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The Role of Anti-DNA Antibodies in the Development of Lupus Nephritis: An alternative, or complementary, viewpoint?

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Lupus nephritis (LN) is probably the clinical feature most closely associated with autoantibody (autoAb) production in SLE, ever since Koffler's seminal observation of complement-fixing IgG depositing in glomeruli of patients with LN.[1] Together with the observation that double stranded (ds) DNA ab levels often correlate with disease activity, the notion emerged that these abs were pathogenic.[2] However, subsequent studies failed to show a clear correlation between circulating anti-DNA Ab and the type or severity of renal disease in individual patients.[3] One explanation for this apparent contradiction may lie in that circulating immunoglobulins (Ig) are distinct from those eluted from nephritic kidneys. The latter were found to (i) react with multiple auto-antigens, including cell surface, matrix, and glomerular basement membrane (GBM) antigens, (ii) have higher isoelectric points, and (iii) have different avidities to DNA, while serum Ab are more directed at DNA and nucleoproteins and display less cross-reactivity.[4–8] The broad antigenic specificities of kidney-eluted autoAb may explain some of the disease variability seen in LN patients. Interestingly, although anti-DNA Ab represent an important fraction of deposited Ig in the kidneys of LN patients, the majority of the latter do not bind DNA.[8,9] Thus, it was unclear how anti-dsDNA Ab (really more broadly anti-nuclear Ab) are involved in the initiation and propagation of LN; the search for the answer to this question has engaged the lupus research community for some time, and remains a challenge highly relevant to the management of SLE patients.[10]

The pathogenic properties of anti-dsDNA Ab have been attributed to *(i)* glomerular binding of circulating preformed complexes of nucleosomes and anti-DNA IgG,[11–13] *(ii)* direct binding to the GBM or cell surface antigens by cross-reactive anti-DNA Ab,[14–17] and *(iii)* the obligatory requisite of anti-DNA Ab being bound to chromatin or nucleosomes in order to bind to the GBM or mesangial matrix *in situ*.[18–24] The latter mechanism stems from morphological studies demonstrating chromatin and IgG co-localizing in glomerular sub-

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endothelial and sub-epithelial electron-dense deposits (EDS) in nephritic kidneys from lupus patients and lupus-prone murine models.[25,26]

In this issue, Pedersen *et al*. summarize an extensive series of elegant studies from their group exploring the pathogenesis of murine and human LN.[27] They report that intra-glomerular EDS containing extracellular chromatin fragments are *in vivo* targets of nephritogenic Ab and describe a two-step process in the pathogenesis of LN in lupus-prone NZB/W F1 mice,[28–33] beginning with mild mesangial proliferation and culminating in membranoproliferative nephritis with immune complex deposition.[34] The authors propose that human LN follows a parallel progressive pattern from WHO class II LN (deposition of immune complexes in the mesangium) to class IV (diffuse proliferative GN). Disease progression in this model is attributed to a loss of renal DNase I activity, in conjunction with an increase in matrix metalloproteinase (MMP) 2 activity.[35–38] Specifically, the loss of DNase I leads to deficient chromatin fragmentation, resulting in larger chromatin fragments being retained in the GBM and becoming accessible to immune cells via activation of MMPs.[39–43]

The centrality of DNAse I in the pathogenesis of LN postulated by Pederson *et al*. can be examined directly in genetically engineered murine strains. Indeed, the mechanistic relationship between DNase I deficiency, the development of anti-dsDNA Ab, and nephritis was further investigated by Jacob *et al*. using a *dnase I*-deficient mouse. The absence of the enzyme did result in higher titers of Ab directed against nucleosomes; however the majority of *dnase I*-deficient mice only developed subclinical nephritis.[44] Jacob *et al.* went on to compare this model to NZB/W F1 mice, and noted that the *dnase I*-deficient mice failed to develop the high anti-dsDNA ab titers exhibited in the spontaneous lupus strain. Moreover, *dnase-I* does not coincide with any of the known lupus susceptibility loci in NZB/W F1 mice. Furthermore, as noted by Pedersen *et al.*, previous attempts to rescue murine models of LN by exogenous administration of DNase I yielded contradictory results, and has also not been a successful approach in LN patients.[45,46] Therefore, while Pedersen *et al.* propose that basement-membrane bound chromatin in LN is not accessible to extracellular DNAse as an explanation for the lack of efficacy with exogenous administration,[27] one also needs to consider the possibility that the loss of DNase I observed in murine LN does not directly contribute to the pathogenicity of anti-dsDNA Ab (at least not initially) and it is perhaps rather a consequence of complex ongoing immune mechanisms as nephritis progresses. Interestingly, upregulation of MMPs is not limited to LN and can occur in several types of acute or chronic kidney injury, also in the absence of glomerular Ab deposition.[47]

