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Induction of IgA Deposits and Glomerulonephritis by IgA Rheumatoid Factor

Jan Novak
Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama

In this issue of JASN, Otani et al.1 describe an interesting murine model for studies of IgA-associated GN. The authors explore the nephrinopathy potential of two different monoclonal IgA rheumatoid factors (designated as 6-19 IgA and 46-42 IgA) specific for murine IgG2a in relation to potential differences in glycosylation of these IgA antibodies.

Previous research by this group showed that 6-19 IgG3 anti-IgG2a rheumatoid factor monoclonal antibody, derived from lupus-prone MRL-Faslpr mice, induces glomerular lesions. These lesions are characterized by IgG subendothelial deposits of the IgG3 cryoglobulin. To assess the role of the IgG3 heavy...
chain in the generation of cryoglobulins and associated tissue lesions, the authors established an IgG1 class-switch variant from the 6-19 hybridoma. The IgG1 switch variant lost its cryoglobulin activity but retained the rheumatoid factor activity and, consequently, did not have the capacity to generate skin and glomerular lesions. This IgG1 switch variant exhibited significantly lower rheumatoid factor activity than the IgG3 6-19 monoclonal antibody, suggesting the self-associating property of the IgG3 isotype promotes antibody-binding activity. Thus, the IgG3 heavy chain was a key determinant in the pathogenicity of 6-19 IgG3 anti-IgG2a rheumatoid factor monoclonal antibody. This conclusion is consistent with the general observation regarding the pathogenic importance of autoantibodies of the IgG3 subclass in murine systemic lupus erythematosus.

In other studies, murine 6-19 IgG3 anti-IgG2a rheumatoid factor monoclonal antibodies with cryoglobulin activity are able to induce skin leukocytoclastic vasculitis and GN resembling “wire-loop” glomerular lesions in normal mice. The results suggest that the cryoglobulin activity of IgG3 rheumatoid factor monoclonal antibodies is responsible for the generation of glomerular lesions and that both rheumatoid factor and cryoglobulin activities are necessary for skin vascular lesions. Some IgG3 cryoglobulins, however, do not exhibit nephritogenic activity, thus suggesting that qualitative features of cryoglobulins are critical for their pathogenic activities.

The 6-19 IgG3-induced lesions are associated with infiltration by polymorphonuclear leukocytes. Interactions of polymorphonuclear leukocytes with endothelial cells mediated by leukocyte function-associated antigen 1 and intercellular adhesion molecule 1 are required for the development of skin leukocytoclastic vasculitis but not for glomerular lesions. With the absence of the polymorphonuclear leukocytes in the glomerular infiltration, 6-19 IgG3-induced glomerular lesions do not have subendothelial deposits, but have intracapillary thrombi and mesangial deposits.

An additional factor, glycosylation of the IgG molecules, was also found to co-determine the nephritogenic activity of IgG3 cryoglobulins. Notably, IgG3 antibody lacking the capacity to induce severe GN displays an increased proportion of galactosylated heavy chains. The lack of nephritogenicity of the more galactosylated variant has been attributed to changes in conformation, reduction cryoglobulin activity, or enhanced clearance. These observations parallel another one regarding the capacity of IgG autoantibodies specific for collagen to induce arthritis only when the autoantibody is enzymatically degalactosylated. In this latter model of collagen-induced arthritis, the lectin complement pathway is involved.

In the present study in JASN, the authors apply their expertise in using immunoglobulin switch variants and other manipulations to determine factors involved in pathogenicity of rheumatoid antibodies. In this elegant study using an IgG3–IgA switch variant of rheumatoid factor (6-19 IgA), the IgA switch variant produced IgA-containing mesangial deposits with IgG2a and C3 co-deposits with many characteristics resembling human IgA nephropathy. Interestingly, another IgA switch variant of rheumatoid factor of the same allotype (46-42 IgA) did not have the capacity to induce any significant immune deposits or glomerular lesions. Control experiments using null mice (IgG3 − and C3 −) observed that both IgG2a (autoantigen, target of the rheumatoid factor) and complement C3 are required for the development of significant immune deposits and glomerular lesions with 6-19 IgA rheumatoid factor.

