Review

Lupus Nephritis

The Central Role of Nucleosomes Revealed

Elin S. Mortensen,^{*†} Kristin A. Fenton,[‡]
and Ole P. Rekvig^{†§}

From the Departments of Pathology^{*} and Biochemistry,[‡] Institute of Medical Biology, Medical Faculty, University of Tromsø, Tromsø; and the Departments of Rheumatology[§] and Pathology,[†] University Hospital of Northern Norway, Tromsø, Norway

Systemic lupus erythematosus (SLE) is an autoimmune syndrome characterized by autoantibodies to nuclear constituents. Some of these antibodies are diagnostically important, whereas others act as disease-modifying factors. One clinically important factor is autoantibodies against dsDNA and nucleosomes, which have overlapping diagnostic and nephritogenic impact in SLE. Although a scientific focus for 5 decades, the molecular and cellular origin of these antibodies, and why they are associated with lupus nephritis, is still not fully understood. A consensus has, however, evolved that antibodies to dsDNA and nucleosomes are central pathogenic factors in the development of lupus nephritis. In contrast, no agreement has been reached as to which glomerular structures are bound by nephritogenic anti-nucleosome antibodies *in vivo*. Mutually contradictory paradigms and models have evolved simply because we still lack precise and conclusive data to provide definitive insight into how autoantibodies induce lupus nephritis and which specificity is critical in the nephritic process(es). In this review, data demonstrating the central role of nucleosomes in inducing and binding potentially nephritogenic antibodies to DNA and nucleosomes are presented and discussed. These autoimmune-inducing processes are discussed in the context of Matzinger's danger model (Matzinger P: Friendly and dangerous signals: is the tissue in control? *Nat Immunol* 2007, 8:11–13; Matzinger P: The danger model: a renewed sense of self. *Science* 2002, 296:301–305; Matzinger P: Tolerance, danger, and the extended family. *Annu Rev Immunol* 1994, 12:991–1045) and Medzhitov's and Janeway's (Medzhitov R, Janeway CA Jr: Decoding the patterns

of self and nonself by the innate immune system. *Science* 2002, 296:298–300; Medzhitov R, Janeway CA Jr: How does the immune system distinguish self from nonself? *Semin Immunol* 2000, 12:185–188; Janeway CA Jr, Medzhitov R: Innate immune recognition. *Annu Rev Immunol* 2002, 20:197–216) distinction of noninfectious self (NIS) and infectious nonself (INS). The mechanisms leading to production of potentially nephritogenic anti-nucleosome antibodies and to overt lupus nephritis are interpreted in the context of these paradigms. (*Am J Pathol* 2008, 172:275–283; DOI: 10.2353/ajpath.2008.070563)

SLE and Lupus Nephritis

SLE is a prototype of an autoimmune syndrome that is characterized by various clusters of organ manifestations, and most organs in the body may be involved. SLE is classified according to 11 American College of Rheumatology criteria.¹ These involve organ manifestations and hematological and immunological parameters. Any combination of 4 of these 11 criteria is sufficient to classify SLE.¹ SLE is further characterized by a wide variety of organ-specific and organ-nonspecific autoantibodies. Of these, some are diagnostically important, whereas others may act as disease-modifying factors.^{2–4} One clinically important factor is autoimmunity to double-stranded (ds)DNA and nucleosomes, which has overlapping diagnostic and pathogenic impact. *In situ* binding of anti-dsDNA antibodies is associated with lupus nephritis,^{3,5} cerebral lupus,⁶ and lupus dermatitis.^{2,7} It is therefore important to understand the cellular and molecular origin of anti-dsDNA antibodies, and why they are associated with organ affections in SLE.

Supported by The Health and Rehabilitation Organization Norway, The Northern Norway Regional Health Authority Medical Research Program (grants SFP-100-04, SFP-101-04), and the University of Tromsø (milieu support given to O.P.R.).

Accepted for publication November 21, 2007.

Address reprint requests to Elin S. Mortensen, Department of Pathology, Institute of Medical Biology, The Medical Faculty, University of Tromsø, N-9037 Tromsø, Norway. E-mail: elin.mortensen@fagmed.uit.no.

Lupus nephritis is one of the most serious manifestations of SLE. This organ manifestation has traditionally been characterized by the World Health Organization classification criteria, which focuses on histological parameters. This classification system of lupus nephritis has recently been revised under the auspices of International Society of Nephrology and Renal Pathology Society.^{8,9} The organ disease is separated into six different classes from subclinical (class I, mild proteinuria) to end-stage disease (class VI). A central classification criterion is detection of immune complexes in glomerular basement membranes and in the mesangial matrix. This demonstrates that autoimmunity plays a major role in the pathogenesis of lupus nephritis. Whether glomerular-bound antibodies are part of immune complex deposits or directly bound to inherent renal structures has been an unsolved issue and is not mentioned in the World Health Organization classification system or in the revised classification criteria.

