Experimental Insights Into the Tubulointerstitial Disease Accompanying Primary Glomerular Lesions

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ABSTRACT
Although chronic progressive tubulointerstitial (TI) disease plays a critical role in the outcome of patients with primary glomerular lesions, the basic mechanisms that generate the TI damage remain unclear. This review focuses on recent insights into this process that originate primarily from studies of animal models of glomerular injury. The acute phase, which is often clinically silent, is characterized by tubular epithelial cell injury and interstitial inflammation. Proposed mediators of tubular injury include antibodies, lysosomal enzymes, obstruction, reactive oxygen metabolites, and complement. Damaged tubules may regenerate or undergo necrosis or apoptosis. The identification of the molecular mediators of mononuclear cell recruitment to the interstitium is of current interest because of evidence that monocytes/macrophages play a key role in progressive interstitial scarring through the release of fibrosis-promoting cytokines, particularly transforming growth factor-β1 (TGF-β1). Events linked to the initiation of interstitial inflammation include the deposition of antibodies or immune complexes along the tubular basement membranes, T cell-dependent mechanisms, glomerular factors, and factors linked to proteinuria. Several molecules likely regulate the interstitial migration of circulating monocytes, although the critical mediators are presently unknown. Candidates include chemotactic factors such as intercrines, growth factors, complement, lipid factors, osteopontin, and monocyte adhesion molecules (β1 integrins, β2 integrins, and L-selectins). The hallmark of the chronic phase of TI damage is interstitial fibrosis. Of the several candidate fibrogenic cytokines, to date, only TGF-β1 has been studied in any detail. TGF-β1 is produced by interstitial inflamma-

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In 1970, Schainuck and colleagues (1) emphasized the importance of chronic tubulointerstitial (TI) disease when they reported a significant correlation between histologic variables of interstitial disease and decline in renal function. This observation was initially made 2 yr earlier by Risdon et al. (2) in their study of patients with chronic glomerular disease. This relationship has been reconfirmed by several studies, in particular, in those performed by Bohle and colleagues (3-5). Although many hypotheses have been proposed to explain the basis of this relationship, it is still not fully understood. Marcusen (6), by performing serial sections through entire glomeruli in chronically damaged kidneys, observed that chronic TI damage ultimately leads to tubular atrophy and the generation of atubular glomeruli. Although at first glance, such glomeruli may appear histologically intact, the absence of tubular elements renders such glomeruli functionless. Because approximately 80% of renal volume is occupied by tubules, it should not be surprising that chronic tubular damage associated with interstitial fibrosis is so tightly linked to overall renal function.

Just how the cascade of progressive tubulointerstitial injury evolves when the initial renal injury is targeted to glomeruli is still unknown, although studies over the past 5 yr have begun to provide some insights. There is increasing evidence that the acute phase of injury, characterized by damaged tubules and interstitial inflammation, represents the first...
phase in a sequence of events that terminates with tubular atrophy and interstitial fibrosis. Unfortunately, the acute phase is often clinically silent, yet it may play a vital role in the fibrogenic cascade. In this review, I will focus discussion on three aspects of this process: (1) tubular injury, (2) recruitment of mononuclear cells to the interstitium, and (3) the role of tubular cells and interstitial mononuclear cells in interstitial fibrogenesis. A schematic overview is presented in Figure 1.

TUBULAR INJURY ASSOCIATED WITH PRIMARY GLOMERULAR DISEASE

On careful histologic evaluation, tubular injury frequently coexists with early glomerular disease. The presence of tubular damage can be inferred by the increased mitotic activity of tubular epithelial cells. Szabolcs et al. (7) have observed a two- to fivefold increase in the number of proliferating tubular cells in various human glomerular diseases. Increased numbers of proliferating cells have been documented by autoradiography in rats with puromycin aminonucleoside (PAN) nephrosis (Figure 2A). Tubular cell regeneration can also be recognized by the transient expression of vimentin intermediate filaments (Figure 2B) that are normally present in developing but not mature renal tubules (8). Several mediators of this tubular injury have been proposed, including antibodies, lysosomal enzymes, reactive oxygen metabolites, and complement. Tubular obstruction by cellular debris or proteinaceous casts (Figure 2C) may also cause tubular damage.

Immunologic Mechanisms of Tubular Injury

Antibody-mediated tubular injury is rare but may occur. For example, in passive Heymann nephritis, a rat model of membranous nephropathy, membrane antigens present on the surface of rat proximal tubular cells interact with Heymann antibodies to form immune aggregates along the subepithelial aspect of the tubular basement membrane (TBM). This is similar to events that occur in the glomerulus of rats with Heymann nephritis (Figure 3) (9). In the presence of complement, this interaction causes tubular cell injury, at least in vitro. During states of glomerular proteinuria, potentially damaging antibodies may also be filtered into the urinary space, where they may interact with tubular antigens present on the apical surface. For example, rats immunized with Heymann antigens develop active Heymann nephritis that is characterized by antibody binding to brush-border antigens and a 14-fold increase in the mitotic activity of proximal tubular epithelial cells (10-12). These events are limited to the phase of heavy proteinuria and are likely triggered by the passage of Heymann antibodies into the glomerular ultrafiltrate, thereby exposing them to brush-border antigens.

