Mechanism of the Antiproteinuric Effect of Cyclosporine in Membranous Nephropathy

Siva Ambalavanan, Jean-Pierre Fauvel, Richard K. Sibley, and Bryan D. Myers

ABSTRACT

Forty-one patients with a nephrotic syndrome and biopsy-proven membranous nephropathy were administered a 3 to 6-month course of cyclosporine (CsA; 4 to 5 mg/kg per day). Differential solute clearances were used to evaluate glomerular function before and after therapy. CsA lowered median proteinuria by 56%, from 7.3 to 3.2 g/24 h ($P < 0.0001$). Corresponding mean increments in serum albumin, immunoglobulin G, and oncotic pressure values were 31, 32, and 26%, respectively (all $P < 0.0001$). Arterial pressure, GFR, and renal plasma flow remained constant, but CsA restored the dextran-sieving curve toward normal, lowering the computed fraction of shunt-like pores by 25% ($P < 0.05$). In 14 instances, a cross-over design was used to randomly assign patients to 3 months of CsA versus 3 months of enalapril (10 to 30 mg daily), separated by a 1-month washout interval. Although enalapril lowered arterial pressure by 8 mm Hg ($P < 0.01$), it had no effect on proteinuria, plasma protein composition, filtration dynamics, or dextran sieving (all $P = n$ significant). CsA dependence of proteinuria, indicated by relapsing nephrosis after CsA withdrawal, required additional courses of CsA to maintain proteinuria subnephrotic in most patients. In six patients with declining GFR during prolonged CsA treatment, a repeat biopsy showed more prominent immune deposits and a thicker glomerular basement membrane than at baseline. It was concluded that: (1) CsA lowers proteinuria in MN in part, by enhancing barrier size-selectivity; (2) lack of comparable efficacy of enalapril suggests that the antiproteinuric effect of CsA is related to its immunosuppressive rather than glomerulodepressor properties; but (3) judged by repeat biopsy, CsA does not prevent continuing autoantibody formation in this disorder.

Key Words: Barrier function, filtration dynamics, glomerular morphology, angiotensin-converting enzyme inhibition

A growing body of evidence suggests that treatment with cyclosporine (CsA) can substantially lower the level of proteinuria in nephrotic patients with membranous nephropathy (MN) (1–6). Shared, albeit less pronounced, antiproteinuric effects of other immunosuppressants in this disorder (7–9) raise the possibility that the favorable effect of CsA on glomerular barrier function is a consequence of its immunosuppressive properties. In Heymann's nephritis, an analogue of MN in the rat, the glomerular injury is mediated by autoantibodies that are directed against an antigen in the cell membrane of glomerular epithelial podocytes (10). The ensuing proteinuria has been attributed to activation of complement and the formation of membrane attack complexes (11). CsA, however, does not inhibit either immunoglobulin production by B cells or an activated complement system (12). Thus, the precise mechanism by which it exerts its antiproteinuric effect in MN remains obscure.

It has been suggested that CsA could reduce proteinuria via its renal hemodynamic actions (13). CsA is a selective constrictor of afferent arterioles, and thereby lowers glomerular capillary hydraulic pressure (14). It is possible therefore, that the antiproteinuric effect of CsA in MN is related to its glomerulodepressor rather than its immunosuppressive properties. In keeping with this possibility is the frequent finding that the lower level of proteinuria achieved during therapy can revert promptly to massive pretreatment levels when CsA is withdrawn (1–6).

In an effort to characterize the extent and reproducibility of its proteinuria-lowering effect, we have administered one or more 3 to 6-month courses of CsA to 41 nephrotic patients with MN. In the hope that the contribution of its hemodynamic actions might be clarified, we compared the magnitude of the response to CsA with that of an angiotensin-converting enzyme inhibitor in a subset of the patients. We also subjected a second subset of patients to a repeat renal biopsy to determine whether long-term CsA therapy can suppress the intraglomerular deposition of autoantibodies. Our findings form the basis of this report.

