Combining an Antiproteinuric Approach with Mycophenolate Mofetil Fully Suppresses Progressive Nephropathy of Experimental Animals

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Abstract. Chronic renal diseases progress to organ insufficiency, which may require replacement therapy within one to three decades even independently of the type of initial insults. In the majority of cases, the degrees of proteinuria and interstitial leukocyte infiltration and scarring are strictly correlated with the rate of disease progression. This study tests the hypothesis that excess intrarenal protein traffic may cause leukocyte-dependent interstitial injury that, while not fully controlled by antiproteinuric therapy, can be further inhibited by concomitant immunosuppression. A primarily nonimmune model was used to reproduce progressive renal disease due to a critical loss of nephron mass. Angiotensin-converting enzyme (ACE) inhibitor limited proteinuria, interstitial inflammation, MHC class II antigen expression, and severe lesions. Combined treatment with ACE inhibitor and a specific antilymphocyte agent, mycophenolate mofetil, dramatically attenuated macrophage and T cell infiltration, MHC-class II overexpression, dendritic cells, and all manifestations of the disease. Evidence of lymphocyte-mediated renal injury in the setting of excess protein traffic provides the basis for combining ACE inhibition and immunosuppression to halt progression of proteinuric kidney disease and minimize the need for dialysis or transplantation.

The accumulation of macrophages and T lymphocytes into the renal interstitium is a common feature of proteinuric nephropathies leading to end-stage fibrosis and organ failure. Cytokine-mediated pathways underlying interstitial inflammation can be activated by the abnormal intrarenal protein traffic that is also responsible for proteinuria, the main clinical predictor of progressive renal disease regardless of etiology (1–3). The specific role for abnormal protein traffic as a common trigger of tubulointerstitial injury was suggested by studies with proximal tubular epithelial cells in culture showing that albumin and other proteins that have trafficked across the glomerular barrier into the urinary tubule can stimulate the cells to synthesize and release mediators responsible for macrophage and lymphocyte recruitment (monocyte chemoattractant protein-1 [MCP-1], RANTES) (4,5) and fibrosis (endothelin) (6). Both in nonimmune and immune models in the rat, including the extensive ablation of the renal mass (7–9), puromycin amionucleoside nephrosis (10), or passive Heymann nephritis (11), such cytokine (7,10) and endothelin pathways (8,9,11) were activated in the kidney as shown by detection of high mRNA and/or protein levels in the stages of interstitial leukocyte infiltration. The pathologic significance of cytokine-recruited leukocytes was strengthened by evidence that the treatment with neutralizing MCP-1 antibodies attenuated the severity of inflammation and renal function impairment (10).

Among antihypertensive drugs, angiotensin-converting enzyme inhibitors (ACEi) have potent antiproteinuric and renoprotective actions both in laboratory animals and in human renal diseases. Thus, in proteinuric patients, the greater the effect of ACEi on proteinuria, the greater the rate of reduction of progressively declining GFR (2). In rat models, ACEi were also found to consistently prevent severe structural injury (12) including interstitial lesions (13–15), presumably by the drug’s ability to limit excess protein traffic across the altered glomerular barrier and its deleterious consequences. However, the ultimate step of halting renal disease progression in the long term is not expected to be achieved with ACEi alone. We have reported previously that along with proteinuria and macrophage accumulation into the interstitium, the expression of MHC class II antigen (MHC II) is markedly enhanced in the rat kidney in the peritubular interstitium at the sites of excess protein traffic, even after a primary nonimmune insult (16). This led us to hypothesize that in proteinuric diseases only partially responsive to ACEi, protein-dependent stimuli persist and may trigger MHC-II overexpression and T lymphocyte-dependent interstitial reactions. This process should be targeted successfully by concomitant immunosuppressive therapy.

In the present study, we tested the possibility that immune mechanisms have a role in the proteinuric nephropathy of rats
after reduction of renal mass, in which the progressive renal disease is caused by a nonimmune insult to the kidney. A potentially important role can be played by dendritic cells, highly immunogenic cells of the immune system that capture and process antigens in peripheral organs and initiate immune responses (17). We characterized the mononuclear cell populations infiltrating the interstitium, and compared the effects of combining ACEi and an antilymphocyte agent versus either drug alone against disease. Both lymphocytes and cells positive for a dendritic cell antigen participate in the interstitial cellular infiltrate. The combined ACEi and immunosuppressive approach abrogates the disease, suggesting the underlying activation of protein-dependent immune mechanisms of renal injury.

