Oxidative stress as a common pathway to chronic tubulointerstitial injury in kidney allografts

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Submitted 19 January 2007; accepted in final form 23 April 2007

Djamali A. Oxidative stress as a common pathway to chronic tubulointerstitial injury in kidney allografts. Am J Physiol Renal Physiol 293: F445–F455, 2007—A major challenge for kidney transplantation is to dissect out the identifiable causes of chronic allograft tubulointerstitial fibrosis and to develop cause-specific treatment strategies. There has been a recent interest in the role of oxidative stress (OS) as a mediator of injury in chronic allograft tubular atrophy (TA) and interstitial fibrosis (IF). A review of the literature and data from my laboratory studying chronic allograft TA/IF in rat, rhesus monkey, and human kidneys suggests that OS is increased in graft-infiltrating macrophages, activated myofibroblasts, interstitium, and areas of tubular injury. Chronic allograft OS may be induced by inflammation, abnormal tissue oxygenation, immunosuppressant drugs, and comorbid clinical conditions including diabetes, hypertension, proteinuria, anemia, and dyslipidemia. Moreover, OS-induced chronic TA/IF is associated with signaling pathways including inflammation, apoptosis, hypoxia, and epithelial-to-mesenchymal transition. Most of these injury pathways participate in a self-perpetuating cycle with OS. In conclusion, evidence suggests that OS is a common mechanism of injury in chronic allograft TA/IF. However, most available data demonstrate a correlation and no causal relationship. Furthermore, the extent to which TA/IF is dependent on OS is unknown. These questions may be answered by prospective randomized placebo-control trials examining the role of select antioxidants in the prevention of chronic allograft TA/IF.

chronic allograft tubular atrophy/interstitial fibrosis (TA/IF) is a major cause of late allograft loss (21, 22, 40, 77). It is a chronic, progressive, nonspecific, and irreversible histopathological entity that is common, occurs early after transplantation (40, 69, 77), and is associated with significant patient morbidity and mortality (40, 50, 62, 104). A major challenge to the future of kidney transplantation is to dissect out the identifiable causes of chronic allograft TA/IF and to develop cause-specific treatment strategies (22).

Oxidative stress (OS) is an interesting candidate at the intersection between injury and histopathology in kidney transplantation. OS is a term that denotes damage to cells, tissues, and organs caused by reactive oxygen species (ROS). ROS are generated exogenously and intracellularly and include superoxide anion (O2•−), hydrogen peroxide (H2O2), hydroxyl radicals (OH·) and peroxynitrite (ONOO−) (Fig. 1). One- and two-electron reductions of O2 generate O2•− and H2O2, respectively (11, 32). Hydroxyl radicals are generated from H2O2 and O2•− in the presence of transition metals (iron and copper in particular) and are extremely reactive. Peroxynitrite is another potent oxidizing agent that is generated when submicromolar concentrations of nitric oxide (NO) compete for O2•− with endogenous superoxide dismutase enzymes (SOD) (Fig. 1) (11, 32). The principal intracellular sources of ROS include the mitochondrial electron transport system, peroxisomes, cytochrome P-450, and NADPH oxidase enzymes (11, 32), whereas commonly described exogenous factors involved in the generation of ROS are represented by inflammatory cytokines, chemotherapeutic drugs, and toxins (32). Antioxidants constitute the defense mechanism against OS injury and include both enzymes and nonenzymatic molecules. Copper-zinc and manganese superoxide dismutase (CuZnSOD and MnSOD), catalase, and glutathione peroxidase (GPX) are key antioxidant enzymes that reduce O2•− to H2O2 and water in a stepwise fashion (11, 32) (Fig. 1). In contrast, glutathione and vitamins A, C, and E constitute the major nonenzymatic antioxidant molecules (32).