Proteinuria

An interesting hypothesis is that severe proteinuria itself may cause tubular injury. Several mechanisms
Figure 3. Interstitial disease in rats with passive Heymann nephritis. Focal infiltrates of mononuclear cells are usually found adjacent to immune deposits along the subepithelial aspect of TBM. The arrows identify the same tubules in serial histologic sections. Cell surface markers identified most of the infiltrating cells as macrophages with a small infiltrate of CD8+ T cells. The summary graph indicates the average increase in the number of T1 cells compared with that of normal animals. Part of this figure was reproduced from Eddy et al. (9), with copyright permission from Academic Press Inc., Orlando, FL.

Figure 2. Histologic evidence of tubular damage during primary glomerular disease. (A) Autoradiograph of a kidney from a rat with PAN nephrosis, illustrating an increase in the number of mitotically active tubular epithelial cells labeled with tritiated thymidine (black nuclei). (B) Abnormal tubular expression of vimentin intermediate filaments in the kidney of a rat with protein-overload proteinuria is evidence of tubular regeneration after recent injury: normal mature tubular cells do not express vimentin. Vimentin-positive mononuclear cells are also present in the abnormally expanded interstitial space. (C) Proteinaceous casts may obstruct tubules, leading to tubular dilation, as illustrated in this biopsy of an infant with nephrotic syndrome whose initial biopsy at the time of presentation did not show these obstructive changes. Magnifications: A, x260; B, x240; C, x75. Panel C was provided by Dr. R. Baumal, Department of Pathology, The Hospital for Sick Children, University of Toronto.

Reactive Oxygen Metabolites

A part of the tubular-injury story of primary glomerular disease that is likely to be important but has not been examined in great detail is the role of ischemia and tubular injury mediated by the generation of reactive oxygen metabolites. Alfrey and coworkers (16) have suggested a relationship between proteinuria changes.
and hydroxyl-radical formation, noting that one of the urinary proteins in rats with nephrotic serum nephritis is transferrin, the protein carrier for iron. Iron catalyzes the Haber-Weiss reaction that leads to the generation of hydroxyl radicals. They were able to show that iron-deficient animals had significantly less chronic renal interstitial injury because of nephrotic serum nephritis than did iron-replete animals. A relationship between tubular iron accumulation and the generation of reactive oxygen species and tubular damage has also been reported in the rat remnant kidney model (17). Studies by Zager et al. (18) suggest a degree of synergism between ischemia and protein-mediated tubular toxicity. They showed that tubules exposed to 10 min of ischemia before the infusion of the low-molecular-weight protein ribonuclease had much worse damage than did the nonischemic tubules. The recent recognition that macrophages (19,20) and tubular cells (21,22) synthesize endothelin and nitric oxide makes it likely that regional alterations in TI perfusion occur during the early phase of injury.

Complement Cascade

Tubular damage might be mediated by complement through the formation of the membrane-attack complex. Complement proteins may be lost into the urine during glomerular injury. C3 deposits are frequently observed along the apical surface of renal tubules in nephrotic rats (8,23). In humans, a correlation between the degree of proteinuria and the amount of soluble C5b-9 in the urine has been reported (24). The C5b-9 complex is probably formed by in situ complement activation, possibly at the tubular level, because it is too large to be filtered by the glomerulus (25). A new dimension to the complement story that may prove to be important is the observation that tubular cells can be stimulated to synthesize C3. The in situ hybridization studies of Welch et al. (26) identified C3 mRNA in the tubules of diseased human kidneys, usually restricted to areas of interstitial inflammation. In vitro studies by Camussi et al. (27) have shown that tubular epithelial cells can activate the alternative complement cascade. C3 may also be activated by amidation after exposure to ammonia in the peritubular fluid (28).

Tubular Response to Injury

Very little is currently known about the tubular response to the injury that accompanies primary glomerular disease. However, there is reason to believe that it will involve events that promote interstitial fibrosis (29). Also unclear is the reason that the tubules ultimately become atrophic. Potential mechanisms include ischemia necrosis, programmed cell death or apoptosis, or failure of the remodeling process. Tubular regeneration likely involves complex interactions between cell-cell and cell-matrix adhesion molecules (e.g., integrin family) and other adhesion-promoting molecules such as clusterin, which is frequently up-regulated in areas of tubular injury.

Integrins are heterodimeric cell-membrane components that mediate adhesion to other cells and to extracellular matrix molecules. Currently, proximal tubular epithelial cells are believed to express several integrins, including a3b1 (binding to fibrinectin, laminin, and collagen), a6b1 (laminin receptor), and a5b3 (binding to vitronectin, fibrinogen, osteopontin, thrombospondin, and von Willebrand factor), whereas distal tubules express abundant a2b1 (receptor for collagen and laminin) in addition to a3b1 and a6b1 (30–32). The potential role of these integrin receptors in acute tubular necrosis has recently been investigated (32,33). It has been suggested that integrin redistribution (especially a3) from a normal basalateral to an abnormal apical position results in tubular desquamation from the basement membrane. This is followed by tubular obstruction as the shed cells attach to downstream tubular epithelium, aided by the displaced integrin receptors. The recent recognition of a b1 integrin on rat glomerular epithelial cells as a ligand for nephrotic serum antiserum (34) and antifX1A antibody (35) and the recognition that Heymann antigen GP330 mediates arginine-glycine-aspartate-independent binding of rat proximal tubular cells to fibronectin, laminin, and collagen (36) suggest that matrix-binding tubular receptors may also be targets of antibody-mediated renal injury.

