Circulating permeability factors may be important in idiopathic nephrotic syndrome (INS) including focal segmental glomerulosclerosis (FSGS) and in recurrence after renal transplantation. Evidence for plasma factors includes posttransplant recurrence of proteinuria and its response to plasmapheresis or immunoabsorption and induction of proteinuria in experimental animals by infusion of patient plasma or its fractions. The authors and other investigators have used proteomic techniques to seek pathogenic molecules. The authors have recently proposed cardiotrophin-like cytokine-1 (CLC-1) as an active factor in FSGS. Other potential permeability factors include hemopexin and vascular permeability factor in minimal change nephrotic syndrome (MCNS) and soluble urokinase receptor in FSGS. In the authors’ studies, in vitro plasma permeability activity is blocked by diverse substances that may decrease levels of active molecules or block the effects of circulating permeability factors. It has been shown that the simple sugar galactose blocks the effect of FSGS serum on albumin permeability in vitro and decreases permeability activity when administered to patients. Because identities of permeability factors and their mechanisms of action are not well defined, therapy of INS/FSGS is empiric. Corticosteroids are the mainstay of initial therapy whereas calcineurin inhibitors such as cyclosporine A (CsA) and immunosuppressive medications provide adjunctive therapy. Nonspecific therapies such as blocking the renin-angiotensin system and controlling blood pressure and plasma lipids may also diminish proteinuria and slow progression. Identification of molecules that initiate proteinuria and application of findings from in vitro studies may lead to development of new treatments to arrest progression and prevent recurrence after transplantation.

Clinical syndromes of idiopathic nephrotic syndrome (INS) include minimal change nephrotic syndrome (MCNS) and focal segmental glomerulosclerosis (FSGS): INS describes a clinical syndrome that includes heavy proteinuria, edema, hypoalbuminemia, and hyperlipidemia in the absence of evident systemic disease. A kidney biopsy is required to further classify INS. Terminology used in the literature may be confusing because routine practice regarding biopsy varies among patient age groups, clinical presentation, and geographical area. Patient groups that may not routinely undergo biopsy include children, some patients with steroid-resistant nephrotic syndrome (NS) and progression to renal failure, and some patients with recurrent NS after transplantation. The most common histologic diagnoses among patients with INS who undergo biopsy are MCNS and FSGS. Investigators occasionally include membranous nephropathy in reports concerning INS. The question of whether MCNS and FSGS are distinct entities or are variants of a single disease has been debated extensively. However, as genetic and viral etiologies of NS have been identified, it has become apparent that neither entity reflects a single pathophysiological process. In this review, we have considered reports concerning circulating “factors” in NS and in recurrent NS after renal transplantation regardless of the terminology used. In the future, it is possible that measurement of such factors may be used to refine various diagnostic categories. Evidence supporting the presence of circulating factors in NS is summarized in Table 1.

Clinical Course and Therapy

MCNS generally has a fairly benign course and is usually steroid responsive. Renal disease progresses slowly even in patients with frequent relapses or steroid dependence. Treatment and clinical responses have been reviewed (1). In contrast, FSGS has a highly variable course. No randomized controlled trials of sufficient numbers of patients are available to provide high-quality information to guide therapy of FSGS in native kidneys or renal allografts. Current therapy results in full or partial remission in only approximately 50% of patients. Treatments that have been used include corticosteroids with or without cyclophosphamide (1,2), cyclosporine (3), mycophenolate mofitil (MMF) (4), and rituximab (5,6). If proteinuria can be diminished by these agents or by nonspecific therapies such as angiotensin converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), and/or lipid lowering agents, progression of renal dysfunction is slowed (7,8). Rapid progression to ESRD is associated with an increased incidence of posttransplant...
plant recurrence of proteinuria and FSGS. Therapy for MCNS in children and adults and of FSGS has been recently reviewed (9–11).

Regardless of debates regarding the etiology of NS, current and proposed therapies include general strategies such as (1) identifying and reversing the primary cause of renal injury, (2) decreasing proteinuria by interventions relating to hemodynamic and/or glomerular cellular responses, and (3) slowing renal fibrosis by the action of nonspecific agents. Current research using animal models and clinical trials is aimed at identifying novel therapies that will address each of these strategies.

