An Antigen-Independent Important Hormone: Intrarenal Angiotensin II (AII) as a Key to Understanding Chronic Allograft Nephropathy

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Graft 2002; 5; 199

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Chronic allograft nephropathy (CAN) is a multifactorial lesion. There has been a gradually increasing recognition of the importance of antigen-independent factors influencing the development and progression of CAN. One important antigen-independent hormone in this setting is angiotensin II (AII), which is associated with progressive kidney disease. Although the pathogenesis of native kidney disease may differ from CAN, the chronic progressive nature of both entities raises the hypothesis that AII also has a role in CAN. However, AII also serves as a theoretical focal point, linking many of the factors involved in CAN. Fortunately, we have therapies, angiotensin converting enzyme inhibitors (ACE-I), and type 1 angiotensin II receptor (AT_1R) antagonists that can alter the effects of AII in grafts. We can treat renal transplant recipients with such therapies in the hopes of preserving graft function. At the same time, given AII's apparent central role in CAN, we can potentially glean novel information as to the pathogenesis and optimal treatment of CAN.

ABBREVIATIONS

CAN	Chronic allograft nephropathy
AII	Angiotensin II
AT1R	Type 1 Angiotensin II receptor
ACE-1	Angiotensin converting enzyme inhibitors
RAS	Renin-angiotensin system
RAAS	Renin-Angiotensin-Aldosterone system
LDL	Low-density lipoprotein
PBL	Peripheral blood lymphocyte

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Why Does It Makes Sense to Consider AII as a Key to Understanding Chronic Allograft Nephropathy?

Chronic allograft nephropathy (CAN) is a multifactorial lesion with varied roles for ischemia-reperfusion injury, acute rejection, chronic inflammation, aberrant matrix deposition, hypertension, hyperlipidemia, and accelerated senescence.[1] Any graft, after overcoming acute insults—for example, ischemia-reperfusion or acute rejection—has to "heal." In that context, any other form of chronic graft injury (Table 1) becomes problematic. Intraglomerular hypertension is one such insult, and as Paul recently noted, it may well contribute to CAN-associated injury.[2] Given the role of intrarenal angiotensin II (AII) in regulating glomerular hemodynamics,[3] it is reasonable to explore its particular impact in CAN. However, CAN is also characterized by tubular epithelial cell pathology. Here again, however, intrarenal AII has an integral role in regulating cellular activity[4] and could lead to many of the changes involved in the epithelial cell transformation occurring in CAN. Thus, AII is potentially an important hormonal, antigen-independent mediator of CAN.

Experimental studies have demonstrated a high level of renin-angiotensin system (RAS) activity in the kidney following transplantation, at a time when the systemic RAS is quiescent.[5] AII could be functioning to maintain fluid and electrolyte homeostasis and regulate blood pressure. Yet, intrarenal AII can assume additional roles, stimulating cytokine production and altering matrix deposition in the allograft, in the setting of any inflammatory stimulus in the allograft. Moreover, allograft-level AII generation can affect not only the organ but also the blood elements circulating through it as AII can stimulate peripheral blood

Table 1 | **Factors Compounding Chronic Graft Injury**

Shear stress
Antibody deposition
Intraglomerular hypertension
Albuminuria

lymphocyte (PBL) cytokine production and PBL activation through type 1 AII receptors (AT_1R)[6,7] via a calcineurin-dependent mechanism.

AII alters the other processes that mediate CAN, aside from hypertension. AII is involved in lipid-mediated injury[8] either through receptor interactions and changes in oxidized low-density lipoprotein (LDL) uptake or by modifying lipids itself and triggering oxidant-mediated damage.[9] Although not yet definitively proven, this represents another avenue of potential AII-mediated effects in an allograft that could lead to the evolution of CAN. Thus, AII is theoretically poised to influence nearly all of the antigen-independent processes that contribute to CAN.

