Glomerular hemodynamic changes associated with arteriolar lesions and tubulointerstitial inflammation

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Glomerular hemodynamic adaptations to loss of renal mass are thought to be the initiating factor of progression to renal failure; however, tubulointerstitial (TI) injury correlates better with progression than with glomerular damage. Thus, it is conceivable that tubulointerstitial alterations participate in the pathophysiology of renal disease progression by modifying the adaptive responses of glomerular hemodynamics.

In experimental models of progressive renal disease, suppressing tubulointerstitial inflammatory cell infiltration with anti-inflammatory drugs reduces renal damage despite persistence of systemic hypertension. In recent studies in rats with subtotal renal ablation, we found that treatment with polysulphate pentosan (PPS) and with mycophenolate mofetil (MMF) prevented proteinuria, glomerular hypertension, and hyperfiltration, despite persisting arterial hypertension due to higher afferent resistance. In addition, arteriolopathy was significantly attenuated by MMF, suggesting preservation of vascular structure and function.

Association of vascular injury of afferent arterioles, glomerular hemodynamic changes, and renal lesions has been described in other conditions such as hyperuricemia, protein overload, fawn-hooded rats, and aging spontaneously hypertensive rats (SHR).

Arteriolopathy results in a maladaptive function that permits the transmission of systemic hypertension to glomerular capillaries. Glomerular hypertension results in mechanical damage to the capillary wall and increased filtration of proteins to tubular lumen. Enhanced tubular reabsorption induces synthesis of proinflammatory and profibrotic factors, resulting in tubulointerstitial inflammation and fibrosis. In conditions in which there is overactivity of the renin-angiotensin system (RAS), such as mild hyperuricemia and protein overload, arteriolopathy is associated with increased glomerular pressure and reduced glomerular plasma flow that results in post-glomerular ischemia and tubulointerstitial injury.

Most chronic nephropathies are characterized by progressive renal failure. The first unifying hypothesis describing the nature of this condition suggested that surviving nephrons activate hemodynamic changes in order to preserve renal function that ultimately causes structural damage in a self-perpetuating circle [1]. More recently, numerous studies established that hypertension and proteinuria play an important role during the progression of renal failure [2–4]. In fact, therapies that lower blood pressure and reduce proteinuria effectively retard progression, especially drugs that interfere with the effects of the renin-angiotensin system (RAS) [5–9].

In experimental animals, a reduction of renal mass results in glomerular hypertension and hyperfiltration of the remnant nephrons in an attempt to preserve whole kidney function, but causes, in the long term, the development of glomerulosclerosis [1]. In addition, the rise in glomerular pressure alters glomerular permeability to proteins, which in turn are filtered in excessive quantities to the tubular lumen [2, 7, 10]. The secondary process of reabsorption of increased amounts of filtered proteins can initiate renal interstitial injury by activating proinflammatory and profibrotic factors that result in infiltration of inflammatory cells [10], tubular ischemia, oxidative stress [11], and epithelial mesenchymal transdifferentiation [12], which eventually lead to tubulointerstitial fibrosis. The inflammatory process contributes to renal injury even when the initiating insult is not mediated by immunologic factors such as the renal lesions associated with overactivity of the RAS [13, 14], subtotal renal ablation [15–17], mild hyperuricemia [18], protein overload [19–24], and other experimental models. It has been shown that glomerular hemodynamic disturbances may lead to glomerulosclerosis; however, the rate of progression of renal failure correlates better with the severity and extension of the tubulointerstitial injury than with glomerular damage [25–27]. One of the mechanisms thought to account for these findings is the development of atubular nephrons [28, 29]. However,
the possibility that tubulointerstitial inflammation participates in the pathophysiology of renal disease progression by modifying the adaptive responses of glomerular hemodynamics has not received attention.