Lupus Nephritis and the Role of Antibodies to DNA and Nucleosomes

Shortly after their detection in 1957,^{10–12} antibodies to dsDNA were associated with renal manifestation of SLE, and anti-dsDNA antibodies have been eluted from affected glomeruli.^{13–17} At the time when the nephritogenic potential of antibodies to dsDNA was revealed, their binding in glomeruli was logically claimed to depend on extracellular DNA. This DNA was thought to be bound *in situ* in glomeruli where it was targeted by the antibodies. This assumption derived from two facts: DNA bound glomerular collagen^{18,19} and the antibodies were specific for DNA.^{13,20}

This model has, however, been difficult to validate by experimental results and is today critically challenged by alternative models implying that antibodies bind to cross-reacting glomerular antigens such as α -actinin, laminin, or cell surface structures.^{17,21–29} Thus, data from different types of experiments and analytical strategies have resulted in different models explaining how anti-DNA antibodies induce nephritis. However, although the models are attractive, none have been validated beyond any doubt, although the dominant specificity of nephritogenic antibodies for dsDNA may point at the most obvious target structures in nephritic kidneys—nucleosomes released from dead cells.

One problem with the cited literature is that most of the current models are explained relative to the way the experiments were performed. Generally, dual specificity of a given antibody does not reveal the real target molecule(s) in a pathophysiological context. For example, the fact that anti-dsDNA antibodies eluted from kidneys cross-react with non-DNA/nucleosome glomerular structures, such as laminin^{14,30} or α -actinin,^{17,21} does not at all inform about the nature of the *de facto* target structures *in vivo*.

Thus, there is currently no definitive, firm, and objective distinction that separates nonpathogenic from pathogenic antibodies.^{3,4,31,32} One experimental result illustrates this: an antibody against nucleosomes may bind

glomeruli when in complex with nucleosomes, whereas noncomplexed antibodies do not bind.³³ This may be one example of glomerular antibody binding because the homologous antigen is made available, not because of a unique property of the antibody (see below). Thus, there must exist cellular processes and/or available molecular structures that determine anti-dsDNA antibody binding in the kidney and, thereby, their nephritogenic potential. Basically, irrespective of which parameters are important, release or exposure of relevant antigens recognized by nephritogenic anti-dsDNA antibodies must be crucial events making target molecules available for the antibodies,³ if the central antigens are not naturally available as, eg, glomerular basement membrane structures. To solve these problems, we must consider the cellular and structural origin of the immune response to dsDNA and nucleosomes and trace the biochemical nature and origin of target structures for such induced, potentially nephritogenic antibodies.

In this review, antibodies to dsDNA and nucleosomes are discussed in the context of their origin, fine molecular specificity, and nature of their glomerular target structures. The surprising perception of these lines of experiments, observations, and considerations is that there exist no principal nephritogenic subpopulations among affinity-maturated antibodies to dsDNA and nucleosomes. Their nephritogenic potential is determined by release and subsequent *in situ* binding of nucleosomes or chromatin fragments in glomerular vascular membranes and in the mesangial matrix or by forming complexes in circulation. Only in such situations will antibodies bind in glomeruli and exert their pathogenic activity.³³

Origin of Nephritogenic Anti-Nucleosome Antibodies—Specificity of B Cells

Although antibodies to dsDNA were discovered 50 years ago,^{10–12} the processes responsible for their induction *in vivo* are still poorly understood. Because DNA and nucleosomes have been regarded as weak immunogens,³⁴ a dogma evolved that such antibodies are induced by cross-reactive antigens rather than by DNA or by nucleosomes (see below).^{35,36} The observed manifold cross-reactions of monoclonal anti-dsDNA antibodies supports this notion.^{36–38}

This is further evident from the elegant study of Wellmann and colleagues.³⁹ They used site-directed mutagenesis to systematically revert the somatic mutations of monoclonal anti-dsDNA antibodies from SLE patients and determined the changes in antigen-binding pattern.³⁹ The data demonstrated that high-affinity antibody binding to nucleosomes and to surface structures of apoptotic cells were acquired by the same somatic mutations that generated high-affinity dsDNA binding. Fully reverted antibodies with germ-line heavy chain variable (VH) regions did not bind DNA but phospholipids, such as phosphatidylserine.³⁹ A similar study by Li and colleagues⁴⁰ demonstrated a comparable transformation in antibody specificity. By substituting a key arginine residue with glycine in the variable region of an anti-DNA

