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Pathogenesis of Proliferative Lupus Nephritis from a Historical and Personal Perspective

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Introduction

In a historical perspective for lupus nephritis (LN) from 1968 to 1998, JS Cameron provided an excellent brief review of lupus nephritis up to 1998 (1). Since then, there has been significant progress in our understanding of the pathogenesis of lupus nephritis. However many issues remain to be debated. Some of these issues are the subject of this perspective with an emphasis on the data generated from the authors' laboratory. This review will address selective aspects of the pathogenesis of proliferative lupus nephritis.

Multiple immune complex systems are involved in lupus nephritis

Figure 1 shows the pathology of class IV diffuse proliferative lupus glomerulonephritis (PLGN). On light microscopy, wire-loop lesions first described by Baehr et al (2) are evident in H&E stained renal biopsy sections (figure 1A). Masson trichrome stain highlights the wire-loop subendothelial deposit (figure 1B). Immunofluorescence (IF) studies show Ig and complement deposits (figure 1C). By electron microscopy (EM), the dense deposits in subendothelial and subepithelial spaces (figure 1D) correlate with the presence of wire-loops. The nature of the deposits in the wire-loops seen by light microscopy and the dense deposits in EM has been the subject of extensive investigation for many decades.

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The dense deposits were shown to be immune complexes (IC) by Vazquez and Dixon (3). With circulating anti-nuclear Ab (ANA) demonstrated to be a dominant Ab in systemic lupus erythematosus (SLE) and the description of anti-DNA Ab to be a component of ANA by Holman and Kunkel (4), ANA and anti-DNA Ab were shown to be enriched in the eluates of post mortem kidneys from patients with LN (5–7). *Although predicted by Kunkel (7) that multiple Ab-Ag systems are involved in these immune complexes, the anti-DNA-DNA IC system has received an inordinately large amount of attention since the early 1970's.* This emphasis on the anti-DNA-DNA system was perhaps influenced by the remarkable paper by Tan et al (8) in 1966 with the following statement in the summary of the paper: “serial studies with the sera of certain patients with SLE indicated that DNA was present at one time, whereas antibodies to DNA were present at another. Such sera interacted with each other by precipitin and complement fixation reactions. The possibility is raised that DNA and anti-DNA antibodies may have occurred simultaneously, giving rise to antigen-antibody complexes of potential significant in renal lesions”. This finding is shown in figure 2. The importance of anti-DNA Ab in the pathogenesis of SLE has been recently reviewed by Marion and Postlethwaite (9). We have reviewed the recent literature to seek support that anti-DNA Ab are the most specific Abs for the diagnosis of SLE. Nevertheless, we concluded that with the evolution of methods in the measurement of anti-DNA Ab, anti-DNA Ab is just one of the many clinically measured lupus-related Ab, repudiating the notion that anti-DNA (or dsDNA) Ab are the most important auto-Ab in SLE (10). It is of historical interest that Henry Kunkel did not consider that the DNA/anti-DNA IC were either the only IC or the most important IC in his writing and his communication with SM Fu. Regarding the role of other IC systems in the pathogenesis of LN, Mannick et al (11) showed that anti-dsDNA Ab accounted for less than 10% of Ab activities eluted from post-mortem kidneys. Ab to C1q, SSA, SSB, Sm and chromatin were enriched in the kidney eluates. In addition Ab to 14 autoantigens accounted for less than 50% of Ab activity in the eluates with the conclusion that multiple Ab-Ag IC were involved in LN. This conclusion fulfilled the predictions made by Henry Kunkel in 1966 (7).

Breaking tolerance to double stranded DNA, nucleosome, and other nuclear antigens is not required for the pathogenesis of lupus GN

The issue regarding the relative importance of DNA/anti-DNA and nucleosome/anti-nucleosome ICs in the pathogenesis of LN was once again raised by two recent review papers (12, 13). Pedersen et al (12) reviewed their studies that emphasized the importance of the nucleosome/anti-nucleosome IC in the pathogenesis of LN while Goilav and Putterman (13) were more conciliatory with the conclusion that both IC systems were complementary. Our 2004 publication in the Journal of Experimental Medicine (14) cited by Goilav and Putterman (13) is relevant to this issue. The paper has by large been ignored by those who favor the important role of ANA and anti-dsDNA Ab in the pathogenesis of SLE in general and LN in particular. The major findings of this paper will be reviewed.