Pedersen *et al.* further describe the role of heparin as a chaperone protein that enhances chromatin degradation and prevents large chromatin fragments from being presented to the immune system,[27] an effect mediated via its affinity for histone tails.[48,49] Indeed, treatment of NZB/W F1 mice with heparin delayed anti-dsDNA Ab production and reduced ab titers and the number of EDS.[50] Although Naparstek could not replicate this finding in NZB/W F1, treating MRL-lpr/lpr mice with low-dose heparin starting at 6 weeks of age similarly resulted in fewer mice developing nephritis and a reduction in glomerular subepithelial EDS.[51] However, Faaber *et al.* previously reported that the beneficial effect of heparin is mediated by its being a sulfated glycosaminoglycan, which (like the GBM) is a target of

anti-dsDNA Ab cross-reactivity.[52] Indeed, van Bruggen *et al.* confirmed that heparin interferes with the binding of immune complexes to the GBM, delaying the development of LN in the MRL-lpr/lpr strain.[53] Similarly, Naparstek *et al.* showed that heparin inhibits binding of DNA to human (patient serum) and mouse (MRL-lpr//lpr kidney eluted) anti-dsDNA Ab.[51] Desulfation of the heparin abolished the cross-reactivity between anti-dsDNA Ab and heparin. Therefore, whether the dominant mechanism underlying the therapeutic effect of heparin is the capacity to enhance chromatin breakdown or rather its structural similarity with GBM components which interferes with anti-DNA Ab binding remains to be determined.

Pedersen *et al.* emphasize that chromatin antigenic material is required in EDS, to which anti-nuclear Ab bind.[27] Nevertheless, left mostly unexplained by this model are the many studies indicating that pathogenic anti-DNA antibodies can directly cross-react with glomeruli in binding interactions *not* mediated by nuclear antigens. Ex vivo induction of nephritis in isolated rat kidneys, which were perfused with either purified polyclonal IgG fractions from sera of LN patients or a pathogenic murine anti-DNA mAb, could be prevented by DNA pre-incubation.[16] Electron microscopic examination of kidney tissue in this model did not demonstrate significant anatomic changes, suggesting that EDS are not required for anti-dsDNA Ab binding. Similarly, Budhai *et al.* demonstrated that the strong binding of anti-dsDNA Ab derived from patients with active nephritis to isolated rat glomeruli was unaffected by pre-treatment with DNase.[54] More recently, Krishnan *et al.* observed in LN the presence of autoAb within the EDS despite the absence of chromatin.[55] In addition, they found that only anti-DNA Ab that bind to components of the GBM, but not antibodies that bound nuclear material alone, were able to form immune deposits, activate complement, and induce proteinuria. Finally, perhaps even more intriguing is the study by Waters *et al.* describing a congenic lupus model, NZM.C57Lc4, which develops chronic glomerulonephritis and severe proteinuria in the absence of circulating ANA, anti-dsDNA, and anti-nucleosome Ab, or detectable glomerular EDS.[56]

If chromatin is not necessary for glomerular binding, what is the target antigen for nephritogenic lupus autoAb?[57–59] While space constraints prevent a more detailed treatment of this topic, it is important to point out, as mentioned earlier, that elution studies from LN kidneys showed that anti-dsDNA Ab only account for 10%–20% of kidney deposited IgG overall.[60] Hence, IgG not recognizing DNA represents the majority of nephritogenic Ab.[7,8,61–66] Several alternative targets for pathogenic antibodies in lupus kidneys have been identified, including laminin, α-enolase, annexin AI, annexin II, and α-actinin. Removal of anti-laminin Ab by extracorporeal immunabsorption has beneficial effects in both murine models and patients with LN.[57] Bruschi *et al.*, utilizing micro-elution and proteomic approaches on laser capture micro-dissected kidney biopsies from patients with LN, identified antibodies to podocyte α-enolase and annexin AI.[67] The same Abs could also be detected in circulation and were altered with therapy.[67] Furthermore, analysis of sera from LN patients revealed Ab against annexin A2.[68] Another glomerular antigen, α-actinin, had been described as a major target for cross-reactive anti-dsDNA Ab in murine lupus models and human disease.[62,69–76] Most recently, Seret *et al.* demonstrated that sera from LN patients distinguish themselves from those with other autoimmune diseases by circulating

anti-cell-membrane Ab that predominantly target α-actinin.[76] Interestingly, the binding of anti-cell-membrane Ab was not affected by pre-treatment with *DN*ase I.

Conclusions

Our understanding of the pathogenic role and molecular targets of nephritogenic anti-dsDNA Ab within lupus kidneys continues to evolve. There is strong scientific support for both major views, i.e. chromatin mediated binding and direct cross reactivity to glomerular components. Perhaps both models are correct, and binding to chromatin and direct cross-reactivity may be involved, yet temporally separated. It is conceivable that cross-reactive anti-dsDNA Ab bind to glomerular structures and cause inflammation, which leads to the formation of EDS. In turn, anti-nucleosome Ab bind to EDS and further amplify the inflammatory process. Another option to reconcile these views is to postulate that while both pathways are possible, a given mechanism is the most relevant for a particular pathogenic antibody, mouse strain, or point in time. Perhaps the optimal way to advance in our treatment of LN is to target both of these pathogenic mechanisms. Moreover, murine models, being an imperfect phenocopy of human LN, can yield controversial data. To achieve more definitive results, researchers should always consider studying several murine models side by side. Last but not least, kidney tissue from LN patients should continue to be methodically studied by applying the ever-advancing imaging and molecular biology technologies available to researchers, to further advance our understanding of this major complication of SLE.

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