Both IgA rheumatoid factors contained similar proportions of monomeric and polymeric forms. Notably, the rheumatoid factor activity is associated with polymeric forms, and the apparent affinities were similar for both IgA rheumatoid factors. Next, the authors assessed whether the hinge-region sequence, PTPPPPITIPSC, of the IgG3-2a allotype of both IgA rheumatoid factors could be O-glycosylated. Mass spectrometric analyses revealed that both IgA rheumatoid factors contained a hinge region with a single O-glycan. The 6-19 IgA rheumatoid factor had about half of the molecules O-glycosylated, whereas the 46-42 IgA rheumatoid factor had only a small proportion of the molecules O-glycosylated. Furthermore, only 6-19 IgA rheumatoid factor contained among the O-glycosylated variants terminal N-acetylgalactosamine (GalNAc).

The authors also analyzed N-glycans of both IgA rheumatoid factors in the context of amino acid sequence and concluded there are substantial differences between the two IgA rheumatoid factors. N-glycans of the CH1 domain of the nephritogenic IgA 6-19 have complex glycans, whereas those of 46-42 nonnephritogenic IgA contain hybrid glycans. The CH3 domain has two N-glycans at Asn438 and Asn453. The Asn438 site of both IgA proteins was glycosylated with similar mixtures of high-mannose and complex bi- and tri-antennary glycans mostly galactosylated, although the nephritogenic IgA had more complex glycans fucosylated. The Asn453 site exclusively contains high-mannose glycans on both IgA proteins.

Based on these intriguing results, the authors speculate that differential O-glycosylation of the murine IgA rheumatoid factor may be a factor that determines the potential nephritogenicity of the formed IgA–IgG2a immune complexes. This model may also be of great interest for studies of factors that drive nephritogenicity of IgA–IgG immune complexes. Presumably, future studies might involve site-directed mutagenesis to remove potential O-glycosylation sites to provide a proof of the involvement of O-glycans. Such studies would provide additional insight into the characteristics of IgA-containing immune complexes with nephritogenic potential.

Other murine models of IgA nephropathy and IgA-associated glomerular lesions have been developed and tested, each with their own advantages and challenges. This current model uses well-characterized murine IgA monoclonal antibodies that are O-glycosylated, but the implantation of hybridoma cells requires pristine treatment and immunosuppression with anti-CD4 and -CD8 antibodies.
It should be noted, however, that unlike in human IgA nephropathy where the aberrantly O-glycosylated IgA1 with terminal GalNAc is an autoantigen, here the IgA rheumatoid factor is an autoantibody. Nevertheless, the intriguing differences in N- and O-glycosylation will allow us to answer some important questions concerning the role of glycans in effector function of these antibodies.

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DISCLOSURES

None.

REFERENCES


See related article, “O-Glycosylated IgA Rheumatoid Factor Induces IgA Deposits and Glomerulonephritis,” on pages 438–446.

Illuminating the Glomerular Filtration Barrier, Two Photons at a Time

William H. Fissell
Biomedical Engineering, Cleveland Clinic, Cleveland, Ohio


The mechanisms by which the kidney retains albumin and larger proteins in the circulation while excreting smaller solutes remain enigmatic despite intense investigation. Although the controversy largely plays out in academic journals, the problem is anything but academic, because the health of millions of adults hinges on effective treatment of proteinuric CKD. Proteinuria is associated with cardiovascular and all-cause mortality.1,2 Indeed, albuminuria has emerged as the single best predictor of progression of CKD, and control of proteinuria is clearly associated with preservation of GFR.3,4 To illustrate the public health burden of CKD, the work by Coresh et al.7 used National Health and Nutrition Examination Surveys data to estimate that nearly 8% of the US population had moderate or severe reductions in GFR and slightly more than 8% had microalbuminuria. Almost all of the increased prevalence of albuminuria over the prior decade could be explained by increased prevalence of hypertension, diabetes, and obesity, suggesting that increased prosperity in the so-called developing world will bring an increased burden of proteinuric CKD.5,6

To illustrate the public health burden of CKD, the work by Sandoval et al.10 presents a detailed description of factors influencing the accuracy of a recently developed technique (two-photon intravital microscopy) that has unexpectedly lent support to a controversial