The Renin-Angiotensin-Aldosterone System (RAAS)

The RAAS exerts its renal effects primarily via production of AII, though a growing body of data now suggests a unique role for aldosterone as well.[10] However, for the purposes of this article, we will focus on AII. This peptide acts at its renal receptors to effect changes in renal sodium and water transport, acidification, and vasomotor tone.[11] It also affects cellular hypertrophy, intrarenal scarring, and apoptosis.[12,13] There are 2 well-defined AII receptors, AT_1R and type 2 AII receptors (AT_2R). AT_1R mediate most of the classical actions of the AII at the level of the kidney.[14] AT_2R do have some renal effects, most in opposing the actions of AT_1R as well as mediating anti-proliferative effects in renal tissue.[15,16] Interestingly, AT_2R may also modulate chemokine release in glomeruli.[17] There are a variety of stimuli for intragraft AII in CAN. These range from hemodynamic shear stress to intrarenal antibody deposition. However, AII alone does not lead to CAN. Rather, the downstream effects of AII are the dangerous events in the at-risk-transplanted kidney.

As noted, AII is an important "nontraditional" cytokine as suggested by Nataraj et al.[18] Inhibition of AII leads to a reduction in cytokine expression in CAN tissue including a decrease in the generation of interleukin-1, interleukin-6, TGF-β, TNF-α, and RANTES.[19,20] Each of these obviously can perpetuate inflammatory events and stimulate immune responses in the graft (Fig. 1). However, AII also can stimulate vascular endothelial growth factor,[21] plasminogen activator inhibitor-1,[22] platelet-activating factor,[23] and platelet-derived growth factor[24] in renal cells and renal tissue. Each of these too has been implicated in CAN. AII thus affects many of the factors that represent the molecular underpinnings of injury in CAN (Fig. 1).

AII and TGF-β

Locally generated AII, via AT_1R, stimulates intrarenal TGF-β mRNA and protein expression. The increase in TGF-β expression correlates with renal fibrogenesis through (a) an increase in the synthesis of matrix proteins, (b) a reduction in matrix degradation, and (c) a change in integrin expression.

The changes also take on a different vein in considering the effects of AII and TGF-β on cellular hypertrophy. This discrete cellular change may presage a transition toward fibrosis and potentiate the development of glomerulosclerosis.[25] These events are, by themselves, ones that may arise through a variety of complex stimuli, but it is striking that they do occur as part of the histologic appearance of CAN. Hence, factors that precipitate them may well deserve investigation as they may play a significant role, not only in native kidney disease but also in the evolution of CAN. AII, by itself and in concert with TGF-β, stimulates transcription but not translation of $p27^{Kip1}$.[25] This protein associates with CdK4-cyclin D complexes and inhibits their kinase activity. This leads to a decrease in phosphorylation of the retinoblastoma gene product and retention of E2F with cell cycle arrest in the G_1 phase, hypertrophy, and increased synthesis of extracellular matrix proteins.