Clusterin is another molecule that appears to be involved in the tubular injury associated with progressive renal disease, although its exact role is not yet clear (37). Clusterin is a glycoprotein that has been isolated from several tissues and given a variety of names (SP-40,40 is probably best known to nephrologists; it was first identified as a protein present in the glomeruli of patients with immune-complex glomerulonephritis) (38). In addition to its association with the soluble complement complex SC5b-9, clusterin may also be synthesized by damaged tubules in the absence of complement proteins. It was initially thought that tubular clusterin expression might be linked with apoptosis, especially when it was associated with ureteric obstruction (39) or tubular ischemia (37). However, the tubular expression of clusterin has been dissociated from apoptosis in other situations, for example, in kidneys with focal segmental glomerulosclerosis (Figure 4), in renal cystic disease (37) and tubular ischemia (40). This observation raises the possibility that clusterin plays a beneficial role in tubular regeneration, perhaps functioning to promote cell aggregation, its first recognized function (41,42).

INTERSTITIAL INFLAMMATION ASSOCIATED WITH PRIMARY GLOMERULAR DISEASE

With the exception of minimal-lesion nephrotic syndrome, increased numbers of interstitial mononuclear cells are present in virtually all human glomerular diseases (Table 1) (43,44). Currently, the relationship
of interstitial inflammation to tubular injury and vice versa is not clear, although the two events often occur together. My colleagues and I have been particularly interested in the mechanisms of interstitial monocyte recruitment. Although the potential importance of lymphocytes in this process should not be underestimated, in the interest of space, this review will focus on cells of the monocyte/macrophage lineage.

A population of macrophages normally resides in the renal interstitium. Theoretically, mitogenic stimuli may lead to interstitial hypercellularity because of the proliferation of resident macrophages. However, in the model of PAN nephrosis, we have shown that the interstitial nephritis likely represents a cellular infiltrate from extrarenal sources. High-dose irradiation delivered locally to one kidney to prevent the proliferation of intrinsic interstitial cells had no effect on the severity of the interstitial inflammation when the irradiated kidney was compared with the contralateral nonirradiated kidney (45).

**Immunologic Mechanisms of Monocyte Recruitment**

Several events may trigger the recruitment of monocytes to the renal interstitium, including the deposition of anti-TBM antibodies or TBM-immune complexes, T cell–dependent mechanisms, putative glomerular factors, and factors linked to proteinuria. In very rare cases, tubular immune deposits may play a role. For example, in our studies of rats with antiglomerular basement membrane (GBM) nephritis (46), foci of interstitial cells appeared in areas adjacent to linear TBM deposits of heterologous anti-GBM antibody (Figure 5). In the model of massive Heymann nephritis, we found focal deposits of immune complexes along the subepithelial aspect of some TBM, in addition to the widespread presence of immune complexes along the subepithelial aspect of the GBM (9). Interstitial inflammation was often found in foci adjacent to these TBM deposits (Figure 3). In both anti-GBM nephritis and passive Heymann nephritis, the interstitial infiltrate is patchy, is usually adjacent to TBM-immune deposits, and is unaltered by complement depletion. It is possible that interactions between monocyte Fc receptors and the antibody itself play a role in interstitial monocyte recruitment, a mechanism that participates in the intraglomerular influx of monocytes in rats during the autologous (planted-antigen) phase of anti-GBM nephritis (47).

An exciting and potentially important pathway, the full scope of which I cannot adequately review here, is

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**TABLE 1. Interstitial inflammation in various human glomerular diseases compared with normal kidneys and with kidneys with primary interstitial nephritis**

<table>
<thead>
<tr>
<th>Renal Disease</th>
<th>Total Interstitial Leukocytes (Relative Increase)</th>
<th>Interstitial Macrophages (Relative Increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Interstitial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allograft rejection</td>
<td>ND to 18-fold</td>
<td>ND to 18-fold</td>
</tr>
<tr>
<td>Interstitial nephritis</td>
<td>14- to 17-fold</td>
<td>11- to 20-fold</td>
</tr>
<tr>
<td>Primary Glomerular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>6 to 13-fold</td>
<td>4- to 15-fold</td>
</tr>
<tr>
<td>Membranous</td>
<td>2- to 9-fold</td>
<td>1.4- to 11-fold</td>
</tr>
<tr>
<td>SLE</td>
<td>4-fold to ND</td>
<td>4-fold to ND</td>
</tr>
<tr>
<td>FSGS</td>
<td>3- to 8-fold</td>
<td>2- to 9-fold</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>ND to 17-fold</td>
<td>ND to 16-fold</td>
</tr>
</tbody>
</table>