Clinical impact of antibodies produced against dsDNA

Figure 1. A model to indicate how affinity maturation may affect the diagnostic and pathogenic impact of induced anti-dsDNA antibodies. Although short-lived stimuli result in antibodies that should be regarded as transient epiphenomenons, antibodies resulting from recurrent or sustained stimulation by immunogenic DNA (nucleosomes) may have strong clinical impact. (Modified from Moens U, Rekvig OP: Molecular biology of BK virus and clinical and basic aspects of BK virus renal infection. Human Polyomaviruses—Molecular and Clinical Perspectives. Edited by Khalili K, Stoner GL. New York, Wiley-Liss, 2001, pp 359–408).

transgene, they observed reduced affinity for dsDNA, and complete reversion of this antibody to germline configuration enhanced affinity for phosphatidylserine. Many anti-DNA antibodies show cross-reactions with phospholipids,³⁶ and some can bind to apoptotic cells,^{41,42} most likely through an interaction with phosphatidylserine that is exposed on the surface of apoptotic cells.^{43,44} Whether phosphatidylserine is able to induce antibodies that may somatically mutate toward dsDNA without participation of DNA in secondary immune responses is still unproven.

On the other hand, numerous reports have demonstrated that antibodies to dsDNA can be induced by experimental immunization with dsDNA, provided it is in complex with an immunogenic carrier protein.^{4,45–50} Consistent with this model is the demonstration of nucleosome- and histone-specific T cells in both murine and human SLE, with the potential to provide cognate help for DNA-specific B cells.^{51–55}

It is clear from such observations that immunogenicity of DNA depends on complex formation with immunogenic peptides. This creates a complex able to stimulate DNA-specific B cells and peptide-specific T cells in analogy to a hapten-carrier model for induction of anti-hapten antibodies. This has been experimentally demonstrated by immunizing mice with mammalian dsDNA in complex with the *Trypanosoma cruzii*-derived DNA-binding peptide Fus 1^{45,46} or with the DNA-binding polyomavirus encoded large T antigen (T-ag).^{49,50,56} Immunization of normal mice with bacterial DNA in complex with methylated bovine serum albumin resulted in anti-dsDNA antibodies with a dominant specificity for the bacterial DNA used as immunogen, whereas binding to mammalian dsDNA was less pronounced.^{48,57} In all these experimental systems, the immunization regime resulted in lupus-like nephritis with proteinuria and glomerular IgG deposits.^{45,48,58} Thus, for a long time DNA was erroneously regarded as a poor immunogen, and experimental immunization did not result in anti-DNA antibodies similar to those spontane-

ously produced in murine and human SLE.^{34,59–63} This paradigm changed with the results cited above.

Given that stimulation of the immune system by immunogenic DNA is sustained, the IgG antibodies affinity mature and gain higher clinical impact. This process is principally outlined in Figure 1. These considerations relate to the ability of B cells to respond to dsDNA. The crucial point in this advanced insight was that responsiveness to dsDNA was primarily controlled by T cells with specificity for certain proteins associated with DNA.^{45,49,50,54,64–66} These may be either infectious-derived, like the Fus 1 peptide or T-ag (see above), or purely autologous, like nucleosomal proteins.^{51,53–55} This distinction needs further clarification.

Autoimmunity versus Immunity to Nucleosomes—Specificity of T-Helper Cells

B cells may have a lower capacity to develop tolerance to nucleosomes than T cells, as is evident from the fact that it is relatively easy to induce high-affinity anti-dsDNA antibodies by immunizing normal mice with DNA-carrier protein complexes.^{45,49,50,67,68} Such autoimmune B cells may bind autologous nucleosomal DNA and present peptides from, eg, nucleosome-bound viral proteins, to T cells committed to respond to such peptides. The result of cognate interaction between autoimmune B cells and viral peptide-specific T cells may in fact be production of somatically mutated autoantibodies similar to those seen in SLE. Thus, in this situation, true SLE-related autoantibodies may be produced on a nonautoimmune background.

However, humoral autoimmunity to components of nucleosomes may not be the only outcome of this self (dsDNA)-nonself (viral protein) model for stimulation of autoimmune B cells. B cells that recognize the DNA component of viral protein-nucleosome complexes will process the complex and present peptides derived from

both autologous proteins (like histones) and viral proteins. In this situation, T cells committed to respond to viral peptides will recognize peptide-HLA II complexes. Provided sufficient co-stimulatory signals, this will result in mutual stimulation of nucleosome-specific B cells and viral peptide-specific T cells. The former may proceed into plasma cells producing antibodies to nucleosomes, whereas the latter may transform into effector T cells and secrete interleukins (ILs), particularly IL-2 in this context.^{54,55} This establishes a scenario that might influence tolerance of autoimmune T cells with specificity for autologous nucleosome-derived peptides. Activation of autoimmune T cells by linked presentation of self and nonself molecules have been documented in several experimental systems.^{54,55,69–73}

The most likely explanation for this is that IL-2, produced by, eg, responder viral DNA-bound peptide-specific T cells, by itself terminates histone-specific T-cell anergy by inducing proliferation of the anergic T cells.^{74,75} This process is outlined in Figure 2.^{54–56} In this situation T cells may further expand in response to the presented histone peptide-HLA class II complexes.

