Permeability Factors in Focal Segmental Glomerulosclerosis

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The pathologic diagnosis of focal segmental glomerulosclerosis (FSGS) is associated with a syndrome of steroid-resistant nephrotic syndrome and progressive renal insufficiency. The incidence of FSGS has increased in recent years. Known causes of FSGS include genetic abnormalities, viral infections, decreased nephron number, and hyperperfusion/hyperfiltration. The etiology is unknown in the majority of cases. FSGS recurs after initial renal transplantation in as many as 30% to 50% of patients. Recent studies have verified the hypothesis that plasma of patients with FSGS contains a factor or factors that increase permeability of glomerular capillaries and cause proteinuria after injection into rats. Patients who experience posttransplant recurrence of FSGS and those with rapidly progressive disease exhibit this activity. Permeability activity has been verified in functional assays and defined by measurement of albumin permeability (P_{ab}) or glomerular volume variation (GVV). Permeability activity is decreased by plasmapheresis or immunoadsorption and can be recovered from discarded plasma or eluate from adsorption materials. Studies from our laboratory indicate that permeability activity is carried by small, highly glycosylated, hydrophobic protein(s)/peptide(s). Normal plasma contains substances capable of blocking or inactivating the FSGS permeability factor. Pharmacologic agents including cyclosporine, indomethacin, and derivatives of Trypterigium wilfordii also block permeability activity in vitro. The observation that permeability activity can be blocked by diverse agents raises hope that specific therapy may be designed for FSGS. Future investigations will permit identification of the active FSGS permeability factor, of mechanisms that initiate and perpetuate proteinuria, and of interventions to prevent renal failure in native kidneys and recurrence of disease in renal allografts.

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belief in the nephrology community that removal of circulating permeability-inducing substances improves clinical outcomes.

Work to clarify the relationship of a circulating proteinuric factor to FSGS remained dormant until the early 1990s when seminal observations from several centers provided strong evidence that an etiologic factor was present in a number of patients with recurrent FSGS. In 1994, we reported that incubation in medium containing serum or plasma from patients with recurrent FSGS increased albumin permeability (P_{\text{ Alb}}) of glomeruli isolated from normal rats and that the capacity to increase P_{\text{ Alb}} was reduced by plasmapheresis.\textsuperscript{4,14} During that same year, other investigators reported that injection of eluate obtained from plasma of patients with recurrent FSGS and eluted from protein A-immunoadsorbent material induced albuminuria in rats.\textsuperscript{3} These investigators have since modified our technique for studying permeability activity in vitro and they and others have confirmed our initial reports that such activity is present in plasma of patients with FSGS.\textsuperscript{15,16} In vitro testing using functional assays of permeability based on glomerular volumetric responses to oncotic gradients has been essential to advancing our understanding of permeability factors in FSGS. This review focuses on the clinical correlates, physiologic effects, and identification of FSGS permeability factor(s) and speculations about future directions in diagnosis and therapy of FSGS.

**INCREASED INCIDENCE OF PRIMARY AND RECURRENT FSGS**

The incidence of primary FSGS has increased markedly during the past 20 years. This increase appears to represent a real change in the disease rather than merely a change in nomenclature. In a review of 1,000 biopsy examinations of adults with nephrotic syndrome from 1976 to 1979 and 1995 to 1997, FSGS increased from 15% to 35%, whereas the incidence of MCNS and of membranoproliferative glomerulonephritis declined.\textsuperscript{17,18} In African Americans, FSGS accounted for 50% of patients with nephrotic syndrome. It appears that the increase in the diagnosis of FSGS in adult patients may be related, in part, to a decrease in the biopsy diagnosis of minimal change nephrotic syndrome and to the emergence of collapsing glomerulopathy, a severe variant of FSGS. A review of articles indexed by the National Library of Medicine indicated that the number of reports describing recurrent FSGS after transplantation during the past 10 years was more than 4-fold greater than the number during to the prior 10 years. It is likely that part of this increase indicated enhanced interest in recurrent FSGS because many reports described risk factors, diagnostic tests, or postulated etiologic mechanisms of recurrence. In addition, it is possible that the incidence of FSGS recurrence actually has increased. The lack of a formal means for reporting FSGS recurrence precludes accurate assessment of its incidence using databases such as the United Network for Organ Sharing.