* Results are expressed as a ratio: the number of cells in diseased kidneys to the number of cells in normal kidneys with the first number based on data in a table from Hooke et al. (43) and the second number adapted from data in a figure by Cameron (44). Copyright permission was obtained from Springer-Verlag. ND, not done; SLE, systemic lupus erythematosus; FSGS, focal segmental glomerulosclerosis; Ig, Immunoglobulin.
the possibility that interstitial monocyte recruitment occurs via a T cell-dependent pathway analogous to a delayed hypersensitivity reaction. This possibility gained popularity when it was learned that proximal tubular cells express major histocompatibility complex class II antigens up-regulated by γ-interferon and that these cells could function as antigen-presenting cells (48-51). There are several possible scenarios in which antigens delivered in the glomerular ultrafiltrate, via the peritubular circulation or expressed by tubules themselves in response to injury, might trigger this cascade. Heat shock proteins (HSP) are an example of candidate antigens. HSP73 accumulates in the tubular cytoplasm of rats treated with PAN (52) or toxic doses of gentamicin (53). HSP70-reactive T cells isolated from the kidneys of mice with cadmium-induced nephrotoxicity are cytotoxic to stressed renal tubular cells (54). Molecular mimicry has also been proposed as a mechanism of interstitial nephritis whereby a primary immune response to an extrarenal antigen causes renal disease because of the expression of cross-reactive antigens in the tubulointerstitium. Although these hypotheses are appealing, they are still speculative rather than established mechanistic pathways. In two nonimmune models of glomerular injury that we have studied, PAN nephrosis (45) and protein-overload proteinuria (8), the complete elimination of CD5 lymphocytes with monoclonal antibody therapy failed to alter the intensity of the monocytic infiltration.

In some glomerular diseases, the interstitial inflammatory response is primarily periglomerular. In a model of accelerated nephrotoxic serum nephritis in rabbits, Eldredge and colleagues (55) were able to reproduce this phenomenon. The early periglomerular infiltration, which ultimately becomes more diffuse, occurred in the absence of extraglomerular immune deposits, suggesting that chemotactic factors of glomerular origin initiated this cascade of events. In a similar model in rats, Lan et al. (56) identified the perivascular sheath of hilar arterioles as the initial site of periglomerular inflammation. In another study (57), those investigators reported the reversal of interstitial macrophage accumulation by the administration of an interleukin-1 (IL-1) receptor antagonist.

**Monocyte Recruitment Linked to Proteinuria**

In T1 disease, a parallel has been made between the degree of proteinuria and the intensity of the monocytic cell infiltrate, which my colleagues and I first observed in rats with PAN-induced nephrosis (45). A similar correlation was found in rats with protein-overload proteinuria induced by daily injections of BSA (8) and in studies of adriamycin glomerulopathy performed by Bertani et al. (58,59). Homologous protein-overload interstitial disease has also been observed in rats given daily injections of rat albumin (8) and in rats with a pituitary tumor that increased endogenous albumin synthesis (60). The reduction of proteinuria in nephrotic rats by dietary protein restriction from 27 to 8% attenuates interstitial inflammation (45). In that study, there was a strong positive correlation between the number of interstitial macrophages and the degree of proteinuria. In virtually all human glomerular diseases, persistent high-grade proteinuria is a bad prognostic feature, although few human studies have been done to compare interstitial disease with proteinuria severity and duration. Yoshioka et al. (61) have recently reported a significant positive correlation between the number of IL-6-positive interstitial cells and the degree of proteinuria in patients with immunoglobulin A nephropathy.

When proteinuria exists, urinary albumin itself may not be the culprit in the genesis of T1 disease. This could account for the unique absence of interstitial disease in children with minimal-lesion nephrotic syndrome in whom albumin accounts for most of the excreted protein. The vindication of albumin is supported by observations in rats with a genetic absence of albumin (62). When these Nagase analbuminemic rats are treated with adriamycin, they develop significantly less proteinuria than do Sprague Dawley rats because of the absence of albumin. However, the degree of proteinuria in the Nagase rats is still elevated compared with that of control rats. Despite this difference in proteinuria, the severity of interstitial disease, assessed as the percentage of microscopic fields, with tubular casts or interstitial fibrosis was similar.

**Monocyte Chemoattraction**

In this exciting era of discovery of new chemoattractant and adhesion molecules that may participate in monocyte infiltration, identifying the mediators of monocyte recruitment in vivo has proved difficult. It may be that there is so much redundancy in the various systems that the blockade of a single pathway will have no effect. Candidate chemoattractant families include intercrines (monocyte chemoattractant protein-1 [MCP-1] and regulated upon activation normal T cell expressed and secreted [RANTES]); growth factors (transforming growth factor-β1 [TGF-β1], and platelet-derived growth factor [PDGF]); complement (C5a); lipid factors (oxidized low-density lipoproteins, leukotrienes, and essential fatty acid-derived factors); and osteopontin. The C-C intercrines, which include MCP-1 and RANTES, are a family of small structurally related chemokines (63). Both of these chemokines are monocyte chemoattractants that are produced by proximal tubular and mesangial cells (63-67). However, despite increased renal MCP-1 mRNA levels and the de novo appearance of the MCP-1 protein in some of the tubules of rats with PAN nephrosis, the administration of an MCP-1-neutralizing antiserum failed to attenuate the interstitial influx of monocytes (Eddy et al., unpublished observation). The role of RANTES in interstitial monocyte recruitment has not been determined, although the expression of RANTES is increased in kidneys with interstitial inflammation be-
cause of HIV nephropathy (68) and allograft rejection (69).