METHODS

Patient Population

The subjects of our study were 41 of 45 adult patients who presented consecutively during a 5-yr period (1989 to 1994) to our nephrology clinic at the Stanford University Medical
Center with a nephrotic syndrome and a biopsy diagnosis of MN. The remaining four patients were excluded from further study because initial evaluation revealed their GFR to be below 15 mL/min per 1.73 m². In 12 members of the patient population, the MN was associated with positive serology for systemic lupus erythematosus. Two additional patients also had secondary forms of MN, one associated with gold therapy and the other with hepatitis B virus infection. In the remaining 27 individuals, no known associations of glomerular subepithelial immune complex formation were discernible, and the MN was categorized as idiopathic.

The patients varied in age from 18 to 76 yr (median age, 42 yr) and 23 of 41 were men. The median duration of the nephrotic episode for which patients were referred was 8 months (range, 2 to 96). In 30 cases, the patient had received a course of therapy with prednisone for a median duration of 4 months (range, 1 to 9 months) before referral. The prednisone dosage was 50 to 60 mg daily and in nine instances was combined with a cytotoxic agent (cyclophosphamide, chlorambucil, or azathioprine). Cytotoxic agents were withdrawn and prednisone was tapered to 0 to 10 mg daily in patients receiving such therapy before proceeding with the CsA trial. All patients entering the trial consented to submit to one or more of three protocols, which had been approved previously by the Panel for Research in Human Subjects at Stanford University.

**CsA Therapy**

**Initial CsA Treatment.** The first 30 patients in this series were given a 3-month course of CsA. Because such therapy was followed by a high relapse rate, initial therapy was lengthened to 6 months in the final 11 individuals that entered the study. After a baseline evaluation of proteinuria and glomerular function, CsA treatment was administered by using a regimen that has been shown by us previously to lower proteinuria to normal levels in minimal change nephropathy and to subnephrotic levels in MN (5). Treatment was initiated in a dosage of 4 mg/kg per 24 h given in two divided doses. Immunoassayable trough levels of CsA in serum (iCsA) were determined at 2 to 4-wk intervals throughout the study, by using a fluorescence polarization assay that measures both the parent compound and its metabolites. Serum creatinine levels were determined simultaneously. The daily CsA dose was adjusted to maintain iCsA between 50 and 150 ng/mL. Downward adjustments in CsA dosage were made when the serum creatinine concentration increased by >25% above baseline, irrespective of the prevailing iCsA. On average, the mean CsA dosage during the initial course of treatment was 5 mg/kg per day (range, 3 to 6.5). Proteinuria was reevaluated during the last day of therapy and glomerular function was redetermined 12 to 18 h after the last CsA dose.

**Crossover Study, CsA versus Enalapril.** In 14 consecutive patients (Patients 17 to 30 in the series), the effects of a 3-month course of CsA on proteinuria were compared with a corresponding course of the angiotensin-converting enzyme inhibitor enalapril (Figure 1). Patients were randomized to CsA or enalapril in a 2:1 ratio, resulting in nine patients commencing the cross-over study with a 3-month course of CsA, and the remaining five patients with enalapril. Of the nine patients that began the cross-over study with CsA, three had earlier been placed on angiotensin-converting enzyme inhibitor therapy. This therapy was withheld for 1 month before commencement of the CsA therapy. The successive 3-month courses of treatment were separated by a 1-month interval to allow the first agent used to be completely washed out before commencement of treatment with the second agent (Figure 1).

CsA treatment was administered and monitored as described above. Hypertension was treated with a calcium channel blocker, and edema with loop diuretics, as required. Enalapril was given in an initial daily dose of 10 mg. If there was no hyperkalemia or incremental azotemia after 2 wk of enalapril therapy, the enalapril dosage was increased up to 30 mg daily, depending on the prevailing level of arterial pressure. On average, the mean enalapril dose was 20 mg daily (range, 10 to 30 mg).

**Chronic CsA Therapy.** Four wk after completion of either the initial course of CsA or the cross-over study, proteinuria had returned to the baseline level in 31 patients. The first 11 patients to relapse in this series remained nephrotic during a 6 to 12-month period of subsequent follow-up, but were not offered additional treatment with CsA. Subsequently, the remaining 20 relapsed patients were offered and consented to further therapy with up to three additional 6-month courses of CsA, each of which was followed by a 4-wk interval of washout (Figure 1). Relapsing nephrosis at the end of the washout period served as an indication for commencement of the next 6-month cycle of CsA therapy, until a maximum of 21 months of this therapy had been administered (initial course + three subsequent 6-month cycles). Serum creatinine levels, 24-h urinary protein excretion rates, and iCsA were monitored at 1 to 2-month intervals. Glomerular function was formally evaluated at the end of each 6-month treatment cycle. Six patients who exhibited relapsing nephrosis were subjected to a repeat biopsy because of a progressive decline in the GFR during chronic CsA therapy.

