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Molecular Pathogenesis of Membranous Nephropathy

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neutral endopeptidase, phospholipase A2 receptor, PLA2R, thrombospondin domain-containing 7A, THSD7A, genome-wide association studies, GWAS, HLA-D risk variants, membrane attack complex of complement

Abstract

Membranous nephropathy is a noninflammatory autoimmune disease of the kidney glomerulus, characterized by the formation of immune deposits, complement-mediated proteinuria, and risk of renal failure. Considerable advances in understanding the molecular pathogenesis have occurred with the identification of several antigens [neutral endopeptidase, phospholipase A2 receptor (PLA_2R), thrombospondin domain-containing 7A (THSD7A)] in cases arising from the neonatal period to adulthood and the characterization of antibody-binding domains (that is, epitopes). Immunization against PLA2R occurs in 70% to 80% of adult cases. The development of highly specific and sensitive assays of circulating antibodies has induced a paradigm shift in diagnosis and treatment monitoring. In addition, several interacting loci in *HLA-DQ*, *HLA-DR*, and *PLA2R1*, as well as classical human leukocyte antigen (HLA)-D alleles have been identified as being risk factors, depending on a patient's ethnicity. Additionally, mechanisms of antibody pathogenicity and pathways of complement activation are now better understood. Further research is mandatory for designing new therapeutic strategies, including the identifying triggering events, the molecular bases of remission and progression, and the T cell epitopes involved.

1. INTRODUCTION

Membranous nephropathy (MN) is a rare disease affecting the kidney glomerulus, more specifically the podocytes, which play a key role in controlling the kidney's permeability to proteins. In healthy people, albumin and higher molecular weight (MW) proteins are minimally filtered, whereas in a condition called nephrotic syndrome, large amounts of proteins escape in the urine, resulting in a decrease in serum albumin and the development of generalized edema. MN is the most common cause of nephrotic syndrome in Caucasian adults, accounting for approximately 30% of cases (annual incidence, 1.7 per 100,000) (1, 2), with a significant male preponderance (sex ratio, 2:1) and a peak incidence in individuals aged 50 to 60 years. It is rare in children.

MN is characterized by the accumulation of immune deposits on the subepithelial (outside) aspect of the glomerular capillary wall, close to the podocytes, which causes a membrane-like thickening, with the expansion of the extracellular matrix leading to the formation of spikes. The immune deposits consist of immunoglobulin (Ig) G, of antigens that have long eluded identification, and of the membrane attack complex (MAC) of complement. Podocytes are the major targets of the immunological response, which most often involves podocyte antigens. IgG4 is usually the prominent deposited subclass in idiopathic MN (iMN), while IgG1, IgG2, and IgG3 exceed IgG4 in cases of secondary MN (3–6). The formation of subepithelial immune deposits and complement activation are responsible for the functional impairment of the glomerular capillary wall, causing proteinuria in the absence of inflammatory cells in the glomerulus.

Despite a common histopathological pattern, MN is a heterogeneous disease, occurring either in the absence of an established cause (80% of cases) or in association with clinical conditions such as infections (hepatitis B), lupus erythematosus, cancer, or drug intoxication. Heterogeneity is also illustrated by the variable clinical outcome. Approximately 40% of patients with iMN will undergo spontaneous remission (7), while another 30% will have a poor response to immunosuppressive therapy and will progress to end-stage renal disease requiring dialysis or transplantation (8). About 40% of patients who receive a kidney graft will develop recurrence, and about 45% of those will lose their graft (9). Treatment with costly and potentially toxic drugs remains controversial and challenging (10, 11). The key to rationalized therapy is the identification in individual patients of the exact etiology and pathogenesis of the disease.

This review covers most aspects of the molecular pathogenesis of MN. There are few diseases in nephrology and beyond in which advances in understanding the molecular pathomechanisms have been so huge and translation to the bedside so fast, occurring in less than a decade, during which MN has entered a new era. Such progress was engendered by studies of experimental models of MN, such as Heymann nephritis in the rat (12–14) and the cationic bovine serum albumin (BSA) model in the rabbit (15, 16). **Figure 1** shows the common thread of this review, from experimental MN to human MN.

2. EXPERIMENTAL MODELS

2.1. Heymann Nephritis: Megalin (LRP2) Is the Target Podocyte Antigen

Heymann nephritis was first described in 1959 by a pediatrician from Cleveland and his team (12). Because the antigenic preparation used in the study did not contain glomerular extracts, this rat MN was initially thought to be caused by the deposition of circulating immune complexes containing the immunizing antigen from the brush border of the proximal tubule (FX1A) and the corresponding antibodies. Two seminal studies by the Couser and Hoedemaeker groups (13, 14), using ex vivo and isolated perfused kidney systems, showed that the disease was induced by in situ

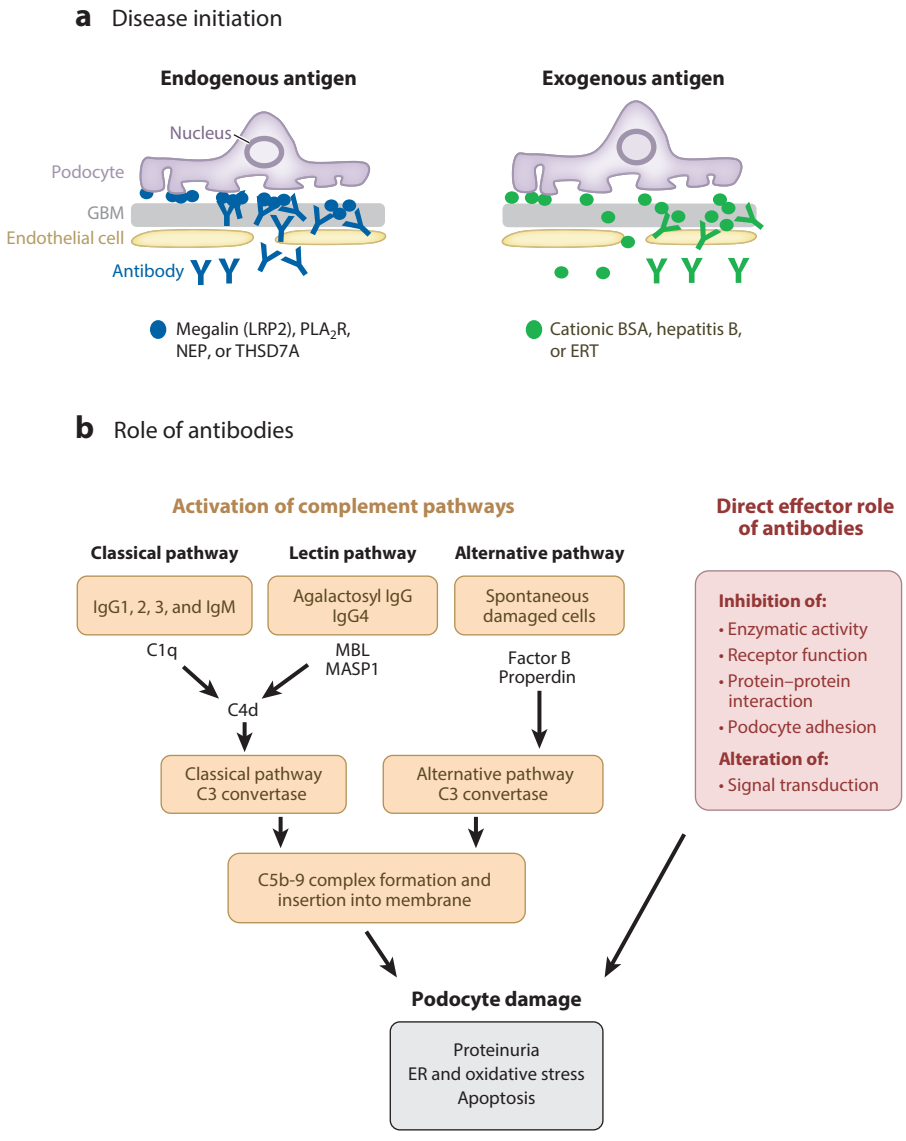

Figure 1

Mechanisms of antibody-mediated immune podocyte injury in membranous nephropathy. (*a*) The in situ formation of immune complexes is initiated by binding of circulating antibodies to antigens that are endogenous integral membrane proteins of the podocyte or to exogenous antigens planted in the glomerular basement membrane. (*b*) Complement can be activated by three different pathways, all of which converge toward the formation of the C5b-9 complement attack complex. In addition, antipodocyte antibodies can directly alter the function of target antigens. Together, these processes trigger cell damage in the absence of inflammation. Abbreviations: BSA, bovine serum albumin; ER, endoplasmic reticulum; ERT, enzyme replacement therapy; GBM, glomerular basement membrane; Ig, immunoglobulin; LRP2, low-density lipoprotein receptor–related protein 2 (new name for megalin); MBL, mannose-binding lectin; NEP, neutral endopeptidase; PLA_2R, phospholipase A_2 receptor; THSD7A, thrombospondin domain-containing 7A.

formation of subepithelial immune complexes that were initiated by the binding of circulating antibodies to an antigenic target located on podocytes. This antigen was identified by Kerjaschki & Farquhar (17, 18) in 1982 and 1983 as the podocyte membrane protein megalin (LRP2; low-density lipoprotein receptor–related protein 2), a member of the low-density lipoprotein receptor superfamily with an MW of about 600 kDa. Epitope mapping showed that full-blown disease, that is, proteinuria, required intramolecular epitope spreading from a specific epitope located in a small glycosylated N-terminal fragment to more distal domains (19).

Epitope: small antigen domain recognized by antibodies

Epitope spreading: phenomenon whereby additional epitopes are recognized within the same or a different molecule during evolution of the immune response

LRP2 is not implicated in human MN, although it may be expressed in human podocytes (20): It has not been detected in subepithelial immune deposits, and circulating anti-LRP2 antibodies have not been found in patients with MN. However, anti-LRP2 antibodies were detected in a cohort of patients with anti–brush border antibody disease, which is characterized by renal failure, proximal tubule damage, and immune deposits in the tubular basement membrane containing LRP2 and IgG (21). Despite the difference in the antigenic targets, Heymann nephritis has substantially contributed to the understanding of human MN.

2.2. Cationic Bovine Serum Albumin as a Model of Antigen Planted in the Glomerular Capillary Wall

In the wake of studies devoted to the mechanisms of immune complex deposition in the glomerulus, Border and colleagues (15, 16) hypothesized in the early 1980s that, given the negative charge of the glomerular capillary wall, antigen charge could be a key factor in the formation of subepithelial deposits. They showed that rabbits repeatedly immunized with cationic BSA developed subepithelial deposits of IgG and C3, whereas those receiving anionic or native (neutral) BSA mainly had mesangial deposits. The same model was later developed in dogs, mice, and rats (22–24). The concept of a planted antigen was confirmed using perfused kidneys, with cationic BSA binding first to anionic heparin sulfate proteoglycans of the basement membrane, followed by binding of the antibody (25).

3. NEONATAL DISEASE INDUCED BY NEUTRAL ENDOPEPTIDASE ALLOIMMUNIZATION

A singular form of MN is observed in neonates born with nephrotic syndrome, which is sometimes associated with acute renal failure and extrarenal manifestations (26). The disease is caused by maternal antibodies against neutral endopeptidase (NEP) that cross the placenta and bind to fetal glomerular podocytes. NEP first appears in the S-shaped body stage and persists in the podocytes of the mature kidney (27). The disease is transient in the neonate because of the limited half-life of maternal antibodies, although advanced chronic kidney disease can develop later, most likely because of nephron loss during the antenatal period. The anti-NEP antibodies produced by the mother cause the infant's MN: The disease can be transferred to pregnant rabbits by maternal, but not by paternal, IgG (26).