Tubulointerstitial injury and glomerular hemodynamics in subtotal renal ablation

Recent studies in rats with extensive renal ablation evaluated the role of inflammation by suppressing inflammatory cells infiltration with different drugs [15–17, 30–33]. In several studies, administration of mycophenolate mofetil (MMF), an immunosuppressive drug that exerts a selective antiproliferative activity on activated B and T cells and inhibits glycosylation of adhesion molecules [34], prevented lymphocyte and macrophage proliferation and infiltration and reduced progressive renal damage [15–17, 32]. In every study the beneficial effects of MMF treatment occurred despite the persistence of systemic hypertension, and in some studies regardless of proteinuria, suggesting that renal protection was not mediated by amelioration of glomerular hemodynamic disturbances [15, 17, 32]. Fujihara et al [15] measured glomerular capillary pressure (Pgc) and found that the rise in glomerular pressure was not changed after 4 weeks of MMF therapy. However, after 8 weeks, when rats had considerably higher values of glomerular pressure, MMF therapy was associated with partial reduction of Pgc and proteinuria [15]. On the other hand, treatment with pentoxyfiline, an anti-inflammatory drug that blocks nuclear factor kappa B (NF-κB), activation also reduced proteinuria and renal damage independent of changes in blood pressure, suggesting preservation of glomerular function [33].

Thus, although the renal protective effect of interrupting the inflammatory process by different maneuvers is well established, it is still controversial if these maneuvers also prevent the glomerular hemodynamic disturbances. In this regard we recently studied if the prevention of renal injury by suppression of the inflammatory process in subtotal renal ablation rats could be partially due to improvement in glomerular hypertension and hyperfiltration in remnant nephrons [31]. We treated 5/6 nephrectomy (Nx) rats with polysulphate pentosan (PPS), a heparinoid with anti-inflammatory effects. Polysulphate pentosan prevented proteinuria, reduced inflammatory cell infiltration, and significantly attenuated renal injury. Glomerular function in remnant nephrons was characterized by hyperfiltration, increased plasma flow, and glomerular hypertension due to inability of the afferent arteriole to prevent the transmission of systemic hypertension to glomerular tuft. Polysulphate pentosan treatment prevented glomerular hypertension and hyperfiltration despite persisting arterial hypertension, due to a rise in afferent arteriolar resistance [31]. These results suggested that suppression of inflammation reduced vascular injury and improved arteriolar functional capacity.

To further test this hypothesis we designed a study in which we evaluated the effects of suppressing the inflammatory process with MMF on glomerular hemodynamics, arteriolar structural changes, and renal histologic injury in the same model of chronic renal failure [35]. Treatment with MMF suppressed inflammatory cells infiltration without decreasing arterial pressure. Despite persistent arterial hypertension, glomerular pressure was normalized, glomerular hyperfiltration was reduced substantially, and the rise in glomerular plasma flow was significantly attenuated due to a higher afferent resistance. Arteriolar structural changes evaluated by morphometry with image computer analysis in 5/6 Nx rats showed hypertrophy of afferent arteriolar wall evidenced by a significant increase of media/lumen ratio, which was significantly attenuated by MMF treatment. These results suggested that preservation of vascular structure was associated with preserved vascular function as indicated by a higher afferent resistance [35]. Although other authors have proposed that beneficial effects of MMF are independent of glomerular function, they have used different strains of rats [15, 17] and more importantly, lower doses of MMF [15, 17, 32]. Thus, it is conceivable that a lesser immunosuppressive effect was insufficient to prevent arteriolopathy and to normalize glomerular hemodynamics.