Recurrence rates in pediatric centers have been reported to be as low as 15%\textsuperscript{19} and as high as 52% to 59%.\textsuperscript{20} A prospective study of recurrence has been initiated by the members of the Recurrent Allograft Disease Registry. Results of this study will provide a needed estimate of the incidence of recurrence in patients with FSGS diagnosed by renal biopsy examination. The importance of recurrent disease as a determinant of renal allograft loss has increased as strategies to prevent and control transplant rejection have become more effective. When recurrent glomerular disease occurs, it markedly decreases the longevity of renal transplants.\textsuperscript{21-23} Recurrence has been identified recently as the third most frequent cause of allograft failure in a cohort of kidney transplant recipients in Australia.\textsuperscript{24} The only clinical predictors of relative risk for FSGS recurrence in pediatric recipients of first transplants are age at diagnosis\textsuperscript{19} and rapid progression to renal failure in native kidneys.\textsuperscript{16} These may each represent measures of the severity of the disease. The prior loss of allograft to recurrent FSGS is associated with recurrence in subsequent transplants of 80% to 100%. Analysis of United Network for Organ Sharing data indicated that Caucasian recipients, donor African-American kidney in Caucasian recipients, younger recipients, and recipients treated for rejection were at increased risk for allograft loss to recurrence.\textsuperscript{25} The relative risk for recurrence has not been related to sex or pathologic parameters on biopsy examination of native kidney. Pretransplant testing for serum permeability activity has not been applied widely although its predictive value is supported by some reports.\textsuperscript{14,16}
TREATMENT OF FSGS

The apparent efficacy of plasmapheresis in reducing proteinuria and prolonging allograft function provides evidence that a plasma component plays an important role in inducing proteinuria in recurrent FSGS. Remission of proteinuria and stabilization of transplant function has been reported in several series from individual transplant centers.\(^5\)\(^-\)\(^13\) The concept of removing a substance or substances from plasma has been extended to the use of extracorporeal treatment with adsorbent materials including protein A and polyclonal anti-human immunoglobulin antibodies. Each of these decreases the activity of residual plasma to increase glomerular permeability during in vitro testing. Low-density lipoprotein apheresis also may be effective in reducing proteinuria but its effect on the \(P_{\text{alb}}\) activity of serum has not been studied.\(^12\) Reports of responses to extracorporeal therapy are based on experience with a limited number of patients; results are not uniform and not all patients with FSGS in native or transplanted kidneys respond to plasmapheresis by decreasing proteinuria.\(^4\)\(^,\)\(^26\) Common themes are that treatment before the onset of visible glomerular scarring is associated with a higher probability of remission, that the concurrent use of plasmapheresis and immunoadsorption may be helpful, and that many patients have relapses of proteinuria and are dependent on repeated treatments. Alternate therapies for treatment of FSGS in both native kidney disease and posttransplant recurrence include indomethacin,\(^27\) cyclophosphamide,\(^28\) and cyclosporine or tacrolimus,\(^29\)\(^-\)\(^32\) in addition to corticosteroids. The relationship between the efficacy of these agents and the presence of a circulating permeability factor in individual patients has not been defined.

The observation that extracorporeal treatment to remove putative permeability factors is most effective in the absence of histologic changes is consistent with the notion that a permeability factor initiates proteinuria whereas glomerular scarring may perpetuate proteinuria independent of the continuing effects of a circulating factor. This postulate is supported by observations in a series of patients with FSGS and established glomerular scarring and renal insufficiency. In these patients, plasmapheresis markedly reduced the in vitro permeability activity of serum but did not decrease proteinuria.\(^26\) In contrast, pharmacologic approaches have been successful in inducing remission of proteinuria without removal of plasma substances. These include treatment with cyclosporine, tacrolimus, and cyclophosphamide. We have shown that remissions of proteinuria that are induced by cyclosporine occur without alteration in serum permeability activity. In adult patients with FSGS and steroid-resistant nephrotic syndrome, treatment with cyclosporine did not affect \(P_{\text{alb}}\).\(^31\) Serial samples from children also had stable \(P_{\text{alb}}\) during remission of posttransplant proteinuria induced by increased cyclosporine or tacrolimus dosing.\(^32\) We have interpreted these findings as evidence that cyclosporine or tacrolimus can protect the glomerular filtration barrier despite continued presence of a permeability factor.

