REVIEW

Future Perspectives on Pathogenesis of Lupus Nephritis

Facts, Problems, and Potential Causal Therapy Modalities

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Divergent incommensurable models have been developed to explain the pathogenesis of lupus nephritis. Most contemporary models favor a central role for anti-chromatin antibodies. How they exert their pathogenic effect has, however, endorsed conflicts that at least for now preclude insight into definitive pathogenic pathways. The following paradigms are contemporarily in conflict with each other: i) the impact of anti-double-stranded DNA (dsDNA) antibodies that cross-react with inherent renal antigens, ii) the impact of anti-dsDNA antibodies targeting exposed chromatin in glomeruli, and iii) the impact of relative antibody avidity for dsDNA, chromatin fragments, or cross-reacting antigens. Aside from these three themes, the pathogenic role of T cells in lupus nephritis is not clear. These different models should be tested through a collaboration between scientists belonging to the different paradigms. If it turns out that there are different pathogenic pathways in lupus nephritis, the emerging pathogenic mechanism(s) may be encountered with new individual causal therapy modalities. Today, therapy is still unspecific and far from interfering with the cause(s) of the disorder. This review attempts to describe what we know about processes that may cause lupus nephritis and how such basic processes may be affected if we can specifically interrupt them. Secondary inflammatory mechanisms, cytokine signatures, activation of complement, and other contributors to inflammation will not be discussed herein; rather, the events that trigger these factors will be discussed. (*Am J Pathol* 2016, 186: 2772–2782; <http://dx.doi.org/10.1016/j.ajpath.2016.06.026>)

Pathogenesis—Background and Present Status

Lupus nephritis represents the arrowhead among pathogenic manifestations in systemic lupus erythematosus (SLE),^{1–4} because it is dangerous, but also because it is scientifically challenging to comprehend its nature.^{1,5–7} This situation prevents us from developing therapy strategies that attack the basic pathogenic processes beyond today's therapy regimens.^{8–10} In the upcoming sections, contemporary status of the pathogenesis of SLE and lupus nephritis will be reviewed and discussed, and new causal therapy modalities will be suggested.

Pathogenesis of the Autoimmune Syndrome SLE—A Central Role for Anti-dsDNA Antibodies?

One central element when we discuss pathogenic processes in SLE is antibodies to double-stranded DNA (dsDNA) and chromatin structures. Anti-DNA antibodies were, however, first described in 1938 to 1939 in patients with

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infections,^{11–14} as reviewed by Rekvig.¹⁵ Approximately two decades later, they were described in the autoimmune syndrome SLE.^{16–19} Since then, their role in SLE, rather than in infections and malignancies,¹⁵ has been extensively studied in two contexts: diagnostics and pathogenicity.¹⁵ However, the pathogenesis of SLE is still poorly understood,^{20,21} and may even remain unclear because SLE, as classified today, is not linked to any unifying origin or pathogenic process. In fact, if we use the 1982 American College of Rheumatology classification criteria,²² and classify SLE by randomly selecting 4 of these 11 defined American College of Rheumatology criteria (a minimum requirement to classify a disease as SLE, according to Tan et al²²), theoretically 330 different clinical phenotypes embrace the term SLE. Does this mean that we, in fact, are dealing with a pile of unrelated disorders and manifestations that today is called SLE—and can we define biomarkers for SLE on this basis?

Recently, Pisetsky²³ characterized anti-dsDNA antibodies as quintessential biomarkers for SLE. In light of the heterogenic image of SLE, and also because anti-dsDNA antibodies occur at various frequencies in different forms of

cancers^{24,25} and infections,¹⁵ the statement that anti-dsDNA antibodies serve as a quintessential biomarker for SLE is difficult to comprehend. In fact, the first successful experiments that resulted in induction of anti-mammalian B helical (dsDNA) were performed by immunizing mice with dsDNA/chromatin fragments in complex with a peptide from *Trypanosoma cruzii* (Fus 1²⁶), or with a complex of polyomavirus T antigen and dsDNA/chromatin fragments (Figure 1A). The experimental details for this model have been described previously.^{27,28} In this experimental context, it is worthwhile to remember that the first discovery of anti-dsDNA antibodies in a natural context was achieved in sera from patients with bacterial infections six decades before the successful immunization experiments with complexes of mammalian chromatin and infectious-derived peptides,^{11–14} and also two decades before their discovery in SLE.¹⁵ Later, data demonstrating that pure chromatin fragments by themselves have the potential to induce diverse antibodies to chromatin have been demonstrated. These may represent antibodies to dsDNA, histones, non-histones, and complex determinants. The cellular processes responsible for these responses are, however, still poorly understood,¹⁵ although they are