Materials and Methods

Experimental Design

Male Sprague Dawley rats (Charles River Italia, Calco, Italy) with initial body weights of 275 to 320 g were used in these studies. Animal care and treatment were conducted in accordance with the institutional guidelines that are in compliance with national (D.L. n.116, G.U., suppl 40, 18 febbraio 1992, Circolare No.8, G.U., 14 luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJL358, December 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996). All animals were housed in a constant-temperature room with a 12-h dark/12-h light cycle and fed a standard diet. Renal mass reduction (RMR) was obtained by right nephrectomy and ligation of two or three branches of the left renal artery under anesthesia according to Olson et al. (18). The first set of experiments was designed to characterize the time course and the cell types infiltrating the remnant interstitium and to analyze the effect of ACEi on proteinuria and interstitial leukocyte infiltration. Four groups of animals (n = 4 each) were sacrificed on days 7, 14, 30, and 60 after surgery. Four sham-operated rats served as controls. A group of four RMR rats treated with ACEi (lisinopril, given from day 7 at the daily dose of 25 mg/L in the drinking water; Merck Sharp & Dohme, Rome, Italy) was sacrificed on day 60. Twenty-four-hour urine samples were collected in metabolic cages for the determination of urinary protein excretion.

The second set of experiments was designed to compare the effect of lisinopril, mycophenolate mofetil (MMF), or their combination on proteinuria, leukocyte infiltration, and renal structural and functional damage in this model. Sixty Sprague Dawley rats underwent 5/6 nephrectomy. Seven days after surgery, they were divided into four groups receiving vehicle (0.5% carboxymethylcellulose, n = 15), lisinopril (25 mg/L in the drinking water, n = 12), MMF (Roche, 20 mg/kg in 0.5% carboxymethylcellulose by gavage, n = 18), and lisinopril + MMF (n = 15) until day 60. Systolic BP, urinary protein excretion, and serum creatinine were assessed at each time point (days 0, 7, 14, and 60). At the end of the study, the rats were anesthetized and the kidneys were removed for histology and immunohistochemistry as described below.

Systolic BP

Systolic BP was recorded by tail plethysmography in conscious rats (19).

Urinary Protein Excretion

Twenty-four-hour samples were collected using metabolic cages, and proteinuria was determined by the modified Coomassie blue G dye-binding assay for proteins with bovine serum albumin as standard (20).

Serum Creatinine

Blood was collected from the tail vein of anesthetized animals. Serum was obtained after whole blood clotting and kept frozen at −20°C until assayed. Creatinine was measured by the alkaline picrate method (21).

Renal Histology and Immunohistochemistry

Light Microscopy. Fragments from remnant kidneys were taken from the center of noninfarcted areas. The specimens were fixed for 6 h in Dubosq-Brazil, dehydrated in alcohol, and embedded in paraffin. Sections (3 μm) were stained with Masson’s trichrome, hematoxylin and eosin, and periodic acid-Schiff reagent (PAS stain). Tubular (atrophy, casts, and dilation) and interstitial (fibrosis and inflammation) changes were graded on a scale of 0 to 4+ (0, no changes; 1+, changes affecting <25% of the sample; 2+, changes affecting 25 to 50% of the sample; 3+, changes affecting 50 to 75% of the sample; 4+, changes affecting 75 to 100% of the sample). At least 100 glomeruli were examined for each animal, and the extent of glomerular damage was expressed as the percentage of glomeruli presenting focal or global sclerotic lesions. All renal biopsies were analyzed by the same pathologist who was unaware of the nature of the experimental groups.