EVIDENCE FOR MULTIPLE ETIOLOGIES FOR FSGS

FSGS is associated with a wide range of clinical presentations. FSGS may be present at any age, from infancy to old age. Nephrotic syndrome may or may not be a prominent feature. Widely varying responses to therapy support the interpretation that several different processes may be manifest by common clinical and pathologic responses. The histologic lesion of FSGS is associated with morbid obesity, ureteral reflux, aging, and viral infections such as hepatitis C or parvovirus infections.\(^33\)\(^,\)\(^34\) These associations are consistent with the idea that there are multiple causes of focal capillary injury and subsequent glomerular sclerosis. Additional evidence for the contribution of multiple etiologies arises from reports of association of familial nephrotic syndrome and FSGS with genetic defects in proteins of the podocyte. Among those that have been identified in humans are defects in nephrin,\(^35\) podocin,\(^36\) \(\alpha\)-actinin-4,\(^37\) and \(\beta_4\)-integrin.\(^38\) In mice, a defect in CD2AP results in proteinuria.\(^39\) This protein is present in human podocytes in association with nephrin and appears to be important in determining the permeability of the slit diaphragm; no human disease related to it has been described. Additional genetic defects have been localized in humans but have not been completely characterized.\(^40\)\(^,\)\(^41\) We have studied 15 patients with familial FSGS with genetic linkage to chromosome 11. Only 1 of the patients had significant \(P_{\text{alb}}\) activity and this patient had recurrence of proteinuria after transplantation.\(^42\) Others have reported permeability activity in several patients...
with a defect in podocin; recurrence of proteinuria in renal allograft and a good therapeutic response to plasmapheresis were associated in 2 of 5 patients. The concurrence of defined genetic defects and circulating permeability activity is not understood completely; the association suggests that complex interactions among several mechanisms may occur.

EXPERIMENTAL EVIDENCE FOR CIRCULATING FSGS PERMEABILITY FACTOR

Advances in identification and isolation of the permeability factor in FSGS as well as identification of clinical associations and therapeutic potentials have been possible primarily because of the introduction of in vitro assays of glomerular permeability. We developed the first of these assays as a tool for the study of mechanisms of injury to the filtration barrier. It is complementary to clearance methods that use dextran sieving for the study of glomerular disease. Our assay of $P_{\text{ alb}}$ is based on the principle that a transmembrane gradient of a solute across a semipermeable membrane exerts an oncotic force. The oncotic force is the product of the chemical gradient, $\Delta \Pi$, and the reflection coefficient of the filtration barrier, $\sigma$. We showed that the expansion of glomerular capillaries after the application of an oncotic gradient is proportional to the effective oncotic gradient. We have used this principle to calculate the albumin reflection coefficient, $\sigma_{\text{ alb}}$, and $P_{\text{ alb}}$ of glomeruli isolated from normal kidneys and incubated under control or experimental conditions. Glomerular volume is estimated from video images and capillary expansion from the increment in glomerular volume ($\Delta V$).

$P_{\text{ alb}}$ is calculated by the formula: $P_{\text{ alb}} = 1 - \sigma_{\text{ alb}}$, where $\sigma_{\text{ alb}} = (\Delta V_{\text{ experimental}}/\Delta V_{\text{ control}})$. We have used this assay to show that a number of experimental manipulations and agents increase glomerular $P_{\text{ alb}}$. The $P_{\text{ alb}}$ assay is both sensitive and reproducible. However, it is not specific because $P_{\text{ alb}}$ is increased after treatment with many agents. These include protamine, reactive oxygen species, complement-mediated injury in glomeruli treated with anti-Fx1A, cytokines including tumor necrosis factor $\alpha$ transforming growth factor $\beta$, platelet-activating factor, matrix metalloprotease-3 (stromalysin), antibodies to $\beta$ integrin or glomerular epithelial protein 1 (both of which are complement independent), ionizing radiation or eicosanoids, PGE$_2$, PGF$_{2\alpha}$ and thromboxane, and nitric oxide. Some parameters that distinguish the glomerular responses to several stimuli and agents that block the increase in permeability are listed in Table 1.

