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Idiopathic Membranous Nephropathy: An Autoimmune Disease

Sudesh P. Makker, MD and Alfonso Tramontano, PhD

Department of Pediatrics, University of California Davis School of Medicine, Davis, California

Summary

For more than 50 years researchers have debated the evidence for an autoimmune basis of human idiopathic membranous nephritis (MN). Work published in the past 2 years has substantially strengthened the belief that MN is indeed an autoimmune disease of the kidney. Autoantibodies of the IgG4 subclass to at least three podocyte membrane proteins including phospholipase A₂-receptor, aldose reductase, and manganese superoxide dismutase have been detected by immunoblotting in sera as well as in acid eluates prepared from renal biopsy tissue of patients with this disease, using either whole tissue or microdissected glomeruli from frozen sections. In each case the podocyte antigen has been shown to co-localize with the subepithelial glomerular immune deposits in renal tissue of the same patients. It is not certain if any of these podocyte proteins is an inciting/primary autoantigen or whether they are secondary antigens recruited by intermolecular epitope-spreading, initiating from a yet-to-be-discovered autoantigen. Although it is clear that autoantibodies to podocyte membrane proteins are elicited in idiopathic MN and contribute to the formation of the subepithelial deposits, many questions remain concerning the triggers for their development and their contribution toward proteinuria and progression of the disease.

The term membranous nephropathy (MN), or membranous glomerulonephritis, is used to describe a chronic glomerular disease that on light, immunofluorescence (IF), and electron microscopy (EM) study of renal tissue shows a set of distinct morphologic features in glomeruli, including thickened glomerular basement membrane (GBM) and GBM spikes, granular staining for IgG and complement along the periphery of glomerular all capillary loops, and electron-dense subepithelial deposits corresponding to the granular IgG staining. The term *idiopathic* or *primary*, as opposed to *secondary*, is used when no cause can be deduced from the history, physical examination, and laboratory tests commonly performed to assess a patient with proteinuric renal disease or glomerulonephritis. The secondary form can be associated with infections, neoplasia, or autoimmunity and is not the subject of this review.

Clinically, most patients present with nephrotic syndrome or have proteinuria detected on a routine urinalysis. Idiopathic MN occurs in all age groups and races and both sexes all over the world and is a leading cause of nephrotic syndrome among Caucasian adults. Spontaneous remission of the disease is common in children¹ but also occurs in adults.^{2,3} Although several immunosuppressive drugs often are used to treat individual patients, the treatment of idiopathic MN remains empiric at this time and, with or without treatment, nearly a third of patients progress to end-stage renal disease.⁴ It is becoming increasingly

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Address reprint requests to Sudesh Paul Makker, MD, Department of Pediatrics, University of California Davis, One Shields Ave, Surge 1 Room 1121, Davis, CA 95616. spmakker@ucdavis.edu.

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clear and more widely accepted that idiopathic MN is an organ-specific autoimmune disease involving the kidney. Starting with the background of the origin of the autoimmune concept, this brief review focuses on recent developments that have considerably strengthened the evidence for an autoimmune basis of idiopathic MN.

HEYMANN MODELS

Most of what we know today about the pathogenesis of idiopathic MN, reviewed at length recently by Glassock,⁵ has been learned from the study of Heymann models of this disease experimentally induced in rat. Therefore, to put these recent developments in perspective, it is helpful to appreciate how these models are induced in the laboratory, and how various concepts of the pathogenesis of MN evolved over time from the study of these models by many investigators in this field. There are two models of Heymann nephritis (HN), active and passive.

The active HN (AHN) model, originally described by Heymann et al⁶ in 1959 by immunizing rats with an emulsion of whole rat renal cortex⁶ in complete Freund's adjuvant, also can be induced by similar immunizations with a crude fraction of renal cortex called Fx1A,⁷ with purified complex of a 600-kd glycoprotein (gp600/gp330/LRP-2/megalin) and the bound 39- to 45-kd receptor-associated protein (RAP),⁸⁻¹¹ with purified megalin without RAP,¹² or even with a small (60 kd) N-terminal proteolytic fragment of native megalin.¹³ These studies are enumerated here to highlight the fact that a single autoantigen or even a small fragment of it that contains the immunodominant epitopes can induce a full spectrum of MN including the nephrotic syndrome, a concept that could be relevant to therapy or even prevention of human idiopathic MN by vaccination. After immunization with any of these antigen preparations the rats develop serum autoantibodies to megalin within 2 weeks, typical immune deposits of MN in glomeruli at 3 to 4 weeks, proteinuria at 6 to 8 weeks, and a nephrotic syndrome at about 12 weeks. Once established, the proteinuria persists until the rats die at about 12 to 15 months of age, usually from infections or complications of the nephrotic syndrome.

