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Murine and Human Lupus Nephritis: Pathogenic Mechanisms and Theoretical Strategies for Therapy

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Summary: Lupus nephritis is one of the most serious manifestations of systemic lupus erythematosus, and represents one of the criteria implemented to classify systemic lupus erythematosus. Although studied for decades, no consensus has been reached related to the basic cellular, molecular, and immunologic mechanism(s) responsible for lupus nephritis. No causal treatments have been developed; therapy is approached mainly with nonspecific immunosuppressive medications. More detailed insight into disease mechanisms therefore is indispensable to develop new therapeutic strategies. In this review, contemporary knowledge on the pathogenic mechanisms of lupus nephritis is discussed based on recent data in murine and human lupus nephritis. Specific focus is given to the effect of anti–double-stranded DNA/antinucleosome antibodies in the kidneys and whether they bind exposed chromatin fragments in glomeruli or whether they bind inherent glomerular structures by cross-recognition. Overall, the data presented here favor the exposed chromatin model because we did not find any indication to substantiate the anti–double-stranded DNA antibody cross-reacting model. At the end of this review we present data on why chromatin fragments are expressed in the glomeruli of patients with lupus nephritis, and discuss how this knowledge can be used to direct the development of future therapies.

Keywords: Systemic lupus erythematosus, murine/human lupus nephritis, DNase I, heparin, chaperone molecules, therapeutic strategies

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by various aberrant clinical and biological parameters.1–4 A characteristic phenomenon in SLE is the presence of autoantibodies against double-stranded DNA (dsDNA), histones, nucleosomes, and chromatin.1,5,6 Renal accumulation of antinuclear antibodies by direct binding to intrinsic renal antigens, or in complex with chromatin antigens, induces severe kidney inflammation analogous to a type II or type III immune-mediated hypersensitivity reaction.7–10 To understand the nature of the processes that account for lupus nephritis and to develop new specific treatment modalities, we need to determine the nature of the renal target structures for anti-dsDNA antibodies and the processes that account for their exposure. Since 1957, the year anti-dsDNA antibodies were discovered in an autoimmune context,9,12 they have been linked to SLE3,4,13,14 and to lupus nephritis.7,15 International consensus has concluded that this autoantibody family is central to the pathogenesis of lupus nephritis.16,17 However, how these antibodies participate in the pathogenesis of lupus nephritis has been and remains controversial.7 The reason for this is simple. Many patients produce anti-dsDNA antibodies, however, of these patients, many do not develop lupus nephritis. Therefore, a unique property must exist among the antibodies that make them nephritogenic, or, as an alternative, all anti-dsDNA antibodies have nephritogenic potential, but this is manifest only in individuals in whom the chromatin fragments, the target for anti-dsDNA antibodies, are exposed and accessible in glomeruli.18

These alternatives have resulted in two main directions in the study of the pathogenesis of lupus nephritis. One is dominated by evidence that the anti-dsDNA antibodies cross-react with intrinsic renal antigens, such as phospholipids,19–21 laminin or the extracellular matrix,22–25 entactin,26 α-actinin,27 annexin II,28 ribosomal P protein,29 vimentin,30 or others. Whether the antilaminin antibodies detected in the urine of lupus nephritis patients31,32 really cross-reacts with DNA was not investigated, however, in other studies, such cross-reactions have been suggested.33,34 Lupus nephritis may develop only in patients with such cross-reacting anti-dsDNA antibodies.

In the alternative model, antibodies comprising the whole spectrum of specificities of dsDNA, as found in chromatin fragments, such as elongated or highly bent DNA,31,32 may initiate lupus nephritis, but only when
chromatin fragments are exposed in the glomeruli. This is not considered in the Witebsky criteria that classify a disease as autoimmune in nature.\textsuperscript{33} These criteria require three types of evidence of the pathogenicity of an autoimmune factor: direct evidence from the transfer of a pathogenic antibody and/or a T cell, indirect evidence based on replication of the autoimmune disease in experimental animals, and circumstantial evidence from clinical parameters. If the alternative chromatin model is correct then an additional Witebsky criterion may include showing that chromatin fragments are exposed in affected organs.