Others have adapted our in vitro methods and have used them in the study of FSGS. Dall’Amico et al have followed our protocol closely and have used computerized measurements of glomerular size to calculate $P_{\text{ alb}}$. Godfrin et al have modified the method for assessing glomerular responses to an oncotic gradient and have measured the glomerular size distribution of control and experimental glomeruli in low albumin medium. They have calculated a parameter termed $\text{glomerular volume variation (GVV)}$; GVV is diminished after incubation with FSGS sera. Calculation of GVV appears to be subject to more experimental variation than $P_{\text{ alb}}$. However, both GVV and $P_{\text{ alb}}$ activity are present in plasma of patients with FSGS recurrence and are reduced by plasmapheresis and by immunoadsorption, and both appear to reflect comparable changes in capillary barrier function.

ASSOCIATIONS BETWEEN $P_{\text{ alb}}$ AND CLINICAL PARAMETERS

We have studied over 500 serum or plasma specimens from patients with renal diseases. We generally have excluded specimens from patients with known inflammatory renal diseases or active infections because a number of substances commonly associated with inflammation caused a marked increase in glomerular $P_{\text{ alb}}$. Figure 1 depicts the $P_{\text{ alb}}$ activity of sera of patients with FSGS and other conditions associated with proteinuria or renal failure. $P_{\text{ alb}}$ activity of individual patients with FSGS varies widely but average $P_{\text{ alb}}$ of FSGS patients’ sera is higher than values for dialysis patients with non-glomerular causes of renal failure. $P_{\text{ alb}}$ of patients with posttransplant recurrence or rapid progression to renal failure, and of patients with collapsing glomerulopathy, are significantly higher than $P_{\text{ alb}}$ of the entire sample of FSGS patients studied.

$P_{\text{ alb}}$ activity is relatively constant in the majority of patients whom we have followed-up for months or years. $P_{\text{ alb}}$ varied by less than 0.3 in 85% of repeated samples from 34 patients studied during the past several years. Treatment with plasmapheresis or immunoadsorption markedly decreased activity in 90% of patients. $P_{\text{ alb}}$ activity has been studied as a predictor of posttransplant recurrence. Measurements of $P_{\text{ alb}}$ activity predicted
recurrence among pediatric patients.\textsuperscript{16} In other studies, GVV values did not appear to predict posttransplant recurrence.\textsuperscript{15} The reasons for the discrepancy between findings using several methods are not clear. The GVV method is based on light scattering by replicate samples of glomeruli. Thus, the size distribution of a sample of glomeruli incubated in control medium is compared with that of a sample incubated in experimental medium containing FSGS serum or other test agent. Composition of incubation medium used, concentration of serum, and conditions of incubation are slightly different from those in Palb assays. These factors or conditions of serum collection and storage may explain the differences among results. In our retrospective studies, the incidence of recurrence increased with each quartile of P\textsubscript{abl} and the risk for recurrence and of allograft loss increased with increasing P\textsubscript{abl}.\textsuperscript{14} The association between high P\textsubscript{abl} and the incidence of recurrence is evident even among high-risk patients.\textsuperscript{45} These patients all had pretransplant P\textsubscript{abl} levels of 0.7 or greater. Those with P\textsubscript{abl} levels of 0.8 or greater had a higher incidence of recurrence than those with values less than 0.8 (Fig 2).

At the moment, P\textsubscript{abl} testing does not appear to predict the clinical course or sensitivity to therapy of newly diagnosed nephrotic syndrome in child-