Although growth factors such as TGF-β1 and PDGF are monocyte chemoattractants in vitro, their ability to direct monocyte recruitment to the renal interstitium has yet to be demonstrated. The observation that the overexpression of TGF-β1 in glomeruli (70) and iliac arteries (71) by gene-transfer therapy resulted in significant fibrosis in the absence of an influx of monocytes raises questions about the role of TGF-β1 as a monocyte chemoattractant in vivo. Similarly, activated complement components, especially C5a, elicit potent monocyte chemoattraction in vitro, yet in virtually all experimental models of glomerulonephritis characterized by an infiltrate of monocytes, complement deficiency or depletion does not attenuate the degree of inflammation (72). In the models of acute PAN nephrosis (45) and passive Heymann nephritis (Figure 3) (9), complement depletion with cobra venom factor had no effect on the severity of the interstitial nephritis.

Perhaps lipid factors will surface as the key soluble mediators of interstitial monocyte recruitment. Rats depleted of essential fatty acids from infancy fail to mount a monocyte inflammatory response to noxious stimuli. The interstitial infiltrate of monocytes is abolished in essential fatty acid-deficient rats with PAN nephrosis (Table 2) (73,74). The essential fatty acid-derived product that is necessary for monocyte recruitment has not yet been identified, although alterations in eicosanoid metabolism (leukotriene B4 and thromboxane B2) have been implicated (75). The possible role of the chemoattractant properties of oxidized lipoproteins in the early phase of atherogenesis has been studied recently (76–80). In preliminary studies, the lipid-lowering drugs probucol and lovastatin did not decrease the severity of the interstitial disease in rats with PAN nephrosis (81).

One of the more promising candidate chemoattractants comes from the work of Kees-Folts et al. (82). Using the rat model of BSA-induced overload proteinuria, they suggested the existence of a novel lipid that is chemotactic for monocytes. Cultured rat tubular epithelial cells showed a dose-dependent release of a monocyte chemoattractant after exposure to BSA, but this factor disappeared if BSA depleted of lipid was used. They suggested that this lipid is bound to urinary proteins, endocytosed by proximal tubules during proteinuric state, and metabolized by these cells to produce the chemotactic factor.

Osteopontin, another promising monocyte chemoattractant, is a glycoprotein that was originally isolated as a bone-matrix molecule in 1989 (83,84). Osteopontin injected sc into mice elicits a macrophage-rich inflammatory response (85). Pichler et al. (86) have demonstrated increased tubular expression of osteopontin mRNA in rats with anti-Thy-1 glomerulonephritis, passive Heymann nephritis, and PAN nephrosis compared with that in normal rats (Figure 6). The appearance of osteopontin protein in tubules was followed by an influx of monocytes into the adjacent interstitial space. Further studies are needed to establish whether osteopontin actually functions as a chemoattractant within the kidney, especially because it is most abundantly expressed on the apical membrane of tubules.

### Monocyte Adhesion

Monocytes express several membrane-adhesion molecules, including members of the β1 integrin family (very late antigen [VLA]-4, VLA-5), the β2 or leukocyte integrin family (CD11/CD18), the immunoglobulin supergene family (intercellular adhesion molecule [ICAM]-1, ICAM-2), and the selectin family or selectin

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Interstitial Macrophages on Day 10 (M/HPF)</th>
<th>Inulin Clearance at 18 wk (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats (EFA⁺)</td>
<td>0.8 ± 0.2</td>
<td>1.75 ± 0.15</td>
</tr>
<tr>
<td>Nephrotic rats (EFA⁺)</td>
<td>13.6 ± 3.2b</td>
<td>0.92 ± 0.12b</td>
</tr>
<tr>
<td>Nephrotic rats (EFA⁻)</td>
<td>0.7 ± 0.6c</td>
<td>1.51 ± 0.13c</td>
</tr>
</tbody>
</table>

a This table was prepared from data published by Harris et al. (73) and Diamond et al. (74) with permission from The Rockefeller University Press and the American Physiological Society. Me, interstitial macrophages; HPF, high-power field.
b P < 0.05 versus control EFA⁺.  
c P < 0.05 versus nephrotic EFA⁺.