Central to understanding these two models is identifying the characteristics of a pathogenic anti-dsDNA antibody, and determining the origin and characteristics of cross-reacting renal antigens and/or chromatin fragments retained in the glomeruli and targeted by anti-dsDNA antibodies. Linked to this is defining the exact role of the silencing of renal DNase I in progressive lupus nephritis.\textsuperscript{34–36} This is potentially important because silencing \textit{DNASE1} gene expression is central to chromatin exposure in kidneys.\textsuperscript{37,38} Both the cross-reacting and the chromatin models are attractive, and provide a basis to explain how anti-dsDNA antibodies may initiate and maintain lupus nephritis.

In this review, we present data that favor the exposed chromatin model. Experimental and observational data developed in the autoimmune, lupus-prone (NZB × NZW)F1 mouse strain will be compared with analyses of renal biopsy specimens from patients with lupus nephritis. The data are discussed in the context of new causal therapy modalities that are expected to eventually confirm the chromatin model.

**SYSTEMIC LUPUS ERYTHEMATOSUS: ONE OR SEVERAL DISEASES?**

The etiology of SLE is unknown. Moreover, one may raise the provocative question as to whether human SLE is one disease entity, or a mixture of individual, etiologically unrelated organ manifestations as defined by the American college of Rheumatology\textsuperscript{3} or the Systemic Lupus International Collaborating Clinics classification criteria\textsuperscript{4} for SLE. The classification criteria do not appear to reflect a common pathogenic process, so it is not clear how genetic aberrancies and biomarkers can be associated with SLE when SLE represents such a divergent mixture of phenotypes. For example, evidence of autoimmunity to nucleosomes, particularly to the individual components of nucleosomes, such as native (ds)DNA and histones, is an important diagnostic criteria for SLE.\textsuperscript{3,4,39} In addition, autoantibodies to dsDNA have the potential to induce nephritis.\textsuperscript{40,41} However, although anti-dsDNA antibodies have a strong pathogenic potential in SLE, this potential appears to be related only to lupus nephritis (see studies by Seredkina et al,\textsuperscript{7} Krishnan et al,\textsuperscript{26} Van Bruggen et al,\textsuperscript{42} and Berden et al\textsuperscript{43}), lupus dermatitis,\textsuperscript{44–46} and possibly certain forms of cerebral lupus.\textsuperscript{47–49}

**MURINE AND HUMAN LUPUS NEPHRITIS: NEW INSIGHTS**

We studied the evolution of lupus nephritis by serial examination of kidneys of lupus-prone (NZB × NZW) F1 and observed a two-step process in the pathogenesis of murine lupus nephritis. First, a mild mesangial nephritis developed simultaneously with the appearance of anti-dsDNA antibodies. Later, the disease progressed to a membranoproliferative nephritis with chromatin-IgG immune-complex deposition in the glomerular basement membrane (GBM). As the disease progressed, the \textit{DNASE1} gene was silenced, followed by a profound increase of proteinuria.\textsuperscript{34}

In human lupus nephritis, it is not clear whether classes II through IV represent different directions of lupus nephritis, or if the natural course of lupus nephritis is to progress from one class to another, similar to the steady progression seen in the (NZB × NZW)F1 mouse. Mesangial proliferative nephritis (class II) generally has been considered a mild form without progression, and with a 10-year renal survival rate of 100%.\textsuperscript{50} However, two recent studies assessing the course of class II lupus nephritis showed progression from class II to class III or IV despite treatment. Lee et al\textsuperscript{51} found progression from class II to class III or IV in 5 of 15 patients over a mean of 5 years, and, earlier, Tam et al\textsuperscript{52} described poor prognosis, reported as progression in 9 of 19 patients originally diagnosed with class II nephritis. Although these were small studies, and the exact progression rate of class II lupus nephritis has yet to be determined, these data support the continuous progressive model in at least some patients with lupus nephritis.