Figure 1 Cognate interaction of nucleosome-specific B cells and infectious-derived (A) or autoimmune-derived (B) peptide-specific T cells. The figure presents classic hapten-carrier-like models to explain linked production of chromatin-reactive antibodies. **A:** In this model, chromatin plays the role as a hapten, whereas heterologous (infectious-derived DNA-binding proteins like polyomavirus large T antigen) peptides play the role as carrier protein. In this model, primed T cells recognize T-antigen–derived peptides presented by B cells specific for different nucleosome structures. This model describes two features typical for systemic lupus erythematosus (SLE), production of affinity-maturated anti-dsDNA antibodies and linked production of antibodies to dsDNA, histones, and nonhistone chromatin-associated proteins. However, in this context, the individual may not at all experience SLE—rather the individual may experience infection. The principal paradigm for the hapten-carrier models presented in this figure is based on strong experimental evidence (see *Pathogenesis of the Autoimmune Syndrome SLE—A Central Role for Anti-dsDNA Antibodies?* for details). **B:** A hapten-carrier-like model is presented where chromatin represent the hapten, whereas chromatin-derived peptides represent the carrier protein. At difference from the model in A, T-cell tolerance to nucleosomes is terminated. As in A, this model also describes linked production of antibodies reactive with chromatin constituents. In this situation, T-cell tolerance to nucleosomal proteins is terminated, and the immune response is truly autoimmune. The cognate interaction of chromatin-specific B cells and immune (A) or autoimmune (B) peptide-specific T cells may explain the origin of the comprehensive repertoire of chromatin-reactive IgG antibodies in human patients. Used with permission from Springer Science and Business Media.²¹ HLA, human leukocyte antigen.

assumed to be based on a T-cell–dependent immune response, as indicated in [Figure 1B](#).

The recent Systemic Lupus International Collaborating Clinics criteria did not solve this problem.²⁹ Experts on SLE implemented clinical and laboratory parameters based on their individual insight, experience, clinical data, and intuition.²⁹ These criteria therefore represent empirical, rather than scientific, approaches to understand SLE. From this, we cannot deduce the pathogenesis of lupus nephritis from the indistinct and poorly described pathogenesis of SLE per se. If lupus nephritis is one distinct disorder or not is presently unclear, and is discussed below.

Lupus Nephritis—A Single Disease Entity?

Theoretically, the approach to study the basic disease pathogenesis of lupus nephritis has a greater probability to succeed than to perform similar studies on the syndrome SLE.

However, there is currently no obvious reason to be optimistic because incommensurable models and contradicting data yet preclude international consensus. It is imperative to initiate new collaborations across the different models and paradigms to prepare consensus study protocols to solve the problem whether lupus nephritis is one disease entity with a dominant pathogenesis, or whether different pathogeneses are based on different incommensurable hypotheses, as indicated in [Figure 2](#). The contemporary

models of lupus nephritis are dependent on scientific interpretations of experimental data that emerge from different hypotheses. For each hypothesis, study protocols are selected and tailored to optimally analyze the validity of hypotheses. A theoretical model of these conflicting hypotheses and data sets is shown in [Figure 2](#). The emerging concepts and models developed from divergent hypotheses are clearly visible in the conflicting interpretations of the pathogenesis, as described by one set of researchers^{6,7} versus another set of researchers.^{1,2,30} The following discussion has one perspective: to define lupus nephritis pathogenesis and key points for specific, causal therapy strategies.