The passive HN (PHN) model is induced by injecting systemically normal rats with a heterologous antiserum to rat Fx1A¹⁴⁻¹⁶ or antiserum to rat megalin.¹⁷ In the former, typical glomerular deposits of MN can be seen within minutes, with proteinuria developing between 4 and 7 days after the injection. This phase of disease has been called the *heterologous phase* because it results from the glomerular deposits formed by the heterologous antibody. The heterologous phase is followed in 2 to 4 weeks by the autologous phase when glomerular deposition of rat IgG (directed against the injected heterologous IgG) and worsening of proteinuria are noted.¹⁴ Much of the work in PHN has been performed on the heterologous phase of the disease, mostly because it is easier to control experimental conditions over the shorter time frame.

ORIGIN OF THE ORGAN-SPECIFIC AUTOIMMUNITY CONCEPT

Soon after his description of the AHN model Heymann noted that the disease could not be induced by immunizations with homogenates of the other rat organs (liver, muscle, lung), but could be induced in a unilaterally nephrectomized rat with the rat's own kidney. Thus, he concluded that the disease had an autoimmune basis and therefore called it *autoimmune nephrosis*.^{18,19} Glassock et al⁷ in 1968 noted that although the disease could be induced equally well with either rat or human Fx1A, only the rat but not the human antigen in Fx1A could be detected in the glomerular deposits whether the rats were immunized with rat or human Fx1A. This observation supported the concept that antibodies in AHN were directed against autologous renal antigens. Heymann originally observed thickening of the GBM on light microscopy. In subsequent work he further noted the remarkable similarity of the

model with the human idiopathic MN by IF and EM. In fact, the appearances of glomerular lesions as analyzed by light, IF, and EM were indistinguishable in the two diseases.²⁰ Thus, the notion that the human idiopathic MN could very well be an autoimmune disease of the kidney was born from this analogy. To my knowledge the name *Heymann nephritis* was first used by Ben Spargo from the University of Chicago in the late 1970s.

The fact that MN was occasionally present as a second disease in patients who had another organ-specific autoimmune disease such as type I diabetes mellitus, myasthenia gravis, pemphigus, Graves disease, primary biliary cirrhosis, celiac disease, and so forth, or even another autoimmune disease in the kidney such as the tubulointerstitial renal disease associated with autoantibodies to a 59-kilodalton proximal tubular antigen was viewed as “interesting” and raised additional suspicion that MN may be an autoimmune disease. This was based on the assumption that these individuals perhaps had an autoimmune predisposition and had developed autoantibodies to tissue antigens in two separate organs and that the autoimmunity in the first organ was not responsible for the MN in the kidney. Whether this assumption is correct remains to be explored. Such studies could be expected to shed light on the mechanisms of autoimmunity in MN. It also is possible that such cases of MN are, in fact, of the secondary variety and result from immune complexes and/or antigens released by immune damage in the first organ. In that instance, the released immune complexes could dissociate in glomerular capillaries and re-associate in the subepithelial space, or form with released antigens that were planted in the subepithelial space in glomeruli. Only after some of the autoantigens in patients with idiopathic MN are discovered would it be possible to differentiate between these two possibilities among patients of this type of MN. It is interesting to note that we could detect megalin in the region of subepithelial deposits in a child with MN associated with autoantibodies to the 59-kd tubular basement membrane antigen, where the disease actually preceded the development of the latter autoantibodies, even though autoantibodies to megalin or RAP were not detected in the serum.²¹