**Murine Lupus Nephritis**

Given the fact that nephritis is a serious manifestation of SLE,\textsuperscript{40,41,53} it is important to determine by which pathways anti-dsDNA antibodies act as pathogenic factors. Parameters that historically have been regarded as important in determining the nephrogenicity of anti-dsDNA antibody subpopulations are antibody avidity for DNA, specificity for unique DNA or nucleosomal structures,\textsuperscript{41,52,54–57} as well as cross-reactivity with inherent renal or non-nucleosomal DNA molecules.\textsuperscript{23,24,27,58–62}

The murine data were reviewed recently.\textsuperscript{2,7,63} In one central study, we focused on the pathogenic processes in kidneys taken at time intervals from lupus-prone (NZB × NZW)F1 mice. For these studies we developed high-resolution techniques that provided evidence
that nephritogenic anti-dsDNA and antinucleosome antibodies selectively recognize intraglomerular extracellular chromatin fragments in vivo. These chromatin fragments appear as electron-dense structures (EDS) by electron microscopy and are associated with the GBM. Autoantibody deposits detected in vivo are localized strictly to these structures.

EDS in glomeruli were first described by Comerford et al in 1968, and confirmed in 1975 and 1979 by Dillard et al and Ben-Bassat et al, respectively. These studies did not characterize the content of EDS. However, according to recent findings, it is fair to suggest that the three pioneering studies performed by Comerford et al, Dillard et al, and Ben-Bassat et al represent high-resolution studies on the nature of immune complexes seen in glomeruli in lupus nephritis. In line with this, in our studies IgG bound in vivo in EDS. This observation lead us to hypothesize that EDS contained an ectopic target structure for the nephritogenic anti-dsDNA antibodies because we assumed that intrinsic renal antigens are not electron-dense. This assumption derives from the fact that in Goodpasture syndrome, the nephritis component of the syndrome is caused by binding of anticollagen autoantibodies to collagen 4 within the GBM. This binding appears linear by direct immunofluorescence, not granular as in lupus nephritis, and EDS have not been reported in Goodpasture syndrome. Because antibodies bound within EDS and not outside, this argued against the cross-reaction model, and suggested that murine lupus nephritis is initiated and maintained by antibodies recognizing structures exposed in chromatin fragments.

Central Role of Anti-dsDNA Antibodies, Renal DNase I, Chromatin Fragments, and Matrix Metalloproteases in the Evolution of Murine Lupus Nephritis

Anti-DNA antibodies, renal DNase I, and matrix metalloproteinase (MMP) messenger RNA levels and enzyme activities are instrumental in early and late events in murine lupus nephritis. Early phases of nephritis are associated with chromatin–IgG complex deposition in the mesangial matrix, which correlates with the appearance of anti-dsDNA antibodies and clinically silent or mild mesangial nephritis. Subsequently, renal DNase I message and enzyme activity increases, whereas MMP2 message and enzyme activity increase. A reduction of renal DNase I levels is coincident with deficient fragmentation of chromatin from dead cells and the retention and accumulation of chromatin in the GBM. Similar observations have not been described in other experimental nuclease deficiencies. Increased expression of MMP2 may be explained by chromatin stimulation of Toll-like receptors in, for example, dendritic cells. MMPs are collagenases with the potential to disintegrate basement membranes, and this could facilitate deposition of large immune complexes in the GBM.

What Does IgG Target in the Glomeruli of Lupus-Prone Mice?

