporine or tacrolimus have a synergistic effect on acute CNI nephrotoxicity, with decreases in renal plasma flow and GFR (329–331). This effect can be explained by the role of cyclo-oxygenase in the vascular effects of calcineurin inhibition (see above). However, although these studies demonstrate an additive pharmacodynamic effect, certain NSAIDs like diclofenac also interact with CNI pharmacokinetics and augment cyclosporine but decrease tacrolimus blood levels (330,331). Whether chronic use of NSAIDs in combination with CNIs would also lead to more rapid progression of chronic CNI nephrotoxicity is currently not known.

Fourth, studies in rats have clearly demonstrated the importance of salt depletion and RAS activation in the development of CNI nephrotoxicity (see above). It could be hypothesized that underlying RAS activation associated with relative salt depletion augments renal susceptibility to CNI nephrotoxicity also in a human setting (332), although this remains to be proven. The potential success of RAS inhibition in preventing CNI nephrotoxicity supports this hypothesis (see below).

Finally, genetic polymorphisms in genes involved in the pathogenesis of CNI nephrotoxicity have been associated with the risk for chronic CNI nephrotoxicity. It was demonstrated that a *TGF-β* polymorphism in codon 10 was associated with renal function in both adult and pediatric heart transplant recipients (333–335), although a study in liver transplant recipients could not confirm this association (336). In renal transplantation, *TGF-β* variants in the recipient were not associated with renal allograft outcome in a very large study (337), but this could be explained by the possibility that local TGF-β production could depend more on donor genotype than on recipient genotype. Angiotensin converting enzyme (*ACE*) gene polymorphisms have been associated with serum ACE levels (338). Because this enzyme appears to be pivotal in the development of CNI nephrotoxicity (see above), *ACE* variants could partly determine the individual risk for CNI nephrotoxicity associated

with the use of cyclosporine or tacrolimus. In a first study, no association between the *D* allele in the *ACE* gene and renal allograft function was found (339). However, in high-risk patients and pediatric kidney allograft recipients, a significant association between this *ACE* variant in recipients and renal allograft outcome was demonstrated (340,341). In nonrenal transplantation, this *ACE* variant has also been associated with renal dysfunction after bone marrow and heart transplantation (342,343).

Given the above evidence, it could be anticipated that older patients with native kidneys, or transplanted kidneys from older donors, could benefit most from a CNI-free immunosuppressive regimen. In addition, determining a patient's or donor's genotype of drug-metabolizing genes like *CYP3A5* and *ABCB1* (see above), or of molecules involved in CNI nephrotoxicity like TGF-β and *ACE*, could provide a reasonable tool to determine which patients are most susceptible for CNI nephrotoxicity.

Prevention and Treatment of CNI Nephrotoxicity

In this section, we will address the prevention and treatment of acute and chronic CNI nephrotoxicity. A summary of the available evidence is given in Table 3.

CNI Avoidance, Withdrawal, and Minimization

An in-depth overview of the clinical trials addressing this topic is beyond the scope of this article. Briefly, it is obvious that complete avoidance of CNIs in immunosuppressive protocols will fully prevent the development of CNI nephrotoxicity. However, the exclusion of CNIs from the immunosuppressive regimens falls short of preserving allograft function due to inadequate acute rejection prophylaxis with the other immunosuppressive regimens (222,262,344,345). Therefore, CNI withdrawal may be a better option, through delivery of CNIs during the early high rejection risk period after transplantation followed by secondary conversion to less nephrotoxic agents, before significant irreversible renal damage occurs (344–346). Finally, novel non-nephrotoxic immunosuppressive regimens, for example, the combination of mycophenolate mofetil and costimulatory blockade (belatacept), offer excellent short-term results (347), but longer term follow-up in more patients is needed to validate these first positive results with complete CNI avoidance.

There is increasing interest in CNI minimization protocols in which the doses of cyclosporine or tacrolimus are adjusted to lower target levels, both for *de novo* immunosuppressive protocols from time of transplantation and for rescue therapy after detection of histologic damage or renal dysfunction attributed to CNI nephrotoxicity (recently reviewed in 344). These approaches appear to be relatively safe (262,344,345), especially when mycophenolate mofetil is combined with tacrolimus, as was recently demonstrated in a large multicenter randomized trial in renal transplantation (222). By minimizing CNI levels, CNI nephrotoxicity might be partially avoided, but it has become clear that the increased risk of allograft rejection could annihilate these positive effects and even explain why the tacrolimus group has a better outcome than the other groups. To date, no randomized studies are available assessing the impact of CNI minimization protocols on the histologic evolution of CNI nephrotoxicity, and the relative contribution of rejection phenomena *versus* CNI nephrotoxicity is not known from minimization protocols. Given the finding that chronic CNI nephrotoxicity also occurs in psoriasis patients treated with low-dose cyclosporine (348), it can be expected that persistent nephrotoxicity will occur as long as the CNIs are continued.

Other Approaches

An alternative approach could be to keep CNIs in the immunsuppressive regimens for their excellent rejection prevention effects, and add more targeted therapy to prevent or treat CNI nephrotoxicity. Given the gradual elucidation of the molecular and physiologic mechanisms underlying CNI nephrotoxicity (see above), more targeted therapy is becoming available.

Calcium Antagonists. Because vasoconstriction of the afferent arterioles appears to play a pivotal role in acute and chronic CNI nephrotoxicity (see above), many groups have studied the potential role of vasodilatory agents for the avoidance of CNI nephrotoxicity. Calcium antagonists like nifedipine appear to improve GFR in rats (349). In human studies after renal transplantation, the calcium antagonists nitrendipine and later lacidipine were shown to prevent the fall in renal plasma flow and GFR associated with cyclosporine administration (209,350,351). In a first randomized trial in renal allograft recipients, it was shown that patients treated with the combination of cyclosporine and nifedipine had better renal function with the same degree of BP control (352). In another randomized trial, a similar effect was demonstrated for lacidipine: the renal allograft recipients treated with concomitant lacipidine had significantly better renal allograft function from 1 yr onward, independent of the effects on BP (353).

Likewise, in nonrenal solid organ transplantation, treatment for 1 wk with the dihydropyridine nifedipine normalized BP and improved renal function, for example, in cardiac allograft recipients with mild hypertension and renal dysfunction (354). More recently, these positive effects of calcium antagonist use on CNI nephrotoxicity have been confirmed by a randomized study with dihydropyridine amlodipine after heart transplantation (355). Also a randomized trial with verapamil in heart and lung transplantation demonstrated beneficial effects of verapamil on renal function (356). These last results should however be interpreted cautiously, as cyclosporine dose requirements also decreased significantly with cyclosporine treatment, and alterations in cyclosporine metabolite levels could have contributed to the observed effects (see above). In apparent contrast with these positive studies, a long-term follow-up study in heart transplant recipients did not find a protective effect of calcium channel blockers in the prevention of CNI nephrotoxicity (267). In this last study, it should, however, be emphasized that the type of calcium antagonists used is not specified, and different calcium antagonists have different effects on renal vascular resistance, and perhaps even more importantly, on CNI pharmacokinetics (306).

Table 3. Therapeutic options to prevent or treat calcineurin inhibitor nephrotoxicity

Option	Rationale	Effect in Animal Studies	Effect in Human Studies	Comment
Decrease exposure to calcineurin inhibitors				
CNI avoidance	Completely avoid exposure to CNIs	NA	+/−	Increased rejection risk, maybe not with costimulatory blockers (*e.g.*, belatacept)
CNI withdrawal	Exposure to CNIs for only a limited time, to bridge the high rejection risk period early after transplantation	NA	+/−	Increased rejection risk
CNI minimization	Lower but continuous exposure to CNIs	NA	+	Safe on short-term; long-term results not known
Decrease exposure to calcineurin inhibitor metabolites				
Inhibitors of CYP3A (*e.g.*, ketoconazole)	Lower exposure to potentially toxic cyclosporine or tacrolimus metabolites	?	+	More frequent monitoring because of risk for overdosing/cost saving, but only very few studies
Decrease local renal susceptibility to CNI nephrotoxicity				
Dihydropyridine calcium antagonists (nifedipine, lacidipine, amlodipine)	Counteract the vasoconstrictory mechanisms of CNI use	+	+	Positive effects both on GFR and on long-term outcome
ACE inhibitors and angiotensin II receptor blockers	Counteract the pivotal role of the RAS in the development of CNI nephrotoxicity	+	+/−	RAS inhibition in itself causes renovascular changes, GFR is unaltered but chronic damage may be avoided
Spironolactone	Counteract the pivotal role of aldosterone in the development of CNI nephrotoxicity	+	?	No human studies available
Vasodilatory prostanoids	Counteract the vasoconstrictory mechanisms of CNI use	+	No effect	Only very few human studies available
NO donors	Counteract the vasoconstrictory mechanisms of CNI use	+	No effect	Only very few human studies available
Anti-oxidants	Counteract the pivotal role of oxidative stress in the development of CNI nephrotoxicity	+	?	No human studies available
Anti-TGF-β antibodies	Counteract the pivotal role of TGF-β in the development of CNI nephrotoxicity	+	?	No human studies available
Statins	Effects on vascular smooth-muscle cells and on the progression of kidney diseases	+	?	No human studies available
Magnesium supplementation	Counteract the potential influence of hypomagnesemia on acute and chronic CNI nephrotoxicity	+	?	No human studies available

References are provided in the text. NA, not appropriate; CNI, calcineurin inhibitor.

RAS Inhibition. The pivotal role of RAS activation in the pathophysiology of CNI nephrotoxicity could suggest that RAS inhibition will prevent its development. The effects of ACE inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) on CNI nephrotoxicity have been studied extensively. In rats, it was shown that ACEIs and ARBs can prevent cyclosporine-induced interstitial fibrosis and improve renal function (145,357–359). Also, in humans, ACE inhibition reduced CNI nephrotoxicity associated with cyclosporine use (360) and improved alterations of the cardiovascular system generally observed in renal transplant patients (361). Again in humans, the ARB losartan was shown to significantly decrease the plasma levels of TGF-β and endothelin (362,363). Creatinine clearance, however, tended to be lower with the addition of losartan, likely through the hemodynamic effects of angiotensin II receptor blockade (362,363). It is, therefore, not clear whether cotreatment with ARBs is able to slow the progression of CNI nephrotoxicity in a human setting. Finally, spironolactone treatment could also abrogate many RAS effects and aldosterone-mediated effects of CNI treatment, at least in rats (364,365). For an extensive review of the potential role of spironolactone therapy for CNI nephrotoxicity, we refer to the review by Bobadilla *et al.* (41). No human studies with spironolactone to prevent the development of CNI nephrotoxicity are available.

In a randomized trial comparing the ACEI lisinopril with the calcium antagonist nifedipine, Mourad *et al.* found that each drug was associated with a similar degree of renal protection in long-term cyclosporine treated patients (366). However, another randomized trial comparing nifedipine to lisinopril for the prevention of CNI nephrotoxicity found that in the first 2 yr after transplantation, graft function increased only with nifedipine (367).

Vasodilatatory Prostanoids. Only a limited number of studies have evaluated the use of vasodilatatory prostanoids on CNI nephrotoxicity. In rats, it was shown that prostaglandin E2 reduces nephrotoxicity of cyclosporine, but this was likely mediated by an interaction between prostaglandin E2 and intestinal cyclosporine absorption (368). Misoprostol, a prostaglandin E1 analog, reduced CNI nephrotoxicity in vitro and in rats treated with chronic cyclosporine (369,370), and in a human study, misoprostol was shown to improve renal function (371). This effect in humans was, however, associated with a lower incidence of acute rejection with misoprostol treatment, misoprostol in itself did not prevent the development of CNI nephrotoxicity in humans (371). Likewise, another study in patients with rheumatoid arthritis did not observe any effect of misoprostol treatment on acute CNI nephrotoxicity (372).

NO Donors. Another approach has been to use L-arginine or molsidomine for the prevention of CNI nephrotoxicity. In rats, L-arginine greatly ameliorated kidney dysfunction induced by cyclosporine (373,374), but not in all studies (375). In humans, no effect of L-arginine was observed (376). Molsidomine was not assessed in human studies for CNI nephrotoxicity.

Other. Finally, other therapeutic approaches are promising for the prevention of CNI nephrotoxicity, such as anti-TGF-β antibodies(133,377), antioxidants (129,378–381), statins (358), and magnesium supplementation (42,382,383). However, no human studies with these approaches are available.

Conclusion

The use of the CNIs cyclosporine and tacrolimus has led to major advances in the field of transplantation, with excellent short-term outcome. However, the chronic nephrotoxicity of these drugs is the Achilles' heel of current immunosuppressive regimens. Chronic CNI nephrotoxicity is associated with mostly irreversible histologic damage to all compartments of the kidneys, including glomeruli, arterioles, and tubulo-interstitium, but the nonspecificity of most lesions makes the differential diagnosis with other injurious processes cumbersome. The pathophysiologic mechanisms underlying CNI nephrotoxicity are partly elucidated, although the main question whether nephrotoxicity is secondary to the actions on the calcineurin-NFAT pathway remains largely unanswered. It becomes clear that local renal factors are more important for susceptibility to CNI nephrotoxicity than systemic exposure to cyclosporine and tacrolimus. These factors include variability in P-glycoprotein and CYP3A4/5 expression or activity, older kidney age, salt depletion, the use of NSAIDs, and genetic polymorphisms in genes like *TGF-β* and *ACE.* Prevention and eventually therapy for CNI nephrotoxicity is aimed at lowering total systemic blood levels and decreasing local renal exposure to the CNIs or their metabolites. In addition, better insight into the mechanisms underlying CNI nephrotoxicity might pave the way toward more targeted therapy or prevention of CNI nephrotoxicity.

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Disclosures

None.

