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Three pathogenic determinants in immune nephritis – anti-glomerular antibody specificity, innate triggers and host genetics

Tianfu Wu, Chandra Mohan

Department of Internal Medicine (Rheumatology) and the Center for Immunology, University of Texas Southwestern Medical School, Dallas, TX 75235

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1. ABSTRACT

The prevailing notion is that lupus nephritis is mediated by autoantibodies, particularly those that bind to DNA and /or glomeruli. However it has become apparent that the development of immune-mediated renal disease is contingent upon additional factors including innate stimuli and host genetics. The purpose of this review is to evaluate our current understanding of three factors that can potentially influence immune-mediated renal disease: (1) Anti-glomerular/DNA antibodies (Abs), (2) Innate triggers, including Toll-Like Receptor (TLR) stimulation, and (3) the genetic makeup of the host.

2. INTRODUCTION

Lupus nephritis is a major concern in Rheumatology and Nephrology, due to its associated high incidence of morbidity and mortality. Ample evidence exists to support the notion that lupus nephritis is mediate by autoantibodies, particularly those that bind to DNA and /or glomeruli. However it has become apparent that the development of immune-mediated renal disease is contingent upon additional factors including innate stimuli and host genetics. The purpose of this review is to evaluate our current understanding of three factors that can potentially influence immune-mediated renal disease: (1) Anti-glomerular/DNA antibodies (Abs), (2) Innate triggers, including Toll-Like Receptor (TLR) stimulation, and (3) the genetic makeup of the host, as diagramed in Figure 1.

3. THE CONTRIBUTION OF ANTI-GLOMERULI/DNA ANTIBODY SPECIFICITY TO IMMUNE NEPHRITIS

Over the past 40 years, several studies have focused on the mechanisms of autoantibody mediated immune nephritis (1-10). Among those studies, passive transfer of antibodies has become a powerful tool for elucidating the pathogenic potential of antibodies. These

Table 1. The contribution of antinuclear antibody reactivity to the severity of renal disease

Ab specificity						
Nucleosome	○	●	●	●	●	●
ssDNA	○	○	●	●	●	●
dsDNA	○	○	○	○	●/○	●
Glomeruli	○	○	○	●	○	●
Severity of renal disease	–	–	–	+	+	+++

Open circle represents absence of antibody reactivity, closed circle represents presence of antibody reactivity. "+++" means severe renal disease, "+" means mild renal disease and "-" means no renal disease. The severity of the renal disease was gauged from the degree of proteinuria and BUN (11) or SLEDAI or renal SLEDAI indices in lupus patients (12).

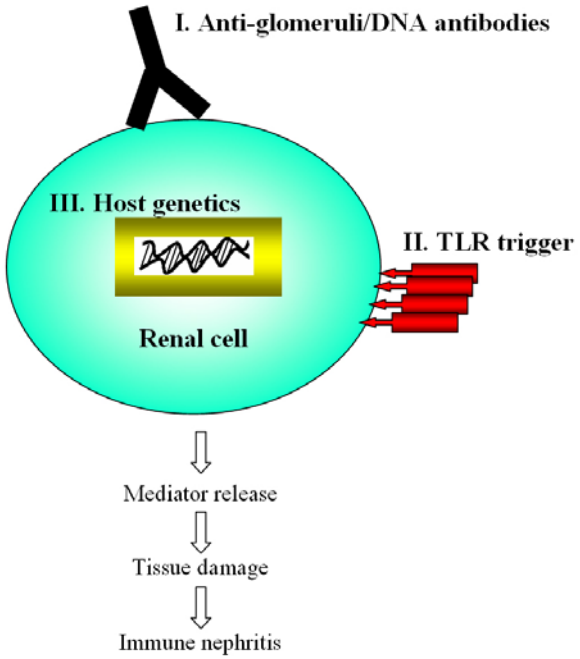

Figure 1. An overview of the three factors that can potentially impact the degree of renal damage in immune nephritis.

