

# Complement Pathways in Membranous Nephropathy: Complex and Multifactorial



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**M**embranous nephropathy (MN) is a rare, but severe autoimmune kidney disease and a major cause of nephrotic syndrome in adults. Clinically defined by nephrotic syndrome, which is persistent in approximately two-thirds of untreated patients, it manifests histologically by subepithelial immune deposits at the glomerular basement membrane in the kidney containing IgGs.

MN can be idiopathic (or primary, iMN) without any identified cause (70%–80% of cases), or secondary (sMN) to clinical disorders such as hepatitis B, systematic lupus erythematosus, cancer, or drug side effects. Etiology of iMN has remained elusive for decades, until recently when several groups discovered different podocyte antigens, such as a neutral endopeptidase in rare cases in the neonate in 2002, M-type phospholipase A2 receptor (PLA2R1) in 70% of patients with iMN in 2009, thrombospondin type-1 domain-

containing 7A in 2% to 5% of patients with iMN in 2014, exostosin 1 and exostosin 2 (EXT1 and EXT2, respectively) in 30% to 40% of patients with sMN in 2019, and NELL-1 in 2% to 7% of patients with iMN in 2020.<sup>1</sup>

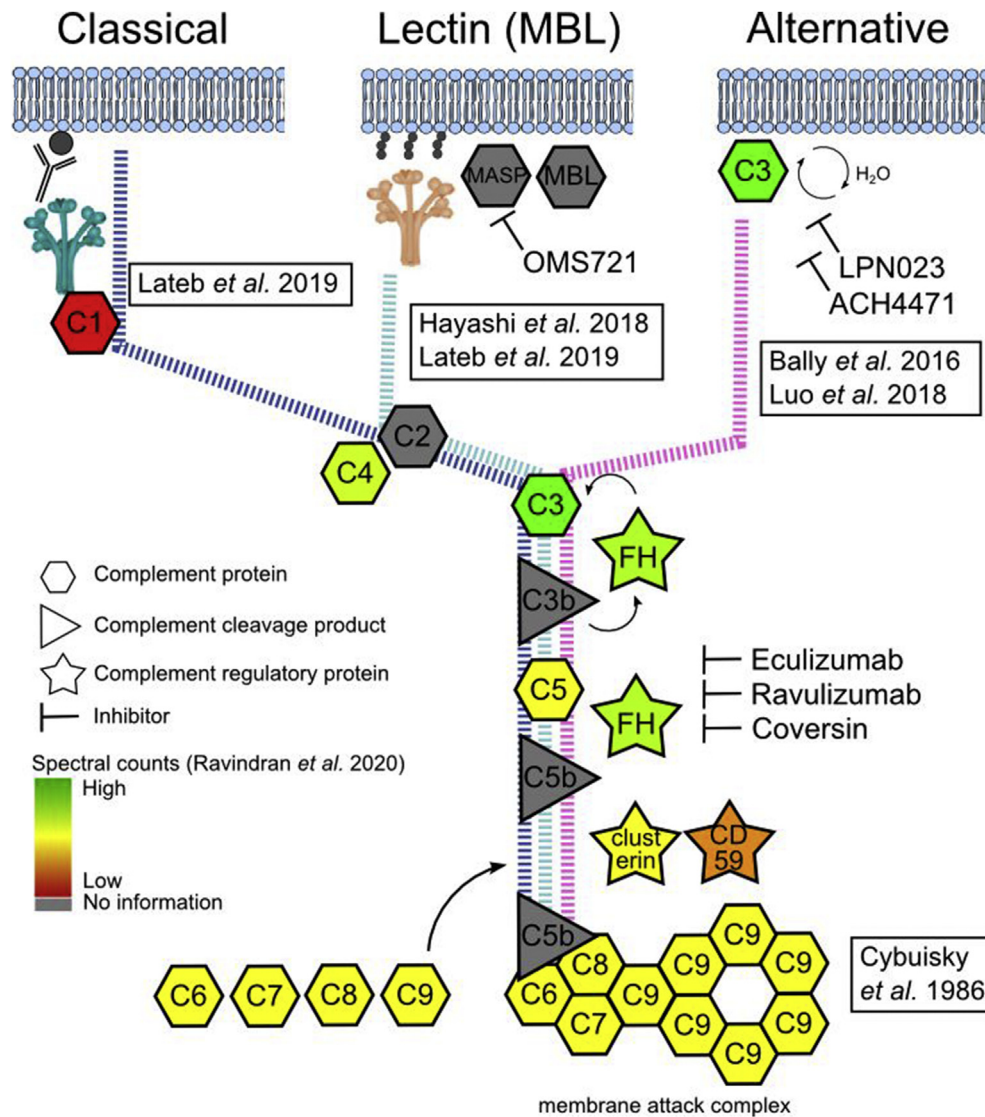
Since the discovery of antibodies against these antigens, much has been learned about their prognostic value, and several genome-wide association studies have discovered disease-related variants associated with a higher risk of MN; however, the pathogenicity of MN remains largely unexplored.