The balance between ROS production and antioxidant defenses defines the degree of OS in a given tissue (32). Whereas ROS play an important role as signaling and regulatory molecules in cell proliferation, differentiation, and apoptosis (37, 42, 98), a prooxidant milieu can alter and denature nucleic acids, carbohydrates, lipids, and proteins, resulting in cell toxicity. The deleterious effects of OS have
Fig. 1. Reactive oxygen species (ROS) include superoxide anion (O$_2^•$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radicals (OH$^•$), and peroxynitrite (ONOO$^•$). One- and two-electron reductions of O$_2$ generate O$_2^•$ and H$_2$O$_2$, respectively. Hydroxyl radicals are extremely reactive species generated from H$_2$O$_2$ and O$_2^•$ in the presence of transition metals (iron and copper in particular). Peroxynitrite is another potent oxidizing agent, generated when submicromolar concentrations of nitric oxide (NO$^•$) compete for O$_2^•$ with endogenous superoxide dismutase (SOD) enzymes. Peroxynitrite can result in protein and lipid damage by protein nitration and lipid peroxidation, respectively. In that regard, nitrotyrosine and malondialdehyde (MDA) may be considered as biomarkers of oxidative stress (OS)-mediated protein and lipid damage, respectively. In the kidney, NO$^•$ is mainly produced by endothelial and inducible nitric oxide synthase enzymes (eNOS and iNOS, respectively). Antioxidants are the defense mechanism against OS injury and include both enzymes and nonenzymatic molecules. CuZnSOD and MnSOD, catalase, and glutathione peroxidase are key antioxidant enzymes that reduce O$_2^•$ to H$_2$O$_2$ and water. Glutathione, selenium, and vitamins C and E are the common nonenzymatic antioxidant molecules. Selenium and glutathione contribute to the reduction of H$_2$O$_2$ to H$_2$O, whereas vitamins C and E can prevent lipid peroxidation. Vitamin E can also inhibit O$_2^•$ generation by inhibiting the assembly of NADPH oxidase subunits. The balance between pro- and antioxidant molecules determines the OS profile.

Fig. 2. Superoxide anion (O$_2^•$; A, B, E, and F) and nitrotyrosine expression (C, D, G, and H) in murine (A–D) and nonhuman primate (E–H) models of chronic allograft tubular atrophy and interstitial fibrosis (TA/IF). O$_2^•$ was stained with dihydroethidine, and nitrotyrosine was stained with a goat anti-rabbit antibody. Both O$_2^•$ and nitrotyrosine were increased in the tubules and interstitium of allografts with chronic TA/IF (B, D, F, and H). Yellow arrows indicate greater tubular expression of nitrotyrosine (D and H).
long been reported in the pathophysiology of aging (11, 32), neoplastic (52), hypertensive (65, 100), cardiovascular (39, 48, 72), and chronic kidney diseases (CKD) (43–45, 73). OS is also involved in the pathogenesis of native kidney tissue injury and fibrosis in experimental models of hypertensive (109), diabetic (55), obstructive (78), and CKD (20, 99).

Systemic biomarkers of OS are increased in kidney transplant recipients (15, 23, 81, 91, 92, 103), similar to patients with native CKD. However, to examine the role of OS in the pathogenesis of chronic allograft TA and IF, one would have to:

1) demonstrate whether OS is increased specifically in allografts with chronic TA/IF,
2) determine the sources of OS in the allograft, and
3) analyze the molecular mechanisms involved in OS-mediated chronic allograft TA/IF. It is concluded that OS is a common mechanism of injury in chronic allograft TA/IF, and yet most available data demonstrate a correlation and no causal relationship. Furthermore, the extent to which TA/IF is dependent on OS is unknown. The ideal approach to address these questions is to design prospective randomized placebo-control trials to examine the role of select antioxidants in the prevention of chronic allograft TA/IF.

IS OS INCREASED IN CHRONIC TA/IF?

Albrecht et al. (4) showed that H2O2-positive cells were increased in the interstitium of human kidney allografts with chronic TA/IF. Similarly, my group has shown greater O2•− levels in graft-infiltrating and tubular cells of rat and rhesus allografts with chronic TA/IF compared with syngeneic and normal controls, respectively (Fig. 2) (25). Briefly, in the rat model of chronic TA/IF, Fisher344 kidney allografts were placed in Lewis recipients receiving cyclosporine for 10 days to prevent graft loss from acute rejection. The allograft undergoes progressive sclerosis with TA, IF, and various degrees of inflammation and vascular and glomerular changes. Most of these changes were established by 6 mo posttransplant, when the allograft was harvested and compared with syngeneic transplants (24, 25). In the rhesus monkey model, immunosuppressive therapy (mostly anti-CD3 immunotoxin) was discontinued 4 to 12 mo after transplant, and allografts were harvested within months to years following immunosuppression withdrawal. These allografts demonstrated similar features of TA, IF, and chronic inflammation, with various levels of glomerulopathy and vasculopathy (96).

