Case presentation

A 63-year-old white male was admitted to the hospital because of renal insufficiency. He had been treated at another hospital for bleeding peptic ulcer disease. He had had two or three episodes of gross hematuria dating back to approximately 10 years prior to admission and had had a gradual decline in renal function over the previous 3 years. The medical history was remarkable for a previous episode of upper gastrointestinal bleeding secondary to duodenal bulb ulcer, and hypertension for at least the previous 10 years that had been controlled intermittently with medication. The family history was negative for renal disease.

Physical examination revealed a blood pressure of 170/105 mm Hg; he was afebrile. Urinalysis showed 3+ protein; 20—30 red blood cells/high-power field, and rare white blood cells; the 24-hour urine protein was 1.6 g. Other laboratory values included blood urea nitrogen (BUN), 39 mg/dl; serum creatinine, 3.4 mg/dl; glucose, 82 mg/dl; sodium, 135 mEq/liter; potassium, 3.7 mEq/liter; hematocrit, 32.5%; hemoglobin, 6.8 mg/dl; and platelet count, 340,000 mm$^3$. The PT, PTT, and liver enzymes were normal. A repeat 24-hour urine collection revealed a creatinine clearance of 14.4 ml/min and 2.3 g protein. The C3, C4, and CH50 were normal, and the sedimentation rate was 40 mm/hr. The rheumatoid factor and ANA were negative, as were the antineutrophilic cytoplasmic antibodies.

A renal biopsy specimen examined by light microscopy revealed 8 of 13 glomeruli to be sclerosed. Three glomeruli showed mesangial hyperplasia, matrix expansion, and crescents with syncytia. Many glomerular capillaries were narrowed or obliterated. Immunofluorescent examination revealed 3+ granular IgA, 3+ IgG, and 2+ C3 deposits.

Electron microscopy revealed electron-dense deposits in the mesangial matrix. Areas of modest interstitial cell infiltrate, extensive interstitial fibrosis, and tubular atrophy were present. A diagnosis of IgA nephropathy was made.

The patient's blood pressure was controlled with medications, but his renal function continued to deteriorate. One year after discharge, the BUN and serum creatinine were 55 mg/dl and 5.0 mg/dl, respectively.

Discussion

DR. HANNA E. ABBoud (Professor and Chief, Division of Nephrology, The University of Texas Health Science Center, and Staff Physician, Audie Murphy Veterans Administration Hospital, San Antonio, Texas): This patient has IgA nephropathy, a disease that accounts for approximately one-third of all cases of primary glomerular disease and is regarded as the most common form of primary glomerulonephritis worldwide. IgA nephropathy is thought to be an immune-complex-mediated disease in which circulating IgA-containing immune complexes are deposited in the mesangium. Autoimmune mechanisms recently have been incriminated in this and other forms of glomerulonephritis. IgA nephropathy is associated with a proliferative form of glomerulonephritis, which is characterized by hypercellularity and expansion of the mesangium; it can be accompanied by crescent formation. Recent studies have clearly documented the infiltration of activated lymphocytes and monocytes-macrophages in the glomerulus and the interstitium. Interstitial cell infiltration occurs in most if not all patients; glomerular infiltration with these inflammatory cells is present predominantly in patients with crescent formation. Poor prognostic indicators of the disease include heavy proteinuria, hypertension, and severe mesangial cell proliferation with crescent formation. The utility of laboratory tests as prognostic indicators remains unproven; serum IgA concentrations and levels of IgA-containing circulating immune complexes do not correlate well with clinical outcome [1].

The immune-mediated, inflammatory glomerular diseases are characterized by a spectrum of morphologic and functional changes that involve both the glomerulus and the interstitium [1—3]. These diseases, progressive in nature, distort the normal architecture of the nephron and eventually reduce renal function [4, 5]. The pathogenesis of this group of diseases has been under intensive investigation in the last decade, and a clear role both for humoral and cell-mediated immune mechanisms is becoming increasingly evident [3, 6]. Unfortunately, despite these advances, therapy for this group of diseases remains empiric, risky, and often futile. The pathologic manifestations of glomerular diseases are diverse, ranging from subtle morphologic changes to severe cellular inflammatory reactions. In...
many instances, however, these diseases are relentlessly progressive and result in a complete loss of renal function. Mesangial matrix expansion and some hypercellularity occur commonly in all chronic progressive glomerular diseases; this pattern of glomerular pathology is seen in advanced renal failure of any cause [2, 4, 5]. Several mediators have been incriminated in the pathogenesis of these morphologic and functional changes [3, 4, 7]. However, in most instances, the presumption of a pathogenetic role is based on a descriptive association or on the known effects of these mediators in other systems. Among the mediators that have attracted recent attention are polypeptide growth factors or cytokines [8-16]. Throughout my discussion, I will use these two terms interchangeably.

Evidence is emerging that cytokines are involved in the mediation of the structural and functional abnormalities that occur in glomerulonephritis and progressive forms of glomerular and interstitial injury. The cytokines constitute a group of low-molecular-weight (<80 kD), potent regulatory peptides with multiple and often overlapping biologic activities. They interact in complex cell- and tissue-specific networks. Their effects are mediated through high-affinity receptors on target cells; these receptors are expressed constitutively or are inducible [8-16]. Cytokines play a role in a wide variety of biologic processes including hypertrophy; proliferation; regulation of matrix synthesis and degradation; immune inflammatory responses; development and differentiation, and regulation of vascular tone. When one considers the multiple biologic effects of these ubiquitous compounds, their potential involvement in the glomerular and interstitial abnormalities of glomerulonephritis comes as no surprise. Glomerular involvement during the course of glomerulonephritis entails one or more of the following: hypercellularity due to intrinsic cellular proliferation, hypercellularity due to infiltration and proliferation of inflammatory cells, and an increase in the mesangial cell matrix due to injury to one or more types of glomerular cells (for example, epithelial or mesangial). The glomerular lesions usually are accompanied by an increase in capillary permeability, which is manifested clinically as proteinuria. In addition, hemodynamic abnormalities are usually present. Depending on the severity and stage of the disease, these abnormalities can decrease or increase glomerular blood flow, filtration rate, and hydrostatic pressure. The interstitium is involved very early in almost all glomerular diseases [2]. Cellular infiltrates in the interstitium are often more pronounced than they are in the glomerulus. Tubular and vascular dysfunction and injury eventually progress to interstitial fibrosis and tubular atrophy [2, 16]. The recent availability of renal, and specifically glomerular, cells in culture has stimulated an exploration of the role of cytokines in glomerular disease [17, 18]. Table 1 lists the potential role of cytokines in renal pathologic manifestations of glomerulonephritis.

Among the polypeptide growth factors, platelet-derived growth factor (PDGF) [19, 20], transforming growth factor beta (TGF8) [13, 21], and insulin-like growth factor-1 (IGF1) [22] are prime candidates as mediators of acute and chronic glomerular injury. Excellent evidence also exists indicating that interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor alpha (TNFa) mediate, in part, immune-mediated glomerular and/or interstitial injury [16]. The results of recent in-vitro studies also suggest that some cytokines such as monocyte chemotactic peptide (MCP-1), macrophage colony-stimulating factor (M-CSF), interleukin-8 (IL-8), and gamma interferon (y-IFN) are involved in the early phase of renal injury [16]. Additional in-vitro studies utilizing cultured glomerular cells and tubular epithelial or interstitial cells suggest that epidermal growth factor (EGF) [23-25], transforming growth factor alpha (TGF8) [11], and basic fibroblast growth factor (bFGF) [11, 25, 26] also can mediate renal injury.