Pathogenesis of Lupus Nephritis—Incommensurable Models

There are no unifying concepts available to explain lupus nephritis as it appears in murine models or in humans with SLE. Some recently reviewed concepts^{1,7} claim that anti-dsDNA antibodies are pathogenic because they recognize exposed chromatin in the mesangium or in glomerular basement membrane ([Figure 3A](#)). Others indicate that antibodies target cross-reacting antigens that appear as normal constituents in glomeruli^{1,15} ([Figure 3B](#)), or that chromatin-IgG complexes derive from circulation.^{31–33} These models may be valid irrespective of whether the antibodies are induced by

Figure 2 Studies of a defined biological problem, like a disease pathogenesis, may result in incommensurable models. The study object, in our context lupus nephritis, promotes testable hypotheses to describe its pathogenesis. In the left part of the figure, a hypothesis A is developed, and it is tested by a strategy assumed to be relevant for the study. Then, the emerging data are interpreted, and translated into a pathogenic model A. In another context, the same study object may promote an alternative hypothesis, hypothesis B (right part of the figure). This alternative hypothesis is analyzed by another strategy still assumed to be relevant to solve the problem. Again, data are analyzed and interpreted as being a pathogenic model B. The realities of model A and model B, although intended to describe the same study object, differ in facts and consequences. Therefore, facts described in one experimental context cannot exclude that facts are somewhat different in another experimental context, although both models (A and B) are generated to solve the same problem. Thus, the incommensurable models A and B may promote further studies, but they may also split and disorganize scientific approaches in a way that hampers consensus. This relates also to the realities emerging from studies of the pathogenesis of lupus nephritis.

Figure 3 Pathogenicity of anti-dsDNA antibodies—do they target chromatin fragments or cross-reactive renal antigens? In **A**, DNA-specific B cells are stimulated by chromatin fragments and histone-specific T helper cells. The emerging anti-dsDNA antibodies bind exposed chromatin in glomeruli and initiate lupus nephritis. In this context, antibodies bind their homologous antigen(s). In **B**, the B and T cells are stimulated by chromatin. The emerging antibodies cross-react with normal inherent structures in glomeruli, and may induce a type II immune-mediated renal inflammation. Whether this inflammation is restricted to kidneys (solid line in **B**), or may also occur in lungs or other organs (dotted lines in **B**), in analogy to anti-collagen IV antibodies in Goodpasture syndrome, has not yet been investigated, but is principally possible. This is particularly relevant for anti-dsDNA antibodies that cross-react with laminin or entactin (see *A Possible Role of Cross-Reacting Anti-dsDNA Antibodies in Lupus Nephritis* for details). In **C**, the B cells specific for an inherent glomerular structure, like laminin or entactin, respond by producing cross-reactive anti-dsDNA/anti-chromatin antibodies. These antibodies may bind exposed chromatin fragments (**C**, left side) or homologous, inherent antigens in kidneys, lungs, and other organs (**C**, right side). In both situations, inflammation may be initiated, either as a type III immune-mediated renal inflammation (**A**) or as a type II immune-mediated renal inflammation (**B**). HLA, human leukocyte antigen.

chromatin fragments (Figure 3, A and B) or by a cross-reactive antigen (Figure 3C).

In the first model, we need to know the role of the dominant renal endonuclease DNase I in lupus nephritis because renal DNase I is almost completely lost during disease progression.^{1,34,35} This seems to reduce clearance of chromatin from dead cells, and to promote harmful accumulation of undigested chromatin in glomeruli, which are targeted by chromatin-reactive IgG antibodies, either *in situ* or in circulation.^{1,34,35} In the second model, we need not to worry about extracellular chromatin and renal chromatin metabolism, because

the antibodies are claimed to bind inherent renal structures.

Low Proportion of Anti-dsDNA Antibodies among Antibodies Eluted from Nephritic Kidneys—What Does That Tell Us?

In several studies,^{7,36–38} antibodies have been eluted from nephritic kidneys, and the relative amount of anti-dsDNA antibodies has been calculated to comprise up to 20% of the total eluted IgG.³⁹ This may indicate that dominant amounts

of IgG bind non-dsDNA antigens, like inherent renal antigens, or antigens released from resident cells.^{7,38–40}

A sound basis to discuss these assumptions and observations derives from an article published by Craft and Hardin⁴¹ in 1987: “Linked sets of antinuclear antibodies: what do they mean?” The meaning could be that complex structures, like chromatin, simultaneously stimulate B cells specific for individual components or complex structures presented by chromatin fragments. This would explain linked production of potentially large arrays of anti-chromatin antibodies other than those uniquely specific for dsDNA¹⁵ (Figure 1).