Thus, humoral autoimmunity to dsDNA or nucleosomes may be induced provided cognate contribution of T-helper cells specific for nonself DNA-bound proteins or of T cells specific for autologous proteins constitutively present in nucleosomes (like histones). Both pathways may be operational *in vivo*, as is evident from the studies of Andreassen and colleagues,^{54–56} analyzing the contribution of viral T-ag-specific T cells in providing help for DNA-specific B cells in SLE patients and normal human individuals, whereas the contribution of autoimmune, nucleosome-specific T cells have been described in SLE by Datta and colleagues.^{51,52,76}

Functional T cells specific for histones or for nucleosomes have been characterized in both murine and human SLE,^{51,53,77} demonstrating that autoimmune T cells undergo antigen-specific activation *in vivo*. Although formal requirements for activation of autoimmune nucleosome-specific T cells have been determined, the basis for termination of T-cell tolerance *in vivo* in SLE has not been established. In one situation autoimmune T cells may be activated when nonself and self molecules are processed and presented in the context of HLA class II molecules by the same antigen-presenting cells. On the other hand, autoimmune nucleosome-specific T cells may be directly activated by exposed necrotic chromatin *in vivo*. This pathway is still unproven but may be explained by Matzinger's danger model (see below).^{78–80}

Necrotic Nucleosomes—Potential Inducer and Target Structures for Nephritogenic Autoantibodies

Although formal cellular and molecular requirements for anti-DNA antibody production have been revealed (see above),^{53,56,76} we still lack relevant insight into the requirements for these processes *in vivo* in the context of

Figure 2. A model to explain how a nonself molecule such as the DNA-binding polyomavirus T antigen, when physically linked to histones through complex formation with nucleosomes, may initiate processes that result in induction of antibodies to DNA. Expressed T antigen forms complexes with nucleosomes.^{56,68} DNA-specific B cells binding and processing such complexes may simultaneously present peptides derived from T antigen and histones. T cells not tolerant for T antigen will respond, produce IL-2, and proliferate. IL-2 has the potential to induce proliferation of anergic cells and terminate the state of anergy.^{74,75} During this process, anergic histone-specific T cells receive two different stimuli, the first as a nonspecific, bystander stimulus by IL-2 produced by T-antigen-specific responder T cells and the other by a conventional, antigen-specific stimulus by presented histone peptides. In this situation, T-cell anergy for histones may be terminated. Thus, if DNA-specific B cells present both T antigen and histones to responder T cells, activation of autoimmune, histone-specific T cells and production of anti-DNA antibodies will be the final result. If this process is sustained, affinity maturation of the anti-DNA antibodies may transform them into high-affinity anti-dsDNA antibodies as a consequence of somatic mutations, particularly within the variable CDR regions of the heavy chain^{65,67,97} (modified from Andreassen et al⁵⁴). T cells marked in red indicate they are activated.

spontaneous SLE. However, in recent years, ideas^{78–83} and results^{84–88} have provided new perspectives to understand the basis for such autoimmune processes *in vivo*.

Basically, we should consider two apparently incompatible paradigms: Matzinger's^{78–80} danger model, leaving the distinction between self versus nonself irrelevant, and Janeway's and Medzhitov's^{81–83} distinction between INS and NIS. A relevant example to approach this discussion is the biological consequence of transformation of apoptotic to secondary necrotic cells and release of (necrotic) chromatin by disrupting apoptotic blebs.