NZM2328 was shown to be an excellent model for human PLGN (15). By 12 months of age, 70% of the female mice of this strain have severe proteinuria and early mortality. These kidneys show severe IC mediated nephritis resembling that of human lupus. Most male mice

do not have severe proteinuria and early death although they have IC deposition and complement activation in their kidneys. We determined that nephritis in female NZM2328 mice presented in two stages; acute GN (aGN) and chronic GN (cGN) as shown in figure 3. aGN was defined as diffuse proliferative GN with hyper-cellular glomeruli, with many exudative mononuclear cells within the capillary lumen, and normal tubules without interstitial nephritis. Female mice with aGN had 1+ to 2+ proteinuria by the dipstick tests. cGN showed severe and diffuse GN with high chronicity index. Glomeruli in CGN showed expansion of the mesangial space with increased matrix material and necrosis. Tubules in cGN were dilated with proteinaceous casts. In addition there was interstitial fibrosis. Female mice with cGN had 3+–4+ proteinuria. Severe proteinuria correlated with early mortality. These two stages were recognized histologically and represented two distinct phenotypes. Genetic studies involving a cohort of female (NZM2328XC57L/J)XNZM2328 mice identified in lupus-prone NZM2328 mice a locus *Cgnz1* on chromosome 1 that was linked to cGN, severe proteinuria, and early mortality in females and a locus *Adnz1* on chromosome 4 linked to ANA and anti-dsDNA Ab production (15).

As shown in figure 4A, two congenic strains, NZM2328.C57L/Jc1 (NZM.C57Lc1) and NZM2328.C57L/Jc4 (NZM.C57Lc4), were generated by replacing the respective genetic intervals containing either *Cgnz1* or *Adnz1* with those from C57L/J, a non lupus-prone strain (14). The NZM.C57Lc1 females had markedly reduced incidence of cGN and severe proteinuria. NZM.C57Lc4 females had cGN and severe proteinuria (figure 4B) without circulating ANA, anti-dsDNA, and anti-nucleosome/nuclear Ab (figure 4C). The kinetics of development of renal disease was similar to that of the parental strain (figure 4B). The diseased NZM.C57Lc4 kidneys had immune complexes by IF and EM. Elution of the diseased kidneys from the parental strain documented enrichment of anti-dsDNA Ab in the kidney eluates (figure 4D). Such enrichment was not detected in the eluates of NZM.C57Lc4 kidneys. Similar results were obtained regarding the ANA IC. These results reaffirm that anti-dsDNA and related Ab production and cGN are under independent genetic control. These findings provide genetic evidence that LN occurs in the absence of ANA and anti-dsDNA Ab. They should have provided the proper perspective as to the importance of DNA/anti-DNA and nucleosome/anti-nucleosome ICs in the pathogenesis of LN.

Multiple auto-Ab may initiate LN

There have been continuing debates as to which auto-Ab initiates LN. From the above discussion of the phenotype of NZM.C57Lc4, it should be concluded that both anti-dsDNA and anti-nucleosome Ab need not be the initiating auto-Ab in LN. Two papers by Bruschi et al (16, 17) would add support to the above conclusion. In their studies, the glomeruli of 20 renal biopsies from LN patients (one class II, three class III, nine class IV, two class IV+V, three class V and two undetermined) were microdissected and captured. The captured glomeruli were acid treated and the antibody reactivities were determined using MALDI-MA and LC-MS. Eleven of the eluates reacted with α -enolase while 10 of them with annexin A1. A total of 11 intrinsic podocyte Ags were identified by the twenty eluates (16). In the second paper, these authors found 55% of the eluates reactive with DNA, 55% with histone and 70% with C1q (17). DNA, histone and C1q were considered as extrinsic antigens planted in the glomerular basement membrane. The lack of anti-DNA Ab in 50% of

the biopsies and the presence of circulating anti-DNA Ab without their detection in the biopsies in many patients suggest that anti-DNA and anti-nucleosome Ab may not be the initiating auto-Ab in many of these patients. In addition it was shown that the anti- α -enolase and anti-annexin Abs did not cross react with DNA. Recently circulating Abs to multiple membrane components were preferentially demonstrated in patients with LN (18), suggesting that these Ab may participate in the initiation of LN. In summary, multiple studies suggest that auto-Ab of diverse specificity are capable of initiating LN.