This idea was tested in our laboratory by injecting plasmids expressing the chromatin-binding polyomavirus BK large T antigen.^{27,28} Our prediction was that expressed T antigen bound host cell chromatin, and that chromatin–T antigen complexes were released from dying cells. Subsequently, they could stimulate a large repertoire of chromatin-specific B cells, and T-antigen peptide-specific T helper cells.^{27,28} The prediction was correct, as plasmid-injected mice simultaneously produced various antibodies to DNA, histones, and transcription factors in addition to T antigen in a timely linked manner (Figure 1A). This model is not fully dependent on non-self-proteins to activate non-tolerant T helper cell. As indicated in Figure 1B, true autoimmune T helper cells (herein specific for histone peptides in the context of human leukocyte antigen II molecules) provide the same effect as T-antigen–specific T helper cells described above, which again may explain a simultaneous production of various anti-chromatin antibodies.

This indicates that among the IgG eluted from nephritic kidneys, some bind dsDNA³⁹ but also that an (yet undetermined) amount of eluted IgG may bind other

Figure 4 Individual models of lupus nephritis pathogenesis lead to divergent causal therapy modalities. The principal structure of a glomerulus is presented with the mesangium on the left side and glomerular basement membrane on the right side. Line 1 represents a glomerulus section seen from the side. In line 2, complexes of chromatin fragments and IgG accumulate in the mesangial matrix as part of early nephritis. Mesangial nephritis is followed by down-regulation of renal DNase I, which promotes a dramatic progression of the disease, with accumulation of large chromatin fragments in complex with IgG in glomerular basement membrane (GBM; line 3). Because of loss of DNase I enzyme activity, chromatin from secondary necrotic cells is not appropriately degraded and accumulates instead in GBM in complex with IgG (line 4). In contrast to the model depicted in lines 2 to 4, anti-dsDNA antibodies may bind directly to components of the mesangial matrix and GBM (line 5). This would be equivalent to the binding of antibodies to collagen IV in glomeruli in context with Goodpasture syndrome (demonstrated in line 6). The chromatin model (lines 2 to 4) gives hints to causal, aim-directed therapy modalities. One approach is to elute chromatin-IgG complexes from the membranes by flushing the kidneys with chaperone molecules (line 7, see *Future Therapy—A Perspective*). This procedure has been shown to be beneficial for both mesangial as well as progressive lupus nephritis. A causal approach to treat progressive lupus nephritis may be to prevent loss of renal DNase I in the kidneys. This intervention would result in clearance of chromatin fragments from dead and dying cells (line 8). The cross-reactive model will most likely not be affected by these therapeutic approaches.

components of chromatin that are not tested for in the referred studies (Figure 3). Therefore, low proportions of eluted anti-dsDNA antibodies can theoretically not represent an argument for a dominant presence of non-chromatin-specific cross-reactive IgG antibodies. However, if some of these antibodies really are cross-reacting, this does not inform about the potential target antigens that really bound the antibodies *in vivo* (see below for further discussion of this problem).

Renal Targets for Nephritogenic Anti-DNA Antibodies—A Scientific Dilemma

Two models have been developed that might explain the nephritogenic effect of anti-dsDNA antibodies. These are discussed in detail below.

A Possible Role for Exposed Chromatin in Lupus Nephritis

Clearance capacity of apoptotic cells is claimed to be reduced in SLE.^{42–44} This is assumed to be harmful because chromatin is not cleared, but instead chromatin fragments, consisting of the basic nucleosome structure and a vast amount of non-histone proteins and RNA molecules,^{45,46} bind glomerular laminin and collagen by fairly high affinities.^{47–49} This is consistent with successive accumulation of chromatin fragments in the mesangial matrix and glomerular basement membrane *in vivo*³⁴ (Figure 4), as shown by the following studies.