References

1. Calne RY, White DJ, Thiru S, Evans DB, McMaster P, Dunn DC, Craddock GN, Pentlow BD, Rolles K: Cyclosporin A in patients receiving renal allografts from cadaver donors. *Lancet* 2: 1323–1327, 1978
2. Calne RY, Rolles K, White DJ, Thiru S, Evans DB, McMaster P, Dunn DC, Craddock GN, Henderson RG, Aziz S, Lewis P: Cyclosporin A initially as the only immunosuppressant in 34 recipients of cadaveric organs: 32 kidneys, 2 pancreases, and 2 livers. *Lancet* 2: 1033–1036, 1979
3. Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramman R, Jain A: FK 506 for liver, kidney, and pancreas transplantation. *Lancet* 2: 1000–1004, 1989
4. Fung JJ, bu-Elmagd K, Todo S, Shapiro R, Tzakis A, Jordan M, Armitage J, Jain A, Alessiani M, Martin M: Overview of FK506 in transplantation. *Clin Transpl* 115–121, 1990
5. Andreoni KA, Brayman KL, Guidinger MK, Sommers CM,

Sung RS: Kidney and pancreas transplantation in the United States, 1996–2005. *Am J Transplant* 7: 1359–1375, 2007
6. Takahashi N, Hayano T, Suzuki M: Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. *Nature* 337: 473–475, 1989
7. Fischer G, Wittmann-Liebold B, Lang K, Kiefhaber T, Schmid FX: Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. *Nature* 337: 476–478, 1989
8. Harding MW, Galat A, Uehling DE, Schreiber SL: A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase. *Nature* 341: 758–760, 1989
9. Siekierka JJ, Hung SH, Poe M, Lin CS, Sigal NH: A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. *Nature* 341: 755–757, 1989
10. Flanagan WM, Corthesy B, Bram RJ, Crabtree GR: Nuclear association of a T-cell transcription factor blocked by FK-506 and cyclosporin A. *Nature* 352: 803–807, 1991
11. Jain J, McCaffrey PG, Miner Z, Kerppola TK, Lambert JN, Verdine GL, Curran T, Rao A: The T-cell transcription factor NFATp is a substrate for calcineurin and interacts with Fos and Jun. *Nature* 365: 352–355, 1993
12. Shaw KT, Ho AM, Raghavan A, Kim J, Jain J, Park J, Sharma S, Rao A, Hogan PG: Immunosuppressive drugs prevent a rapid dephosphorylation of transcription factor NFAT1 in stimulated immune cells. *Proc Natl Acad Sci USA* 92: 11205–11209, 1995
13. Clipstone NA, Crabtree GR: Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. *Nature* 357: 695–697, 1992
14. O'Keefe SJ, Tamura J, Kincaid RL, Tocci MJ, O'Neill EA: FK-506- and CsA-sensitive activation of the interleukin-2 promoter by calcineurin. *Nature* 357: 692–694, 1992
15. Emmel EA, Verweij CL, Durand DB, Higgins KM, Lacy E, Crabtree GR: Cyclosporin A specifically inhibits function of nuclear proteins involved in T cell activation. *Science* 246: 1617–1620, 1989
16. Macian F: NFAT proteins: Key regulators of T-cell development and function. *Nat Rev Immunol* 5: 472–484, 2005
17. Su Q, Zhao M, Weber E, Eugster HP, Ryffel B: Distribution and activity of calcineurin in rat tissues. Evidence for post-transcriptional regulation of testis-specific calcineurin B. *Eur J Biochem* 230: 469–474, 1995
18. Halloran PF, Helms LM, Kung L, Noujaim J: The temporal profile of calcineurin inhibition by cyclosporine in vivo. *Transplantation* 68: 1356–1361, 1999
19. Kung L, Batiuk TD, Palomo-Pinon S, Noujaim J, Helms LM, Halloran PF: Tissue distribution of calcineurin and its sensitivity to inhibition by cyclosporine. *Am J Transplant* 1: 325–333, 2001
20. Rao A, Luo C, Hogan PG: Transcription factors of the NFAT family: Regulation and function. *Annu Rev Immunol* 15: 707–747, 1997
21. Liu EH, Siegel RM, Harlan DM, O'Shea JJ: T cell-directed therapies: Lessons learned and future prospects. *Nat Immunol* 8: 25–30, 2007
22. Myers BD, Ross J, Newton L, Luetscher J, Perlroth M: Cyclosporine-associated chronic nephropathy. *N Engl J Med* 311: 699–705, 1984
23. Palestine AG, Austin HA, III, Balow JE, Antonovych TT, Sabnis SG, Preuss HG, Nussenblatt RB: Renal histopathologic alterations in patients treated with cyclosporine for uveitis. *N Engl J Med* 314: 1293–1298, 1986
24. Ojo AO, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young EW, Arndorfer J, Christensen L, Merion RM: Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med* 349: 931–940, 2003
25. Olyaei AJ, de Mattos AM, Bennett WM: Nephrotoxicity of immunosuppressive drugs: New insight and preventive strategies. *Curr Opin Crit Care* 7: 384–389, 2001
26. Homan WP, Fabre JW, Williams KA, Millard PR, Morris PJ: Studies on the immunosuppressive properties of cyclosporin a in rats receiving renal allografts. *Transplantation* 29: 361–366, 1980
27. Homan WP, French ME, Millard P, Denton TG, Fabre JW, Morris PJ: Studies on the effects of cyclosporin A upon renal allograft rejection in the dog. *Surgery* 88: 168–173, 1980
28. Klintmalm GB, Iwatsuki S, Starzl TE: Nephrotoxicity of cyclosporin A in liver and kidney transplant patients. *Lancet* 1: 470–471, 1981
29. Morris PJ, French ME, Dunnill MS, Hunnisett AG, Ting A, Thompson JF, Wood RF: A controlled trial of cyclosporine in renal transplantation with conversion to azathioprine and prednisolone after three months. *Transplantation* 36: 273–277, 1983
30. Bennett WM, Pulliam JP: Cyclosporine nephrotoxicity. *Ann Intern Med* 99: 851–854, 1983
31. Klintmalm G, Bohman SO, Sundelin B, Wilczek H: Interstitial fibrosis in renal allografts after 12 to 46 months of cyclosporin treatment: Beneficial effect of low doses in early post-transplantation period. *Lancet* 2: 950–954, 1984
32. Farnsworth A, Hall BM, Duggin GG, Horvath JS, Tiller DJ: Interstitial fibrosis in renal allografts in patients treated with cyclosporin. *Lancet* 2: 1470–1471, 1984
33. Starzl TE, Fung J, Jordan M, Shapiro R, Tzakis A, McCauley J, Johnston J, Iwaki Y, Jain A, Alessiani M: Kidney transplantation under FK 506. *JAMA* 264: 63–67, 1990
34. Randhawa PS, Shapiro R, Jordan ML, Starzl TE, Demetris AJ: The histopathological changes associated with allograft rejection and drug toxicity in renal transplant recipients maintained on FK506. Clinical significance and comparison with cyclosporine. *Am J Surg Pathol* 17: 60–68, 1993
35. Dieperink H, Starklint H, Leyssac PP, Kemp E: Glomerulotubular function in cyclosporine-treated rats. A lithium clearance, occlusion time/transit time and micropuncture study. *Proc Eur Dial Transplant Assoc Eur Ren Assoc* 21: 853–859, 1985
36. Murray BM, Paller MS, Ferris TF: Effect of cyclosporine administration on renal hemodynamics in conscious rats. *Kidney Int* 28: 767–774, 1985
37. Barros EJ, Boim MA, Ajzen H, Ramos OL, Schor N: Glomerular hemodynamics and hormonal participation on cyclosporine nephrotoxicity. *Kidney Int* 32: 19–25, 1987
38. Laskow DA, Curtis J, Luke R, Jones P, Barber H, Deierhoi M, Diethelm A: Cyclosporine impairs the renal response to volume depletion. *Transplant Proc* 20: 568–571, 1988
39. Laskow DA, Curtis JJ, Luke RG, Julian BA, Jones P, Deierhoi MH, Barber WH, Diethelm AG: Cyclosporine-induced changes in glomerular filtration rate and urea excretion. *Am J Med* 88: 497–502, 1990
40. English J, Evan A, Houghton DC, Bennett WM: Cyclospor-

ine-induced acute renal dysfunction in the rat. Evidence of arteriolar vasoconstriction with preservation of tubular function. *Transplantation* 44: 135–141, 1987
41. Bobadilla NA, Gamba G: New insights into the pathophysiology of cyclosporine nephrotoxicity: A role of aldosterone. *Am J Physiol Renal Physiol* 293: F2–F9, 2007
42. Burdmann EA, Andoh TF, Yu L, Bennett WM: Cyclosporine nephrotoxicity. *Semin Nephrol* 23: 465–476, 2003
43. Ramirez C, Olmo A, O'Valle F, Masseroli M, Aguilar M, Gomez-Morales M, Revelles F, Garcia-Chicano MJ, Arrebola F, Reguero ME, del Moral RG: Role of intrarenal endothelin 1, endothelin 3, and angiotensin II expression in chronic cyclosporin A nephrotoxicity in rats. *Exp Nephrol* 8: 161–172, 2000
44. Nakahama H: Stimulatory effect of cyclosporine A on endothelin secretion by a cultured renal epithelial cell line, LLC-PK1 cells. Eur J Pharmacol 180: 191–192, 1990
45. Kon V, Sugiura M, Inagami T, Harvie BR, Ichikawa I, Hoover RL: Role of endothelin in cyclosporine-induced glomerular dysfunction. *Kidney Int* 37: 1487–1491, 1990
46. Textor SC, Burnett JC, Jr., Romero JC, Canzanello VJ, Taler SJ, Wiesner R, Porayko M, Krom R, Gores G, Hay E: Urinary endothelin and renal vasoconstriction with cyclosporine or FK506 after liver transplantation. *Kidney Int* 47: 1426–1433, 1995
47. Perico N, Dadan J, Remuzzi G: Endothelin mediates the renal vasoconstriction induced by cyclosporine in the rat. *J Am Soc Nephrol* 1: 76–83, 1990
48. Kurtz A, Della BR, Kuhn K: Cyclosporine A enhances renin secretion and production in isolated juxtaglomerular cells. *Kidney Int* 33: 947–953, 1988
49. Ruster C, Wolf G: Renin-angiotensin-aldosterone system and progression of renal disease. *J Am Soc Nephrol* 17: 2985–2991, 2006
50. Lee DB: Cyclosporine and the renin-angiotensin axis. *Kidney Int* 52: 248–260, 1997
51. Lassila M: Interaction of cyclosporine A and the renin-angiotensin system; new perspectives. *Curr Drug Metab* 3: 61–71, 2002
52. Tufro-McReddie A, Gomez RA, Norling LL, Omar AA, Moore LC, Kaskel FJ: Effect of CsA on the expression of renin and angiotensin type 1 receptor genes in the rat kidney. *Kidney Int* 43: 615–622, 1993
53. Iijima K, Hamahira K, Kobayashi A, Nakamura H, Yoshikawa N: Immunohistochemical analysis of renin activity in chronic cyclosporine nephropathy in childhood nephrotic syndrome. *J Am Soc Nephrol* 11: 2265–2271, 2000
54. Bohle A, Christensen J, Meyer DS, Laberke HG, Strauch M: Juxtaglomerular apparatus of the human kidney: Correlation between structure and function. *Kidney Int Suppl* 12: S18–23, 1982
55. Sugimoto T, Haneda M, Sawano H, Isshiki K, Maeda S, Koya D, Inoki K, Yasuda H, Kashiwagi A, Kikkawa R: Endothelin-1 induces cyclooxygenase-2 expression via nuclear factor of activated T-cell transcription factor in glomerular mesangial cells. *J Am Soc Nephrol* 12: 1359–1368, 2001
56. Hocherl K, Dreher F, Vitzthum H, Kohler J, Kurtz A: Cyclosporine A suppresses cyclooxygenase-2 expression in the rat kidney. *J Am Soc Nephrol* 13: 2427–2436, 2002
57. Hocherl K, Kees F, Kramer BK, Kurtz A: Cyclosporine A attenuates the natriuretic action of loop diuretics by inhibition of renal COX-2 expression. *Kidney Int* 65: 2071–2080, 2004
58. Duque J, Fresno M, Iniguez MA: Expression and function of the nuclear factor of activated T cells in colon carcinoma cells: Involvement in the regulation of cyclooxygenase-2. *J Biol Chem* 280: 8686–8693, 2005
59. Ichihara A, Imig JD, Navar LG: Cyclooxygenase-2 modulates afferent arteriolar responses to increases in pressure. *Hypertension* 34: 843–847. 1999
60. Rossat J, Maillard M, Nussberger J, Brunner HR, Burnier M: Renal effects of selective cyclooxygenase-2 inhibition in normotensive salt-depleted subjects. *Clin Pharmacol Ther* 66: 76–84, 1999
61. Komers R, Anderson S, Epstein M: Renal and cardiovascular effects of selective cyclooxygenase-2 inhibitors. *Am J Kidney Dis* 38: 1145–1157, 2001
62. Hackenthal E, Paul M, Ganten D, Taugner R: Morphology, physiology, and molecular biology of renin secretion. *Physiol Rev* 70: 1067–1116, 1990
63. Schnermann J: Juxtaglomerular cell complex in the regulation of renal salt excretion. *Am J Physiol* 274: R263–R279, 1998
64. Hortelano S, Castilla M, Torres AM, Tejedor A, Bosca L: Potentiation by nitric oxide of cyclosporin A and FK506-induced apoptosis in renal proximal tubule cells. *J Am Soc Nephrol* 11: 2315–2323, 2000
65. Diederich D, Skopec J, Diederich A, Dai FX: Cyclosporine produces endothelial dysfunction by increased production of superoxide. *Hypertension* 23: 957–961, 1994
66. Zhong Z, Arteel GE, Connor HD, Yin M, Frankenberg MV, Stachlewitz RF, Raleigh JA, Mason RP, Thurman RG: Cyclosporin A increases hypoxia and free radical production in rat kidneys: Prevention by dietary glycine. *Am J Physiol* 275: F595–F604, 1998
67. Navarro-Antolin J, Lopez-Munoz MJ, Klatt P, Soria J, Michel T, Lamas S: Formation of peroxynitrite in vascular endothelial cells exposed to cyclosporine A. *FASEB J* 15: 1291–1293, 2001
68. Oriji GK, Schanz N: Nitric oxide in CsA-induced hypertension: Role of beta-adrenoceptor antagonist and thromboxane A2. *Prostaglandins Leukot Essent Fatty Acids* 65: 259–263, 2001
69. Kou R, Greif D, Michel T: Dephosphorylation of endothelial nitric-oxide synthase by vascular endothelial growth factor. Implications for the vascular responses to cyclosporin A. *J Biol Chem* 277: 29669–29673, 2002
70. Lungu AO, Jin ZG, Yamawaki H, Tanimoto T, Wong C, Berk BC: Cyclosporin A inhibits flow-mediated activation of endothelial nitric-oxide synthase by altering cholesterol content in caveolae. *J Biol Chem* 279: 48794–48800, 2004
71. Roullet JB, Xue H, McCarron DA, Holcomb S, Bennett WM: Vascular mechanisms of cyclosporin-induced hypertension in the rat. *J Clin Invest* 93: 2244–2250, 1994
72. Moss NG, Powell SL, Falk RJ: Intravenous cyclosporine activates afferent and efferent renal nerves and causes sodium retention in innervated kidneys in rats. *Proc Natl Acad Sci USA* 82: 8222–8226, 1985
73. Elzinga LW, Rosen S, Burdmann EA, Hatton DC, Lindsley J, Bennett WM: The role of renal sympathetic nerves in experimental chronic cyclosporine nephropathy. *Transplantation* 69: 2149–2153, 2000
74. Zhang W, Victor RG: Calcineurin inhibitors cause renal