I believe that additional evidence beyond the mere presence of a given cytokine must exist before it can be invoked as a mediator of the structural and functional changes in glomerulonephritis (Table 2). For example, does the cytokine in question elicit a similar biologic effect to that postulated for the kidney in some other tissue or cultured cell type? Second, is there evidence for the presence of functional receptors known to mediate certain biologic effects of a particular cytokine in freshly isolated glomeruli, cortical tissue, or cultured glomerular or extraglomerular cells? Third, does the cytokine induce one or more of the pathologic manifestations when it is administered in vivo or when it is overexpressed in transgenic animals? Fourth, is there evidence of a temporal change (increase or decrease) in the expression of the active cytokine and/or its receptor in vivo appropriate to the postulated pathologic manifestations? Fifth, and perhaps most definitive, does administration of an antagonist or neutralizing antibody of the cytokine or its receptor attenuate or abolish the disease manifestation? The best evidence to date comes from studies in

**Table 1. Established or postulated role of cytokines in renal pathology**

<table>
<thead>
<tr>
<th>Growth regulation: hypertrophy of the glomerulus and/or glomerular or tubular cells, proliferation of glomerular or tubulointerstitial cells or infiltrating inflammatory cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulation of matrix component synthesis or degradation including changes in basement membranes, and development and progression of fibrosis</td>
</tr>
<tr>
<td>Modulation of the immune inflammatory response through chemotaxis, migration, activation, or suppression of inflammatory or intrinsic glomerular cell responses</td>
</tr>
<tr>
<td>Regulation of vascular tone with subsequent changes in blood flow and glomerular filtration rate</td>
</tr>
<tr>
<td>Development and differentiation</td>
</tr>
</tbody>
</table>

**Table 2. Suggested criteria implicating a particular cytokine in renal pathology**

| Ability to elicit a biologic function relevant to renal pathology |
| Presence of functional receptors for a cytokine in freshly isolated tissue such as glomeruli, tubules, or cultured glomerular and tubular cells |
| Cytokines can induce one or more pathologic manifestations upon in-vivo administration or when overexpressed, as in transgenic animals |
| Temporal relationship between the expression of cytokines and pathologic manifestation of the disease process |
| Attenuation or abolition of disease manifestation upon neutralization of biologic effect of cytokines |
human renal tissue and in animal models of renal disease; these
studies suggest that PDGF, TGFβ, IGF1, IL-1, IL-6, and TNF
mediate hypercellularity, matrix expansion, and other patho-
logic manifestations of some forms of glomerulonephritis or
progressive glomerular injury. In many instances, however,
the evidence is largely descriptive and relies simply on the asso-
ciation of increased or decreased expression of cytokines during
the course of a disease.

The precise source of the polypeptide growth factors impli-
cated in various renal diseases is important because this infor-
mation likely has therapeutic as well as pathogenetic implica-
tions. Most of these compounds are stored or synthesized in
infiltrating inflammatory cells [26-30]. In addition, many of
these peptides are synthesized by intrinsic glomerular cells [16,
31-37]. Hence these compounds could play a role both in
immune inflammatory and noninflammatory renal diseases.
Such a distinction, however, is becoming arbitrary now that
careful immunohistochemical techniques using specific markers
have identified monocytic and lymphocytes, in traditionally “noninflam-
atory” lesions [28]. Activated platelets, monocytes, tissue macro-
phages, and lymphocytes “passing through” or infiltrating the
kidney could release substantial amounts of these peptides. On
the other hand, cytokine release by intrinsic glomerular cells or
extraglomerular cells suggests their involvement even in late
phases of glomerulonephritis when the acute inflammatory
process has subsided, or even in the so-called “noninflamma-
tory” glomerular diseases [5]. Table 3 lists the site of produc-
tion of some of the cytokines. Whereas cytokines such as IL-1,
TNF, IL-6, EGF, and IGF, are found in the systemic circula-
tion, mounting evidence suggests that they do not primarily
function in an endocrine manner, but rather in an autocrine or
paracrine manner.

I will focus the remainder of my discussion on the role of
selected cytokines. Because our primary interest is in under-
standing the pathogenesis of individual disease entities, the
ideal format would be to discuss a given disease and the role of
various cytokines in mediating its pathologic manifestations.

**Platelet-derived growth factor**

Biologically active PDGF, the most potent mitogen for me-
sangial cells, is a disulfide-linked dimer composed of two
polypeptide chains, designated A and B, that are encoded by
two separate genes [14, 15]. Recent evidence disclosed that
PDGF exists in three biologically active forms: PDGF AB, AA,
and BB [38]. The PDGF stored in human platelet alpha granules
is predominantly PDGF AB (approximately 80%); less than
20% is PDGF BB [38]. Activated monocytes or tissue macro-
phages, as well as glomerular mesangial and endothelial cells,
release PDGF [27, 36-40]. Among the several peptide growth
factors, as I noted, PDGF stands out as the most potent mitogen
for mesangial cells [25, 36, 37]. Mesangial cells not only respond
to PDGF but, as I said, they also are a source of this peptide
[32, 36, 37]. Mesangial cells isolated from glomeruli of several
species, including the human, rat, and mouse, express PDGF
A- and B-chain mRNA and produce PDGF [37, 41]. The PDGF
mRNA and protein also are highly expressed in microvascular
endothelial cells isolated from human kidneys [39, 40]. Serum,
as well as several purified recombinant growth factors, stimu-
late DNA synthesis and induce PDGF A- and B-chain mRNAs
in mesangial cells [37]. The induction of PDGF genes and the
production of PDGF in response to several peptide growth
factors raise the possibility that endogenous PDGF derived
from mesangial cells mediates the mitogenic effect of these
peptides. Two distinct PDGF receptors are encoded by two
separate genes. Molecular cloning of mouse and human PDGF
receptor cDNA have shown structural similarity with other
protein tyrosine kinases receptors [11, 42-44]. The two genes
code two separate receptors, termed alpha and beta receptors
[42-44]. The PDGF A and B chains bind the alpha receptor
subunit, whereas only the PDGF B chain binds the beta
receptor subunit [42]. We recently compared the mitogenic

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**Table 3. Biologic sources of growth factors**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Monocytes-macrophages</th>
<th>Platelets</th>
<th>Lymphocytes</th>
<th>Renal tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF</td>
<td>Activated monocytes or tissue macrophages</td>
<td>Release primarily PDGF AB (80%)</td>
<td>HIV infected</td>
<td>Mesangial cells express A &amp; B chains and release predominantly AA isoform</td>
</tr>
<tr>
<td>IGF1</td>
<td>Activated monocytes and macrophages</td>
<td></td>
<td></td>
<td>Mesangial and endothelial cells release latent TGF-beta</td>
</tr>
<tr>
<td>IL-1</td>
<td>Activated monocytes and macrophages</td>
<td></td>
<td>Release IL-1 beta</td>
<td>Mesangial and endothelial cells, collecting duct cells</td>
</tr>
<tr>
<td>IL-6</td>
<td>Activated monocytes</td>
<td></td>
<td>When activated</td>
<td>Activated mesangial cells</td>
</tr>
<tr>
<td>TNF</td>
<td>Activated monocytes and macrophages</td>
<td></td>
<td></td>
<td>Mesangial cells stimulated with lipopolysaccharide</td>
</tr>
</tbody>
</table>

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effect of all three isoforms in human mesangial cells; our data indicate the predominance of beta receptors in mesangial cells [45]. The mitogenic signals that transduce the effects of PDGF receptor activation are similar to those reported in other cell systems, although they have been less extensively studied in mesangial cells [46]. It is likely that the PDGF beta receptor is the major target for PDGF in the glomerulus and specifically in mesangial cells. The PDGF receptors are subject to regulation by PDGF itself as well as by other peptides [47-49]. Therefore PDGF, as well as its receptor, might amplify an inflammatory response to PDGF itself or to other cytokines.

Platelet-derived growth factor also is a chemotactrant for mesangial cells [50]. This property is likely to be an important mechanism for intraglomerular migration of mesangial cells following mesangiolysis or necrosis. PDGF, like other cytokines, has vasoactive properties (Table 4). As a potent vasoconstrictor [51], PDGF elicits contraction of mesangial cells, a property that might regulate ultrafiltration coefficient [52]. Preliminary micropuncture studies in the rat, in which PDGF was infused into the renal circulation, demonstrated a marked decrease in renal blood flow and glomerular filtration rate, an increase in efferent arteriolar resistance, and a fall in the ultrafiltration coefficient [53]. Infusion of PDGF into the isolated microperfused glomerulus markedly increases intraglomerular capillary pressure and is associated with a decrease in flow rate and a marked increase in vascular resistance (Abboud H, Oggood R, unpublished observations). PDGF may act as a vasoconstrictor in certain vascular beds. Vessels with endothelial cells that express PDGF receptors undergo vasodilation in response to the BB isoform because of a release of endothelin-derived relaxing factor [54]. PDGF regulates matrix metabolism in mesangial cells either directly or through the release of other growth factors such as TGF beta [33, 55, 56]. Although PDGF does stimulate the production of decorin as well as biglycan, it is less potent than is TGF beta [55]. Indirect in-vitro evidence suggests that PDGF mediates the increased expression of type-IV collagen mRNA in response to advanced glycosylated end products that accumulate in diabetes [56].