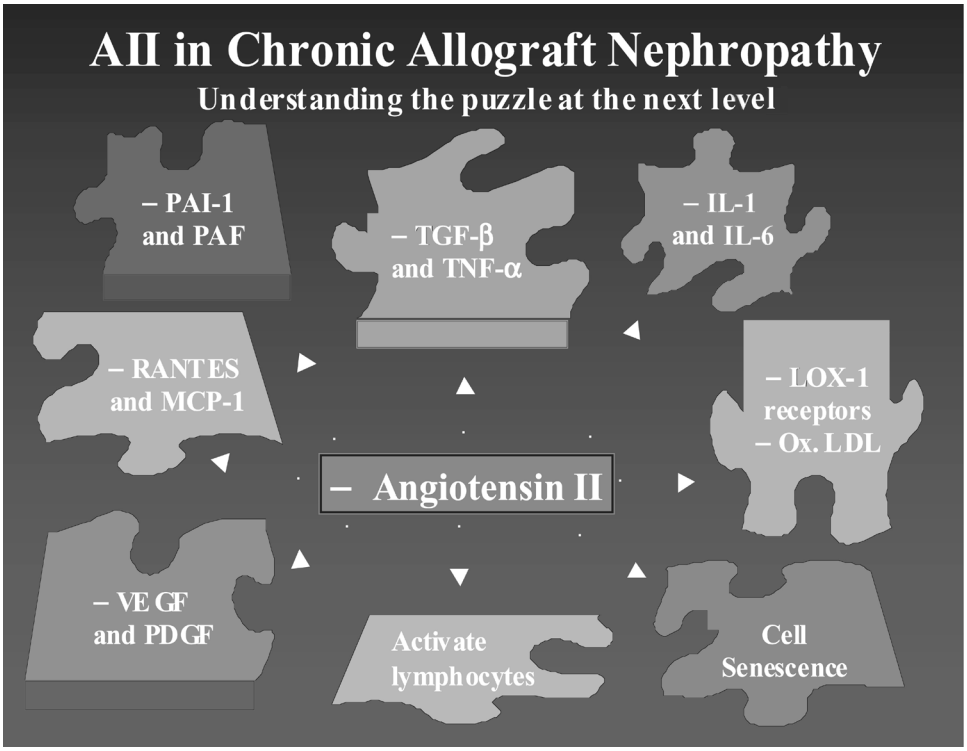

Figure 1. Cytokines, vasoactive substances, and pro-fibrotic molecules implicated in the genesis of chronic allograft nephropathy.

AII, the Chemokine

Indirect evidence also suggests that AII promotes monocyte and macrophage infiltration into renal tissue through AII-stimulated monocyte chemoattractant protein-1 (MCP-1). AII stimulates MCP-1 expression in several renal cell types.[25,26] Interestingly, AII also stimulates monocyte and macrophage infiltration through biochemical effects. Compromised renal tissue increases acid secretion through AII-mediated changes in bicarbonate reabsorption and ammoniagenesis. The AII-induced increase in ammoniagenesis activates complement and also triggers monocyte and macrophage infiltration into renal tissue.[27]

AII and Senescence

Halloran and associates hypothesized that senescence plays an important role in the evolution of CAN.[28] Aging, in general, will induce a number of changes in hormonal responsiveness, including a suppression of systemic RAAS activity. In contrast, however, there is an apparent increase in the renal RAAS, akin to the changes seen in transplantation.[29] A number of different studies in models of aging have indirectly identified the importance of AII as an effector of disease processes. In each, angiotensin converting enzyme inhibitors (ACE-I) reduced proteinuria and slowed progressive kidney disease.[30,31] Anderson has postulated that renal vascular responses are enhanced in the aging kidney as a result of AT_1R up-regulation.[29] Moreover, the tubulointerstitial RAS demonstrates very high levels of AII in the aged state. Thus, AII may play a significant role in the senescent kidney, either native or transplanted.

AII in CAN—Human Studies

Published data in animal models of CAN report that AT_1R blockade protects the transplanted kidney from fibrosis.[19,32] However, whether this also occurs in human renal transplantation is unknown. We recently evaluated 42 renal transplant patients who underwent protocol renal transplant biopsy.[33] No patient was taking an ACE-I or AT_1R antago-

nist at study-entry. ACE and AT_1R gene expression predicted graft outcome in association with changes in inflammatory and cytokine mRNA expression in the biopsy samples.[33]

Recently, we also evaluated the effects of ACE-I and AT_1R antagonists in our chronic renal transplant population. We examined data for 212 transplant recipients who received either an ACE-I or AT_1R antagonist and evaluated renal function in all treated individuals after 6 months of graft function. Change in renal function was assessed with general linear mixed modeling of the change in slope of serum creatinine (Scr). This value, the Δslope Scr, was analyzed prior to ACE inhibitor or AT_1R antagonist therapy then during treatment with the medications. A positive Δslope Scr indicated declining renal function, whereas a negative Δslope Scr indicated improving renal function. ACE-I and AT_1R antagonists were associated with a negative Δslope Scr (Table 2), and improvements in renal function compared to the immediate pretreatment Δslope Scr. More important, long-term ACE-I or AT_1R antagonism stabilized renal function over time, suggesting that they played a role in preventing progressive loss of function.

