

# Alternative Therapies and Future Intervention for Treatment of Membranous Nephropathy

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Despite a multitude of investigation over the last 2 decades, the treatment of membranous nephropathy remains both controversial and suboptimal. Recent progress in the molecular pathways of inflammation and immunologic regulation holds the promise of offering futuristic alternatives and/or supplements to the standard regimen of glucocorticoids and alkylating agents. Several potential points of intervention along the path of disease development and expression have been identified: modulation of the immune response to the pathogenetic antigen; inactivation of the inflammatory pathways responsible for B and T cell activation; blockade of pathogenetic antibody formation by B and T cells; blockade of the complement cascade; blockade of lipid peroxidation of glomerular basement membrane components; and blockade of renal fibrosis resulting from proteinuria, lipiduria, and/or inflammation. These points of intervention form the basis for our discussion of such varied potential therapies for membranous nephropathy as: vaccines, inhibitors of tissue plasminogen activator, humanized monoclonal antibodies, mycophenolate mofetil, pentoxifylline, and others.

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MEMBRANOUS NEPHROPATHY has been the subject of extensive investigation pertaining to its pathogenesis, natural history, and response to treatment regimens. In fact, there are more randomized, controlled, clinical treatment trials concerning membranous nephropathy than for any other glomerular disease. One would expect then that the treatment of this glomerular disease would be standardized and straightforward. Yet, when confronted with a patient with membranous nephropathy, the treating physician wonders whether the patient will have a spontaneous remission, a response to angiotensin converting enzyme (ACE) inhibition or angiotensin receptor blockade, or, rather, is a candidate for more aggressive treatment using glucocorticoids and alkylating agents. Complicating the decision is the knowledge that these immunosuppressive treatments often do not work to attain remission, or fail to provide a sustained remission. Furthermore, most agents carry with them substantial attendant risks. Treatment of membranous nephropathy, as with most glomerular diseases, has been stuck in an era dependent on the use of glucocorticoid and alkylating agents, and, more recently, on immunosuppressive therapies borrowed from the transplant experience.

A new age is dawning on the treatment of all autoimmune diseases. Recent progress in under-

standing the molecular pathways involved in inflammation and immunologic regulation has been coupled with huge strides in biotechnology expertise. As a consequence, a number of biologic immunomodulatory products have been designed specifically to affect selected cell types or molecular pathways involved in the pathogenesis of various diseases. Because of their selective effect on targeted pathogenic pathways, these products hold the promise of therapeutic efficacy while limiting the adverse effects of nonselective immunosuppression. These advances are more than theoretical because highly successful advances in the therapy of rheumatoid arthritis and ankylosing spondylitis have been made by the use of agents that alter the interaction of tumor necrosis factor (TNF) with its receptor. Humanized monoclonal antibodies and fusion proteins targeting T and B cells have improved the therapeutic armamentarium in transplantation and in cancer therapy.

The application of new biologic agents requires an understanding of the pathogenesis of the disease. Thus, it is important to reflect on certain key target areas for therapeutic intervention in the pathogenesis of membranous nephropathy. In brief, membranous nephropathy is caused by immune complex localization in the subepithelial area of the glomerulus. It is likely that many endogenous or exogenous antigens are the focus for deposition in the subepithelial zone as either preformed or circulating immune reactants. The resultant immune response of binding of antibody to the targeted and deposited antigen results in the development of the typical picture of membranous nephropathy by immunofluorescence microscopy. Antibody formation is dependent on proliferation

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0270-9295/03/2303-0006\$30.00/0