The complement system plays an important role in a variety of autoimmune diseases, including MN. A number of proteins are involved and sequentially activated following an immune trigger, finally leading to the formation of the membrane attack complex C5b-9. Three major complement pathways have been described: classical and lectin pathways are usually activated following an immune trigger, whereas the alternative pathway is continuously activated at low level (Figure 1).<sup>2–8</sup>

Proteinuria in patients with MN is probably a result of podocyte injury and complement activation, notably because of the destructive action of C5b-9 at the glomerular basement membrane, as has been nicely demonstrated in Heymann nephritis, a rat model of MN.<sup>2</sup> The pathogenic nature of the antibodies in MN has recently been demonstrated by Tomas *et al.*,<sup>3</sup> who showed that injection with human anti-thrombospondin type-1 domain-containing 7A antibodies forms glomerular deposits and induces proteinuria in mice, but the complement pathways involved have not been studied.

As the complement pathways in MN have remained unexplored, so has the pathogenicity of different subclasses of IgG antibodies. In most iMN cases, IgG4 is the predominant subclass, whereas IgG1 and IgG3 are present in a minority of patients. As IgG4 cannot bind C1q, a major precursor in the classical pathway, it is believed that IgG4 cannot activate the complement and therefore cannot be pathogenic. Accordingly, most patients with iMN present very weak C1q deposits. These observations suggest that the alternative and/or the lectin pathways might be involved in complement activation in iMN. Bally *et al.*<sup>4</sup> reported that iMN can develop in patients with complete mannose-binding lectin deficiency with complement mostly activated by the alternative pathway. In line with this theory, mice without a functional alternative pathway did not exhibit glomerular deposition of C5b-9 and did not develop albuminuria, arguing that the alternative and not the classical or the lectin pathway may be crucial for the onset of proteinuria.<sup>5</sup> However, only a genetic approach was used in this study, and the

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**Figure 1.** Complement system in patients with membranous nephropathy. Classical and lectin pathways are activated following an immune trigger, whereas the alternative pathway is constitutively activated at low level. Classical, lectin, and alternative pathways converge on C3, eventually forming a membrane attack complex. Studies arguing for the predominance of each pathway are cited in rectangles.<sup>2-6,8</sup> Spectral counts from Ravindran *et al.*<sup>7</sup> are presented as a heat map for each protein studied. Inhibitors available or in clinical trials targeting different complement proteins and complement-regulating proteins are listed next to their targets. MASP, mannan-binding lectin-associated serine protease; MBL, mannose-binding lectin; FH, complement factor H. Adapted with permission from and courtesy of The Binding Site Group Ltd.

planted antigen method used to induce proteinuria is more similar to sMN than to iMN.