A subset of these patients treated with an AT_1R antagonist, losartan or valsartan (n = 10) (mean CsA 133 ± 36 ng/dl; mean Scr 1.78 ± 1.08 mg/dl), underwent protocol biopsies. Intragraft TGF-β and TNF-α mRNA expression were compared in biopsy tissue from these patients with biopsy samples obtained from 10 patients with similar CsA (148 ± 31 ng/dl) and Scr (1.9 ± 0.86 mg/dl) who were not receiving treatment with an AT_1R antagonist or ACE-I for their blood pressure. Chronic AT_1R antagonist therapy was associated with a decrease in intragraft TGF-β mRNA (P = 0.02) and TNF-α mRNA (P = 0.05) levels compared to no AT_1R-antagonist therapy (Fig. 2). These data suggest that AT_1R antagonism may have discrete intragraft immunomodulatory effects in addition to their effects on blood pressure and proteinuria.

Safety concerns have been the biggest obstacle toward RAAS antagonism with ACE-I or AT_1R antagonists in the transplant setting. However, Stigant et al. recently noted that such medications could be used with safety in renal transplant recipients[34] with a minimum of side effects. Our analysis mirrored their results with a 4.6% incidence of significant anemia (hematocrit values declining > 5%) and 1.4% of significant hyperkalemia (serum potassium > 6 mEq/L).

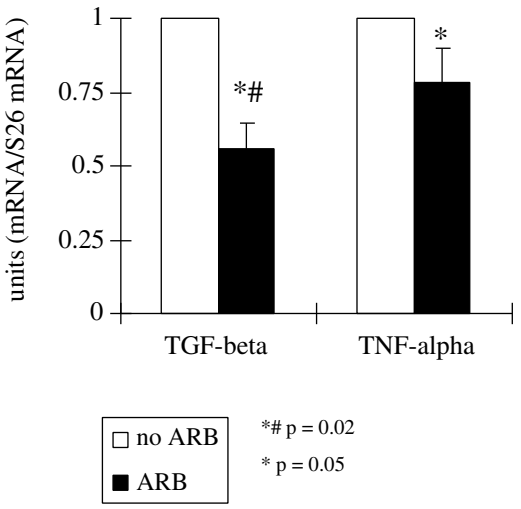

Figure 2. Allograft-level semi-quantitative reverse transcription polymerase chain reaction (RT-PCR). Semi-quantitative RT-PCR was performed as described in reference 33. S26, a ribosomal housekeeping gene, was used as an internal control. α-actin mRNA expression was used as a positive control. TGF-β and TNF-α mRNA levels were normalized to values in untreated biopsy samples. AT_1R antagonism with losartan was associated with a decrease in TGF-β(P = 0.02) and TNF-α (P = 0.05) mRNA levels compared to untreated individuals (n = 10 for each cohort).

AII and CAN—The Future

AII is positioned at the theoretical precipice of CAN. It represents a unique link in coalescing all of the factors involved in this process and, thus, is a key hormone in educating us about the true pathogenesis of CAN. Yet, we are tremendously fortunate. We have available therapies to counter this hormone, apparently so integral to the process of CAN. By using AT_1R antagonists and ACE-I clinically, we can potentially protect allografts from long-term damage. Moreover, in the context of well-constructed studies with AT_1R antagonist and ACE-I therapy, with patients willing to pursue translational investigations, we are likely to uncover novel aspects of CAN.

Table 2 | ΔSLOPE SCR IN RENAL TRANSPLANT RECIPIENTS WHO RECEIVED ACE-I OR AT_1R ANTAGONIST

TREATMENT DAY	DIFFERENCE
(–) 100- (+) 100	–0.001
(–) 100- (+) 200	–0.0006
(–) 100- (+) 300	–0.0002
(–) 100- (+) 400	–0.001
(–) 100- (+) 500	–0.0008

Acknowledgments

This work was supported in part by a Merck Medical School Grant (BNB), a grant from Novartis (BNB), and by NIH grant 1RO1-AI49285-02 (BNB).

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