Abstract. Protein A immunoadsorption (IA) has proved effective in reducing proteinuria in patients with nephrotic syndrome after recurrence of focal and segmental glomerulosclerosis (FSGS) in kidney transplants. The effect of IA in nephrotic syndrome of other etiologies remains unknown. Nine patients with nephrotic syndrome secondary to membranous nephropathy (four cases), diabetes mellitus (one case), IgA nephropathy (two cases), and amyloidosis (two cases) had three to five IA of 2.5 plasma volumes over 4 to 8 d. Patients received no concomitant immunosuppressive treatment, and antihypertensive drugs were left unchanged. Proteinuria decreased from 12.64 ± 5.49 to 3.35 ± 2.2 g/24 h (mean ± SD) in all patients after three to five IA. Hematocrit decreased from 37.32 to 32.64% (12.5% hemodilution) and serum albumin from 25.43 to 18.6 g/L (26.4% decrease). Proteinuria returned to baseline levels within 1 mo, as described in recurrent FSGS following transplantation. When serum albumin balance was controlled by albumin infusion after IA in two patients, comparable decreases in proteinuria were observed. Therefore, IA is effective in producing short-term reduction of proteinuria in nephrotic syndromes related not only to FSGS but also to membranous and IgA nephropathies, diabetes mellitus, and amyloidosis, which suggests that IA removes a nonspecific circulating hemodynamic-altering or permeability-increasing factor.

A preliminary study showed that protein A immunoadsorption (IA) can produce a short-term decrease in urinary protein excretion in patients with heavy proteinuria after recurrence of focal and segmental glomerulosclerosis (FSGS) in kidney transplants (1). It was suggested that IA might remove a glomerular permeability factor since protein A eluates obtained in FSGS patients induced proteinuria when injected into rats (1). However, these protein A eluates showed no glomerular permeability-increasing properties in isolated glomeruli (2), and the mechanism of proteinuria decline after IA remains unknown.

In this context, we designed a prospective study to test the effect of IA on proteinuria in patients with nephrotic syndrome following primary membranous nephropathy or various other etiologies.

Materials and Methods

Patients

Ten patients with proteinuria over 3 g/d and a serum albumin level below 35 g/L were selected. Patients did not receive immunosuppressive drugs, intravenous IgG, or angiotensin-converting enzyme inhibitors, and their antihypertensive regimen was left unchanged. One patient received only one IA because of vascular access problems and was excluded from the study. Patients 1, 2, 3, and 4 had membranous nephropathy (four cases), diabetes mellitus (one case), IgA nephropathy (two cases), and amyloidosis (two cases) had three to five IA of 2.5 plasma volumes over 4 to 8 d. Patients received no concomitant immunosuppressive treatment, and antihypertensive drugs were left unchanged. Proteinuria decreased from 12.64 ± 5.49 to 3.35 ± 2.2 g/24 h (mean ± SD) in all patients after three to five IA. Hematocrit decreased from 37.32 to 32.64% (12.5% hemodilution) and serum albumin from 25.43 to 18.6 g/L (26.4% decrease). Proteinuria returned to baseline levels within 1 mo, as described in recurrent FSGS following transplantation. When serum albumin balance was controlled by albumin infusion after IA in two patients, comparable decreases in proteinuria were observed. Therefore, IA is effective in producing short-term reduction of proteinuria in nephrotic syndromes related not only to FSGS but also to membranous and IgA nephropathies, diabetes mellitus, and amyloidosis, which suggests that IA removes a nonspecific circulating hemodynamic-altering or permeability-increasing factor.
series of three IA was followed by albumin infusion to keep serum albumin constant.

This study was conducted according to the provisions of the Helsinki Declaration and was approved by the ethics committee of our institution.

Study Variables

Daily 24-h urine collections were obtained for proteinuria determination before, during, and after IA. Serial blood collections monitored serum creatinine and albumin levels, hematocrit, Ig G, A, and M, complement fractions (C3, C4), fibrinogen, and total cholesterol to check the biocompatibility of the system before and after the first and last IA.

The main outcome variable was the rate of proteinuria decline after IA. Secondary outcomes were the rate of proteinuria increase after IA discontinuation and the biocompatibility of the IA procedure in nephrotic patients.

Results

Primary Outcome Variables

The mean proteinuria of the nine patients who completed the study decreased from 12.64 ± 5.49 before IA to 3.35 ± 2.2 g/24 h (mean ± SD) at the end of the IA series (Figure 1). The mean decrease in proteinuria was 75.4% (range, 62.1 to 92.4%). There was a nonsignificant negative correlation between the percentage of decrease in proteinuria and proteinuria level before treatment ($r = -0.23$).

Secondary Outcome Variables

Proteinuria tended to return to baseline levels within 1 mo after IA discontinuation, following a kinetics similar to that observed in the previous IA study of FSGS recurrence in kidney transplants (1) (Figure 2).

The efficacy of IA was demonstrated by the decrease of total serum IgG level from 5.54 ± 4.31 g/L before IA to 0.89 ± 1.1 g/L (83.9% decrease) at the end of the IA series. There were also decreases of hematocrit from 37.32 ± 7.01% to 32.64 ± 6.02% (12.5% mean hemodilution) and of serum albumin level from 25.43 ± 7.16 to 18.6 ± 4.04 g/L (26.4% mean decrease).