The above cited studies (16–18) regarding auto-Ab that may initiate LN provide useful information regarding the distribution of IgG subclasses of these Ab. It has been emphasized that anti-cell membrane Ab such as anti- α enolase, anti-laminin Ab and anti-C1q auto-Abs are predominantly IgG2 and anti-dsDNA Ab are IgG2 and IgG3. Small amounts of IgG1 and IgG3 anti-membrane Ab are readily found in some of the patients with LN. While it is of interest to note that IgG1 and IgG3 are more efficient in complement fixation and Fc binding to monocytes, IgG2 does fixed complement though less efficient. It is difficult to ascertain which Ab are more pathogenic.

IC deposition and complement activation are not sufficient for LN to progress from aGN to cGN and end stage renal disease

It has been assumed that IC deposition with complement activation will inevitably lead to deteriorating renal function. Many investigators with an interest in LN would not have expected that aGN and cGN are distinct phenotypes under separate genetic control. In our paper published in 2004 (14), we proposed the hypothesis that separates autoimmunity i.e. the presence of auto-Ab and autoreactive T cells from autoimmune diseases that involve end organ damage. The hypothesis is depicted in figure 5 as published in Current Opinion in Immunology (19). The hypothesis assumes that the target organ is not an idle player and that it participates in the pathogenesis that leads to its destruction. It is also implicit that there is interaction between the innate and adaptive immune system with the target organs.

Nine years later we have accumulated sufficient data to validate the hypothesis. The data will be briefly reviewed. In figure 4A, the segment of chromosome 1 that was replaced in NZM.C57Lc1 included both *Cganz1* that linked to cGN and *Agnz1* one of the three loci linked to aGN (14). We generated multiple intrachromosomal recombinant lines to further characterize *Cganz1* (figure 6A). One of these lines, NZM.C57Lc1R27 (R27) was characterized extensively and the phenotypes of this line are summarized in figures 7 and 8 (20). In R27, the region of 8 Mb excluding *Agnz1* on chromosome 1 in NZM2328 is replaced by that from C57L/J. The female mice of R27 develop aGN without progression to cGN and end stage renal failure (figure 7). These mice develop anti-kidney Ab as early as 10 weeks old. Their kidneys have deposits of all subclasses of IgG and complement activation detected by IF and the IF intensities of the staining in R27 kidneys were comparable to those in the parental line (figure 8). These mice have mild proteinuria (1–2 plus by dipsticks). It is of interest to note that no differences have been detected in renal cytokines and cellular infiltration between NZM2328 and R27. Many of these cytokines and cellular infiltration have been considered important in the pathogenesis of LN (21, 22). R27 female mice were

shown to be resistant to nephrotoxic anti-GBM serum induced proteinuria, renal failure and death (19). Adeno-IFN α accelerated the development of GN in NZM2328. Similarly treated R27 female mice were resistant to this induction of GN (23) although Adeno-IFN α did induce much higher titers of anti-dsDNA Ab and more IC deposition and more intense staining for C3 in the treated R27 mice. The latter observation further supports our hypothesis as depicted in figure 5. These observations support our conclusion that the *Cgnz1* allele confers kidney resistance to damage preventing progression of IC-mediated aGN.

As depicted in figure 6A, we generated many intrachromosomal recombinant lines of NZM.C57Lc1. The phenotypes of R290-2 and R570-2 narrow the region where *Cgnz1* resides to be blockaded in figure 6A. The recombinant sites of these two recombinant lines were determined (24). These results indicate that *Cgnz1* is located in a region of 1.34 Mb where 45 genes are located. The region of interest maps with the *Sle1b* (*Sle1* genetics has been reviewed by L Morel in 25). As discussed by us (20), the postulated *Sle1d* (26) that contributes to GN, the phenotype of the original studies by Morel et al (27) in 1994, in NZM2410 is likely to be identical to *Cgnz1*. In addition, it is tempting to speculate that *Agnz1* may be located within *Sle1c*.

In summary, our genetic data supports the conclusion that IC deposition and complement activation are not sufficient for LN to progress from aGN to cGN and end stage renal disease. This conclusion has significant implications in our understanding of the pathogenesis of PLGN and the heterogeneity of our patients' clinical course and varied responsiveness to therapy.