Our early analyses to elucidate the nature of the targets for nephritic anti-dsDNA antibodies were founded on the hypothesis that only anti-dsDNA antibodies that cross-react with inherent glomerular structures were pathogenic. To prove this we used high-resolution techniques to trace where and to what antigens nephritogenic anti-dsDNA antibodies bound in glomeruli. These techniques included transmission electron microscopy, immune electron microscopy (IEM), co-localization IEM, and co-localization terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling (TUNEL) IEM, in addition to classic immunologic assays, measurements of apoptosis in renal cells, and studies of renal endonucleases. Although we anticipated finding evidence for the cross-reacting model, the results instead showed that IgG antibodies in lupus nephritis targeted chromatin fragments irrespective of whether they were in the mesangium or GBM. In vivo–bound IgG co-localized with the following: structures that bound experimental dsDNA antibodies, histones, and transcription factors in vitro, and with TUNEL–positive material that contained nicked DNA. IgG molecules eluted from the kidneys were specific for DNA, histones, and nucleosomes.

We did not observe in vivo–bound IgG outside EDS, or in association with regular basement membrane structures, as is the case in Goodpasture syndrome. Because antibodies bound within EDS and not outside, this argued against the cross-reaction model, and suggested that murine lupus nephritis is initiated and maintained by antibodies recognizing structures exposed in chromatin fragments.

HUMAN LUPUS NEPHRITIS: THE SAME PATHOGENESIS AS FOR MURINE LUPUS NEPHRITIS?

Our studies of human lupus nephritis were based on these findings from murine disease. We analyzed biopsy specimens from patients with lupus nephritis using the same strategies that had been applied to murine lupus nephritis (Table 1 shows the comparative analytic approaches in murine and human lupus nephritis). Transmission electron microscopy and IEM showed that EDS were present in the mesangial matrix and...
GBM of class IV biopsy specimens. Glomerular IgG was strictly confined to these EDS. Co-localization IEM showed that when experimental antibodies against dsDNA, histones, transcription factor–associated TATA-binding protein, and cyclic adenosine monophosphate response element-binding protein were added to sections of human kidney, they co-localized with glomerular in vivo–bound IgG autoantibodies. One problem with the co-localization assays is that the experimental antibodies, similar to true autoantibodies, may be cross-reactive. The results from co-localization IEM were supported by an antibody-independent assay, co-localization TUNEL IEM (Table 1). This approach showed that in vivo–bound IgG was binding to structures that contained nicked DNA, similar to murine lupus nephritis.

One striking observation that emerged from the murine studies was the selective silencing of the renal DNase I gene. In human kidney biopsy specimens we observed an analogous silencing of renal DNase I, together with accumulation of chromatin fragments in the mesangium and GBM. DNase I is the dominant renal endonuclease and accounts for 80% of the total endonuclease activity in the kidneys. In mice, the loss of DNase I endonuclease activity was associated with a marked reduction in chromatin fragmentation, and the subsequent accumulation of large chromatin fragments in glomeruli. Because this event occurred in the kidneys, and DNase I and other endonuclease activities were normal in other organs, it seemed reasonable to assume that exposed chromatin fragments found in glomeruli derive from the kidneys themselves.

MURINE AND HUMAN LUPUS NEPHRITIS: TRANSLATING PATHOGENESIS TO THERAPY

The data obtained in our studies of murine and human lupus nephritis provide an approach to understand basic molecular and immunologic processes accounting for antibody-mediated nephritis in human SLE. Notably, all elements of the mechanisms that account for murine lupus nephritis have been shown to be operational in human lupus nephritis. The pathogenesis of the chromatin model may be simplified to a two-step process. First, early anti-dsDNA antibodies accumulate in the mesangium and create a local and silent inflammation. After this, the renal DNase I gene is silenced, followed by reduced fragmentation of chromatin from dead and dying cells. Therefore, instead of silent clearance of chromatin, chromatin is retained in glomeruli and binds to basement membranes and matrices as a complex with IgG. This will promote more severe inflammation and, consequently, the progression of lupus nephritis into severe organ failure.

This model suggests that preventing the silencing of the renal DNase I gene and/or preventing chromatin binding to basement membranes and matrices may be relevant therapeutic approaches for human lupus nephritis. Maintaining renal DNase I activity may hamper progressive lupus nephritis, whereas interfering with chromatin binding may prevent the development of both mesangial and progressive nephritis (Fig. 1).