SITE OF AUTOANTIGENS IN THE GLOMERULUS

Heymann believed that the subepithelial deposits in AHN resulted from the binding of autoantibodies to a fixed tissue antigen in the glomerulus and not from circulating immune complexes. This is evident from his 1965 article in which he stated, “the granular deposits in autoimmune nephrosis may not be related to deposition of circulating antigen-antibody complexes, but perhaps result from reaction with a fixed tissue antigen in the kidney with a characteristic histologic distribution in the glomerular tuft.”²² However, the idea that circulating immune complexes were the source of granular glomerular immune deposits in various glomerulonephritides, as proposed by Germuth et al²³ and Dixon et al²⁴ based on their studies in chronic serum sickness model in rabbits, was entrenched at that time and was extended to explain the granular deposits in AHN as well.⁷ Many investigators using indirect immunofluorescence microscopy on normal rat kidney sections found that the antibodies from serum or even from glomerular eluates of AHN kidneys stained only the brush border of proximal tubules (where the antigen is abundant) and not the glomerulus.

Heymann's concept of a “fixed” tissue antigen in the glomerulus received a new life after studies in the PHN model by Van Damme et al¹⁵ and Couser et al.¹⁶ These investigators showed that ex vivo perfusion of a bloodless kidney (thus excluding circulating antigen and immune complexes) with heterologous anti-Fx1A antiserum led to the formation of the subepithelial deposits, supporting the idea that complexes in glomerulus were formed in situ by reaction with antigen present in that location rather than arriving there from circulation as preformed antigen-antibody complexes. It is now established that the fixed tissue autoantigen Heymann speculated about is indeed present in the glomerulus,²⁵ and is a 600-

kd podocyte membrane glycoprotein (variously named as gp600, gp330, LRP-2, and megalin), and that similar to PHN the glomerular immune deposits in the AHN model also are formed in situ²⁶ by the binding of circulating autoantibodies.

Once megalin was identified it seemed reasonable to search for the same autoantigen in the glomerulus in the human kidney. However, its presence could not be shown in the normal human glomerulus by IF microscopy with various antimegalin antibodies, including heterologous antibodies or autoantibody to rat kidney megalin, a monoclonal antibody to human parathyroid megalin,²⁷ or with heterologous antibodies to human kidney megalin (unpublished observations). However, these antibodies did react with the parietal glomerular epithelial cells (unpublished observations), which can be precursors of podocytes both in mice²⁸ and human beings.²⁹ Consequently, many investigators believed that other antigens expressed on the surface of podocytes could be the target of circulating autoantibodies in idiopathic MN and the search for such antigens was pursued in several laboratories. This belief was boosted by the elegant studies of Debiec et al³⁰ and Ronco and Debiec³¹ in an experiment of nature involving differential expression of neutral endopeptidase (NEP), a podocyte membrane protein, in the mother and fetus that led to alloimmunization of mother with fetal NEP and development of MN in the newborn from transplacentally transferred NEP alloantibodies. These alloantibodies bound to fetal NEP on podocytes and induced MN similar to the mechanism seen in HN.

SEARCH FOR AUTOANTIBODIES IN SERUM TO PODOCYTE ANTIGENS

Early attempts by many investigators, including us, tested sera of patients with idiopathic MN for antibodies on normal human kidney sections by indirect IF microscopy. These studies universally failed to show reaction with the glomerulus. It needs to be pointed out that there is always some background fluorescence in glomeruli owing to the presence of endogenous human IgG in normal human kidney sections, which may mask the reaction of test sera from patients, and also the technique is not very sensitive. Later, with the availability of Western blotting, efforts again were made to detect antibodies in sera using human kidney homogenates as the substrate. It is important to point out here that antimegalin autoantibodies deposited in the AHN model fail to react with megalin in Western blots run under reducing conditions, indicating that those autoantibodies recognize conformational epitopes.³² Therefore, we have used both reducing and nonreducing conditions to search for the human autoantibodies. Although the Western blotting technique is more sensitive than indirect IF microscopy we and many other investigators were unable to detect autoantibodies in sera from patients with idiopathic MN. Retrospectively, part of the reason for this may have been because there is always more background in the blots from nonreducing gels than from reducing gels, particularly in the molecular weight region of intact IgG (160 kd), because there is always enough endogenous IgG in the human kidney homogenates. This made it almost impossible to differentiate a reaction with antigens in the 150- to 200-kd region from the background reaction of endogenous IgG.