afferent activation in rats: A novel mechanism of cyclosporine-induced hypertension. *Am J Hypertens* 13: 999–1004, 2000
75. Hausberg M, Lang D, Levers A, Suwelack B, Kisters K, Tokmak F, Barenbrock M, Kosch M: Sympathetic nerve activity in renal transplant patients before and after withdrawal of cyclosporine. *J Hypertens* 24: 957–964, 2006
76. Snyder SH, Lai MM, Burnett PE: Immunophilins in the nervous system. *Neuron* 21: 283–294, 1998
77. Hemenway CS, Heitman J: Calcineurin. Structure, function, and inhibition. *Cell Biochem Biophys* 30: 115–151, 1999
78. Steiner JP, Dawson TM, Fotuhi M, Snyder SH: Immunophilin regulation of neurotransmitter release. *Mol Med* 2: 325–333, 1996
79. Zhang W, Li JL, Hosaka M, Janz R, Shelton JM, Albright GM, Richardson JA, Sudhof TC, Victor RG: Cyclosporine A-induced hypertension involves synapsin in renal sensory nerve endings. *Proc Natl Acad Sci USA* 97: 9765–9770, 2000
80. Mihatsch MJ, Thiel G, Basler V, Ryffel B, Landmann J, von Overbeck J, Zollinger HU: Morphological patterns in cyclosporine-treated renal transplant recipients. *Transplant Proc* 17: 101–116, 1985
81. Mihatsch MJ, Ryffel B, Hermle M, Brunner FP, Thiel G: Morphology of cyclosporine nephrotoxicity in the rat. *Clin Nephrol 25*: S2–S8, 1986 [suppl 1]
82. Kim JY, Suh KS: Light microscopic and electron microscopic features of cyclosporine nephrotoxicity in rats. *J Korean Med Sci* 10: 352–359, 1995
83. Mihatsch MJ, Kyo M, Morozumi K, Yamaguchi Y, Nickeleit V, Ryffel B: The side-effects of ciclosporine-A and tacrolimus. *Clin Nephrol* 49: 356–363, 1998
84. Morozumi K, Takeda A, Uchida K, Mihatsch MJ: Cyclosporine nephrotoxicity: how does it affect renal allograft function and transplant morphology? *Transpl Proc* 36: S251–S256, 2004
85. Naesens M, Kambham N, Concepcion W, Salvatierra O, Jr., Sarwal M: The evolution of non-immune histological injury and its clinical relevance in adult-sized kidney grafts in pediatric recipients. *Am J Transplant* 7: 2504–2514, 2007
86. Legendre C, Thervet E, Page B, Percheron A, Noel LH, Kreis H: Hydroxyethylstarch and osmotic-nephrosis-like lesions in kidney transplantation. *Lancet* 342: 248–249, 1993
87. Randhawa PS, Saad RS, Jordan M, Scantlebury V, Vivas C, Shapiro R: Clinical significance of renal biopsies showing concurrent acute rejection and tacrolimus-associated tubular vacuolization. *Transplantation* 67: 85–89, 1999
88. Lee HT, Jan M, Bae SC, Joo JD, Goubaeva FR, Yang J, Kim M: A1 adenosine receptor knockout mice are protected against acute radiocontrast nephropathy in vivo. *Am J Physiol Renal Physiol* 290: F1367–F1375, 2006
89. Heyman SN, Brezis M, Reubinoff CA, Greenfeld Z, Lechene C, Epstein FH, Rosen S: Acute renal failure with selective medullary injury in the rat. *J Clin Invest* 82: 401–412, 1988
90. Cantu TG, Hoehn-Saric EW, Burgess KM, Racusen L, Scheel PJ: Acute renal failure associated with immunoglobulin therapy. *Am J Kidney Dis* 25: 228–234, 1995
91. Haas M, Sonnenday CJ, Ciccone JS, Rabb H, Montgomery RA: Isometric tubular epithelial vacuolization in renal allograft biopsy specimens of patients receiving low-dose intravenous immunoglobulin for a positive crossmatch. *Transplantation* 78: 549–556, 2004
92. Bollee G, Anglicheau D, Loupy A, Zuber J, Patey N, Gregor DM, Martinez F, Mamzer-Bruneel MF, Snanoudj R, Thervet E, Legendre C, Noel LH: High-dosage intravenous immunoglobulin-associated macrovacuoles are associated with chronic tubulointerstitial lesion worsening in renal transplant recipients. *Clin J Am Soc Nephrol* 3: 1461–1468, 2008
93. Pallet N, Rabant M, Xu-Dubois YC, Lecorre D, Mucchielli MH, Imbeaud S, Agier N, Hertig A, Thervet E, Legendre C, Beaune P, Anglicheau D: Response of human renal tubular cells to cyclosporine and sirolimus: A toxicogenomic study. *Toxicol Appl Pharmacol* 229: 184–196, 2008
94. Mihatsch MJ, Basler V, Curschellas E, Kyo M, Duerig M, Huser B, Landmann J, Thiel G: Giant mitochondria in "zero-hour" transplant biopsies. *Ultrastruct Pathol* 16: 277–282, 1992
95. Simon N, Morin C, Urien S, Tillement JP, Bruguerolle B: Tacrolimus and sirolimus decrease oxidative phosphorylation of isolated rat kidney mitochondria. *Br J Pharmacol* 138: 369–376, 2003
96. Servais H, Ortiz A, Devuyst O, Denamur S, Tulkens PM, Mingeot-Leclercq MP: Renal cell apoptosis induced by nephrotoxic drugs: Cellular and molecular mechanisms and potential approaches to modulation. *Apoptosis* 13: 11–32, 2008
97. Healy E, Dempsey M, Lally C, Ryan MP: Apoptosis and necrosis: Mechanisms of cell death induced by cyclosporine A in a renal proximal tubular cell line. *Kidney Int* 54: 1955–1966, 1998
98. Lally C, Healy E, Ryan MP: Cyclosporine A-induced cell cycle arrest and cell death in renal epithelial cells. *Kidney Int* 56: 1254–1257, 1999
99. Camara NO, Matos AC, Rodrigues Da, Pereira AB, Pacheco-Silva A: Early detection of heart transplant patients with increased risk of ciclosporin nephrotoxicity. *Lancet* 357: 856–857, 2001
100. Camara NO, Silva MS, Nishida S, Pereira AB, Pacheco-Silva A: Proximal tubular dysfunction is associated with chronic allograft nephropathy and decreased long-term renal graft survival. *Transplantation* 78: 269–275, 2004
101. Burdmann EA, Andoh TF, Rosen S, Lindsley J, Munar MY, Elzinga LW, Bennett WM: Experimental nephrotoxicity, hepatotoxicity and pharmacokinetics of cyclosporin G versus cyclosporin A. *Kidney Int* 45: 684–691, 1994
102. Yamauchi H, Kobayashi E, Sugimoto K, Tsuruoka S, Yabana M, Ishii M, Fujimura A: Time-dependent cyclosporine A-induced nephrotoxicity in rats. *Clin Exp Pharmacol Physiol* 25: 435–440, 1998
103. Yuan CM, Bohen EM, Musio F, Carome MA: Sublethal heat shock and cyclosporine exposure produce tolerance against subsequent cyclosporine toxicity. *Am J Physiol* 271: F571–F578, 1996
104. Paslaru L, Rallu M, Manuel M, Davidson S, Morange M: Cyclosporin A induces an atypical heat shock response. *Biochem Biophys Res Commun* 269: 464–469, 2000
105. Stacchiotti A, Rezzani R, Angoscini P, Corsetti G, Bianchi R: Distribution of heat shock proteins in kidneys of rats after immunosuppressive treatment with cyclosporine A. *Acta Histochem* 103: 167–177, 2001
106. Lima R, Serone AP, Schor N, Higa EM: Effect of cyclo-

sporin A on nitric oxide production in cultured LLC-PK1 cells. *Ren Fail* 23: 43–52, 2001
107. Gordjani N, Epting T, Fischer-Riepe P, Greger RF, Brandis M, Leipziger J, Nitschke R: Cyclosporin-A-induced effects on the free Ca2+ concentration in LLC-PK1-cells and their mechanisms. *Pflugers Arch* 439: 627–633, 2000
108. Carvalho da Costa M, de Castro I, Neto AL, Ferreira AT, Burdmann EA, Yu L: Cyclosporin A tubular effects contribute to nephrotoxicity: Role for Ca2+ and Mg2+ ions. *Nephrol Dial Transplant* 18: 2262–2268, 2003
109. Franz M, Regele H, Schmaldienst S, Stummvoll HK, Horl WH, Pohanka E: Posttransplant hemolytic uremic syndrome in adult retransplanted kidney graft recipients: Advantage of FK506 therapy? *Transplantation* 66: 1258–1262, 1998
110. Yango A, Morrissey P, Monaco A, Butera J, Gohh RY: Successful treatment of tacrolimus-associated thrombotic microangiopathy with sirolimus conversion and plasma exchange. *Clin Nephrol* 58: 77–78, 2002
111. Lin CC, King KL, Chao YW, Yang AH, Chang CF, Yang WC: Tacrolimus-associated hemolytic uremic syndrome: A case analysis. *J Nephrol* 16: 580–585, 2003
112. Bren A, Pajek J, Grego K, Buturovic J, Ponikvar R, Lindic J, Knap B, Vizjak A, Ferluga D, Kandus A: Follow-up of kidney graft recipients with cyclosporine-associated hemolytic-uremic syndrome and thrombotic microangiopathy. *Transplant Proc* 37: 1889–1891, 2005
113. Ponticelli C: De novo thrombotic microangiopathy. An underrated complication of renal transplantation. *Clin Nephrol* 67: 335–340, 2007
114. Bennett WM, DeMattos A, Meyer MM, Andoh T, Barry JM: Chronic cyclosporine nephropathy: The Achilles' heel of immunosuppressive therapy. *Kidney Int* 50: 1089–1100, 1996
115. Nankivell B, Richard J, Borrows R, Fung CL-S, O'Connell PJ, Allen R, Chapman JR: The natural history of chronic allograft nephropathy. *N Engl J Med* 349): 2326–2333, 2003
116. Collins BS, Davis CL, Marsh CL, McVicar JP, Perkins JD, Alpers CE: Reversible cyclosporine arteriolopathy. *Transplantation* 54: 732–734, 1992
117. Morozumi K, Thiel G, Albert FW, Banfi G, Gudat F, Mihatsch MJ: Studies on morphological outcome of cyclosporine-associated arteriolopathy after discontinuation of cyclosporine in renal allografts. *Clin Nephrol* 38: 1–8, 1992
118. Elzinga LW, Rosen S, Bennett WM: Dissociation of glomerular filtration rate from tubulointerstitial fibrosis in experimental chronic cyclosporine nephropathy: Role of sodium intake. *J Am Soc Nephrol* 4: 214–221, 1993
119. Young BA, Burdmann EA, Johnson RJ, Andoh T, Bennett WM, Couser WG, Alpers CE: Cyclosporine A-induced arteriolopathy in a rat model of chronic cyclosporine nephropathy. *Kidney Int* 48: 431–438, 1995
120. Graef IA, Chen F, Chen L, Kuo A, Crabtree GR: Signals transduced by Ca(2+)/calcineurin and NFATc3/c4 pattern the developing vasculature. *Cell* 105: 863–875, 2001
121. Amberg GC, Rossow CF, Navedo MF, Santana LF: NFATc3 regulates Kv2.1 expression in arterial smooth muscle. *J Biol Chem* 279: 47326–47334, 2004
122. Nieves-Cintron M, Amberg GC, Nichols CB, Molkentin JD, Santana LF: Activation of NFATc3 down-regulates the beta1 subunit of large conductance, calcium-activated K+ channels in arterial smooth muscle and contributes to hypertension. *J Biol Chem* 282: 3231–3240, 2007
123. Lopez-Ongil S, Saura M, Rodriguez-Puyol D, Rodriguez-Puyol M, Lamas S: Regulation of endothelial NO synthase expression by cyclosporin A in bovine aortic endothelial cells. *Am J Physiol* 271: H1072–H1078, 1996
124. Djamali A: Oxidative stress as a common pathway to chronic tubulointerstitial injury in kidney allografts. *Am J Physiol Renal Physiol* 293: F445–F455, 2007
125. Wolf A, Clemann N, Frieauff W, Ryffel B, Cordier A: Role of reactive oxygen formation in the cyclosporin-A-mediated impairment of renal functions. *Transplant Proc* 26: 2902–2907, 1994
126. Wang C, Salahudeen AK: Lipid peroxidation accompanies cyclosporine nephrotoxicity: Effects of vitamin E. *Kidney Int* 47: 927–934, 1995
127. Longoni B, Boschi E, Demontis GC, Ratto GM, Mosca F: Apoptosis and adaptive responses to oxidative stress in human endothelial cells exposed to cyclosporin A correlate with BCL-2 expression levels. *FASEB J* 15: 731–740, 2001
128. Jennings P, Koppelstaetter C, Aydin S, Abberger T, Wolf AM, Mayer G, Pfaller W: Cyclosporine A induces senescence in renal tubular epithelial cells. *Am J Physiol Renal Physiol* 293: F831–F838, 2007
129. Galletti P, Di Gennaro CNI, Migliardi V, Indaco S, Della RF, Manna C, Chiodini P, Capasso G, Zappia V: Diverse effects of natural antioxidants on cyclosporin cytotoxicity in rat renal tubular cells. *Nephrol Dial Transplant* 20: 1551–1558, 2005
130. Wolf G, Killen PD, Neilson EG: Cyclosporin A stimulates transcription and procollagen secretion in tubulointerstitial fibroblasts and proximal tubular cells. *J Am Soc Nephrol* 1: 918–922, 1990
131. Prashar Y, Khanna A, Sehajpal P, Sharma VK, Suthanthiran M: Stimulation of transforming growth factor-beta 1 transcription by cyclosporine. *FEBS Lett* 358: 109–112, 1995
132. Johnson DW, Saunders HJ, Johnson FJ, Huq SO, Field MJ, Pollock CA: Cyclosporin exerts a direct fibrogenic effect on human tubulointerstitial cells: Roles of insulin-like growth factor I, transforming growth factor beta1, and platelet-derived growth factor. *J Pharmacol Exp Ther* 289: 535–542, 1999
133. Islam M, Burke JF, Jr., McGowan TA, Zhu Y, Dunn SR, McCue P, Kanalas J, Sharma K: Effect of anti-transforming growth factor-beta antibodies in cyclosporine-induced renal dysfunction. *Kidney Int* 59: 498–506, 2001
134. Khanna A, Plummer M, Bromberek C, Bresnahan B, Hariharan S: Expression of TGF-beta and fibrogenic genes in transplant recipients with tacrolimus and cyclosporine nephrotoxicity. *Kidney Int* 62: 2257–2263, 2002
135. Roos-van Groningen MC, Scholten EM, Lelieveld PM, Rowshani AT, Baelde HJ, Bajema IM, Florquin S, Bemelman FJ, de Heer E, de Fijter JW, Bruijn JA, Eikmans M: Molecular comparison of calcineurin inhibitor-induced fibrogenic responses in protocol renal transplant biopsies. *J Am Soc Nephrol* 17: 881–888, 2006
136. Vieira JM, Jr., Noronha IL, Malheiros DM, Burdmann EA: Cyclosporine-induced interstitial fibrosis and arteriolar TGF-beta expression with preserved renal blood flow. *Transplantation* 68: 1746–1753, 1999
137. Border WA, Noble NA: Transforming growth factor {beta} in tissue fibrosis. *N Engl J Med* 331: 1286–1292, 1994