THERAPY AIMED TO PREVENT SILENCING OF THE RENAL DNASE I GENE: BACKGROUND INFORMATION AND THERAPEUTIC STRATEGIES

Aberrations in DNase I expression have been associated with SLE and the production of antinuclear antibodies at least since 1968. Since then, reduced expression of the DNase I enzyme or mutations in the DNASE1 gene have been linked to SLE. Our discovery of an acquired silencing of the renal DNASE1 gene in the context of lupus nephritis that was followed consistently by deposition of large chromatin fragments in complex with IgG antichromatin antibodies in the GBM and mesangial matrix is consistent with these early observations. Loss of renal DNase I may be the

<p>| Table 1. Analytic Approach to Study Loci and Composition of Immune Complexes in Murine and Human Lupus Nephritis |</p>
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<th>Assay</th>
<th>Murine Lupus Nephritis</th>
<th>Human Lupus Nephritis</th>
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<tr>
<td>Antibodies bound in glomeruli in vivo</td>
<td>Confocal microscopy/ DIF/IHC</td>
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<td>Immune complex characterization</td>
<td>TEM, IEM</td>
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<td>Analyses of TLR7-TLR9, MMP2, and MMP9</td>
<td>Protein expression in situ/qPCR/IHC</td>
<td>Protein expression in situ/qPCR/IHC</td>
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<td>Proinflammatory cytokines</td>
<td>qPCR, IHC, WB</td>
<td>qPCR, IHC</td>
</tr>
<tr>
<td>Renal DNase I and Trap 1 expression</td>
<td>qPCR</td>
<td>qPCR</td>
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<td>Determination of affinity between chromatin fragments and membrane structures</td>
<td>Surface plasmon resonance</td>
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Abbreviations: DIF, direct immunofluorescence; IHC, immunohistochemistry; TEM, transmission electron microscopy; qPCR, quantitative polymerase chain reaction; WB, Western blot; Trap 1, tumor necrosis factor–receptor–associated protein-1.
adverse event that promotes progression of (class II) lupus nephritis.

DNase I is an endonuclease that cleaves DNA in chromatin. It exerts its dominant role in degradation of chromatin in the context of cell death. Traditionally, it has been linked to apoptosis, but it also plays a role in necrosis. In addition, DNase I also is secreted, so it could have a role in the digestion of extracellular DNA. For example, Nagata and Kawane point out that undigested DNA in macrophages, whether autologous or viral, activates the innate immune system and causes strong inflammation, resulting in inflammatory diseases.

One way to analyze the effect of DNase I in lupus nephritis would be to inject the enzyme and to determine if this reduces exposure of chromatin in the kidneys. This has been tested in both experimental animals and in patients with lupus nephritis. One promising result was published by Macanovic et al. They injected DNase I in young prenephritic (NZB × NZW)F1 mice and observed that disease progression was retarded. Treatment of nephritic mice reduced levels of proteinuria and serum creatinine, however, these observations were not pursued. In another study, injection of DNase I in (NZB × NZW)F1 mice did not improve early or late stages of murine lupus nephritis. In a human study performed by Davis et al., patients with lupus nephritis classes III and IV were given intravenous or subcutaneous recombinant human DNase I. This treatment did not show any effect on kidney function or disease activity. These findings show that exogenously administered DNase I has little or no influence on chromatin fragmentation in dying renal cells, and suggest that intracellular DNase I is responsible for the safe and effective degradation of chromatin in dying cells.

The reason why extracellular DNase I may not be effective for degradation of extracellular chromatin could be explained by two facts. First, chromatin possesses a fairly high affinity for laminin and collagens. Second, extrapolating from information provided by DNase footprinting assays, the interaction of chromatin with proteins may protect chromatin from being degraded enzymatically.