In the seven mothers from five families reported so far, the mothers of the affected children were apparently healthy despite high titers of circulating anti-NEP antibodies. However, the mothers were found to be NEP deficient because of truncating mutations in the *MME* gene coding for NEP, located in exon 7 (detected in all mothers) and in exon 15 (detected only in the first mother, who was a heterozygous compound) (28, 29). These mutations resulted in the absence of the protein and of NEP-specific enzymatic activity. Alloimmunization occurred after previous spontaneous miscarriages or during the course of the ongoing pregnancy, during which the mother's immune system was first exposed to NEP of paternal origin on syncytiotrophoblastic

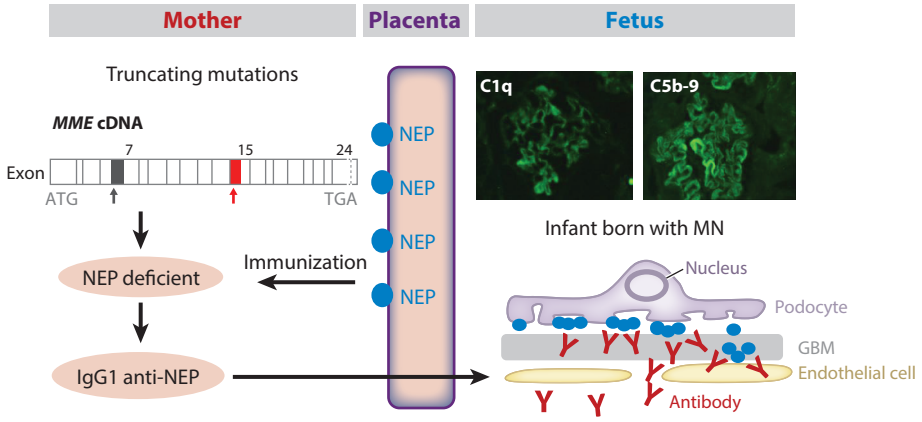

Figure 2

NEP-related alloimmune neonatal glomerulopathy. NEP serves as a pathogenic antigen in the podocyte cell membrane. Antibodies to this protein originate in women who genetically lack NEP because of truncating mutations in the *MME* gene (the gene coding for NEP). Immunization occurs during pregnancy when the mother's immune system is first exposed to NEP, which is strongly expressed by placental cells. From about the eighteenth week of gestation, maternal antibodies of the IgG class are actively transported across the placenta to the fetus, where they bind to the NEP antigen expressed on podocytes. Complement-fixing anti-NEP IgG1, which also inhibits NEP enzymatic activity, is necessary for the disease to develop. The micrographs show deposits of complement components C1q and C5b-9. The five reported families affected by this disorder are from five different countries. Abbreviations: cDNA, complementary DNA; GBM, glomerular basement membrane; Ig, immunoglobulin; MN, membranous nephropathy; NEP, neutral endopeptidase. Adapted from figure 1 in Reference 153.

cells (30). Although the mothers of affected children did not show renal or extrarenal manifestations at the time of pregnancy, probably because of compensation by other enzymes, after 10 to 20 years they developed a peripheral neuropathy similar to Charcot-Marie-Tooth disease (P. Ronco, manuscript in preparation).

These rare cases of neonatal MN provided important information about the mechanisms of human MN. First, they provided the proof of concept that a human podocyte antigen could serve as target for circulating nephritogenic antibodies. Second, they provided insight into the mechanisms of the disease. Although gene mutations were detected in all of the mothers who produced anti-NEP antibodies, the expression of renal disease was variable, being determined by the mother's antibody response. Maternal production of anti-NEP IgG1 seemed necessary for the disease to develop; if only anti-NEP IgG4 were produced, then proteinuria did not result (28, 29). Contrary to adult MN, IgG1 and C1q deposits were detected in the kidneys of most affected children, which suggested activation of the classical complement pathway by IgG1. In addition, IgG1 also inhibited NEP enzymatic activity, whereas IgG4 had a weak inhibitory potency. Thus, lesions may result from both complement activation and the accumulation of peptides such as endothelin, which is normally degraded by NEP. This hypothesis is supported by the vascular and glomerular ischemic lesions seen in the more severe forms of neonatal MN (26, 28). The anti-NEP subclass may be driven by the route of exposure (for instance, sudden, massive alloimmunization due to miscarriage versus low-level, prolonged exposure due to the presence of NEP in sperm), a mother's specific human leukocyte antigen (HLA) class II repertoire, or other genetic or environmental differences (31). The basic concept of NEP-related alloimmune neonatal glomerulopathy is summarized in **Figure 2**.

Detecting families with this disease is of utmost importance because subsequent pregnancies put the fetus at high risk (32), neonatal MN can lead to renal failure, and NEP-deficient individuals may develop peripheral neuropathy.

4. AUTOIMMUNE DISEASE INDUCED BY PODOCYTE ANTIGENS: PLA_2R AS A MODEL ANTIGEN

4.1. PLA_2R: The M-type Phospholipase A_2 Receptor

The search for target antigens in autoimmune iMN was unsuccessful for many years. Beck et al. (33) used a combination of glycoproteins purified from glomerular extracts followed by Western blotting of the extracts in nonreducing conditions to detect antibodies that were reactive with a 185-kD protein in about 70% of samples from patients with iMN. The lack of reactivity in reduced conditions suggests that the epitope is conformational, requiring intact disulfide bonds. Both the fully glycosylated and the deglycosylated forms are recognized by patients' antibodies. This protein was identified by mass spectrometry as the type-M phospholipase A_2 receptor (PLA_2R). PLA_2R had initially been cloned in a search for receptors that bound secreted phospholipase A_2 enzymes (34). The M designation indicates that it was the muscle-derived receptor, as opposed to the N, or neuronal, receptor. PLA_2R is detected in normal human glomeruli, specifically in podocytes. PLA_2R and IgG4, the main IgG subclass carrying anti-PLA_2R activity, as shown by Western blotting, are colocalized within subepithelial deposits. Furthermore, IgG eluted from biopsy samples reacts with recombinant PLA_2R.

PLA_2R is a member of the mannose receptor family, which in mammals also includes the cation-dependent mannose-6-phosphate receptor, the C-type mannose receptor 2, Endo 180, and the dendritic cell receptor DEC-205. Its ortholog in birds is the avian yolk sac IgGY receptor FcRY (35, 36) (**Figure 3**). Members of this family are transmembrane proteins with a similar domain structure, including an N-terminal cysteine-rich (CysR, or ricin B) domain, a single fibronectin type II (FnII) domain, and 8 to 10 C-type lectin-like domains (CTLDs). They have a short cytoplasmic domain that contains motifs that enable constitutive endocytotic recycling in clathrin-coated pits between the plasma membrane and the endosomal machinery, leading to the internalization of extracellular ligands (37). They can present with at least two pH-dependent configurations: an extended conformation, with the N-terminal CysR domain pointing outward from the cell surface; or a bent conformation, in which the N-terminal domain folds back to interact with the CTLDs at the middle of the structure. This conformational change is likely necessary to allow the binding of ligand at physiological pH and the release of the cargo into the more acidic pH of endosomes and lysosomes before return of the receptor to the cell surface (35). Great progress has been achieved with the determination of the structure of human M-PLA_2R by cryo-electron microscopy (38). The ectodomain has high internal flexibility and forms a compact, dual-ring-shaped conformation at acidic pH, and it adopts extended conformations at basic pH. Several of the major epitopes have been located in the CysR, CTLD1, and CTLD7 domains (39–41), and they may be more accessible in the extended conformation of PLA_2R under physiological or basic pH.

PLA_2R can also be expressed as an alternatively spliced variant that is predicted to be truncated in the middle of CTLD8, and this may encode a secreted, soluble isoform (42). There is evidence that this form can be detected in the circulation, a finding compatible with a role in pathogenesis. Watanabe et al. (43) showed that human recombinant soluble PLA_2R bound to collagen I probably through the FnII domain and inhibited the interaction of collagen I with the extracellular domain of integrin-β1 on the cell surface, thereby suppressing the collagen-I-induced–integrin-β1-mediated migratory response.

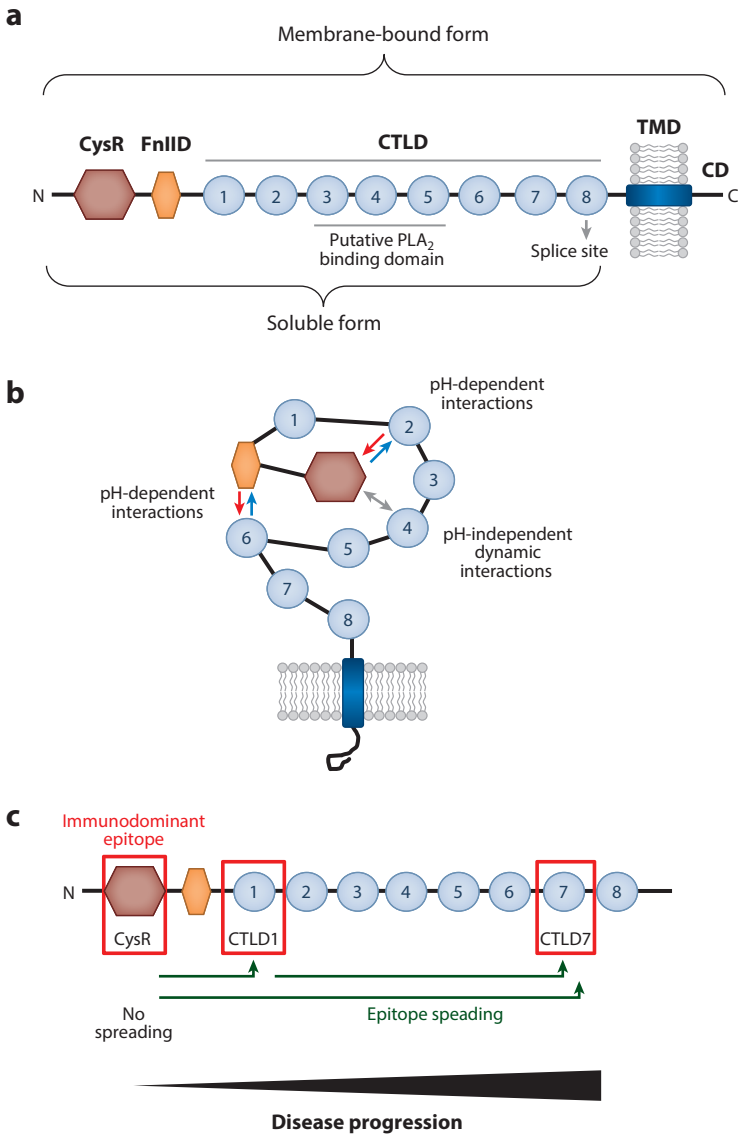

Figure 3

(*a*) Schematic of phospholipase A_2 receptor (PLA_2R). This protein is a type I transmembrane glycoprotein and contains an N-terminal cysteine-rich (CysR) region, a fibronectin-like type II domain (FnIID), a tandem repeat of eight C-type lectin-like domains (CTLDs), a transmembrane domain (TMD), and a short intracellular C-terminal domain (CD). (*b*) A ball-and-stick model of the domain arrangement of PLA_2R from cryo-electron microscopy. Interactions between CysR and CTLD2, and FnIID and CTLD6, are controlled by pH. In acidic conditions (*blue arrow*), the ectodomain can adopt a compact dual-ring-shaped conformation, while at basic pH (*red arrow*), it can adopt an extended conformation. Panel adapted with permission from Reference 38. (*c*) Epitope spreading in the PLA_2R antigen and correlation with clinical outcome.

4.2. PLA_2R Epitopes and Spreading

Immunodominant epitope: epitope that is recognized by the majority of antibodies raised during an immune response against an antigen

Putative linear epitopes have been identified with the use of a high-throughput capture immunoassay (44). However, these linear epitopes only partially reflect the repertoire of conformational epitopes. Therefore, a different approach based on the production of truncated domains of PLA_2R was taken by two research groups who each identified important B cell epitope–containing domains in the N terminus of PLA_2R (39, 40). Because of the approach used, the identified domain largely exceeded the size of a B cell epitope, and so should instead be called a macroepitope. Kao et al. (40) showed that the smallest macroepitope contained CysR–FnII–CTLD1. Fresquet et al. (39) narrowed this macroepitope to CysR, where they identified a 31–amino acid sequence that blocked much of the autoantibody binding, possibly localizing the B cell epitope to this region. Seitz-Polski et al. (41) confirmed that the immunodominant epitope of PLA_2R lies within CysR and is recognized in 100% of patients with circulating anti-PLA_2R.

And again, Heymann nephritis opened the way to investigate epitope spreading in human MN. Using a series of PLA_2R deletion mutants covering the extracellular domains, Seitz-Polski et al. (41) identified reactive epitopes in the CysR, CTLD1, and CTLD7 domains. In their retrospective study of 69 patients, those patients with anti-CysR-restricted activity were younger, had less proteinuria, and exhibited a higher rate of spontaneous remission and lower rate of progression to renal failure. Those with antibodies to all three epitopes had the most severe disease. The authors suggested that the immune response started from the immunodominant CysR and then spread in the more severely affected patients, although this remains to be demonstrated by careful longitudinal studies. Because the most relevant antibodies are deposited in the glomerulus, the next step should be analyzing the reactivity of immunoglobulins eluted from kidney biopsies from patients with different profiles of circulating antibodies (45). A subsequent, prospective study comparing the efficacy of rituximab and antiproteinuric therapy showed that although epitope spreading strongly correlated with PLA_2R antibody titers, epitope spreading at baseline was associated with a decreased remission rate at month 6 and at last follow-up, independently of age, sex, baseline PLA_2R antibody level, and treatment group (46). However, the predictive value of epitope spreading remains a matter of intense controversy.

