Mechanisms of tubulointerstitial injury in IgA nephropathy

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Background. IgA nephropathy (IgAN) runs a highly variable clinical course, with frequent involvement of tubulointerstitial damage. A subgroup of IgAN with severe tubulointerstitial damage is often associated with the most rapid progression to end-stage renal failure. In IgAN, mesangial sclerosis and tubulointerstitial damage were found to be correlated with the increase in pore size of the glomerular barrier.

Methods. The direct toxicity of proximal tubular epithelial cells (PTEC) by IgA in IgAN is still unresolved. Activation of PTEC by mediators released from infiltrating cells or resident kidney cells that induce tubular inflammation is the common final pathway in most chronic renal diseases. We hypothesize that mediators released from human mesangial cells (HMC) triggered by IgA deposition may lead to PTEC activation.

Results. We found that IgA binding to PTEC was less than one tenth that of HMC. The binding was nonspecific and exhibited no increased cell proliferation or enhanced synthesis of cytokines or adhesion molecules. However, when PTEC were cultured with IgA-HMC spent medium prepared from IgAN patients, there was enhanced proliferation of PTEC and increased synthesis of cytokines and adhesion molecules.

Conclusion. These findings implicate a glomerulotubular cross-talk with mediators released from the mesangium, contributing to the pathogenesis of tubulointerstitial damage in IgAN. There are preliminary data to suggest that the expression of angiotensin II subtype-1 receptor and angiotensin II subtype-2 receptor in PTEC differs from that of HMC. These novel findings may provide clinicians new therapeutic approaches for selective blockade of the tubulointerstitial injury in IgAN.

IgA nephropathy (IgAN), the most common primary glomerulonephritis worldwide, is associated with a substantial risk of progression to end-stage renal failure (ESRF) [1]. Proteinuria is a well-recognized risk factor for progression in different glomerular diseases. However, the prognostic value of high-grade proteinuria is more complicated in IgAN. First, heavy proteinuria is not common in IgAN [2]; nephrotic syndrome, an unusual presenting symptom, occurs in only 5% of all IgAN [3]. Second, it has been reported that the severity of proteinuria may not always bear any significant correlation with the severity of renal histopathologic changes [4, 5]. One of the possible explanations is the existence of a variant of IgAN associated with a nephrotic syndrome that resembles lipid nephrosis in its responsiveness to steroid [6].

Certain clinical, laboratory, and pathologic parameters have been identified as predictors of poor outcome in IgAN. These include older onset of disease, arterial hypertension, high glomerular histopathologic scores, the extent of global glomerular and interstitial sclerosis, tubulointerstitial fibrosis, persistent microscopic hematuria, nephrotic-range proteinuria, and renal insufficiency at the time of first diagnosis [7, 8]. These factors are concluded from retrospective analysis of large cohorts of patients. Indeed, for most patients with IgAN, these prognostic indicators are weak on an individual basis. Notably, most nephrologists observed renal progression correlates more closely with the severity of tubulointerstitial lesions than with the degree of glomerular lesions [9, 10]. With marked tubular atrophy, the remaining time to ESRF was 3.5 ± 2.7 for those with and 8.2 ± 4.2 years for those without [11]. In addition, infiltration of circulating inflammatory cells, including mononuclear and polymorphonuclear leukocytes, to the renal interstitium is one of the early and prominent histopathologic changes preceding the induction of glomerular or tubulointerstitial injury. Inflammation elicited by these infiltrating cells plays an important role in subsequent development of glomerular and tubulointerstitial damage [12].

WHAT LEADS TO TUBULOINTERSTITIAL CHANGES: PROTEINURIA, MONOCYTIC/MACROPHAGE INFILTRATION, TUBULAR IGA DEPOSITION, OR OTHER MECHANISMS?

The pathogenetic cascade of IgAN can be conveniently divided into 3 phases: (1) synthesis of “pathogenetic IgA;” (2) mesangial IgA deposition and mesangial inflammatory injury; and (3) tubulointerstitial injury. While the pathogenetic significances of abnormal glycosylation of the IgA molecule and the mesangial binding of IgA via known IgA receptors still remain unclear, IgA deposited in the mesangium clearly induces local release
of cytokines, complement, and angiotensin II (Ang II), leading to inflammatory injury [3, 13]. The question that has not been explored is how would mesangial IgA deposition lead to tubulointerstitial injury in IgAN? Four pathogenetic mechanisms of tubulointerstitial injury may operate independently or synergistically, namely: monocytic/macrophage infiltration, proteinuria, direct inflammatory effect of IgA, and a glomerulotubular cross-talk.