doi:10.1016/S0270-9295(03)00000-0

**Table 1. Summary of Therapeutic Targets and Potential Agents in the Treatment of Membranous Nephropathy**

Pathogenic Mechanism	Target Pathway	Intervention or Agent
Allo- or autoimmune response Inflammatory mediators	Induction of tolerance	"Vaccination"
	Multiple pathways	Corticosteroids
	TNF- $\alpha$	Etanercept, infliximab (anti-TNF- $\alpha$ ), pentoxifylline
	IL-2	Cyclosporine
T & B cell proliferation and activation	PAI-1	PAI-1 inhibitor
	"Nonspecific" anti-proliferative agent	Cyclophosphamide, chlorambucil
	"More specific" T and B cells inhibitors	Mycophenolate mofetil
	Blockers of co-stimulation	Anti-CD40L, CTLA4-Ig
Complement activation	Anti-B cell	Rituximab (anti-CD20)
	Complement cascade	Eculizumab (anti-C5a), compstatin, pentosan polysulfate
Epithelial and GBM damage Chronic fibrosis, scarring, TI damage, progression	Lipid peroxidation, oxygen radicals	Probuocol
	Proteinuria	ACE inhibitors
	Lipiduria	HMG CoA reductase inhibitors
	PAI-1	PAI-1 inhibitor
	TGF- $\beta$	Anti TGF Antibodies
	Complement activation	Eculizumab (anti-C5a), compstatin, pentosan polysulfate

of B-cell clones. The studies of passive and active Heymann nephritis have shown that once the immune complex has formed, the activation of the complement cascade leads to the formation of the C5b-9 membrane attack complex. Complement-induced glomerular injury is therefore another likely mechanism of glomerular damage. Production of reactive oxygen species and lipid peroxidation of the cell membrane proteins and type IV collagen also play a substantial role. Similarly, thickening of the basement membrane in membranous nephropathy may be caused, in part, by a decrease in fibrinolytic activity owing to the stabilization of active plasminogen activator inhibitor-1 (PAI-1).

Several potential points of intervention along the path of disease development and expression can be identified conceptually. These include: (1) modulating the immune response to the pathogenic target auto- or alloantigen involved in the pathogenesis of membranous nephropathy; (2) blocking the activation of inflammatory pathways responsible for the B- and T-cell activation; (3) blocking the activation of B and T cells and the production of pathogenic antibodies; (4) blocking T-cell costimulatory pathways; (5) blocking the activation of the complement cascade; (6) blocking the lipid peroxidation of glomerular basement membrane components; and (7) blocking the progression of renal scarring that results from the chronic effects of inflammation, proteinuria, and/or lipiduria.

The therapeutic regimen currently used in the treatment of membranous nephropathy target some

of these steps (Table 1). The following sections describe currently available, developing, and hypothetical therapeutic options in the management of membranous nephropathy based on these points of intervention.

#### MODULATING THE IMMUNE RESPONSE TO THE PATHOGENIC TARGET AUTO- OR ALLOANTIGEN INVOLVED IN THE PATHOGENESIS OF MEMBRANOUS NEPHROPATHY

The nature of the antigen involved in immune complex deposition of membranous nephropathy remains unknown. If it were known, specific strategies to remove the antigen (void it either from the diet or from environmental exposures) would be the most appropriate way of preventing membranous nephropathy. There are futuristic approaches to engendering tolerance to specific known antigens. Our usual consideration of the term *vaccination* is based on the administration of infectious agents that either have been altered substantially or killed to manipulate the immune system to develop protective antibodies. This term is no longer restricted to this process, but applies to the manipulation of the immune system resulting in the suppression or regulation of inflammation. In fact, a more general use of the term *vaccination* can be considered as the induction of an immune response beneficial to the host. Antigen-specific alterations of the immune system may be the most specific and least toxic way to manipulate the immune

system. Although these types of antigen vaccination approaches never have been tried in glomerular disease, antigen-specific therapies have been developed for a mouse model of multiple sclerosis (the experimental autoimmune encephalomyelitis model) The most successful of these so far is the use of glatiramer acetate, or copolymer-1.<sup>1</sup> This antigen is a random copolymer of 4 amino acids that was designed to mimic myelin basic protein and to induce the experimental autoimmune encephalomyelitis model. This vaccination works by an antigen-specific manner to suppress this encephalopathic disease by generating regulatory T cells. These regulatory cells secrete anti-inflammatory cytokines including interleukin 10 and transforming growth factor (TGF)  $\beta$ .<sup>2</sup> Anti-inflammatory cytokines depress inflammation caused by T cells of different specificity.