Clearance of apoptotic cells is reduced in SLE.^{5,86,87} This defect transforms the silent, noninflammatory re-

removal of apoptotic cells into release of chromatin structures meant to be hidden within blebs until taken up by phagocytic cells. Because chromatin particles generated in the context of apoptosis are normally encapsulated within blebs presenting eat me (friendly as opposed to danger) signals, this ensures that they are cleared in a fast and discreet way. Retained and exposed tissue-distributed chromatin may break this silence and become a problem for the immune system by initiating inflammation,^{85,86} because they, according to Matzinger's danger model,^{78–80} may provide signals (by eg, CpG motifs) that may initiate dendritic cell maturation and exposure of co-stimulatory signals (when CpG binds Toll like receptor 9). In addition, apoptosis-induced changes in chromatin may enhance immunogenicity and pathogenicity of chromatin *in vivo*.⁸⁹ Thus, danger signals and secondary structural alterations linked to apoptosis and necrosis may contribute to inflammation and activation of dendritic cells. The dendritic cells may in other words become armed and effective antigen-presenting cells with the potential to activate relevant nucleosome-specific T cells provided they are not deleted in the thymus. However, Andreassen and colleagues^{54,55} have clearly demonstrated that even immunologically normal individuals harbor autoimmune nucleosome-specific T cells in their circulation and that they are easily activated to be functional T-helper cells. Is there really a need for a nonself, nucleosome-bound component to activate such autoimmune T cells?

In Matzinger's⁷⁹ context, the immune system may be more concerned with structures that provide danger signals, than with those that are foreign. In that sense, NIS molecules may be as harmful as INS structures. Thus, purely autologous, secondary necrotic structures provide danger signals (like Toll-like receptor engagement, up-regulation of co-stimulatory molecules, and engagement of appropriate autoimmune T cells). This scenario may result in dendritic cell maturation and presentation of, eg, nucleosome-derived peptides for relevant T cells. These may be fully activated and provide cognate help for DNA-specific B cells.^{78,79,84,86} This is consistent with the fact that Toll-like receptors bind autologous structures like the CpG motif, present in all mammalian DNA, and, eg, HSP60 and HSP70 (see Matzinger⁷⁹ for discussion).

Thus, there is no obvious need for a self-nonself hapten-carrier model to initiate and maintain humoral and cellular autoimmunity to nucleosomes, at least not in SLE. That model may, however, explain the appearance of anti-nucleosome antibodies in healthy individuals who process apoptotic cells appropriately. This response is, however, transient in nature and will die out on termination of the infection.

The Danger Model—Predicting Pathogenic Autoimmunity

In the viral infection-dependent self-nonself (hapten-carrier) model, the discrimination between INS and NIS is set aside, as B cells recognize the self component, while processing and presenting the nonself component to

nontolerant T cells (see Figure 2). This intermolecular cognate interaction provides a basis for initiation and affinity maturation of humoral autoimmunity. This model is operational^{149,54,66,73,90} and provides an exception from Medzhitov's and Janeway's^{81–83} distinction between INS and NIS to control autoimmunity, in which indeed INS is the factor that renders NIS immunogenic. We therefore predict that autoimmunity induced by complexes of INS and NIS is nonpathogenic simply because the structures initiating the autoimmune response are not available for antibody binding in peripheral tissue because of their normal processing and removal. The autoimmune response is transient because the complex formation of INS and NIS is terminated when the infection is healed. Thus, this model does not relate to impaired clearance of dead cells. That defect confers to the danger model.

The probability that autoimmunity induced in context of Matzinger's^{78–80} danger model is pathogenic is higher just because of the nature of the model. The immunogenic (necrotic) chromatin is retained in tissue, provides danger signals, arms the innate immune system, and subsequently induces affinity-maturated IgG antibodies to (the necrotic) nucleosomes. Retained necrotic chromatin in peripheral tissue (like in glomeruli) represents the partner that renders such induced IgG autoantibodies pathogenic because they are available *in vivo* for antibody binding. Thus, we see the Janus face of secondary necrotic chromatin, which activates the innate and, in the next round, the adaptive immune system and represents the available target structures for the induced autoantibodies, as was also previously discussed.^{5,87,91} In consequence, secondary necrotic chromatin induces and binds the antibodies and is therefore the central partner in the evolution of the pathogenic process. Lupus nephritis may well be an organ disorder that fits with this paradigm.

Pathogenic Antibodies to dsDNA and Nucleosomes—What Do They Recognize in Vivo

There is compelling evidence for a central role of anti-dsDNA antibodies in the pathogenesis of lupus nephritis. However, until now no consensus has been reached whether only a subpopulation of these antibodies is really nephritogenic. For this reason the structural basis for the pathogenic function of both murine and human anti-dsDNA antibodies has yet to be determined. If nucleosomes indeed drive the immune response against nucleosomes and DNA, then one may assume that 1) nucleosomes are exposed *in vivo* at a level that is not reached in normal physiology, 2) nucleosomes by themselves are immunogenic for both B cells and T cells, and 3) exposed nucleosomes are targeted *in situ* (in eg, glomeruli) or in circulation by the same antibodies they induce. If this is correct, lupus nephritis is induced by antibodies that recognize and bind nucleosomes in affected glomeruli (ie, homologous recognition). In fact, recent theoretical considerations, experimental data, and

clinical analyses are all consistent with this recognition principal and inconsistent with the idea that nephritogenic antibodies recognize cross-reacting glomerular antigens.