The involvement of infiltrating inflammatory cells in the tubulointerstitium is important in mediating tubular injury and renal fibrosis [14]. As a consequence, resident kidney cells can become activated during the inflammatory process. In recent years, much attention has focused on the role of proximal tubular epithelial cells (PTEC) in orchestrating the infiltration of inflammatory cells and the renal fibrosis via production of inflammatory mediators upon activation. Mediators released by infiltrating cells are directly responsible for the activation of PTEC, which, in turn, may amplify the inflammatory cascade by local production of chemotactic mediators that attract even more inflammatory competent cells. The outcome of such a chain reaction is the generation of a positive feedback loop of activation that may lead to the overproduction of extracellular matrix components, resulting in fibrosis and ultimately loss of kidney function. Therefore, a cytokine “cross-talk” network between PTEC and interstitial immunocompetent cells can be envisaged to be the major driving force of tubulointerstitial injury. The key role of tubular epithelial cells in progressive renal diseases have been reviewed, and collective data from the literature have clearly demonstrated the ability of tubular epithelial cells in producing a wide variety of inflammatory mediators [15]. Proteinuria is the major stimulus of PTEC activation and subsequent chemotaxis of infiltrating immunocompetent cells in most glomerular diseases [16]. Our novel finding that “conditioned” supernatant from PTEC stimulates the proliferation of mesangial cells provides experimental evidence to suggest a tubuloglomerular “cross-talk” mechanism, involving different soluble factors, is likely to operate in different glomerular and interstitial nephritis [17]. However, proteinuria may be one of the several contributory factors in tubulointerstitial injury in IgAN because heavy or nephrotic-range proteinuria is uncommon [2, 3].

The other possible contributory factor is the direct toxic effect following tubular binding of IgA. IgAN patients have increased urinary IgA concentration that correlates with serum creatinine concentration, as well as the urinary protein excretion [18]. By immunofluorescence, tubulointerstitial deposits were previously reported in 10% to 35% of proximal tubules, consisting of C3 in all, and IgA in 11% of IgAN patients in one study [19]. However, other investigators noted that IgA deposits are rarely detected in the tubulointerstitium [20, 21]. The scarcity of tubular IgA deposition in IgAN is illustrated in Figure 1.

**DOES URINARY IGA BIND TO TUBULAR EPITHELIAL CELLS TO EXERT INFLAMMATORY INJURY IN IgAN?**

The increase in glomerular barrier pore size that allowed the passage of proteins to the tubular lumen was observed in various glomerular diseases [22]. Normally, the glomerular barrier is impermeable to proteins. However, it is possible that the tubular lumen could be exposed to the high-molecular-weight IgA from patients with IgAN, especially when the glomerular size barrier was impaired in IgAN [23]. Hence, it remains interesting and important to ascertain in IgAN whether IgA is capable of binding to PTEC, and to elicit similar inflammatory responses as those observed in human mesangial cells (HMC).

Our preliminary data demonstrated that there was minimal binding of IgA from IgAN patients to cultured PTEC [24]. The amount of IgA binding to PTEC was less than 10% that of mesangial cells. The representative immunofluorescence staining of IgA binding to HMC or PTEC is shown in Figure 2. As for the expression of documented IgA receptors in PTEC, there was absence of known IgA receptors in cultured PTEC except the transferrin receptor. Competitive binding assay of IgA to PTEC using different ligands (including transferrin) for known IgA receptors further confirms that known IgA receptors were not expressed in PTEC. The lack of cell proliferation and absence of inflammatory mediator production from PTEC stimulated with IgA from IgAN patients suggest that the low level of IgA bound to the PTEC is a nonspecific binding in the cell culture experiment. Our data support the previous observation that IgA deposits are rarely detected in the tubulointerstitium in IgAN [20].