Figure 6. Immunofluorescence photomicrograph illustrating the relationship between the de novo tubular expression of osteopontin and an interstitial infiltrate of monocytes in the kidney of a rat with anti-Thy-1 glomerulonephritis. In contrast to an area of normal tubules to the left, the brush border of several proximal tubules to the right illustrates the de novo expression of osteopontin. With a second antibody-labeling system, several interstitial cells adjacent to the osteopontin-positive tubules (black cytoplasmic stain) are shown to express the ED-1 macrophage marker. Photomicrograph kindly provided by Dr. Rick Johnson, University of Washington, Seattle (magnification, ×400).
counter receptors (reviewed by Brady [87]). Studies are just beginning to evaluate the renal expression of the ligands of monocyte-adhesion molecules. The studies that are necessary to prove that any of these molecules participate in interstitial monocyte recruitment have not yet been performed. The selectins are a group of molecules with a similar extracellular structure (>60% identical), namely, an N-terminal lectin-like domain, an epidermal growth factor repeat, and a variable number of complement regulatory-like modules [88]. The three selectin families are distinguished according to the cell type on which they were originally identified: endothelial (E)-selectins (including endothelial leukocyte adhesion molecule-1), lymphocyte (L)-selectins, and platelet (P)-selectins. E-selectin and P-selectin, which are expressed by endothelial cells, may be involved in the initial transient adhesion of circulating monocytes by low-affinity interactions with carbohydrate structures, particularly sialyl-Lewis x and sialyl-Lewis a. In contrast, L-selectins are expressed by leukocytes (monocytes, neutrophils, and lymphocytes) and interact with sialylated glycans on endothelial cells. These interactions are thought to enable leukocytes to roll along the endothelial surface [89]. Although E-selectins are not expressed in normal human kidneys, tubular epithelial cells are E-selectin positive in patients with acute glomerular diseases [90].

The primary role of selectins may be to tether circulating leukocytes, facilitating their interaction with other adhesion molecules that ultimately support their diapedesis into the perivascular tissue. Monocytes may adhere to endothelial cells through integrins: VLA-4 (α4β1), which binds to vascular cell adhesion molecule-1 (VCAM-1) and fibronectin, and VLA-5, which binds to fibronectin [91,92]. VLA-4 is particularly interesting: it is known to play a major role in monocyte migration [93] and is absent from neutrophils, which could explain the paucity of neutrophils in the majority of kidneys with chronically inflamed interstitia. VCAM-1 is present in normal tubules and small interstitial vessels; its expression is increased on tubules and interstitial capillaries of renal allografts with acute rejection [94–97] and in a murine model of lupus nephritis [98]. The tubular expression of VCAM-1 has been positively correlated with the number of transferrin receptor-positive interstitial cells in human kidney biopsy specimens from patients with primary glomerular diseases [99]. Anti-VLA-4 antibodies have been reported to prevent interstitial disease in rats with mercuric chloride–induced nephritis [100].

Alternatively, monocytes may bind by means of the leukocyte (B2) integrins, which are also known as the CD11/CD18 complex. CD11b/CD18 binds to ICAM-1 and ICAM-2, as well as to the activated complement component IC3b [91,92]. On the basis of numerous in vitro and in vivo studies, CD11b/CD18 appears to be essential for neutrophil migration [101]. ICAM-1 is expressed on normal renal interstitial cells and vessels. De novo expression has been described on tubular epithelial cells in murine lupus nephritis [102], in renal allografts with rejection [103,104], and in a variety of human glomerular diseases, especially in areas of tubular damage [90,105–107]. Antibodies to ICAM-1 have decreased interstitial inflammation in renal allograft rejection [108], in rats with autoimmune anti-GBM nephritis [109], and in the kidney strain of mice with autoimmune interstitial nephritis [110]. However, in those experiments, anti-ICAM-1 antibody therapy was likely targeted to ICAM-1-positive T cells. In rats with accelerated anti-GBM nephritis, Hill et al. [111] observed an up-regulation of ICAM-1 expression, not only on the brush border of proximal tubules but also in capillary endothelium and fibroblast-like interstitial cells. VCAM-1 and ICAM-1 are also expressed by mononuclear cells, suggesting a possible role for these molecules in the amplification phase of interstitial nephritis. Clearly, much remains to be learned about the molecular basis of monocyte recruitment to the renal interstitium.

**INTERSTITIAL FIBROSIS IN PRIMARY GLOMERULAR DISEASES**

**Monocytes in Interstitial Fibrosis**

This review has emphasized the monocyte/macrophage family because the evidence is almost overwhelming that these cells play an active and important role in interstitial fibrosis. At this time, it is important to remember that the net effect of interstitial monocyte recruitment may still be a beneficial one. Several studies that have used PAN nephrosis as a model support a role for monocytes in fibrogenesis. Foci of interstitial fibrosis are evident by 2 to 3 wk (Figure 7) [112]. The depletion of circulating monocytes by systemic irradiation has been shown to prevent the interstitial influx of monocytes in rats during...
the acute phase of PAN nephrosis (113). Eighteen weeks later, these irradiated animals were reported to have less interstitial fibrosis. Tissue infiltration by monocytes can also be prevented by a diet deficient in essential fatty acids. If rats are depleted of essential fatty acids before treatment with PAN, interstitial invasion by monocytes is prevented (73). Eighteen weeks later, the rats fed the essential fatty acid-deficient diet had better renal function and were also reported to have less severe chronic TI damage (Table 2) (74). Saito and Atkins (114) have reported a beneficial effect of methylprednisolone: rats given repeated injections of PAN and protamine sulfate develop severe interstitial inflammation and chronic TI damage. If these rats are also treated with prednisolone, not only is the number of interstitial mononuclear cells reduced, but the rats have significantly less interstitial fibrosis. Nakamura et al. (115) reported a decrease in renal medullary a1(I) procollagen mRNA levels in rats with acute PAN nephrosis that were treated with methylprednisolone. Barash et al. (116) have obtained beneficial results by treating rats or mice that have polycystic kidney disease with methylprednisolone.