studies have revealed that autoantibodies may induce renal damage through several different mechanisms. Firstly, autoantibodies may engage their glomerular antigenic targets through intermediating "antigen bridges". In this case, these autoantibodies may bind glomerular surfaces through nucleosomal antigens or other glomerular substrates. Secondly, some autoantibodies, such as anti-nuclear antibodies, may bind directly to glomerular antigens through cross-reactivity. Through an in vivo antibody transfer experiment, for example, Katz et al reported that dsDNA-binding autoantibodies can mediate nephritis (5). They found that the loss of DNA binding activity eliminated the pathogenicity of the antibody. In reviewing all of the anti-dsDNA antibody transfer experiments carried out thus far, 95% of these studies were associated with antibody deposition in the renal glomeruli, 60% with proteinuria and 42% with azotemia and/or renal pathology, as summarized elsewhere (11). Interestingly, among the antibodies that do bind glomerular/GBM antigens, none have been demonstrated to be clearly DNA-independent (11); on the other hand, several studies have conclusively demonstrated glomerular binding to be DNA-dependent (6-10).

In our previous work, we have rescued a panel of 56 anti-nuclear and 47 non-nuclear binding monoclonal antibodies from four seropositive NZM2410 lupus mice (10). The monoclonals showed different reactivity pattern to nucleosome, ssDNA, dsDNA, and glomerular substrate. A large number of these monoclonal antibodies clearly demonstrated polyreactivity (to DNA, histones, and glomerular antigens) apparently due to bound, DNase-1 sensitive nuclear antigenic material as summarized in Table 1. In that study, the pathogenic potential of these different antibodies were tested by adoptively transferring them into young healthy recipients. Interestingly, we observed that anti-nucleosome Abs of both IgM and IgG isotypes were relatively innocuous, in comparison to anti-dsDNA antibodies. Moreover, although dsDNA-reactive Abs appeared to be fairly pathogenic, the presence of any concomitant reactivity to glomerular substrate significantly boosted their pathogenic potential, as signified by elevated proteinuria and azotemia. Thus, our findings are consistent with the prevailing notion that the glomerular reactivity of autoantibodies may predict pathogenic potential in lupus. It is reasonable to hypothesize that the Abs with nephrophilicity (irrespective of whether or not their glomerular binding is mediated by nuclear antigenic bridges) may be the most pathogenic because they may possess the greatest potential to bind to the glomerular basement membrane or matrix.

Based on the observation that glomerular binding antibodies are the most pathogenic, a recent proteomic study has explored the fine specificities of glomerular binding antibodies further using a newly fabricated "glomerular proteome array" (12). Basically, these are glass-slides coated with a spectrum of glomerular antigens. These arrays have been used to study sera from mice and patients with lupus nephritis. Compared to normal serum, serum from B6.*Sle1.lpr* lupus mice (C57BL/6 mice homozygous for the NZM2410/NZW allele of *Sle1* as well as the FAS^{lpr} defect) exhibited high levels of IgG and IgM anti-glomerular as well as anti–double-stranded DNA/chromatin Abs and variable levels of Abs to α-actinin, aggrecan, collagen, entactin, fibrinogen, hemocyanin, heparan sulphate, laminin, myosin, proteoglycans, and histones. The use of these glomerular proteome arrays also revealed 5 distinct clusters of IgG autoreactivity in the sera of lupus patients. Importantly, 2 of these IgG reactivity clusters (DNA/chromatin/glomeruli and laminin/myosin/Matrigel/vimentin/ heparan sulphate) showed good association with disease activity and renal SLEDAI scores. On the other hand, the presence of several other antigenic specificities was not associated with renal disease (12).