**HYPOTHESIS: A GLOMERULOTUBULAR CROSS-TALK IN IgAN VIA HUMORAL FACTORS**

Failing to demonstrate a specific binding of IgA to PTEC and the infrequent occurrence of high-grade proteinuria in IgAN, we postulate a new mechanism that may be operative upon mesangial IgA deposition that subsequently leads to tubulointerstitial atrophy and fibrosis in IgAN. It has been documented that inflammatory cytokines, including Ang II, are released from mesangial cells following binding to IgA in IgAN. We hypothesize that these mediators alter the glomerular barrier pore size that allows the passage of these inflammatory mediators to the tubular lumen. These mediators then activate the PTEC, which in turn may amplify the inflammatory cascade by local production of chemotactic...
mediators, which attract even more inflammatory competent cells. This glomerulotubular cross-talk will generate a positive feedback loop of activation in the renal tubules that leads to the overproduction of extracellular matrix components, resulting in fibrosis and ultimately loss of kidney function. We tested our hypothesis by conducting an experiment in which PTEC was cultured with spent medium prepared from mesangial cells incubated with IgA from IgAN patients [24]. Contrary to the absent stimulatory effect on PTEC upon direct incubation with IgA, we observed an increased proliferation and enhanced expression of inflammatory mediators (including interleukin-6, tumor necrosis factor-α, soluble intercellular adhesion molecule-1, and Ang II) in PTEC cultured with spent medium prepared from mesangial cells incubated with IgA from IgAN patients [24]. Despite the presence of gene encoding for renin, angiotensinogen, and ACE in renal mesangial cells [28], little is known about the intrarenal RAS in IgAN. Both AT1R and AT2R are expressed in the kidney from healthy subjects and from patients with glomerular disease [29]. The histologic distribution of these receptors

DIFFERENTIAL EXPRESSION OF ANG II SUBTYPE-1 RECEPTOR (AT1R) AND ANG II SUBTYPE-2 RECEPTOR (AT2R) IN MESANGIAL CELLS AND TUBULAR EPITHELIAL CELLS

The renin-angiotensin system (RAS) is a key factor in the progression of chronic renal failure. Ang II plays a pivotal role as a mediator of glomerular hemodynamic adaptation, and also in immunologic injury. We have demonstrated that polymeric IgA (pIgA) from IgAN patients up-regulates the synthesis and signal transduction of transforming growth factor-β in human mesangial cell via the RAS by inducing the synthesis of Ang II [15]. Pharmacologic blockade of this system, either by angiotensin-converting enzyme (ACE) inhibitor or AT1R antagonist, retards the progression of glomerulosclerosis [27]. Despite the presence of gene encoding for renin, angiotensinogen, and ACE in renal mesangial cells [28], little is known about the intrarenal RAS in IgAN. Both AT1R and AT2R are expressed in the kidney from healthy subjects and from patients with glomerular disease [29]. The histologic distribution of these receptors

Fig. 1. Representative immunohistologic staining in kidney biopsy from patients with IgAN. (A) Immunofluorescence study revealing mesangial IgA deposition with IgA cast in tubular lumen (solid arrow) and absence of tubular IgA deposits (open arrow) (magnification ×400). (B) Immunoperoxidase study (visualized by the Dako Envision plus system) revealing mesangial IgA deposition and absence of tubular IgA deposits (magnification ×400). (C) Immunoperoxidase study (visualized by the Dako Envision plus system) revealing mesangial and tubular deposition of TGF-β (magnification ×250). (D) Immunoperoxidase study revealing no IgA deposition in control kidney tissue (magnification ×400).
implicates that both receptor may have a physiologic role in human normal and diseased kidneys.

Our recent studies on the mesangial expression of AT1R and AT2R following the release of Ang II by pIgA from patients with IgAN revealed a reduction of AT1R expression after the acute exposure of the cells to pIgA [30]. The initial down-regulation of AT1R expression mirrors an adaptive response to high intrarenal Ang II level in human glomerulonephritis, including IgAN. An immediate down-regulation of mesangial AT1R expression will ameliorate the proliferative and inflammatory changes. However, we found that such adaptive changes might gradually be lost following prolonged exposure of mesangial cells to pIgA in IgAN. The supernatant concentration of Ang II (10^{-11} to 10^{-10} mol/L) in HMC released by incubating with pIgA was too low to induce apoptosis via activation of the AT2R. In contrast, similar concentration of Ang II was able to stimulate AT1R expression and maintain proliferative activity. Hence, the failure to suppress the AT1R expression continuously in the presence of defective AT2R activation is likely to permit the development of proliferative and inflammatory processes in the glomerular mesangium that may perpetuate a glomerulotubular cross-talk that finally leads to progression of renal deterioration in IgAN. More interestingly, our preliminary data showed that Ang II at a concentration of 10^{-10} mol/L is able to induce apoptosis in PTEC, and this concentration is 10-fold lower than that in a previous study [31]. We also observed an up-regulation of gene expression of both AT1R and AT2R in PTEC cultured with spent medium prepared from HMC incubated with IgA from IgAN patients. These findings suggest a differential expression of AT1R and AT2R in HMC and PTEC in response to raised intrarenal Ang II concentration following exposure to pIgA that may play a significant role in the pathogenesis of IgAN. Interestingly, in renal proximal tubular cells exposed to Ang II, the growth-stimulatory effects through AT1R may be counterbalanced by AT2R-mediated apoptosis and growth inhibition [32, 33].