By immune electron microscopy analyses applied to kidneys from mice and humans with lupus nephritis, we hardly observed lupus nephritis in absence of electron-dense structures¹ in the mesangial matrix, typical for mesangial nephritis, and in glomerular basement membrane, typical for membranoproliferative nephritis, respectively.^{50,51} These electron-dense structures were first described more than three decades ago, and at that time linked to lupus nephritis.^{52–54} Detailed analyses revealed that electron-dense structures contained chromatin fragments, as indicated by detection of terminal deoxy-nucleotidyl transferase dUTP nick end labeling—positive DNA, histones, and transcription factors.^{50,51} These chromatin fragments colocalized with *in vivo*—bound IgG. *In vivo* binding of IgG directly to regular glomerular basement membrane and matrix structures, which could indicate a cross-reaction with normal membrane/matrix structures, was not observed during these analyses.^{15,50}

The progression of the disease coincided with a nearby complete loss of renal *DNase I* gene expression and endonucleolytic activity.^{34,35,55,56} The expression of renal *DNase I* gene was selectively down-regulated among renal genes examined, and only in kidneys and not in other organs.³⁵ DNase I constitutes >80% of the total renal endonuclease activity. Accordingly, loss of this endonuclease resulted in

accumulation of large chromatin fragments in glomeruli, and correlated in time with progressive renal inflammation.^{35,50,57} Thus, chromatin-IgG complexes may be involved in both early and late stages of lupus nephritis. This contrasts the nephritic processes possibly induced by cross-reactive anti-dsDNA antibodies.

A Possible Role of Cross-Reacting Anti-dsDNA Antibodies in Lupus Nephritis

Another aspect that was considered to be important in the pathogenesis of lupus nephritis was the promiscuous tendency of anti-dsDNA antibodies to cross-react with non-DNA structures.^{30,58–60} This could imply that anti-dsDNA antibodies have the potential to cross-react with intrinsic renal structures.⁷

Why Did Cross-Reactivity Become a Focus in a Search for the Pathogenesis in Lupus Nephritis?

This idea evolved decades ago when scientists tried to understand the molecular and cellular origin of anti-dsDNA antibodies. During the 1970s and 1980s, target structures for naturally and/or experimentally induced anti-DNA antibodies were described, like double-stranded Z-DNA, natural and synthetic single-stranded DNA and dsDNA sequences, bent and elongated dsDNA, cruciform structures, RNA-DNA hybrid double strands, and mammalian B-helical dsDNA.^{61–64}

At the same time, scientists tried to understand if molecules with a certain degree of mimicry to DNA could induce anti-dsDNA antibodies,^{65–68} because mammalian dsDNA itself was considered nonimmunogenic.^{63,69} Indeed, many cross-reactions between DNA and non-DNA structures have been described.^{15,30} Thus, cross-reaction may have a twofold meaning in this context: an idea to understand the origin of cross-reactive anti-dsDNA antibodies that transformed into the idea to understand the role of cross-reacting antibodies in the pathogenesis of lupus nephritis.

Anti-dsDNA antibodies cross-react with a large spectrum of antigens to intracellular structures, renal cell membranes, or extracellular matrices.^{15,30} A common denominator for many of these studies is that anti-dsDNA antibodies bind inherent glomerular antigens whether they are part of membranes and matrices or released from resident renal cells.^{1,30}

Relative Avidity for the Structures Recognized by Nephritogenic Antibodies—Theoretical Thoughts

The Intrinsic Characteristics of Anti-dsDNA Antibodies

When we discuss the role of intrinsic affinity, avidity, and specificity of an anti-dsDNA antibody, we traditionally do not consider whether high or low avidity relates to

cross-reactivity between different DNA structures that are present in chromatin, or between DNA and non-DNA structures. We have not taken into account what is known among basic scientists who analyze DNA structures like elongated or highly bent B-helical or Z-helical dsDNA^{70,71} within chromatin or in protein-free DNA.¹⁵ Therefore, it is wise to consider what we mean by the term avidity applied to anti-dsDNA antibodies.

Avidity

A formal definition of avidity in the context of ligand interactions is the accumulated strength of multiple affinities of noncovalent binding interactions between a polyvalent structure and a polyvalent ligand. Thus, avidity is distinct from intrinsic affinity, which describes the strength of a single, monovalent interaction like the molecular forces involved in monomeric Fab-epitope interactions.^{72,73} Avidity is therefore the accumulated strength of multiple affinities and is much stronger than the sum of individual monomeric intrinsic affinities. How does this translate into the understanding of avidity in the context of anti-dsDNA antibodies and lupus nephritis?