Clinical implications

Thus far the homolog of *Cgnz1* in man has not been identified and none of the 45 genes have been shown to link to SLE or LN in GWA studies. As shown in figure 6B, the human homolog of the region where *Cgnz1* is located is highly conserved in that with the exception of one gene, all the genes in the human chromosome 1 in this region have the exact gene order as that of the murine homolog (19). Thus this region is highly conserved over evolution. The region must have certain evolution advantage that confers advantage in survival. With the advance of genomic sciences and selection of suitable patient populations, it is likely that the human homolog of *Cgnz1* will be identified. In addition, it is predicted that more genetic loci will be identified with functions similar to *Cgnz1*.

Recently considerable interest has been centered on the genetics of *ApoL1* (reviewed in 28). The G1 and G2 haplotypes have been shown to be high risk genotypes for end stage renal disease (ESRD) in African American populations in a recessive inheritance pattern. These haplotypes were also shown to be significantly associated with ESRD (odds ratio of 2.72) in African Americans with lupus nephritis. In contrast, this association was not found in a smaller study of Brazilian patients with mixed ethnicity. The mechanism of this association remains to be clarified. However, a recent study in Zebrafish suggests that ApoL1 plays a role in glomerular filtration and affects the expression of nephrin suggesting that ApoL1 plays a role in glomerular integrity (29). This conclusion is not supported by the observation that an *ApoL1*^{-/-} individual in India has no renal disease (30).

Our genetic studies discussed above have significant clinical implications. They provide an explanation for the variable clinical course in patients. In some patients the renal function declines rapidly while in other patients it remains relatively stable. It may also explain why some patients respond very well to therapy while others do not. Thus it is reasonable to have individualized therapeutic programs for each patient based on our understanding of the genetic of LN.

Concluding remarks

The interest on the contribution of targeted organs in autoimmune diseases has steadily increased in the past several years. In this review, contributions from other renal intrinsic cells such as endothelial cells, mesangial cells, renal tubular cells and cells in the interstitium in the pathogenesis of LN have not been discussed. Their contributions have been discussed in a chapter authored by us (31). Undoubtedly they will be discussed by other contributors in this special issue of Clinical Immunology. It should be remembered that local factors play a crucial role in the pathogenesis of LN and that the complexity of its pathogenesis should be appreciated. It is unlikely that a single hypothesis can explain the clinical course and explain the heterogeneity of patients' response to therapy.

It is appropriate to conclude our remarks once again by citing the introduction in an editorial by WL Whittier and J Reiser and entitled "Lupus Nephritis: Through the Looking Glass" (32):

Lupus Nephritis Through the Looking Glass

"If I had a world of my own, everything would be nonsense. Nothing would be what it is, because everything would be what it isn't. And contrary wise, what is, it wouldn't be. And what it wouldn't be, it would. You see?"

– Alice

In *Through the Looking Glass*, Lewis Carroll's sequel to *Alice in Wonderland*, Alice learns that things on the other side of the metaphorical mirror (looking glass) are not always what they seem. She enters a world of riddles and challenges, and learns that she cannot always expect to find logic in the situations she encounters. Over the last 60 years, as we similarly "reflect" on the history of our understanding of lupus nephritis, our world has been consumed with the obvious immune aggregates seen in the glomeruli on immunofluorescence microscopy and likewise tries to explain many, if not all, of the clinical manifestations of this disease by these immune complexes. In fact, lupus nephritis is commonly known as the "prototypical immune complex mediated disease." The unwillingness to look beyond the immune aggregates has made alternative views on pathophysiology of this complicated disease difficult to appreciate.