138. Wolf G: Renal injury due to renin-angiotensin-aldosterone system activation of the transforming growth factor-beta pathway. *Kidney Int* 70: 1914–1919, 2006
139. Liu Y: Epithelial to mesenchymal transition in renal fibrogenesis: Pathologic significance, molecular mechanism, and therapeutic intervention. *J Am Soc Nephrol* 15: 1–12, 2004
140. Slattery C, Campbell E, McMorrow T, Ryan MP: Cyclosporine A-induced renal fibrosis: A role for epithelial-mesenchymal transition. *Am J Pathol* 167: 395–407, 2005
141. Feldman G, Kiely B, Martin N, Ryan G, McMorrow T, Ryan MP: Role for TGF-beta in cyclosporine-induced modulation of renal epithelial barrier function. *J Am Soc Nephrol* 18: 1662–1671, 2007
142. Hertig A, Verine J, Mougenot B, Jouanneau C, Ouali N, Sebe P, Glotz D, Ancel PY, Rondeau E, Xu-Dubois YC: Risk factors for early epithelial to mesenchymal transition in renal grafts. *Am J Transplant* 6: 2937–2946, 2006
143. Hertig A, Anglicheau D, Verine J, Pallet N, Touzot M, Ancel PY, Mesnard L, Brousse N, Baugey E, Glotz D, Legendre C, Rondeau E, Xu-Dubois YC: Early epithelial phenotypic changes predict graft fibrosis. *J Am Soc Nephrol* 19: 1584–1591, 2008
144. Hazzan M, Hertig A, Buob D, Noel C, Copin MC, Rondeau E, Xu-Dubois YC: Cyclosporin induces epithelial to mesenchymal transition in renal grafts. *Am J Transplant* 8: 214, 2008 [abstract]
145. Pichler RH, Franceschini N, Young BA, Hugo C, Andoh TF, Burdmann EA, Shankland SJ, Alpers CE, Bennett WM, Couser WG: Pathogenesis of cyclosporine nephropathy: Roles of angiotensin II and osteopontin. *J Am Soc Nephrol* 6: 1186–1196, 1995
146. Shihab FS, Bennett WM, Tanner AM, Andoh TF: Angiotensin II blockade decreases TGF-beta1 and matrix proteins in cyclosporine nephropathy. *Kidney Int* 52: 660–673, 1997
147. Andoh TF, Burdmann EA, Lindsley J, Houghton DC, Bennett WM: Enhancement of FK506 nephrotoxicity by sodium depletion in an experimental rat model. *Transplantation* 57: 483–489, 1994
148. Remuzzi G, Cattaneo D, Perico N: The aggravating mechanisms of aldosterone on kidney fibrosis. *J Am Soc Nephrol* 19: 1459–1462, 2008
149. Thomas SE, Andoh TF, Pichler RH, Shankland SJ, Couser WG, Bennett WM, Johnson RJ: Accelerated apoptosis characterizes cyclosporine-associated interstitial fibrosis. *Kidney Int* 53: 897–908, 1998
150. Shihab FS, Andoh TF, Tanner AM, Yi H, Bennett WM: Expression of apoptosis regulatory genes in chronic cyclosporine nephrotoxicity favors apoptosis. *Kidney Int* 56: 2147–2159, 1999
151. Yang CW, Faulkner GR, Wahba IM, Christianson TA, Bagby GC, Jin DC, Abboud HE, Andoh TF, Bennett WM: Expression of apoptosis-related genes in chronic cyclosporine nephrotoxicity in mice. *Am J Transplant* 2: 391–399, 2002
152. Young BA, Burdmann EA, Johnson RJ, Alpers CE, Giachelli CM, Eng E, Andoh T, Bennett WM, Couser WG: Cellular proliferation and macrophage influx precede interstitial fibrosis in cyclosporine nephrotoxicity. *Kidney Int* 48: 439–448, 1995
153. Li C, Yang CW, Park JH, Lim SW, Sun BK, Jung JY, Kim SB, Kim YS, Kim J, Bang BK: Pravastatin treatment attenuates interstitial inflammation and fibrosis in a rat model of chronic cyclosporine-induced nephropathy. *Am J Physiol Renal Physiol* 286: F46–F57, 2004
154. Charuk JH, Wong PY, Reithmeier RA: Differential interaction of human renal P-glycoprotein with various metabolites and analogues of cyclosporin A. *Am J Physiol* 269: F31–F39, 1995
155. Nankivell BJ, Borrows RJ, Fung C L-S, O'Connell PJ, Allen RD, Chapman JR: Evolution and pathophysiology of renal-transplant glomerulosclerosis. *Transplantation* 78: 461–468, 2004
156. Nankivell BJ, Borrows RJ, Fung C L-S, O'Connell PJ, Chapman JR, Allen RD: Calcineurin-inhibitor nephrotoxicity: Longitudinal assessment by protocol histology. *Transplantation* 78: 557–565, 2004
157. Harris RD, Steffes MW, Bilous RW, Sutherland DE, Mauer SM: Global glomerular sclerosis and glomerular arteriolar hyalinosis in insulin dependent diabetes. *Kidney Int* 40: 107–114, 1991
158. Gandhi M, Olson JL, Meyer TW: Contribution of tubular injury to loss of remnant kidney function. *Kidney Int* 54: 1157–1165, 1998
159. Chevalier RL, Forbes MS: Generation and evolution of atubular glomeruli in the progression of renal disorders. *J Am Soc Nephrol* 19: 197–206, 2008
160. Meehan SM, Pascual M, Williams WW, Tolkoff-Rubin N, Delmonico FL, Cosimi AB, Colvin RB: De novo collapsing glomerulopathy in renal allografts. *Transplantation* 65: 1192–1197, 1998
161. Woolley AC, Rosenberg ME, Burke BA, Nath KA: De novo focal glomerulosclerosis after kidney transplantation. *Am J Med* 84: 310–314, 1988
162. Cosio FG, Frankel WL, Pelletier RP, Pesavento TE, Henry ML, Ferguson RM: Focal segmental glomerulosclerosis in renal allografts with chronic nephropathy: Implications for graft survival. *Am J Kidney Dis* 34: 731–738, 1999
163. Goes NB, Colvin RB: Case records of the Massachusetts General Hospital. Case 12–2007. A 56-year-old woman with renal failure after heart-lung transplantation. *N Engl J Med* 356: 1657–1665, 2007
164. Heering P, Grabensee B: Influence of ciclosporin A on renal tubular function after kidney transplantation. *Nephron* 59: 66–70, 1991
165. Heering P, Ivens K, Aker S, Grabensee B: Distal tubular acidosis induced by FK506. *Clin Transplant* 12: 465–471, 1998
166. Lin HY, Rocher LL, McQuillan MA, Schmaltz S, Palella TD, Fox IH: Cyclosporine-induced hyperuricemia and gout. *N Engl J Med* 321: 287–292, 1989
167. Clive DM: Renal transplant-associated hyperuricemia and gout. *J Am Soc Nephrol* 11: 974–979, 2000
168. Alexander RT, Hoenderop JG, Bindels RJ: Molecular determinants of magnesium homeostasis: Insights from human disease. *J Am Soc Nephrol* 19: 1451–1458, 2008
169. Lea JP, Sands JM, McMahon SJ, Tumlin JA: Evidence that the inhibition of Na+/K(+)-ATPase activity by FK506 involves calcineurin. *Kidney Int* 46: 647–652, 1994
170. Deppe CE, Heering PJ, Tinel H, Kinne-Saffran E, Grabensee B, Kinne RK: Effect of cyclosporine A on Na+/K(+)-ATPase, Na+/K+/2Cl- cotransporter, and H+/K(+)-ATPase in MDCK cells and two subtypes. *C7 and C11 Exp Nephrol* 5: 471–480, 1997
171. Naesens M, Steels P, Verberckmoes R, Vanrenterghem Y,