**Evaluation of Glomerular Function**

A detailed evaluation of glomerular function was performed before commencement, and again on the day after initial CsA or enalapril therapy. The same evaluation was also repeated on the day after each subsequent 6-month course of CsA (Figure 1). After urine collections for estimation of the 24-h urinary protein excretion rate, patients were admitted to a general clinical research center for clearance studies that commenced at 8:00 a.m. After blood samples were taken for determination of the plasma oncotic pressure (φo2) and levels of albumin, immunoglobulin G (IgG), and cholesterol in
serum, recumbent blood pressure was measured. Water loading (10 mL/kg per 60 min) was then begun, and the patients were primed with inulin and para-amino hippuric acid (PAH), followed by a sustaining infusion of each marker. A simultaneous priming and sustaining infusion of dextran 40 (Rheomacrodex; Pharmacia Fine Chemicals, Uppsala, Sweden) was given along with inulin and PAH in the case of the 14 patients who participated in the cross-over study (15). After an equilibration period of 60 minutes after prime, four successive 20 to 30-min urine collections were made by spontaneous voiding. Each was bracketed by a venous blood sample. An automated assay, which has been described previously, was used to determine the concentrations of inulin, PAH, and dextran (16). Plasma oncotic pressure was measured by membrane osmometry, and plasma albumin and IgG concentrations by nephelometry, as described in detail elsewhere (17).

The GFR was expressed as the average value for the four timed inulin clearances. The rate of RPF was determined by division of the corresponding PAH clearance by an estimated PAH extraction ratio of 0.8 in those patients with normal GFR, and 0.7 in those patients in whom GFR was depressed (16,17). After separating dextran in plasma and urine into discrete 2Å fractions by gel-permeation chromatography, the fractional clearances (θ) of discrete dextran fractions over the 24 to 60 Å radius interval were calculated by division of the clearance of each narrow dextran fraction by that of inulin. The θ for each dextran was then used to compute the radius of restrictive glomerular pores (rθ), as well as ωθ, a measure of the fraction of filtrate volume passing through a parallel pathway of nondiscriminatory shunt-like pores within the glomerular capillary wall. This "isoporous + shunt" representation of the glomerular size-selective barrier has been shown by us to depict accurately the glomerular capillary wall in MN, and has been described in detail elsewhere (19,20). The model separates the effects of pore parameters on θ dextran from those that result from purely hemodynamic changes, by using GFR, RPF, ωθ, and ΔP as input values (19). Because ΔP cannot be measured, we have assumed it to be 40 mm Hg before therapy, and to have declined to 35 mm Hg during therapy with either agent, both of which are known to be glomerulodepressors (14,21). To take into account the possibility that CsA and enalapril failed to lower ΔP in MN, we also examined the effects of constancy of ΔP on membrane parameters during either therapy.

Serial Morphology

An analysis of glomerular, interstitial, and arteriolar structure was performed in the six patients who underwent serial renal biopsy. The percentage of glomeruli that were sclerosed with the fraction of cortex occupied by interstitium were determined by light microscopy of cross-sections stained with periodic acid-Schiff reagent (15,16). All glomerular arterioles in the tissue available for light microscopy were also carefully examined for the presence of subintimal hyaline deposits that are associated with chronic CsA-induced nephropathy (22,23).