### Permeability Factors in MCNS

It has long been postulated that a T cell-derived humoral factor damages the glomerular permeability barrier in MCNS. Candidates include a vascular permeability factor (VPF) and hemopexin. VPF is a lymphokine that is elaborated by concanavalin A-stimulated T lymphocytes from patients with INS. VPF acts on systemic capillaries and on the glomerular permeability barrier (12). Its secretion is enhanced by IL-2, IL-15, IL-12, and IL-18 and is inhibited by TGFβ1 (13). Hemopexin is a protease that activates protein kinase B and Rho A and induces nephrin-dependent reorganization of the actin cytoskeleton in cultured podocytes (14). It reduces endothelial glycocalyx and increases albumin diffusion across glomerular endothelial cell monolayers (14). Injection into rats causes proteinuria and glomerular alterations characteristic of MCNS (15). It is found in the urine of children with steroid-responsive NS and disappears during remission (16). Studies of VPR and hemopexin in patients with FSGS have not been reported.

### Permeability Factors in FSGS

We have been studying permeability activity in serum and plasma of FSGS patients for more than 20 years. Details of our observations are given below. Investigators have recently identified soluble urokinase receptor (suPAR) in the plasma of patients with recurrent FSGS. They postulate that it may be a cause of proteinuria in FSGS (17). Induction of urokinase receptor (uPAR) signaling in podocytes leads to foot process effacement and urinary protein loss via a mechanism that includes lipid-dependent activation of α5β3 integrin (18). The relationship between these factors and the FSGS factor that we have been studying is not known.

### Differentiation between MCNS and FSGS

Early in the course of INS, histologic changes may not be present, making the distinction between MCNS and FSGS problematic. None of the permeability factors proposed for MCNS or FSGS are currently measured in clinical laboratories. In the future, it may be possible to examine patient specimens for a panel of circulating substances. Similarly, diagnostic tests of urine may be developed. Urinary CD80 has been proposed as a diagnostic test to distinguish between MCNS and FSGS (19,20). CD80 appears to arise from podocytes and is found in urine, but it has not been shown in circulation.

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**Table 1. Evidence for circulating factor(s) in nephrotic syndrome caused by MCNS and FSGS**

<table>
<thead>
<tr>
<th>Clinical Observations/Identified Mediators</th>
<th>Experimental Findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MCNS</strong></td>
<td></td>
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<tr>
<td>vascular permeability activity of serum of INS patients</td>
<td>Generation of permeability factor by T lymphocytes (VPF)</td>
<td>12</td>
</tr>
<tr>
<td>increased urinary protease, hemopexin, in active INS</td>
<td>Proteinuria after hemopexin injection into rats. Altered nephrin expression and glomerular endothelial cell monolayer permeability by hemopexin</td>
<td>14 to 16</td>
</tr>
<tr>
<td><strong>FSGS</strong></td>
<td></td>
<td></td>
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<tr>
<td>immediate recurrence of proteinuria after transplantation; efficacy of plasmapheresis, immunoadsorption in reducing proteinuria</td>
<td>Induction of proteinuria in rat after injection of FSGS serum or plasma; increased glomerular permeability after incubation with plasma/fraction</td>
<td>2, 21, 22, 24, 28, 30 to 35, 38 to 40</td>
</tr>
<tr>
<td>suPAR increased in FSGS plasma CLC-1 in active fraction of plasmapheresis fluid from recurrent FSGS patients</td>
<td>suPAR activates uPAR CLC-1 increases glomerular permeability, decreases nephrin expression, FSGS permeability activity blocked by anti-CLC-1</td>
<td>17, 18 52</td>
</tr>
</tbody>
</table>
Posttransplant Recurrence of NS/FSGS