Immunohistochemical Analysis. Mouse monoclonal antibodies were used for the immunohistochemical detection of the following antigens: (1) ED1 antigen present in rat monocytes and macrophages (Chemicon, Temecula, CA); (2) a rat MHC class II antigen monomorphic determinant (OX6) (Serotec, Oxford, United Kingdom); (3) CD4+ cell surface glycoprotein, a 55-kD molecule expressed by helper T cells, thymocytes, and macrophages (W3/25) (Serotec); (4) rat CD8+ cell surface glycoprotein expressed by T suppressor cells (OX8) (Chemicon); and (5) an α-like integrin subunit on rat dendritic cells (OX62) (Serotec) (17.22). Detection of ED1 antigen was performed on paraffin sections using an alkaline phosphatase-Fast Red technique as described (23). The slides were heated twice for 5 min in 0.01 mol/L sodium citrate buffer, pH 6.0, at an operating frequency of 2450 MHz and 600-W power output of a microwave oven. They were allowed to cool for 15 min, then rinsed in distilled water twice and in phosphate-buffered saline (PBS) for 5 min. The sections were then incubated with the primary antibody for 1 h at room temperature. After washing in PBS, biotinylated sheep anti-mouse IgG (Boehringer Mannheim Biochemica, Mannheim, Germany) was applied for 30 min at room temperature. Sections were washed with PBS and incubated with alkaline phosphatase-conjugated streptavidin (Boehringer Mannheim Biochemica) for 30 min at room temperature, followed by washes with PBS and by development with Fast Red substrate (Boehringer Mannheim Biochemica). Sections were counterstained with Harris-type hematoxylin and mounted using an aqueous mounting medium (Bio-Optica, Milan, Italy).

Other leukocyte markers and MHC class II antigens were analyzed by indirect immunofluorescence technique. The tissue fragments were frozen in liquid nitrogen. Tissue sections (5 μm thick) were cut using a Mikrom 500 O cryostat (Walldorf, Germany). The sections were blocked with PBS/1% BSA, incubated overnight at 4°C with the primary antibody (W3/25, 40 μg/ml; OX8, 1:100; OX6, 5 μg/ml; OX62, 10 μg/ml), washed with PBS, and then incubated with Cy3-conjugated donkey anti-mouse IgG antibodies (affinity-purified, absorbed with rat IgG, 5 μg/ml in PBS; Jackson ImmunoResearch, West Grove, PA) for 1 h at room temperature. For each marker, the number
of cells was counted in at least 10 randomly selected high-power microscopic fields (×400) for each animal.

Statistical Analyses
Results are expressed as mean ± SEM. Data were analyzed using the nonparametric Kruskal–Wallis test for multiple comparisons. The statistical significance level was defined as \( P < 0.05 \).

Results
Analysis of Immune and Inflammatory Cellular Infiltrates in the Remnant Kidney and Effects of Antiproteinuric Drug Therapy
The time course of inflammatory cell infiltration into the interstitium, its relationship with proteinuria, and the effect of lisinopril on these parameters are shown in Figure 1. Rats with RMR developed progressively high levels of urinary protein excretion that were significantly increased compared with sham-operated rats starting from day 7 after surgery. The increase in urinary protein excretion over time was associated with the accumulation of increasingly high numbers of cells expressing MHC class II antigen into the cortical interstitium, confirming our previous findings in this model (16). ED1-positive cells (monocytes/macrophages) were a major component of the interstitial cellular infiltrate, and their numbers increased significantly from day 14 after surgery. Increased numbers of cells with CD4 or CD8 phenotype were found in the remnant kidney interstitium from day 7, together with the appearance of OX62-positive cells, which were absent in the kidneys of sham-operated rats. The numbers of all of these cell types further increased at subsequent time points, in parallel with increasing levels of proteinuria.

Lisinopril given from day 7 after surgery attenuated the increases both in urinary protein excretion and in infiltrating macrophage and T cell numbers compared with untreated RMR rats (RMR vehicle versus lisinopril, ED1: 40 ± 5 versus 12 ± 2, CD4: 59 ± 6 versus 25 ± 3, CD8: 17 ± 2 versus 10 ± 1 cells/high-power field; \( P < 0.05 \)). The numbers of MHC II-positive cells and of dendritic cells were also reduced significantly in RMR rats treated with lisinopril (OX6: 49 ± 5 versus 26 ± 3, OX62: 10 ± 2 versus 2 ± 1; \( P < 0.05 \)).

Comparison of the Effects of Lisinopril, MMF, and Their Combination on the Renal Disease of Rats with Remnant Kidney
These experiments were designed to assess whether concomitant immunosuppression from day 7 after surgery may further attenuate lymphocyte-dependent injury that was not fully prevented by antiproteinuric therapy alone.

Rats with RMR given vehicle, MMF, or combined lisinopril and MMF gained weight in a comparable manner (day 60, vehicle: 428 ± 15; MMF: 411 ± 15; lisinopril + MMF: 412 ± 10 g). Rats treated with lisinopril tended to grow more than the other groups (468 ± 7 g, \( P < 0.05 \)).