4.3. Pathogenesis: Disease Initiation, Restriction to the Kidney, Relapse

At variance with LRP2 and NEP, PLA_2R is not present in rodent glomeruli, which is a bottleneck for transfer experiments. The early recurrence of MN in kidney transplantation recipients who have circulating anti-PLA_2R antibodies, which echoes passive Heymann nephritis, supports a pathogenic role for these antibodies (47–50). In one patient, recurrence was caused by transfer to the graft of an anti-PLA_2R monoclonal IgG3-κ that was colocalized with PLA_2R, both in the native tissue and in the kidney graft biopsy (50). Whether immune deposits result from the binding of circulating anti-PLA_2R autoantibodies to podocyte PLA_2R remains controversial because PLA_2R is only weakly expressed in the normal podocyte, and there is evidence that PLA_2R circulates in blood. Therefore, the contribution of PLA_2R–anti-PLA_2R IgG4 circulating immune complexes should be considered.

The mechanisms causing disease initiation relatively late in life and the site of initial exposure of PLA_2R to the immune system are unknown. Although the highest expression of PLA_2R mRNA is in the kidney, it is also expressed in lung, placenta, liver, and skeletal muscle (42). A role for the lung should be carefully considered, given the increase in MN incidence in provinces in China that have the highest levels of pollution with particulate matter 2.5 particles (51). It remains to be established whether environmental factors act directly on the lung, which could be the site of antigen presentation in the inflamed tissue, or through epigenetic modifications that

could induce increased PLA_2R expression, including in podocytes (52). Antigen mimicry has also been hypothesized to occur by the Brenchley group (39) because they found a linear amino acid sequence (LTLENCK) in the CysR domain of PLA_2R that also exists in D-alanyl-D-alanine carboxypetidase, an enzyme in the cell wall of several bacterial strains. To explain the late onset of disease, which cannot be accounted for by genetic risk alleles, a multi-hit mechanism has been proposed (53). This hypothesis is supported by the detection of anti-PLA_2R antibodies in some patients before they develop clinical manifestations of MN (54).

As with many organ-specific autoimmune diseases, it is also not clear why the disease is limited clinically to the target organ, for example, the kidney (42, 55). Restriction of the disease to the glomerulus might be explained by the level of protein expression at the podocyte membrane in certain circumstances, differences in glycosylation or other posttranslational modifications, or the ability of autoantibodies to reach the antigen because of endothelial cell fenestrations.

The reasons underlying the variability in disease resolution are also largely unknown. The outcome of iMN is unpredictable, and the occurrence of spontaneous remission (in 40% of patients) and relapses seems random, although some correlation has been established with PLA_2R antibody levels (56). The role of regulatory T cells has been suggested by the Ronco group (57), who showed that patients with MN had a lower percentage of regulatory T cells within the $CD4^+$ T cell population at baseline when compared with healthy controls. Patients who responded clinically to rituximab demonstrated not only lower levels at baseline than nonresponders but also a significant increase in the percentage of regulatory T cells as early as day eight after treatment. There is no study on the role of regulatory T cells in patients who have undergone spontaneous remission, but similar changes may occur.

Genome-wide association study (GWAS): method that screens the entire genome for genetic polymorphisms (SNPs) that occur more frequently in cases than in ethnically matched controls

Single nucleotide polymorphism (SNP): the most common type of genetic variation in the human genome, defined by a change in a single base/nucleotide, usually not coding

4.4. Genetics

It has been known since 1979 that MN is strongly associated with HLA-DR3 in European Caucasians (58) and since 1989, with HLA-DQA1 (59). Considerable progress in understanding has been made possible in the past 10 years by the development of genome-wide association studies (GWASs). Two years after the identification of PLA_2R, a GWAS conducted by a European consortium revealed that two series of alleles in the *HLA-DQA1* immune response gene (chromosome 6) and in the *PLA2R1* gene (chromosome 2) were strongly associated with iMN (60). Thus, this report showed, first, that a GWAS could be successful despite the small size of the cohorts. Second, it confirmed by an unbiased approach the discovery of PLA_2R by the Salant group (33). Third, the odds ratios were amazing for common alleles in a multigenic disease, for which they usually have only a small effect size. Homozygosity for the lead risk alleles in *HLA-DQA1* and *PLA2R1* increased the odds ratios for MN by, respectively, 20-fold and 4-fold, and the odds ratio was increased by 80-fold when they were combined, due to an interaction between the two loci. These findings were confirmed by subsequent studies in Caucasians and South Asians (61, 62). Because the most significant risk allele in *PLA2R1* is intronic, it is unlikely to change PLA_2R autoreactivity. A follow-up study by the same consortium did not reveal mutations or rare variants in *PLA2R1* coding sequence and splice sites that could have accounted for changes in PLA_2R conformation and antigenicity (63). Because variants in coding regions are common (64, 65), a current hypothesis used to explain genetic susceptibility implicates single nucleotide polymorphisms (SNPs) in promoter or regulatory regions that could influence the level or site of expression of PLA_2R (66), although one cannot exclude that common missense variants may alter peptide binding to HLA class II molecules.

Several groups have confirmed the presence of risk alleles in or near *HLA-DQA1* (61, 62, 67–69) and established correlation between the specific *HLA-DQA1*0501* allele and the presence

of circulating anti-PLA_2R antibodies (62, 67, 70). However, the situation is more complex because of the high degree of linkage disequilibrium that leads to commonly inherited HLA-D haplotypes (71). Two studies from China (72, 73), two from Japan (66, 74), and one from Europe (61) shed new light on this important issue. Studies of Han Chinese from Beijing (72) identified *DRB1*1501* and *DRB1*0301* as independent risk alleles for MN, while a study from Nanjing (73) confirmed the strong association with *DRB1*1501* and revealed another independent, strong association with *DRB3*0202*, which probably accounts for the *DRB1*0301* signal since they are on the same haplotype. Participants with either risk allele *DRB1*1501* or *DRB3*0202* had a 99-fold increased risk of developing PLA_2R-related MN compared with those without a risk allele, but it must be noted that 44% of healthy controls also inherited one of the two haplotypes. In the first study, from Beijing, the identified alleles were associated with circulating anti-PLA_2R antibodies. In the second study, from Nanjing, *DRB1*1501* was associated with an earlier age at onset. In the Japanese population (66, 74), *DRB1*1501* and *DQB1*0602* (same haplotype) were associated with iMN, whereas in the European population (61), iMN was associated with the *DRB1*0301–DQA1*0501–DQB1*0201* haplotype common to Han Chinese participants, but not with the Asian-specific haplotype *DRB1*1501–DQA1*0102–DQB1*0602*.

The strong gene–gene interaction between the lead SNP in the *PLA2R1* locus (rs4664308) and *HLA-DRB1*1501* and *DRB1*0301* in the Beijing study (72) suggests a physical interaction between DRβ1 molecules and PLA_2R epitope(s) during antigen presentation. Amino acid positions 13 and 71 in the major histocompatibility complex (MHC) DRβ1 chain independently associate with MN. Structural models showed that arginine 13 and alanine 71, encoded by *DRB1*1501*, and lysine 71, encoded by *DRB1*0301*, facilitate interactions with T cell epitopes of PLA2R. Using predictive algorithms to identify which fragments of PLA_2R would best fit within these variants, the authors identified sequences within CTLD1 and CTLD7, potentially linking the T cell and B cell epitopes of PLA_2R (**Figure 4**). These results need to be confirmed by functional studies.

In further studies, Wang et al. (75) from the Beijing group identified *DRB1*1502*, which differs from *DRB1*1501* by a single amino acid, as a potential modifier with a strong predictive value when associated with HLA risk alleles. Although *DRB1*1502* is not a risk allele, it was associated with a lower estimated glomerular filtration rate at baseline and last follow-up and with a worse renal outcome, with a higher risk of end-stage renal disease.

In summary, recent advances in MN genetics have clearly demonstrated interactions between the *HLA-D* and *PLA2R1* loci in patients with PLA_2R-related MN. A better understanding of these interactions and of the role of noncoding SNPs will be necessary to unravel the initial steps of the disease through the use of expression quantitative trait loci studies (76) and a combination of GWASs and epigenomic and chromatin conformation data.

5. THSD7A, EXOSTOSIN, AND OTHER PODOCYTE ANTIGENS

5.1. THSD7A

In 2010, Beck and colleagues (77) detected IgG4 antibodies against an unknown high MW glomerular antigen in a patient with MN and prostate cancer. It took 4 more years for a consortium composed of Beck, Salant, Lambeau, and Stahl to identify this antigen as being the glomerular glycoprotein THSD7A. One reason for this delay is that, at variance with PLA_2R, which is associated with about 70% of cases of iMN, THSD7A-associated MN is rare, being observed in less than 5% of patients with iMN (78–80). THSD7A shares some characteristics with PLA_2R: It is a large, transmembrane, 250-kDa glycoprotein expressed by podocytes and has a wide tissue distribution; it is composed of 21 thrombospondin domains; the antigen is destroyed

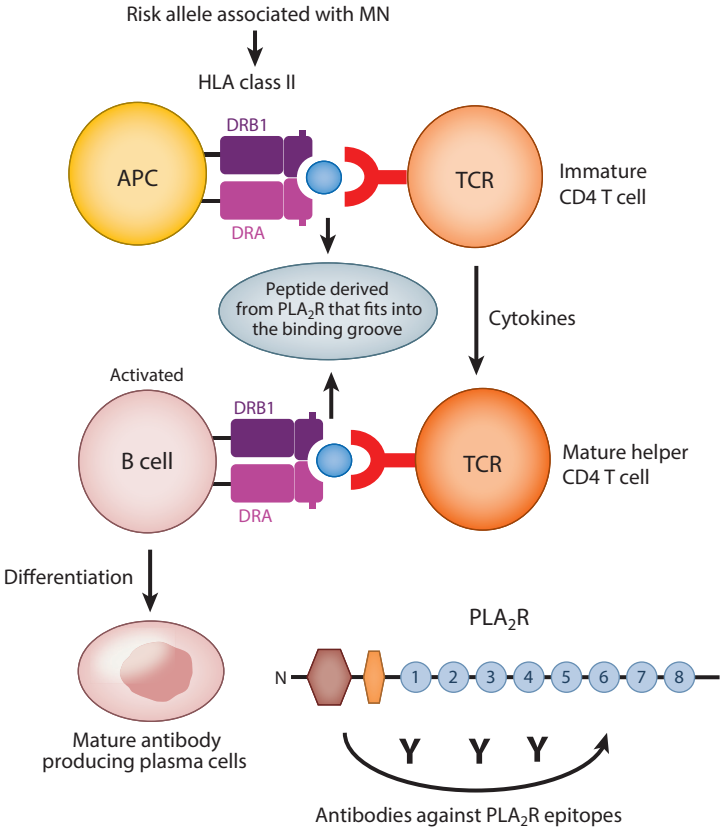

Figure 4

Flow of the immune response to PLA_2R antigen in individuals with HLA class II risk alleles. MN risk alleles at the HLA-D locus expressed on APCs can present linear peptides of PLA_2R (*blue circles*) to the T cell receptor on immature CD4 T cells. Then, developed mature helper CD4 T cells drive the differentiation of activated B cells into plasma cells, which produce antibodies to the distinct epitopes of the PLA_2R antigen. Abbreviations: APC, antigen-presenting cell; HLA, human leukocyte antigen; MN, membranous nephropathy; PLA_2R, phospholipase A_2 receptor; TCR, T cell receptor.

by reduction of the disulfide bonds; and the immune response is mostly carried by IgG4 (78). Both PLA_2R and THSD7A have potentially soluble forms, resulting from alternative splicing (PLA_2R) or proteolytic cleavage (THSD7A and possibly PLA_2R as well).

However, there are also substantial differences. In contrast to PLA_2R, which is expressed in humans and primates, but not in rodents, THSD7A was detected in most species studied. It was first identified as a novel endothelial protein (81) involved in endothelial cell migration and tube formation (81, 82). In zebrafish, it was described as a neural protein required for angiogenic patterning during development (83). The Stahl group (84) took advantage of THSD7A expression in rodent podocytes to demonstrate that patient-derived anti-THSD7A antibodies induce MN in mice. They also developed a heterologous model of THSD7A-related MN in mice injected with rabbit antibodies and coimmunized with human and mouse THSD7A complementary DNAs (85).

Compared with PLA_2R, specific THSD7A epitope domains are less well defined. Stoddard et al. (86) built a homology structural model of the extracellular portion of THSD7A and predicted that 18 domains (domains 1–17 and 19) would contain epitope sites, which corresponded well

to the regions identified by Seifert et al. (87), except for domains 4 and 5. Although patients' autoantibodies preferentially bind to the most N-terminal region of THSD7A (domains 1 and 2), additional reactivity is scattered along the antigen, suggesting a multivalent immune response (87; G. Lambeau, unpublished data).

As for PLA_2R, the questions regarding the source of the antigen, triggering events, and induction of relapses remain largely unresolved, except for in the few cases with a clear relation to cancer, in which THSD7A was found in the tumor and metastatic lymph node cells, dendritic cells of the lymph node, and subepithelial immune deposits (79, 88). THSD7A was also found in the skin and tumor of patients with, respectively, neurofibromatosis type 1 (89) and benign schwannoma (90). The relationship of THSD7A immunization to cancer (and female sex), which was suspected in the first report (78), was not confirmed in three studies of patients with different ethnic and geographical origins (80, 91, 92). However, a Dutch study performed before THSD7A was described and that did not include detection of PLA_2R in biopsies showed that the absence of anti-PLA_2R was associated with a 37% chance of developing a malignancy, often early after the diagnosis of MN, whereas only 9% of anti-PLA_2R-positive patients developed a cancer, often long after the diagnosis of MN (93), a finding that calls for long-term patient monitoring.

