Serial Estimates of Serum Permeability Activity and Clinical Correlates in Patients with Native Kidney Focal Segmental Glomerulosclerosis

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Abstract. A serum or plasma factor in certain patients with focal segmental glomerulosclerosis (FSGS) has been found to increase glomerular albumin permeability (P Alb) and causes proteinuria in experimental animals. High P Alb is associated with recurrence of FSGS after transplantation, but serial studies of P Alb activity in patients with native kidney FSGS have not been performed, and the relationship between P Alb and remission of proteinuria is not known. This study was designed to determine P Alb activity before, during, and after 24 wk of treatment with cyclosporine or placebo given as part of a randomized controlled trial in steroid-resistant FSGS patients with nephrotic range proteinuria. Pretreatment P Alb averaged 0.36 ± 0.22 and was not significantly different between treatment groups and was not altered during or after the test medication. There was no association between P Alb activity and remission or relapse in proteinuria. The average P Alb activity in native kidney FSGS was lower than in previously reported patients with posttransplant recurrence of the disease, and its level did not vary during the course of the study. The antiproteinuric effect of cyclosporine appeared independent of changes in P Alb. This finding is consistent with a direct effect of cyclosporine on glomerular barrier function and/or that within this group of patients the variations in proteinuria are not reflected in changes in P Alb because of its limits in terms of reproducibility and responsiveness.

Focal segmental glomerulosclerosis (FSGS) is the most common primary glomerulonephritis that leads to end-stage renal disease (1,2). It was originally reported almost half a century ago from a postmortem study of children with nephrotic syndrome (3). The pathology of the disease has been well described since then and it now includes a number of histologic variants (4–6). It appears to be a heterogeneous condition (7) that is not universally responsive to immunosuppression and is frequently characterized by steroid resistance (8–24). Hypertension and renal dysfunction are commonly found at presentation. Thirty to fifty percent of patients will respond to prolonged treatment with corticosteroids, but a high percentage of nonresponders will progress to renal failure (10,12,19,25).

Many patients who do progress to end-stage renal disease (ESRD) will subsequently receive a renal transplant. The disease process, including both the clinical manifestations and the focal and segmental lesions on pathology will recur in up to 40% of these renal allografts, especially in patients whose disease course was rapid in their native kidney (26–28). In many cases, the proteinuria can be immediate and massive. The propensity for recurrence, reports of a positive response to plasmapheresis posttransplantation in such patients, and in vitro and in vivo studies examining the effects of infusion of patients plasma into rats have led to research focused on the identification of a circulating factor that alters glomerular permeability (29–35). Savin et al. have developed an in vitro method for assessing the permeability of glomeruli to albumin (P Alb) (33,35), and they have shown that this method is useful in assessing risk of recurrence of FSGS after transplantation. However, P Alb activity changes over time and in relationship to treatment in native kidney FSGS have not been measured.

In the present study, P Alb was assessed in serum samples of adults with steroid-resistant nephrotic syndrome secondary to FSGS who were involved in a clinical trial to test the efficacy of cyclosporine. Cyclosporine has been shown to block the in vitro effects of FSGS on glomerular permeability (36,37), but its effect in vitro on P Alb levels over time in patients with native kidney FSFG have not been reported.

Materials and Methods

Study Subjects

A subset of 27 patients representing approximately 50% of those enrolled in a randomized controlled trial were studied. Additional details of the study protocol and results of the parent trial have been reported (24). All patients must have failed to achieve a remission of proteinuria after a minimum of 8 wk of ≥1 mg/kg oral prednisone treatment. Other entry criteria included continued proteinuria of ≥50 mg/kg or ≥3.5g/d, creatinine clearance (CrCl) ≥ 42 ml/min per
1.73m², BP ≤ 135/85 mmHg and dietary protein intake ≤ 0.8 g/kg. A nephropathologist masked to patient assignment also had to confirm the pathologic diagnosis. Eighteen of the patients in the current study had been assigned cyclosporine treatment, and nine patients placebo. These patients were arbitrarily selected by the principle investigator of the parent trial, but treatment assignment and outcome were unknown to laboratory staff assessing the $P_{\text{alb}}$ activity. Twice as many cyclosporine-treated patients as placebo patients were chosen; the cyclosporine group had a higher remission rate, and we anticipated that the largest changes in $P_{\text{alb}}$ would be seen in the patients with the greatest changes in proteinuria.