These in-vitro studies provided a major impetus for an exploration of the role of PDGF in vivo.

Evidence is accumulating in humans and experimental animals that PDGF is heavily involved in inflammatory and proliferative glomerular diseases. An increased expression of PDGF has been demonstrated histochemically in glomeruli from patients with lupus nephritis [57]. Immunohistochemical studies demonstrated that expression of PDGF beta receptor is increased in rejected kidney transplants and in human renal biopsy specimens obtained from patients with proliferative glomerulonephritis [58, 59]. Working with our group, Gesualdo demonstrated an increased expression of PDGF in experimental animals with proliferative glomerular disease as well as in renal biopsy tissue obtained from patients with a variety of renal lesions [60]. In two models of IgA nephropathy induced by the administration of charged dextran, histochemical techniques and solution hybridization analysis, respectively, demonstrated the increased expression of PDGF and PDGF B-chain mRNA in kidneys from diseased mice; PDGF was localized primarily within mesangial areas of glomeruli and to a lesser extent in the interstitium [60]. The increased PDGF expression correlated with the degree of hypercellularity and clinical features of the disease. Given that glomeruli occupy less than 5% of renal mass, the detection of the increase in PDGF mRNA in whole-kidney tissue and the presence of PDGF in extraglomerular structures clearly illustrate the contribution of the interstitium to the expression of PDGF. Potential sources of PDGF in the interstitium include infiltrating mononuclear cells, vascular endothelial and smooth muscle cells, tubular epithelial cells such as inner medullary collecting duct cells [61], and likely interstitial cells such as fibroblasts. In addition to lupus nephritis, increased expression of PDGF has been demonstrated in glomeruli from patients with IgA nephropathy and, interestingly, focal glomerulosclerosis but not minimal-chain disease [62]. Iida and colleagues demonstrated a marked increase in the expression of PDGF B-chain as well as PDGF beta-receptor mRNA and their translated proteins in glomeruli from a rat model of proliferative glomerulonephritis [63]. The rats had severe proliferative lesions induced by anti-thy 1 antibodies. The increased expression of both PDGF and its receptor indicate that PDGF probably participates in the pathogenesis of cellular proliferation of this disease. In-situ hybridization studies in the same model localized PDGF mRNA transcripts to mesangial cells [64]. More recently, the same investigators demonstrated that infusion of antibody to PDGF reduced the late phase of mesangial cell proliferation by more than 50% during the course of this disease [65]. Complement depletion or platelet depletion significantly reduced cell proliferation and PDGF expression. Additional in-vivo evidence suggesting that PDGF provokes renal and glomerular injury is provided by studies in a remnant kidney model in the rat [66]. In this model, mesangial cell proliferation was documented before the development of glomerulosclerosis and was associated with an increased expression of glomerular PDGF mRNA and protein. The researchers found proliferation of mesangial cells within 3 to 5 days of renal ablation and, to a lesser degree, of endothelial cells starting at day 5 and persisting up to 4 weeks. The increased glomerular cell proliferation was associated with an increased expression of PDGF B-chain by immunohistochemistry. In-situ hybridization revealed the induction of PDGF

<table>
<thead>
<tr>
<th>Growth factor/Cytokine</th>
<th>Hemodynamic effect</th>
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<tbody>
<tr>
<td>PDGF</td>
<td>Constricts aortic rings</td>
</tr>
<tr>
<td></td>
<td>Contracts mesangial cells, reduces Kf, increases efferent arteriolar resistance</td>
</tr>
<tr>
<td></td>
<td>Dilates certain vascular beds whose endothelium expresses PDGF receptors due to release of EDRF</td>
</tr>
<tr>
<td>EGF, TGF-alpha</td>
<td>Constricts aortic rings</td>
</tr>
<tr>
<td></td>
<td>Contracts mesangial cells, constricts afferent and efferent arterioles, reduces Kf, decreases renal blood flow and GFR (rat)</td>
</tr>
<tr>
<td>IGF_1</td>
<td>Increases renal blood flow and GFR</td>
</tr>
<tr>
<td>bFGF</td>
<td>Vasodilates systemic and renal vasculature due to release of EDRF</td>
</tr>
<tr>
<td>IL-1</td>
<td>Inhibits contraction of aortic rings; lag period requires protein synthesis; not dependent on endothelium</td>
</tr>
<tr>
<td>TNF</td>
<td>Shock with systemic hypotension</td>
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</table>
B-chain mRNA between weeks one and four. In addition, the increased expression of PDGF beta-receptor mRNA and receptor protein was demonstrated by Northern blot analysis and immunohistochemistry. The onset of glomerular cell proliferation was associated with mild glomerular platelet accumulation as well as with fibrin deposition. Proteinuria, glomerular sclerosis, and leukocyte infiltration followed cellular proliferation. These data strongly suggest that PDGF causes mesangial cell proliferation and possibly contributes to the initiation and progression of renal injury in this experimental model.

Given its chemotactic and vasoactive properties, PDGF also is likely to influence other aspects of glomerular pathology besides proliferation [67]. PDGF can activate neutrophils and monocytes to release lysosomal enzymes and neutral proteases, and fibroblasts to release collagenase, thereby influencing tissue remodeling [67-69]. Shear stress stimulates PDGF expression [70], thus providing a potential link between hemodynamic and metabolic mechanisms of glomerular injury.

**Transforming growth factor β**

Transforming growth factor β is secreted from most cells, and is released from platelets in an inactive or latent form [71-74]. The latent form of TGFB released by human or rat platelets consists of three distinct components. (1) Mature TGFB1, the active component, is a 25 kD molecule comprising two identical disulfide-linked polypeptide chains. (2) The N-terminal remnant of the TGFB1 is denoted “TGFB1 latency-associated peptide” because it is sufficient to confer latency. (3) A novel type of protein, denoted the “latent TGFB1-binding protein,” associates with the TGFB1 rapidly inside the cells, and this association appears important for the proper assembly and secretion of TGFB1. A post-translational modification of TGFB1 takes place before secretion; this modification is distinct from the activation events required for the generation of biologically active TGFB from the latent molecule [72]. Given that most cells in culture release latent TGFB in various forms, and that latent TGFB does not bind to the TGFB receptor(s), an understanding of the regulation of the release of the active form and the mechanism of activation of TGFB is crucial for delineating the biologic role of this peptide [75-77]. Latent TGFB1 can be converted into the active form by strong acid treatment in vitro. The biologic relevance of this method of activation is doubtful, however. Purified latent TGFB also can be activated by plasmin. Plasmin appears to act by cleaving within the amino terminal region of the TGFB1, thereby destabilizing the latent complex and releasing active TGFB [75]. Two recent reports described the conversion of latent TGFB to the active species in co-cultures of endothelial cells and pericytes or smooth muscle cells [76-78]. Pericytes or smooth muscle cells inhibit endothelial cell mitosis and movement because they form active TGFB. Activation of TGFB requires cell-cell contact or very close apposition of the two different cell types. Inhibitors of plasmin block the formation of active TGFB. The active TGFB produced induces the synthesis of plasminogen activator inhibitor, which blocks the subsequent conversion of plasminogen to plasmin and thereby further suppresses the activation of latent TGFB [77]. It is interesting to note that co-culture of endothelial and mesangial cells inhibits endothelial cell DNA synthesis, whereas the addition of medium conditioned by mesangial cells does not affect endothelial cell DNA synthesis [79]. It is likely that contact of endothelial and mesangial cells activates TGFB released by either cell type. The mechanism of activation of TGFB is probably related to the pathogenesis of renal pathology; active TGFB is released both by cortical tissue and by isolated glomeruli from several models of proliferative glomerulonephritis, including the anti-thymocyte model and an anti-glomerular basement membrane model [80, 81]. Gamma interferon and retinoids stimulate macrophages to release active TGFB [82].