Urinary Protein Excretion. Lisinopril reduced urinary protein excretion both over vehicle and MMF alone at a statistically significant extent (lisinopril 64 ± 15 versus MMF 1544 Journal of the American Society of Nephrology J Am Soc Nephrol 10: 1542–1549, 1999
damage (score: 0.9) (Figure 2). MMF had a mild lowering effect on proteinuria detectable at 60 d after surgery that, however, did not reach significance. In contrast, both at 30 and 60 d the combined lisinopril and MMF therapy reduced urinary protein excretion more effectively than single drugs to very low levels comparable to baseline (16 ± 4 mg/d, P < 0.01 versus the other groups) (Figure 2).

**Immunohistology.** The results of the analysis of mononuclear cell subpopulations at day 60 are summarized in Table 1. Lisinopril markedly reduced the numbers of infiltrating ED1+, CD4+, CD8+, or OX62+ cells, as well as cells expressing MHC II antigen compared with vehicle. MMF treatment was also effective in reducing the numbers of infiltrating cells into the interstitium. However, MMF caused a less marked reduction of ED1+, MHC II+, and OX62+ cells (P < 0.05 versus lisinopril); in contrast, CD8+ cells and CD4+ cells were more effectively reduced by MMF (P < 0.05 versus lisinopril).

The combined lisinopril and MMF treatment dramatically attenuated the interstitial accumulation of all infiltrating cell types as well as the overexpression of MHC II antigen at the end of the study more effectively than any other treatment (Table 1).

**Renal Function and Pathology.** In RMR rats given lisinopril or the combined lisinopril and MMF treatment, serum creatinine levels at 60 d after surgery were lower than in the vehicle group (P < 0.05 and P < 0.01 versus vehicle, respectively). In contrast, in animals given MMF, serum creatinine values remained similar to those measured in vehicle rats (Table 2).

The inspection of periodic acid-Schiff-stained sections of kidney cortex of vehicle-treated rats at 60 d after surgery revealed the presence of glomerular sclerotic lesions, tubular dilation, atrophy, proteinaceous casts in tubular lumina, and areas of interstitial hypercellularity and fibrosis. Sclerotic lesions were present in 62% of glomeruli, and the mean score for tubulointerstitial damage was 2.4 in rats receiving vehicle.

Table 1. Effect of lisinopril, mycophenolate mofetil (MMF), and their combination on immunostaining of ED1+ macrophages, CD4+ and CD8+ cells, OX6+ (MHC II+) cells, and OX62+ cells a

<table>
<thead>
<tr>
<th>Group</th>
<th>ED1+</th>
<th>CD4+</th>
<th>CD8+</th>
<th>OX6+</th>
<th>OX62+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>42 ± 4</td>
<td>49 ± 4</td>
<td>16 ± 3</td>
<td>50 ± 4</td>
<td>10 ± 1.3</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>11 ± 1 c</td>
<td>22 ± 1 b</td>
<td>7 ± 0.6</td>
<td>26 ± 2.0 d</td>
<td>2 ± 0.3 c</td>
</tr>
<tr>
<td>MMF</td>
<td>17 ± 2 b</td>
<td>18 ± 1 b e</td>
<td>3 ± 0.3 b e</td>
<td>33 ± 1.0 d</td>
<td>7 ± 0.5 d</td>
</tr>
<tr>
<td>Lisinopril + MMF</td>
<td>5 ± 1 f</td>
<td>13 ± 1 f</td>
<td>1 ± 0.1 f</td>
<td>16 ± 1.0 f</td>
<td>1 ± 0.1 g</td>
</tr>
</tbody>
</table>

Table 2. Serum creatinine and renal histology in rats with remnant kidney untreated or treated with lisinopril and MMFe

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Creatinine (mg/dl)</th>
<th>Glomeruli with Sclerotic Changes (%)</th>
<th>Tubulointerstitial Damage (score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>1.6 ± 0.2</td>
<td>62 ± 9</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>1.0 ± 0.1 b</td>
<td>9 ± 2 c</td>
<td>0.9 ± 0.2 e</td>
</tr>
<tr>
<td>MMF</td>
<td>1.3 ± 0.2</td>
<td>41 ± 9</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Lisinopril + MMF</td>
<td>0.8 ± 0.1 d, e</td>
<td>2 ± 1 f</td>
<td>0.5 ± 0.1 c</td>
</tr>
</tbody>
</table>

P values are mean ±SEM at day 60.