Appreciating the earlier-described obstacle, Salant et al³³ depleted endogenous IgG from homogenates of kidney and sieve-isolated glomeruli by absorption on an anti-human IgG affinity column. Accordingly, they succeeded in detecting a protein in the region of 185 kd reacting with sera from 26 of 37 patients participating in their study in Boston. Later, they confirmed their findings in 15 of 19 patients from another geographic location (The Netherlands). Proteomic analysis of the reactive band identified the protein phospholipase A₂ receptor (PLA₂-R) from the database of human proteins. More recently, Prunotto et al,³⁴ from Italy, using cultured human podocytes³⁵ instead of sieve-isolated human glomeruli, and proteomic analysis from two-dimensional gel blots, identified autoantibodies to aldose reductase (AR) and manganese superoxide dismutase (SOD2) in sera of patients with

idiopathic MN. AR and SOD2 also could be shown by immunofluorescence microscopy on membrane of cultured podocytes. Although SOD2 was recognized with a greater number of sera (10 of 24), several other podocyte proteins including α -enolase, secretin hydrolase, ubiquitin hydrolase, and ser/thr protein phosphatase 2A also were recognized by some sera in the two-dimensional blots. It should be noted that the autoantibodies against SOD2 and AR³⁴ could be detected under both reducing and nonreducing conditions, whereas autoantibodies to PLA₂-R,³³ similar to the antimegalin autoantibodies in AHN,³² could be detected only under nonreducing conditions, indicating reaction to conformational epitopes. It is interesting to appreciate that in both these studies the reactive autoantibodies were predominantly³³ or exclusively³⁴ IgG₄, a subclass that does not activate complement.³⁶ It is worth pointing out that autoantibodies to α -enolase also were identified previously by a group from Japan in sera of patients with both idiopathic as well as secondary MN (owing to systemic lupus erythematosus [SLE] or rheumatoid arthritis).³⁷ Interestingly, those antibodies were IgG1 and IgG3 and not the IgG4 subclass. Prunotto et al³⁴ did not describe the subclass of α -enolase antibodies they detected. In any case, this finding in the Japanese study could be another example of intermolecular epitope spreading (see later) possibly seen in both idiopathic and secondary MN. Also, Ronco and Debiec³⁸ recently stated in a review article that they found significantly higher levels of autoantibodies to NEP by enzyme-linked immunosorbent assay in sera of patients with idiopathic MN compared with controls. To summarize, it appears that multiple podocyte antigens could participate in the pathogenesis of idiopathic MN.

There is also the possibility that serum is an inadequate source for the search of the various autoantibodies because some of the autoantibodies may become undetectable in serum with time, as is noted in AHN. This could occur by depletion of serum autoantibodies owing to deposition in the kidney or by periodic suppression of the autoimmune responses, as is often seen in many autoimmune diseases. The latter, we believe, could be the reason for the spontaneous remission seen in some patients with idiopathic MN. However, the quiescent B cells that produced these antibodies presumably survive in circulation. A genetic and functional representation of natural and pathogenic antibodies could be accessed in phage display libraries of Fab fragments constructed from the circulating B cells. Each phage particle displays a monoclonal antibody on its surface and, therefore, a library generated from MGN patients could include autoantibodies relevant to the disease. We have generated such a library and have tested the phage particles and the expressed soluble Fabs on normal human kidney and the cultured human podocyte line obtained from Saleem et al,³⁵ the same line used by Prunotto et al.³⁴ Both polyclonal phages and several clones showed reaction with podocytes (Fig. 1). By using this library and the conventional proteomic approach we expect to confirm the autoantigens identified from serum studies and possibly identify other autoantigens that could reveal the progression of the autoimmune response in idiopathic MN.