Proteinuria recurs after initial renal transplantation in approximately 30% of patients whose underlying diagnosis is FSGS (2,21). Recurrence may exceed 85% in patients with a history of allograft loss because of recurrence. Risk factors for recurrence include young age, rapid course of primary disease (i.e., renal failure within 3 years of diagnosis of primary disease), previous recurrence in an allograft, and elevated permeability activity in an in vitro assay (22). Renal allografts are often biopsied to establish a diagnosis in the setting of recurrent proteinuria. However, recurrent FSGS is the presumptive diagnosis in patients with primary FSGS and proteinuria in the early posttransplant period. Biopsies in the first hours after proteinuria have no morphologic changes. Subsequent biopsies may show only foot process effacement, but glomerulosclerosis eventually appears unless a remission can be induced. Premature allograft loss after recurrence is common (23,24). Therapy of recurrent FSGS includes early plasmapheresis intended to remove injurious substances(s). Possible alternative interpretations of the observed benefit include the addition of a salutary substance or immunomodulation. High doses of calcineurin inhibitors often improve proteinuria and stabilize renal function. These agents likely have multiple targets (25–27), but they do not decrease circulating permeability activity (27). We speculate that long-term remissions after relatively short courses of plasmapheresis may also be related to protective effects of calcineurin inhibitors or other agents. It is also possible that susceptibility to injurious agents is enhanced by ischemic or immunological injury at the time of transplant, and recovery confers some degree of resistance.

Prevention of Recurrence

Pretransplant plasmapheresis appears to prevent or delay recurrence in high-risk patients (28). Encouragement regarding pretransplant immunotherapy comes from a trial in which transplant patients received hematopoietic donor cells after a non ablative preconditioning regimen. Early recurrence of FSGS was significantly reduced, although only minimal donor-derived engraftment occurred (29). The precise mechanism for this protection is not well defined but might include suppression of generation of a permeability factor by conditioning regimen or by the generation of a chimeric state.

Permeability Factors in FSGS and Posttransplant Recurrence

Investigators have hypothesized the presence of one or more circulating substances injurious to the glomerulus. This hypothesis is supported by observations that proteinuria may begin immediately after transplantation and that injection of patient plasma or its fractions into rats causes proteinuria (30–32). Additionally, plasmapheresis may improve proteinuria and lessen the risk of development of progressive FSGS. Our work (31,33–37) and that of several other groups (38–40) has confirmed that serum or plasma from patients with recurrent FSGS contains one or more substances that injure the filtration barrier and/or cause proteinuria. We and others have studied plasma from patients with recurrent FSGS in an attempt to identify the injurious substance. Our efforts have been aided by the use of an in vitro functional assay of glomerular permeability (41). Isolated rat glomeruli are briefly incubated with patient serum, plasma, or plasma fraction. An albumin oncotic gradient across the glomerular wall is established by replacing the isolation medium with one of lower oncotic pressure. When the protein permeability barrier is intact and uninjured, water flows into the capillaries, causing the glomeruli to increase in size. Incubation with injurious substances such as sera from patients with recurrent FSGS causes a decrease in the albumin reflection coefficient of the membrane \((\sigma_{\text{alb}})\) and a decrease in the effective oncotic gradient. The increase in glomerular volume that occurs in response to an albumin oncotic gradient is then diminished compared to the response of control glomeruli. Because the concept of reflection coefficient has no intuitive relationship to clinical disease, we have calculated the convective albumin permeability, \(P_{\text{conv}}\), as \((1 - \sigma_{\text{alb}})\), a dimensionless parameter that ranges from 0 in normal glomeruli to 1.0 with maximal injury. The reproducibility of the \(P_{\text{conv}}\) assay is high with a correlation of 0.72 \((P < 0.001)\) (33), and an agreement of repeated values within 0.3 in >83% of patients in whom repeated samples have been tested (37). The assay reflects injury to the glomerular protein permeability barrier and is not specific to FSGS serum/plasma. For example, agents as diverse as TNFα, superoxide, or antibodies to protein tyrosine phosphatase receptor and β1 integrin increase \(P_{\text{alb}}\) (42–46).