This problem is complicated by the fact that antibody specificity and avidity are not well-defined entities. Mammalian dsDNA comprises different structures, like single-stranded (ss)DNA regions, Z-DNA, highly bent or elongated mammalian B-helical DNA, and cruciform structures.^{61,62,64} B cells recognize these structures with high precision.^{15,69,74,75} This may also mean that if an anti-dsDNA antibody is induced by elongated nucleosomal linker dsDNA, this will represent the specificity of that given immune response—and it may be of high avidity.

Assume that this antibody cross-reacts with bent dsDNA as in the Crithidia kinetoplast or in a plasmid used in Farr assays, and, if they are not heteroclitic,⁷⁶ the avidity may be lower. Does then this antibody have a phenomenologically high avidity (for elongated dsDNA) or low avidity (for cross-reactive bent dsDNA)?

That an antibody bind in high salt, as in the Farr assay, may not at all relate to avidity. The latter assumption derives from the fact that antibodies against Z-DNA, but not B-DNA, bind at high salt (2 to 4 mol/L NaCl).^{77,78} Thus, it is not clear whether antibodies that bind DNA in the Farr assay possess unique specificity for DNA structures shaped in high salt concentration, or have a high avidity. An overview of the forces involved in antibody-antigen interactions has previously been published.⁷³

It is in the context important to make clear that many antibodies, when binding chromatin, will bind to the DNA structure that fits best, because chromatin fragments present the whole universe of mammalian DNA structures.⁴⁵ Therefore, an antibody that binds in an enzyme-linked immunosorbent assay, but not in Farr, may still have a

high avidity for its target antigen—exposed chromatin structures.

From this, one may assume that all kinds of anti-dsDNA antibodies reactive with mammalian dsDNA as part of chromatin may be pathogenic, independent of the magnitude of avidity or intrinsic affinity as long as they bind accessible dsDNA in chromatin fragments like those exposed in kidneys in the context of lupus nephritis.¹⁵

Likewise, we do not know whether anti-dsDNA antibodies cross-react with renal antigens with high avidity, or at lower avidity than toward dsDNA in chromatin fragments. Their relative avidities may in fact direct the antibodies toward the structures recognized by the highest relative avidity. This may question whether heterologous (cross-reactive) or homologous (recognition of chromatin) binding of the antibodies determines their pathogenic impact.

Pathogenesis of Lupus Nephritis Linked to Cross-Reactive Anti-dsDNA Antibodies and to Chromatin Autoimmunity—Facts and Problems

Several models are generated to understand lupus nephritis, and they are all easy to comprehend. However, each model raises problems that need to be solved, preferentially by collaboration across the models.

The Cross-Reactive Model—Facts and Problems

There is no doubt that anti-dsDNA antibodies cross-react with structurally unrelated renal antigens^{15,30,61} (see also the thoughtful review by Goilav and Putterman⁷). However, several problems that may hamper the understanding of the effect of cross-reactive anti-dsDNA antibodies can be identified. Therefore, further investigations are required. For example, there is a tendency that each scientific report focuses on a new cross-reacting antibody without emphasizing the impact of the other reported cross-reactive antibodies in comparative studies.

Theoretically, the cross-reactive pattern of an anti-dsDNA antibody may not prevail in the body when the immune responses affinity mature. The heavy and light chain V-region structures are degenerate and structurally unstable.^{15,79,80} This implies that over time, the specificity may converge toward the immunogen with loss of cross-specificity^{15,80} because of stochastic somatic mutations.

Furthermore, is antibody avidity for the cross-reacting antigens (eg, dsDNA and entactin) similar? Will the highest avidity direct the antibodies to chromatin or to the renal antigen?

Relevant to the latter question is whether antibodies monospecific for the non-DNA cross-reacting antigen will bind in glomeruli on injection as, for example, purified biotinylated IgG antibodies? This experiment will inform

whether antibodies to cross-reactive non-DNA antigens can bind *in vivo* in a way similar as the Goodpasture-related anticollagen IV antibody. In many of the studies referred to above, antibodies bound *in vivo* in glomeruli were not eluted from kidney specimens. A central question in this context will consequently be whether a cross-reactive antibody population is present in the eluates, and whether the relative avidities for dsDNA and the cross-reactive antigen differ in magnitude. If so, high avidity for one of the ligands may in fact point at targeted antigen *in vivo*. For example, we observed that antibody avidity for dsDNA was higher in the eluted antibody population compared to the antibodies in circulation.⁸¹