Kuypers D: Bartter's and Gitelman's syndromes: From gene to clinic. *Nephron Physiol* 96: 65–78, 2004
172. Stahl RA, Kanz L, Maier B, Schollmeyer P: Hyperchloremic metabolic acidosis with high serum potassium in renal transplant recipients: A cyclosporine A associated side effect. *Clin Nephrol* 25: 245–248, 1986
173. Tumlin JA, Sands JM: Nephron segment-specific inhibition of Na+/K(+)-ATPase activity by cyclosporin A. *Kidney Int* 43: 246–251, 1993
174. Younes-Ibrahim M, Barnese M, Burth P, Castro-Faria MV: Inhibition of purified human kidney Na+,K+-ATPase by cyclosporine A: A possible mechanism for drug human nephrotoxicity. *Ann N Y Acad Sci* 986: 633–635, 2003
175. Heering PJ, Klein-Vehne N, Fehsel K: Decreased mineralocorticoid receptor expression in blood cells of kidney transplant recipients undergoing immunosuppressive treatment: Cost efficient determination by quantitative PCR. *J Clin Pathol* 57: 33–36, 2004
176. Chang CT, Hung CC, Tian YC, Yang CW, Wu MS: Ciclosporin reduces paracellin-1 expression and magnesium transport in thick ascending limb cells. *Nephrol Dial Transplant* 22: 1033–1040, 2007
177. Miura K, Nakatani T, Asai T, Yamanaka S, Tamada S, Tashiro K, Kim S, Okamura M, Iwao H: Role of hypomagnesemia in chronic cyclosporine nephropathy. *Transplantation* 73: 340–347, 2002
178. Holzmacher R, Kendziorski C, Michael HR, Jaffery J, Becker B, Djamali A: Low serum magnesium is associated with decreased graft survival in patients with chronic cyclosporin nephrotoxicity. *Nephrol Dial Transplant* 20: 1456–1462, 2005
179. Wilson AJ, Jabr RI, Clapp LH: Calcium modulation of vascular smooth muscle ATP-sensitive K(+) channels: Role of protein phosphatase-2B. *Circ Res* 87: 1019–1025, 2000
180. Lim SW, Ahn KO, Sheen MR, Jeon US, Kim J, Yang CW, Kwon HM: Downregulation of renal sodium transporters and tonicity-responsive enhancer binding protein by long-term treatment with cyclosporin A. *J Am Soc Nephrol* 18: 421–429, 2007
181. Higgins R, Ramaiyan K, Dasgupta T, Kanji H, Fletcher S, Lam F, Kashi H: Hyponatraemia and hyperkalaemia are more frequent in renal transplant recipients treated with tacrolimus than with cyclosporin. Further evidence for differences between cyclosporin and tacrolimus nephrotoxicities. *Nephrol Dial Transplant* 19: 444–450, 2004
182. Aker S, Heering P, Kinne-Saffran E, Deppe C, Grabensee B, Kinne RK: Different effects of cyclosporine a and FK506 on potassium transport systems in MDCK cells. *Exp Nephrol* 9: 332–340, 2001
183. Watanabe S, Tsuruoka S, Vijayakumar S, Fischer G, Zhang Y, Fujimura A, Al-Awqati Q, Schwartz GJ: Cyclosporin A produces distal renal tubular acidosis by blocking peptidyl prolyl cis-trans isomerase activity of cyclophilin. *Am J Physiol Renal Physiol* 288: F40–F47, 2005
184. Kambham N, Nagarajan S, Shah S, Li L, Salvatierra O, Sarwal MM: A novel, semiquantitative, clinically correlated calcineurin inhibitor toxicity score for renal allograft biopsies. *Clin J Am Soc Nephrol* 2: 135–142, 2007
185. Greenberg A, Egel JW, Thompson ME, Hardesty RL, Griffith BP, Bahnson HT, Bernstein RL, Hastillo A, Hess ML, Puschett JB: Early and late forms of cyclosporine nephrotoxicity: Studies in cardiac transplant recipients. *Am J Kidney Dis* 9: 12–22, 1987
186. Marcussen N, Olsen TS, Benediktsson H, Racusen L, Solez K: Reproducibility of the Banff classification of renal allograft pathology. Inter- and intraobserver variation. *Transplantation* 60: 1083–1089, 1995
187. Solez K, HANSEN HE, Kornerup HJ, Madsen S, Sorensen AW, Pedersen EB, Marcussen N, Benediktsson H, Racusen LC, Olsen S: Clinical validation and reproducibility of the Banff schema for renal allograft pathology. *Transplant Proc* 27: 1009–1011, 1995
188. Furness PN, Taub N: International variation in the interpretation of renal transplant biopsies: Report of the CERT-PAP project. *Kidney Int* 60: 1998–2012, 2001
189. Gough J, Rush D, Jeffery J, Nickerson P, McKenna R, Solez K, Trpkov K: Reproducibility of the Banff schema in reporting protocol biopsies of stable renal allografts. *Nephrol Dial Transplant* 17: 1081–1084, 2002
190. Furness PN, Taub N, Assmann KJ, Banfi G, Cosyns JP, Dorman AM, Hill CM, Kapper SK, Waldherr R, Laurinavicius A, Marcussen N, Martins AP, Nogueira M, Regele H, Seron D, Carrera M, Sund S, Taskinen EI, Paavonen T, Tihomirova T, Rosenthal R: International variation in histologic grading is large, and persistent feedback does not improve reproducibility. *Am J Surg Pathol* 27: 805–810, 2003
191. Sis B, Dadras F, Khoshjou F, Cockfield S, Mihatsch MJ, Solez K: Reproducibility studies on arteriolar hyaline thickening scoring in calcineurin inhibitor-treated renal allograft recipients. *Am J Transplant* 6: 1444–1450, 2006
192. Solez K, Axelsen R, Benediktsson H, Burdick J, Cohen A, Colvin R, Croker B, Droz D, Dunnill MS, Halloran PF, Hayry P, Jennette JC, Keown P, Marcussen N, Mihatsch MJ, Morozumi K, Myers B, Nast C, Olsen S, Racusen L, Ramos E, Seymour R, Sachs D, Salomon DR, Sanfilippo F, Verani H, von Willebrand E, Yamaguchi Y: International standardization of criteria for the histologic diagnosis of renal allograft rejection: The Banff working classification of kidney transplant pathology. *Kidney Int* 44: 411–422, 1993
193. Racusen L, Solez K, Colvin R, Bonsib S, Castro M, Cavallo T, Croker B, Demetris A, Drachenberg C, Fogo A, Furness P, Gaber L, Gibson I, Glotz D, Goldberg J, Grande J, Halloran P, Hansen H, Hartley B, Hayry P, Hill C, Hoffman E, Hunsicker L, Lindblad A, Marcussen N, Mihatsch M, Nadasdy T, Nickerson P, Olsen T, Papadimitriou J, Randhawa P, Rayner D, Roberts I, Rose S, Rush D: The Banff 97 working classification of renal allograft pathology. *Kidney Int* 55: 713–723, 1999
194. Racusen L, Colvin RB, Solez K, Mihatsch MJ, Halloran PF, Campbell PM, Cecka MJ, Cosyns JP, Demetris AJ, Fishbein MC, Fogo A, Furness P, Gibson I, Glotz D, Hayry P, Hunsickern L, Kashgarian M, Kerman R, Magil AJ, Montgomery R, Morozumi K, Nickeleit V, Randhawa P, Regele H, Seron D, Seshan S, Sund S, Trpkov K: Antibody-mediated rejection criteria-An addition to the Banff '97 classification of renal allograft rejection. *Am J Transplant* 3: 708–714, 2003
195. Solez K, Colvin RB, Racusen LC, Sis B, Halloran PF, Birk PE, Campbell PM, Cascalho M, Collins AB, Demetris AJ, Drachenberg CB, Gibson IW, Grimm PC, Haas M, Lerut E, Liapis H, Mannon RB, Marcus PB, Mengel M, Mihatsch MJ, Nankivell BJ, Nickeleit V, Papadimitriou JC, Platt JL, Randhawa P, Roberts I, Salinas-Madriga L, Salomon DR, Seron D, Sheaff M, Weening JJ: Banff '05 Meeting Report:

Differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am J Transplant* 7: 518–526, 2007
196. Solez K, Colvin RB, Racusen LC, Haas M, Sis B, Mengel M, Halloran PF, Baldwin W, Banfi G, Collins AB, Cosio F, David DS, Drachenberg C, Einecke G, Fogo AB, Gibson IW, Glotz D, Iskandar SS, Kraus E, Lerut E, Mannon RB, Mihatsch M, Nankivell BJ, Nickeleit V, Papadimitriou JC, Randhawa P, Regele H, Renaudin K, Roberts I, Seron D, Smith RN, Valente M: Banff 07 classification of renal allograft pathology: Updates and future directions. *Am J Transplant* 8: 753–760, 2008
197. Nicholson ML, Bailey E, Williams S, Harris KP, Furness PN: Computerized histomorphometric assessment of protocol renal transplant biopsy specimens for surrogate markers of chronic rejection. *Transplantation* 68: 236–241, 1999
198. Grimm PC, Nickerson P, Gough J, McKenna R, Stern E, Jeffery J, Rush DN: Computerized image analysis of Sirius Red-stained renal allograft biopsies as a surrogate marker to predict long-term allograft function. *J Am Soc Nephrol* 14: 1662–1668, 2003
199. Solez K, Vincenti F, Filo R: Histopathologic findings from 2-year protocol biopsies from a US multicenter kidney transplant trial comparing tacrolimus versus ciclosporine. *Transplantation* 66: 1736–1740, 1998
200. Rowshani AT, Scholten EM, Bemelman F, Eikmans M, Idu M, Roos-van Groningen MC, Surachno JS, Mallat MJ, Paul LC, de Fijter JW, Bajema IM, ten B, I, Florquin S: No difference in degree of interstitial Sirius red-stained area in serial biopsies from area under concentration-over-time curves-guided cyclosporine versus tacrolimus-treated renal transplant recipients at one year. *J Am Soc Nephrol* 17: 305–312, 2006
201. Sigal NH, Dumont F, Durette P, Siekierka JJ, Peterson L, Rich DH, Dunlap BE, Staruch MJ, Melino MR, Koprak SL: Is cyclophilin involved in the immunosuppressive and nephrotoxic mechanism of action of cyclosporin A? *J Exp Med* 173: 619–628, 1991
202. Papp K, Bissonnette R, Rosoph L, Wasel N, Lynde CW, Searles G, Shear NH, Huizinga RB, Maksymowych WP: Efficacy of ISA247 in plaque psoriasis: A randomised, multicentre, double-blind, placebo-controlled phase III study. *Lancet* 371: 1337–1342, 2008
203. Gaber O, Busque S, Mulgaonkar S, Gaston R, Jevnikar A, Meier-Kriesche HU: ISA247: A phase-IIB multicenter, open-label, concentration-controlled trial in de novo renal transplantation [Abstract]. *Am J Transplant* 8: 336, 2008
204. Busque S, Mateo R, Kapur S, Hartmann EL, Klintmalm G, Laftavi M, Gaber AO: Voclosporin (ISA 247): The phase 2 'promise' de novo renal transplant trial demonstrates efficacy and an improved safety profile across a wide therapeutic range. *Transplantation* 86: 292–293, 2008 [abstract]
205. Bagnis C, Deray G, Dubois M, Adabra Y, Jacquiaud C, Jaudon MC, Jacobs C: Comparative acute nephrotoxicity of FK-506 and ciclosporin in an isolated in situ autoperfused rat kidney model. *Am J Nephrol* 17: 17–24, 1997
206. Epstein A, Beall A, Wynn J, Mulloy L, Brophy CM: Cyclosporine, but not FK506, selectively induces renal and coronary artery smooth muscle contraction. *Surgery* 123: 456–460, 1998
207. Gardiner SM, March JE, Kemp PA, Fallgren B, Bennett T: Regional haemodynamic effects of cyclosporine A, tacrolimus and sirolimus in conscious rats. *Br J Pharmacol* 141: 634–643, 2004
208. Klein IH, Abrahams A, van ET, Hene RJ, Koomans HA, Ligtenberg G: Different effects of tacrolimus and cyclosporine on renal hemodynamics and blood pressure in healthy subjects. *Transplantation* 73: 732–736, 2002
209. Nankivell BJ, Chapman JR, Bonovas G, Gruenewals S: Oral cyclosporine but not tacrolimus reduces renal transplant blood flow. *Transplantation* 77: 1457–1459, 2004
210. Jain S, Bicknell G, Nicholson ML: Tacrolimus has less fibrogenic potential than cyclosporin A in a model of renal ischaemia-reperfusion injury. *Br J Surg* 87: 1563–1568, 2000
211. Israni A, Brozena S, Pankewycz O, Grossman R, Bloom R: Conversion to tacrolimus for the treatment of cyclosporine-associated nephrotoxicity in heart transplant recipients. *Am J Kidney Dis* 39: E16, 2002
212. Kobashigawa JA, Miller LW, Russell SD, Ewald GA, Zucker MJ, Goldberg LR, Eisen HJ, Salm K, Tolzman D, Gao J, Fitzsimmons W, First R: Tacrolimus with mycophenolate mofetil (MMF) or sirolimus vs. cyclosporine with MMF in cardiac transplant patients: 1-year report. *Am J Transplant* 6: 1377–1386, 2006
213. Lucey MR, Abdelmalek MF, Gagliardi R, Granger D, Holt C, Kam I, Klintmalm G, Langnas A, Shetty K, Tzakis A, Woodle ES: A comparison of tacrolimus and cyclosporine in liver transplantation: Effects on renal function and cardiovascular risk status. *Am J Transplant* 5: 1111–1119, 2005
214. Canales M, Youssef P, Spong R, Ishani A, Savik K, Hertz M, Ibrahim HN: Predictors of chronic kidney disease in long-term survivors of lung and heart-lung transplantation. *Am J Transplant* 6: 2157–2163, 2006
215. O'Grady JG, Burroughs A, Hardy P, Elbourne D, Truesdale A: Tacrolimus versus microemulsified ciclosporin in liver transplantation: The TMC randomised controlled trial. *Lancet* 360: 1119–1125, 2002
216. Jurewicz WA: Tacrolimus versus ciclosporin immunosuppression: Long-term outcome in renal transplantation. *Nephrol Dial Transplant* 18: i7–i11, 2003 [suppl 1]
217. Kaplan B, Schold JD, Meier-Kriesche HU: Long-term graft survival with neoral and tacrolimus: A paired kidney analysis. *J Am Soc Nephrol* 14: 2980–2984, 2003
218. Vincenti F, Friman S, Scheuermann E, Rostaing L, Jenssen T, Campistol JM, Uchida K, Pescovitz MD, Marchetti P, Tuncer M, Citterio F, Wiecek A, Chadban S, El-Shahawy M, Budde K, Goto N: Results of an international, randomized trial comparing glucose metabolism disorders and outcome with cyclosporine versus tacrolimus. *Am J Transplant* 7: 1506–1514, 2007
219. Webster AC, Woodroffe RC, Taylor RS, Chapman JR, Craig JC: Tacrolimus versus ciclosporin as primary immunosuppression for kidney transplant recipients: Meta-analysis and meta-regression of randomised trial data. *BMJ* 331: 810, 2005
220. Cantarovich D, Renou M, Megnigbeto A, Giral-Classe M, Hourmant M, Dantal J, Blancho G, Karam G, Soulillou JP: Switching from cyclosporine to tacrolimus in patients with chronic transplant dysfunction or cyclosporine-induced adverse events. *Transplantation* 79: 72–78, 2005
221. Shihab FS, Waid TH, Conti DJ, Yang H, Holman MJ, Mulloy LC, Henning AK, Holman J, Jr., First MR: Conversion from cyclosporine to tacrolimus in patients at risk for

