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Breaking the Barrier–Glomerular C5b-9 as a Prognostic Marker in Membranous Nephropathy

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Inspite of recent informative clinical trials in membranous nephropathy (MN), the optimal treatment of this disease remains a major challenge. Immunosuppressive drugs such as alkylating agents, calcineurin inhibitors, and rituximab lead to remission in more than 50% of patients.[1] A substantial number of patients do not have a lasting response, however, and adverse events with these medications are relatively common. Furthermore, even patients with nephrotic range proteinuria have greater than 30% likelihood of spontaneous remission with conservative care.[2] Given these considerations, the risks and benefits of treating MN patients with immunosuppressive drugs are not always clear. New biomarkers might help to identify those patients most likely to progress without treatment, as well as those patients likely to respond to a particular therapy.

Complement activation fragments are seen in the glomeruli of most cases of MN, indicating that this this immune cascade is activated by the deposited immune complexes. Full complement activation leads to generation of C5b-9, also referred to as the membrane attack complex. C5b-9 is a multimeric protein complex that forms pores in the outer membrane of target cells, leading to cell injury or lysis. Investigators have used animal models of MN to test whether glomerular C5b-9 is pathogenic in MN, but the results have been inconsistent. Using the Heymann nephritis rat model, for example, an early study showed that depletion of C6, which prevents the formation of C5b-9, reduced proteinuria.[3] More recently, however, Spicer *et al.*[4] demonstrated that C6-deficient rats were not protected in this model. Clinical studies have also raised questions about the pathophysiologic importance of C5b-9 in MN. Eculizumab is a therapeutic antibody that blocks the cleavage of C5, preventing the generation of C5a and C5b-9. In a randomized controlled trial in 200 patients with MN, treatment with eculizumab did not significantly reduce proteinuria. The drug may not have been adequately dosed in this study; however, the results have not been formally published. Studies have also shown that the soluble complement activation fragment C3a contributes to proteinuria in MN.[5] Therefore, even if C5b-9 formation causes podocyte injury, it may be only one of several downstream mechanisms of complement-mediated damage.

Whether or not C5b-9 is pathogenic in MN, studies have found that it can serve as a useful biomarker. Disease activity is usually defined using serum creatinine and urine protein measurements; however, these readouts are also affected by irreversible scarring of the kidney. C5b-9 is too large to filter into the urine from plasma, so its presence in urine presumably reflects ongoing complement activation within the kidney. Investigators have shown C5b-9 in the urine of patients with MN correlates with ongoing immune complex deposition and complement activation ("immunologically active" disease), and is associated with worse outcomes.[6]

In this issue of *Kidney International Reports*, Teisseyre *et al.*[7] investigate the clinical significance of glomerular C5b-9 deposits in MN. The investigators immunostained kidney biopsies from patients with MN for C4d, C5b-9, and the M-type phospholipase A2 receptor 1. Sixty-four samples were analyzed, including 45 from patients with primary MN and 19 from patients with secondary disease. They found that

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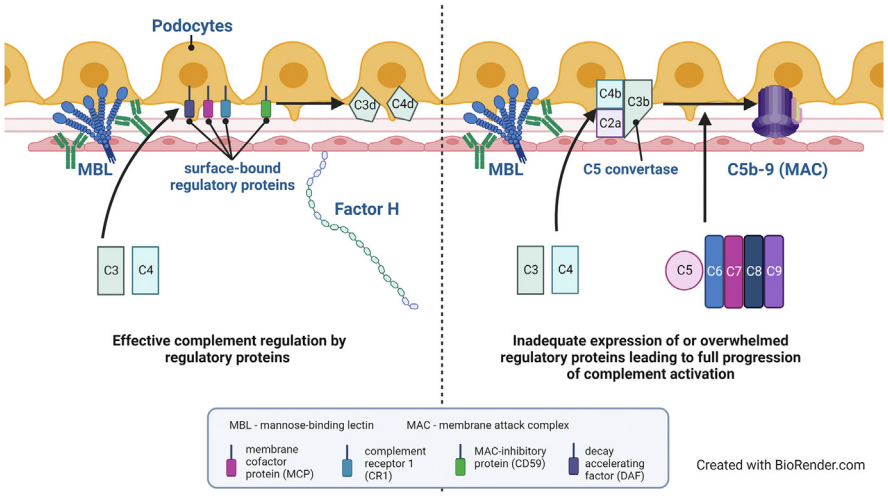