<table>
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<tr>
<th>Class of Mediators</th>
<th>Agent Studied</th>
<th>Onset of P\textsubscript{abl} Increase</th>
<th>Known or Proposed Intermediaries</th>
<th>Known Blockers</th>
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<tr>
<td>FSGS plasma</td>
<td>70% supernatant Affinity purified fraction Eluate from Protein A or immuno-adsorption</td>
<td>2 minutes</td>
<td>Eicosanoids</td>
<td>Indomethacin\textsuperscript{67} T wilfordii\textsuperscript{73} Normal plasma\textsuperscript{67} Apolipoproteins\textsuperscript{68} Cyclosporine\textsuperscript{70,71} Tacrolimus\textsuperscript{72} cAMP analogs\textsuperscript{71} T wilfordii\textsuperscript{73}</td>
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<tr>
<td>Polycations</td>
<td>Protamine\textsuperscript{44}</td>
<td>30 minutes</td>
<td>Reactive oxygen species\textsuperscript{44}</td>
<td>T wilfordii\textsuperscript{73} Superoxide dismutase\textsuperscript{45} Dimethyliiourea\textsuperscript{46} Genistein\textsuperscript{55}</td>
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<td>Reactive oxygen species</td>
<td>Superoxide\textsuperscript{45}</td>
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<td>Hydroxyl ion\textsuperscript{46} NO\textsuperscript{55}</td>
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<td>10 minutes</td>
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<td>Complement</td>
<td>Anti-Fx1A + complement\textsuperscript{47}</td>
<td>10 minutes</td>
<td>Membrane attack complex, C5b-9\textsuperscript{47}</td>
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<td>Cytokines</td>
<td>TNF-\alpha\textsuperscript{50}</td>
<td>10 minutes</td>
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<td>T wilfordii\textsuperscript{73} Anti-TNF-\alpha antibody\textsuperscript{60} Superoxide dismutase\textsuperscript{50} Dimethyliiourea\textsuperscript{51}</td>
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<td>Cross linking of integrins\textsuperscript{48}</td>
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<td>HIV accessory protein Vpr</td>
<td>Synthetic Vpr\textsuperscript{54}</td>
<td>10 minutes</td>
<td>Eicosanoids\textsuperscript{54}</td>
<td>Indomethacin\textsuperscript{54}</td>
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<td>PGF\textsubscript{2\alpha}</td>
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<td>Thromboxane \textsuperscript{53}</td>
<td>10 minutes</td>
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Abbreviations: Fx1A, fraction 1A; TNF-\alpha, tumor necrosis factor-\alpha; TGF-\beta, transforming growth factor-\beta; GLEPP-1, glomerular epithelial protein-1.
ren and its use in the follow-up of such patients is uncertain. In contrast, a high P Alb level in pretransplant serum appears to be predictive of posttransplant recurrence in patients who have already reached ESRD owing to FSGS. As noted earlier, the highest P Alb levels (eg, ≥ .80), coupled with the clinical risk factors for rapid progression and/or prior recurrence, are associated with a risk for recurrence of greater than 90% in the patients we have studied. A low P Alb level, however, does not ensure that a patient will be free of recurrence after transplantation.

**ANIMAL MODELS OF FSGS**

Serum, plasma, or their derivatives derived from FSGS patients have been injected into experimental animals to attempt to identify an etiologic factor and to develop a model of FSGS. This model would be useful especially for studying mechanisms of injury and developing therapy for FSGS and other proteinuric renal diseases. Initial studies were performed by using unmodified serum from patients with recurrent FSGS. More recently, we and other investigators have studied the renal responses to injection of material derived from FSGS plasma. We have prepared plasma fractions that are highly enriched in P Alb activity and have studied the effects of preparations of increasing doses of these preparations. A single intravenous injection resulted in proteinuria and albuminuria beginning after about 12 hours and persisted for about 48 hours. The magnitude of proteinuria was proportional to the dose injected and was greater when the material with higher in vitro activity was used. Other investigators also have shown that injection of material derived from plasma by elution from adsorbent material and subsequent manipulation caused albuminuria or proteinuria.
MOLECULAR CHARACTERIZATION OF FSGS PERMEABILITY FACTOR

The availability of a reliable functional assay has permitted considerable advances in characterizing a permeability factor in the circulation of patients with FSGS. We have focused on patients with posttransplant recurrence, as have other investigators. This strategy is based on the postulate that patients with recurrence represent a population with the most aggressive disease who are likely to exhibit high levels of the responsible substance in their plasma. In addition, the use of plasmapheresis and immunoadsorption in the therapy of recurrent FSGS has made large quantities of material available for testing and purification. We have performed biochemical manipulations on plasmapheresis fluid and have determined several characteristics of the FSGS permeability factor. Plasma has been subjected to sequential precipitation, affinity chromatography, high-pressure liquid chromatography, 1-dimensional and 2-dimensional chromatography, and mass spectrometry. The active material in highly enriched preparations carries an anionic charge, is hydrophobic, and elutes in a narrow pH range from hydrophobic interaction material. It consists of highly glycated proteins/peptides with an apparent molecular weight of less than 30 kd. These glycoproteins are absent from comparable preparations of normal plasma and also are absent in sera obtained after therapeutic plasmapheresis. Representative 2-dimensional gels displaying components of the active fraction of FSGS plasma and comparable, inactive, fractions of normal plasma are shown in Figure 3.