In humans, a tolerogenic strategy has been attempted in the treatment of systemic lupus erythematosus with the use of a compound, LJP394. It was designed to induce tolerance in anti-DNA B cells by cross-linking their surface antibodies in the absence of costimulatory signals from T cells.<sup>3,4</sup> LJP394 is composed of 4 double-stranded oligonucleotides linked to a common platform. In human trials, LJP 394 was shown to decrease circulating anti-dsDNA antibodies,<sup>5</sup> but not to reduce the incidence of disease flares.<sup>4</sup>

Could such tolerogenic strategies be used in membranous nephropathy? The answer, of course, is yes if the nature of the antigen(s) were established. The substantial work aimed at understanding the antigen GP-330 in Heymann nephritis has provided understanding of the nature of this specific protein. To date, the evidence that this antigen is the target of the human membranous immune response never has been established. However, strategies that may generally induce regulatory T cells and anti-inflammatory cytokines derived from other kinds of vaccines may prove to be nonspecific anti-inflammatory approaches.

#### BLOCKING THE ACTIVATION OF INFLAMMATORY PATHWAYS RESPONSIBLE FOR B- AND T-CELL ACTIVATION

Corticosteroids, frequently used as a first-line drug in the management of membranous nephropathy and other immune-mediated glomerulonephritides, act in part by inhibiting several proinflammatory pathways that lead to the activation of

T and B cells as well as the recruitment of mononuclear cells to the site of injury.

TNF- $\alpha$ , a potent 17-kd proinflammatory cytokine produced by monocytes, macrophages, and possibly glomerular mesangial cells, is implicated in the pathogenesis and progression of a number of autoimmune diseases,<sup>6</sup> including glomerulonephritides.<sup>7</sup> TNF- $\alpha$  induces the expression of major histocompatibility complex class I and II molecules, endothelial adhesion molecules, and the release of other proinflammatory molecules such as interleukin-1 $\beta$ , and TGF- $\beta$ . The role of TNF- $\alpha$  in the pathogenesis of membranous glomerulopathy<sup>8-10</sup> is supported by evidence of high urinary levels of this cytokine in patients with this disease. Three therapeutic agents capable of blocking TNF- $\alpha$  recently have drawn attention in the treatment of membranous nephropathy. One older agent, pentoxifylline, has preliminary human data, whereas 2 newer agents, the monoclonal antibodies infliximab and the fusion protein etanercept, each have a strong theoretical basis for efficacy.

The methylxanthine derivative, pentoxifylline, is a phosphodiesterase inhibitor that is best known for its use in symptomatic peripheral vascular disease. It has strong anti-inflammatory properties such as reducing the production of TNF- $\alpha$ , as well as other cytokines in normal and in disease states.<sup>11-15</sup> Ducloux et al<sup>16</sup> selected 10 patients with biopsy examination-proven idiopathic membranous nephropathy and persistent nephrotic syndrome despite maximum-dose treatment with ACE inhibitors. All 10 patients were treated with oral pentoxifylline at 1,200 mg/d for a total of 6 months. Changes in proteinuria and in plasma and urinary TNF- $\alpha$  were assessed. Importantly, patients did not receive any immunosuppressive therapy during the treatment period. At the end of 6 months of follow-up, 9 patients were in remission in proteinuria (though not defined), and the average proteinuria changed from 11 g/24 h to 1.8 g/24 h. The average plasma TNF- $\alpha$  changed from 12 pg/mL to 0.5 pg/mL, and the average urine TNF- $\alpha$  changed from 2.5 pg/mL to 0.3 pg/mL.

Etanercept (Enbrel; Immunex Corporation, Seattle, WA), a fusion protein of a TNF receptor (p75) with the Fc fragment of human immunoglobulin (Ig)G,<sup>17</sup> currently is approved for the treatment of rheumatoid arthritis. Infliximab (Remicade; Centocor, Inc., Malvern, PA) is a chimeric mouse/human monoclonal antibody directed against TNF- $\alpha$ . It is approved for the treatment of

active and fistulizing Crohn's disease and rheumatoid arthritis. Both biologic agents are potent immunosuppressive agents associated with serious risks (life-threatening infections, neurologic events, and demyelinating reactions,<sup>18</sup> and the development of antinuclear antibodies as well as skin vasculitis<sup>19</sup>). Neither etanercept nor infliximab thus far have been evaluated in the treatment of membranous nephropathy.