Electron photomicrographs of two patent glomeruli from each biopsy were then used to determine the thickness of the glomerular basement membrane (GBM) and filtration slit frequency (FSF) at a magnification of ×11,280. The GBM thickness was measured by the orthogonal intercept method (24). FSF was determined by division of the total number of epithelial filtration slits by the total length of GBM captured on the electron photomicrographs (24). The relative promi-
TABLE 1. Effect of CsA on glomerular functiona

<table>
<thead>
<tr>
<th>Value</th>
<th>Pre-CsA</th>
<th>Post-CsA</th>
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<tbody>
<tr>
<td>Proteinuria (g/day)b</td>
<td>7.3</td>
<td>3.2c</td>
</tr>
<tr>
<td>Serum Albumin (mg/L)</td>
<td>179 ± 1</td>
<td>235 ± 11c</td>
</tr>
<tr>
<td>Serum mgG (mg/L)</td>
<td>58 ± 6</td>
<td>76 ± 6c</td>
</tr>
<tr>
<td>Serum Cholesterol (mg/dL)</td>
<td>331 ± 18</td>
<td>254 ± 12c</td>
</tr>
<tr>
<td>πa (mm Hg)</td>
<td>13.9 ± 0.6</td>
<td>17.5 ± 0.6c</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>106 ± 2</td>
<td>108 ± 2</td>
</tr>
<tr>
<td>GFR (ml/min per 1.73 m²)</td>
<td>58 ± 4</td>
<td>56 ± 5</td>
</tr>
<tr>
<td>RPF (nl/min per 1.73 m²)</td>
<td>613 ± 45</td>
<td>569 ± 44</td>
</tr>
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</table>

a Values are mean ± SE.
b Median (range).
c P < 0.0001.

arms of the cross-over study. Specifically, baseline median proteinuria in g/24 h was 9.8 (range, 3.6 to 26) versus 9.2 (range, 2.6 to 28), and serum albumin was 186 ± 73 versus 186 ± 64 mg/L, respectively. The effects observed during initial CsA therapy of the entire population were replicated in the 14 patients who participated in the cross-over study (Figure 3). In contrast, minor trends after 3 months of enalapril toward less proteinuria and improved plasma protein composition all failed to reach statistical significance (Figure 3). Neither GFR nor RPF changed significantly during either CsA or enalapril therapy. The coadministration of a calcium channel blocker during CsA therapy maintained MAP constant, at 106 ± 11 mm Hg at baseline versus 110 ± 10 mm Hg at the end of therapy (P = not significant [NS]). In contrast, monotherapy with enalapril lowered MAP from 104 ± 11 mm Hg at baseline to 96 ± 10 mm Hg at the end of therapy (P < 0.01).

The dextran-sieving curve in the patients with MN was indistinguishable at the onset of each therapy (Figures 4 and 5). It differed from the sieving curve in healthy volunteers with normal glomeruli in that it was depressed at the low-radius end and elevated at the high-radius end (Figures 4 and 5). CsA therapy was associated with significant alteration in the dextran-sieving curve. This was characterized by a selective elevation of the low-radius end of the sieving curve toward normal values; θ for dextrans >50Å radius did not change significantly (Figure 4). In contrast, enalapril therapy failed to change the dextran-sieving curve significantly (Figure 5). According to the isoporous + shunt model, the elevation of the low-radius end of the sieving curve during CsA therapy is attributable to an increase above initially depressed values of restrictive-pore radius (r0) and the ultrafiltration coefficient, Kf (Table 2). The selective failure of θ for large nearly impermeant dextrans of >52Å radius to increase in parallel is attributed to a reduction in the prominence of the shunt pathway, such that ω0 declined by 25% (P < 0.05; Table 2). Whereas enalapril failed to increase either Kf or r0, a trend toward lower values for θ of dextran at the high-radius end of the curve is also attributed by the model to a reduction in ω0, in this case by 22%. This latter change, however, failed to reach statistical significance (Table 2).
patients whose proteinuria continued to relapse after completion of three courses of CsA to date (Table 4). Each exhibited a decline in final GFR by 23 to 43% below baseline. Repeat biopsies were also performed in an additional two patients after only 6 and 9 months of CsA, respectively, because relapsing nephrosis was associated in each case with a marked reduction in the GFR; from 91 to 9 and 137 to 56 mL/min per 1.73 m², respectively. The findings are summarized in Table 4, in which normal control values from 16 healthy kidney-transplanted donors are also provided for comparison.

Judged by the absence of afferent arteriolar hyalnosis or striped interstitial fibrosis, unequivocal evidence of chronic CsA nephropathy was not observed in any case in this small series of repeat biopsies (22,23). Among serial structural changes attributable to MN per se was an increasing prevalence of global sclerosis and a numerical increase in fractional interstitial area between the two biopsies (P = NS; Table 4). Notably, however, the latter increase was a consequence of generalized interstitial fibrosis, a phenomenon that is known to accompany progressive membranous nephropathy (16), and not of the patchy stripes of interstitial fibrosis that typically accompany chronic CsA nephrotoxicity.