Acknowledgments

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Abbreviations

PLGN	Proliferative lupus glomerulonephritis
IC	Immunocomplexes
aGN	acute glomerulonephritis
cGN	chronic glomerulonephritis

References

1. Cameron JS. Lupus nephritis: an historical perspective 1968–1998. *J Nephrol.* 1999; 12(Suppl 2):s29–41. [PubMed: 10688400]
2. Baehr G, Klemperer P, Schifren A. A diffuse disease of the peripheral circulation (usually associated with lupus erythematosus and endocarditis). *Trans Assoc Am Phys.* 1935; 50:139–55.
3. Vazquez JJ, Dixon FJ. Immunohistochemical study of lesions in rheumatic fever, systemic lupus erythematosus, and rheumatoid arthritis. *Lab Invest.* 1957; 6:205–17. [PubMed: 13429841]
4. Holman HR, Kunkel HG. Affinity between the lupus erythematosus serum factor and cell nuclei and nucleoprotein. *Science.* 1957; 126:162–3. [PubMed: 13442661]
5. Koffler D, Schur PH, Kunkel HG. Immunological studies concerning the nephritis of systemic lupus erythematosus. *J Exp Med.* 1967; 126:607–24. [PubMed: 4168098]
6. Koffler D, Agnello V, Carr RI, Kunkel HG. Variable patterns of immunoglobulin and complement deposition in the kidneys of patients with systemic lupus erythematosus. *Am J Pathol.* 1969; 56:305–16. [PubMed: 4186204]
7. Kunkel HG. Mechanisms of renal injury in systemic lupus erythematosus. *Arthritis Rheum.* 1966; 9:725–6. [PubMed: 4162757]
8. Tan EM, Schur PH, Carr RI, Kunkel HG. Deoxybonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. *J Clin Invest.* 1966; 45:1732–40. [PubMed: 4959277]
9. Marion CTN, Postlethwaite AE. Chance, genetics, and the heterogeneity of disease and pathogenesis in systemic lupus erythematosus. *Semin Immunopathol.* 2014; 6:495–517.
10. Fu SM, Dai C, Zhao Z, Gaskin F. Anti-dsDNA Antibodies are one of the many autoantibodies in systemic lupus erythematosus. *F1000Res.* 2015 Oct 14:939. (F1000 Faculty Rev). eCollection 2015. doi: 10.12688/f1000research.6875.1 [PubMed: 26594353]
11. Mannik M, Merrill CE, Stamps LD, Wener MH. Multiple autoantibodies form the glomerular immune deposits in patients with systemic lupus erythematosus. *J Rheumatol.* 2003; 30:1495–504. [PubMed: 12858447]
12. Pedersen HL, Horvei KD, Thiagarajan D, Seredkina N, Rekvig OP. Murine and human lupus nephritis: pathogenic mechanisms and theoretical strategies for therapy. *Semin Nephrol.* 2015; 35:427–38. [PubMed: 26573545]
13. Goilav B, Putterman C. The role of anti-DNA antibodies in the development of lupus nephritis: A complementary, or alternative, viewpoint? *Semin Nephrol.* 2015; 35:439–43. [PubMed: 26573546]
14. Waters ST, McDuffie M, Bagavant H, Deshmukh US, Gaskin F, Jiang C, et al. Breaking tolerance to double stranded DNA, nucleosome and other nuclear antigens is not required for the pathogenesis of lupus glomerulonephritis. *J Exp Med.* 2004; 199:255–64. [PubMed: 14718514]
15. Waters ST, Fu SM, Gaskin F, Deshmukh US, Sung S-sJ, Kannapell CC, et al. NZM2328: a new mouse model of systemic lupus erythematosus with unique genetic susceptibility loci. *Clin Immunol.* 2001; 100:372–83. [PubMed: 11513551]
16. Bruschi M, Sinico RA, Moroni G, Pratesi F, Migliorini P, Galetti M, et al. Glomerular autoimmune multicomponents of human lupus nephritis in vivo: α -enolase and annexin AI. *J Am Soc Nephrol.* 2014; 25:2483–98. [PubMed: 24790181]