It is important to recall that cyclosporine exerts its immunosuppressive effect by inhibiting production of interleukin-2, interleukin-3, and interferon- $\gamma$ , resulting in inhibition of T-lymphocyte helpers/inducers and cytotoxic cell function. Cyclosporine has been investigated as a therapy for membranous nephropathy, resulting in improvement in proteinuria and stability of renal function in two thirds of patients.<sup>20-22</sup> Interestingly, repeat biopsy examinations obtained from cyclosporine-responsive patients revealed the persistence of immunoglobulin and complement deposits,<sup>23</sup> suggesting that the disease process was not halted.

#### BLOCKING THE B- AND T-CELL ACTIVATION AND THE PRODUCTION OF ANTIBODIES DIRECTED AGAINST THE MEMBRANOUS OFFENDING ANTIGENS

Removal of offending autoantibodies has long been the mainstay of our treatment. Several anti-inflammatory and immunosuppressive agents abrogate this step in the pathogenesis of immune-mediated diseases. Among such agents are cyclophosphamide and chlorambucil, which represent the traditional approach to the management of membranous glomerulopathy. More recently, mycophenolate mofetil (MMF) has been the focus of interest in the management of this and other glomerulonephritides. In addition, removal of autoantibodies with plasmapheresis has been tried in membranous glomerulonephritis with little data to commend it. Similarly, pooled intravenous immunoglobulins have been considered for this, as well as all other autoimmune disease with only anecdotal evidence for its efficacy.

One of the most specific drugs in the current armamentarium that alters antibody production is MMF. It is the esterified, prodrug of mycophenolic acid, an agent first purified from the fungus *Penicillium* in 1919. MMF is a potent, noncompetitive, and reversible inhibitor of the eukaryotic inosamine monophosphate dehydrogenase.<sup>24</sup> This enzyme is crucial in the pathway for de novo

synthesis of guanine nucleotides. Lymphocytes are dependent on both the de novo and salvage pathways for purine biosynthesis. Most other cells can survive with only the salvage pathway. Hence, MMF is a rather selective inhibitor of B and T lymphocytes.

MMF has shown efficacy in other disease states in which T-cell activation appears to have a central pathogenetic mechanism. These diseases include acute allograft rejections,<sup>25,26</sup> the treatment of lupus nephritis,<sup>27</sup> and chronic allograft rejection.<sup>28</sup> There is considerable animal data reporting a favorable effect of MMF in various types of experimental glomerulopathy.<sup>29,30</sup> Animal data examining the effect of MMF in experimental membranous nephropathy is sparse, but worth reviewing.<sup>31,32</sup> Penny et al<sup>31</sup> showed the efficacy of MMF in preventing active Heymann nephritis among rats when treated at the beginning of the disease course. MMF given to rats from 0 to 4 weeks prevented the occurrence of Heyman nephritis during that period of treatment. Furthermore, these rats never developed significant proteinuria during the entire 16 weeks of follow-up. Interstitial infiltrates of T cells, natural killer cells, and macrophages were not observed in the cortex of these animals treated from 0 to 4 weeks. MMF, given at the other times, did not have a beneficial effect. In another study of rats with active Heymann nephritis,<sup>32</sup> those animals given MMF at the beginning of the disease process developed less proteinuria than the animals not given MMF. Furthermore, MMF significantly muted the production of antibodies to gp330 at 4 weeks compared with no treatment with MMF. This attenuated antibody response remained after cessation of MMF therapy. Yet, histologic evaluation of the rat kidneys revealed that MMF did not significantly lower the glomerular deposition of IgG.

In contrast to the shown effectiveness of early dosing of MMF in animal studies, human clinical studies examined the effect of MMF among individuals with established membranous nephropathy. It therefore comes as little surprise that these studies have shown only modest efficacy.<sup>33-35</sup> Furthermore, these studies are confounded by several factors. First and foremost, how can a clinician identify an individual early in the course of membranous nephropathy, analogous to the time course in experimental Heyman nephritis? Second, these human studies represented a highly selective group of individuals with membranous nephropathy—those resistant to any previous cytotoxic agents. Third, a signifi-

cant number of individuals were prescribed steroids, cyclosporin, HMG-CoA reductase inhibitors, and/or ACE inhibitors concurrently. Fourth, the studies had important design limitations—lack of a control group and they were retrospective.