The biologic actions of TGFB are diverse [72-85]. In the main, this factor suppresses cell proliferation and fosters cell adhesion [72]. These effects are accomplished by enhanced synthesis and deposition of extracellular matrix components, decreased proteolysis, and modification of cell-surface-adhesion receptors known as integrins. The integrins in turn control migration, homing, and settlement during tissue remodeling. Integrins are one of the best-characterized families of cell-adhesion receptors [72, 85]. Many integrins function as adhesion receptors for extracellular matrix components such as fibronectin, collagen, laminin, vitronectin, fibrinogen, von Willebrand factor, and others. This effect of TGFB on extracellular matrices is likely to play a major role in the development, morphogenesis, tissue repair, and pathogenesis of fibrotic and sclerotic states in the kidney as well as in other tissues. Further, TGFB elevates the expression of many matrix protein components, including fibronectin, type-I collagen, alpha I and alpha II chains, and collagen types III, V, and VI. Some of these effects have been demonstrated in mesangial and epithelial cells [56, 72, 86, 87]. Other matrix glycoproteins in which synthesis is induced with TGFB include osteopontin, osteonectin, tenascin, thrombospondine, and the chondroitin dermatan sulfate proteoglycans known as biglycan and decorin. Induction of matrix synthesis is a major response observed also following the in-vivo local injection of TGFB1 in animals. TGFB also inhibits proteolysis of extracellular matrix. In mesangial cells, plasminogen activator inhibitor I and tissue inhibitor of metalloprotease, two inhibitors of extracellular matrix-degrading enzymes, also are strongly upregulated by TGFB. One study also reported that TGFB decreases the expression of collagenase, stromelysin, plasminogen activator, and certain proteases [72].

The cellular mechanisms of action of TGFB are unknown despite a great deal of study over the past decade. None of the pathways for intracellular signaling, including phospholipid turnover, modulation of intracellular cyclic AMP level, and tyrosine phosphorylation, has been directly linked to TGFB action [72]. The likely candidates are three cell-surface proteins that have been identified through their ability to bind and be chemically cross-linked to radioiodinated TGFB. They are termed type I, type II, and type III receptors, and have molecular weights of 55, 88, and 280 kD respectively. The TGFB type II and type III receptors recently were cloned and are likely to clarify signal transduction mechanisms involved in the action of TGFB [88].

Since TGFB is stored in high concentrations in platelets [72] and is expressed in activated monocytes and macrophages found within the kidney [72, 89], it is likely to be a major factor in inflammatory diseases of the kidney. In this setting, TGFB is likely a promoter of extracellular matrix deposition and probably regulates cell migration and development. Also, TGFB is an
extremely potent chemoattractant for monocytes, and to a lesser extent fibroblasts, and therefore might attract these cells to sites of inflammation and repair in the kidney. Excellent experimental evidence indicates that TGFβ plays an important role in mediating some of the pathologic changes observed in the kidney [83, 90-95]. High levels of TGFβ1 mRNA and protein are expressed in glomeruli as compared with whole-kidney tissue [90, 91]. TGFβ2 mRNA, on the other hand, is present only in glomeruli and is not detected in whole kidney. Binding proteins (receptors) in rat glomeruli are unique and differ from those described in cultured cells in their affinities to the various forms of TGFβ [91, 92]. Although the function of these binding proteins remains to be defined, they might store TGFβ and therefore regulate its bioavailability.

Immunohistochemical distribution of TGFβ1 does not always match the distribution of the corresponding mRNA, and this discrepancy could reflect (1) diffusion and accumulation of the protein away from its site of synthesis, (2) a lack of translation of the mRNA, or (3) immune cross-reactivity with other forms of TGFβ. Freshly isolated glomeruli and glomerular cells in culture, including mesangial, epithelial, and endothelial cells, express receptors for TGFβ [86].

In general, TGFβ is a potent inhibitor of glomerular cell proliferation including mesangial, endothelial, and epithelial cells [25, 86]. The effect of TGFβ on cell proliferation is influenced by several factors including the target cell, the presence of other cytokines, the type of matrix, and the cell density. For example, mesangial cells plated at high density respond in a positive fashion to TGFβ [86]. The inhibitory effect of TGFβ is markedly attenuated when cells are plated at high density and allowed to grow in the presence of serum (personal observations). Whether these in-vitro events are relevant in vivo remains to be determined. TGFβ also stimulates matrix production both in mesangial and epithelial cells [56, 83, 86, 87]. It significantly increases the production of collagen and fibronectin by glomerular mesangial cells, but only fibronectin production is augmented in glomerular epithelial cells in response to TGFβ. In addition, TGFβ increases the production of biglycan and decorin, and stimulates the deposition of fibronectin on the mesangial cell surface [56, 83, 87].

These in-vitro observations prompted in-vivo studies indicating that TGFβ contributes to the mesangial matrix expansion that occurs in glomerular disease [92-95]. Okuda et al demonstrated increased expression of TGFβ mRNA and de-novo synthesis of TGFβ protein in glomeruli from an experimental rat model of glomerulonephritis induced by injection of antithymocyte serum [93]. In this model, mesangial expansion coincided with elevated proteoglycans synthesis and the secretion of active TGFβ by glomeruli. More recently the same group of investigators demonstrated amelioration of matrix expansion in glomeruli after treatment of the diseased rats with antibody to TGFβ [94]. Most recently, dietary maneuvers such as protein restriction reduced both matrix expansion and TGFβ expression in the same model [95]. Further, TGFβ might promote the glomerular pathology observed in anti-glomerular basement membrane disease in the rabbit [81]. Cortical tissue from diseased kidneys releases active TGFβ into conditioned medium that, in turn, stimulates collagen synthesis in cultured rabbit mesangial cells. The secretion of TGFβ is accompanied by an increase in renal cortical TGFβ mRNA content. Isolated glomeruli from diseased animals release active TGFβ; isolated glomeruli from control animals release latent TGFβ. The increased release of TGFβ was accompanied by an increased expression of TGFβ mRNA [81]. We recently demonstrated that cultured mesangial cells release latent TGFβ constitutively [33]. In addition, PDGF and EGF stimulate the release of latent TGFβ and stimulate the expression of TGFβ mRNA [33]. Data from these studies and in-vivo studies that compared the effects of PDGF and TGFβ infused subcutaneously [96, 97] led us to believe that the relative expression of PDGF and EGF, taken together with the activation of TGFβ, probably determines the nature and progression of the inflammatory lesion in glomeruli. Expression of PDGF or EGF, as well as other positive growth regulators initially, might result in increased cell proliferation. However, as the lesion progresses, the activation and enhanced TGFβ production acting on mesangial cells, or other cells that may invade the glomerulus, such as fibroblasts, increase the synthesis of matrix components and eventually cause fibrosis. Thus, the differential expression of these peptide growth factors might determine the ultimate outcome of the glomerular lesion, proliferation, or fibrosis (Fig. 1).

TGFβ is a potent negative and positive modulator of the immune system. The antiproliferative action of TGFβ on B-lymphocytes, T-lymphocytes, and thymocytes is among the immunosuppressive activities displayed by this factor in vitro and in vivo [98, 99]. Like cyclosporine, TGFβ inhibits the release of several cytokines, including γ-interferon, and TNFβ, from peripheral blood mononuclear cells and tissue macrophages [99]. Since mononuclear infiltration is a prominent histologic feature both in glomeruli and interstitium during many forms of glomerulonephritis, TGFβ might act as an endogenous immunosuppressive agent at some stage of renal injury.