There is also indirect evidence for increased peroxynitrite activity. MacMillan-Crow et al. (60) demonstrated that allo-
graft tubular MnSOD is nitrated and inactivated in human kidneys with chronic TA/IF (60). The same authors later showed that MnSOD and cytochrome c nitration occur before the onset of kidney allograft dysfunction, suggesting that protein nitration and inactivation of antioxidant enzymes are early events in the pathogenesis of chronic tubulointerstitial injury (61). These observations have been confirmed by other groups, showing that peroxynitrite formation and protein nitration (e.g., nitrotyrosine in Fig. 1) are increased in tubular and graft-infiltrating cells in rat, rhesus, and human allografts with chronic TA/IF (4) (Fig. 2). There is also evidence of lipid peroxidation, since greater intragraft malondialdehyde (MDA) levels have been found in experimental chronic allograft TA/IF (34).

Interstitial (4, 25, 66) and tubular (25) levels of inducible nitric oxide synthase (iNOS) are also increased in chronic allograft TA/IF. Oligonucleotide microarray analyses of rhesus kidney allografts with chronic TA/IF showed that heat shock protein 27 (HSP27), heat shock factor-1 (HSF-1), and iNOS levels were all significantly increased (252-, 91- and 3.4-fold, respectively), whereas SOD1 and catalase levels were decreased by more than twofold (Fig. 3A). HSP27 can be induced by HSF-1 under conditions of stress (110). This stress protein is a cytoprotective molecule with antioxidant characteristics, including the maintenance and/or upregulation of reduced glutathione levels within the cell (7, 8, 24). Conversely, iNOS is an important prooxidant enzyme that generates NO•, especially under inflammatory conditions (13, 59). NO• competes with antioxidant enzymes (MnSOD and CuZnSOD) for O2•− to generate the potent free radical ONOO− (Fig. 1) (13, 59). In patients with chronic allograft TA/IF, semiquantitative real-time PCR analyses of GPX, hypoxemia-inducible factor-1α (HIF-1α), and heme-oxygenase-2 (HO-2) showed that GPX and HIF-1α levels were significantly increased and that, conversely, HO-2 levels were decreased in TA/IF (Fig. 3B).

The association of OS and TA/IF does not imply a causal relationship. Moreover, OS and stress-response pathways participate in a self-perpetuating cycle, and it is difficult to separate the independent and specific contribution of OS to kidney allograft injury on the basis of observational studies. Nevertheless, these observations together demonstrate that OS and stress-response pathways are activated in kidney allografts with chronic TA/IF.

![Fig. 4. Graft-infiltrating T lymphocytes and macrophages were studied with anti-CD3 and anti-CD68 antibodies. NADPH oxidase expression was examined through its Gp91 subunit with mouse anti-Gp91 antibodies. Immunofluorescent secondary antibodies (Alexa 594 red and Alexa 488 green) were used for double-staining experiments. CD3-Gp91 studies are shown in A–F, and CD68-Gp91 experiments are shown in G–L. Merged digital image analyses (A, B, G, and H) demonstrate that CD68+ cells (macrophages) and not CD3+ cells (T lymphocytes) were an important source of NADPH oxidase in allografts with chronic TA/IF as indicated by greater intracytoplasmic expression of Gp91 in CD68+ cells (yellow).](image-url)
WHAT ARE THE SOURCES OF OS IN KIDNEY ALLOGRAFTS WITH CHRONIC TA/IF?

As mentioned earlier, the principal intracellular sources of ROS include the mitochondrial electron transport system, peroxisomes, cytochrome P-450, and NADPH oxidase enzymes (11, 32), whereas putative exogenous sources ROS are represented by inflammatory cytokines, chemotherapeutic drugs, and toxins (32). How does that apply to chronic allograft TA/IF? Inflammation has long been considered a contributing factor to chronic allograft injury (40, 70, 80). However, only circumstantial evidence suggested a role for inflammation in the generation of ROS in kidney allografts with chronic TA/IF. Data was limited to the observation that graft-infiltrating monocyte/macrophages produced iNOS and proinflammatory cytokines, including monocyte chemotactic protein-1 (MCP-1) and IL-6 in the Fisher to Lewis model (9, 24, 66). My group recently addressed this question and examined NADPH oxidase enzymes and graft-infiltrating cells in human and nonhuman primate kidney allografts undergoing chronic TA/IF (Fig. 4). NADPH oxidases are major sources of superoxide in vascular cells, myocytes, and phagocytes (16, 36). Double-staining immunofluorescent experiments and merged digital image analyses demonstrated that CD68⁺ cells (macrophages) and not CD3⁺ cells (T lymphocytes) were an important source of NADPH oxidase on the basis of greater intracytoplasmic levels of Gp91. Thus my group’s data suggest that macrophages contribute to OS through upregulation of iNOS (24) and NADPH oxidase enzymes.