**Fibrogenic Cytokines**

The ability of macrophages to produce the fibrogenic cytokines TGF-β1, PDGF, IL-1, IL-6, fibroblast growth factor, and Tumor necrosis factor α (117) appears to be critical in this process of progressive interstitial fibrosis. Proximal tubular cells can also be stimulated to produce all of these cytokines, with the possible exception of IL-1 (118–125). For renal interstitial fibrosis, TGF-β1 is the only member of this family that has been investigated in any detail. TGF-β1 elicits a variety of responses that promote fibrosis, including the stimulation of several extracellular matrix genes, whereas matrix turnover is blocked by both the reciprocal down-regulation of matrix-degrading metalloproteinase enzymes and the up-regulation of metalloproteinase inhibitors (126–129). TGF-β1 may also function as a monocyte chemokine to amplify the inflammatory response (130). In acute PAN nephrosis, my colleagues and I (112) observed a significant increase in renal TGF-β1 mRNA at the peak of interstitial inflammation. In a subsequent study (131), when rats were given repeated injections of PAN to sustain the nephrotic state and interstitial nephritis, persistently increased TGF-β1 mRNA levels were seen. Some tubular epithelial cells also produce TGF-β1, even in normal rats. However, TGF-β1-positive interstitial monocytes could be found only in the interstitium of nephrotic and not normal kidneys (132).

Our study (132) of dietary protein restriction suggests that interstitial inflammatory cells are an important intrarenal source of TGF-β1 in experimental nephrosis. Dietary restriction to 8% protein reduced the intensity of the inflammatory response (Table 3). This effect was associated with the normalization of renal TGF-β1 mRNA levels (Figure 8). Renal TGF-β1 mRNA levels showed a significant positive correlation with the number of interstitial macrophages ($r = 0.76$). Furthermore, protein-restricted nephrotic rats appeared to benefit biologically. Renal mRNA levels for several extracellular matrix proteins were normalized and by 3 wk, nephrotic rats on the low-protein diet had significantly less renal collagen than did rats on the standard 27% protein diet (Table 3). Dietary protein restriction appears to attenuate interstitial fibro-

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**TABLE 3. Dietary protein restriction in nephrotic rats**

<table>
<thead>
<tr>
<th>Dietary Protein</th>
<th>Urinary Albumin on Day 10 (mg/day)</th>
<th>Interstitial Macrophages on Day 10 (cells/1,000 TI cells)</th>
<th>Kidney Collagen on Day 21 (mg/kidney)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27%</td>
<td>401 ± 44</td>
<td>188 ± 15</td>
<td>5.3</td>
</tr>
<tr>
<td>8%</td>
<td>186 ± 50</td>
<td>79 ± 38</td>
<td>4.5</td>
</tr>
<tr>
<td>PAN Rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27%</td>
<td>0.3 ± 0.2</td>
<td>18 ± 2</td>
<td>3.9</td>
</tr>
<tr>
<td>8%</td>
<td>0.2 ± 0.3</td>
<td>10 ± 3</td>
<td>3.7</td>
</tr>
</tbody>
</table>

$a$ This table was based on data published by Eddy (132), with permission from the American Physiological Society.

$b$ $p < 0.05$ compared with nephrotic rats fed a 27% protein diet. Controls were non-nephrotic rats fed a 27% protein diet.

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![Figure 8. The effect of a low-protein diet on renal mRNA levels for TGF-β1 and the interstitial matrix proteins procollagen α1(I), procollagen α1(III), and fibronectin. kb kilobases. The graphs below the northern blots present the mean densitometric score ± 1 SD, expressed in arbitrary units after correction for any inequality in RNA loading. Reading from left to right, the bars represent PAN rats fed 27% protein, control rats fed 27% protein, PAN rats fed 8% protein, and control rats fed 8% protein. * $p < 0.05$ compared with PAN rats fed 27% protein. Reproduced from Eddy (132), with permission from the American Physiological Society.](image_url)
sis by diminishing the number of interstitial macrophages present to produce TGF-β. Why fewer monocytes are recruited remains unknown.

In the last couple of years, it has become apparent that angiotensin II may stimulate the production of fibrogenic cytokines. Proximal tubular cells have recently been shown to produce TGF-β1 after exposure to angiotensin II (133). It is possible that the ability of angiotensin-converting enzyme inhibitors to preserve renal function may be related to the down-regulation of fibrogenic cytokine production. For example, rats with chronic PAN nephrosis that were treated with enalapril had significantly less interstitial fibrosis than did untreated rats (Figure 9) (134). In a rat model of renovascular hypertension, Mai et al. (135) have shown that, within 7 days, there is an interstitial influx of mononuclear cells and an interstitial accumulation of extracellular matrix proteins in hypertensive rats. The continuous infusion of angiotensin II into rats produces focal TI inflammation and fibrosis within 14 days (136).