![Fig. 1. Schematic depiction of the role of cytokines in proliferation and fibrosis](image-url)
Interleukin-6

Interleukin-6 (IL-6) is another cytokine that might be involved in proliferative glomerular diseases [100, 101]. A 26 kD protein originally identified in the culture supernatants of stimulated peripheral mononuclear cells, IL-6 is produced by B- and T-lymphocytes, endothelial cells, fibroblasts, and epithelial cells. Whole-kidney tissue expresses IL-6 mRNA. Horii et al first demonstrated that cultured rat mesangial cells release biologically active IL-6 and express IL-6 mRNA as determined by Northern blotting and in-situ hybridization [102]. Moreover, the same investigators demonstrated that IL-6 induces DNA synthesis in rat mesangial cells in the presence of fetal calf serum. Coleman, Reuf, and colleagues also demonstrated that rat mesangial cells release IL-6 and express IL-6 mRNA [101, 103]. Physicochemical characterization of mesangial cell IL-6 demonstrated similarity to IL-6 from other sources. Rat mesangial cells also respond to IL-6 in the presence of a low concentration of fetal calf serum. Several factors stimulate IL-6 release from rat mesangial cells; these factors include PDGF, IL-6, fetal calf serum, lipopolysaccharide, and TNFα [101, 104]. In fibroblasts, glucocorticoids inhibit IL-6 production. Certain matrix components, such as collagen type I, also inhibit IL-6 release in mesangial cells in a dose-dependent fashion. More definitive evidence for the potential role of IL-6 in glomerulonephritis comes from the demonstration that overexpression of IL-6 in mice transgenic for this cytokine results in mesangial proliferative glomerulonephritis [105].

Utilizing a monoclonal antibody to IL-6, Horii and colleagues localized the cytokine to mesangial cells in biopsy specimens from patients with proliferative glomerulonephritis, but not from patients with membranous nephropathy, minimal-change disease, or normal tissue [102]. Other studies also have demonstrated that the expression of IL-6 by immunofluorescence techniques correlates with an increase in glomerular cell number in patients with proliferative glomerulonephritis [106]. The precise cellular origin of IL-6 remains to be determined, however. Urine from patients with proliferative glomerulonephritis, but not from patients with minimal-change or membranous nephropathy, also contains large amounts of IL-6. One study demonstrated a positive correlation between the urinary level of IL-6 and the severity of the proliferative lesions [102, 106]. Glomerular tissue from patients with advanced sclerosis demonstrated reduced binding of IL-6 [102, 106]. A definitive role for IL-6 in inducing proliferative glomerular diseases awaits further studies demonstrating that the administration of IL-6 receptor antagonists or an antibody to the protein or its receptor attenuates or blocks the proliferative process in the glomerulus.

Interleukin-1, TNF, and γ-interferon

The role of cytokines in general, and of these three cytokines in particular, in glomerulonephritis was reviewed recently [16]. Evidence suggests that these cytokines are involved in the initiation stage or in perpetuating certain forms of immune-mediated renal injury. Most of the pioneering work on cytokines and mesangial cell biology focused on the role of IL-1 [107–109]. Human and rat mesangial cells synthesize IL-1 and express IL-1α and IL-1β mRNA transcripts. Serum, PDGF, and EGF stimulate IL-1 production in mesangial cells [108]. TNFα is synthesized by mesangial cells, and its synthesis is stimulated by lipopolysaccharide and inhibited by prostaglandin E₂ and cAMP [110, 111]. IL-1, TNF, and γ-IFN exert several biologic activities in mesangial cells, all of which are relevant to the pathologic manifestations of glomerular and interstitial involvement in glomerulonephritis [16]. Effects elicited by IL-1 in mesangial cells include DNA synthesis, release of proteases, and collagen synthesis [107, 109]. TNF also activates mesangial cells to release procoagulant activity, plasminogen activator, and plasminogen activator inhibitor [111]. TNF and γ-interferon also induced expression of MHC-I and -II in mesangial cells [16]. Increased expression of TNF and IL-1 have been demonstrated in vivo in several animal models of glomerulonephritis [112–115]. Increased IL-1β mRNA has been demonstrated in membranous nephropathy [112], IgA nephropathy [16], and puromycin aminonucleoside nephrosis in the rat [116].

No studies exploring the role of IGF₁ in immune-mediated glomerulonephritis are available as yet. However, evidence does exist that overexpression of IGF₁ contributes to the progression of glomerular injury [124–126]. Renal, and specifically glomerular, hypertrophy has been incriminated as a risk factor for the progression of renal disease in patients and experimental animals following significant loss of renal mass, irrespective of the primary renal disease [4, 5, 10]. Some investigators have suggested that hypertrophy, even without loss of renal mass, is a prerequisite for mesangial expansion and sclerosis of the glomerular microvascular bed [4, 5, 7, 10]. Peptide growth factors might be involved in glomerular hypertrophy, as has been suggested for IGF₁ [124–126]. Recent studies utilizing mice transgenic for growth hormone (GH), growth-hormone-releasing factor (GHRF), and IGF₁ demonstrated that only mice transgenic for GH and GHRF develop hypertrophy and sclerosis [126]. Mice transgenic for IGF₁ did develop some hypertrophy but little sclerosis, even though circulating levels of IGF₁ in the animals transgenic for IGF₁ were higher than in those transgenic for GH and GHRF. Glomerular levels of IGF₁ were not reported, however, and...
local release of IG_once might have been responsible for the hypertrophy [124–126]. Alternatively, additional growth factors other than IGF might mediate the effect of GH. Of particular interest in these studies was the disproportionate hypertrophy of glomeruli compared with total-kidney or total-body weight of the animals. A potential interesting role for IG_F, in mediating the increased renal blood flow and glomerular filtration rate in remnant nephrons is suggested by the observation that infusion of IG_F in fasting rats increases renal blood flow and glomerular filtration rate [127, 128].

Additional evidence suggests that IG_F is involved in the pathogenesis of diabetic nephropathy. Mesangial cells from genetically diabetic mice, db/db, express higher levels of IG_F receptors than do mesangial cells from control db/m mice [129]. Both IG_F and insulin receptor mRNA expression were increased in db/db mesangial cells grown in the presence of high-glucose medium. Abnormalities in IG_F receptor regulation in this as well as other models of glomerular injury should be explored. In vitro studies showed that glomerular mesangial, epithelial, and endothelial cells produce IG_F, and IG_F-binding proteins. IG_F binds to specific receptors in these cells and stimulates DNA synthesis [130–133]. Moreover, IG_F is released from monocytic cell lines and from macrophages [134]. These studies suggest a potential role for IG_F, as a mediator of matrix expansion and hypercellularity in glomerulonephritis.

**Cytokines and inflammatory cells**

Circulating inflammatory cells might be a target for immune processes that initiate and propagate renal injury in glomerulonephritis. In some diseases, such as lupus nephritis and IgA nephropathy, evidence exists for activation of the various mononuclear cell types including monocytes, T- and B-lymphocytes, and platelets [135]. Such activation may result in the release of several cytokines either into the circulation or locally in the kidney, or into specialized tissue such as lymphoid organs, where cell proliferation and phenotypic modulation of these cells can occur. Increased expression and release of these cytokines probably affects the severity of the renal lesions. It is very unlikely that PDGF or TGFβ secreted by circulating mononuclear cells would be readily accessible to the glomerulus, interstitium, or even blood vessel wall, since these cytokines would be rapidly inactivated in the circulation by complexing with plasma proteins. However, IG_F, IL-1, IL-6, and TNF are some of the cytokines that probably gain access to target organs not only through autocrine or paracrine pathways but also through the systemic circulation [116]. Mononuclear cells that are trapped in an inflammatory lesion within the glomerular microvascular bed or the interstitium may be in direct contact with each other and with glomerular or interstitial cells. Monocyte entry and macrophage exit from renal lesions might be a continuous and direct process, as has been demonstrated in experimentally induced atherosclerotic lesions [137]. The possibility therefore exists that circulating mononuclear cells are activated during their journey in the glomerular microvascular bed and interstitium and result in the local release of PDGF, TGFβ, or other cytokines. Such a scenario is consistent with observations of a positive correlation between PDGF B-chain expression in peripheral blood mononuclear cells and histopathologic changes and urinary protein excretion in a recent study of IgA nephropathy [138, 139]. The demonstration of an increased expression of these cytokines in monocytes, macrophages, or lymphocytes infiltrating renal tissue would provide more direct evidence for a role for mononuclear cells in glomerular and interstitial lesions. In this setting, it is likely that monocytes and macrophages as well as T-cells interact with each other and with intrinsic glomerular or interstitial cells. This interaction leads to the release of multiple cytokines that result in cell proliferation, matrix expansion, and eventual fibrosis.