Few patients present with dual positivity for PLA_2R and THSD7A (91, 92). Zaghrini et al. (80) identified eight double-positive patients in a large series of 49 patients with THSD7A-related MN. No major clinical differences were observed between the single- and double-positive patients. These studies could not determine whether the rare occurrence of both anti-PLA_2R1 and anti-THSD7A autoantibodies is coincidental or linked through intermolecular epitope spreading or whether one autoantibody precedes the other.

5.2. Exostosins 1 and 2: Antigens or Biomarkers?

The target antigens in so-called double-negative MN, that is, MN without immunization against PLA_2R and THSD7A, remain elusive. It is likely that given the low prevalence of THSD7A immunization, several additional antigens are involved. Sethi et al. (94) used an original combination of laser microdissection, tandem mass spectrometry (MS/MS), and immunohistochemistry (IHC) techniques to identify two novel proteins, exostosin (EXT) 1 and EXT2, that accumulate in the subepithelial immune deposits in a subset of PLA_2R-negative patients with MN. They studied 224 cases with biopsy-proven PLA_2R-negative MN and 102 control cases, including 47 cases with PLA_2R-associated MN, in a discovery cohort and 48 cases with PLA_2R-negative primary and lupus MN in a validation cohort. They identified 21 cases with PLA_2R-negative MN who showed high total spectral counts for both EXT1 and EXT2, which were absent in all cases with PLA_2R-associated MN and in control cases. EXT1 and EXT2 were localized by IHC to granular deposits along the glomerular basement membrane (GBM). Clinical and biopsy findings revealed features of autoimmune disease. Altogether, 80% of the patients were women, and the average age was 36 years. And 70% of patients had one or several antibodies against nuclear antigens, double-stranded DNA, Smith proteins, Sjögren syndrome–related antigen A or B, or ribonucleoprotein. Altogether, 35% of the patients had a clinical diagnosis of lupus, and 12% had mixed connective tissue disease. In 85% of patients, kidney biopsy revealed features suggestive of secondary MN related to autoimmune disease, including immunofluorescence (IF) staining for C1q or IgA or IgM, or a combination of these; subendothelial and mesangial deposits; and tubuloreticular inclusions in endothelial cells on electron microscopy. Furthermore, IgG1 was the dominant IgG, with spectral counts significantly higher than those of IgG4 and than the spectral counts of IgG1 in cases with PLA_2R-associated MN. Validation studies in a French cohort established that EXT1or

EXT2 staining was detected in about half of cases with pure class V lupus nephropathy and in primary MN associated with signs of autoimmunity (antinuclear antibodies without anti-DNA), whereas cases of mixed class V and III/IV lupus nephritis were rarely positive for EXT1 or EXT2. In total, 39 cases of EXT1- or EXT2-associated MN have been identified.

Exostosins are glycosyltransferases responsible for the synthesis of the heparin sulfate backbone. They add glycosaminoglycan residues to the core protein, resulting in the generation of complex polysaccharides (95, 96). Among the products of the five genes that encode the EXT proteins, only EXT1 and EXT2 were detected by MS/MS. EXT1 and EXT2 can exist as heterodimers, thus increasing their activity and stability (97, 98), which probably explains why the two proteins are found together by MS/MS and IHC. EXT proteins have wide tissue and cell distribution, including in podocytes. They are endoplasmic reticulum transmembrane proteins, which raises the question of whether the EXT1 or EXT2 detected in the immune deposits are full-length proteins, shed partial or truncated proteins, or proteins with posttranslational modifications. Mutations in *EXT1* and *EXT2* are associated with autosomal dominant, hereditary multiple exostoses, which are among the most common inherited skeletal disorders (96, 99).

One intriguing question is why in a small number of samples there is an absence of circulating antibodies identified by Western blotting under nonreducing conditions or by enzyme-linked immunosorbent assay (ELISA) and slot blot under native conditions. However, this finding does not exclude a role for anti-EXT1 or anti-EXT2 antibodies: There may be low circulating amounts of antibodies that are cleared rapidly from the blood by the deposited antigen (the so-called sink effect), or they may be highly conformation-sensitive epitopes or neoepitopes not expressed by the recombinant proteins. Alternatively, the absence of detectable antibodies raises the possibility that EXT1 and EXT2 proteins may represent biomarker proteins for MN associated with autoimmune disease rather than target antigens. Further studies are required to address these issues.

5.3. Lecithin–Cholesterol Acyltransferase

Seven cases of acquired lecithin–cholesterol acyltransferase (LCAT) deficiencies caused by anti-LCAT antibody have been reported so far, all but one in the Japanese population (100). Among them, two patients developed MN (100, 101). LCAT was detected along segments of the glomerular capillary wall, which suggested that LCAT could be the target antigen. Treatment with corticosteroids resulted in complete remission of the nephrotic syndrome, normalization of serum LCAT activity and HDL level, and disappearance of foam cell accumulation in renal tissue.

5.4. Cytoplasmic Antigens

Using a proteomic approach to identify antigens recognized by serum and glomerular eluates from patients with MN, the Ghiggeri group (102, 103) identified antibodies against cytoplasmic antigens, such as superoxide dismutase 2, aldose reductase, and α-enolase, which are not or are only weakly expressed in the normal glomerulus but can be induced and routed to the podocyte membrane under stress conditions. When exposed upon initial insult to podocytes, for instance by anti-PLA_2R antibodies, these antigens might then be the trigger for and the target of a second wave of antibodies. This hypothesis is supported by Kimura et al. (104), who showed the presence of anti-α-enolase antibodies in 70% of cases of both primary (mostly IgG1 and IgG4) and secondary (mostly IgG1 and IgG3) MN. There is controversy regarding the presence (103) or absence (104) of α-enolase in subepithelial immune deposits and its role in the formation of those deposits. However, the absence of α-enolase from the deposits would not exclude that anti-α-enolase antibodies could bind to endothelial or podocyte cell surfaces and, subsequently, alter cell functions.

6. EXOGENOUS ANTIGENS: CATIONIC BOVINE SERUM ALBUMIN, ENZYME REPLACEMENT THERAPY, AND HEPATITIS B

6.1. Cationic Bovine Serum Albumin as a Cause of Early Childhood MN

The description of cationic BSA–related MN in children aged <5 years (105) was regarded as a breakthrough in the field of kidney diseases because this was the first report of a food antigen being responsible for an immune-mediated glomerulonephritis. Again, this description was inspired by the experimental model described by Adler et al. and Border et al. (15, 16) about 30 years earlier. Some young children with what was considered to be idiopathic MN were found to have high serum titers of anti-BSA antibodies (both IgG1 and IgG4) that reacted primarily with one peptide region in BSA but not with the homologous peptide in human serum albumin. The key finding was the identification in children's serum of cationic BSA migrating in the basic range of pH, while in adult patients with MN and circulating BSA, immune-purified BSA migrated in the neutral region as native BSA. Furthermore, BSA could specifically be detected in subepithelial immune deposits, while the staining for PLA_2R was negative, and the Ig eluted from the patients' glomeruli reacted with BSA (carried by IgG1 and IgG4), not with human serum albumin. These cases are the translation of the so-called planted antigen concept (15, 16) to human pathology, involving the binding of cationic BSA to the negatively charged glomerular capillary wall, followed by the formation of in situ immune complexes. The exact source of the cationic antigen is unknown. Cow's milk and beef are the major sources of BSA in young children. It is hypothesized that the change in electric charge might be caused by the industrial processing of milk or modifications by the intestinal microbiota or by the gut epithelium during intestinal absorption of the entire BSA molecule (106, 107). As yet, cationic BSA-related MN has been reported only in children who have increased permeability of the intestinal barrier and frequent gastroenteritis episodes. These findings open the way for discoveries regarding other food or nondietary antigens.

6.2. Enzyme Replacement Therapy as a Cause of Alloimmune MN

A similar scenario involving a planted antigen may be at play in patients receiving enzyme replacement therapy (ERT) for a lysosomal storage disease. Cases of MN were described in patients with Pompe disease and mucopolysaccharidosis type VI who received, respectively, recombinant human α-glucosidase (108) or arylsulfatase B (rhASB) (109). Because of the absence or only low levels of enzyme in many patients, therapeutic proteins may be recognized as alloantigens. Alloantibodies may be without clinical significance or may lead to hypersensitivity reactions, decreased bioavailability, and reduced efficacy of the therapeutic proteins. More rarely, patients develop MN, a diagnosis that should not be missed because of the therapeutic implications. In a patient with MN caused by rhASB therapy (109), IF staining showed that subepithelial immune deposits contained rhASB colocalized with IgG, and IgG eluted from the biopsy specimen reacted specifically with rhASB. Because of deterioration in the patient's clinical condition on discontinuation of ERT (while proteinuria decreased), induction of tolerance to rhASB was initiated by high-dose immunosuppressive therapy. ERT was resumed 8 weeks after initiation of immunosuppressive therapy without inducing a rebound of anti-rhASB antibody titers or an increase in proteinuria.

6.3. Other Nonpodocyte Antigens

In patients with secondary MN, hepatitis B, hepatitis C, and *Helicobacter pylori* antigens; tumor antigens; thyroglobulin; and DNA-containing material were detected in subepithelial deposits (110, 111). Although the presence of these antigens was initially attributed to their being trapped

in the glomerular capillary wall, several of these cases have recently been revisited. Most cases of hepatitis B–related MN are actually associated with immunization against PLA_2R (112, 113).

7. EFFECTOR MECHANISMS: ONE OR SEVERAL PATHWAYS?

It is generally considered that formation of the MAC of complement is the end product of the immune conflict occurring in the glomerulus in MN, inducing massive proteinuria and nephrotic syndrome. However, studies of Heymann nephritis had already shown that anti-LRP2 (antimegalin) antibody could be harmful by inhibiting the receptor function of LRP2 (114). Actually, all target antigens identified have biological functions that may be altered by the relevant antibody, but the importance of complement activation and blockade of biological function is often difficult to establish. Another conundrum is the predominance of the IgG4 subclass in the deposits, although this subclass has a low potential for antigen clustering and complement activation (if any).

7.1. Biology of Target Antigens and Direct Effector Role of Antibodies

The four major antigens in MN (LRP2, NEP, PLA_2R, and THSD7A) are transmembrane proteins. The formation of immune deposits implies that antigen–antibody complexes are clustered at the podocyte cell surface, then released into the extracellular space, where they most likely adhere to GBM components. Such a scenario was nicely demonstrated by Kerjaschki et al. (115), who studied the early events in the formation of immune deposits in passive Heymann nephritis. They showed that immune complexes form at the clathrin-coated pits located at the bases of the foot processes where LRP2 is expressed. Immune deposits become firmly fixed to the GBM 15 min after IgG injection, and they remain associated with the coated pits for up to 8 days after IgG injection. Notably, both the antigen and the antibody are present throughout the deposits at all time points. Later, it was shown in the proximal tubule that LRP2 could undergo proteolytic cleavage, leading both to the shedding of the ectodomain and the release of an intracellular fragment translocated to the nucleus, a finding compatible with regulated intramembrane proteolysis (RIP) (116, 117). RIP is an evolutionarily conserved process that links transmembrane proteins to cell signaling and gene transcription (118). Whether this process occurs in the podocyte, participates in the formation of immune deposits through the release of the ectodomain, and alters podocyte biology remains to be established. The case of PLA_2R seems more complex because there are both an alternative spliced, soluble form that contains epitopes of the parent molecule (42) and a protease cleavage form, at least in the mouse, potentially associated with RIP (119). Theoretically, both forms can contribute to the deposits. THSD7A may also be cleaved at a juxtamembrane site (82). The release of soluble THSD7A during antibody-mediated injury may influence podocyte adhesion, and thereby contribute to foot process effacement. The nature of the enzymes (sheddases) that cleave PLA_2R and THSD7A is still elusive. Further studies are needed to map the binding sites of the enzymes to specific epitopes and to establish whether antibody binding perturbs this process. It remains to be established whether the released soluble forms of LRP2, NEP, PLA_2R, and THSD7A antigens can play a role in triggering the immunization process.

In addition to forming immune complexes that result in complement activation, antipodocyte antibodies can directly alter podocyte biology. Both LRP2 and PLA_2R are cell surface receptors, and NEP cleaves biologically active peptides at the cell surface. In the proximal tubule, LRP2 is critical for the reuptake of numerous ligands, including albumin and low-MW proteins, lipoproteins, sterols, vitamin-binding proteins, and hormones, although its role in the rat podocyte is unknown (120). The Kerjaschki group (114) showed that anti-LRP2 antibodies inhibited the uptake of lipoproteins, the accumulation of which within immune deposits, together with the production of reactive oxygen species, resulted in the formation of toxic lipid peroxidation products.