Other investigators have produced partially purified fractions of plasma or plasmapheresis fluid or material eluted from protein A affinity material by using protocols similar to ours. They have reported the identification of several components of the active fraction including orosomucoid and several other proteins, specifically, fibulin, apolipoprotein J, vitronectin, albumin isoforms, gamma...
chain fibrinogen, and mannan-binding lectin-associated serine protease, but have not documented that any of these carries permeability activity. They have not shown that these proteins increase glomerular permeability in vitro or that they cause proteinuria after injection into experimental animals.

SUBSTANCES THAT PREVENT INCREASED GLOMERULAR PERMEABILITY

We have shown that serum or plasma of normal humans or other mammals has the capacity to protect the glomerular filtration barrier from the increased $P_{ab}$ caused by FSGS plasma or active plasma fractions. Complete protection of the filtration barrier is conferred when normal and FSGS plasma are present in equal concentrations. Lower relative concentrations of normal serum provide partial protection; neither albumin nor immunoglobulins in commercially prepared fractions have any protective effect. Normal serum also is protective when glomeruli are incubated with it before introduction of FSGS material but not when glomeruli are first exposed to FSGS plasma or its derivatives. Other investigators have identified some of the plasma components that may be responsible for protection as apolipoproteins (apo) E2 and E4 (allelic variants), apo J, apo L, and a 28-kd fragment of apo A-IV. The relevance of these substances to the renal responses of individuals with FSGS has not been defined.

We have tested the protective effects of several pharmacologic agents. Inhibitors of cyclooxygenase, calcineurin inhibitors, including cyclosporine and tacrolimus, exogenous cyclic adenosine monophosphate (cAMP) analogs, and derivatives of a Chinese herbal medicinal plant, Trypterigium wilfordii, each protect the glomerular filtration barrier (Fig 4). The mechanisms by which protection occurs may vary with the agent used. Cyclooxygenase inhibition may have its effects by eliminating synthesis of prostaglandins ($\text{PG}E_2$, $\text{PGF}_{2\alpha}$, and thromboxane $A_2$), because these substances each increase macromolecular permeability. Alternatively, it may be effective because of actions of other affected compounds such as leukotrienes or hydroxyeicosatetraenoic acids.

Cyclosporine increases glomerular epithelial cell cAMP and cAMP; these changes and consequent cellular responses may be sufficient to maintain the barrier because incubation with 8-Br cAMP also provides protection. Alternatively, the mechanism of protection by cyclosporine and tacrolimus may be related to inhibition of the serine/threonine phosphatase activity of calcineurin. The mechanism by which certain glycosides of Trypterigium confer protection is unknown. The documentation of protection by several different and apparently unrelated substances acting on disparate pathways indicates that the cellular functions required to maintain the filtration barrier depend on complex metabolic processes. The fact that complete protection of the filtration barrier can be conferred during in vitro studies lends credence to the idea that intervention to protect or reverse FSGS in its early stages may be effective.

CELLULAR RESPONSES AND MECHANISM OF GLOMERULAR PERMEABILITY FACTORS

The nature of the abnormalities leading to proteinuria has been the subject of investigations for many years. Nephrotic proteinuria appears to arise from abnormalities in the function of the glomerular filtration barrier although lesser degrees of proteinuria may be consequent to impaired tubular
The processing of normally filtered proteins. The extracellular matrix of the capillary wall was first described as the primary barrier to protein filtration and its anionic components proposed as the source of the charge specificity. More recently, the essential roles of the podocyte and of the specific molecular structures of the slit-diaphragm have become evident. This understanding is shown by the identification of nephrin as a major component of the slit-diaphragm. The absence of nephrin results in Finnish congenital nephrotic syndrome in infants. Other glomerular epithelial cell proteins also appear to be essential to maintenance of the filtration barrier. Recurrent FSGS is associated with transdifferentiation of glomerular epithelial cells.