Lupus Nephritis—A Nucleosome-Mediated Disease

As a disease process, lupus nephritis may be fully explained by the danger model of Matzinger.^{78–80} Until recently, our understanding of how antibodies to dsDNA were involved in lupus nephritis was inconsistent and incoherent. Because it has been difficult to agree on the mode of glomerular antibody binding, several models have been described. One model implies glomerular antibody binding to exposed chromatin structures (Figure 3, A–D) in which chromatin in capillary membranes is visualized as electron-dense structures (EDSs, Figure 3A). As seen in

Figure 3. A principal outline of the impact of chromatin in lupus nephritis. In **A**, a typical transmission electron microscopy observation in lupus nephritis is glomerular basement membrane (GBM)-associated EDSs. By performing immune electron microscopy, it is evident that these EDSs, and only these, contain targets for autoantibodies *in vivo* (**B**, bound antibodies are stained with gold particles). In **C**, the EDSs are shown as dark unique structures. In **D**, we show how nucleosomes bind glomerular capillary membranes or the mesangial matrix (GBM) where they are observed as EDSs. These are in complex with antibodies reactive with nucleosomes (homologous recognition). Whether these have bound nucleosomes *in situ*, or whether nucleosomes and antibodies bound in the glomeruli as preformed immune complexes, has not been determined. In sum, this model is consistent with recently published data that demonstrate that the EDSs bind antibodies *in vivo*, that they bind experimental antibodies to dsDNA, histones, or transcription factors *in vitro*, and that they contain TUNEL-positive DNA.^{93,95} There is no evidence that nephritogenic anti-dsDNA antibodies cross-react with inherent glomerular constituents such as membrane structures or α -actinin *in vivo* (see text for details).

Figure 3, B–D, antibodies bound to glomerular membrane-associated chromatin are visible by transmission electron microscopy as EDSs.^{15,92,93} The other model suggests that antibodies bind through cross-reactions with different inherent renal nonchromatin constituents (not shown because this model lacks firm evidence).^{14,17,21,23,25,27,94}

Recently, we have published a series of studies on these mechanisms. Data generated by transmission electron microscopy, immune electron microscopy (IEM), and co-localization IEM analyses using experimental antibodies specific for dsDNA, histones, transcription factors, or laminin identified exposed glomerular basement membrane-associated nucleosomes as target structures for nephritogenic autoantibodies *in vivo*.^{15,92,93} Furthermore, terminal deoxynucleotidyl-transferase (TdT)-mediated dUTP nick end-labeling (TUNEL) assay and activated caspase 3 staining demonstrated that murine lupus nephritis was linked to accumulation of apoptotic cells in glomeruli and chromatin in glomerular capillary membranes and in the mesangial matrix.^{92,93} The data suggest that apoptotic nucleosomes can be released, bound to glomerular membranes, but not cleared. In this situation, they may be targeted by pathogenic anti-nucleosome antibodies (see details in Figure 3).

Data obtained by the newly developed co-localization TUNEL IEM assay, in which extracellular DNA was traced by TdT-mediated introduction of biotinylated nucleotides and glomerular *in vivo*-bound autoantibodies simultaneously by IEM, demonstrated that TUNEL-positive DNA co-localized with autoantibodies in glomerular membrane-associated EDSs in both murine⁹⁵ and human⁹⁴ nephritic kidneys. This confirms earlier results from the co-localization IEM assay, in which *in vivo*-bound glomerular autoantibodies co-localized perfectly with binding of experimental antibodies to dsDNA, histones, or transcription factors constitutively bound to nucleosomes. Both *in vivo*-bound and experimental antibodies bound exclusively in EDSs (Figure 3),^{92,93} and they did not co-localize with, eg, anti-laminin antibodies.⁹²

These results generally demonstrate two properties of extracellular nucleosomes. First, in situations in which nucleosomes are not cleared, they may be modified, unmasked from apoptotic blebs, and then gain immunogenic power by providing danger signals. Thereby, they become able to activate autoimmune nucleosome-specific T cells and to induce antibodies reactive with nucleosomes and individual components of nucleosomes, such as dsDNA, histones, and nonhistone proteins.^{5,87,96} Second, they associate with glomerular membranes in complex with nephritogenic antibodies.^{15,92,93} Thus, apoptotic, but retained secondary necrotic nucleosomes may represent both inducer and target structures for nephritogenic autoantibodies in SLE.