NEP is involved in the catabolism of a number of regulatory peptides with vasoactive properties, and thus it plays an important role in turning off peptide signaling events at the cell surface (121). The Ronco group (29) showed that in children with alloimmune MN, NEP-specific activity was inhibited in a dose-dependent manner by anti-NEP IgG1, whereas IgG4 had no effect. Thus, some of the deleterious effects of anti-NEP antibody might be mediated by the blockade of enzymatic activity, resulting in alterations of glomerular hemodynamics, endothelial permeability, and tubular function. These alterations might be the cause of the unusual, severe ischemic lesions observed in some children's biopsies (26, 29). Because the affected children also showed signs of classical pathway complement activation caused by IgG1 (C1q deposits in biopsy samples), it is difficult to determine whether enzymatic blockade or complement activation contributed more to disease pathogenesis. It is remarkable that despite a common genetic cause, differences in the anti-NEP IgG subclass driven by T helper type 1 cells (IgG1) or T helper type 2 cells (IgG4) modulate disease severity through complement activation and enzyme inhibition (31).

PLA_2R is known as the multifunctional M-type 180-kDa receptor of secretory PLA_2 ($sPLA_2$), a potent proinflammatory enzyme. The putative $sPLA_2$ binding site is CTLD5. Actually, the exact function of PLA_2R remains unknown. In theory, PLA_2R can function as an $sPLA_2$ clearance receptor or as a positive regulator of $sPLA_2$ by inducing a variety of biological responses, such as initiating mitogen-activated protein kinase activation, producing lipid mediators, producing reactive oxygen species, and activating DNA damage pathways (leading to senescence) (122, 123). Because markers of senescence are overexpressed in podocytes from patients with MN, a potential role for agonistic activity of anti-PLA_2R should be considered (124). It has also been shown that PLA_2R FnII and CTLDs could modulate binding and migratory responses to collagen (125), although this is controversial (126), and that anti-PLA_2R antibodies could interfere with this function (127). Antibodies can alter PLA2R functions in many ways, and studies are needed to establish which one may be of pathogenic significance.

The role of THSD7A seems more well established. There is accumulating evidence favoring a role for THSD7A in cell (podocyte) adhesion, most likely through the thrombospodin type 1 domains. THSD7A is restricted to the basal aspect of foot processes, closely following the meanders of the slit diaphragm in humans and mice. In primary cultured murine glomerular epithelial cells, anti-THSD7A, but not control IgG, induces marked cytoskeletal rearrangement (84). In cultured human podocytes, THSD7A increases cell size, enhances adhesion, and reduces detachment from collagen type IV–coated plates, and it also decreases the ability to migrate (128). Based on their localization to the slit diaphragm in vivo, autoantibodies to THSD7A might structurally and functionally alter the permeability of the slit diaphragm to protein. This finding is compatible with the absence of C3 and C5b-9 deposits in the early proteinuric phase of mouse disease induced by injection of human anti-THSD7A antibodies (84). Interestingly, THSD7A expression begins upon vascularization, during slit diaphragm formation in glomerular development, and it is colocalized with focal adhesion proteins in endothelial cells, where it mediates cell migration and tube formation (81–83).

7.2. Pathogenicity of Immunoglobulin G4

Although IgG4 is the most prevalent subclass in most cases of PLA_2R- and THSD7A-related MN, both in the circulation and in immune deposits, its pathogenic role remains elusive because of its unusual properties. First, IgG4 is a subclass that does not fix complement C1q. Second, IgG4 antibodies can behave like monovalent antibodies because they may undergo Fab-arm exchange, with each arm recognizing a different antigen. Consequently, their ability to cross-link the antigen and induce its clustering is much less than it is for other IgG subclasses (129). Actually, IgG4 is most

often associated with smaller amounts of other subclasses (particularly IgG1), as shown by IF and mass spectrometry analyses of immune deposits (94); to demonstrate that IgG4s are pathogenic, it will be necessary to purify patients' IgG4 so that it is free from other Ig subclasses and infuse this into animals that present PLA_2R or THSD7A at the podocyte surface.

Yet IgG4 autoantibodies are pathogenic in other IgG4-associated autoimmune diseases in which the effect of monovalent IgG4 might be to disrupt receptor–ligand or enzyme–substrate interactions (129). However, one cannot exclude a protective effect due to IgG4 atypical rheumatoid factor activity and binding to the IgG1 Fc region through Fc–Fc interactions (130, 131). Such interactions may interfere with IgG1 lattice formation and C1q binding, thus downregulating complement activation in the deposits.

7.3. Complement Activation and Complement Regulatory Proteins: Which Pathway is Trodden?

There is strong evidence from studies on passive Heymann nephritis that complement activation leading to MAC formation is a major mediator of proteinuria (132, 133). The Kerjaschki group (134) showed that C5b-9 was inserted into the immune deposits, then endocytosed in multivesicular bodies before being exocytosed in the urinary space. Sublytic C5b-9 activates a variety of downstream pathways, including those of protein kinases, lipid metabolism, reactive oxygen species, growth factors and gene transcription, matrix metalloproteinases, endoplasmic reticulum stress, and the ubiquitin–proteasome system, and C5b-9 impacts the integrity of the cytoskeleton and slit diaphragm proteins (135). In active Heymann nephritis, proteinuria develops only if complement regulatory proteins (CRPs) are neutralized by anti-Crry (complement receptor 1–related protein/gene y) antibodies (136). However, the role of complement has been questioned by the development of nephrotic syndrome in C6-deficient rats with active (137) and passive (138) Heymann nephritis.

In human primary MN, the role of complement is more debated. On the one hand, C4, C3, and C5b-9 are detected in the immune deposits, and C5b-9 is excreted in the urine and has been considered to be a biomarker of disease activity (139); on the other hand, after passive transfer of patients' anti-THSD7A antibodies to mice, IgG4 does not activate C1q, and the development of proteinuria precedes the deposition of C3 (84). Additionally, complement deposits were not observed in a heterologous model of THSD7A-associated MN induced by the injection of rabbit anti-THSD7A, but one likely explanation is that these antibodies fail to activate complement, at least in vitro (85).

Actually, the three complement pathways can be activated in patients with MN, depending on the nature of the antigen, the subclass of IgG antibody, and other antibody specificities directed to CRPs. In neonatal alloimmune MN, the classical pathway is activated by maternal IgG1, which is found in samples from kidney biopsies from the most severely affected children together with C1q, C3, and C5b-9 in the absence of IgG4 (29).

Activation of the classical pathway can also occur in cases of adult MN. The Ronco group (50) reported a patient who produced a monoclonal anti-PLA_2R IgG3-κ antibody, inducing recurrence in an allograft. Kidney biopsy showed exclusive IgG3-κ deposits associated with components of the classical pathway (C1q, C3, C5b-9) in the absence of mannose-binding lectin (MBL). Sethi et al. (94) described a subset of patients with seemingly primary MN but signs of autoimmunity and EXT1 or EXT2 subepithelial deposits in biopsy samples, which also showed signs of classical pathway activation (C1q deposits). These patients may later develop full-blown systemic lupus erythematosus.

However, in most adult cases of primary MN, the absence of C1q in the deposits practically rules out the classical pathway. In this context, the frequent presence of C4d favors activation of

the MBL pathway. In contrast, the presence of factor B and properdin points to activation of the alternative pathway (AP). Thus, it is generally considered that both MBL and AP are implicated in most patients, although there may be exceptions. Cases of MN with MBL deficiency (140) and with ficolin 3 deficiency (141) have been reported, suggesting that formation of the MAC complex resulted mostly from hyperactivation of AP.

Triggers of activation of MBL and AP in primary MN are beginning to be understood. There is some indication that abnormally glycosylated IgG4 might activate the MBL pathway. MBL and the ficolins bind to acetyl glycans, typically limited to microbial carbohydrates and apoptotic host cells, and form complexes with MBL-associated serine proteases to cleave and activate C4 (142). It was shown that in rheumatoid arthritis, glycosylation of IgG is altered, which can create a new mode of interaction with MBL (143). More specifically, when the Fc region of IgG lacks galactose and terminates with an *N*-acetylglucosamine residue, MBL can bind to IgG and activate the lectin pathway, as shown by nuclear magnetic resonance and X-ray analyses (143). Similar alterations have been reported in patients with other autoimmune diseases (144, 145). There is in vitro evidence that anti-PLA2R IgG4 is able to bind MBL and activate C4 and, at least in some cases, is hypogalactosylated (146, 147).

To address the pathogenic relevance of AP, the Borza group (148) used a model of MN induced by immunization with the noncollagenous domain of the α-3 chain of collagen in complement factor B–deficient mice, which lack a functional AP. Unlike wild-type mice, those lacking factor B did not develop albuminuria or exhibit glomerular deposition of C5b-9, despite similar amounts of deposited IgG. Thus, this model provides direct evidence that AP is necessary for complement activation by subepithelial immune complexes.

In healthy individuals, AP activation is controlled by CRPs. Accumulating evidence shows that genetic defects or autoimmunity against CRPs play an important role in disease conditions by causing AP hyperactivation. The Ronco group (149) reported the case of a patient with PLA_2R-related primary MN in whom anti-complement factor H (CFH) autoantibodies developed and who subsequently had impaired renal function despite the disappearance of anti-PLA2R antibodies. The IgG3 isotype antibody recognized the C-terminal domain, which suggested that CFH binding to the cell surface could be affected. Interestingly, whereas in the first biopsy intense staining of CFH contrasted with weak staining of properdin, the reverse was observed in the second biopsy, a finding suggestive of slowly evolving hyperactivation of AP. An analysis of 92 serum samples from a retrospective cohort of patients revealed that two additional patients had anti-CFH antibodies. Inhibition of CFH activity by autoantibodies at the podocyte cell surface might contribute to hyperactivation of AP and accelerated disease progression in the absence of thrombotic microangiopathy.

8. TRANSLATION TO THE BEDSIDE

Less than 2 years after the discovery of PLA_2R in 2009, an IF assay followed by an ELISA was developed to detect and measure PLA_2R antibodies. These assays have a specificity of close to 100% and a sensitivity close to 70% (56). They have induced a paradigm shift in the standard of care, both for diagnosis, thus challenging the utility of kidney biopsy (150), and for treatment monitoring. Proteinuria, which for a long time was the only marker of disease activity, has been outperformed by antibody titers in monitoring the early stages of treatment because immunological remission (defined by the disappearance of antibodies) precedes clinical remission, while later on, the reappearance of or an increase in antibodies usually precedes relapses. Recently, an IF assay (79) and an ELISA (80) were also developed to assess anti-THSD7A antibodies.

The discovery of MN antigens has provided the molecular bases for a new pathophysiological classification of MN based on serology and the identification of antigens in immune deposits;

accordingly, MN can be defined as PLA_2R-, THSD7A-, EXT1-, or EXT2-related or as triple-negative. This new classification should substitute for the classical distinction between primary and secondary MN, which can occur with any of these antibody specificities. Thus, immunization against PLA_2R can be induced by different triggers, such as hepatitis B and sarcoidosis, while THSD7A may in exceptional cases be associated with cancer. Antigen- and biomarker-based classification has important diagnostic and therapeutic implications (56).

9. UNMET CHALLENGES AND PERSPECTIVES

Despite considerable advances in understanding the pathophysiology of MN, a lot remains to be done, particularly in the field of new therapies. The main challenges are summarized in the Future Issues section (below). Addressing these challenges is of the utmost importance for ensuring therapeutic advances.

There are two major issues that need to be resolved. The first relates to the still-controversial role of complement activation in human MN. Complement inhibitors are now in the pipeline at a number of companies, but do we have any arguments for launching costly clinical trials? Unpublished results from a trial using anti-C5 were disappointing, most likely because of loss of the antibody in the urine; a post hoc analysis in patients who showed signs of C5 neutralization suggested that anti-C5 was efficacious. More work needs to be done to further dissect the pathways of complement activation and select the most appropriate inhibitors, such as small-molecule inhibitors of C5b-9 assembly. Should this be achieved, there are two windows of opportunity for complement inhibitors: The first is during the interval occurring after the start of immunosuppressive therapy and before full treatment efficacy is reached (at 3 to 6 months, based on the effect of treatment on anti-PLA_2R antibody titers); the second is in the 30% or more of patients who do not respond, or respond only partially, to immunosuppression.