17. Moroni G, Bonanni A, Radice A, et al. Glomerular autoimmune multicomponents of human lupus nephritis *in vivo* (2): planted antigens. *J Am Soc Nephrol*. 2015; 26:1905–24. [PubMed: 25398787]
18. Seret G, Cañas F, Pougnet-Di Costanzo L, Hanrotel-Saliou C, Jousse-Joulin S, Le Meur Y, Saraux A, Valeri A, Putterman C, Youinou P, Rojas-Villarraga A, Anaya JM, Renaudineau Y. Anti-alpha-actinin antibodies are part of the anti-cell membrane antibody spectrum that characterize patients with lupus nephritis. *J Autoimmun*. 2015; 61:54–61. [PubMed: 26071203]
19. Dai C, Deng Y, Quinlan A, Gaskin F, Tsao BP, Fu SM. Genetics of systemic lupus erythematosus: immune responses and end organ resistance to damage. *Curr Opin Immunol*. 2014; 31:87–96. [PubMed: 25458999]
20. Ge Y, Jiang C, Sung S-sJ, Bagavant H, Dai C, Wang H, et al. *Cgnz1* allele confers kidney resistance to damage preventing progression of immune complex-mediated acute lupus glomerulonephritis. *J Exp Med*. 2013; 210:2387–401. [PubMed: 24101379]
21. Schiffer L, Bethunaickan R, Ramanujam M, Huang W, Schiffer M, Tao H, et al. Activated renal macrophages are markers of disease onset and disease remission in lupus nephritis. *J Immunol*. 2008; 180:1938–47. [PubMed: 18209092]
22. Bethunaickan R, Berthier CC, Ramanujam M, Sahu R, Zhang W, Sun Y, et al. A unique hybrid renal mononuclear phagocyte activation phenotype in murine systemic lupus erythematosus nephritis. *J Immunol*. 2011; 186:4994–5003. [PubMed: 21411733]
23. Dai C, Wang H, Sung S-sJ, Sharma R, Kannapell C, Han W, et al. Interferon alpha on NZM2328.Lc1R27: enhancing autoimmunity and immune complex-mediated glomerulonephritis without end stage renal failure. *Clin Immunol*. 2014; 154:66–71. [PubMed: 24981059]
24. Ge, Y. Doctoral Dissertation PHD Microbiology. 2009. Prevention of Chronic Fatal Lupus Nephritis in NZM2328 Mice by Replacing the Chronic-Glomerulonephritis-Associated *Cgnz1* Locus and Identification of Candidate *Cgnz1* Genes; p. 97-101. Retrieved from UVA Libra
25. Morel L. Mapping lupus susceptibility genes in the NZM2410 mouse model. *Adv Immunol*. 2012; 115:113–39. [PubMed: 22608257]
26. Morel L, Blenman KR, Croker BP, Wakeland EK. The major murine systemic lupus erythematosus susceptibility locus, *Sle1*, is a cluster of functionally related genes. 2001. *Proc Natl Acad Sci USA*. 2001; 98:1787–1792. [PubMed: 11172029]
27. Morel L, Rudofsky UH, Longmate JA, Schiffenbauer J, Wakeland EK. Polygenic control of susceptibility to murine systemic lupus erythematosus. *Immunity*. 1994; 1:219–229. [PubMed: 7889410]
28. Chen TK, Estrella MM, Parekh RS. The evolving science of apolipoprotein-L1 and kidney disease. *Curr Opin Nephrol Hypertens*. 2016; 25:217–25. [PubMed: 27023839]
29. Kotb AM, Simon O, Blumenthal A, Vogelgesang S, Dombrowski F, Amann K, et al. Knockdown of ApoL1 in Zebrafish Larvae Affects the Glomerular Filtration Barrier and the Expression of Nephhrin. *PLoS One*. 2016; 11:e0153768. [PubMed: 27138898]
30. Johnstone DB, Shegokar V, Nihalani D, Rathore YS, Mallik L, Ashish, Zare V, Ikizler HO, Powar R, Holzman LB. APOL1 null alleles from a rural village in India do not correlate with glomerulosclerosis. *PloS One*. 2012; 7:e51546. [PubMed: 23300552]
31. Fu, SM., Dai, C., Wang, H., Sung, S-sJ, Gaskin, F. Mechanisms of renal damage in systemic lupus erythematosus. In: Tsokos, GC., editor. *Systemic Lupus Erythematosus: Basic, Applied and Clinical Aspects*. 1. Elsevier Inc; London, UK: 2016. p. 283-293.
32. Whittier WL, Reiser J. Lupus nephritis: through the looking-glass. *Lupus*. 2015; 24:533–5. [PubMed: 25721124]

Highlights

Multiple immune complexes are involved in lupus nephritis.

DNA/anti-dsDNA and nucleosome/anti-nucleosome are neither the only nor the most important immune complexes in lupus nephritis.

Lupus nephritis may be initiated by autoantibodies of several or multiple specificities.