Although the fibrosis-promoting effects of TGF-β1 are undisputed, what is currently unclear is whether any of the other putative fibrogenic cytokines play a significant role in progressive fibrosis of the renal interstitium. The direct transfer of the TGF-β1 gene into the iliofemoral arteries of pigs (71) or into the glomeruli of rats (70) results in significant fibrosis in vessel walls and glomeruli, respectively. The attenuation of glomerular matrix production in rats with anti-Thy-1 glomerulonephritis (a model of antibody-complement-mediated mesangial injury) by treatment with the small proteoglycan decorin (137) or a TGF-β1-neutralizing antibody (138) provides further support for the role of TGF-β1. TGF-β1 binds to the core protein of decorin, an interaction that results in its neutralization. In vitro studies suggest that, in addition to growth factors and cytokines, other inflammatory mediators such as C5b-9 (139) may also stimulate extracellular matrix production.

**Interstitial Fibroblasts**

What are the principal TGF-β1 target cells that produce these matrix proteins? Although tubular cells are capable of synthesizing several matrix proteins (140–142), in states of progressive interstitial fibrosis, interstitial cells rather than tubular cells appear to be the primary matrix-producing cells. In PAN nephrosis, in situ hybridization for procollagen α1(I) identifies interstitial cells, likely fibroblasts/myofibroblasts (112). In situ hybridization for procollagen α2(I) in rats with nephrotoxic serum nephritis performed by Wiggins et al. (143) highlights interstitial perivascular cells.

We are just beginning to learn more about the biology of interstitial fibroblasts. Müller and coworkers (144–148) have used a series of markers to identify three mitotically active fibroblast progenitor cells and three postmitotic fibroblasts, with Stage VI being the terminally differentiated cell. They have shown that interstitial fibroblasts isolated from kidneys with interstitial fibrosis are more likely to be in the highly proliferative Stage I than are those of normal kidneys, and at all stages, interstitial fibroblasts from scarred kidneys synthesize more total collagen, but different relative amounts of the interstitial collagens I, III, and V than do the interstitial fibroblasts of normal kidneys. Alvarez and coworkers (149) have shown that even interstitial fibroblasts from normal kidneys show different proliferative and collagen secretion responses after exposure to cytokines than do fibroblasts isolated from other parts of the body such as the skin. It has recently been appreciated that a population of α-smooth muscle actin-producing cells, presumably myofibroblasts, appear at sites of interstitial injury (150). A smaller number of these cells can be identified in the interstitium of normal human but not rat kidneys.

**Interstitial Matrix Remodeling**

To end on a note of potential optimism, my colleagues and I have some preliminary evidence that suggests that early interstitial fibrosis is reversible, as illustrated by the transient accumulation of collagen I, collagen III, and fibronectin in the interstitium of rats given a single injection of PAN (Figure 10) (112). How might this occur? A series of matrix-degrading metalloproteinase enzymes (interstitial collagenase, gelatinase, stromelysin), as well as metalloproteinase inhibitors, are present in renal tissue, although currently, little is known about their production by TI cells (151–158). In addition, the plasmin pathway has also
Is Early Interstitial Fibrosis Reversible?

*Figure 10. Immunofluorescence microscopic evaluation of the percentage of TI fields with increased deposits of the interstitial matrix proteins collagen I, collagen III, and fibronectin in rats with acute PAN nephrosis suggests that, without a sustained injury, early interstitial fibrosis may be reversible. *P < 0.05 compared with normal control animals. Figure based on data published by Jones et al. (112), with copyright permission from the American Society of Investigative Pathology.*

been implicated in the turnover of renal matrix (159–161). Not only does plasmin activate latent metalloproteinase enzymes, but plasmin may directly degrade several extracellular matrix proteins (151,153). Increased enzyme activity, especially early in the disease before a highly organized scar is formed, may reverse the fibrotic process. In the model of acute PAN nephrosis at the peak of the disease, we observed a significant increase in mRNA levels for the tissue inhibitor of metalloproteinase-1 (TIMP-1) that reverses during the recovery phase, but little change in the metalloproteinase enzymes (112). It is conceivable that the reversal of interstitial fibrosis in this model was due to the return of intrinsic metalloproteinase activity once the enzyme inhibitor TIMP was turned off. Studies of glomerulosclerosis have implicated the overexpression of plasminogen activator inhibitor (PAI-1) in progressive renal scarring (162). It is interesting that the expression of both TIMP-1 and PAI-1 is up-regulated by TGF-β1 (163–165). If, in fact, inhibition of matrix turnover is important in renal fibrosis, the relative contribution of the metalloproteinase and plasmin pathways needs to be determined.

Considerable progress has been made in the last few years. The notion that TI disease is an ischemic sequela of glomerular sclerosis is clearly naive. Overall, TI disease associated with injury primarily targeted to the glomerulus is likely to emerge as a complex network involving interactions between a variety of cells and molecules (Figure 1).

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