RELATIONSHIP OF AUTOANTIBODIES IN SERUM TO IMMUNE DEPOSITS IN GLOMERULUS

Both Beck et al³³ and Prunotto et al³⁴ showed that IgG reactive with the podocyte antigens they identified (PLA₂-R,³³ SOD2, and AR³⁴) could be eluted from renal biopsy tissue from patients with idiopathic MN using either whole tissue³³ or microdissected glomeruli from frozen sections.³⁴ Prunotto et al³⁴ found that only IgG₄ was present in the eluted autoantibodies, whereas Beck et al³³ did not test eluted IgG for subclasses. It would be interesting to know if the IgG eluted by Beck et al³³ belonged to the IgG₄ subclass. This could be important because Beck et al³³ found small amounts of IgG₁, IgG₂, and IgG₃ in the PLA₂-R autoantibodies from patient sera, which could indicate that IgG₄ autoantibodies were selectively deposited in glomeruli. It is also worth noting that no IgG₄ was found in the

glomerular eluates of SLE patients in the study by Prunotto et al.³⁴ However, these investigators did not state whether those SLE patients had secondary MN, which would have been interesting to know.

Further, colocalization of these podocyte antigens and IgG4 in the subepithelial immune deposits of patients with idiopathic MN, but not other glomerular diseases, was shown by confocal immunofluorescence microscopy for PLA₂-R and by confocal immunofluorescence microscopy and immunoelectron microscopy for SOD2 and AR. It is interesting to note that although PLA₂-R could be detected on podocytes in normal human glomeruli in frozen sections, SOD2 and AR were detected only in the renal biopsies of idiopathic MN patients, indicating that SOD2 and AR were expressed *de novo* on podocytes during the disease process in idiopathic MN. Why AR and SOD2 could be detected on membranes of cultured podocytes and not on normal glomeruli in frozen sections is not clear, but it could relate to differences in antigen expression under the culture conditions. However, the findings in both studies strongly indicate that autoantibodies to all three identified podocyte antigens bind *in vivo* to their respective antigens and are responsible at least in part for the subepithelial immune deposits seen in idiopathic MN. In this regard, it may be noted that PLA₂-R autoantibodies (subclass not described) were postulated for the development of MGN in the renal transplant in a patient whose original disease was MGN and who had been on dialysis for 5 years and had these autoantibodies in serum before the transplant.³⁹ Whether these autoantibodies were responsible for the development of MGN labeled as “recurrent” is not clear.

It should be noted that in addition to AR and SOD2 the autoantibodies eluted by Prunotto et al.³⁴ detected several other reactive proteins in a membrane extract of cultured podocytes. A total of eight bands in the molecular weight range of 10 to 120 kd were seen under reducing conditions. Three were identified by mass spectrometry as AR, SOD2, and vimentin. The other five bands were not characterized. Interestingly, the strongest reaction was seen with vimentin, a cytoskeletal protein. Under nonreducing conditions a total of 11 bands were seen, which included the 8 identified under reducing condition and three more bands in the molecular weight range of 150 to 200 kd (Table 1). The proteins in the 150- to 200-kd range probably did not contain human IgG because these proteins came from membranes of podocytes cultured in the absence of human serum proteins. One of these three bands easily could be PLA₂-R, as pointed out by the authors. Thus, the study by Prunotto et al.³⁴ indicated that the eluted IgG4 reacted with several, perhaps as many as 11, podocyte proteins. Although the preparation used in these blots was a membrane fraction of podocytes the possibility that some contamination with cytoplasmic proteins occurred during isolation of the membrane fraction cannot be ruled out. How do deposits of IgG4 autoantibodies, which do not activate complement, lead to the production of proteinuria? Do they produce proteinuria at all? These questions will have to wait until suitable animal models can be established that use these antigen-antibody systems. In this regard the following should be noted: (1) proteinuria levels in AHN correlate with IgG1 (equivalent of IgG4 in human beings) in glomerular deposits,⁴⁰ and (2) staining of deposits for IgG4 decreased in five patients and disappeared in two patients in follow-up renal biopsies of 7 patients with idiopathic MN who achieved remission after treatment with rituximab (monoclonal antibody to CD20 on B cells) as recently shown by Ruggerenti et al.⁴¹