chronic renal allograft failure: 60-month results of the CRAF Study. *Transplantation* 85: 1261–1269, 2008
222. Ekberg H, Tedesco-Silva H, Demirbas A, Vitko S, Nashan B, Gurkan A, Margreiter R, Hugo C, Grinyo JM, Frei U, Vanrenterghem Y, Daloze P, Halloran PF: Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 357: 2562–2575, 2007
223. Keown PA, Stiller CR, Ulan RA, Sinclair NR, Wall WJ, Carruthers G, Howson W: Immunological and pharmacological monitoring in the clinical use of cyclosporin A. *Lancet* 1: 686–689, 1981
224. Klintmalm G, Sawe J, Ringden O, von BC, Magnusson A: Cyclosporine plasma levels in renal transplant patients. Association with renal toxicity and allograft rejection. *Transplantation* 39: 132–137, 1985
225. Henny FC, Kleinbloesem CH, Moolenaar AJ, Paul LC, Breimer DD, van Es LA: Pharmacokinetics and nephrotoxicity of cyclosporine in renal transplant recipients. *Transplantation* 40: 261–265, 1985
226. Laskow DA, Vincenti F, Neylan JF, Mendez R, Matas AJ: An open-label, concentration-ranging trial of FK506 in primary kidney transplantation: A report of the United States Multicenter FK506 Kidney Transplant Group. *Transplantation* 62: 900–905, 1996
227. Vincenti F, Laskow DA, Neylan JF, Mendez R, Matas AJ: One-year follow-up of an open-label trial of FK506 for primary kidney transplantation. A report of the U.S. Multicenter FK506 Kidney Transplant Group. *Transplantation* 61: 1576–1581, 1996
228. Kershner RP, Fitzsimmons WE: Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. *Transplantation* 62: 920–926, 1996
229. Ptachcinski RJ, Burckart GJ, Venkataramanan R: Cyclosporine concentration determinations for monitoring and pharmacokinetic studies. *J Clin Pharmacol* 26: 358–366, 1986
230. Fahr A: Cyclosporin clinical pharmacokinetics. *Clin Pharmacokinet* 24: 472–495, 1993
231. Staatz CE, Tett SE: Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin Pharmacokinet* 43: 623–653, 2004
232. Maes BD, Lemahieu W, Kuypers D, Evenepoel P, Coosemans W, Pirenne J, Vanrenterghem YF: Differential effect of diarrhea on FK506 versus cyclosporine A trough levels and resultant prevention of allograft rejection in renal transplant recipients. *Am J Transplant* 2: 989–992, 2002
233. Lemahieu W, Maes B, Verbeke K, Rutgeerts P, Geboes K, Vanrenterghem Y: Cytochrome P450 3A4 and P-glycoprotein activity and assimilation of tacrolimus in transplant patients with persistent diarrhea. *Am J Transplant* 5: 1383–1391, 2005
234. Kuypers DR, de Jonge H, Naesens M, Lerut E, Verbeke K, Vanrenterghem Y: CYP3A5 and CYP3A4 but not MDR1 single-nucleotide polymorphisms determine long-term tacrolimus disposition and drug-related nephrotoxicity in renal recipients. *Clin Pharmacol Ther* 82: 711–725, 2007
235. Naesens M, Salvatierra O, Li L, Kambham N, Concepcion W, Sarwal M: Maturation of dose-corrected tacrolimus predose trough levels in pediatric kidney allograft recipients. *Transplantation* 85: 1139–1145, 2008
236. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K, Boguski MS, Schuetz E: Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* 27: 383–391, 2001
237. MacPhee I, Fredericks S, Tai T, Syrris P, Carter N, Johnston A, Goldberg L, Holt D: Tacrolimus pharmacogenetics: Polymorphisms associated with expression of cytochrome P4503A5 and P-glycoprotein correlate with dose requirement. *Transplantation* 11: 1486–1489, 2002
238. Hesselink D, van Schaik R, van der Heiden I, van der Werf M, Gregoor PJH, Lindemans J, Weimar W, van Gelder T: Genetic polymorphisms of the CYP3A4, *CYP3A5,* and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther* 74: 245–254, 2003
239. Tsuchiya N, Satoh S, Tada H, Li Z, Ohyama C, Sato K, Suzuki T, Habuchi T, Kato T: Influence of CYP3A5 and MDR1 (ABCB1) polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. *Transplantation* 78: 1182–1187, 2004
240. Haufroid V, Mourad M, Van Kerckhove V, Wawrzyniak J, De Meyer M, Eddour D, Malaise J, Lison D, Squifflet JP, Wallemacq P: The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. *Pharmacogenetics* 14: 147–153, 2004
241. Macphee IA, Fredericks S, Mohamed M, Moreton M, Carter ND, Johnston A, Goldberg L, Holt DW: Tacrolimus pharmacogenetics: The CYP3A5*1 allele predicts low dose-normalized tacrolimus blood concentrations in whites and South Asians. *Transplantation* 79: 499–502, 2005
242. Kuzuya T, Kobayashi T, Moriyama N, Nagasaka T, Yokoyama I, Uchida K, Nakao A, Nabeshima T: Amlodipine, but not MDR1 polymorphisms, alters the pharmacokinetics of cyclosporine A in Japanese kidney transplant recipients. *Transplantation* 76: 865–868, 2003
243. Hesselink DA, van Gelder T, van Schaik RHN, Balk A, van der Heiden IP, van Dam T, van der Werf M, Weimar W, Mathot R: Population pharmacokinetics of cyclosporine in kidney and heart transplant recipients and the influence of ethnicity and genetic polymorphisms in the MDR-1, CYP3A4, and CYP3A5 genes. *Clin Pharmacol Ther* 76: 545–556, 2004
244. Anglicheau D, Thervet E, Etienne I, Hurault De Ligny B, Le Meur Y, Touchard G, Buchler M, Laurent-Puig P, Tregouet D, Beaune P, Daly A, Legendre C, Marquet P: CYP3A5 and MDR1 genetic polymorphisms and cyclosporine pharmacokinetics after renal transplantation. *Clin Pharmacol Ther* 75: 422–433, 2004
245. Anglicheau D, Verstuyft C, Laurent-Puig P, Becquemont L, Schlageter MH, Cassinat B, Beaune P, Legendre C, Thervet E: Association of the multidrug resistance-1 gene single-nucleotide polymorphisms with the tacrolimus dose requirements in renal transplant recipients. *J Am Soc Nephrol* 14: 1889–1896, 2003
246. Mai I, Perloff ES, Bauer S, Goldammer M, Johne A, Filler G, Budde K, Roots I: MDR1 haplotypes derived from exons 21 and 26 do not affect the steady-state pharmacokinetics of tacrolimus in renal transplant patients. *Br J Clin Pharmacol* 58: 548–553, 2004
247. Hesselink DA, van GT, van Schaik RH: The pharmacoge-

netics of calcineurin inhibitors: One step closer toward individualized immunosuppression? *Pharmacogenomics* 6: 323–337, 2005
248. Anglicheau D, Legendre C, Beaune P, Thervet E: Cytochrome P450 3A polymorphisms and immunosuppressive drugs: An update. *Pharmacogenomics* 8: 835–849, 2007
249. Oellerich M, Armstrong VW: The role of therapeutic drug monitoring in individualizing immunosuppressive drug therapy: Recent developments. *Ther Drug Monit* 28: 720–725, 2006
250. Kuypers DRJ: Immunosuppressive drug monitoring-What to use in clinical practice today to improve renal graft outcome. *Transplant Int* 18: 140–150, 2005
251. Canadian Neoral Renal Transplantation Study Group: Absorption profiling of cyclosporine microemulsion (neoral) during the first 2 weeks after renal transplantation. *Transplantation* 72: 1024–1032, 2001
252. International Neoral Renal Transplantation Study Group: Cyclosporine microemulsion (Neoral) absorption profiling and sparse-sample predictors during the first 3 months after renal transplantation. *Am J Transplant* 2: 148–156, 2002
253. Nashan B, Bock A, Bosmans JL, Budde K, de Fijter H, Jaques B, Johnston A, Luck R, Midtvedt K, Pallardo LM, Ready A, Salame E, Salizzoni M, Suarez F, Thervet E: Use of Neoral C2 monitoring: A European consensus. *Transplant Int* 18: 768–778, 2005
254. Knight SR, Morris PJ: The clinical benefits of cyclosporine C2-level monitoring: A systematic review. *Transplantation* 83: 1525–1535, 2007
255. Kuypers DRJ, Claes K, Evenepoel P, Maes B, Coosemans W, Pirenne J, Vanrenterghem Y: Time-related clinical determinants of long-term tacrolimus pharmacokinetics in combination therapy with mycophenolic acid and corticosteroids: A prospective study in one hundred de novo renal transplant recipients. *Clin Pharmacokinet* 43: 741–762, 2004
256. Scholten EM, Cremers SCLM, Schoemaker RC, Rowshani AT, van Kan EJ, den Hartigh J, Paul LC, de Fijter JW: AUC-guided dosing of tacrolimus prevents progressive systemic overexposure in renal transplant recipients. *Kidney Int* 67: 2440–2447, 2005
257. Gwinner W, Hinzmann K, Erdbruegger U, Scheffner I, Broecker V, Vaske B, Kreipe H, Haller H, Schwarz A, Mengel M: Acute tubular injury in protocol biopsies of renal grafts: Prevalence, associated factors and effect on long-term function. *Am J Transplant* 8: 1684–1693, 2008
258. Nakamura T, Nozu K, Iijima K, Yoshikawa N, Moriya Y, Yamamori M, Kako A, Matsuo M, Sakurai A, Okamura N, Ishikawa T, Okumura K, Sakaeda T: Association of cumulative cyclosporine dose with its irreversible nephrotoxicity in Japanese patients with pediatric-onset autoimmune diseases. *Biol Pharm Bull* 30: 2371–2375, 2007
259. di Paolo S, Teutonico A, Stallone G, Infante B, Schena A, Grandaliano G, Battaglia M, Ditonno P, Schena PF: Cyclosporin exposure correlates with 1 year graft function and histological damage in renal transplanted patients. *Nephrol Dial Transplant* 19: 2107–2112, 2004
260. Seron D, Moreso F, Fulladosa X, Hueso M, Carrera M, Grinyo JM: Reliability of chronic allograft nephropathy diagnosis in sequential protocol biopsies. *Kidney Int* 61: 727–733, 2002
261. Naesens M, Lerut E, Damme BV, Vanrenterghem Y, Kuypers DR: Tacrolimus exposure and evolution of renal allograft histology in the first year after transplantation. *Am J Transplant* 7: 2114–2123, 2007
262. Ekberg H, Grinyo J, Nashan B, Vanrenterghem Y, Vincenti F, Voulgari A, Truman M, Nasmyth-Miller C, Rashford M: Cyclosporine sparing with mycophenolate mofetil, daclizumab and corticosteroids in renal allograft recipients: The CAESAR Study. *Am J Transplant* 7: 560–570, 2007
263. Kahan BD, Welsh M, Urbauer DL, Mosheim MB, Beusterien KM, Wood MR, Schoenberg LP, Dicesare J, Katz SM, Van Buren CT: Low intraindividual variability of cyclosporin A exposure reduces chronic rejection incidence and health care costs. *J Am Soc Nephrol* 11: 1122–1131, 2000
264. Waiser J, Slowinski T, Brinker-Paschke A, Budde K, Schreiber M, Bohler T, Hauser I, Neumayer HH: Impact of the variability of cyclosporin A trough levels on long-term renal allograft function. *Nephrol Dial Transplant* 17: 1310–1317, 2002
265. Myers BD, Newton L, Boshkos C, Macoviak JA, Frist WH, Derby GC, Perlroth MG, Sibley RK: Chronic injury of human renal microvessels with low-dose cyclosporine therapy. *Transplantation* 46: 694–703, 1988
266. Zietse R, Balk AH, vd Dorpel MA, Meeter K, Bos E, Weimar W: Time course of the decline in renal function in cyclosporine-treated heart transplant recipients. *Am J Nephrol* 14: 1–5, 1994
267. Lindelow B, Bergh CH, Herlitz H, Waagstein F: Predictors and evolution of renal function during 9 years following heart transplantation. *J Am Soc Nephrol* 11: 951–957, 2000
268. van Gelder T, Balk AH, Zietse R, Hesse C, Mochtar B, Weimar W: Renal insufficiency after heart transplantation: A case-control study. *Nephrol Dial Transplant* 13: 2322–2326, 1998
269. Soin AS, Rasmussen A, Jamieson NV, Watson CJ, Friend PJ, Wight DG, Calne RY: CsA levels in the early posttransplant period–Predictive of chronic rejection in liver transplantation? *Transplantation* 59: 1119–1123, 1995
270. Yoshida EM, Marotta PJ, Greig PD, Kneteman NM, Marleau D, Cantarovich M, Peltekian KM, Lilly LB, Scudamore CH, Bain VG, Wall WJ, Roy A, Balshaw RF, Barkun JS: Evaluation of renal function in liver transplant recipients receiving daclizumab (Zenapax), mycophenolate mofetil, and a delayed, low-dose tacrolimus regimen vs. a standard-dose tacrolimus and mycophenolate mofetil regimen: A multicenter randomized clinical trial. *Liver Transpl* 11: 1064–1072, 2005
271. Iwasaki K, Shiraga T, Matsuda H, Teramura Y, Kawamura A, Hata T, Ninomiya S, Esumi Y: Absorption, distribution, metabolism and excretion of tacrolimus (FK506) in the rat. *Drug Metab Pharmacokinet* 13: 259–265, 1998
272. Andoh TF, Lindsley J, Franceschini N, Bennett WM: Synergistic effects of cyclosporine and rapamycin in a chronic nephrotoxicity model. *Transplantation* 62: 311–316, 1996
273. Kahan BD: Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: A randomised multicentre study. The Rapamune US Study Group. *Lancet* 356: 194–202, 2000
274. Kahan BD, Camardo JS: Rapamycin: Clinical results and future opportunities. *Transplantation* 72: 1181–1193, 2001
275. Vitko S, Margreiter R, Weimar W, Dantal J, Kuypers D, Winkler M, Oyen O, Viljoen HG, Filiptsev P, Sadek S, Li Y,