The epithelial foot processes were similarly broadened in the baseline and repeat biopsies, with the result that the FSF was similarly lowered below the normal value in each biopsy (Table 4). The most striking alteration between the two biopsies was in the median thickness of the GBM. Compared with normal, the GBM was thickened by 69% to 866 nm at the baseline biopsy (Table 4). This increased further by twofold to fourfold to 2048 nm at the time of the second biopsy, a highly significant difference (P < 0.001). The GBM thickening was in part the result of enhanced deposition of extracellular matrix. Also contributing to the thicker basement membrane in the repeat biopsy, however, was a striking increase in the prominence of the electron-dense immune deposits, compared with baseline. As shown in the paired illustrations for each patient in Figure 7, many of the immune deposits were in a subepithelial location. Others were walled off from the sole of epithelial foot processes by a layer of extracellular matrix, and were thus in an intramembranous location. Comparison of the repeat biopsy to the baseline biopsy reveals that the immune deposits were both more numerous and larger in the wake of CsA therapy, compared with before such therapy (Figure 7).

**DISCUSSION**

We have shown that CsA exerts a rather uniform and reproducible effect to rapidly lower proteinuria in MN. Whether CsA therapy is superior in this respect to more conventional immunosuppressive treatment of MN is difficult to ascertain from the reported literature. In five studies that provide a comparison to
TABLE 2. Membrane parameters\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Assumed (\Delta p) (mmHg)</th>
<th>(K_I) (ml/min/mmHg)</th>
<th>(r_0) (\AA)</th>
<th>(\sigma_0)</th>
</tr>
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<tbody>
<tr>
<td>Pre-CsA</td>
<td>40</td>
<td>2.3 ± 0.4</td>
<td>53.9 ± 0.5</td>
<td>0.008 ± 0.002</td>
</tr>
<tr>
<td>Post-CsA</td>
<td>35</td>
<td>3.4 ± 0.6(b,d)</td>
<td>55.8 ± 0.6(b,d)</td>
<td>0.004 ± 0.001(c)</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>2.5 ± 0.4</td>
<td>55.9 ± 0.6(b,d)</td>
<td>0.006 ± 0.001(c)</td>
</tr>
<tr>
<td>Pre-Enalapril</td>
<td>40</td>
<td>2.3 ± 0.3</td>
<td>54.7 ± 0.6</td>
<td>0.009 ± 0.002</td>
</tr>
<tr>
<td>Post-Enalapril</td>
<td>35</td>
<td>2.9 ± 0.5</td>
<td>54.1 ± 0.8</td>
<td>0.007 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>2.2 ± 0.4</td>
<td>54.1 ± 0.8</td>
<td>0.007 ± 0.001</td>
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</table>

\(^a\) Values are mean ± SE.
\(^b\) \(P < 0.01\).
\(^c\) \(P < 0.05\) versus pre-CsA.
\(^d\) \(P < 0.05\) versus corresponding values post-enalapril.

TABLE 3. Chronic CsA therapy and proteinuria

<table>
<thead>
<tr>
<th>Patients (N)</th>
<th>Reduction in Median Proteinuria (%)</th>
<th>Post-Washout Relapse (N/%)</th>
<th>Sustained Remission (N/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Course I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>56</td>
<td>31 (75%)</td>
<td>10 (24%)</td>
</tr>
<tr>
<td>Course II</td>
<td></td>
<td>14/18(^a) (78%)</td>
<td>4/18(^b) (22%)</td>
</tr>
<tr>
<td>20</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Course III</td>
<td></td>
<td>4/6(^a) (67%)</td>
<td>2/6(^b) (33%)</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td></td>
<td></td>
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</table>

\(^a\) Total number completing post-CsA washout.
\(^b\) Total number completing 12 months of follow-up post-CsA.