Immunosuppressive drugs represent another potential source of ROS generation in chronic allograft TA/IF. Cyclosporine-treated rats have greater lipid peroxidation and decreased antioxidant (glutathione) levels in the kidney (95). Similarly, rat proximal tubular epithelial cells exposed to cyclosporine accumulate intracellular ROS and lipid peroxidation products, along with an altered glutathione redox state (33). Ramzy et al. (83) compared the effects of sirolimus and cyclosporine on OS in rat thoracic aortic segments and showed that cyclosporine increased isoprostane production (8-isoprostane is a marker of lipid peroxidation and therefore OS-mediated molecular damage). Another recent study examined the effect of mycophenolic acid (MPA) effect on platelet-derived growth factor (PDGF)-induced cellular ROS generation in rat vascular smooth muscle cells and showed that MPA inhibited PDGF-induced cellular ROS measured by flow cytometry (76). These experimental studies suggest a prooxidant role for cyclosporine A and antioxidant properties for MPA. However, the exact contribution of these molecules to the OS balance in chronic allograft TA/IF has yet to be determined.

Tissue hypoxia could also contribute to ROS generation in kidney allografts with chronic TA/IF, because cellular metabolism in hypoxic or anoxic conditions results in the production

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**Fig. 5.** Blood oxygen level-dependent (BOLD) MRI analyses of kidney allografts from patients undergoing chronic TA/IF were compared with those of native kidneys in healthy volunteers. Representative color R² maps in the coronal planes are shown in A and B. Blue represents the lowest R² value (lowest deoxyhemoglobin concentration), and green, yellow, and red show increasing R² values. Bar graphs in C represent average R² levels. Medullary and cortical R² levels were significantly decreased in allografts with chronic TA/IF, suggestive of decreased intrakidney deoxyhemoglobin levels. Data in D displays the correlation between serum and urine OS biomarkers and medullary and cortical R² levels, suggesting that there is an association between intrakidney oxygenation and ROS production. [Adapted from Djamali et al. (26)].
of ROS. Consistent with this hypothesis, renal activity and mRNA levels of CuZnSOD and MnSOD enzymes were increased in the cortex and medulla of rats subjected to hypoxia (18). Similarly, lipid peroxidation and MDA levels were increased in rat kidney tissue homogenates when animals were exposed to chronic hypobaric hypoxia (67). My group recently studied intrarenal oxygenation in human kidney allografts with chronic TA/IF, using blood oxygen level-dependent (BOLD) MRI. These studies showed that medullary and cortical R2* levels (corresponding to deoxyhemoglobin concentrations) were significantly decreased in allografts with chronic TA/IF (Fig. 5, A–C) (26). The BOLD signal is caused by field distortion from paramagnetic deoxyhemoglobin contained in red blood cells. In other words, this noninvasive imaging technique uses deoxyhemoglobin as an endogenous contrast agent (26, 27). Deoxyhemoglobin levels correlate with tissue oxygenation when capillaries are intact, because oxygen can diffuse freely toward the tissue. However, chronic allograft TA/IF is associated with a significant loss of peritubular capillaries (46). It is therefore likely that tissue capillary loss, together with interstitial fibrosis, results in limited tissue oxygen extraction and lower deoxyhemoglobin levels (26). Interestingly, serum H2O2 and HSP27 levels were significantly increased, whereas urine total antioxidant potential and NO levels were decreased in patients with chronic allograft TA/IF (26). There was also a significant correlation between medullary and cortical oxygenation (R2* levels) and serum/urine biomarkers of OS, suggesting that abnormal intrarenal oxygenation may be involved in the generation of ROS (Fig. 5D) (26).

Congruent with these findings, we observed an upregulation of tissue biomarkers of hypoxia (HIF-1α) and OS (HSP27, MnSOD, and CuZnSOD) in kidney allografts with chronic TA/IF in the Fisher to Lewis model (24).

Finally, proteinuria (106, 112), diabetes (14, 38), anemia (15, 68), dyslipidemia (17, 97), high salt intake (2, 51), and hypertension (63, 86, 87, 108) have all been associated with OS and TA/IF in experimental models of native kidney disease. Specific studies demonstrating an association between these conditions and OS in chronic allograft TA/IF are lacking to date, but these comorbidities are common in kidney trans-
plant recipients (28), and it is plausible that they contribute to intragraft generation of ROS.