Thus, despite its basic biologic potential, its efficacy in the transplant experience, and the impressive animal data, MMF has proven of only modest value in the treatment of human membranous nephropathy. Clinical studies need to be performed in membranous nephropathy in a prospective and randomized fashion of unselected patients to determine its efficacy in any measure, let alone as salvage therapy. It is most likely that the pathogenetic forces interrupted by MMF in the transplant setting are not operating in membranous nephropathy.

A novel approach to removing B cells has been made possible with the introduction of a chimeric monoclonal antibody known as rituximab. This agent contains a human Fc IgG1 region, and a murine variable region specific for the CD20 B-cell antigen. Despite nearly 6 years of postmarketing experience, the exact mechanism by which rituximab works *in vivo* has not been discerned fully. The most important likely mechanism of action is the ligation of membrane receptor CD 20, resulting in inhibition of B-cell activation, proliferation, differentiation, and reduced immunoglobulin secretion.<sup>36-40</sup>

Data on the efficacy of rituximab in membranous nephropathy are preliminary and available only in humans. Remuzzi et al<sup>41</sup> selected a group of 8 patients with biopsy examination–proven idiopathic membranous nephropathy, persistent proteinuria ( $>3.5$  g/24 hours for at least 6 mo; range, 4.8–16.0 g/24 hr), and treated with full-dose ACE inhibition. All 9 study subjects received intravenous infusions of rituximab ( $375$  mg/m<sup>2</sup>) every 4 weeks for a total of 20 weeks. Subjects had various clinical variables, including the primary outcome proteinuria, assessed every 4 weeks. During the treatment period, the patients experienced a significant, nonlinear reduction in proteinuria. Two individuals achieved a full remission in proteinuria as defined by protein excretion of 1 g/24 hr or less, and 3 individuals achieved a partial remission, proteinuria of 3.5 g/24 hr or less. None of the remaining subjects had any worsening of their proteinuria. The average reduction in proteinuria was 62% from baseline. Randomized controlled trials using this promising agent need to be performed.

#### BLOCKADE OF T-CELL COSTIMULATORY PATHWAYS

T-cell activation is dependent on engagement of 2 signals. Signal 1 depends on the interaction of the T-cell receptor with the major histocompatibility complex on the antigen-presenting cell. A second costimulatory signal is necessary for the optimal activation of a T cell, without which it is rendered anergic.<sup>42</sup> In fact, the type and degree of T-cell activation depends on the strength of the T-cell receptor–mediated signal 1 and a balance of stimulatory and inhibitory second signals. This mechanism is thought to be fundamental in maintaining tolerance to self-antigens; and its dysregulation has been implicated in the development of autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis.<sup>43,44</sup> Improved understanding of the cell surface proteins and the molecular mechanisms involved in costimulatory signals paves the way to the development of therapeutic agents capable of modulating an immune response by affecting these pathways. Two major costimulatory pathways, the B7-CD28/CTLA4 pathway and the CD40-CD40L pathway, are well characterized. The interaction of CD40 ligand (CD40L, CD154) on the T-helper cell surface with CD40 on the antigen-presenting cell or B cell is crucial for B-cell activation, proliferation, differentiation, antibody isotype switching, and the generation of B-cell memory. It also is important for T-cell activation either by up-regulation of B-cell molecules B7-1 and B7-2, which in turn provide costimulatory message for T-cell activation, or by a direct signal delivered to T cells via CD40L itself.<sup>45</sup> The interaction of B7-1 and B7-2 with CD28 on the T cell results in an activation of the latter, whereas their engagement of CTLA4 mediates an inhibitory signal.<sup>46</sup> The CD40-CD40L and the B7-CD28/CTLA4 pathways therefore present interesting targets for the modulation of the immune response to self-antigens. Two humanized monoclonal antibodies directed against CD40L were generated, one of which is under evaluation in the treatment of systemic lupus erythematosus and lupus nephritis whereas the phase 2 trial of the other was interrupted because of thrombotic complications. CTLA4-Ig, a fusion protein composed of the extracellular domain of CTLA4 and the Fc portion of IgG2a, blocks the interaction of B7 with CD28. CTLA4-Ig has been evaluated in a phase I trial in the treatment of psoriasis.<sup>47</sup> Whether either

costimulatory pathway is implicated in the pathogenesis of membranous nephropathy is unknown.