Deposition of immune complexes with complement activation may not be sufficient to induce progression of acute glomerulonephritis to chronic glomerulonephritis.

Local factors may determine the outcomes of immune-complex mediated lupus proliferative glomerulonephritis.

Figure 1. Pathology of class IV diffuse proliferative lupus glomerulonephritis

(A) H&E shows frequent round capillary luminal deposits, referred to as hyaline thrombi. In addition, numerous large confluent subendothelial deposits, known as wire-loop lesions are present. (B) Masson trichrome stain can highlight the wire-loop subendothelial deposits (bright red). (C) Immuno-fluorescence in proliferative lupus glomerulonephritis shows numerous glomerular capillary loop deposits, wire loops by light microscopy, and extraglomerular deposits involving tubular basement membranes and interstitium. (D) EM shows numerous subendothelial and mesangial deposits with well-developed basement membrane duplication. There are scattered subepithelial deposits with basement membrane response in the form of spikes. (Composite photographs from Atlas of Medical Renal Pathology by Dr. Stephen M. Bonsib, published in 2013 by Springer, New York. Permission to use these photographs has been obtained from both the publisher and Dr. Bonsib).

Figure 2. Course of a patient with SLE initially showing DNA antibody consecutive serum samples

Exacerbation of illness with very high fever and increased proteinuria coincided with appearance of DNA in serum and disappearance of antibody. DNA was not detected after acute illness had subsided. (8)

Figure 3. Renal histopathology of NZM2328 mice

(A and B) The kidney sections of a 12-month-old female mouse with no proteinuria. Shown are glomeruli with normal cellularity (A) and normal tubules without interstitial cellular infiltrate, fibrosis, or tubular atrophy (B). (C and D) Renal pathology of a 12-month-old female mouse with diffuse proliferative glomerulonephritis without severe proteinuria shows hypercellular glomeruli with many exudative mononuclear cells within the capillary lumen (C) and normal tubules without interstitial nephritis (D). (E and F) The kidney sections of a 6-month-old female mouse, which was morbid-bound and sacrificed, show severe and diffuse glomerulonephritis with high chronicity index. In (E), glomeruli show expansion of the mesangial space with increased matrix material, designated m, and one necrotic glomerulus designated n. In (F), dilated tubules, d, proteinaceous casts, c, and interstitial fibrosis, f, are shown (hematoxylin and periodic acid Schiff stain with 200X magnification (15).

Figure 4. Characterization of congenic lines NZM.C57Lc1 and NZM.C57Lc4

(A) NZM.C57Lc1 and NZM.C57Lc4 congenic lines were derived by replacing the genetic intervals in NZM2328 with those from C57L/J (filled bars). The genetic intervals with SLE susceptibility genes in NZM2328 delineated by informative microsatellite markers are shown (open bars). Chromosome intervals are not drawn to scale. (B) Development of severe proteinuria in females of NZM2328 and NZM.C57Lc4 but not in those of NZM.C57Lc1. Upper panel: Incidence of severe proteinuria in each strain of mice. Lower panel: Kinetics of proteinuria development. (C) Marked reduction of circulating anti-dsDNA, antinuclear, and antinucleosome Ab in NZM.C57Lc1 and NZM.C57Lc4 congenic lines in comparison with NZM2328. Staining of HeLa cell nuclei by DAPI are seen in A, C, and E. The right side of the figure shows the presence of ANA in the sera of NZM2328 (B) but not in the sera of NZM.C57Lc1 (D) and NZM.C57Lc4 (F). Although not shown, the majority of the sera from C57L/J were not positive for ANA. On the bottom, frequencies of the presence of anti-dsDNA, antinuclear, and antihistone/DNA Ab in these strains are summarized. (D) Anti-dsDNA Ab in sera and kidney eluates from NZM2328, NZM.C57Lc1, NZM.C57Lc4, and C57L/J females at 11–12 mo. Abs to dsDNA were present in sera of NZM2328 and they were enriched in their kidney eluates. These Abs were rarely detected in the sera and the kidney eluates of the other three strains. P-values indicate significant differences of the respective strain as compared with NZM2328 (Modified from figures in 14).