HOW CAN OS RESULT IN CHRONIC TA/IF?

Interstitial fibroblasts are the principal source of kidney fibrosis (31, 47). Under stress, interstitium fibroblasts expand by cell division and generate profibrotic molecules. Up to one-third of all disease-related fibroblasts can originate from tubular epithelia at the site of injury through epithelial-to-mesenchymal transition (EMT) (47). EMT can contribute to native (49, 57) and transplant kidney injury, including chronic allograft TA/IF (25, 41, 85, 102). Transforming growth factor-β1 (TGF-β1) can initiate and maintain EMT by activating signaling pathways and transcriptional regulators involved in tissue fibrosis (107).

What is the evidence that OS is involved in the pathogenesis of EMT in chronic allograft TA/IF? First, in vitro studies in proximal tubular epithelial cells have shown that ROS play an important role in TGF-β1-induced EMT through activation of MAPK and Smad pathways (84). Second, my group (25) has demonstrated that OS is associated with EMT in experimental chronic allograft TA/IF. These studies showed increased α-smooth muscle actin (α-SMA) and collagen type I and III levels together with reduced E-cadherin expression, confirming the presence of EMT. EMT was associated with OS, since tubulointerstitial O$_2^•$ and iNOS levels were also significantly increased (25). Third, my group recently examined the role of myofibroblasts and interstitial fibroblasts in the OS pathway in human allografts with chronic TA/IF. Immunofluorescent studies for α-SMA, S100A4, and Gp91 were performed to study myofibroblasts, fibroblasts, and the NADPH oxidase enzyme, respectively (Fig. 6). Myofibroblasts had significantly greater intracytoplasmic Gp91 expression compared with fibroblasts (Fig. 6, A and D). Next, a subset of tubular epithelial cells showed intracytoplasmic staining for α-SMA and Gp91, suggestive of early EMT and OS (Fig. 6A). S100A4 staining was also present in atrophic tubular epithelial cells (Fig. 6, D and F–H). Double-staining studies for α-SMA and S100A4 demonstrated nearly no overlap (Fig. 6, G–I), confirming previous reports that these markers separate two fibroblast phenotypes. Myofibroblasts are the activated form of fibroblasts and can be better identified by the presence of α-SMA (54, 79, 94). Results of studies by my group are congruent with recent reports of EMT in patients with TA/IF (41, 85, 102) and support the involvement of EMT in the pathogenesis of chronic allograft tubulointerstitial fibrosis. However, the extent of EMT’s contribution to allograft fibrogenesis remains unknown.

OS can also contribute to tubular atrophy through apoptosis (6, 10, 24, 101) and inflammation (19, 56, 58, 100). In vitro studies suggest that ROS can induce tubular epithelial cell apoptosis through activation of caspases and endonucleases (6, 10) and downregulation of antiapoptotic proteins (101). In-

Fig. 7. OS is associated with local inflammation in the rat (Fisher to Lewis) model of chronic TA/IF, as suggested by increased tubular, interstitial, and whole kidney expression of phosphorylated p38-MAPK (A and B) together with greater α-SMA and HSP72 levels (B). C: normal human kidney (iii and iv) and allografts with chronic TA/IF (v and vi) stained for MnSOD and Gp91 (purple and brown, respectively; iii and v) and α-SMA and e-cadherin (brown and purple, respectively; iv and vi). Chronic allograft TA/IF was associated with greater interstitial expression of Gp91 and α-SMA, together with reduced tubular expression of MnSOD and e-cadherin.
creased OS and apoptosis, together with upregulation of FasL, Bax, and HSP27, have been observed in areas of tubular injury in kidney allografts with chronic TA/IF (24, 25), suggesting a proapoptotic role for OS. OS may also result in activation of proinflammatory pathways including c-Jun NH2-terminal kinase, p38-MAPK (82, 98), nuclear factor-κB (56, 111), and activator protein-1 (89). Resulting inflammation, in turn, can lead to ROS production by leukocytes and resident cells. This becomes a chronic self-perpetuating form of injury (40, 70, 80). Figure 7 shows that phosphorylated p38 MAPK (activated form of p38 MAPK), HSP72 [a protective heat shock protein involved in stress-response (71)], and α-SMA are all increased in experimental chronic allograft TA/IF (Fig. 7, A and B). This was associated with downregulation of MnSOD and E-cadherin and upregulation of Gp91, suggesting that OS may contribute to chronic allograft TA/IF through inflammatory injury.