#### BLOCKING COMPLEMENT ACTIVATION

Inhibiting the activation of the complement cascade and the formation of the membrane attack complex or the membrane attack complex-mediated glomerular and tubulointerstitial cell injury has been the focus of extensive research efforts. The recognition of several complement regulatory proteins provides as many therapeutic opportunities to interfere with this mechanism of injury. These include soluble forms of MCP, DAF, CR1, and CD59.<sup>48</sup> This approach in the treatment of immune-complex mediated glomerular diseases is supported by studies in rodents.<sup>49-51</sup> The search for soluble inhibitors of complement activation also is pursued actively and several compounds are at various stages of investigation.<sup>51</sup> Compstatin is a 13-mer synthetic peptide that binds C3, C3b, and C3c, and reversibly inhibits complement activation through the classic and alternate pathways.<sup>52</sup> In vivo, inhibition of complement activation was shown in primates using the heparin/protamine complex model.<sup>53</sup> Pentosan polysulfate is an orally active mucopolysaccharide similar in structure to heparin that is Food and Drug Administration approved for the symptomatic treatment of interstitial cystitis.<sup>54</sup> Pentosan polysulfate is shown to inhibit complement activation in vitro,<sup>55</sup> and limit complement-mediated myocardial injury.<sup>56,57</sup> Whether effective complement inhibition, without significant anticoagulation, can be achieved in vivo from oral dosing remains to be determined. No information is available as to the possible effects of this drug on membranous nephropathy or other glomerular diseases.

Eculizumab (Alexion Pharmaceuticals Inc., Cheshire, CT) is a humanized monoclonal antibody that binds complement factor C5, preventing the generation of C5a anaphylatoxin and the formation of the C5b-9 membrane attack complex, while preserving the ability of generating C3b, critical for the opsonization of pathogenic microorganisms and the clearance of immune complexes. Activation of the complement cascade and the formation of the C5b-9 membrane attack complex are implicated in the pathogenesis of glomerular diseases characterized by the deposition or in situ formation of antibody-antigen immune complexes, such as membranous nephropathy<sup>58</sup> and lupus nephritis. In animal models of complement-mediated inflammatory disease, the use of a murine

antibody to C5 resulted in salutary effects.<sup>59</sup> In a phase II placebo-controlled trial of Eculizumab in the treatment of idiopathic membranous nephropathy, few patients achieved effective persistent complement inhibition at the dose used in this trial. Nevertheless, the data currently available suggest a beneficial effect on proteinuria, hyperlipidemia, and hypoalbuminemia, without adverse effects on glomerular filtration rate, especially in those patients in whom inhibition of the complement cascade could be documented.<sup>60</sup> These encouraging results need confirmation in further trials of this new agent. Because Eculizumab is not thought to have any effect on the production of the pathogenic antibodies, or the formation of immune complexes, it is conceivable that the most benefit of this agent may be in combination with agents targeting this more proximal step in the pathogenesis of disease.

#### Intravenous Immunoglobulin

High-dose pooled immunoglobulin (IgIV) has been used in the treatment of various autoimmune diseases. IgIV likely affords its immunomodulatory effects through several possible Fc- and F(ab)-mediated mechanisms.<sup>61,62</sup> These include Fc-mediated blockade of Fc $\gamma$  receptors on macrophages, modulation of synthesis and release of cytokines, modulation of T- and B-cell function and proliferation, variable region-mediated neutralization of autoantibodies by introducing anti-idiotypic antibodies that inhibit the action of the offending autoantibodies,<sup>63,64</sup> and modulation of the antibody immune repertoire. There is also evidence that IgIV interferes with complement-mediated immune damage by binding to C3b and C4b, and interfering with their binding to target cells. This latter mechanism may be particularly pertinent to membranous nephropathy as suggested by a study in passive Heymann nephritis whereby treatment with systemic immunoglobulin resulted in decreased proteinuria, associated with decreased glomerular deposition of C3c and C5b-9, but no change in the amount, size, or distribution of the subepithelial immune complexes.<sup>65</sup>