**Conclusion**

The interest in cytokines as regulators of cultured glomerular cells has grown rapidly and now includes active exploration of the role of cytokines in glomerular injury in vivo. Their role in mediating interstitial changes, which invariably accompany glomerular disease, is less well researched but currently is being explored. The availability of recombinant cytokines, neutralizing antibodies, and receptor antagonists should facilitate studies exploring the role of cytokines in mediating renal injury in experimental models of glomerulonephritis and other models of glomerular injury. Detailed chronologic studies in experimental animals might establish a cascade of events specific for certain mechanisms of injury. In addition, such studies will help establish temporal relationships between cytokine expression, inflammatory cell infiltration, cell proliferation, matrix expansion, and changes in renal function. Logistic difficulties will be encountered in assessing the role of cytokines in human subjects. However, a concerted application of techniques such as immunohistochemical staining and in situ hybridization to renal biopsy specimens from patients with different diseases is likely to advance our understanding of cytokine involvement in human renal pathology very quickly. The results of such studies also might bring us a greater understanding of the mechanisms of renal injury; we might even be forced to redefine what constitutes an active lesion warranting continuous treatment. The expression in a renal biopsy specimen of certain phenotypic markers denoting cell activation (for example, a cytokine, or an intermediate filament such as alpha smooth muscle actin) may well turn out to be a more sensitive index of disease activity than is a simple assessment of cellularity or crescent formation. The participation of other inflammatory cells, such as eosinophils and mast cells, remains unexplored. There might be therapeutic implications for cytokine expression in intrinsic glomerular cells versus infiltrating inflammatory cells. An initial regimen of immunosuppression could be followed by maintenance courses of less toxic agents that result in the “deactivation” of mononuclear cells and thereby decrease expression of cytokines. Identifying potential inhibitors of glomerular cell proliferation and cytokine release or action in vitro might have important applications in vivo. These agents also might improve glomerular pathology by a direct inhibitory effect on mesangial cells as well as inhibition of growth factor release from platelets, monocytes, or lymphocytes. I believe there is a good rationale for suppressing the immune system and at the same time attempting to inhibit glomerular and interstitial cell activation, proliferation, and matrix expansion. It makes perfect sense to use chronic regimens of less toxic drugs that can inhibit or deactivate intrinsic glomerular cells as well as the remaining infiltrating inflammatory cells, and thereby prevent the development of otherwise progressive renal failure.
Questions and answers

DR. F. PAOLO SCHEMA (Chief of Nephrology, University of Bari, Bari, Italy): Circulating immune complexes, or "in-situ" formed immune complexes, activate the complement system at the glomerular level. Over the last few years, investigators have demonstrated the presence of some complement components, such as C5b-9, in the active phase of some glomerulonephritides (lupus nephritis, IgA nephropathy, and membranous nephropathy) [140—142]. Moreover, other investigators recently have shown that C5b-9 induces cytokine production in cultured mesangial cells [143]. Is there a correlation between the complement system and growth factors?

DR. ABBoud: Lovett et al first reported that C5b-9 membrane attack complex stimulates IL-1 production in mesangial cells [144]. The study you are citing demonstrated that C5b-9 stimulated the synthesis of IL-1, IL-6, and TNF by mesangial cells [143]. Immune complexes acting through Fc receptors, however, can directly activate mesangial cells to release cytokines, specifically colony-stimulating factor (CSF-1) and monocyte-specific chemoattractant protein (MCP-1); this effect was demonstrated recently by Hora et al [145].

DR. JORDAN J. COHEN (Dean of Medicine, State University of New York at Stony Brook, Stony Brook, New York, USA): You stated that in only a few instances thus far has inhibition of a cytokine been shown to retard the development of disease. In the absence of more data, how confident can we be that the mere presence of a given cytokine in the tissue, or even of the mRNA for a given cytokine, has pathogenetic significance, given the welter of these cytokines that are present?

DR. ABBoud: It is true that the association of overexpression or underexpression of a particular cytokine with a renal lesion does not necessarily establish a pathogenetic role. To establish a cause-and-effect relationship, one has to rely on antibody or receptor antagonist studies and demonstrate that neutralizing the biologic effect of the cytokine ameliorates or abolishes the structural or functional consequence of a disease process. Neutralizing antibodies for TGF-β and PDGF recently have been utilized to establish the role of these two cytokines in mesangial cell proliferation and matrix expansion in a rat model of acute proliferative glomerulonephritis [65, 94]. Changes in the expression of a cytokine, however, might be helpful as a diagnostic tool for determining the state of activation or differentiation of infiltrating inflammatory cells or intrinsic glomerular cells. Most inflammatory cells express cytokines only when they are activated [27]. Expression of cytokines by intrinsic glomerular cells also might be a sign of activation.

DR. R. BERNDT STERZEL (Professor of Medicine, University of Erlangen-Nürnberg, Germany): I am concerned about how cytokines might be influenced by the variable degree of sclerosis present in these glomerular diseases. It seems to me that sclerosis itself might affect the presence of cytokines in at least two ways. One, cytokines might be sequestered passively in the sclerotic material, and two, the matrix material in the sclerotic areas might modulate the phenotype of the mesangial cell, altering PDGF expression.

DR. ABBoud: This is an important issue that I would like to discuss further. Changes in the amount, nature, and composition of matrix can have profound effects on mesangial cell behavior [18, 85, 146]. There is a continuous and dynamic process of communication between matrix and cell-surface receptors. Matrix components also facilitate cell-to-cell communication. Certain cytokines, such as TGF-β or basic fibroblast growth factor, bind to matrix and are released slowly, thereby influencing the surrounding cellular elements. Therefore, one mechanism by which extracellular matrix material might influence behavior of cells is via sequestration and subsequent release of cytokines. This process could be regulated by inflammatory mediators [147].

Extracellular matrix could modulate mesangial cell behavior by additional mechanisms. Specific receptors for matrix proteins are present on most cells. For example, receptors for the integrin family of adhesion molecules are present on glomerular cells and may mediate the binding of matrix proteins such as laminins, collagens, and fibronectin [18]. Matrix protein might influence growth and differentiation of cells as well as expression of cytokines and/or cytokine receptors. Matrix modulates the release of IL-6, the expression of PDGF receptors, and the synthesis of collagen by mesangial cells [18]. This area deserves more exploration in vitro as well as in vivo. Particularly relevant is the identification of the effect of the matrix on glomerular cells in vivo. For example, what is the interaction between matrix components and glomerular cells in patients with a moderate degree of sclerosis? Studies in experimental models of progressive glomerular disease provide evidence for the presence of activated and proliferating cells around such lesions, even when total cellularity is decreased [148]. This observation raises questions about the traditional interpretation of human renal biopsies and about our current ability to distinguish between active disease, on the one hand, and chronic and/or inactive disease, on the other. I believe that special techniques should be applied to identify markers for disease activity such as the expression of intermediate filaments or cytokines [149]. Cytokines have diverse and occasionally opposite biologic effects. Therefore, different cytokines can be expressed at different stages of a disease process.

DR. COHEN: Dr. Striker, would you like to comment?

DR. GARY STRIKER (Director, Division of Kidney, Urologic, and Hematologic Diseases, National Institutes of Health, Bethesda, Maryland, USA): A couple of findings have turned out to be a major surprise for us. One is that examination of histologic sections is not sufficient to determine proliferation. We are reasonably good at counting dots, but that gives us no idea about cell turnover; proliferation must be assessed directly [148]. I agree with Dr. Sterzel that the kind of matrix that one finds needs to be defined more carefully. Pathologists can stain tissue with PAS, but that method doesn’t tell us much about the amount, type, or mixture of various matrix components, all of which are likely to be very important. I am very impressed, Dr. Abboud, with your notion of a rheostat—of up-regulating one and down-regulating another. I agree that the balance of growth factors will be very important. Finally, I believe we have to stage these complex processes carefully. Antibody studies alone might be misleading; for example, TGF-β at one stage is probably quite important, as you’ve shown, whereas PDGF might be important at a later stage.