The Critical Role of Nucleosomes in Lupus Nephritis

While analyzing specificity and strength of the antibodies eluted from diseased kidneys, data demonstrated that they generally bound DNA and nucleosomes similar to or

much better than they bound, eg, α -actinin, laminin, or collagen.^{15,14,17} This harmonizes nicely with the fact that *in vivo*-bound antibodies co-localize with experimental antibodies against chromatin constituents and with TUNEL-positive DNA in capillary membranes and mesangial matrix, the central loci for immune complex deposits.^{92,93} Exposure and accessibility of chromatin *in situ* according to such observations is consistent with nucleosomes as target structures for *in vivo*-bound antibodies.

If nucleosomes indeed represent the glomerular structures binding nephritogenic antibodies *in vivo*, then why are the nucleosomes localized to capillary membranes and mesangial matrix? Recently, we analyzed if nucleosomes and DNA possessed affinity for glomerular laminin, collagen IV, and the mesangial matrix heparan sulfate proteoglycan perlecan by surface plasmon resonance. Kinetic analyses demonstrated that nucleosomes bound collagen IV and laminin at high affinity, but they did not bind perlecan.⁹⁵ Collectively, these results provide firm evidence that dominant target structures for nephritogenic autoantibodies are constituted by TUNEL-positive chromatin associated with glomerular capillary membranes and the mesangial matrix at high affinity. These data demonstrate that antibodies reactive with nucleosomes exert their pathogenic effect only in the context of exposure of their homologous target structures, ie, nucleosomes.

Central Unsolved Problems

The data and considerations discussed here open for new, hypothesis-directed studies that may provide information regarding how to approach the rational for causal therapy as, eg, to avoid nucleosome or antibody-nucleosome complex binding in glomeruli. Central questions include why chromatin is released at all and why it is preferentially deposited in glomerular membranes and mesangial matrix. Where do the chromatin fragments come from—through circulation or are they released within the kidney? Is lupus nephritis an isolated organ disease or part of a systemic disorder? We are currently approaching these questions by morphological, genetic, and biological studies on tissue from autoimmune, lupus-prone mice and from human SLE patients.

Concluding Remarks

The considerations given here link the danger model of Matzinger^{78–80} to persistent nephritogenic autoimmunity and the NIS-INS model of Medzhitov and Janeway^{81–83} to transient nonpathogenic autoimmunity (in the situation in which NIS is in complex with INS). Autoimmunity induced by the former process, but not the latter, is by nature persistent attributable to a sustained clearance deficiency in SLE patients. In both situations, however, dendritic cells may be activated by the complexes (NIS-INS complex or necrotic chromatin), establish germinal centers, and provide the scenario for antibody production. However, only in individuals with reduced clearance

of, eg, apoptotic cells does secondary necrotic chromatin accumulate in tissue where it serves as *in situ* target structures for such antibodies. Autoantibodies to dsDNA or to nucleosomes become pathogenic only in that situation. Without exposed chromatin, they remain nonpathogenic.