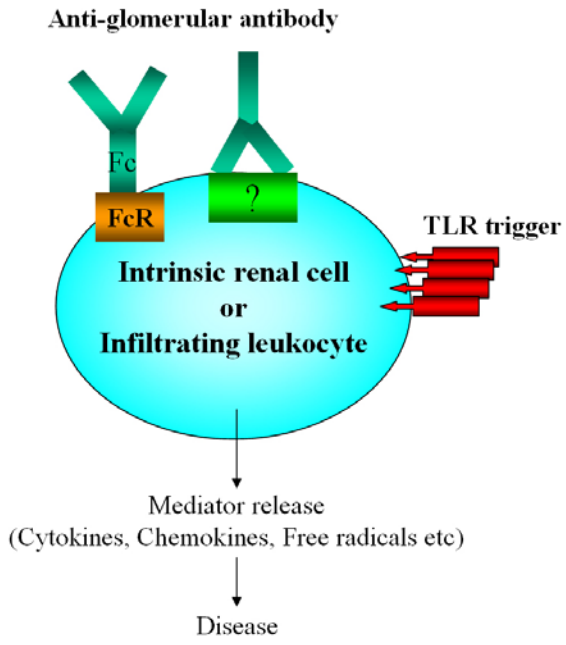

Figure 2. Schematic representation of the development of immune-mediated renal disease accelerated by innate immune stimuli. The glomerular targeted antibodies may be stimulating intrinsic renal cells or infiltrating leukocytes in one of 2 ways, as depicted.

These early studies need to be expanded and confirmed with larger numbers of SLE patients and more comprehensive glomerular proteome arrays. Collectively, the antibody transfer studies and the serum proteomic studies clearly indicate that the antigenic fine-specificity of the anti-glomerular/DNA Abs is one important determinant of pathogenicity in immune and lupus nephritis.

4. THE CONTRIBUTION OF INNATE IMMUNITY TO RENAL DISEASE

It has been known for quite some time that whereas the active immunization with anti-glomerular Abs in adjuvant leads to severe immune nephritis, the passive transfer of glomerular-targeting antibodies alone elicits only minimal renal disease. Several follow-up studies have revealed that the concomitant delivery of innate stimuli together with the anti-glomerular Abs could trigger severe nephritis (13, 14). Savige et al. found the administration of lipopolysaccharide (LPS) to Sprague-Dawley rats 24 h before the induction of immune nephritis resulted in the earlier appearance of larger numbers of glomerular neutrophils compared to animals injected with nephrotoxic globulin alone.

In our recent work (14), we found that triggering either TLR2, TLR3, TLR4, or TLR5, using peptidoglycan, poly I:C, LPS, or flagellin, respectively, could also facilitate anti-glomerular antibody elicited nephritis in mice. Moreover, our genetic studies revealed that whereas the innate trigger was dependent upon Toll-receptor/IRAK-mediated signaling, the immune component was contingent upon FcR-mediated signals as summarized in Figure 2. Importantly, infiltrating leukocytes as well as intrinsic glomerular cells may both serve to integrate these diverse signals. We speculate that in spontaneous immune-mediated nephritis, the adaptive immune system may be important in generating end-organ targeting antibodies, while the extent of renal damage inflicted by these antibodies may be greatly dependent on cues from the innate immune system. Currently, the source of the innate trigger in spontaneous lupus nephritis remains a mystery. Although endogenous TLR ligands such as heat shock proteins and fibronectins have been described, it remains to be established if these do indeed play a pathogenic role in amplifying antibody-mediated glomerulonephritis. Finally, ample evidence exists to support the notion that an additional mediator of innate immunity, complement, also plays an important role in immune-mediated renal disease, as reviewed elsewhere (15).

5. THE CONTRIBUTION OF HOST GENETICS TO RENAL DISEASE

There are several hints indicating that autoantibody formation and end-organ disease may be under distinct genetic control in lupus, as listed below:

A. Discordance between anti-nuclear antibodies and glomerulonephritis have been documented in murine, as well as in human lupus, as reviewed (16, 17, 18).

B. In experimental models, strongly nephrophilic seropositivity can be uncoupled from renal disease. Thus, for example, the absence of key molecular mediators (e.g., FcR, MCP-1, complement, TNF-α, ICAM-1) in the kidneys can ameliorate Ab-mediated disease, despite the presence of potentially pathogenic, autoAbs (19-24).