Tubulointerstitial injury and glomerular hemodynamics in hyperuricemia

Tubulointerstitial scarring, glomerulosclerosis, and vascular disease are prominent findings in renal injury associated with hyperuricemia and gout [18, 36, 37]. Recently, Mazzali et al [18] reported an experimental model of mild hyperuricemia in the rat. Administration of oxonic acid mixed in a low-salt diet blocked urate oxidase, the enzyme responsible for the degradation of uric acid to allantoin in inferior mammals. This maneuver produced a slight increment of serum uric acid levels that were associated with hypertension, and renal damage characterized by tubulointerstitial inflammatory infiltration without urate crystal deposition; in addition, there was overexpression of osteopontin, collagen III, and renin overexpression in juxtaglomerular cells [18]. Hyperuricemia also induced medial vascular thickening of the preglomerular vessels that occurred independent of blood pressure but was partially dependent of RAS [37]. In cultured vascular smooth muscle cells (VSMCs) uric acid induced the expression of chemotactic cytokines [monocyte chemoattractant protein-1 (MCP-1)], oxidative stress, and stimulated proliferation mediated by platelet-derived growth factor (PDGF) A [38] [abstract; Kanellis J, et al: J Am Soc Nephrol 13:60A, 2002; Susumu W, et al: J Am Soc Nephrol 13:514A, 2002].

To further evaluate the impact of tubulointerstitial injury and arteriolopathy of afferent arterioles on glo-
merular hemodynamics we studied rats with oxonic acid-induced mild hyperuricemia [39]. We found that rats treated with oxonic acid plus low-salt diet developed hyperuricemia, arterial hypertension, and significant elevation of intraglomerular pressure. Morphometric analysis revealed arteriopathy of preglomerular vessels indicated by increased arteriolar area and media/lumen ratio. Simultaneous treatment with allopurinol prevented the rise in serum uric acid, arterial and glomerular hypertension, as well as arteriopathy of afferent arterioles. Within individual rats there was a striking correlation between serum uric acid, arteriolar thickening, and systemic blood pressure with glomerular hydrostatic pressure [39]. Moreover, in 5/6 Nx rats, hyperuricemia worsens the course of renal damage progression mediated by increasing the rise of blood pressure, proteinuria, renal hypertrophy, preglomerular arteriopathy, fibrosis, and glomerulosclerosis associated with a greater expression of renin and cyclooxygenase (COX)-2 [40]. In this regard, we have found in rats with subtotal renal ablation that hyperuricemia is related with severe renal vasoconstriction characterized by decreased glomerular plasma flow, single nephron glomerular filtration rate (GFR), and elevations in pre- and post-glomerular resistances associated with increased arteriolar media/lumen ratios and more tubulointerstitial fibrosis (unpublished observations). These findings suggest that arteriolar disease may have contributed to the transmission of the systolic blood pressure in glomerular capillaries. Proliferation of VSMCs and increased collagen deposition might be expected to increase rigidity of the vascular wall and thus limit its capacity to contract in response to higher perfusion pressure.

**Tubulointerstitial injury and glomerular hemodynamics in albumin overload**

Systemic protein overload (AO) in rats is characterized by marked proteinuria and tubulointerstitial (TI) infiltration of macrophages and T lymphocytes [19–24]. There is increased expression of MCP-1 and transforming growth factor-β (TGF-β) [19], production of regulated upon activation, normal T cell expressed and secreted (RANTES) [22], interleukin (IL)-8 [41], and tubular activation of NF-κB [21–23]. In addition, cells producing Ang II and reactive oxygen species (ROS) are present [24] and up-regulation of angiotensinogen and angiotensin-converting enzyme has been demonstrated, suggesting an increase in intrarenal generation of Ang II [20, 21]. Contrasting with the tubulointerstitial lesions, protein overload is associated with relatively minor glomerular changes [24]. Thus, this experimental model seems appropriate to evaluate the alterations of glomerular hemodynamics associated with tubulointerstitial injury. Increased production of Ang II [20, 21], ROS [24], and other vasoactive molecules [21] can induce changes in glomerular hemodynamics and impair permselectivity of the capillary wall, thereby contributing to the enhancement of proteinuria and renal damage.