**PODOCYTES IN IgAN**

Information of podocyte dysfunction in IgAN is extremely scarce. The concomitant observation of podocyte loss and increasing disease severity in IgAN suggest that podocyte loss is involved in causing or contributing to the progressive proteinuria, glomerular sclerosis, and filtration failure [34]. The analysis of podocyte injury by morphometric analysis has provided additional prognostic information in IgAN [35]. Recent data suggest a process in which the elaboration of regulatory factors by podocytes may play a direct role in the activation of mesangial cell proliferation in IgAN [36]. An overexpression of Bcl-2 (death suppressor) protein observed in early stages of IgAN may confer protection against apoptotic injury to glomerular cells by counteracting the opposing activities of Bax (death promotor) protein.
Fig. 3. Schematic model of the variable mechanisms operating between the HMC, podocytes, and PTEC following mesangial IgA deposition in the development of tubulointerstitial injury in progressive IgAN.

Table 1. New therapeutic options in reducing tubulointerstitial injury in IgAN

<table>
<thead>
<tr>
<th>Target cells</th>
<th>Mechanism</th>
<th>Therapeutic options</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Mesangial cell</td>
<td>Reduction of IgA binding due to electrostatic interaction</td>
<td>Polyanion—heparan, poly-L-aspartic acid, poly-L-glutamic acid</td>
<td>[37]</td>
</tr>
<tr>
<td></td>
<td>Decrease of Ang II release by IgA</td>
<td>ACE inhibitor or AT1R antagonist</td>
<td>[13]</td>
</tr>
<tr>
<td>Podocyte</td>
<td>Antagonism to the proliferative effect of PDGF</td>
<td>PDGF antagonist—PDGF-B aptomer</td>
<td>[38]</td>
</tr>
<tr>
<td>Tubular epithelial cell</td>
<td>Protection of apoptosis due to down-regulation of Bcl-2 and up-regulation of Bax</td>
<td>Antiapoptotic action of peroxisome</td>
<td>[39]</td>
</tr>
<tr>
<td></td>
<td>Reduce Ang II-induced renal vascular constriction</td>
<td>Proliferation of cytochrome P450 2C23-mediated epoxygenase activities—peroxisome</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>Induction of apoptosis and G1 phase arrest</td>
<td>Proliferation of receptor alpha agonist—L-805645</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
<td>Antagonizing the proliferative and proinflammatory effect of AT1R expression</td>
<td>ACE inhibitor or AT1R antagonist</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>Enhancing the antiproliferative and apoptotic effect of AT2R expression</td>
<td>AT2R agonist—CGP-42112A, L-162,313</td>
<td>[43, 43]</td>
</tr>
</tbody>
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Down-regulation of Bcl-2, associated with an increased ratio of Bax/Bcl-2 by glomerular epithelial cells, correlates with the severity of glomerulosclerosis. The issue of how IgA affects podocyte function remains unknown and deserves further study, as this will provide insight to other pathogenetic mechanism of IgAN.

FUTURE THERAPEUTIC TARGETS FOR IgAN

Until now, there has been no effective or specific treatment for IgAN. Reducing the rate of disease progression is the main strategy in intervening the progression of this nephropathy. Figure 3 summarizes the disease mechanisms of IgAN revealed by recent studies. Because it remains difficult to target the upstream of the pathogenetic cascade (i.e., synthesis of underglycosylated IgA and blockade of specific IgA receptors), attention is now focused in the downstream, aiming to reduce the tubulointerstitial injury. This can potentially be achieved at different cellular levels—mesangial binding of IgA, podocyte dysfunction leading to proteinuria and glomerulosclerosis, and reduction of inflammatory injury of tuberulointerstitium. The rationale and therapeutic options are outlined in Table 1. Most of these therapeutic options remain experimental or at the laboratory levels, yet these novel directions represent a new paradigm to intervene the progression of renal failure in IgAN by reducing the tubulointerstitial injury.
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REFERENCES