Figure 1. Complement activation on the podocyte. Antibodies reactive with M-type phospholipase A2 receptor 1 or other podocyte antigens binds to the surface of the podocye. IgG typically activates the classical pathway of complement, but in membranous nephropathy anti-phospholipase A2 receptor 1 seems to bind mannose-binding lectin and activate the lectin pathway. Lectin pathway activation fixes soluble C3 and C4 molecules to the cell surface. Podocytes express several complement regulators on their surface, including membrane cofactor protein (or CD46) and complement receptor 1. They may also express decay accelerating factor or CD55. These proteins control complement activation on the cell. Podocytes also express CD59, which prevents formation of C5b-9 (also called the membrane attack complex). Factor H is a soluble complement regulator that may also limit complement activation on the cells. After cleavage of the deposited C3 and C4, the C3d and C4d fragments remain bound to the podocyte surface. Effective regulation of the complement system is shown on the left. If the regulatory proteins are overwhelmed by the deposited immune complexes, then activation proceeds to the level of the C5 convertase (C4bC2aC3b) which cleaves C5, allowing generation of C5b-9 (shown on the right). This may happen in patients in whom the regulatory proteins are downregulated on injured podocytes, or who have genetic variants in the complement regulatory genes that reduce their function. MBL, mannose-binding lectin; MCP, membrane cofactor protein.

95.3% of the biopsies stained positively for C4d, and glomerular C5b-9 was detected in 45.3% of the biopsies. Glomerular C5b-9 deposition was associated with higher levels of proteinuria and serum creatinine; and correlated with lower rates of remission and greater progression to kidney failure, even after correction for the initial glomerular filtration rate and level of proteinuria. The presence of C5b-9 in biopsies was reported as positive or negative, and the authors indicate that there was concordance between 2 pathologists in all cases. The results suggest that formation of C5b-9 in the glomeruli is a binary process; complement activation is either controlled or it is not. Nevertheless, quantitative methods might have been able to distinguish between differences in the abundance of C5b-9 deposits among the patients and between individual glomeruli.

Antiphospholipase A2 receptor 1 is predominantly IgG4, an isotype that does not activate the classical pathway of complement. Studies have shown, however, that glycan side chains on antiphospholipase A2 receptor 1 IgG4 antibodies bind to mannose-binding lectin, activating complement through the lectin activation pathway.[8] The pattern of glomerular complement fragments detected in this study is consistent with activation through the lectin pathway. C4d, which is activated by both the classical and lectin pathways, was seen in most cases of primary and secondary disease, whereas C1q, which is deposited during activation of the classical pathway, was only seen in 20% of the biopsies from patients with primary MN. Perhaps the most surprising aspect of this study is that C3 and C4d were deposited in >90% of the biopsies; however, C5b-9 was seen in fewer than 50%. This suggests that glomerular complement activation is controlled at the level of C3 in many patients and does not reach the level of C5b-9 formation.

Several complement regulatory proteins are expressed in the glomeruli, including decay accelerating factor (or CD55), membrane cofactor protein (or CD46,) complement receptor 1, or CD35, and CD59.[9] These proteins could halt activation at the level of C3 or in the case of CD59, block the final step of C5b-9 formation. Full progression of complement activation within the glomerulus might signify that deposited immune complexes are sufficient to overwhelm these regulators, in which case C5b-9 would reflect the magnitude of the autoimmune response (Figure 1). Complement regulation might also become impaired in damaged glomeruli, in which case glomerular C5b-9 would indicate that injury was sufficient to disrupt local regulation and reflect the severity of

tissue damage. Finally, congenital differences in the function of the regulatory proteins might determine whether activation reaches C5b-9 formation, in which case glomerular C5b-9 would be a marker of a patient's genetic susceptibility to complement-mediated injury.

Detection of C5b-9 in kidney biopsies of patients with MN provides important prognostic information and can aid in assessing the patient's risk of progression. Unfortunately, this study only analyzed C5b-9 in biopsies, but did not measure it in urine from the study subjects. It would be useful to measure C5b-9 in biopsies and urine samples collected from patients with MN at the same time, as such a comparison might reveal whether analysis of urine C5b-9 can be used to serially (and noninvasively) monitor complement activation within the glomeruli. It is also not clear from this study whether glomerular C5b-9 can be used to select patients for treatment. Patients with C5b-9 deposits were less likely to enter remission; however, this was true whether they received immunosuppressive therapy or not. Several new complement inhibitory drugs are currently being evaluated for use in glomerular disease. The presence of glomerular C5b-9 might be particularly useful for stratifying patients to treatment with a complement inhibitory drug. In the eculizumab study discussed above, for example, it is possible that C5 blockade would have been more effective if the study had selected patients with histologic evidence of terminal complement activation. The prospect of creating new regimens that target this subset of patients while sparing them from the adverse effects of currently used agents is an exciting development. Further study will be needed before clinicians can individualize treatment of patients with MN based on the presence of C5b-9 deposits in the biopsy; however, it is clear that complement diagnostics provide important prognostic and pathophysiologic information.

DISCLOSURE

JMT receives royalties from Alexion Pharmaceuticals, Inc. and is a consultant for Q32 Bio, Inc., a company developing complement inhibitors. He also holds stock and may receive royalty income from Q32 Bio, Inc. CT declares no conflict of interest.

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