C. As a corollary, in certain models, high titers of nephrophilic Abs do not seem to be required for renal pathology to ensue. The NZW strain (which is the origin of 75% of the NZM genome) is a classic example of this fact (25-27). An extreme example was shown in lupus-prone mice that lack serum Abs totally, but still bear B-cells (28-29). The study of these mice has shown that certain types of nephritis can still develop in genetically predisposed individuals, even in the absence of autoAbs.

D. Linkage analyses have shown that certain genetic loci are strongly linked to nephritis, but not autoantibodies (30, 31); such loci may contribute directly to renal disease, with little impact on systemic immunity, or anti-nuclear autoantibody (ANA) formation. Similar GN-linked (but not ANA-linked) loci have also been noted in human SLE (32).
E. Reports of familial clustering of primary/idiopathic GN (33-35), and of GN following lupus, diabetes, and hypertension (36-39) further support the potential importance of genetics in determining intrinsic susceptibility to renal disease in lupus, as well as in other diseases.

The notion that host genetics may be an important determinant in immune nephritis is fortified by

Table 2. The development of immune-mediated nephritis is strain-dependent

Strain	Proteinuria	BUN	GN	Glomerular Cresents	TIN
A/J	–	–	–	–	–
C57BL/6	–	–	–	–	–
Balb/c	–	–	–	–	–
AKR	–	–	+	–	–
BUB	+++	++	++	+++	+++
C3H	+	++	+	–	+
DBA1	+++	+	++	+	++
DBA2	+	+	+	–	–
MRL	–	–	–	–	–
NOD	–	–	–	–	–
P/J	–	–	–	–	–
SJL	–	–	–	–	–
SWR	–	–	–	–	–
NZW	+++	+++	+++	+++	+++
129	+++	+++	+++	+++	+++

All strains were challenged with anti-glomerular antibodies and monitored for proteinuria, blood urea nitrogen (BUN), severity of glomerulonephritis (GN) and tubular intestinal nephritis (TIN). Values from low to high are expressed as "-", "+", "++", "+++" accordingly. Please see references 39 and 40 for details.

strain-distribution studies in mice. In this context, we have recently screened 15 different mouse strains to investigate the influence of genetic background on the development of immune-mediated renal disease (40, 41). Anti-glomerular antibodies were transferred into these different strains to determine which ones were most susceptible to nephritis. We found that some of the strains developed more severe immune nephritis than others. Compared to anti-GBM-injected A/J, AKR/J, C3H/HeJ, DBA/2J, MRL/MpJ, NOD/LtJ, P/J, SJL/J, C57BL/6, Balb/c, and SWR/J mice, the anti-GBM-injected BUB/BnJ, DBA/1J, NZW, and 129/svJ mice developed more severe proteinuria and azotemia. Their kidneys exhibited more pronounced glomerulonephritis, with crescent formation, as well as tubulointerstitial disease, with these phenotypes being particularly profound in 129/svJ mice, as depicted in Table 2. However, these strains did not appear to differ in the nature of their xenogeneic immune response to the administered rabbit sera, either quantitatively or qualitatively. Collectively, these findings allude to the presence of genetic elements in the NZW, BUB/BnJ, DBA/1J, and 129/svJ genomes that may potentially confer susceptibility to immune-mediated nephritis. Studies are in progress o define the genetics of immune nephritis using these 4 disease-prone strains.

6. CONCLUSION

For a long time it was believed that the fine specificity of the glomerular-targeting antibodies was the only or main determinant of disease severity in immune/lupus nephritis. It is now clear that the contribution of innate triggers and the genetics of the host kidney are two additional determinants of disease severity in immune nephritis. Further research is warranted to elucidate how the complex interplay of these 3 factors may lead to different degrees of severity and diverse patterns of renal disease in lupus.

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Send correspondence to: Chandra Mohan, MD, PhD, Department of Internal Medicine/Rheumatology, UT Southwestern Medical Center, Mail Code 8884, Y8.204, 5323 Harry Hines Boulevard, Dallas, TX 75390-8884, USA, Tel: 214-648-9675, Fax: 214-648-7995, E-mail: Chandra.mohan@utsouthwestern.edu

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