Figure 5. Interactive model for the pathogenesis of SLE

This model makes the assumption that environmental triggers act on susceptible hosts. The triggers act on both genes controlling immune responsiveness and genes for end organ damage. These are two independent yet interactive pathways. Pathway I leads to the generation of autoantibodies and autoreactive effector T cells. Pathway II provides autoantigens and/or soluble mediators that influence immune responsiveness. Pathways I and II interact at several levels as indicated by III. These interactions can lead to end organ damage. In the context of this chapter the end organ is the kidney and the autoimmune response is the production of autoantibodies to multiple autoantigens that forms immune complexes to be deposited in the kidney. (19)

Figure 6. Fine mapping of *Cgnz1* region on mouse chromosome 1 and the human homologous region

(a) Six intrachromosomal recombinant lines, R314-1, R286-7, R290-2, R507-2, R503-10 and R1205 were generated by generation of heterozygous mice from (R27×N2M2328)F1 × (R27×N2M2328)F1 and the heterozygous mice were bred to generate homozygous lines. R27 has a c1 segment covering both *Sle1a* and *Sle1b* from C57L/Jc1. 314-1 has the whole region of *Sle1a* from C57L/Jc1. R503-10 has only part of the *Sle1a* from C57L/Jc1. R286-7 lacks both *Sle1a* and *Sle1b* from C57L/Jc1. R290-2 contains part of the *Sle1b* from C57L/Jc1. R507-2 has the whole *Sle1b* region from C57L/Jc1. R1205 is a newly generated line with early mortality and it contains 2–19 genes depending on further delineation of the recombinant sites. The red rectangle depicts the region of 1.34 Mb where *Cgnz1* locates. The gray areas denote the intervals in N2M2328 that are replaced with that of C57L/J (modified from [19]). (b) Five intrachromosomal recombinant lines, R314-1, R286-7, R290-2, R507-2 and R503-10 were generated and a female cohort of these lines was followed for 12 months. All but R507-2 were susceptible to cGN. These results provide a genetic region located between microsatellite markers Mit148 and Mit206. This region is within the *Sle1b* region. The data show that the other two *Sle1* subregions, that is, *Sle1a* and *Sle1c*, are not important for cGN. Female mice of R314-1, R286-7, R290-2, and R503-10 develop cGN in larger percentages in comparison with the parental line N2M2328. Almost 100% of R314-1 and R503-10 develop severe proteinuria by seven months of age (modified from [19]). (c) The mouse *Cgnz1* locus is nearly perfectly homologous to a 1.6 Mb locus on

human chromosome 1. The assembled human locus is inverted with respect to the mouse locus; nonetheless the exact gene order is preserved. Dotted lines connect the human and mouse homologs at the boundaries of the mouse and human *Cgzi1* loci (19).

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Figure 7. Characterization of cohorts of R27 female mice showing lack of progression of IC-mediated aGN

(A) Severe proteinuria was markedly reduced in R27 (left). Sera from terminal bleeds from NZM2328 or at 12 mo of age from other strains showed that anti-dsDNA and ANA were markedly reduced in R27. (B) cGN was rarely seen in R27. Acute GN (aGN) was detected in the majority of the older R27 mice. Despite the presence of ICs and complement, renal function of R27 was normal. Horizontal bars show means. **, $P < 0.01$; ***, $P < 0.001$ (20).

Figure 8. Morphological studies on NZM2328 and R27 kidneys

(A) PAS staining of R27 kidneys and H&E staining of NZM2328 kidneys. R27 females without aGN (1) and with aGN (2) are shown. PAS-positive material in the glomerular capillary lumen is shown in 2. Kidneys of NZM2328 at age of 8 wk and NZM2328 at 36 wk are shown in 3 and 4, respectively. Bar, 50 μ m. (B) Immunofluorescent staining for detecting IgG subclasses and C3 in the glomeruli. Bars, 100 μ m. (C) Semiquantitative estimates of IgG subclass staining of kidneys of NZM2328 and in R27 at different ages are shown. Horizontal bars show means. *, $P < 0.05$ is denoted by. (D) Transmission electron micrographs of the kidney of a 12-mo-old R27 mouse (left photo; bar, 1 μ m) and that of an 8-mo-old NZM2328 mouse (right photo; bar, 5 μ m). In the left photo, the arrowheads show subendothelial deposits. Despite the massive deposits, the foot processes are well preserved. In right photo, effacement of the foot processes is shown by the arrows (20).