IgIV was tested in the treatment of 9 patients (5 patients with normal glomerular filtration rate and 4 patients with moderate renal insufficiency) with membranous nephropathy using initially 3 daily doses (0.4 g/kg body weight) repeated 3 times at 21-day intervals and followed by 10 monthly doses. A total of 5 patients achieved total remission and 3 patients attained partial remission. Interest-

ingly, repeat renal biopsy examinations performed on 5 responders revealed the absence of immune deposits and recovery of the glomerular lesions by light microscopy.<sup>66</sup>

A retrospective analysis compared the outcome of 30 patients treated with IgIV (in addition to corticosteroids  $\pm$  immunosuppressants in 16%) compared with 56 control patients who received either no treatment (in 30%) or treatment with either corticosteroids alone or in combination with immunosuppressants.<sup>67</sup> The IgIV regime consisted of 1 to 3 courses of 0.1 to 0.15 g/kg/d for 6 consecutive days. This study revealed a statistically significant higher rate of complete remission (57% versus 10%;  $P = .006$ ) at 6 months among patients treated with IgIV. There was no statistically significant difference in the rate of remission at 12, 24, or 60 months. The benefits of IgIV were limited to the subgroup of patients with a homogeneous (synchronous) pattern of immune deposits.

#### BLOCKING LIPID PEROXIDATION

Once immune complexes are deposited or formed in the subepithelial space, subsequent injury to the epithelial cell membrane and the glomerular basement membrane is mediated, at least in part, by the production of reactive oxygen species and lipid peroxidation of cell membrane proteins and of type IV collagen. The salutary effect of inhibiting such lipid peroxidation is suggested by animal studies whereby treatment of rats with passive Heymann nephritis with an lipoperoxidase (LPO) inhibitor (probucol) led to a  $\sim$ 85% decrease in proteinuria, and in glomerular staining for malondialdehyde. However, probucol did not effect the formation or deposition of immune complexes. Recently, the effects of inhibiting lipid peroxidation with the LPO scavenger probucol were reported in a study of 15 patients with membranous nephropathy resistant to conventional immunosuppressive therapy ( $n = 7$ ) and/or ACE inhibitor treatment ( $n = 12$ ). Probucol (1 g/d)  $\times$  3 months, but not lovastatin (10-20 mg/d orally)  $\times$  3 months was associated with a significant reduction in proteinuria (median [range]: 6.4 [3.8-9.1] g/d versus 4.7 [1.3-16] g/d;  $P < .05$ ) and partial remission in 4 patients.

#### BLOCKING THE PROGRESSION OF RENAL SCARRING THAT RESULTS FROM THE CHRONIC EFFECTS OF INFLAMMATION

The prevention of progressive renal fibrosis is one of the most important issues in all of chronic

kidney disease therapy. This is certainly true for membranous glomerular disease in which the lengthy course and repetitive bouts of inflammation and proteinuria and hyperlipidemia conspire to scar the renal cortex. There are multiple, and perhaps now even conventional, methods for diminishing proteinuria and hyperlipidemia. More experimental approaches to this process are soon to be made available.

Hyperlipidemia commonly is found in patients with membranous nephropathy. Yet the exact role of the lipid abnormalities in progression of membranous nephropathy is still unclear. Among various animal models of kidney disease, but not Heymann nephritis, hyperlipidemia has been shown to cause histologic changes in the kidney. For example, healthy animals made hyperlipidemic developed mesangial fat deposition and/or glomerulosclerosis.<sup>68-71</sup> Diet-induced hyperlipidemia also was associated with the development of glomerulosclerosis in hypertensive, diabetic, or partially nephrectomized rats.<sup>70,72,73</sup> Furthermore, the treatment of hyperlipidemia with a variety of cholesterol-lowering agents reduced the development of glomerulosclerosis compared with no treatment in animals.<sup>74-77</sup> Preliminary data from Rayner et al<sup>78</sup> suggest that treatment of hyperlipidemia may have a role in membranous nephropathy. In that study, 17 patients with membranous nephropathy were treated with a low-cholesterol diet. In addition, 9 of the patients received the HMG-CoA reductase inhibitor, simvastatin. After 19 months of follow-up, the simvastatin group had a significant decrease in serum lipid levels and in proteinuria, whereas there was no change in these parameters for the group given just diet modification. However, simvastatin did not significantly alter the decline of glomerular filtration rate compared with diet therapy alone.