**Randomization, Treatment, and Surveillance**

Patient selection and randomization in the parent study were performed per study protocol (24). Selection and submission of specimens for $P_{\text{alb}}$ assay were performed after the treatment protocol had been completed. The laboratory staff, including those performing the $P_{\text{alb}}$ assay was blinded to treatment assignment and to clinical response. All samples for $P_{\text{alb}}$ were obtained at the same time as the cyclosporine/placebo trough level assessment (i.e., 12 h after dose). Treatment with cyclosporine or placebo, plus low-dose prednisone (0.15 mg/kg) in all patients was continued for 26 wk and was then tapered to 0 over 4 to 8 wk. Patients on an angiotensin-converting enzyme inhibitor (ACEI) or an angiotensin receptor blocker before the treatment were allowed to remain on them during the study, but these classes of drug could not be introduced during the treatment part of the trial.

Clinical and laboratory evaluations were at selected times from pretreatment to 208 wk after study entry. At randomization and after 26 wk of treatment, blinded assessments on serum samples for $P_{\text{alb}}$ were made through the laboratory of one of the collaborators (VS). In 15 patients, a third sample was obtained between week 40 and the end of the observation period, after cyclosporine or placebo treatment had been discontinued for a minimum of 12 wk.

**Outcome Measures**

The primary outcome of the parent study was the number of complete or partial remission in proteinuria by week 26 in the two arms of the trial. This was also assessed at weeks 52, 78, 104 and at the last follow-up. In this study, we used the same end point definitions for outcome: complete remission (CR) was defined as ≤0.3 g/d proteinuria plus stable renal function, and a partial remission (PR) was both a 50% reduction of initial proteinuria and ≤3.5 g/d with stable renal function. Stable renal function was defined as a CrCl that was within 15% of the initial value. Secondary end points included progressive disease (CRI) defined as doubling of initial creatinine, end-stage renal disease (ESRD) defined as a CrCl ≤ 12 ml/min per 1.73m², start of dialysis, or transplantation.

$P_{\text{alb}}$ Assay

The principles of the in vitro assay, the methodology, and preparation of patients’ sera for the assay have been described previously (35). Glomeruli isolated from Sprague-Dawley rats by standard sieving techniques in an isolation medium containing an oncotic agent, bovine serum albumin (BSA, 4 g/dl), are incubated at 37°C with a diluted sample of serum (2% vol/vol) from a study participant. An oncotic gradient across the capillary wall was caused by replacing the medium with fresh medium with BSA (1 g/dl), and the resulting expansion of glomerular capillaries was measured using videomicroscopy. An increase in albumin permeability results in a decrease in effective oncotic gradient and in diminished glomerular capillary expansion ($\Delta V$). $P_{\text{alb}}$ was calculated as $1 - \frac{\sigma_{\text{alb}}}{\sigma_{\text{alb}}}$, where $\sigma_{\text{alb}} = \frac{\Delta V_{\text{experimental}}}{\Delta V_{\text{control}}}$. $P_{\text{alb}}$ values for each specimen were reported as means of values of five or more glomeruli.

**Statistical Analyses**

Prior measurement of $P_{\text{alb}}$ values in normal people after incubation in serum or plasma had a mean value ± SD of 0.00 ± 0.13 to 0.06 ± 0.2 (35,43). In earlier articles, we had defined individual values >0.3 as abnormal, based on the value that best separated recurrent from nonrecurrent FSGS disease after renal transplant and because it represented a value of more than 2 SD above the mean of normal sera (35). This definition was not used in the current data set because we are now aware of the wider range in $P_{\text{alb}}$ in those with native kidney disease, including patients in the part of the FSGS disease spectrum chosen for this study (35,38) and because we were most interested in intrapatient changes in activity over time. For this latter parameter we defined a value of ≥0.3 as a significant difference. This was based on previously published data that had shown agreement on replicate samples of <0.3 in 83% of cases (35). Results are reported as mean ± SD and/or ranges. Averages were compared using ANOVA. Non-parametric tests employed Fischer exact test, and a $P < 0.05$ was considered significant.