The second important issue is the use of targeted immunosuppression, which aims for more efficacy and less toxicity. Until now, treatment has relied on using nonspecific immunosuppressants against B cells instead of targeting only those cells that are responsible for producing anti-PLA_2R and anti-THSD7A antibodies. First, recent attempts to restore self-tolerance to the target antigen, ideally by enhancing the generation of specific T regulatory cells (Tregs) have been successful. Work by the Ronco group (57) has suggested that Tregs may play an important role, including as predictors of a patient's response to rituximab. Studies of several autoimmune diseases have shown that antigen-specific protective immunity can be induced by antigen-derived peptides coupled to the relevant MHC class II molecules through the generation of Tregs and induction of B regulatory cells (151). Another approach is to target antigen-specific B cells. Building on the success of chimeric antigen receptor T cells (CAR-T) used to treat B cell lymphomas and leukemias, chimeric autoantigen receptor expressing T cells (CAAR-T cells) have been advocated for use in autoimmune diseases, with the aim of specifically eliminating the B cells that express the B cell receptor for PLA_2R or THSD7A (152). In MN, significant advances have been achieved in identifying B cell epitopes and HLA-D class II alleles. However, we need to expand our knowledge of B cell microepitopes and epitope spreading and, even more importantly, of T cell epitopes, all of which are mandatory steps for the development of epitope-targeted therapy.

10. CONCLUSIONS

During the past two decades, research on MN has been a success story, both for investigators and for patients. It has created great opportunities for the development of more specific therapies that could benefit patients with MN as well as those with other organ-specific autoimmune diseases. Looking back at the pathophysiological advances that have been achieved, MN can be considered as a paradigm of translational research and precision medicine.

FUTURE ISSUES

1. What are the physiological functions of PLA_2R and THSD7A, and what is the pathogenic role of antibodies?
2. Can additional loci be identified by genome-wide association studies in patients with PLA_2R-related MN? Is it possible to establish a genetic risk score in patients with PLA_2R-related and -unrelated MN?
3. What is the role of EXT1 and EXT2: Are they biomarkers or antigens? Is it possible to identify other antigens in triple-negative patients?
4. What are the real microepitopes in PLA_2R and THSD7A? Is it possible to identify PLA_2R- and THSD7A-specific T cell epitopes? Should epitope spreading be considered when treating patients rather than antibody titers?
5. How, and in which organ, is self-tolerance lost? How does environmental pollution work in MN? Is there a role for antigen mimicry?
6. Is IgG4 pathogenic? Is it protective? Can anti-PLA_2R IgG4 activate the lectin pathway?
7. What is the role of complement? How is complement activated in primary MN? What are the prevalence of and the role of antibodies against complement regulatory proteins (CRPs)? Is there a role for functional polymorphism in CRP genes?
8. What is the podocyte response to injury? What are the mechanisms of glomerular basement membrane disorganization and repair?

NOTE ADDED IN PROOF

PLA_2R and THSD7A are target antigens in about, respectively, 70% and 1–5% of primary cases of MN. In the remaining cases, the target antigen is unknown. In a recent paper, the Sethi and Ronco groups (154) identified a neural tissue-encoding protein with epidermal growth factor (EGF)-like repeats, NELL-1, using laser microdissection of glomeruli followed by mass spectrometry. In a discovery cohort of 126 PLA_2R-negative cases recruited at the Mayo Clinic (Rochester, Minnesota), 29 were positive for NELL-1 by mass spectrometry and tissue IHC. Five additional NELL-1-positive cases were identified out of 84 PLA_2R- and THSD7A-negative cases. Samples from patients with PLA_2R-associated MN and controls stained negative for NELL-1. By confocal microscopy, both IgG and NELL-1 colocalized to the GBM. Western blot analysis showed reactivity to NELL-1 in five serum samples, but no reactivity in control serum samples. Clinical and biopsy findings of patients with NELL-1-positive MN showed features of primary membranous nephropathy, although four of the five European cases were associated with a cancer. Hence, NELL-1 defines a distinct type of primary MN.

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LITERATURE CITED

1. Simon P, Ramée MP, Autuly V, Laruelle E, Charasse C, et al. 1994. Epidemiology of primary glomerular diseases in a French region: variations according to period and age. *Kidney Int*. 46:1192–98
2. Maisonneuve P, Agodoa L, Gellert R, Stewart JH, Buccianti G, et al. 2000. Distribution of primary renal diseases leading to end-stage renal failure in the United States, Europe, and Australia/New Zealand: results from an international comparative study. *Am. J. Kidney Dis.* 35:157–65
3. Imai H, Hamai K, Komatsuda A, Ohtani H, Miura AB. 1997. IgG subclasses in patients with membranoproliferative glomerulonephritis, membranous nephropathy, and lupus nephritis. *Kidney Int*. 51:270–76
4. Kuroki A, Shibata T, Honda H, Totsuka D, Kobayashi K, Sugisaki T. 2002. Glomerular and serum IgG subclasses in diffuse proliferative lupus nephritis, membranous lupus nephritis, and idiopathic membranous nephropathy. *Intern. Med.* 41:936–42
5. Ohtani H, Wakui H, Komatsuda A, Okuyama S, Masai R, et al. 2004. Distribution of glomerular IgG subclass deposits in malignancy-associated membranous nephropathy. *Nephrol. Dial. Transplant*. 19:574–79
6. Segawa Y, Hisano S, Matsushita M, Fujita T, Hirose S, et al. 2010. IgG subclasses and complement pathway in segmental and global membranous nephropathy. *Pediatr. Nephrol.* 25:1091–99
7. Polanco N, Gutiérrez E, Rivera F, Castellanos I, Baltar J, et al. 2012. Spontaneous remission of nephrotic syndrome in membranous nephropathy with chronic renal impairment. *Nephrol. Dial. Transplant*. 27:231–34
8. Glassock RJ. 2003. Diagnosis and natural course of membranous nephropathy. *Semin. Nephrol.* 23:324–32
9. Grupper A, Cornell LD, Fervenza FC, Beck LH Jr., Lorenz E, Cosio FG. 2016. Recurrent membranous nephropathy after kidney transplantation: treatment and long-term implications. *Transplantation* 100:2710–16
10. Hofstra JM, Fervenza FC, Wetzels JF. 2013. Treatment of idiopathic membranous nephropathy. *Nat. Rev. Nephrol.* 9:443–58
11. Cattran D, Brenchley P. 2017. Membranous nephropathy: thinking through the therapeutic options. *Nephrol. Dial. Transplant.* 32(Suppl. 1):i22–29
12. Heymann W, Hackel DB, Harwood S, Wilson SG, Hunter JL. 1959. Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspensions. *Proc. Soc. Exp. Biol. Med.* 100:660–64
13. Van Damme BJ, Fleuren GJ, Bakker WW, Vernier RL, Hoedemaeker PJ. 1978. Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens. V. Fixed glomerular antigens in the pathogenesis of heterologous immune complex glomerulonephritis. *Lab. Investig.* 38:502–10
14. Couser WG, Steinmuller DR, Stilmant MM, Salant DJ, Lowenstein LM. 1978. Experimental glomerulonephritis in the isolated perfused rat kidney. *J. Clin. Investig.* 62:1275–87
15. Border WA, Ward HJ, Kamil ES, Cohen AH. 1982. Induction of membranous nephropathy in rabbits by administration of an exogenous cationic antigen. *J. Clin. Investig.* 69:451–61
16. Adler SG, Wang H, Ward HJ, Cohen AH, Border WA. 1983. Electrical charge. Its role in the pathogenesis and prevention of experimental membranous nephropathy in the rabbit. *J. Clin. Investig.* 71:487–99
17. Kerjaschki D, Farquhar MG. 1982. The pathogenic antigen of Heymann nephritis is a membrane glycoprotein of the renal proximal tubule brush border. *PNAS* 79:5557–61
18. Kerjaschki D, Farquhar MG. 1983. Immunocytochemical localization of the Heymann nephritis antigen (GP330) in glomerular epithelial cells of normal Lewis rats. *J. Exp. Med.* 157:667–86

19. Shah P, Tramontano A, Makker SP. 2007. Intramolecular epitope spreading in Heymann nephritis. *J. Am. Soc. Nephrol.* 18:3060–66
20. Prabakaran T, Nielsen R, Larsen JV, Sørensen SS, Feldt-Rasmussen U, et al. 2011. Receptor-mediated endocytosis of α-galactosidase A in human podocytes in Fabry disease. *PLOS ONE* 6:e25065
21. Larsen CP, Trivin-Avillach C, Coles P, Collins AB, Merchant M, et al. 2018. LDL receptor-related protein 2 (megalin) as a target antigen in human kidney anti-brush border antibody disease. *J. Am. Soc. Nephrol.* 29:644–53
22. Wright NG, Mohammed NA, Eckersall PD, Nash AS. 1985. Experimental immune complex glomerulonephritis in dogs receiving cationized bovine serum albumin. *Res. Vet. Sci.* 38:322–28
23. Koyama A, Inage H, Kobayashi M, Ohta Y, Narita M, et al. 1986. Role of antigenic charge and antibody avidity on the glomerular immune complex localization in serum sickness of mice. *Clin. Exp. Immunol.* 64:606–14
24. Kobayashi M, Muro K, Yoh K, Kondoh M, Iwabuchi S, et al. 1998. Effects of FK506 on experimental membranous glomerulonephritis induced by cationized bovine serum albumin in rats. *Nephrol. Dial. Transplant.* 13:2501–8
25. Koyama A, Inage H, Kobayashi M, Nakamura H, Narita M, Tojo S. 1986. Effect of chemical cationization of antigen on glomerular localization of immune complexes in active models of serum sickness nephritis in rabbits. *Immunology* 58:529–34
26. Debiec H, Guigonis V, Mougenot B, Decobert F, Haymann JP, et al. 2002. Antenatal membranous glomerulonephritis due to anti–neutral endopeptidase antibodies. *N. Engl. J. Med.* 346:2053–60
27. Platt JL, Tucker WL, Michael AF. 1983. Stages of renal ontogenesis identified by monoclonal antibodies reactive with lymphohematopoietic differentiation antigens. *J. Exp. Med.* 157:155–72
28. Debiec H, Nauta J, Coulet F, van der Burg M, Guigonis V, et al. 2004. Role of truncating mutations in *MME* gene in fetomaternal alloimmunisation and neonatal glomerulopathies. *Lancet* 364:1252–59
29. Vivarelli M, Emma F, Pellé T, Gerken C, Pedicelli S, et al. 2015. Genetic homogeneity but IgG subclass–dependent clinical variability of alloimmune membranous nephropathy with anti-neutral endopeptidase antibodies. *Kidney Int.* 87:602–9
30. Ronco P, Debiec H, Guigonis V. 2006. Mechanisms of disease: alloimmunization in renal diseases. *Nat. Clin. Pract. Nephrol.* 2:388–97
31. Beck LH. 2015. Lessons from a rare disease: IgG subclass and disease severity in alloimmune antenatal membranous nephropathy. *Kidney Int.* 87:494–97
32. Nortier JL, Debiec H, Tournay Y, Mougenot B, Nöel JC, et al. 2006. Neonatal disease in neutral endopeptidase alloimmunization: lessons for immunological monitoring. *Pediatr. Nephrol.* 1:1399–405
33. Beck LH Jr., Bonegio RG, Lambeau G, Beck DM, Powell DW, et al. 2009. M-type phospholipase A_2 receptor as target antigen in idiopathic MN. *N. Engl. J. Med.* 361:11–21
34. Lambeau G, Ancian P, Barhanin J, Lazdunski M. 1994. Cloning and expression of a membrane receptor for secretory phospholipases A_2. *J. Biol. Chem.* 269:1575–78
35. East L, Isacke CM. 2002. The mannose receptor family. *Biochim. Biophys. Acta Gen. Subj.* 1572:364–86
36. West AP Jr., Herr AB, Bjorkman PJ. 2004. The chicken yolk sac IgY receptor, a functional equivalent of the mammalian MHC-related Fc receptor, is a phospholipase A_2 receptor homolog. *Immunity* 20:601–10
37. Zvaritch E, Lambeau G, Lazdunski M. 1996. Endocytic properties of the M-type 180-kDa receptor for secretory phospholipases A_2. *J. Biol. Chem.* 271:250–57
38. Dong Y, Cao L, Tang H, Shi X, He Y. 2017. Structure of human M-type phospholipase A_2 receptor revealed by cryo-electron microscopy. *J. Mol. Biol.* 429:3825–35
39. Fresquet M, Jowitt TA, Gummadova J, Collins R, O'Cualain R, et al. 2015. Identification of a major epitope recognized by PLA2R autoantibodies in primary membranous nephropathy. *J. Am. Soc. Nephrol.* 26:302–13
40. Kao L, Lam V, Waldman M, Glassock RJ, Zhu Q. 2015. Identification of the immunodominant epitope region in phospholipase A_2 receptor–mediating autoantibody binding in idiopathic membranous nephropathy. *J. Am. Soc. Nephrol.* 26:291–301
41. Seitz-Polski B, Dolla G, Payré C, Girard CA, Polidori J, et al. 2016. Epitope spreading of autoantibody response to PLA2R associates with poor prognosis in membranous nephropathy. *J. Am. Soc. Nephrol.* 27:1517–33