If proteinuria leads to progressive renal damage, then patients with membranous nephropathy should be a prime group in whom to explore the issue. Disruption of the glomerular basement membrane occurs in most types of glomerulonephritides, including membranous nephropathy. The loss of structural integrity allows for the passage of numerous plasma proteins (albumin, clotting factors, inflammatory cytokines) into the tubular space.<sup>79,80</sup> It has been proposed that tubular cells bathed in serum proteins may up-regulate genes encoding vasoactive and inflammatory substances.<sup>81,82</sup> Subsequently, these active substances are secreted by renal tubular epithelial cells toward the basolateral

compartment.<sup>83-85</sup> This in turn gives rise to an inflammatory reaction in the interstitium postulated to lead to progressive scarring.<sup>86-89</sup>

It follows that proteinuria is considered a strong risk factor for progressive renal disease in many types of nephropathies,<sup>90-92</sup> including in membranous nephropathy.<sup>93-96</sup> Strategies to lower proteinuria have focused on inhibition of angiotensin II. In membranous nephropathy, ACE inhibition has shown beneficial effects in animals with Heymann nephritis. Clinical trials specific to membranous nephropathy have shown the efficacy of ACE inhibition in reducing proteinuria.<sup>97,98</sup> Yet these studies have been limited by small sample size and short follow-up period. Larger studies that have included various forms of chronic kidney disease, including membranous nephropathy, have shown the superiority of ACE inhibition over other types of antihypertensive agents in reducing proteinuria.<sup>99-101</sup> Furthermore, large studies have shown the efficacy of ACE inhibition in reducing proteinuria, and in slowing the decline in renal function.<sup>102,103</sup>

Newer and more experimental approaches to abrogating renal fibrosis are on the horizon. For instance, PAI-1 is a member of the serine proteinase inhibitor family, or serpin, and is responsible for the physiologic inhibitor of tissue-type and urokinase-type plasminogen activators.<sup>104</sup> An ever-enlarging number of factors are reported to increase PAI-1 expression including growth factors, coagulation factors, hormones, environmental agents, and obesity. Although there are known protease inhibitor-dependent actions of PAI-1, a growing number of nonprotease roles for PAI-1 are being discovered, especially in renal disease. In recent years, PAI-1 has emerged as a critical mediator of glomerulosclerosis and renal interstitial fibrosis. PAI-1 could be a possible therapeutic target to delay progressive renal disease. Additionally, PAI-1 may induce disease regression if treatment is begun before glomerulosclerosis occurs. In membranous nephropathy, PAI-1 may play a special role. PAI-1 transcripts have been found in large numbers in membranous nephropathy.<sup>105</sup> The PAI-1 protein has been found within epimembranous deposits to membranous nephropathy.<sup>106</sup> The PAI-1 protein has been found in within epimembranous deposits to membranous nephropathy.<sup>107</sup> Interestingly, megalin (gp300), the target antigen in Heyman nephritis, is a plasminogen receptor. Although any role for PAI-1 in membranous nephropathy remains entirely speculative, inhibitors

of PAI-1 may have a profound effect on progressive renal disease and most specifically in membranous nephropathy.

Two other molecules important in progression of renal disease deserve attention. The fundamental role of TGF- $\beta$  in progressive renal fibrosis has been well documented.<sup>108-112</sup> Antibodies that inhibit TGF- $\beta$  are in early phases of clinical study. Another protein, bone morphogenic protein-7, has been shown to play a role in progressive renal fibrosis.<sup>113-116</sup> Small bone morphogenic protein-7 molecules are available and could be used in clinical studies to protect the kidney from scarring.

## CONCLUSION

In conclusion, futuristic therapies for membranous nephropathy are fast becoming a reality. These agents attempt to target pathogenetic pathways specific to membranous glomerulonephritis. These therapies need to be tested in well-constructed and well-organized randomized clinical trials for us to know how well they perform in practice rather than just on a theoretical basis. Moreover, a more precise measure of disease activity may alter the type of drug combinations that may prove to be the most efficacious in treating these patients. Certainly, understanding the proximate cause of membranous disease in humans, that is, understanding the nature of the antigens and autoantibodies themselves, may lead to vaccinations or other means of inducing long-term tolerance.

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