DR. LORETO GESUALDO (Clinical Fellow, Division of Nephrology, University of Bari): I’ve been interested in the expression of PDGF mRNA in renal biopsies [6]. We have been unable to
find an increased expression of PDGF mRNA in IgA nephropathy during the G5 stage (that is, end-stage renal disease), but we do find it during stages G3 and G4. So maybe Dr. Sterzel's suggestion is correct: PDGF might merely bind to the matrix at late stages and thereby become detectable by immunoperoxidase staining, but its presence might have no pathogenetic significance. I believe, however, that in the early stages of the disease, PDGF alone or in combination with other growth factors could play an important pathogenetic role.

I have a question: since cAMP analogues can reduce proliferation, when do you think therapy should be started with such analogues? IgA nephropathy is a chronic disease. Patients usually do not develop renal insufficiency until the disease has been present for many years. Do we have to begin treatment early, or should we wait until renal insufficiency begins to develop?

DR. ABBOUd: Let me address the first point you raised regarding cytokine localization by immune peroxidase techniques and/or localizing the mRNA by in-situ hybridization. If at an early stage of disease immunoperoxidase or immunofluorescence staining shows that PDGF protein is present, yet in-situ hybridization fails to disclose that mRNA is expressed or up-regulated, one should consider the platelet as a source of the PDGF; platelets have very little machinery for synthesizing proteins and therefore might merely release PDGF and deposit it in extracellular sites. If, by contrast, one finds that PDGF mRNA is expressed and up-regulated, it is likely that macrophages or mesangial or endothelial cells are the sources of PDGF. In advanced stages, the failure to demonstrate PDGF mRNA expression might be due to the presence of very few activated glomerular cells or macrophages. In advanced acellular lesions, obviously one does not expect to detect mRNA.

With respect to treatment using cAMP analogues or adenylate cyclase agonists, I certainly believe that this approach should be considered seriously. The limitation of a protracted course of immunosuppressive drugs is obvious. Prostaglandin analogues that activate adenylate cyclase are potential therapeutic agents. We and others find that prostaglandins inhibit mesangial cell proliferation in vitro. Studies in experimental animals also demonstrated therapeutic benefit in glomerulonephritis [150]. The administration of drugs that can deactivate cells and inhibit cell proliferation may have a "sparing effect," permitting the use of lower doses of immunosuppressive therapy. Compounds that decrease platelet aggregation, which also happen to elevate cAMP levels, deserve further trials because they might be effective in some patients with proliferative glomerular diseases, including those with IgA nephropathy [130].

DR. LEON G. FINE (Professor and Head, Department of Medicine, University College and Middlesex School of Medicine, UK): Your discussion focused primarily on the glomerulus. The patient under discussion had progressive renal disease that was accompanied by extensive interstitial fibrosis and tubular atrophy. As we know, many pathologists with vast experience would argue that what one sees in the glomerulus in progressive renal disease has little to do with whether the disease will progress. Extensive studies by Professor Bohle and colleagues revealed that changes in the tubulointerstitial compartment appear to reflect the progressive nature of the disease better than the glomerular changes do [151]. Why is this the case? I would suggest that the same growth-factor-related damage that you're describing for the glomerulus also could affect peritubular capillaries. It is now known, for instance, that PDGF is produced by some tubular cells—certainly the cells of the collecting duct [61]. I find it very interesting that the fibroblast of the renal interstitium, with its regional responsiveness to PDGF, indicates that paracrine growth systems likely are present in the interstitium. Johnson and colleagues have studied glomerular injury and have shown nicely that tubular expression of PDGF also is present [64]. It's entirely possible, therefore, that the progressive obliterative vascular injury that occurs in the interstitium is in fact mediated by a tubular lesion that is possibly ischemic and which leads not only to the tubular atrophy, but to the release of growth factors. Finally, this release of growth factors sets up a fibrotic response in the interstitium, further aggravating the vascular destruction. Maybe that's the reason these diseases progress. This is clearly only a hypothesis at the moment, but we cannot ignore this possibility when considering the relationship between growth factors and the progression of renal disease.

DR. ABBOUd: I did not discuss interstitial damage in detail, not because it is unimportant; the interstitium obviously is very important, and the careful studies that are now being reported by several groups, including yours, have emphasized the potential role of the interstitium in progressive glomerular injury [9]. In some forms of IgA nephropathy, for example, mononuclear cells are present in the interstitium [138]. Mononuclear cells infiltrate glomeruli only in crescentic lesions. The interstitium undoubtedly affects the glomerulus, and vice-versa. The blood supply to the interstitium passes through the glomerulus. Both units are therefore interdependent, and a primary injury to one segment can have a major influence on the other. Moreover, many pathologic processes often target both segments.

DR. LILIANE STRIKER (Senior Investigator, National Institute of Diabetes and Digestive and Kidney Disease, National Institutes of Health): I think it's a very exciting time for investigators in this field, and I'm very grateful to Dr. Abboud for emphasizing the role of growth factors. For the first time, we are seeing that we can measure proliferation as, for example, Johnson's group has done, using antibodies to cyclin (PCNA) [65]. I'm hoping for a time when we'll be able to analyze biopsy tissue from a patient with IgA nephropathy, for instance, and say that the patient has this many attomoles of type-I collagen, and this many attomoles of PDGF. Therefore, let's start treatment with antisense, for instance, to get rid of the PDGF, or the TGF-β, or the IL-6. I think we are heading towards an era when we are going to be able to produce very specific responses. I would like Emmanuel Peter to comment because he has developed in our laboratory a technique using microdissection combined with competitive polymerase chain reaction (PCR) that I believe holds great promise for quantitating such substances in individual glomeruli or, for that matter, in the interstitium or tubular cells as well.

DR. EMMAuEL PETER (Postdoctoral Research Fellow, National Institutes of Health): With the new technique of competitive PCR applied to microdissected human glomeruli, we have for the first time a quantitative way of assessing specific molecular events in sclerotic glomeruli [152]. Competitive PCR, as opposed to standard PCR, is a very sensitive way of reproducibly quantitating absolute amounts of mRNAs in very...
small samples, that is, in microdissected glomeruli. All the variables that affect a standard PCR reaction, and that have hampered its use as a quantitative method, are controlled in competitive PCR. The basic principle is that the molecule to be quantitiated is co-amplified with an essentially identical synthetic cDNA that will compete with the test cDNA on an equimolar basis. The level of detectability routinely achieved in the laboratory is in the 10⁶ atomole range (1 atomole equals 10¹⁶ mole)! As Dr. Abboud just pointed out, assessing mRNA expression of some of the multiple growth factors and of some of the extracellular matrix proteins yields only a partial view and is short-sighted. Instead, we have to try to integrate the various growth factors suspected of playing a role in glomerular diseases into more global schemes and, in the case of the glomerular extracellular matrix, investigate whether both matrix synthesis and degradation are involved. Such studies utilizing the new tools of molecular biology hopefully will allow us to predict more precisely the outcome of a glomerular disease in a patient by basing such predictions on the level of gene expression in the glomeruli.

DR. COHEN: Is the amount of the renal tissue that we are now obtaining by routine biopsy procedures sufficient to perform these kinds of studies?

DR. PETER: At present, to quantitate one single mRNA species in a human biopsy specimen with quantitative PCR requires as little as 0.6 of a glomerulus. For example, with a human biopsy sample containing 30 glomeruli, the pathologist could use one half of the tissue to make a diagnosis and could use the other half for quantitative PCR assay. With that much material, it is possible to quantitate in absolute terms at least 15 mRNA molecules. We can do a lot with very little tissue. In the near future, we might even be able to use less material; for example, 1/50th of a glomerulus might be sufficient to quantitate some mRNA species by competitive PCR.