Sera from some but not all patients with FSGS cause increased \(P_{\text{alb}}\) in isolated glomeruli. In our initial report, \(P_{\text{alb}} > 0.5\) was found in approximately 30% of FSGS samples tested (33). It should be noted that this was a sample of convenience made up of sera from patients with a wide spectrum of disease collected at multiple centers. In that study, sera from nine patients with steroid-sensitive MCNS were negative. We subsequently reported that 42% (11 of 26) of children at presentation with NS had \(P_{\text{alb}} > 0.5\). \(P_{\text{alb}}\) did not discriminate between steroid-responsive and steroid-resistant patients (47). A prospective study of a larger number of children will be needed to interpret these results. In patients whose specimens have been submitted to our laboratory for evaluation, \(P_{\text{alb}} > 0.5\) was associated with more rapid progression to ESRD (48). In patients at high risk for recurrence, defined by rapid progression or previous graft loss to recurrence, \(P_{\text{alb}}\) was uniformly high. \(P_{\text{alb}}\) was also very high in nearly every patient with collapsing glomerulopathy in two independent samples (49,50).

In vitro assays offer the unique opportunity to study integrated glomerular function in the absence of the influence of systemic humoral, hemodynamic, or rheologic forces. The immediate responses to various mediators can be measured and agents that may have a multitude of systemic effects can be readily studied. In addition, more chronic responses can be studied using glomeruli isolated from animal models of disease or after in vivo manipulations. Glomerular cells maintain structure and interaction with other glomerular cells and the extracellular matrix, features that are increasingly recognized as being crucial. However, like other technically demanding as-
says, appropriate effort and time are required to standardize the assay. Several other laboratories have successfully established this technique. There is potential for selection bias, especially when glomeruli are isolated from animals with established disease. It is thus complementary to other assays of glomeruli function.

We have used various techniques for protein isolation and described characteristics of the substance(s) in FSGS serum/plasma that exhibit activity in the $P_{\text{alb}}$ assay. We have shown that the active substance in FSGS serum or plasma is a small protein with one or more glycation sites. It has high affinity for protein A and also has some affinity for cationic materials. It has a hydrophobic domain as evidenced by affinity in a hydrophobic interaction column. During initial fractionation studies, we used ammonium sulfate precipitation and found that $P_{\text{alb}}$ activity was present in a fraction with an apparent molecular weight of approximately 50 kD. All of the activity of the original specimen was retained in this fraction. $P_{\text{alb}}$ activity per milligram of protein was enriched by more than 10,000 fold compared with the activity of the initial plasma specimen (51).

More recent studies (unpublished data) using affinity chromatography and fractionation by membrane sieving indicate that the $P_{\text{alb}}$ activity is present in a fraction with an apparent molecular weight of $\leq$30 kD. With progressive purification it is likely that noncovalently or otherwise loosely associated proteins are removed, accounting for changes in apparent molecular size of the active substance we are studying. Recently, we have used galactose affinity purification to enrich specimens and have used mass spectrometry to identify the proteins in the active fraction (37). Galactose affinity chromatography has allowed us to achieve a high degree of enrichment of activity in a single step.

We have found cardiotrophin-like cytokine-1 (CLC-1) in the active fraction from galactose affinity chromatography. CLC-1, a member of the IL-6 family, is the only cytokine present. Further studies suggest that CLC-1 may be the permeability factor in recurrent FSGS. It is present in active patient plasma, it mimics the effects of FSGS plasma on $P_{\text{alb}}$, and it decreases nephrin expression by glomeruli and cultured podocytes. Strikingly, a monoclonal antibody to CLC-1 blocks the $P_{\text{alb}}$ effect of active FSGS sera. The concentration of CLC-1 in the circulation of patients with recurrent FSGS may be up to 100 times higher than in normal subjects (52). Studies to confirm and expand these observations are ongoing.

**Functional Properties of FSGS Permeability Factor and Clinical Associations**

The permeability factor in FSGS plasma not only has a strong affinity for galactose as noted above, but its activity in the $P_{\text{alb}}$ assay is blocked by galactose in concentrations of $10^{-12}$ M (37). Activity is also blocked by normal serum or plasma (53). $P_{\text{alb}}$ activity is higher in patients with severe and rapidly progressive disease than in those with slower progression (48). Its removal by plasmapheresis or immunoadsorption is often associated with remission of NS (34,38), and pretransplant plasmapheresis may diminish recurrence (28). Interestingly, activity has been found during posttransplant recurrence in some patients with genetic mutations in podocyte proteins (54,55). We can only speculate about the relationship between podocyte mutations and circulating permeability factors. It may be that the co-occurrence is happenstance (i.e., two relatively common conditions coexist). On the other hand, it may be that podocyte abnormalities predispose to more severe injury by circulating substance(s) and result in severe and recurrent renal disease. A study of many podocyte genes and serum activity in patients with renal disease will be required to understand the relationship between these mechanisms for glomerular injury.