PROGRESSION OF IMMUNE DEPOSITS AND INTRAMOLECULAR AND INTERMOLECULAR EPILOPE SPREADING

In AHN the autoimmune response spreads with time from the immunodominant region in the N-terminus to other regions of megalin, leading to production of multiple specificities of high-affinity antimegalin autoantibodies that progressively accumulate in the immune

deposits. Furthermore, this process of intramolecular epitope spreading temporally correlates with the onset of proteinuria.⁴² In addition to intramolecular epitope spreading there is also evidence for intermolecular epitope spreading in AHN as indicated by the development of autoantibodies to plasminogen,^{43,44} which is a known ligand of megalin. The autoantibodies to plasminogen apparently accumulate in immune deposits because they can be found in the glomerular eluates from AHN rats.⁴⁴ Interestingly, the autoantibodies to plasminogen also are specific for conformational epitopes.⁴⁴ These autoantibodies conceivably could have a role in proteinuria as well as thrombosis reported in the small veins within the AHN kidney.⁴⁵ It would be of interest to search for autoantibodies to plasminogen in patients with idiopathic MGN who develop renal vein thrombosis. Autoantibodies to plasminogen also have been reported recently in patients with antineutrophil cytoplasmic autoantibody-associated glomerulonephritis.⁴⁶

It is possible that PLA₂-R is the inciting antigen and AR, SOD2, and other antigens are recruited through the mechanism of intermolecular epitope spreading. However, it also is possible that autoantibodies to all three podocyte antigens identified to date result from intermolecular epitope spreading and the inciting podocyte autoantigen still remains to be identified. It would not be surprising if additional antigens on podocytes or ligands that bind to PLA₂-R are identified as being involved in the generation of autoantibodies that contribute to the development of immune deposits in this disease.

In AHN the progressive enlargement of subepithelial deposits, a hallmark of the lesion of MN, results from up-regulated expression of megalin by podocytes,⁴⁷ presumably from continuous exposure of podocytes to autoantibodies arriving from circulation and sequestration of megalin in the immune deposits. Therefore, one might expect to find that the progressive enlargement of the subepithelial immune deposits seen in patients with idiopathic MN also occurs by the same phenomenon of increased synthesis of the autoantigens by podocytes.

NATURE OF THE AUTOIMMUNE RESPONSE

It has been known for several years that IgG deposits in the glomerulus in patients with idiopathic MN are predominantly IgG₄,⁴⁸ a subclass that does not activate complement, has low affinity for Fc receptors, and is least abundant in serum (comprising <5% of total IgG). Nevertheless, the concentration of IgG₄ in serum is not increased in patients with idiopathic MN.⁴⁹ Thus, there must be a selective accumulation of IgG₄ in the glomerular deposits. Apparently, development of an IgG₄ response is slow and requires many months of repeated antigen exposure as seen in patients receiving repeated allergen injections for treatment of allergic disorders and in beekeepers exposed to bee venom antigens over many years.⁵⁰ Also, it requires T-helper 2 cell-mediated B-cell stimulation, depends on interleukin (IL)-4/IL-13 for class switch, and on IL-10 for secretion of IgG₄ by B cells.⁵⁰ How and whether these conditions are met in idiopathic MN is difficult to speculate at this time. However, it is interesting to note that IL-10 and IL-13 messenger RNA levels were increased in peripheral blood mononuclear cells, and IL-4 enhanced in vitro production of IgG₄ by B cells of patients with idiopathic MN compared with normal controls.⁵¹

IgG₄ antibodies are reported to engage in dynamic Fab arm exchange on cell surfaces in vivo or in blood, where half of an IgG₄ molecule of a given antigen specificity (heavy chain with attached light chain) is exchanged by another half of an IgG₄ with a different antigen specificity, producing a bi-specific IgG₄ molecule.⁵² The third constant domain, but not the core hinge region, is involved in this exchange, which likely requires a reducing environment on erythrocytes or other cell surfaces.⁵² The exchange is not seen in IgG1 molecules. Theoretically, the bi-specificity of IgG₄ could lead to cross-linking of two