DR. COHEN: Let me ask Dr. Striker about her futuristic speculation. How might one deliver antisense molecules to be sure they get to the right place?

DR. STRIKER: You’ll have to ask a molecular biologist. It’s my hope that this will be achieved. For now, we are just trying to describe what happens in human diseases and hoping that, in the future, some investigators will figure out how to manipulate the system therapeutically.

DR. NAPIER THOMSON (Monash Medical Centre, Melbourne, Australia): I would like to play the devil’s advocate. You stressed the potential damaging effect of various growth factors on the glomerulus. Might some growth factors not have beneficial effects? After all, it’s a whole soup, isn’t it? We’re not talking about a single element acting at a single time.

DR. STEWART CAMERON (Professor of Renal Medicine, Guy’s Hospital, London, England): I was just going to raise the same question. For example, you mentioned the very intriguing, and to me inexplicable, studies by McDevitt, who gave TNF to mice with lupus and made them better [153]. A lot of evidence now exists that a TNF-dependent, murine model of immune injury—allogenic encephalomyelitis—is ameliorated by TGF-β [154, 155]. Would you like to comment on these observations?

DR. ABBoud: These are very important and pertinent questions. Certainly growth factors or cytokines can have beneficial effects. I do not believe that we should classify cytokines as “good” or “bad.” There is tissue specificity in terms of the biologic role of cytokines. More important, within the same tissue and for a given disease entity, it is likely that a given cytokine can play both a beneficial and a deleterious role, depending on the timing of up- or down-regulation of either the cytokine itself or its receptor. For example, PDGF is an important mediator of mesangial cell proliferation in vitro and in vivo. Although mesangial cell proliferation and hypercellularity generally are features of glomerular pathology, the mesangial cell migration and proliferation that follow mesangiolysis could represent a beneficial effect of PDGF, helping to repopulate and restore the cellular architecture of the glomerulus. Similar roles have been postulated for EGF in tubular epithelial cell regeneration after acute tubular necrosis [12]. TGF-β is another protein that elicits cellular responses of opposing nature; it can elicit both immunostimulatory and immunosuppressive effects in vitro as well as in vivo [98, 99], albeit its immunosuppressive effects are more widespread. These latter effects include in vitro inhibition of cytokine production, T- and B-cell proliferation, and the expression of DR antigens and Fc receptors on human lymphocytes. With regard to macrophage functions, TGF-β is basically an antiinflammatory molecule that can deactivate macrophages in vitro by reducing their production of cytokines and H₂O₂ and by reducing their cytotoxicity. These in vitro data provided the rationale for the in vivo studies that you referred to, Dr. Cameron; as you noted, these studies demonstrated a protective effect of TGF-β in experimental autoimmune encephalomyelitis, as well as in acute and chronic arthritis in experimental animals [98].

DR. ARTURO BORSATTI (Professor of Nephrology, and Director, Division of Nephrology, University Hospital of Padova, Padova, Italy): In reviewing the factors controlling mesangial cell growth, you did not mention nitric oxide. I understand that, strictly speaking, nitric oxide is not a growth factor, but it has been shown to retard mesangial cell proliferation [156]. Activated macrophages can synthesize a tremendous amount of nitric oxide [157]. Accordingly, couldn’t infiltration by macrophages be considered potentially beneficial rather than deleterious?

DR. ABBoud: Indeed, nitric oxide and cyclic GMP have been shown to inhibit proliferation both of mesangial cells and smooth muscle cells in vitro. Macrophages do release large amounts of nitric oxide. What I am not sure about is whether “activation” of mononuclear cells entails a generalized mechanism in which the cells release growth factors and nitric oxide simultaneously, or a selective mechanism in which the cells release some but not all macrophage products. Again, whether infiltrating macrophages play a beneficial or a deleterious role likely depends on the stage and nature of the pathologic lesion.

DR. VICKI E. RUBIN-KELLY (Associate Professor of Medicine, Harvard Medical School, Boston, Massachusetts): I would like to make two comments. The first has to do with the administration of cytokines. I think the dose is critical. Dr. McDevitt’s group used extremely high doses of TNF and found improvement not only in animals with lupus [153] but in non-obese diabetic mice as well [158]. Large doses of IL-1 also can retard disease. Since we reported increases in TNF and IL-1 in lupus nephritis [113], we examined the issue by looking at a wide range of doses and found that lower levels of cytokines produced a more physiologic level and, when given at
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a certain point in the disease, accelerated the disease process [115]. I think large doses of TNF, in particular, probably act directly by elevating cyclic AMP levels and turning off cell division, whereas lower doses indirectly induce pro-inflammatory events.

My second comment has to do with antisense. Whereas the delivery of antisense into the cell is a very appealing concept, it turns out to be a tremendous problem. Moreover, the duration of action, that is, the interval over which the target molecule is actually suppressed, is also very limited; after 24 hours, you have to give the antisense again. What’s worse, repeated injections of antisense might not be effective. So even though the concept of using antisense molecules therapeutically is very appealing on theoretical grounds, in practical terms, we’re not there yet.

Dr. Abboad: Your first point is well taken. The concentration of the cytokine certainly has to be taken into consideration. Certain cytokines have biphasic effects, depending on the concentration being achieved. When cytokines are administered systemically, the effects on the immune system in general can differ from the effects exerted locally at target tissues such as the glomerulus or interstitium. Your second point is also well taken. The delivery system is a very important issue that is being addressed by several groups. I would not be surprised if, for therapeutic purposes, targeting proteins proved to be a lot easier than targeting DNA.

Dr. Giuseppe Remuzzi (Head, Laboratory of Kidney Diseases, Mario Negri Institute for Pharmacological Research, Bergamo, Italy): I’d like to make a general comment. I think we are in a very early stage of this new game. We are dealing with a complex problem of interaction between various cells and multiple factors. Using techniques such as in-situ hybridization, we find virtually everything we look for. I doubt that this is the right approach to answering the questions we have. Let me use an analogy. People have wondered for years about the relative importance of coagulation factors, fibrin, and platelets in thrombosis, and in vasoconstriction in patients with myocardial infarction. At least a partial answer to the problem was provided when it became possible to pharmacologically inhibit a single factor at a time. An example is inhibition of thromboxane by the administration of low-dose aspirin. Using such information, researchers have been able to reduce the effects of unstable angina and the mortality rate from reinfarction. In my view, we should develop pharmacologic tools, let’s say molecules that bind to a specific receptor, and see what happens, first using experimental animals and then human beings. In contrast to the problem with myocardial infarction, however, in this case we are dealing with a chronic process and not an acute phenomenon. To make matters worse, these are relatively rare diseases, so it’s very difficult to conduct well-controlled studies that will provide definitive answers. I think it’s going to be very difficult to sort all this out.

Dr. Abboad: It is true that because multiple cytokines might be expressed simultaneously, it is difficult to target a particular cytokine for neutralization. However, serial biopsies from experimental models of renal disease, as well as careful analysis of human renal biopsies at different stages of glomerulonephritis, might identify the predominant expression of a particular cytokine or its receptor that might play a pathogenetic role. It certainly is not going to be an easy task.

Dr. Striker: I have two comments. First, I think it is very important to try to find the right ligand-receptor interactions and to try to locate the region or regions within a given molecule that are responsible for different cell responses by the same ligand-receptor interaction. Cross-talk between cells depends on the cell, the receptor and, of course, the post-receptor signaling, which can differ among individual cells. My second comment is this: cell culture techniques give us very powerful tools, but it’s important for us to recognize that when we take mesangial cells out of their natural habitat and put them in culture, we are creating quite a “cultural shock.” Mesangial cells respond differently in vitro than they do in vivo. For example, one finds different receptor numbers and the expression of different matrix molecules as a function of time [8]. It’s very important, therefore, that we clearly distinguish between “mesangial cell” and “mesangial cell in culture.” Cell culture is a very nice way of looking at certain reactions, but the difficulty in extrapolating from the mesangial cell to the glomerulus and then to the overall function of the organism is going to be a critical issue for us to keep in mind.

Dr. Abboad: I agree.

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