**Observations with Implications for Future Therapy**

We have identified several experimental agents that inhibit $P_{\text{alb}}$ activity (36,56). Inclusion in incubation medium of normal plasma (33), eicosanoids including 20-hydroxyeicosatetraenoic acid (57) or 8,9-epoxyeicosatrienoic acid (58), nitric oxide (59), certain glycosides of Trpetygium wilfordii (60), or cyclosporine (61) prevents the FSGS-induced increase in $P_{\text{alb}}$. Inhibition of cyclooxygenase (62), phosphatases, or kinases also prevents the increase in $P_{\text{alb}}$ (35). These observations may lead to future specific therapeutic interventions in FSGS.

**Standard Therapy and Potential New Therapies**

Steroid therapy is a reasonable first-line therapy and is likely to be effective in the early stages of disease, especially in children. Cyclosporine or MMF may induce remissions in additional patients. The relative effectiveness of these agents is not yet known, but clinical experience suggests that they may have approximately the same likelihood of benefit. Control of blood pressure and use of ACEIs and/or ARBs and control of lipid levels with statins or fibrates provide useful adjunctive measures to decrease proteinuria and slow progression. New agents that block effects of aldosterone, renin, vasopressin, or endothelin may be useful in limiting podocyte activation as well as in decreasing proteinuria and controlling blood pressure, but these agents have not been formally tested.

New therapies for patients with steroid-resistant or recurrent NS/FSGS are sorely needed, and multicenter trials will be required. Design of these trials will depend on understanding of mechanisms of glomerular injury and or progression of renal damage. The FSGS Clinical Trial (FSGS-CT NCT00135811) is an example of such a trial. Entry into this multicenter randomized controlled trial comparing cyclosporine to MMF plus pulse dexamethasone has recently been completed. The study is ongoing and analysis has not yet been reported. A multicenter phase I trial of rosiglitazone (63,64) and of adalimumab has also been completed (64,65). The salutary responses in some patients and the low incidence of adverse effects support the potential clinical utility of such agents. A phase II trial is underway (IND#103,147) to compare standard conservative therapy (lisinopril, losartan, and atorvastatin) alone versus standard therapy plus rosiglitazone, adalimumab, or galactose. The rationale for using adalimumab and rosiglitizone is that their potential antifibrotic activity may slow or prevent progression...
of renal disease independent of their effects on proteinuria. The potential efficacy of galactose therapy is supported by a case report of remission of nephrotic syndrome after oral galactose therapy (66) or complete loss of FSGS permeability activity in a single patient with posttransplant recurrence (37). Other agents under consideration or in small pilot trials include anti-TGF antibodies and rituximab, a monoclonal antibody to the B cell surface marker CD20 that depletes B cells.

Strategies for therapy of NS are summarized in Figure 1. Targets for intervention include circulating substances, the podocyte itself, the glomerular endothelium, and profibrotic processes.

Summary

There is evidence that circulating factors may play a role in MCNS and FSGS in native kidneys and recurrent disease in renal allografts. The precise nature of these factors and the mechanisms by which they cause renal injury is the subject of intense investigation. We strongly believe that ongoing studies to define the nature, prevalence of circulating injurious factors, and mechanisms by which they alter the permeability barrier and result in kidney injury must be supported by the renal community and by funding agencies. Large multicenter cooperative studies will be required to test efficacy of novel therapies. Meanwhile, physicians must continue to follow the availability of clinical trials (www.clinicaltrials.gov and other sites) and refer patients to these trials. Broad participation in clinical trials is essential for significant advances in the care of patients with NS.

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Disclosures

None.

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