Figure 6. Mean level (± SE) of proteinuria before (PRE) and after (POST) successive courses of treatment in 20 patients who have completed two courses of CsA to date. Courses (I) and (II) are separated by a 1-month washout period. ** \(P < 0.001\) before versus after CsA therapy.

baseline after 3 to 6 months of treatment, prednisone monotherapy was reported to lower average proteinuria by only 28 and 39% (7,25), whereas a corresponding reduction by prednisone combined with cytotoxic drug therapy lowered average proteinuria by 28, 31, and 60%, respectively (25,26,27). Our finding that CsA lowers average proteinuria by >50% within 3 months is in keeping with the observations of others (1,6,28) and suggests that it is more potent in this respect than prednisone and cytotoxic agents.

In agreement with the findings of Zietse et al. (28), our analysis of dextran sieving indicates that CsA enhances the size-selective properties of the glomerular barrier (Figure 4, Table 2). The reduced prominence of shunt-like pores per se does not seem to fully explain a proportionately larger decline in proteinuria, however. This raises the possibility that CsA also restores the charge-selective properties of the barrier, whose impairment contributes to the magnitude of albuminuria in MN (29). Our analysis also reveals intrinsic ultrafiltration capacity to be improved during CsA therapy. Depending upon whether \(\Delta p\) declined or remained constant, \(K_I\) is computed to have increased by between 10 and 41% (Table 2). The higher \(K_I\) in turn explains the ability of our patients to maintain GFR constant, notwithstanding a substantial increase in the opposing oncotic pressure (Table 1, Figure 3). Without a repeat biopsy during the partial remission of proteinuria, the precise mechanism by which CsA treatment served to elevate \(K_I\) cannot be determined. We have shown, however, that foot-process broadening and effacement lower the hydraulic permeability of glomerular capillaries in this disorder (24). Reversal of this change in foot-process conformation likely accompanies partial remission of nephrotic-range proteinuria (30). It is tempting to speculate therefore, that CsA enhances \(K_I\) by restoring foot-process architecture toward normal, thereby increasing hydraulic permeability of the glomerular capillary walls.

Our findings suggest that CsA improves barrier function and ultrafiltration capacity independent of its vasoconstrictor effects (Table 1), and also raise the possibility that CsA can directly modulate the intrinsic properties of the injured glomerular capillary wall. Because a similar effect was not observed during therapy with a vasodepressor dose of enalapril, we infer that the membrane-modulating effect of CsA is unlikely to be hemodynamically mediated. Whereas some researchers have also reported similarly modest efficacy of enalapril in MN (31,32), we are cognizant of the fact that others have reported a more profound effect of enalapril on barrier function than that ob-
Cyclosporine in Membranous Nephropathy

TABLE 4. Renal morphology

<table>
<thead>
<tr>
<th></th>
<th>Normal Controls</th>
<th>Membranous Nephropathy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Biopsy 1</td>
</tr>
<tr>
<td>Globally Sclerosed Glomeruli (%)</td>
<td>0 (0 to 0.1)</td>
<td>2(^b) (0 to 15)</td>
</tr>
<tr>
<td>Fractional Interstitial Area (%)</td>
<td>13.0 (8 to 19)</td>
<td>20(^b) (12 to 58)</td>
</tr>
<tr>
<td>Filtration-Slit Frequency (#/mm GBM length)</td>
<td>1410 (1066 to 1594)</td>
<td>763(^c) (312 to 923)</td>
</tr>
<tr>
<td>Basement-Membrane Thickness (nm)</td>
<td>512 (448 to 627)</td>
<td>866(^c) (766 to 1035)</td>
</tr>
</tbody>
</table>

\(^a\) Results expressed as median (range).

\(^b\) \(P < 0.05\) and \(^c\) \(P < 0.001\) MN versus Normal.

\(^d\) \(P < 0.001\) second versus first biopsy in MN.

Figure 7. A comparison of changes in glomerular capillary wall morphology before (A) and after (B) 6 to 18 months of CsA therapy in six patients (Patients 1 through 6) who underwent serial biopsy. All 12 electron photomicrographs were taken at the same magnification (×11,400).