In analogy to the pathogenesis of Goodpasture syndrome, where antibodies to collagen IV bind in basement membranes in glomeruli and alveoli,⁸² one may question whether antibodies that cross-react between dsDNA and laminin or entactin (see above) bind such antigens exposed in other organs (Figure 3B). There is some precedence for this model,⁸³ although deposits in the lungs were not analyzed in that study. This has, to our knowledge, not been investigated so far. Theoretically, antibodies that cross-react with dsDNA and laminin or entactin may bind in other organs where such structures are accessible.

If the cross-reactive antibody is the pathogenic factor in lupus nephritis, how should we then explain their role after successful treatment, when the kidneys restore their function while antibodies remain in circulation even at high titers?^{84,85} An alternative explanation would be that target structures for the antibodies (like chromatin) were cleared.

The Chromatin Model—Facts and Problems

There is no doubt that chromatin in complex with IgG is exposed in glomeruli during lupus nephritis.^{34,50,86,87} In that form, chromatin-IgG complexes initiate severe inflammation, as in lupus nephritis. Still, however, several theoretical problems are connected with this pathogenic model. What is the origin of extracellular chromatin, and why is chromatin exposed and accumulated in glomeruli? The indirect cause may be silencing of the renal *DNase I* gene expression,³⁵ which consequently may cause local accumulation of extracellular chromatin that binds glomerular membranes and matrices with high affinity, irrespective of whether in complex with IgG anti-chromatin antibodies or not.⁴⁷ We do not know the basis for the process that accounts for silencing of the *DNase I* gene expression. However, preliminary data may indicate that glomerular exposed chromatin derives from resident glomerular cells (studies in progress).

Future Therapy—A Perspective

Today, therapeutic intervention in lupus nephritis is not specific and not causal (several studies^{8,88,89} provide an

update on current therapy modalities). Before we can develop causal strategies, we need to know the basic, responsible pathogenic mechanism(s) in lupus nephritis. Figure 4 provides principals of the different models and possible development of causal therapy modalities. We can achieve this if scientists collaborate across the different models to determine which the correct ones are. If, for example, the exposed chromatin model is correct, then an obvious approach would be to promote up-regulation of the renal *DNase I* gene to increase clearance of chromatin in dying cells (Figure 4). It is, however, not sufficient to inject DNase I because intravenous or subcutaneous administration of DNase I has a limited effect,³⁵ probably because chromatin bound to membranes is relatively nuclease resistant.¹ This would not protect against mesangial nephritis, but against progression of the disease (Figure 4).

The reason why extracellular DNase I may not be effective for degradation of extracellular chromatin could be explained as follows. First, chromatin possesses a fairly high affinity for laminin and collagens.^{47,48} Second, extrapolating from information provided by DNase footprinting assays,^{90,91} the interaction of chromatin/dsDNA with proteins may protect chromatin from being enzymatically degraded.

Another approach would be to increase degradation of extracellular chromatin, and to prevent binding of chromatin-IgG complexes to membranes and matrices by infusing chaperone molecules like heparin. For example, heparin has the potential to inhibit binding of chromatin to membrane structures *in vitro* and *in vivo*, and to make the chromatin structure more sensitive to nucleases and proteases^{92,93} (Figure 4).

If, on the other hand, cross-reaction of anti-dsDNA antibodies represents the basis for a pathogenic process, this may be treated with selected peptides from either of the specific determinants. A thoughtful discussion is provided by Monneaux and Muller⁹⁴ and other researchers.^{95–97} If this approach is valid, we face the problem that many cross-reacting peptides are linked to lupus nephritis. Therefore, this therapeutic approach may represent a new version of individualized treatment.

Conclusions

Today, we are confronted with the situation that each of the described pathogenic mechanisms may both be true and both may explain lupus nephritis. However, they are not proved beyond doubt. It is in this context a problem that scientists confer themselves to their own hypotheses in an unreservedly subjective way. This generates the basis for incommensurable models. This critical comment relates to scientists involved in this research field, irrespective of which of the models they advocate: recognition of renal

targets by cross-reaction or antibody recognition of exposed chromatin in the kidneys.

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