**Results**

Baseline demographics and laboratory parameters of the 27 patients in this study are shown in Table 1. The average age was 36 yr, and the group was predominately Caucasian. Sixty-one percent of cyclosporine-treated and 44% of placebo-treated patients were male. Responders and nonresponders in proteinuria, their corresponding medication assignment, and $P_{\text{alb}}$ activity before and after treatment, as well as the absolute difference and direction of change over time in these parameters are given in Table 2. Changes before and after treatments in the laboratory parameters by medication assignment to cyclosporine or to placebo are outlined in Table 3. $P_{\text{alb}}$ values from study patients showed a distribution from 0 to 0.98. The average initial $P_{\text{alb}}$ was 0.36 ± 0.22 and did not differ between the treatment groups. The average was higher than the mean $P_{\text{alb}}$ of normal serum samples but was lower than the $P_{\text{alb}}$ in patients with recurrence of FSGS in renal allografts (35). Changes over the experimental treatment period by medication assignment in serum creatinine, creatinine clearance and proteinuria are shown in Tables 3. Neither serum creatinine nor creatinine clearance were different in either group at the end of the treatment period but 13/18 of the cyclosporine treated patients had a remission in proteinuria versus only 1 of 9 placebo patients ($P = 0.05$) (Table 2). Paired analysis looking at changes in $P_{\text{alb}}$ from before to after treatment compared to changes in urine protein over the same time frame showed no association. Also no association was seen when the groups regardless of treatment assignment were divided into responder and nonresponder. The rates of remission in proteinuria were similar to those found in the parent study. $P_{\text{alb}}$ values overall did not change over time in the entire patient population, 0.36 ± 0.22 (before) versus 0.38 ± 0.27 (after) or by treatment assignment, (cyclosporine, 0.31 ± 0.23 [before] versus 0.46 ± 0.28 [after]; placebo group, 0.41 ± 0.21 [before] versus post 0.36 ± 0.25 [after]).
balance between plasma factors that enhance is supported by data that has suggested Palb activity is a non that Palb activity is reduced by have concluded that it is not a reliable method for assaying this proteinuria to be quite variable and show that injection of sera from patients with recurrent FSGS into rats increased urinary protein excretion (40). Some investigators have reported this proteinuria to be quite variable and have suggested that a circulating factor (or factors) may be involved in the pathogenesis of FSGS. Further evidence is offered by a case report that described the transient occurrence of the nephrotic syndrome in a neonate whose mother had known FSGS (39). In addition, earlier studies have shown that injection of sera from patients with recurrent FSGS both before and after transplant compared with those without recurrence (35) and that Palb activity is reduced by plasmapheresis in some patients with both native kidney FSGS and with recurrent disease (38). Although the circulating factor has not been fully characterized, it appears to be non-Ig, glycosylated, low–molecular weight protein (41). We have tested the capacity of several experimental manipulations to change Palb and have found it increased or decreased after a wide range of agents analogous to those postulated to cause glomerular injury in experimental and human renal disease (42–44). These findings suggest that the Palb assay is a functional assay rather than an assay of a specific substance. This is supported by data that has suggested Palb activity is a balance between plasma factors that enhance versus those that inhibit permeability. We have previously demonstrated that certain factors present in normal sera, e.g., apoprotein J and E

In the 15 patients whose Palb was measured after the drugs had been discontinued for 12 to 40 wk, the mean Palb was 0.49 ± 0.29. In these patients, as in the entire population, there was no relationship between Palb or change in Palb and change in proteinuria or progression to chronic renal insufficiency.