The greater decrease of serum albumin suggested that not only hemodilution was involved but that some albumin was also definitively lost during the procedures. The albumin measured in the waste bag of two patients during six treatments was $5.08 ± 3.15$ g per IA session, which for a 65-kg (2.8 l plasma) patient corresponds to an expected decrease of 1.8 g/L in serum albumin level during each IA session. There was also an 18.9% decrease of C3, a 21.9% decrease of C4, a 38.6% decrease in fibrinogen, and a 20.9% decrease in total cholesterol. All of these changes were greater than the decrease of hematocrit (12.5%), and therefore not only due to hemodilution. In contrast, there was only a 7.9% decrease of serum creatinine level from 1.77 ± 0.67 before IA to 1.63 ± 0.58 mg/dl after the end of the IA series.

Minor side effects were related to vascular access problems. Both systolic and diastolic BP decreased during each IA session. Mean systolic/diastolic BP was 149.7/82.5 mmHg before and 137.25/74.0 mmHg at the end of the first IA session, and decreased again from 146.75/80.5 mmHg before to 137.0/75.3 mmHg at the end of the last IA session. One severe complication was observed in patient 8, a 79-yr-old man with nephrotic syndrome due to amyloidosis who exhibited a severe BP drop with generalized convulsion at the end of the third IA session. Mean urine output was 2417 ± 784 ml before IA and was unchanged at 2373 ± 754 ml after the IA series.

Effect of Albumin Infusion on Proteinuria Decline

Serum albumin decline was prevented by albumin infusion at the end of the second series of three IA sessions in two patients. In patient 7, serum albumin level before IA was 21.1 g/L, had decreased to 17.3 g/L at the end of the first IA series, and was 20.8 g/L after albumin infusion at the end of the second IA series. In patient 9, concentrations were 17.8, 15.6, and 17.3 g/L, respectively, at the same time points. Similar proteinuria reductions were observed with and without albumin replacement after IA (Figure 3).

Discussion

Protein A IA reduces proteinuria not only in patients with recurrence of FSGS after kidney transplantation but also in...
those with membranous and IgA nephropathies, diabetes mellitus, and amyloidosis. In all of these nephrotic conditions, the similarity between a decrease in proteinuria after IA courses and an increase after IA discontinuation suggests a common but still unidentified mechanism.

Protein A IA was associated with several nonspecific effects. Acute reduction of serum albumin level was observed after IA, occasionally with severe BP drop. Albumin did not bind to protein A, but was acutely lowered by plasma dilution after IA and also ended up in the waste bag after multiple column elutions. However, antiproteinuric response after IA was probably not simply related to a reduction of serum albumin since proteinuria decrease was not affected by subsequent albumin infusion. A transient major decrease in total IgG was also observed in our study after IA. This lowering of total IgG might be relevant since albumin reabsorption by the proximal tubule is a saturable process that cannot operate when cells are exposed to high molecular weight protein (e.g., IgG) that normally does not reach the urinary space (4). Yet some of the patients had selective albuminuria, without IgG urinary loss, and still exhibited proteinuria decrease after IA.

Glomerular permeability factors have long been described in minimal change nephrotic syndrome (MCNS) and FSGS (1,5–13), although their molecular characterization has not been determined (2,5). They are not usually found in other nephrotic conditions. Some discrepancies exist between studies considering various disease specificities (MCNS versus FSGS) and molecular characteristics of the factor, which suggests that more than one permeability-increasing substance may be involved. The role of these factors is still unknown since they could be initiators, amplifiers, or late effectors in the disease process, including hemodynamic-altering factors. Because protein A is effective \textit{ex vivo} in depleting sera of relapsing FSGS patients of a factor capable of increasing albumin permeability in isolated glomeruli (2), it is likely that IA does remove this circulating factor, which is subsequently destroyed after acid elution.

If protein A does remove a common permeability factor in nephrotic syndromes present in immune as well as nonimmune glomerulopathies, it must be a very late effector. The immediate antiproteinuric effect of IA suggests that a hemodynamic-altering factor might be involved. Similar proteinuria decreases have been observed in nephrotic patients due to FSGS (14–16), MCNS (15), and membranous nephropathy (15) treated by LDL-apheresis, which also suggests the removal of a factor

Figure 2. Changes in proteinuria level (mg/24 h) after IA discontinuation. Mean and SD of proteinuria before IA, immediately following the end of the IA series of three to five IA sessions, 1 wk after IA discontinuation, and at follow-up for six of the nine patients who did not receive maintenance IA treatment.

Figure 3. Serum albumin infusion did not prevent proteinuria decline after IA in patients 7 and 9. For both patients, the first two bars represent proteinuria level before and after IA without albumin infusion, and the remaining two bars represent proteinuria before and after the IA session that was followed by albumin infusion to keep serum albumin constant.
inducing an acute modification of glomerular hemodynamics and/or permeability. Several hemodynamic-altering factors may be modulated by proteins or lipids passing through the glomerular barrier, including endothelin-1 (17) and nitric oxide (18). However, it is unlikely that the marginal decrease in total cholesterol following IA is responsible for the observed antiproteinuric response after IA. Alternatively, IA and LDL-apheresis might remove a similar hemodynamic-altering factor.

Additional studies are required to identify the nonspecific permeability-increasing or hemodynamic-modifying factor removed by IA. This factor is probably destroyed after acid elution during the IA procedure, which prevents its isolation and molecular characterization.

Acknowledgments

This study was supported by the “Délégation à la Recherche Clinique” of Nantes University Hospital, France. We are grateful to the Western French Society of Nephrology, as well as to our two research nurses, Nathalie Guilbert and Valérie Betou.

References