We recently performed micropuncture (MP) studies in rats with mild albumin overload. Male Sprague-Dawley rats received 1 g/day of bovine serum albumin intraperitoneally for two weeks. Systolic blood pressure increased slightly and urine protein excretion rose by almost 7-fold ($P < 0.05$); this was associated with renal cortical vasoconstriction indicated by a decrease in GFR ($-27\%$, $P = 0.01$), single nephron GFR ($-34\%$, $P < 0.001$), and glomerular plasma flow ($-30\%$, $P < 0.01$). Despite this vasoconstrictive response, there was a significant rise of 5 mm Hg in glomerular pressure ($P < 0.05$) that positively correlated with efferent resistance ($r = 0.58, P < 0.02$); afferent resistance increased by 65% ($P < 0.01$). Histologic examination showed diffuse, mild tubulointerstitial inflammatory infiltration, and mesangial expansion in 24% of glomeruli, as well as mild arteriopathy (Fig. 1). Morphometric analysis of preglomerular vessels stained with alfa actin–specific antibody showed a significant increase in media/lumen ratio. Cortical vasoconstriction and glomerular hypertension were probably mediated by local release of vasoactive factors by infiltrating inflammatory cells. Our results suggest that glomerular hypertension develops when histologic changes are still incipient and contribute to perpetuate renal injury associated with proteinuria.

The studies previously described suggest a close relationship between the inflammatory process, arteriopathy, and glomerular hemodynamics (Table 1). Local release of cytokines, growth factors, vasoactive molecules, and free oxygen radicals, can contribute to development of arteriolar hypertrophy and increased transmission of systemic pressure to glomerular capillaries. Prevention of inflammatory cells infiltration with PPS [31] and with MMF [35] restores arteriolar structure and function, normalizing glomerular pressure despite persisting systemic hypertension. Moreover, in experimental CsA nephrotoxicity, Yang et al [42] recently showed that amelioration of tubulointerstitial inflammation and fibrosis by MMF treatment decreased arteriopathy of afferent arterioles and improved renal function [42]. In oxonic acid–induced hyperuricemia, mild tubulointerstitial lesions are associated with arteriopathy and glomerular hypertension [39]. In albumin overload, proteinuria is associated with over-expression of chemotactic factors [19, 22, 41], influx of inflammatory cells, and activation of intrarenal RAS [19–24]. Despite relatively minor glomerular alterations [24], there is mild arteriopathy and glomerular hypertension.

The association of afferent arteriopathy and glomerular hypertension has been demonstrated in the fawn-hooded rats. This strain constitutes a spontaneous model for chronic renal failure with early systemic and glomeru-
Fig. 1. (A) Tubulointerstitial inflammatory cells infiltrate and (B) arteriolopathy of afferent arteriole in a rat with albumin overload (1 g/day) during two weeks.

### Table 1. Glomerular hemodynamics, arteriolopathy, and tubulointerstitial inflammation

<table>
<thead>
<tr>
<th>Model</th>
<th>MAP</th>
<th>Pgc</th>
<th>SNGFR</th>
<th>M/L</th>
<th>TI MØs</th>
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<tr>
<td>5/6 Nx</td>
<td>↑↑↑</td>
<td>↑↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
<td>KI 63:994, 2003</td>
</tr>
<tr>
<td>5/6 Nx + MMF</td>
<td>↑↑</td>
<td>N</td>
<td>↑</td>
<td>N</td>
<td>↑</td>
<td>KI 63:994, 2003</td>
</tr>
<tr>
<td>Low-salt diet</td>
<td>↑</td>
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<td>↓</td>
<td>N</td>
<td>↑</td>
<td>AJP 283:F1105, 2002</td>
</tr>
<tr>
<td>Low-salt diet + OA</td>
<td>↑↑</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>↑↑</td>
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</tr>
<tr>
<td>Normal condition</td>
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<td>AJP 283:F1132, 2002</td>
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Abbreviations are: OA, oxonic acid; Alb, albumin; MMF, mycophenolate mofetil; Nx, nephrectomy; N, normal; MAP, mean arterial pressure; Pgc, glomerular capillary pressure; TI, tubulointerstitial; MØs, macrophages; SNGFR, single nephron glomerular filtration rate; M/L, media to lumen ratio.