This assumption is strengthened by the strong therapeutic effect of DNase I in the chronic lung disease cystic fibrosis. Extracellular chromatin from massive neutrophil extracellular trap (NET)-osis of recruited neutrophils contributes to increased viscosity of purulent secretions in the respiratory tract. Inhalation of recombinant human DNase I reduces viscosity within minutes, and is a standard chronic treatment for cystic fibrosis to reduce exacerbations. The reason why treatment with exogenous DNase I is efficient in clearing extracellular chromatin in cystic fibrosis, but not beneficial in SLE and lupus nephritis, may lie in the fact that chromatin in cystic fibrosis is embedded in mucus and are more accessible for enzymatic degradation, in contrast to basement membrane–bound chromatin in lupus nephritis.

Exactly how DNase I expression is regulated is not understood, despite the fact that the enzyme was described in 1946 by McCarty. Nonetheless, this information will be critical for developing DNase I as a therapy for lupus nephritis. We recently approached this problem based on the observation that silencing of renal DNase I generally follows mesangial nephritis. This suggested that proinflammatory cytokines could regulate DNase I. We found that hypoxia and tumor necrosis factor α, but not interferon-γ, interleukin (IL)-1β, IL-6, or IL-10, possess the potential to up-regulate DNase I expression in a human tubular cell line.

Figure 1. The exposed chromatin model of lupus nephritis. We propose that the overall mechanism in the exposed chromatin model is similar for both human and murine lupus nephritis. In general, a two-step process occurs. (A) First, a mild mesangial nephritis develops together with the appearance of anti-dsDNA antibodies, followed by membranoproliferative nephritis with chromatin–IgG complex deposition in the GBM. (B) As lupus nephritis progresses into membranoproliferative nephritis, the renal DNase I enzymatic activity is silenced, with subsequent accumulation of chromatin fragments in the glomeruli, and increased proteinuria. Therapy 1 and 2 in the figure refer to the therapy models described in the text. Briefly, therapy 1 is aimed at preventing the down-regulation of DNase I gene expression, whereas therapy 2 targets chromatin structure to prevent binding of chromatin fragments to the mesangial matrix and the GBM.
HEPARIN FUNCTIONS AS A CHAPERONE FOR CHROMATIN AND ENHANCES ENZYMATIC CHROMATIN DEGRADATION

Several factors are involved in chromatin remodeling. For example, it has been shown that chaperone molecules such as nucleosome assembly protein 1, nucleoplasmin, and Hsp90 induce changes in chromatin conformation through the process of assembly/disassembly of chromatin fragments, and in so doing increase the susceptibility of chromatin to enzymatic degradation. In the compact structure of chromatin, digestion with DNase I can degrade linker DNA, but not the core nucleosomal DNA, which is protected by the histone core octamer and by nonhistone proteins. The DNA wrapped around the histone core octamer therefore is not digested. Similarly, the core histones H2A, H2B, H3, and H4 have been shown to resist enzymatic degradation. Trypsinization removed only the N and C terminal histone tails protruding from the nucleosome surface, while the core globular parts and core nucleosome structure remained largely intact. Chaperone molecules, however, can open even this restricted core structure, making it accessible for proteinases and nucleases. As such, chaperone molecules could be therapeutic candidates to enhance enzymatic degradation of ectopic chromatin in vivo, but may not be tolerated in doses necessary to achieve the desired effect.

As an alternative to chaperone therapeutics, it may be possible to exploit the potential of heparin to enhance enzymatic degradation of chromatin fragments. Heparin has been shown to have an effect on chromatin structure similar to that of certain chaperone molecule classes. Heparin binds tightly to histone tails and changes the net surface-oriented charge of the nucleosome. It also may interfere with binding of chromatin fragments, and even immune complexes consisting of chromatin fragments and IgG, to structures within the GBM and the mesangial matrix. These data were obtained using plasmon resonance analyses of chromatin fragments’ affinity for laminins and collagens. Isolated DNA did not bind to these structures. Heparin binds the trypsin-sensitive solvent-phase N and C terminal tails of core histones. The interaction of heparin and core histones increases the sensitivity of nucleosomes and chromatin for nucleases. Furthermore, heparin disrupts the nucleosome structure, and, linked to this effect, increases enhancer–promoter communication by disassembling the chromatin structure.