Chronic hypoxia is another potential mechanism of OS-mediated chronic injury in the allograft (68). O2•− leads to decreased NO+ bioavailability through ONOO− formation. Because NO+ suppresses mitochondrial respiration, depletion of NO+ by OS may stimulate mitochondrial respiration and uncouple it from energy consumption, resulting in tissue hypoxia (3). Ishii et al. (46) showed a significant correlation between progressive loss of peritubular capillaries and development of IF. In addition, HIF-1α studies suggest that there is a correlation between hypoxia and clinical/subclinical rejection (88) and chronic TA/IF (24). These results are actually in line with BOLD MRI studies showing decreased intrarenal deoxyhemoglobin levels in patients with chronic allograft TA/IF (26) (Fig. 5). As stated earlier, chronic allograft TA/IF is associated with a significant loss of peritubular capillaries (46), limiting tissue oxygen extraction. It is also conceivable that the cytokine and inflammatory milieu in chronic allograft TA/IF decreases oxygen uptake from hemoglobin as a result of tubular cell injury. Yet, intricate regulatory mechanisms influence the balance between intrarenal oxygenation and OS, and it is therefore likely that these injury pathways participate in a self-perpetuating cycle in chronic allograft TA/IF (3, 12, 30, 30, 68, 75). Overall, there is at least circumstantial evidence pointing toward an active role for OS in tissue hypoxia in chronic allograft TA/IF.

IS THERE A ROLE FOR ANTIOXIDANTS IN THE PREVENTION OF CHRONIC ALLOGRAFT TA/IF?

Despite experimental (32, 52) and epidemiological (93) studies suggesting that antioxidants may prevent cardiovascular disease, the evidence from prospective randomized controlled trials is either inadequate or conflicting (1, 29, 35, 64, 105). However, in the absence of verification that antioxidant therapy successfully reduces OS, these negative results must be interpreted cautiously (90). As mentioned earlier, ROS are involved in the development of both EMT (24, 25, 74, 84) and chronic TA/IF (4, 24–26, 60, 61, 84). Yet, one critical question remains unanswered: to what extent does OS contribute to chronic allograft TA/IF? Only a few studies have looked at the effect of antioxidants on kidney allograft outcomes (5, 34, 53). Vitamin E supplementation alone was not able to prevent allograft injury in an experimental model of chronic allograft nephropathy (34). However, in the same model, L-arginine was able to attenuate proteinuria and glomerulosclerosis (5). A clinical trial using intraoperative intravenous injections of recombinant human SOD demonstrated that the incidence of acute and chronic rejection decreased significantly (53). Vitamin C (alone or in combination with vitamin E and selenium) preserved kidney structure and prevented interstitial fibrosis in experimental models of native kidney disease (17, 58, 108, 109). To the best of my knowledge, however, there is no data examining the effects of ascorbic acid on chronic kidney allograft injury. It seems therefore that the type, dose, and timing of intervention therapies with antioxidants for the prevention of chronic allograft TA/IF need be determined.

CONCLUSIONS

Together, these data demonstrate that OS is increased in graft-infiltrating macrophages, activated myofibroblasts, interstitium, and areas of tubular injury. It is also shown that chronic allograft OS may be induced by inflammation, abnormal tissue oxygenation, immunosuppressant drugs, and comorbid conditions including diabetes, hypertension, proteinuria, anemia, and dyslipidemia. Finally, OS-induced chronic TA/IF may occur through pathways including inflammation, apoptosis, hypoxia, and EMT. Interestingly, most of these injury pathways participate in a self-perpetuating cycle with OS. In conclusion, evidence suggests that OS is a common mechanism of injury in chronic kidney allograft TA/IF. Yet, association does not imply causation, and the extent of OS-mediated allograft injury remains unknown. These questions may be answered by determining the role of antioxidants in the prevention of chronic allograft tubulointerstitial fibrosis.

ACKNOWLEDGMENTS

I am grateful to many colleagues and collaborators, especially Dr. Stuart J. Knechtle for invaluable expertise with the nonhuman primate studies. Most importantly, particular thanks are due to friends and colleagues in my laboratory, especially Shannon Reese, who has made an enormous contribution.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK067981-3.

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