42. Ancian P, Lambeau G, Mattéi MG, Lazdunski M. 1995. The human 180-kDa receptor for secretory phospholipases A_2: molecular cloning, identification of a secreted soluble form, expression, and chromosomal localization. *J. Biol. Chem.* 270:8963–70
43. Watanabe K, Watanabe K, Watanabe Y, Fujioka D, Nakamura T, et al. 2018. Human soluble phospholipase A_2 receptor is an inhibitor of the integrin-mediated cell migratory response to collagen-I. *Am. J. Physiol. Cell Physiol.* 315:C398–408
44. Behnert A, Fritzler MJ, Teng B, Zhang M, Bollig F, et al. 2013. An anti-phospholipase A_2 receptor quantitative immunoassay and epitope analysis in membranous nephropathy reveals different antigenic domains of the receptor. *PLOS ONE* 8:e61669
45. Debiec H, Ronco P. 2016. Immune response against autoantigen PLA2R is not gambling: implications for pathophysiology, prognosis, and therapy. *J. Am. Soc. Nephrol.* 27:1275–77
46. Seitz-Polski B, Debiec H, Rousseau A, Dahan K, Zaghrini C, et al. 2018. Phospholipase A_2 receptor 1 epitope spreading at baseline predicts reduced likelihood of remission of membranous nephropathy. *J. Am. Soc. Nephrol.* 29:401–8
47. Stahl R, Hoxha E, Fechner K. 2010. PLA2R autoantibodies and recurrent membranous nephropathy after transplantation. *N. Engl. J. Med.* 363:496–98
48. Blosser CD, Ayalon R, Nair R, Thomas C, Beck LH Jr. 2012. Very early recurrence of anti-phospholipase A_2 receptor–positive membranous nephropathy after transplantation. *Am. J. Transplant.* 12:1637–42
49. Debiec H, Martin L, Jouanneau C, Dautin G, Mesnard L, et al. 2011. Autoantibodies specific for the phospholipase A_2 receptor in recurrent and de novo membranous nephropathy. *Am. J. Transplant.* 11:2144–52
50. Debiec H, Hanoy M, Francois A, Guerrot D, Ferlicot S, et al. 2012. Recurrent membranous nephropathy in an allograft caused by IgG3κ targeting the PLA2 receptor. *J. Am. Soc. Nephrol.* 23:1949–54
51. Xu X, Wang G, Chen N, Lu T, Nie S, et al. 2016. Long-term exposure to air pollution and increased risk of membranous nephropathy in China. *J. Am. Soc. Nephrol.* 27:3739–46
52. Zhang XD, Cui Z, Zhao MH. 2018. The genetic and environmental factors of primary membranous nephropathy: an overview from China. *Kidney Dis.* 4:65–73
53. Salant DJ. 2013. Genetic variants in membranous nephropathy: perhaps a perfect storm rather than a straightforward conformeropathy? *J. Am. Soc. Nephrol.* 24:525–28
54. Guerry MJ, Vanhille P, Ronco P, Debiec H. 2016. Serum anti-PLA2R antibodies may be present before clinical manifestations of membranous nephropathy. *Kidney Int.* 89:1399
55. Yu NY, Hallström BM, Fagerberg L, Ponten F, Kawaji H, et al. 2015. Complementing tissue characterization by integrating transcriptome profiling from the Human Protein Atlas and from the FANTOM5 consortium. *Nucleic Acids Res.* 43:6787–98
56. Ronco P, Debiec H. 2015. Pathophysiological advances in membranous nephropathy: time for a shift in patient's care. *Lancet* 385:1983–92
57. Rosenzwajg M, Languille E, Debiec H, Hygino J, Dahan K, et al. 2017. B- and T-cell subpopulations in patients with severe idiopathic membranous nephropathy may predict an early response to rituximab. *Kidney Int.* 92:227–37
58. Klouda PT, Manos J, Acheson EJ, Dyer PA, Goldby FS, et al. 1979. Strong association between idiopathic membranous nephropathy and HLA-DRW3. *Lancet* 2:770–71
59. Vaughan RW, Demaine AG, Welsh KI. 1989. A *DQA1* allele is strongly associated with idiopathic membranous nephropathy. *Tissue Antigens* 34:261–69
60. Stanescu HC, Arcos-Burgos M, Medlar A, Bockenhauer D, Kottgen A, et al. 2011. Risk *HLA-DQA1* and *PLA_2R1* alleles in idiopathic membranous nephropathy. *N. Engl. J. Med.* 364:616–26
61. Sekula P, Li Y, Stanescu HC, Wuttke M, Ekici AB, et al. 2017. Genetic risk variants for membranous nephropathy: extension of and association with other chronic kidney disease aetiologies. *Nephrol. Dial. Transplant.* 32:325–32
62. Ramachandran R, Kumar V, Kumar A, Yadav AK, Nada R, et al. 2016. PLA_2R antibodies, glomerular PLA_2R deposits and variations in *PLA2R1* and *HLA-DQA1* genes in primary membranous nephropathy in South Asians. *Nephrol. Dial. Transplant.* 31:1486–93

63. Coenen MJ, Hofstra JM, Debiec H, Stanescu HC, Medlar AJ, et al. 2013. Phospholipase A_2 receptor (*PLA2R1*) sequence variants in idiopathic membranous nephropathy. *J. Am. Soc. Nephrol.* 24:677–83
64. Kim S Chin HJ, Na KY, Kim S, Oh J, et al. 2011. Single nucleotide polymorphisms in the phospholipase A_2 receptor gene are associated with genetic susceptibility to idiopathic membranous nephropathy. *Nephron Clin. Pract.* 117:c253–58
65. Liu YH, Wan L, Chang CT, Liao WL, Chen W, et al. 2011. Association between copy number variation of complement component C4 and Graves' disease. *J. Biomed. Sci.* 18:71
66. Latt KZ, Honda K, Thiri M, Hitomi Y, Omae Y, et al. 2018. Identification of a two-SNP *PLA2R1* haplotype and *HLA-DRB1* alleles as primary risk associations in idiopathic membranous nephropathy. *Sci. Rep.* 8:15576
67. Lv J, Hou W, Zhou X, Liu G, Zhou F, et al. 2013. Interaction between *PLA2R1* and *HLA-DQA1* variants associates with anti-PLA2R antibodies and membranous nephropathy. *J. Am. Soc. Nephrol.* 24:1323–29
68. Saeed M, Beggs ML, Walker PD, Larsen CP. 2014. PLA2R-associated membranous glomerulopathy is modulated by common variants in *PLA2R1* and *HLA-DQA1* genes. *Genes Immun.* 15:556–61
69. Bullich G, Ballarín J, Oliver A, Ayasreh N, Silva I, et al. 2014. *HLA-DQA1* and *PLA2R1* polymorphisms and risk of idiopathic membranous nephropathy. *Clin. J. Am. Soc. Nephrol.* 9:335–43
70. Kanigicherla D, Gummadova J, McKenzie EA, Roberts SA, Harris S, et al. 2013. Anti-PLA2R antibodies measured by ELISA predict long-term outcome in a prevalent population of patients with idiopathic membranous nephropathy. *Kidney Int.* 83:940–48
71. Mladkova N, Kiryluk K. 2017. Genetic complexities of the HLA region and idiopathic membranous nephropathy. *J. Am. Soc. Nephrol.* 28:1331–34
72. Cui Z, Xie LJ, Chen FJ, Pei ZY, Zhang LJ, et al. 2017. MHC class II risk alleles and amino acid residues in idiopathic membranous nephropathy. *J. Am. Soc. Nephrol.* 28:1651–64
73. Le WB, Shi JS, Zhang T, Liu L, Qin HZ, et al. 2017. *HLA-DRB1*15:01* and *HLA-DRB3*02:02* in PLA2R-related membranous nephropathy. *J. Am. Soc. Nephrol.* 28:1642–50
74. Thiri M, Honda K, Kashiwase K, Mabuchi A, Suzuki H, et al. 2016. High-density association mapping and interaction analysis of *PLA2R1* and *HLA* regions with idiopathic membranous nephropathy in Japanese. *Sci. Rep.* 6:38189
75. Wang HY, Cui Z, Xie LJ, Zhang LJ, Pei ZY, et al. 2018. HLA class II alleles differing by a single amino acid associate with clinical phenotype and outcome in patients with primary membranous nephropathy. *Kidney Int.* 94:974–82
76. Gillies CE, Putler R, Menon R, Otto E, Yasutake K, et al. 2018. An eQTL landscape of kidney tissue in human nephrotic syndrome. *Am. J. Hum. Genet.* 103:232–44
77. Beck LH Jr. 2010. Membranous nephropathy and malignancy. *Semin. Nephrol.* 30:635–44
78. Tomas NM, Beck LH Jr., Meyer-Schwesinger C, Seitz-Polski B, Ma H, et al. 2014. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. *N. Engl. J. Med.* 371:2277–87
79. Hoxha E, Beck LH Jr., Wiech T, Tomas NM, Probst C, et al. 2017. An indirect immunofluorescence method facilitates detection of thrombospondin type 1 domain-containing 7A-specific antibodies in membranous nephropathy. *J. Am. Soc. Nephrol.* 28:520–31
80. Zaghrini C, Seitz-Polski B, Justino J, Dolla G, Payre C, et al. 2019. Novel ELISA for thrombospondin type 1 domain–containing 7A autoantibodies in membranous nephropathy. *Kidney Int.* 95:666–79
81. Wang CH, Su PT, Du XY, Kuo MW, Lin CY, et al. 2010. Thrombospondin type I domain containing 7A (THSD7A) mediates endothelial cell migration and tube formation. *J. Cell. Physiol.* 222:685–94
82. Kuo MW, Wang CH, Wu HC, Chang SJ, Chuang YJ. 2011. Soluble THSD7A is an N-glycoprotein that promotes endothelial cell migration and tube formation in angiogenesis. *PLOS ONE* 6:e29000
83. Wang CH, Chen IH, Kuo MW, Su PT, Lai ZY, et al. 2011. Zebrafish Thsd7a is a neural protein required for angiogenic patterning during development. *Dev. Dyn.* 240:1412–21
84. Tomas NM, Hoxha E, Reinicke AT, Fester L, Helmchen U, et al. 2016. Autoantibodies against thrombospondin type 1 domain–containing 7A induce membranous nephropathy. *J. Clin. Investig.* 126:2519–32
85. Tomas NM, Meyer-Schwesinger C, von Spiegel H, Kotb AM, Zahner G, et al. 2017. A heterologous model of thrombospondin type 1 domain-containing 7A-associated membranous nephropathy. *J. Am. Soc. Nephrol.* 28:3262–77