In other words, heparin may have two beneficial effects in lupus nephritis. First, by facilitating enzymatic degradation of chromatin, heparin may preclude exposure of chromatin to the immune system in vivo, and thereby prevent or reduce the production of pathogenic, chromatin-specific autoantibodies and the accumulation of immune complexes in kidneys. In addition, the chaperone effect of heparin on chromatin structure may preclude binding of chromatin fragment–IgG complexes to central structures in basement membranes and matrices, such as laminin and collagen. The latter process has been tested experimentally by Van Bruggen et al, who showed that interaction of heparin or heparin analogs with immune complexes containing nucleosomal antigens prevents the binding of these immune complexes to the GBM. Importantly, they further showed that nephritis in the MRL/lpr mouse model of lupus nephritis was delayed by treatment with heparins. Their data are consistent with the idea that heparin prevents binding of immune complexes to the GBM and also presumably to the mesangial matrix.

We therefore performed in vitro and in vivo analyses to determine the effects of heparin on enzymatic chromatin degradation, the kinetics of anti-dsDNA antibody production, and on the progression of murine lupus nephritis. These studies showed that heparin increased the sensitivity of chromatin to DNase I digestion (Fig. 2A). Likewise, proteinase digestion of chromatin was faster in the presence of heparin.

These in vitro results then were compared with the effects of heparin in (NZB × NZW)F1 mice. The kinetics of anti-dsDNA antibody production, deposition of chromatin fragment–IgG complexes in GBM, and proteinuria were followed up. The data showed delayed anti-dsDNA antibody production and reduced antibody titers (Fig. 2B). This was accompanied by reduced chromatin fragment–IgG complex accumulation in the GBM and the mesangial matrix. Importantly, these effects occurred at doses below those needed for heparin’s anticoagulant activity.

PREVENTING THE BINDING OF CHROMATIN FRAGMENTS TO GBM OR MESANGIAL MATRIX

In these studies we used surface plasmon resonance to determine if heparin could prevent nucleosome binding to laminin and collagen. The data showed that heparin not only prevented binding of nucleosomes to GBM components in vitro (Fig. 3), but that heparin promoted dissociation of these complexes from laminin and collagen after stable binding. Similarly, binding of nucleosomes in complex with IgG
antichromatin antibodies to membrane components also was inhibited by heparin. In addition, corresponding results were obtained when comparing data from this experimental system using either of the laminin or collagen structures. Notably, isolated DNA had no affinity for these GBM structures, and sets of IgG-chromatin antibodies including anti-dsDNA antibodies did not bind to laminin, as has been reported previously.\textsuperscript{127} These data are consistent with data from other investigators who showed that nucleosomes bind GBM in kidneys in the context of lupus nephritis,\textsuperscript{73,128,129} and that this binding could be inhibited by heparin and heparinoids.\textsuperscript{126,130}

In summary, the beneficial effects of heparin on lupus nephritis are enhanced degradation and elimination of chromatin fragments, reduced anti-dsDNA antibody production in vivo, and prevention of chromatin–IgG complex binding to basement membrane structures. Taken together, all of these effects of heparin may be regarded as ex juvantibus confirmation of the chromatin model of murine lupus nephritis. Because the role of chromatin fragment–IgG complexes in murine lupus nephritis seems to be highly analogous to early and late human lupus nephritis,\textsuperscript{36,38,64,94,131} the promising results of treatment of murine lupus nephritis with low-molecular-weight heparin should foster new therapeutic strategies and clinical trials along these lines (Fig. 1).

CONCLUDING REMARKS
Deeper insight into processes that are operational in progressive lupus nephritis is imperative to develop new causal therapy strategies. Currently, no such treatment modalities are available. As discussed here, we have increased our insights into specific aspects of lupus nephritis, and from these insights we have proposed ways to directly block specific pathogenic processes.
We suggest that studies to investigate these therapeutic approaches in human lupus nephritis are warranted.

REFERENCES

Murine and human lupus nephritis