It appears that the mechanism by which the FSGS factor increases glomerular permeability depends on active cellular metabolism rather than charge neutralization. This conclusion is based on the rapidity of the response, the small quantity of the FSGS factor required, its anionic rather than cationic charge, and the fact that a number of inhibitors prevent the increase in permeability. The mechanism that is most clearly documented relies on the action of cyclooxygenase. The permeability barrier is preserved by the inclusion of indomethacin or the thromboxane synthase inhibitor furegrelate in the incubation medium. Several eicosanoids increase $P_{\text{alt}}$ including $\text{PGE}_2$, $\text{PGF}_{2\alpha}$, and a thromboxane $A_2$ mimetic. The protective effects of cyclosporine, T. wilfordii derivatives, and serum components may depend on other cellular responses.

**RELATIONSHIP BETWEEN FSGS PERMEABILITY FACTOR AND GLOMERULOSCLEROSIS**

The current evidence strongly supports the thesis that a substance present in plasma of certain patients with FSGS alters the glomerular filtration barrier and causes proteinuria. However, the relationship between the FSGS permeability factor and glomerular capillary collapse and scarring is not clear. No laboratory has established an animal model based on injection of a derivative of FSGS plasma that displays the full syndrome of glomerular sclerosis and renal failure. It is possible that the scarring arises from the continued proteinuria itself rather than from the effect of the FSGS permeability factor itself. The observation that angiotensin converting enzyme inhibitors and cyclosporine decrease proteinuria and slow the progression of renal failure without removing the permeability factor suggests that proteinuria plays an important role in sclerosis. Experimental evidence also supports the idea that the FSGS permeability factor may favor sclerotic processes by its effects on glomerular cells. The active fraction impairs the induction of nitric oxide synthase and
production of nitric oxide by mesangial cells in response to norepinephrine, alters glomerular metabolism of arachidonic acid, decreases nephrin expression, and changes phosphorylation of glomerular proteins. Further studies will be required to define the interactions among the early responses that lead to proteinuria and to glomerular scarring.

Recently, cross-strain transplantation in rats that result in glomerulosclerosis has been proposed as a model of posttransplant recurrence. Kidneys from Buffalo/Mna rats, a strain that exhibits glomerulosclerosis during aging, were transplanted into LEW.1W rats and the reverse studies also were performed. Glomerulosclerosis occurred in kidneys transplanted into Buffalo rats and regressed in those transplanted into LEW.1W rats. These studies are consistent with the action of extra-renal mechanisms of renal injury but fail to confirm the presence of circulating substance(s) because no tests of plasma permeability activity were reported.

TREATMENT RECOMMENDATIONS

Nephrotic syndrome in FSGS is notoriously difficult to treat and progression to renal failure is common. Nonetheless, some patients respond to treatment with corticosteroids or cytotoxic agents and reports of uncontrolled series support aggressive and prolonged therapy in FSGS in native kidneys. Responses are more common in children and in patients with early disease. The heterogeneity of responses may be predicated on the wide variety of etiologies of FSGS or to the inherently refractory nature of the lesions that result in the syndrome. About 70% of children will respond by a decrease in proteinuria when challenged with a cocktail of steroids and immunosuppressive medications. The remaining 30% have persistent nephrotic syndrome. Patients who experience a decrease in proteinuria have a more benign course than those who are refractory to therapy. Adult patients have a lower response rate and a higher rate of rapid progression to renal failure despite treatment. Regimens used include combinations of high-dose corticosteroids and cytotoxic agents. The role of cyclosporine has been explored in studies of both children and adults. There is a high rate of partial or complete remission of proteinuria, but relapses are common after discontinuing treatment. This fact, coupled with fears that cyclosporine may induce or hasten scarring, has limited the widespread use of this agent.