### Discussion
The short time to recurrence of FSGS posttransplantation and the beneficial effects of plasmapheresis therapy on their proteinuria supports the hypothesis that a circulating factor (or factors) may be involved in the pathogenesis of FSGS. Further evidence is offered by a case report that described the transient occurrence of the nephrotic syndrome in a neonate whose mother had known FSGS (39). In addition, earlier studies have shown that injection of sera from patients with recurrent FSGS into rats increased urinary protein excretion (40). Some investigators have reported this proteinuria to be quite variable and have concluded that it is not a reliable method for assaying activity (31), but we have found the degree of proteinuria induced depends on the potency of the preparation and on the amount injected (41). Measurements of Palb in vitro in our laboratory are consistent with the hypothesis that a circulating factor increases glomerular permeability. It has also been shown that Palb activity is higher in patients with recurrent FSGS both before and after transplant compared with those without recurrence (35) and that Palb activity is reduced by plasmapheresis in some patients with both native kidney FSGS and with recurrent disease (38). Although the circulating factor has not been fully characterized, it appears to be non-Ig, glycosylated, low–molecular weight protein (41). We have tested the capacity of several experimental manipulations to change Palb and have found it increased or decreased after a wide range of agents analogous to those postulated to cause glomerular injury in experimental and human renal disease (42–44). These findings suggest that the Palb assay is a functional assay rather than an assay of a specific substance. This is supported by data that has suggested Palb activity is a balance between plasma factors that enhance versus those that inhibit permeability. We have previously demonstrated that certain factors present in normal sera, e.g., apoprotein J and E

### Table 1. Baseline demographic and laboratory data

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cyclosporine</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>36 ± 11(^b) [21 to 59](^b)</td>
<td>36 ± 9 [23 to 47]</td>
</tr>
<tr>
<td><strong>Gender (% males)</strong></td>
<td>61</td>
<td>44</td>
</tr>
<tr>
<td><strong>Racial group, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>1 (6)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>White</td>
<td>17 (94)</td>
<td>8 (89)</td>
</tr>
<tr>
<td><strong>Creatinine (mg/dl)</strong></td>
<td>1.2 ± 0.4 [0.5 to 1.2]</td>
<td>1.1 ± 0.6 [0.6 to 2.6]</td>
</tr>
<tr>
<td><strong>Creatinine clearance (ml/min per 1.73 m(^2))</strong></td>
<td>72 ± 24</td>
<td>66 ± 30</td>
</tr>
<tr>
<td><strong>Proteinuria (g/d)</strong></td>
<td>7.2 [3.6 to 14.4]</td>
<td>9.5 [4 to 22.4]</td>
</tr>
<tr>
<td><strong>P(_{alb})</strong></td>
<td>0.31 ± 0.23 [0 to 0.75]</td>
<td>0.41 ± 0.21 [0.09 to 0.78]</td>
</tr>
</tbody>
</table>

\(^a\) ± values are SD.  
\(^b\) Values in brackets are ranges.  
\(^c\) To change to SI units: ×88.4.  
\(^d\) Median.

### Table 2. Proteinuria

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Posttreatment</th>
<th>Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Responders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine group</td>
<td>9.9/0.30</td>
<td>3.2/0.28</td>
</tr>
<tr>
<td>Placebo group</td>
<td>9.5/0.75</td>
<td>1.1/0.98</td>
</tr>
<tr>
<td>Nonresponders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine group</td>
<td>10.2/0.00</td>
<td>0.2/0.21</td>
</tr>
<tr>
<td>Placebo group</td>
<td>3.6/0.30</td>
<td>0.6/0.46</td>
</tr>
<tr>
<td><strong>Responders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine group</td>
<td>4.0/0.33</td>
<td>1.7/0.57</td>
</tr>
<tr>
<td>Placebo group</td>
<td>2.2/0.09</td>
<td>0.4/0.41</td>
</tr>
<tr>
<td><strong>Nonresponders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine group</td>
<td>7.2/0.34</td>
<td>1.7/0.57</td>
</tr>
<tr>
<td>Placebo group</td>
<td>5.8/0.16</td>
<td>1.2/0.18</td>
</tr>
<tr>
<td><strong>Responders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>7.2/0.34</td>
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<td>1.2/0.18</td>
</tr>
</tbody>
</table>

\(^a\) Values in brackets are ranges.
inhibit permeability activity induced by FSGS serum *in vitro* (45). Others have provided data that suggests there is a urinary loss of inhibitory factors of $P_{ab}$ in nephrotic patients. Co-incubation of sera with $P_{ab}$ activity with homologous urine resulted in neutralization of the permeability abnormality *in vitro*. This was not due to urinary levels of apo J or E, suggesting other inhibitors are present (46).