served by us in the study presented here (33,34). We are unable to explain the observed variability of responsiveness among reported studies, but speculate that differences in the stage and severity of the MN and in the dosage regime could be implicated. We note that a similarly wide variation in the responsiveness of proteinuria to angiotensin-converting enzyme inhibitors has been reported in diabetic nephropathy (35). By using a regime identical to that in the study presented here, we have previously examined the efficacy of enalapril in diabetic nephropathy (36). We observed a nonsignificant reduction of proteinuria by 21% and a generalized but minor shift to lower values of dextran-sieving coefficients over the entire range of
sizes examined. Thus, the present findings in MN have essentially replicated, and are internally consistent with, our earlier findings in diabetic nephropathy (36). The purpose of using enalapril in our cross-over study was to examine the effects of an isolated fall in ΔP on barrier function. Taking together the observed reduction in arterial pressure by 8 mm Hg, and the known effect of enalapril to disproportionately dilate the efferent arteriole, it seems probable that we achieved this objective. Given that our observations were paired, we interpret the more beneficial effect of CsA on barrier function in this study, compared with that of enalapril, to indicate that CsA could be acting to improve foot-process architecture via its immunosuppressive properties rather than by a nonspecific glomerulodepressor action.

As stated previously, CsA is thought not to influence antibody formation by B cells or to inhibit an activated complement system (12). Our repeat biopsies strongly suggest that this is true of its use in MN. Although CsA effectively lowered the level of proteinuria in each patient who underwent rebiopsy, the electron-dense immune deposits associated with the glomerular capillary wall were more numerous and larger in the wake of CsA than before such therapy (Figure 7). Because the two biopsies provide only isolated “snapshots” in time, we are unable to exclude the possibility that enhanced deposition of antibody and subsequent shedding of immune complexes into the GBM occurred, in fact, between the baseline biopsy and the initiation of CsA therapy. Such a sequence of events in each of the six rebiopsied patients seems improbable, however. Thus, although they provide only indirect evidence and are confined to patients with severe and progressive MN, we interpret our morphological findings to indicate that CsA does not abolish the autoimmunity that underlies MN.

A possible immunological basis for the efficacy of CsA in MN is related to its ability to inhibit the secretion of cytokines by infiltrating T cells and macrophages (12). The prominence of infiltrating mononuclear cells in the interstitium of rats with Heymann nephritis was first emphasized in an early report of that experimental injury more than 30 yr ago (37). More recently, infiltrating T cells and macrophages have also been demonstrated in the interstitium of humans with MN, and shown to be concentrated in a periglomerular location (38). Further, the density of the infiltrate was shown to be predictive of persistent nephrosis and a progressive decline in GFR (38). The fact that infiltrating cells may be implicated in barrier dysfunction is consistent with the observation that administration of monoclonal antibodies to CD8+ T cells for 6 wk after the induction of Heymann nephritis failed to prevent autoantibody deposition, but largely eliminated the interstitial infiltrate and attenuated the magnitude of proteinuria (39). These findings in the rat with Heymann nephritis can be replicated by substitution of CsA for antiCD8+ antibody therapy, and provide a possible explanation for the proteinuria-lowering effect of CsA in human MN (39–41).

We have found the short-term use of CsA in low dosage in MN to be associated with fewer side effects than corresponding therapy with prednisone and cytotoxic drug therapy. On the basis of what we perceive to be its greater efficacy in lowering proteinuria than the aforementioned agents, we submit that CsA may well be the drug of choice for controlling the acute morbidity associated with the nephrotic syndrome in MN. Its ability to rapidly restore plasma protein composition toward normal is associated with a reversal of edema formation, and should limit other serious sequelae of the nephrotic syndrome, such as phlebothrombosis, protein malnutrition, and enhanced susceptibility to infection.

What remains to be determined, however, is whether CsA is efficacious and safe as a long-term treatment designed to ameliorate progression of this disorder to end-stage renal failure. Imperiale and his coworkers recently concluded from a meta-analysis of controlled trials that insufficient information is presently available to decide whether or not cytotoxic drug therapy is renoprotective in MN (9). On the other hand, Cattran and coworkers have shown in a controlled trial that when CsA treatment is targeted to patients with a rapidly progressive injury, it is more effective than conventional supportive therapy in delaying progression of MN (42). Given our finding that CsA does not seem to abolish increasing deposition of immune complexes in the glomerular capillary wall under these circumstances, the mechanism by which CsA might protect the kidney remains to be elucidated. We thus conclude that additional controlled trials of long duration and adequate sample size are needed to determine the place of CsA in long-term therapy of this disorder.

ACKNOWLEDGMENTS

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