P < 0.05 versus vehicle.

P < 0.01 versus vehicle.

P < 0.05 versus MMF.

P < 0.05 versus lisinopril.

P < 0.01 versus other groups.

P < 0.05 versus other groups.

Discussion

Results of immunohistology in this study show that cells of the immune system accumulate into the renal interstitium in rats after 5/6 nephrectomy, a paradigm model for progressive proteinuria and renal parenchymal injury (24). The feature of lymphocyte and macrophage infiltration is shared with immune

Clear cell subpopulations at day 60 are summarized in Table 1. Lisinopril consistently reduced both the frequency of glomerular sclerosis (9%) and tubulointerstitial damage to 60% of glomeruli, and the mean score for tubulointerstitial damage was 2.4 in rats receiving vehicle.

The combination of lisinopril and MMF fully prevented interstitial cellular infiltration and both glomerular sclerosis (2%; P < 0.01 versus the other groups) and tubulointerstitial damage (score: 0.5; P < 0.01 versus vehicle and MMF) (Figure 3D). No significant histologic changes were found in kidneys of sham-operated rats (not shown).

**Blood Pressure.** Vehicle-treated animals developed hypertension (182 ± 11 mmHg). Lisinopril, but not MMF, controlled systolic BP (139 ± 10 mmHg). Combined administration of lisinopril and MMF had a remarkable antihypertensive effect maintaining systolic BP at 139 ± 10 mmHg, a mean value lower than normal range (110 to 135 mmHg) (Figure 4).
diseases of the kidney that have intrinsic tendency to progress to the loss of renal function. In the remnant kidney and other proteinuric rat models, however, immune cell infiltration and tubulointerstitial injury are not caused by a primary immune insult. Evidence from clinical studies, as well as data on animal models and cell culture systems, led us to suggest that the enhanced passage of proteins across the glomerular barrier due to any insult to the filtration compartment, including the hemodynamic abnormalities found in remnant kidneys, may exert deleterious effects on the kidney and promote disease progression in part by eliciting a mononuclear cell inflammatory reaction. In this respect, we have recently documented that in the early phase of injury, macrophages and other cells expressing high MHC class II levels accumulate into the peritubular interstitium at the sites of tubular epithelial cell loading with proteins that are ultrafiltered in abnormal amounts and reabsorbed by proximal tubules. CD4+ and CD8+ lymphocytes and macrophages were found to accumulate into the renal interstitium in animals in which the hyperfiltration of proteins in the renal tubule was induced by repeated injections of albumin. Here, the early concomitant increases in the numbers of ED1 macrophages, OX62+ dendritic cells, and CD4+ and CD8+ lymphocytes as detected in the interstitium at 7 d, when animals were already proteinuric, point to putative roles for the interaction of lymphocytes, macrophages, and specialized antigen-presenting cells in immunologic processes possibly linked to endogenous protein processing and unfolding.

Proteinuria is the best predictor of disease progression to organ failure and can be uniquely lowered by ACEi independently of the underlying etiology. We found that antiproteinuric therapy by lisinopril given from day 7 after surgery attenuated the increases both in urinary protein excretion and in macrophage and T cell numbers in the interstitium. Enhanced MHC class II expression and numbers of dendritic cells were also limited by lisinopril. No studies found evidence to support direct anti-inflammatory or immunosuppressive effects of lisinopril. ACEi are known to ameliorate the function of the glomerular barrier to protein, thus preventing protein traffic in
proximal tubule (1,28). ACEi also control glomerular capillary pressure, which is increased as a consequence of functional adaptation to loss of functional kidney mass (12), and is in turn a factor leading to excess protein traffic (29). In proteinuric disease elicited by either immune or nonimmune insults, macrophage and T cell accumulation into the renal interstitium can be caused by locally upregulated chemotactic cytokines and other proinflammatory molecules of tubular epithelial cell origin (4,5), and by activated ultrafiltered proteins themselves (30,31). Such pathways, thus limited by antiproteinuric action of ACEi in remnant kidney, include enhanced gene expression for the macrophage, lymphocyte, and dendritic cell attractant MCP-1 (4), as well as for tumor necrosis factor-α (32) and osteopontin (16), and activation of complement C3 (30).