Cretin N, Budde K: Three-year efficacy and safety results from a study of everolimus versus mycophenolate mofetil in de novo renal transplant patients. *Am J Transplant* 5: 2521–2530, 2005
276. Miller DS, Fricker G, Drewe J: p-Glycoprotein-mediated transport of a fluorescent rapamycin derivative in renal proximal tubule. *J Pharmacol Exp Ther* 282: 440–444, 1997
277. Anglicheau D, Pallet N, Rabant M, Marquet P, Cassinat B, Meria P, Beaune P, Legendre C, Thervet E: Role of P-glycoprotein in cyclosporine cytotoxicity in the cyclosporine-sirolimus interaction. *Kidney Int* 70: 1019–1025, 2006
278. Napoli KL, Wang ME, Stepkowski SM, Kahan BD: Relative tissue distributions of cyclosporine and sirolimus after concomitant peroral administration to the rat: Evidence for pharmacokinetic interactions. *Ther Drug Monit* 20: 123–133, 1998
279. Podder H, Stepkowski SM, Napoli KL, Clark J, Verani RR, Chou TC, Kahan BD: Pharmacokinetic interactions augment toxicities of sirolimus/cyclosporine combinations. *J Am Soc Nephrol* 12: 1059–1071, 2001
280. McAlister VC, Gao Z, Peltekian K, Domingues J, Mahalati K, MacDonald AS: Sirolimus-tacrolimus combination immunosuppression. *Lancet* 355: 376–377, 2000
281. Gonwa T, Mendez R, Yang HC, Weinstein S, Jensik S, Steinberg S: Randomized trial of tacrolimus in combination with sirolimus or mycophenolate mofetil in kidney transplantation: results at 6 months. *Transplantation* 75: 1213–1220, 2003
282. Ciancio G, Burke GW, Gaynor JJ, Ruiz P, Roth D, Kupin W, Rosen A, Miller J: A randomized long-term trial of tacrolimus/sirolimus versus tacrolimus/mycophenolate versus cyclosporine/sirolimus in renal transplantation: Three-year analysis. *Transplantation* 81: 845–852, 2006
283. Lloberas N, Torras J, Alperovich G, Cruzado JM, Gimenez-Bonafe P, Herrero-Fresneda I, Franquesa ML, Rama I, Grinyo JM: Different renal toxicity profiles in the association of cyclosporine and tacrolimus with sirolimus in rats. *Nephrol Dial Transplant* 23: 3111–3119, 2008
284. Saeki T, Ueda K, Tanigawara Y, Hori R, Komano T: Human P-glycoprotein transports cyclosporin A and FK506. *J Biol Chem* 268: 6077–6080, 1993
285. Ernest S, Rajaraman S, Megyesi J, Bello-Reuss EN: Expression of MDR1 (multidrug resistance) gene and its protein in normal human kidney. *Nephron* 77: 284–289, 1997
286. del Moral RG, Andujar M, Ramirez C, Gomez-Morales M, Masseroli M, Aguilar M, Olmo A, Arrebola F, Guillen M, Garcia-Chicano MJ, Nogales FF, O'Valle F: Chronic cyclosporin A nephrotoxicity, P-glycoprotein overexpression, and relationships with intrarenal angiotensin II deposits. *Am J Pathol* 151: 1705–1714, 1997
287. Jette L, Beaulieu E, Leclerc JM, Beliveau R: Cyclosporin A treatment induces overexpression of P-glycoprotein in the kidney and other tissues. *Am J Physiol* 270: F756–F765, 1996
288. Koziolek MJ, Riess R, Geiger H, Thevenod F, Hauser IA: Expression of multidrug resistance P-glycoprotein in kidney allografts from cyclosporine A-treated patients. *Kidney Int* 60: 156–166, 2001
289. Joy MS, Nickeleit V, Hogan SL, Thompson BD, Finn WF: Calcineurin inhibitor-induced nephrotoxicity and renal expression of P-glycoprotein. *Pharmacotherapy* 25: 779–789, 2005
290. Hauser IA, Schaeffeler E, Gauer S, Scheuermann EH, Wegner B, Gossmann J, Ackermann H, Seidl C, Hocher B, Zanger UM, Geiger H, Eichelbaum M, Schwab M: ABCB1 genotype of the donor but not of the recipient is a major risk factor for cyclosporine-related nephrotoxicity after renal transplantation. *J Am Soc Nephrol* 16: 1501–1511, 2005
291. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, Gottesman MM: A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science* 315: 525–528, 2007
292. Komar AA: Silent SNPs: Impact on gene function and phenotype. *Pharmacogenomics* 8: 1075–1080, 2007
293. Gow JM, Hodges LM, Chinn LW, Kroetz DL: Substrate-dependent effects of human ABCB1 coding polymorphisms. *J Pharmacol Exp Ther* 325: 435–442, 2008
294. Hebert MF, Dowling AL, Gierwatowski C, Lin YS, Edwards KL, Davis CL, Marsh CL, Schuetz EG, Thummel KE: Association between ABCB1 (multidrug resistance transporter) genotype and post-liver transplantation renal dysfunction in patients receiving calcineurin inhibitors. *Pharmacogenetics* 13: 661–674, 2003
295. Schinkel AH, Wagenaar E, van DL, Mol CA, Borst P: Absence of the MDR1A P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J Clin Invest* 96: 1698–1705, 1995
296. Thevenod F, Friedmann JM, Katsen AD, Hauser IA: Up-regulation of multidrug resistance P-glycoprotein via nuclear factor-kappa B activation protects kidney proximal tubule cells from cadmium- and reactive oxygen species-induced apoptosis. *J Biol Chem* 275: 1887–1896, 2000
297. Roby KA, Shaw LM: Effects of cyclosporine and its metabolites in the isolated perfused rat kidney. *J Am Soc Nephrol* 4: 168–177, 1993
298. Copeland KR, Yatscoff RW, McKenna RM: Immunosuppressive activity of cyclosporine metabolites compared and characterized by mass spectroscopy and nuclear magnetic resonance. *Clin Chem* 36: 225–229, 1990
299. Yatscoff RW, Rosano TG, Bowers LD: The clinical significance of cyclosporine metabolites. *Clin Biochem* 24: 23–35, 1991
300. Copeland KR, Thliveris JA, Yatscoff RW: Toxicity of cyclosporine metabolites. *Ther Drug Monit* 12: 525–532, 1990
301. Christians U, Kohlhaw K, Budniak J, Bleck JS, Schottmann R, Schlitt HJ, Almeida VM, Deters M, Wonigeit K, Pichlmayr R: Cyclosporine metabolite pattern in blood and urine of liver graft recipients. I. Association of cyclosporine metabolites with nephrotoxicity. *Eur J Clin Pharmacol* 41: 285–290, 1991
302. Christians U, Radeke HH, Kownatzki R, Schiebel HM, Schottmann R, Sewing KF: Isolation of an immunosuppressive metabolite of FK506 generated by human microsome preparations. *Clin Biochem* 24: 271–275, 1991
303. Iwasaki K, Shiraga T, Matsuda H, Nagase K, Tokuma Y, Hata T, Fujii Y, Sakuma S, Fujitsu T, Fujikawa A: Further metabolism of FK506 (tacrolimus). Identification and biological activities of the metabolites oxidized at multiple sites of FK506. *Drug Metab Dispos* 23: 28–34, 1995
304. Christians U, Jacobsen W, Benet LZ, Lampen A: Mechanisms of clinically relevant drug interactions associated with tacrolimus. *Clin Pharmacokinet* 41: 813–851, 2002
305. Keogh A, Spratt P, McCosker C, Macdonald P, Mundy J, Kaan A: Ketoconazole to reduce the need for cyclosporine

after cardiac transplantation. *N Engl J Med* 333: 628–633, 1995
306. Campana C, Regazzi MB, Buggia I, Molinaro M: Clinically significant drug interactions with cyclosporin. An update. *Clin Pharmacokinet* 30: 141–179, 1996
307. Ferguson CJ, Griffin PJ, Moore RH, Salaman JR: Effect of pancreas transplantation on glomerular structure in diabetes. *Lancet* 343: 120–121, 1994
308. el-Agroudy AE, Sobh MA, Hamdy AF, Ghoneim MA: A prospective, randomized study of coadministration of ketoconazole and cyclosporine a in kidney transplant recipients: Ten-year follow-up. *Transplantation* 77: 1371–1376, 2004
309. el-Dahshan KF, Bakr MA, Donia AF, Badr A, Sobh MA: Ketoconazole-tacrolimus coadministration in kidney transplant recipients: Two-year results of a prospective randomized study. *Am J Nephrol* 26: 293–298, 2006
310. Dai Y, Hebert MF, Isoherranen N, Davis CL, Marsh C, Shen DD, Thummel KE: Effect of CYP3A5 polymorphism on tacrolimus metabolic clearance in vitro. *Drug Metab Dispos* 34: 836–847, 2006
311. Fukudo M, Yano I, Yoshimura A, Masuda S, Uesugi M, Hosohata K, Katsura T, Ogura Y, Oike F, Takada Y, Uemoto S, Inui K: Impact of MDR1 and CYP3A5 on the oral clearance of tacrolimus and tacrolimus-related renal dysfunction in adult living-donor liver transplant patients. *Pharmacogenet Genomics* 18: 413–423, 2008
312. Joy MS, Hogan SL, Thompson BD, Finn WF, Nickeleit V: Cytochrome P450 3A5 expression in the kidneys of patients with calcineurin inhibitor nephrotoxicity. *Nephrol Dial Transplant* 22: 1963–1968, 2007
313. Klauke B, Wirth A, Zittermann A, Bohms B, Tenderich G, Korfer R, Milting H: No association between single nucleotide polymorphisms and the development of nephrotoxicity after orthotopic heart transplantation. *J Heart Lung Transplant* 27: 741–745, 2008
314. Kubo M, Kiyohara Y, Kato I, Tanizaki Y, Katafuchi R, Hirakata H, Okuda S, Tsuneyoshi M, Sueishi K, Fujishima M, Iida M: Risk factors for renal glomerular and vascular changes in an autopsy-based population survey: The Hisayama study. *Kidney Int* 63: 1508–1515, 2003
315. Remuzzi G, Cravedi P, Perna A, Dimitrov BD, Turturro M, Locatelli G, Rigotti P, Baldan N, Beatini M, Valente U, Scalamogna M, Ruggenenti P: Long-term outcome of renal transplantation from older donors. *N Engl J Med* 354: 343–352, 2006
316. El-Husseini A, Sabry A, Zahran A, Shoker A: Can donor implantation renal biopsy predict long-term renal allograft outcome? *Am J Nephrol* 27: 144–151, 2007
317. Feutren G, Mihatsch MJ: Risk factors for cyclosporine-induced nephropathy in patients with autoimmune diseases. International Kidney Biopsy Registry of Cyclosporine in Autoimmune Diseases. *N Engl J Med* 326: 1654–1660, 1992
318. Bennett WM, Lindsley J, Buss WC: The effects of age and dosage route on experimental cyclosporine nephrotoxicity. *Transplantation* 51: 730–731, 1991
319. Greenfeld Z, Peleg I, Brezis M, Rosen S, Pisanty S: Potential interaction between prolonged cyclosporin administration and aging in the rat kidney. *Ann N Y Acad Sci* 717: 209–212, 1994
320. Legendre C, Brault Y, Morales JM, Oberbauer R, Altieri P, Riad H, Mahony J, Messina M, Pussell B, Martinez JG, Lelong M, Burke JT, Neylan JF: Factors influencing glomerular filtration rate in renal transplantation after cyclosporine withdrawal using sirolimus-based therapy: A multivariate analysis of results at five years. *Clin Transplant* 21: 330–336, 2007
321. Jung FF, Kennefick TM, Ingelfinger JR, Vora JP, Anderson S: Down-regulation of the intrarenal renin-angiotensin system in the aging rat. *J Am Soc Nephrol* 5: 1573–1580, 1995
322. Luke RG: Hypertensive nephrosclerosis: pathogenesis and prevalence. Essential hypertension is an important cause of end-stage renal disease. *Nephrol Dial Transplant* 14: 2271–2278, 1999
323. Long DA, Mu W, Price KL, Johnson RJ: Blood vessels and the aging kidney. *Nephron Exp Nephrol* 101: e95–e99, 2005
324. Schmitt R, Cantley LG: The impact of aging on kidney repair. *Am J Physiol Renal Physiol* 294: F1265–F1272, 2008
325. Melk A, Schmidt BM, Vongwiwatana A, Rayner DC, Halloran PF: Increased expression of senescence-associated cell cycle inhibitor p16INK4a in deteriorating renal transplants and diseased native kidney. *Am J Transplant* 5: 1375–1382, 2005
326. Melk A, Mansfield ES, Hsieh SC, Hernandez-Boussard T, Grimm P, Rayner DC, Halloran PF, Sarwal MM: Transcriptional analysis of the molecular basis of human kidney aging using cDNA microarray profiling. *Kidney Int* 68: 2667–2679, 2005
327. Halloran PF, Melk A, Barth C: Rethinking chronic allograft nephropathy: The concept of accelerated senescence. *J Am Soc Nephrol* 10: 167–181, 1999
328. Sturrock ND, Lang CC, Struthers AD: Indomethacin and cyclosporin together produce marked renal vasoconstriction in humans. *J Hypertens* 12: 919–924, 1994
329. Altman RD, Perez GO, Sfakianakis GN: Interaction of cyclosporine A and nonsteroidal anti-inflammatory drugs on renal function in patients with rheumatoid arthritis. *Am J Med* 93: 396–402, 1992
330. Kovarik JM, Kurki P, Mueller E, Guerret M, Markert E, Alten R, Zeidler H, Genth-Stolzenburg S: Diclofenac combined with cyclosporine in treatment refractory rheumatoid arthritis: Longitudinal safety assessment and evidence of a pharmacokinetic/dynamic interaction. *J Rheumatol* 23: 2033–2038, 1996
331. Soubhia RM, Mendes GE, Mendonca FZ, Baptista MA, Cipullo JP, Burdmann EA: Tacrolimus and nonsteroidal anti-inflammatory drugs: An association to be avoided. *Am J Nephrol* 25: 327–334, 2005
332. Bennett WM: Drug interactions and consequences of sodium restriction. *Am J Clin Nutr* 65: 678S–681S, 1997
333. Baan CC, Balk AH, Holweg CT, van R, I, Maat LP, Vantrimpont PJ, Niesters HG, Weimar W: Renal failure after clinical heart transplantation is associated with the TGF-beta 1 codon 10 gene polymorphism. *J Heart Lung Transplant* 19: 866–872, 2000
334. van de Wetering J, Weimar CH, Balk AH, Roodnat JI, Holweg CT, Baan CC, van Domburg RT, Weimar W: The impact of transforming growth factor-beta1 gene polymorphism on end-stage renal failure after heart transplantation. *Transplantation* 82: 1744–1748, 2006
335. Di Filippo S, Zeevi A, McDade KK, Boyle GJ, Miller SA, Gandhi SK, Webber SA: Impact of TGFbeta1 gene poly-