The question arises whether testing for permeability factor(s) or activity may be used as a guide to therapy. Little information is available to answer this question. We have recently reported measurements recently of $P_{ab}$ using sera from 26 pediatric patients with newly diagnosed and as yet untreated nephrotic syndrome. Average $P_{ab}$ level was $0.45 \pm 0.04$, and this was a wide range of values. In this small series from 2 centers there was no obvious association between steroid responsiveness and $P_{ab}$. These findings stand in marked contrast to our initial findings in established steroid-responsive nephrotic syndrome. They indicate that high $P_{ab}$ activity is present in many children at the onset of proteinuria. It is possible that activity may diminish during the course of the disease or in response to therapy. Alternatively, undefined compensatory mechanisms such as induction of circulating neutralizing or blocking substance(s) or resistance of the glomerular cells to the activity of the permeability factors may permit resolution of nephrotic syndrome. Resistance of glomerular cells to the effects of the circulating factor appears to be the operative mechanism in remissions of proteinuria induced by cyclosporine in native FSGS and after transplantation because circulating $P_{ab}$ level is unchanged by cyclosporine and is only transiently reduced by plasmapheresis. We also have observed spontaneous loss of $P_{ab}$ activity in an individual patient in association with spontaneous resolution of posttransplant proteinuria (unpublished data). In addition, we have documented the loss of activity as well as resolution of proteinuria in a single patient with established FSGS and newly diagnosed Hodgkin’s lymphoma within a few weeks after initiation of successful chemotherapy (unpublished data). Thus, the limited information available supports the potential for remissions induced by decreased serum $P_{ab}$ activity during spontaneous remission, after cytotoxic therapy, or after removal of an active substance by plasmapheresis or immunoadsorption. Observations also confirm the potential for glomerular protection by pharmacologic agents including cyclosporine, tacrolimus, T. wilfordii extracts, and cyclooxygenase inhibitors.

In our opinion, treatment of posttransplant recurrence of FSGS with extracorporeal therapy with plasmapheresis and/or immunoadsorption should
be undertaken as soon as the clinical or biopsy diagnosis of recurrence is made. Extracorporeal therapy also may be justified in patients with FSGS in native kidneys when life-threatening nephrotic syndrome is present. Its efficacy in native FSGS has yet to be established. Immunosuppressive therapy has considerable efficacy in many patients with FSGS in native kidneys. Adjunctive use of agents including angiotensin converting enzyme inhibitors, angiotensin receptor blockers, or cyclooxygenase inhibitors may diminish proteinuria but probably do not alter the underlying disease mechanisms. Cyclosporine may have dual mechanisms of action including resistance to the effects of the FSGS factor, enhancement of the barrier to filtration by diminished glomerular hydraulic conductivity, Lp, and vasoconstriction. The addition of cytotoxic agents such as cyclophosphamide may be useful, although the mechanism by which these may act has not been defined. Aggressive chemotherapy with multiple agents has not been studied in the context of idiopathic FSGS in native or transplanted kidneys. Potential interventions and their proposed mechanisms of action are outlined in Table 2.

SUMMARY

FSGS is a very common cause of nephrotic syndrome and of progressive renal failure in both children and adults. Its relative incidence has increased during the past several decades as other causes of proteinuric renal disease including membranous nephropathy have diminished. The contribution of genetic abnormalities has been established clearly in the case of nephrin, but the role of other genetic abnormalities associated with glomerulosclerosis is not well understood. The presence of circulating permeability factor(s) is widely acknowledged and the characteristics of some of these have been reported but the precise identification of the factor associated with FSGS and with posttransplant recurrence has not been made. Additional unanswered questions include the origin of the FSGS permeability factor, the nature of the responses that lead to proteinuria, and its relationship to glomerular sclerosis. Despite the lack of complete understanding of the genetic, etiologic, and mechanistic aspects of FSGS, treatment of proteinuria caused by FSGS after transplantation now includes plasmapheresis and plasma exchange, use of calcineurin inhibitors such as cyclosporine and tacrolimus, and antimetabolites such as cyclophosphamide. Prospects for the future include measures to prevent the synthesis of the FSGS permeability factor, to enhance its clearance, to block its interaction with glomerular receptors, and to inhibit cell responses that mediate increased permeability and sclerosis. Further genetic, physiologic, and biochemical and cell biologic studies are needed to understand fully the origin, nature, and mechanism of action of FSGS permeability factor(s). This information will permit design of rational therapy and will provide information central to the understanding of the glomerular filtration barrier.

ACKNOWLEDGMENT

Xuili Ge and Fuad Ahmed provided expert technical assistance in studies described here. Ram Sharma has been essential to the design and performance of all the studies of the FSGS activity and factor in our laboratory. Many collaborators have contributed ideas and material for studies of specific patient groups and have permitted us to assist in evaluation of their patients. Our FSGS patients have provided both plasma and personal encouragement that have made our work possible.

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