Two recent articles have tried to resolve this dilemma. The first, by Lateb *et al.*,<sup>6</sup> demonstrated that at least *in vitro* anti-PLA2R1 antibodies, but not antibodies from a healthy donor, are cytotoxic for HEK293 cells overexpressing PLA2R1 antigen in the presence of complement. In addition, the inhibition of classical and lectin pathways highly decreased, but did not completely abolish the

anti-PLA2R1 antibody-mediated cytotoxicity, suggesting that the classical and lectin pathways may nevertheless be the predominant pathways in patients with iMN with anti-PLA2R1 antibodies, thus contradicting the mice study.<sup>5</sup> Sera containing only the IgG4 subclass and/or with a lower anti-PLA2R1 titer were less cytotoxic than the sera containing at least one additional IgG subclass and/or a higher anti-PLA2R1 titer, thereby indirectly confirming the hypothesis that the subclass of IgG antibodies

as well as their quantity may define which complement pathway is activated and to what extent.<sup>6</sup> In iMN, multiple IgG subclasses are detected at disease onset, whereas the IgG4 subclass becomes predominant during the course of the disease, suggesting an IgG subclass switching, as already reported in other IgG4-mediated autoimmune diseases. This switch might explain the heterogeneity of complement activation profiles in patients with iMN at onset and during follow-up.

The newest article, by Ravindran *et al.*,<sup>7</sup> presented in this issue, develops a novel and unique approach to study complement proteins in the biopsies of subjects with MN using mass spectrometry following laser-capture microdissection. Indeed, the number of proteins studied (complement proteins, complement-regulating proteins, and IgGs) and the quantitative data obtained are a major strength of this study, allowing for a careful dissection of complement pathways activated *in situ*. The groups studied include control cases (donor kidney biopsy before transplantation), patients with PLA2R1-associated iMN, and patients with EXT1/EXT2-associated sMN. As expected, PLA2R1-associated iMN presents mostly with IgG4, whereas in the secondary forms of MN, IgG1 is predominant. Despite these clear differences in IgG subclasses, the pathways activated did not seem to clearly differ between PLA2R1- and EXT1/EXT2-associated MN, suggesting that both alternative and classical pathways are activated in patients with MN (mostly detection of C3, C4, and complement-regulating proteins of the alternative pathway, whereas C1 seems to be predominant in patients with EXT1/EXT2-positive sMN). It would be interesting to study the presence of complement proteins depending on the predominant IgG subclass for each individual patient, which could be the reason why there is no difference on the group level, whereas there could be a marked difference on the individual level. No protein from the lectin pathway was analyzed, even though immunostaining of mannose-binding lectin has already been described.<sup>8</sup> The alternative pathway may not trigger but rather maintain

complement activation (high detection of complement-regulating proteins of the alternative pathway) (Figure 1), and this requires further investigation. Although the number of patients per group is small and mass spectrometry may have its limitations, especially because the spectral counts were not confirmed by another method (immunostaining or similar), to this date this is nevertheless the most comprehensive analysis of complement proteins, and further studies both *in vitro* and *in vivo* are needed to corroborate the results presented and to clarify which pathway is activated at what time in the natural history of MN, following what stimuli and whether the pathway(s) activated may change depending on MN etiology, antigen(s) involved, and the severity of MN.

Even though these recent studies suggest somewhat different conclusions, they may nevertheless show that the complement activation is complex and multifactorial and that the 3 pathways are likely redundant in most patients.

These articles also open a way to rethink the treatment of patients with MN, which usually consists of immunosuppressive drugs that may be ineffective in approximately 30% of cases, and the patients may relapse in another 30% of cases. For these unresponsive or relapsing patients, especially if their kidney function is rapidly deteriorating, a complement inhibitor may be a valid alternative. A personalized approach based on the individual patient's dominant complement pathway should be considered when possible, and a broad-scale inhibitor targeting a protein downstream of C3 in all other cases. At the moment, several

inhibitors of complement are being tested in clinical trials,<sup>9</sup> and their efficacy remains to be determined in comparison with the standard immunosuppressive drugs, such as rituximab.

## DISCLOSURE

All the authors declared no competing interests.

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