Although $P_{ab}$ is increased by diverse agents, it has been used by us and others in studies of FSGS as a measure of circulating permeability activity that may be important in causing and perpetuating proteinuria. Serial measurements of $P_{ab}$ over the course of the disease in native kidney FSGS patients have not been previously reported. In our study, $P_{ab}$ did not change in any consistent fashion in either the cyclosporine-treated or placebo-treated patients regardless of the change in proteinuria over the course of the trial. There was no association between partial or complete remission of proteinuria during treatment with cyclosporine or placebo in either initial $P_{ab}$ or change in $P_{ab}$ during treatment (Table 2). This is similar to the lack of correlation seen in $P_{ab}$ activity and clinical outcome after plasmapheresis in native kidney FSGS (38). The activity levels were on average lower than earlier studies, perhaps reflecting a less severely affected population and a different part of the disease spectrum compared with earlier studies that compared $P_{ab}$ activity in posttransplant patients whose native kidney disease was FSGS and in all cases had induced end-stage renal failure (35). In contrast, our selection criteria for the parent study were designed to provide a relatively homogeneous sample of FSGS patients who, although they were resistant to corticosteroid therapy, had stable renal function during the 6-mo pretreatment period. This would result in the exclusion of patients at both extremes of the disease spectrum, *i.e.*, those with steroid-sensitive disease and those with rapid progression to renal failure.

One explanation of the data is that there is a direct protective effect of cyclosporine on the glomerular permeability barrier independent of $P_{ab}$. This interpretation is consistent with previous *in vitro* findings that cyclosporine protects glomeruli from the increase permeability induced by FSGS sera with $P_{ab}$ activity (36).

An alternative or additional explanation relates to the assay in terms of its reproducibility and responsiveness. The reproducibility of the assay in this case relates to the test-retest component. This has been established for $P_{ab}$ in previous studies at <0.3 in 83% of cases (35). This in turn means the responsiveness or the ability of the assay to reflect or be sensitive to change, requires a relatively large difference in values between time points in each individual tested. If we restrict our evaluation to those patients that achieved this value, only five cases were identified (Table 2) and no association with change in proteinuria was observed within this group.

In summary, the sera of patients in the study had increased $P_{ab}$ activity compared with normal sera, but they were lower than that seen in patients with rapidly progressive FSGS disease and in patients with recurrent posttransplant FSGS disease. The $P_{ab}$ did not change significantly during the study in either of the treatment groups and did not decrease during remission or increase during worsening of proteinuria. One possible explanation of this discordance between $P_{ab}$ and proteinuria is that there is a direct protective effect of cyclosporine on the glomerular permeability barrier independent of other factors that may either inhibit or enhance permeability activity (45,46). An alternate or additional explanation is related to the assay’s limits related to reproducibility and responsiveness. These limits as discussed would reduce the likelihood of detecting a relationship between $P_{ab}$ and disease activity, since the required value to be considered biologically relevant (≥0.3) is large relative to the total range of the assay.

Multiple factors influence the pathophysiology of FSGS. The current study of $P_{ab}$ addresses only the capacity of sera to cause immediate increase in glomerular macromolecular permeability. It was not focused on providing information about potential mechanisms of sclerosis or the role of fibrogenic and/or inflammatory agents in the disease pathogenesis (47,48). Our study in this selected group of steroid-resistant nephrotic patients with relatively well-preserved renal function found no relationship between $P_{ab}$ and outcome. Additional studies with larger numbers and a broader spectrum of native kidney FSGS over longer periods of time may be required to determine if there is any correlation between $P_{ab}$ activity and the clinical course of the disease.

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References