morphisms on late renal function in pediatric heart transplantation. *Hum Immunol* 66: 133–139, 2005
336. Jonsson JR, Hong C, Purdie DM, Hawley C, Isbel N, Butler M, Balderson GA, Clouston AD, Pandeya N, Stuart K, Edwards-Smith C, Crawford DH, Fawcett J, Powell EE: Role of cytokine gene polymorphisms in acute rejection and renal impairment after liver transplantation. *Liver Transpl* 7: 255–263, 2001
337. Mytilineos J, Laux G, Opelz G: Relevance of IL10, TGF-beta1, TNFalpha, and IL4Ralpha gene polymorphisms in kidney transplantation: A collaborative transplant study report. *Am J Transplant* 4: 1684–1690, 2004
338. Rigat B, Hubert C, henc-Gelas F, Cambien F, Corvol P, Soubrier F: An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 86: 1343–1346, 1990
339. Beige J, Scherer S, Weber A, Engeli S, Offermann G, Opelz G, Distler A, Sharma AM: Angiotensin-converting enzyme genotype and renal allograft survival. *J Am Soc Nephrol* 8: 1319–1323, 1997
340. Broekroelofs J, Stegeman CA, Navis G, Tegzess AM, De ZD, De Jong PE: Risk factors for long-term renal survival after renal transplantation: A role for angiotensin-converting enzyme (insertion/deletion) polymorphism? *J Am Soc Nephrol* 9: 2075–2081, 1998
341. Barocci S, Ginevri F, Valente U, Torre F, Gusmano R, Nocera A: Correlation between angiotensin-converting enzyme gene insertion/deletion polymorphism and kidney graft long-term outcome in pediatric recipients: A single-center analysis. *Transplantation* 67: 534–538, 1999
342. Juckett MB, Cohen EP, Keever-Taylor CA, Zheng Y, Lawton CA, Moulder JE, Klein J: Loss of renal function following bone marrow transplantation: An analysis of angiotensin converting enzyme D/I polymorphism and other clinical risk factors. *Bone Marrow Transplant* 27: 451–456, 2001
343. Abdi R, Tran TB, Zee R, Brenner BM, Milford EL: Angiotensin gene polymorphism as a determinant of posttransplantation renal dysfunction and hypertension. *Transplantation* 72: 726–729, 2001
344. Srinivas TR, Meier-Kriesche HU: Minimizing immunosuppression, an alternative approach to reducing side effects: Objectives and interim result. *Clin J Am Soc Nephrol* 3: S101–S116, 2008 [suppl 2]
345. Flechner SM, Kobashigawa J, Klintmalm G: Calcineurin inhibitor-sparing regimens in solid organ transplantation: Focus on improving renal function and nephrotoxicity. *Clin Transplant* 22: 1–15, 2008
346. Kasiske BL, Chakkera HA, Louis TA, Ma JZ: A meta-analysis of immunosuppression withdrawal trials in renal transplantation. *J Am Soc Nephrol* 11: 1910–1917, 2000
347. Vincenti F, Larsen C, Durrbach A, Wekerle T, Nashan B, Blancho G, Lang P, Grinyo J, Halloran P, Solez K, Hagerty D, Levy E, Zhou W, Natarajan K, Charpentier B, the Belatacept Study Group: Costimulation blockade with belatacept in renal transplantation. *N Engl J Med* 353: 770–781, 2005
348. Griffiths CE, Dubertret L, Ellis CN, Finlay AY, Finzi AF, Ho VC, Johnston A, Katsambas A, Lison AE, Naeyaert JM, Nakagawa H, Paul C, Vanaclocha F: Ciclosporin in psoriasis clinical practice: An international consensus statement. *Br J Dermatol* 150: 11–23, 2004 [suppl 67]
349. Dieperink H, Leyssac PP, Starklint H, Jorgensen KA, Kemp E: Antagonist capacities of nifedipine, captopril, phenoxybenzamine, prostacyclin and indomethacin on cyclosporin A induced impairment of rat renal function. *Eur J Clin Invest* 16: 540–548, 1986
350. Rahn KH, Barenbrock M, Fritschka E, Heinecke A, Lippert J, Schroeder K, Hauser I, Wagner K, Neumayer HH: Effect of nitrendipine on renal function in renal-transplant patients treated with cyclosporin: A randomised trial. *Lancet* 354: 1415–1420, 1999
351. Ruggenenti P, Perico N, Mosconi L, Gaspari F, Benigni A, Amuchastegui CS, Bruzzi I, Remuzzi G: Calcium channel blockers protect transplant patients from cyclosporine-induced daily renal hypoperfusion. *Kidney Int* 43: 706–711, 1993
352. Morales JM, Rodriguez-Paternina E, Araque A, Andres A, Hernandez E, Ruilope LM, Rodicio JL: Long-term protective effect of a calcium antagonist on renal function in hypertensive renal transplant patients on cyclosporine therapy: A 5-year prospective randomized study. *Transplant Proc* 26: 2598–2599, 1994
353. Kuypers DR, Neumayer H-H, Fritsche L, Budde K, Rodicio J, Vanrenterghem Y, Lacidipine Study Group: Calcium channel blockade and preservation of renal graft function in cyclosporine-treated recipients: A prospective randomized placebo-controlled 2-year study. *Transplantation* 78: 1204–1211, 2004
354. Legault L, Ogilvie RI, Cardella CJ, Leenen FH: Calcium antagonists in heart transplant recipients: Effects on cardiac and renal function and cyclosporine pharmacokinetics. *Can J Cardiol* 9: 398–404, 1993
355. Leenen FH, Coletta E, Davies RA: Prevention of renal dysfunction and hypertension by amlodipine after heart transplant. *Am J Cardiol* 100: 531–535, 2007
356. Chan C, Maurer J, Cardella C, Cattran D, Pei Y: A randomized controlled trial of verapamil on cyclosporine nephrotoxicity in heart and lung transplant recipients. *Transplantation* 63: 1435–1440, 1997
357. Mervaala E, Lassila M, Vaskonen T, Krogerus L, Lahteenmaki T, Vapaatalo H, Karppanen H: Effects of ACE inhibition on cyclosporine A-induced hypertension and nephrotoxicity in spontaneously hypertensive rats on a high-sodium diet. *Blood Press* 8: 49–56, 1999
358. Li C, Sun BK, Lim SW, Song JC, Kang SW, Kim YS, Kang DH, Cha JH, Kim J, Yang CW: Combined effects of losartan and pravastatin on interstitial inflammation and fibrosis in chronic cyclosporine-induced nephropathy. *Transplantation* 79: 1522–1529, 2005
359. Sun BK, Li C, Lim SW, Choi BS, Lee SH, Kim IS, Kim YS, Bang BK, Yang CW: Blockade of angiotensin II with losartan attenuates transforming growth factor-beta1 inducible gene-h3 (betaig-h3) expression in a model of chronic cyclosporine nephrotoxicity. *Nephron Exp Nephrol* 99: e9–16, 2005
360. Hannedouche TP, Natov S, Boitard C, Lacour B, Grunfeld JP: Angiotensin converting enzyme inhibition and chronic cyclosporine-induced renal dysfunction in type 1 diabetes. *Nephrol Dial Transplant* 11: 673–678, 1996
361. Hausberg M, Kosch M, Hohage H, Suwelack B, Barenbrock M, Kisters K, Rahn KH: Antihypertensive treatment in

renal transplant patients–Is there a role for ACE inhibitors? *Ann Transplant* 6: 31–37, 2001
362. Campistol JM, Inigo P, Jimenez W, Lario S, Clesca PH, Oppenheimer F, Rivera F: Losartan decreases plasma levels of TGF-beta1 in transplant patients with chronic allograft nephropathy. *Kidney Int* 56: 714–719, 1999
363. Inigo P, Campistol JM, Lario S, Piera C, Campos B, Bescos M, Oppenheimer F, Rivera F: Effects of losartan and amlodipine on intrarenal hemodynamics and TGF-beta(1) plasma levels in a crossover trial in renal transplant recipients. *J Am Soc Nephrol* 12: 822–827, 2001
364. Feria I, Pichardo I, Juarez P, Ramirez V, Gonzalez MA, Uribe N, Garcia-Torres R, Lopez-Casillas F, Gamba G, Bobadilla NA: Therapeutic benefit of spironolactone in experimental chronic cyclosporine A nephrotoxicity. *Kidney Int* 63: 43–52, 2003
365. Perez-Rojas JM, Derive S, Blanco JA, Cruz C, Martinez de la ML, Gamba G, Bobadilla NA: Renocortical mRNA expression of vasoactive factors during spironolactone: Protective effect in chronic cyclosporine nephrotoxicity. *Am J Physiol Renal Physiol* 289: F1020–F1030, 2005
366. Mourad G, Ribstein J, Mimran A: Converting-enzyme inhibitor versus calcium antagonist in cyclosporine-treated renal transplants. *Kidney Int* 43: 419–425, 1993
367. Midtvedt K, Hartmann A, Foss A, Fauchald P, Nordal KP, Rootwelt K, Holdaas H: Sustained improvement of renal graft function for two years in hypertensive renal transplant recipients treated with nifedipine as compared to lisinopril. *Transplantation* 72: 1787–1792, 2001
368. Ryffel B, Donatsch P, Hiestand P, Mihatsch MJ: PGE2 reduces nephrotoxicity and immunosuppression of cyclosporine in rats. *Clin Nephrol* 25: S95–S99, 1986 [suppl 1]
369. Paller MS: Effects of the prostaglandin E1 analog misoprostol on cyclosporine nephrotoxicity. *Transplantation* 45: 1126–1131, 1988
370. Nast CC, Hirschberg R, Artishevsky A, Adler SG: Misoprostol partially inhibits the renal scarring of chronic cyclosporine nephrotoxicity. *Am J Ther* 2: 882–885, 1995
371. Moran M, Mozes MF, Maddux MS, Veremis S, Bartkus C, Ketel B, Pollak R, Wallemark C, Jonasson O: Prevention of acute graft rejection by the prostaglandin E1 analogue misoprostol in renal-transplant recipients treated with cyclosporine and prednisone. *N Engl J Med* 322: 1183–1188, 1990
372. Boers M, Bensen WG, Ludwin D, Goldsmith CH, Tugwell P: Cyclosporine nephrotoxicity in rheumatoid arthritis: No effect of short term misoprostol treatment. *J Rheumatol* 19: 534–537, 1992
373. Amore A, Gianoglio B, Ghigo D, Peruzzi L, Porcellini MG, Bussolino F, Costamagna C, Cacace G, Picciotto G, Mazzucco G: A possible role for nitric oxide in modulating the functional cyclosporine toxicity by arginine. *Kidney Int* 47: 1507–1514, 1995
374. Chander V, Chopra K: Effect of molsidomine and L-arginine in cyclosporine nephrotoxicity: Role of nitric oxide. *Toxicology* 207: 463–474, 2005
375. Lassila M, Santisteban J, Finckenberg P, Salmenpera P, Riutta A, Moilanen E, Virtanen I, Vapaatalo H, Nurminen ML: Vascular changes in cyclosporine A-induced hypertension and nephrotoxicity in spontaneously hypertensive rats on high-sodium diet. *J Physiol Pharmacol* 52: 21–38, 2001
376. Zhang XZ, Ardissino G, Ghio L, Tirelli AS, Dacco V, Colombo D, Pace E, Testa S, Claris-Appiani A: L-arginine supplementation in young renal allograft recipients with chronic transplant dysfunction. *Clin Nephrol* 55: 453–459, 2001
377. Ling H, Li X, Jha S, Wang W, Karetskaya L, Pratt B, Ledbetter S: Therapeutic role of TGF-beta-neutralizing antibody in mouse cyclosporin A nephropathy: Morphologic improvement associated with functional preservation. *J Am Soc Nephrol* 14: 377–388, 2003
378. Tariq M, Morais C, Sobki S, Al Sulaiman M, Al Khader A: N-acetylcysteine attenuates cyclosporin-induced nephrotoxicity in rats. *Nephrol Dial Transplant* 14: 923–929, 1999
379. Barany P, Stenvinkel P, Ottosson-Seeberger A, Alvestrand A, Morrow J, Roberts JJ, Salahudeen AK: Effect of 6 weeks of vitamin E administration on renal haemodynamic alterations following a single dose of neoral in healthy volunteers. *Nephrol Dial Transplant* 16: 580–584, 2001
380. Jenkins JK, Huang H, Ndebele K, Salahudeen AK: Vitamin E inhibits renal mRNA expression of COX II, HO I, TGF-beta, and osteopontin in the rat model of cyclosporine nephrotoxicity. *Transplantation* 71: 331–334, 2001
381. Lessio C, de Assuncao SF, Gloria MA, Di Tommaso AB, Gori MM, Di Marco GS, Schor N, Higa EM: Cyclosporine A and NAC on the inducible nitric oxide synthase expression and nitric oxide synthesis in rat renal artery cultured cells. *Kidney Int* 68: 2508–2516, 2005
382. Pere AK, Lindgren L, Tuomainen P, Krogerus L, Rauhala P, Laakso J, Karppanen H, Vapaatalo H, Ahonen J, Mervaala EMA: Dietary potassium and magnesium supplementation in cyclosporine-induced hypertension and nephrotoxicity. *Kidney Int* 58: 2462–2472, 2000
383. Asai T, Nakatani T, Tamada S, Kuwabara N, Yamanaka S, Tashiro K, Nakao T, Komiya T, Okamura M, Kim S, Iwao H, Miura K: Activation of transcription factors AP-1 and NF-kappaB in chronic cyclosporine A nephrotoxicity: Role in beneficial effects of magnesium supplementation. *Transplantation* 75: 1040–1044, 2003