lar hypertension [43]. Elevations in renal blood flow, GFR, and glomerular capillary pressure precede the development of renal damage [44]. Arteriolopathy of the preglomerular vessels is characterized by media hypertrophy, coincidental with myocyte degeneration of the innermost media layers [45]. Glomerular pressure is related to arterial pressure, indicating that in these rats the mechanism to maintain constancy of Pgc is less effective, exposing glomerular capillaries directly to variations in systemic blood pressure [46]. Although tubulointerstitial injury has not been studied in this strain, significant lesions can be anticipated because of the high levels of proteinuria exhibited by these animals.

Spontaneous hypertensive rats are relatively resistant to develop renal lesions; however, one-year-old animals have proteinuria, glomerular injury, and impaired renal function [47]. Glomerular hypertension is associated with reduced glomerular plasma flow and increased afferent and efferent resistances. Histologic examination revealed glomerular sclerosis, interstitial inflammatory cells infiltration, and thickening of the afferent arteriolar wall with proliferation of VSMCs [48]. Thus, although afferent resistance increased it was insufficient to prevent the transmission of systemic hypertension to glomerular capillaries.

The mechanism by which vascular hypertrophy impairs the ability of preglomerular vessels to limit the transmission of systemic pressure to glomerular capillaries may be associated with inflammation of the vascular wall. Activation of transcription factors, NF-κB, and activator protein-1 (AP-1) in VSMCs induces a switch of VSMCs to a proliferative phenotype [48]. This change promotes growth, and increased synthesis and rearrangement of extracellular matrix proteins, and decreases the synthesis of contractile proteins, which in turn impair decrease the response of VSMCs to contractile stimulus [49].

However, in the long term, further hypertrophy of VSMCs and expanded extracellular matrix (ECM) on the vascular wall may critically reduce the lumen of preglomerular vessels, inducing the decline of blood flow to glomeruli and post-glomerular ischemia. Renal hypoperfusion stimulates renin secretion and angiotensin II production, eliciting vasoconstriction of efferent arterioles; this tends to preserve the GFR by further increasing
glomerular hyperfiltration. Post-glomerular ischemia is a well-known stimulus to produce tubulointerstitial fibrosis [50]. The process is self-perpetuating, as ischemia promotes inflammation and fibrosis, and the loss of glomerular and peritubular capillaries induced by fibrosis aggravates ischemia [51]. Pre-glomerular arteriolopathy coexisting with loss of glomerular and peritubular capillaries was documented in remnant kidney model [52], nitric oxide inhibition [52], and concomitant hyperuricemia exacerbated these vascular changes [40].

CONCLUSION

Figure 2 illustrates our proposal for the role of arteriolopathy in the progression of renal injury as follows: initial insult to the kidney results in decreased number of nephrons that compensate renal function loss by increasing filtration and glomerular capillary pressure. On one hand, glomerular hypertension induces a mechanical damage to the capillary wall, and on the other hand, it increases filtration of proteins to tubular lumen, leading to increased tubular reabsorption that result in the synthesis of proinflammatory and profibrotic factors. Recruitment of immune cells amplifies the inflammatory process, inducing hypertrophy and proliferation of pre-glomerular VSMCs. Arteriolopathy results in a maladaptive function that permits the transmission of systemic hypertension to glomerular capillaries. In addition, inflammation contributes to glomerulosclerosis and tubulointerstitial fibrosis, further reducing functional nephrons and thereby perpetuating progression.

In conditions in which there is overactivity of the RAS, such as hypertension, hyperuricemia, and albumin overload, arteriolopathy is associated with increased glomerular pressure and reduced glomerular plasma flow that results in post-glomerular ischemia and tubulointerstitial fibrosis, which further contributes to loss of functioning nephrons.

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