different epitopes of a single antigen or of two different antigens, resulting in more stable complexes. However, because of the dynamic nature of the exchange these bi-specific antibodies are transient. Therefore, under most conditions, IgG₄ molecules lose their ability to cross-link antigen(s) to form immune complexes and are functionally monovalent. Accordingly, they are considered anti-inflammatory.⁵² In fact, in a model of myasthenia gravis, another autoantibody-mediated organ-specific autoimmune disease in which cross-linking of antigen (acetylcholine receptor) plays an important role in disease pathogenesis, pre-injection of an IgG₄ antibody directed against the same epitope of acetylcholine receptor as the pathogenic IgG1 antibody decreased cross-linking and protected the animal (rhesus monkey) from disease.⁵² From this analogy one may speculate that the IgG₄ in idiopathic MN deposits represents a protective response (as seen in allergic disorders with repeated allergen injections) against cross-linking of antigen(s) by the IgG1 antibodies that developed earlier and activated complement but later were superseded by IgG₄ antibodies. This would be consistent with the finding of small amounts of IgG1 in some patients and the presence of C3 complement in most patients. Although IgG1 may even disappear with time a fragment of C3 could remain covalently bound to the podocyte cell surface near deposits.

CONCLUSIONS AND FUTURE QUESTIONS

It is clear that autoantibodies (belonging predominantly to the IgG₄ subclass) to podocyte antigens develop in idiopathic MN and those autoantibodies can be present in serum as well as can be eluted from glomeruli of patients. Moreover, these antibodies colocalize with their corresponding antigens in the subepithelial immune deposits. Thus, at least a part of the autoimmune basis of idiopathic MN, represented by a set of podocyte-specific autoantibodies, is beginning to emerge. Nevertheless, many questions about the antigens and the role of T-cell responses in the disease remain to be explored.

What is the inciting podocyte antigen? Is there only one or could multiple primary antigens participate in the initiation? How many antigens are recruited through intermolecular epitope spreading and what are their identities? What are the immunodominant epitopes on the inciting antigens? Are post-translational modifications on antigens involved in breakdown of tolerance as seen with megalin in AHN?⁵³ Which autoantibodies disappear from circulation and how is the immune response controlled in patients who go into spontaneous remissions? What is the mechanism of proteinuria? Can the disease be treated with autoantigen-specific immunotherapy? What are the triggers of autoimmunity? Can the susceptible individuals be diagnosed before the onset of disease? Is vaccination of susceptible individuals possible? These are some of the questions that undoubtedly remain to be studied in the future. Collaborations among clinicians, clinician scientists, and basic researchers are crucial to answer these and other questions that will arise as further progress is made toward the understanding of the fundamental mechanisms in pathogenesis and resolution of this disease. Development of suitable animal models continues to serve as an important research tool. Similar to most other autoimmune diseases both genetic and environmental factors are likely to be involved in the induction of autoimmune MN in which environmental factors such as infections, allergens, or exposure to other chemicals may act as triggers of autoimmunity in susceptible individuals. Once the primary or inciting podocyte protein antigens are established it should be possible to identify shared sequences between these antigens and various infectious agents that could induce autoimmunity through the mechanism of molecular mimicry. Ultimately, it should be possible to develop antigen-specific therapeutic strategies for the treatment of the disease based on this knowledge.

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Figure 1.

A. Frozen section of a normal human kidney is reacted with polyclonal phage from a Fab-phage expression library prepared from pooled peripheral blood B cells of patients with idiopathic MN and studied by indirect immunofluorescence microscopy. Reaction along the periphery of glomerular capillaries (presumably podocytes) can be seen. B.

Paraformaldehyde fixed podocytes grown on cover slips are reacted with the earlier-described polyclonal phage and studied by indirect immunofluorescence microscopy. Reaction of phage on podocytes can be seen. C. Podocytes as in B described previously are

reacted with purified soluble Fab expressed from the earlier-described polyclonal phage and studied by indirect immunofluorescence microscopy. Reaction on podocyte can be seen.

Table 1

Autoantibodies to Podocyte Proteins in Idiopathic MN

Medium	Podocyte Proteins
Serum	M-type phospholipase A ₂ receptor
	Aldolase reductase
	SOD2
	α -enolase
	Secretin hydrolase
	Ubiquitin hydrolase
	Ser/thr protein phosphatase 2A
	Neutral endopeptidase
IgG eluted from glomeruli	M-type phospholipase A ₂ receptor
	Aldolase reductase
	SOD2
	Vimentin
	4 to 7 other unidentified proteins in the 9- to 20-kd range