86. Stoddard SV, Welsh CL, Palopoli MM, Stoddard SD, Aramandla MP, et al. 2019. Structure and function insights garnered from in silico modeling of the thrombospondin type-1 domain–containing 7A antigen. *Proteins* 87:136–45
87. Seifert L, Hoxha E, Eichhoff AM, Zahner G, Dehde S, et al. 2018. The most N-terminal region of THSD7A is the predominant target for autoimmunity in THSD7A-associated membranous nephropathy. *J. Am. Soc. Nephrol.* 29:1536–48
88. Hoxha E, Wiech T, Stahl PR, Zahner G, Tomas NM, et al. 2016. A mechanism for cancer-associated membranous nephropathy. *N. Engl. J. Med.* 374:1995–96
89. Lin F, Zhang D, Chang J, Tang X, Guan W, et al. 2018. THSD7A-associated membranous nephropathy in a patient with neurofibromatosis type 1. *Eur. J. Med. Genet.* 61:84–88
90. Zhang Z, Gong T, Rennke HG, Hayashi R. 2019. Duodenal schwannoma as a rare association with membranous nephropathy: a case report. *Am. J. Kidney Dis.* 73:278–80
91. Wang J, Cui Z, Lu J, Probst C, Zhang YM, et al. 2017. Circulating antibodies against thrombospondin type-I domain-containing 7A in Chinese patients with idiopathic membranous nephropathy. *Clin. J. Am. Soc. Nephrol.* 12:1642–51
92. Larsen CP, Cossey LN, Beck LH. 2016. THSD7A staining of membranous glomerulopathy in clinical practice reveals cases with dual autoantibody positivity. *Mod. Pathol.* 29:421–26
93. Timmermans SA, Ayalon R, van Paassen P, Beck LH Jr., van Rie H, et al. 2013. Anti-phospholipase A_2 receptor antibodies and malignancy in membranous nephropathy. *Am. J. Kidney Dis.* 62:1223–25
94. Sethi S, Madden B, Debiec H, Charlesworth MC, Gross L, et al. 2019. Exostosin1/exostosin2-associated membranous nephropathy. *J. Am. Soc. Nephrol.* 30:1123–36
95. Busse-Wicher M, Wicher KB, Kusche-Gullberg M. 2014. The exostosin family: proteins with many functions. *Matrix Biol.* 35:25–33
96. Ahn J, Lüdecke HJ, Lindow S, Horton WA, Lee B, et al. 1995. Cloning of the putative tumour suppressor gene for hereditary multiple exostoses (*EXT1*). *Nat. Genet.* 11:137–43
97. Busse M, Kusche-Gullberg M. 2003. In vitro polymerization of heparan sulfate backbone by the EXT proteins. *J. Biol. Chem.* 278:41333–37
98. Busse M, Feta A, Presto J, Wilén M, Grønning M, et al. 2007. Contribution of EXT1, EXT2, and EXTL3 to heparan sulfate chain elongation. *J. Biol. Chem.* 282:32802–10
99. Cook A, Raskind W, Blanton SH, Pauli RM, Gregg RG, et al. 1993. Genetic heterogeneity in families with hereditary multiple exostoses. *Am. J. Hum. Genet.* 53:71–79
100. Ishibashi R, Takemoto M, Tsurutani Y, Kuroda M, Ogawa M, et al. 2018. Immune-mediated acquired lecithin–cholesterol acyltransferase deficiency: a case report and literature review. *J. Clin. Lipidol.* 12:888–97
101. Takahashi S, Hiromura K, Tsukida M, Ohishi Y, Hamatani H, et al. 2013. Nephrotic syndrome caused by immune-mediated acquired LCAT deficiency. *J. Am. Soc. Nephrol.* 24:1305–12
102. Prunotto M, Carnevali ML, Candiano G, Murtas C, Bruschi M, et al. 2010. Autoimmunity in membranous nephropathy targets aldose reductase and SOD2. *J. Am. Soc. Nephrol.* 21:507–19
103. Bruschi M, Carnevali ML, Murtas C, Candiano G, Petretto A, et al. 2011. Direct characterization of target podocyte antigens and auto-antibodies in human membranous glomerulonephritis: α-enolase and borderline antigens. *J. Proteom.* 74:2008–17
104. Kimura Y, Miura N, Debiec H, Morita H, Yamada H, et al. 2017. Circulating antibodies to α-enolase and phospholipase A_2 receptor and composition of glomerular deposits in Japanese patients with primary or secondary membranous nephropathy. *Clin. Exp. Nephrol.* 21:117–26
105. Debiec H, Lefeu F, Kemper MJ, Niaudet P, Deschênes G, et al. 2011. Early-childhood membranous nephropathy due to cationic bovine serum albumin. *N. Engl. J. Med.* 364:2101–10
106. Sathe SK, Teuber SS, Roux KH. 2005. Effects of food processing on the stability of food allergens. *Biotechnol. Adv.* 23:423–29
107. Sanchez C, Fremont S. 2003. Consequences of heat treatment and processing of food on the structure and allergenicity of component proteins. *Rev. Fr. Allergol. Immunol. Clin.* 43:13–20
108. Hunley TE, Corzo D, Dudek M, Kishnani P, Amalfitano A, et al. 2004. Nephrotic syndrome complicating α-glucosidase replacement therapy for Pompe disease. *Pediatrics* 114:e532–35

109. Debiec H, Valayannopoulos V, Boyer O, Nöel LH, Callard P, et al. 2013. Allo-immune membranous nephropathy and recombinant aryl sulfatase replacement therapy: a need for tolerance induction therapy. *J. Am. Soc. Nephrol.* 25:675–80
110. Jordan SC, Buckingham B, Sakai R, Olson D. 1981. Studies of immune-complex glomerulonephritis mediated by human thyroglobulin. *N. Engl. J. Med.* 304:1212–15
111. Bhimma R, Coovadia HM. 2004. Hepatitis B virus–associated nephropathy. *Am. J. Nephrol.* 24:198–211
112. Xie Q, Li Y, Xue J, Xiong Z, Wang L, et al. 2015. Renal phospholipase A_2 receptor in hepatitis B virus–associated membranous nephropathy. *Am. J. Nephrol.* 41:345–53
113. Berchtold L, Zanetta G, Dahan K, Mihout F, Peltier J, et al. 2017. Efficacy and safety of rituximab in hepatitis B virus–associated PLA2R-positive membranous nephropathy. *Kidney Int. Rep.* 3:486–91
114. Kerjaschki D, Exner M, Ullrich R, Susani M, Curtiss LK, et al. 1997. Pathogenic antibodies inhibit the binding of apolipoproteins to megalin/gp330 in passive Heymann nephritis. *J. Clin. Investig.*100:2303–9
115. Kerjaschki D, Miettinen A, Farquhar MG. 1987. Initial events in the formation of immune deposits in passive Heymann nephritis: gp330–anti-gp330 immune complexes form in epithelial coated pits and rapidly become attached to the glomerular basement membrane. *J. Exp. Med.* 166:109–28
116. Zou Z, Chung B, Nguyen T, Mentone S, Thomson B, Biemesderfer D. 2004. Linking receptor-mediated endocytosis and cell signaling: evidence for regulated intramembrane proteolysis of megalin in proximal tubule. *J. Biol. Chem.* 279:34302–10
117. Biemesderfer D. 2006. Regulated intramembrane proteolysis of megalin: linking urinary protein and gene regulation in proximal tubule? *Kidney Int.* 69:1717–21
118. McCarthy AJ, Coleman-Vaughan C, McCarthy JV. 2017. Regulated intramembrane proteolysis: emergent role in cell signalling pathways. *Biochem. Soc. Trans.* 45:1185–202
119. Higashino KI, Yokota Y, Ono T, Kamitani S, Arita H, Hanasaki K. 2002. Identification of a soluble form phospholipase A_2 receptor as a circulating endogenous inhibitor for secretory phospholipase A_2. *J. Biol. Chem.* 277:13583–88
120. Nielsen R, Christensen EI, Birn H. 2016. Megalin and cubilin in proximal tubule protein reabsorption: from experimental models to human disease. *Kidney Int.* 89:58–67
121. Turner AJ, Isaac RE, Coates D. 2001. The neprilysin (NEP) family of zinc metalloendopeptidases: genomics and function. *BioEssays* 23:261–69
122. Lambeau G, Gelb MH. 2008. Biochemistry and physiology of mammalian secreted phospholipases A_2. *Annu. Rev. Biochem.* 77:495–520
123. Augert A, Payré C, de Launoit Y, Gil J, Lambeau G, Bernard D. 2009. The M-type receptor PLA2R regulates senescence through the p53 pathway. *EMBO Rep.* 10:271–77
124. Sis B, Tasanarong A, Khoshjou F, Dadras F, Solez K, Halloran PF. 2007. Accelerated expression of senescence associated cell cycle inhibitor $p16^{INK4A}$ in kidneys with glomerular disease. *Kidney Int.* 71:218–26
125. Takahashi S, Watanabe K, Watanabe Y, Fujioka D, Nakamura T, et al. 2015. C-type lectin-like domain and fibronectin-like type II domain of phospholipase A_2 receptor 1 modulate binding and migratory responses to collagen. *FEBS Lett.* 589:829–35
126. Jürgensen HJ, Johansson K, Madsen DH, Porse A, Melander MC, et al. 2014. Complex determinants in specific members of the mannose receptor family govern collagen endocytosis. *J. Biol. Chem.* 289:7935–47
127. Škoberne A, Behnert A, Teng B, Fritzler MJ, Schiffer L, et al. 2014. Serum with phospholipase A_2 receptor autoantibodies interferes with podocyte adhesion to collagen. *Eur. J. Clin. Investig.* 44:753–65
128. Herwig J, Skuza S, Sachs W, Sachs M, Failla A, et al. 2019. Thrombospondin type 1 domain–containing 7A (THSD7A) localizes to the slit diaphragm and stabilizes membrane dynamics of fully differentiated podocytes. *J. Am. Soc. Nephrol.* 30:824–83
129. Koneczny I. 2018. A new classification system for IgG4 autoantibodies. *Front. Immunol.* 9:97
130. Rispens T, Ooievaar-De Heer P, Vermeulen E, Schuurman J, van der Neut Kolfschoten M, Aalberse RC. 2009. Human IgG4 binds to IgG4 and conformationally altered IgG1 via Fc–Fc interactions. *J. Immunol.* 182:4275–81
131. Ito T, Kitahara K, Umemura T, Ota M, Shimozuru Y, et al. 2010. A novel heterophilic antibody interaction involves IgG4. *Scand. J. Immunol.* 71:109–14

132. Cybulsky AV, Rennke HG, Feintzeig ID, Salant DJ. 1986. Complement-induced glomerular epithelial cell injury: role of the membrane attack complex in rat membranous nephropathy. *J. Clin. Investig.* 77:1096–107
133. Couser WG, Johnson RJ, Young BA, Yeh CG, Toth CA, Rudolph AR. 1995. The effects of soluble recombinant complement receptor 1 on complement-mediated experimental glomerulonephritis. *J. Am. Soc. Nephrol.* 5:1888–94
134. Kerjaschki D, Schulze M, Binder S, Kain R, Ojha PP, et al. 1989. Transcellular transport and membrane insertion of the C5b-9 membrane attack complex of complement by glomerular epithelial cells in experimental membranous nephropathy. *J. Immunol.* 143:546–52
135. Takano T, Elimam H, Cybulsky AV. 2013. Complement-mediated cellular injury. *Semin. Nephrol.* 33:586–601
136. Schiller B, He C, Salant DJ, Lim A, Alexander JJ, Quigg RJ. 1988. Inhibition of complement regulation is key to the pathogenesis of active Heymann nephritis. *J. Exp. Med.* 188:1353–58
137. Leenaerts PL, Hall BM, Van Damme BJ, Daha MR, Vanrenterghem YF. 1995. Active Heymann nephritis in complement component C6 deficient rats. *Kidney Int.* 47:1604–14
138. Spicer ST, Tran GT, Killingsworth MC, Carter N, Power DA, et al. 2007. Induction of passive Heymann nephritis in complement component 6–deficient PVG rats. *J. Immunol.* 179:172–78
139. Kon SP, Coupes B, Short CD, Solomon LR, Raftery MJ, et al. 1995. Urinary C5b-9 excretion and clinical course in idiopathic human membranous nephropathy. *Kidney Int.* 48:1953–58
140. Bally S, Debiec H, Ponard D, Dijoud F, Rendu J, et al. 2016. Phospholipase A_2 receptor–related membranous nephropathy and mannan-binding lectin deficiency. *J. Am. Soc. Nephrol.* 27:3539–44
141. Michalski M, Świerzko AS, Pągowska-Klimek I, Niemir ZI, Mazerant K, et al. 2015. Primary Ficolin-3 deficiency—Is it associated with increased susceptibility to infections? *Immunobiology* 220:711–13
142. Thiel S, Gadjeva M. 2009. Humoral pattern recognition molecules: mannan-binding lectin and ficolins. In *Target Pattern Recognition in Innate Immunity*, Vol. 63, ed. U Kishore, pp. 58–73. New York: Springer
143. Malhotra R, Wormald MR, Rudd PM, Fischer PB, Dwek RA, Sim RB. 1995. Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. *Nat. Med.* 1:237–43
144. Bond A, Alavi A, Axford JS, Bourke BE, Bruckner FE, et al. 1997. A detailed lectin analysis of IgG glycosylation, demonstrating disease specific changes in terminal galactose and *N*-acetylglucosamine. *J. Autoimmun.* 10:77–85
145. Parekh R, Isenberg D, Rook G, Roitt I, Dwek R, Rademacher T. 1989. A comparative analysis of disease-associated changes in the galactosylation of serum IgG. *J. Autoimmun.* 2:101–14
146. Ma H, Sandor DG, Beck LH Jr. 2013. The role of complement in membranous nephropathy. *Semin. Nephrol.* 33:531–42
147. Haddad G, Kistler A. 2017. An in vitro model of idiopathic membranous nephropathy reveals PLA2R- and complement-dependent pathways of podocyte injury. *J. Am. Soc. Nephrol.* 28(Suppl.):109
148. Luo W, Olaru F, Miner JH, Beck LH Jr., van der Vlag J, et al. 2018. Alternative pathway is essential for glomerular complement activation and proteinuria in a mouse model of membranous nephropathy. *Front. Immunol.* 9:1433
149. Seikrit C, Ronco P, Debiec H. 2018. Factor H auto-antibodies and membranous nephropathy. *New Engl. J. Med.* 379:2479–81
150. Bobart SA, De Vriese AS, Pawar AS, Zand L, Sethi S, et al. 2019. Noninvasive diagnosis of primary membranous nephropathy using phospholipase A_2 receptor antibodies. *Kidney Int.* 95:429–38
151. Wraith D. 2016. Autoimmunity: antigen-specific immunotherapy. *Nature* 530:422–23
152. Ellebrecht CT, Bhoj VG, Nace A, Choi EJ, Mao X, et al. 2016. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. *Science* 353:179–84
153. Ronco P, Debiec H. 2017. A podocyte view of membranous nephropathy: from Heymann nephritis to the childhood human disease. *Plugers Arch.* 469:997–1005
154. Sethi S, Debiec H, Madden B, Charlesworth MC, Morelle J, et al. 2019. Neural epidermal growth factor-like 1 protein (NELL-1) associated membranous nephropathy. *Kidney Int.* In press. **https://doi.org/10.1016/j.kint.2019.09.014**