Next, because our data suggested that lymphocytes may contribute to tubulointerstitial injury despite no primary immune insult and that the accumulation of lymphocytes into the interstitium can be reduced by antiproteinuric therapy, we assessed the effects of a specific immunosuppressant agent versus ACEi and the potential therapeutic advantage of their combination to simultaneously interfere with lymphocyte function and with the protein traffic determinant of interstitial inflammation. MMF selectively inhibits lymphocyte proliferation among eukaryotic cells by inhibition of purine metabolism (33). MMF has been successfully used in human renal transplantation (34,35), it is well tolerated (34,35), and is being tested for other immune renal diseases in which antiproteinuric therapy may be recommended. We found that MMF greatly limited the accumulation of CD4+ and CD8+ cells. CD8+ lymphocytes were even lowered to control values, an effect predicted by the MMF action of inhibiting the generation of cytotoxic T cells (33). The associated reduction of infiltrating macrophages and MHC class II+ expression by MMF, reminiscent of studies in other models (36,37), can occur by virtue of secondary effects of lymphocyte inhibition with consequent perturbed T cell-dependent macrophage recruitment, as well as by impaired glycosylation of adhesion molecules (33). In contrast to lisinopril, however, MMF had little effect on protein traffic as reflected by persistent high proteinuria, and it was consistently less effective in reducing structural and functional damage at day 60. In particular, the glomerular sclerotic lesions, which often, but not exclusively, develop at the site of abnormal protein ultrafiltration, were only reduced by 30% by MMF vis-a-vis over 80% by lisinopril. MMF had no effect on systemic BP, suggesting that the partial beneficial effect of MMF alone was not due to interference with hemodynamic mechanisms of injury in this model. Comparing the immunohistology data, it is interesting that the numbers of macrophages and remarkably dendritic cells, as well as MHC class II+ expression, were significantly lower in the remnant interstitium of rats receiving lisinopril compared with MMF, suggesting that the upstream mechanism of protein traffic determinant possibly controlled by lisinopril is critical both to initiate and maintain severe inflammation and immune injury.

The combined lisinopril and MMF treatment dramatically lowered urinary protein excretion to normal levels and suppressed interstitial accumulation of macrophages, T cells, dendritic cells, and MHC class II overexpression at the end of the experiment to degrees that were comparable and even lower than those found in RMR rats at 7 d, when treatment was started. Remarkably, the combined treatment also preserved glomerular and tubular structural integrity, as well as renal function, better than lisinopril alone. BP was also lowered from the high levels found at day 7 to normal values in contrast to persistent hypertension of vehicle or MMF rats, an effect that was even more pronounced than that achieved by lisinopril. Thus, antiproteinuric therapy completely suppressed the disease in this model only if combined with MMF, which would suggest that immunologic mechanisms can perpetuate parenchymal damage in the circumstance of enhanced protein traffic. Our data do not exclude, however, that the benefit of the addition of MMF to lisinopril could be linked in part to additional anti-inflammatory effects in the glomerulus accounting for full restoration of the integrity of the glomerular filtration compartment and further amelioration of excess protein traffic, or other independent effects of MMF on glomerular cell function in the proteinuric setting.

Intrarenal processing of self epitopes has a role in immune tubulointerstitial injury (38,39). Central in this process is the interaction between the T cell receptor on lymphocyte surface and peptide epitope/MHC class II complexes on dendritic cells, macrophages (17), and renal tubular epithelial cells endowed with antigen-presenting capacity (40,41). Because the disease of remnant kidney is both primarily nonimmune and protein-dependent, our results suggest that protein traffic may provide a load of self epitopes possibly including ultrafiltered proteins themselves for local immune processing and consequent lesion progression. Regardless of the underlying mechanism with proteinuria, the ultimate consequences of concomitant antigen-independent and antigen-dependent mechanisms of injury are tubular loss and parenchymal scarring that were inhibited altogether by combined therapy.

Combined ACE inhibition and MMF immunosuppression is a novel route to therapy and remission of disease for the many conditions of known or undefined etiology associated with enhanced protein traffic that lead to kidney failure. The current treatment is ACEi, which slow, but may not halt, the progression of the disease, and which may not be effective to the same degree in all patients. A kidney transplant in turn does not lead to lifelong interruption of the process despite available therapies and otherwise noncomplicated course. Patients with a kidney transplant and late graft dysfunction are also candidates for such combined therapy, because their functioning kidney mass is reduced, they are proteinuric, and they need immunosuppression.

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