References

1. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ: The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; 25:1271–1277
2. Fisman S, Rekvig OP, Mortensen E: Pathogenesis of SLE dermatitis—a reflection of the process in SLE nephritis? *Curr Rheum Rev* 2007; 3:1–7
3. Rekvig OP, Kalaaji M, Nossent H: Anti-DNA antibody subpopulations and lupus nephritis. *Autoimmun Rev* 2004; 3:1–6
4. Rekvig OP, Nossent JC: Anti-double-stranded DNA antibodies, nucleosomes, and systemic lupus erythematosus: a time for new paradigms? *Arthritis Rheum* 2003; 48:300–312
5. Berden JH, Licht R, Van Bruggen MC, Tax WJ: Role of nucleosomes for induction and glomerular binding of autoantibodies in lupus nephritis. *Curr Opin Nephrol Hypertens* 1999; 8:299–306
6. Huerta PT, Kowal C, DeGiorgio LA, Volpe BT, Diamond B: Immunity and behavior: antibodies alter emotion. *Proc Natl Acad Sci USA* 2006; 103:678–683
7. Grootsholten C, Van Bruggen MC, van der Pijl JW, de Jong EM, Ligtenberg G, Derksen RH, Berden JH: Deposition of nucleosomal antigens (histones and DNA) in the epidermal basement membrane in human lupus nephritis. *Arthritis Rheum* 2003; 48:1355–1362
8. Weening JJ, D'agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, Balow JE, Bruijn JA, Cook T, Ferrario F, Fogo AB, Ginzler EM, Hebert L, Hill G, Hill P, Jennette JC, Kong NC, Lesavre P, Lockshin M, Looi LM, Makino H, Moura LA, Nagata M: The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int* 2004; 65:521–530
9. Weening JJ, D'agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, Balow JE, Bruijn JA, Cook T, Ferrario F, Fogo AB, Ginzler EM, Hebert L, Hill G, Hill P, Jennette JC, Kong NC, Lesavre P, Lockshin M, Looi LM, Makino H, Moura LA, Nagata M: The classification of glomerulonephritis in systemic lupus erythematosus revisited. *J Am Soc Nephrol* 2004; 15:241–250
10. Ceppellini R, Polli E, Celada F: A DNA-reacting factor in serum of a patient with lupus erythematosus diffusum. *Proc Soc Exp Biol Med* 1957; 96:572–574
11. Robbins WC, Holman HR, Deicher H, Kungel HG: Complement fixation with cell nuclei and DNA in lupus erythematosus. *Proc Soc Exp Biol Med* 1957; 96:575–579
12. Seligmann M: Demonstration in the blood of patients with disseminated lupus erythematosus a substance determining a precipitation reaction with desoxyribonucleic acid. *C R Hebd Seances Acad Sci* 1957; 245:243–245
13. Khalil M, Spatz L, Diamond B: Anti-DNA antibodies. *Systemic Lupus Erythematosus*, ed 3. Edited by Lahita RG. San Diego, Academic Press, 1999, pp 197–217
14. Xie C, Liang Z, Chang S, Mohan C: Use of a novel elution regimen reveals the dominance of polyreactive antinuclear autoantibodies in lupus kidneys. *Arthritis Rheum* 2003; 48:2343–2352
15. Kalaaji M, Sturfelt G, Mjelle JE, Nossent H, Rekvig OP: Critical comparative analyses of anti- α -actinin and glomerulus-bound antibodies in human and murine lupus nephritis. *Arthritis Rheum* 2006; 54:914–926
16. van Bruggen MC, Kramers C, Hylkema MN, Smeenk RJ, Berden JH: Significance of anti-nuclear and anti-extracellular matrix autoantibodies for albuminuria in murine lupus nephritis: a longitudinal study on plasma and glomerular eluates in MRL/l mice. *Clin Exp Immunol* 1996; 105:132–139
17. Deocharan B, Qing X, Lichauro J, Putterman C: Alpha-actinin is a cross-reactive renal target for pathogenic anti-DNA antibodies. *J Immunol* 2002; 168:3072–3078
18. Izui S, Lambert PH, Miescher PA: In vitro demonstration of a particular

- affinity of glomerular basement membrane and collagen for DNA. A possible basis for a local formation of DNA-anti-DNA complexes in systemic lupus erythematosus. *J Exp Med* 1976, 144:428–443
19. Izui S, Lambert PH, Fournie GJ, Turler H, Miescher PA: Features of systemic lupus erythematosus in mice injected with bacterial lipopolysaccharides: identification of circulating DNA and renal localization of DNA-anti-DNA complexes. *J Exp Med* 1977, 145:1115–1130
20. Hahn BH: Antibodies to DNA. *N Engl J Med* 1998, 338:1359–1368
21. Mostoslavsky G, Fischel R, Yachimovich N, Yarkoni Y, Rosenmann E, Monestier M, Baniyash M, Eilat D: Lupus anti-DNA autoantibodies cross-react with a glomerular structural protein: a case for tissue injury by molecular mimicry. *Eur J Immunol* 2001, 31:1221–1227
22. Zhao Z, Weinstein E, Tuzova M, Davidson A, Mundel P, Marambaio P, Putterman C: Cross-reactivity of human lupus anti-DNA antibodies with alpha-actinin and nephritogenic potential. *Arthritis Rheum* 2005, 52:522–530
23. Amital H, Heilweil M, Ulmansky R, Szafer F, Bar-Tana R, Morel L, Foster MH, Mostoslavsky G, Eilat D, Pizov G, Naparstek Y: Treatment with a laminin-derived peptide suppresses lupus nephritis. *J Immunol* 2005, 175:5516–5523
24. Mageed RA, Zack DJ: Cross-reactivity and pathogenicity of anti-DNA autoantibodies in systemic lupus erythematosus. *Lupus* 2002, 11:783–786
25. Raz E, Ben Bassat H, Davidi T, Shlomai Z, Eilat D: Cross-reactions of anti-DNA autoantibodies with cell surface proteins. *Eur J Immunol* 1993, 23:383–390
26. Raz E, Brezis M, Rosenmann E, Eilat D: Anti-DNA antibodies bind directly to renal antigens and induce kidney dysfunction in the isolated perfused rat kidney. *J Immunol* 1989, 142:3076–3082
27. D'Andrea DM, Coupaye Gerard B, Kleiman TR, Foster MH, Madaio MP: Lupus autoantibodies interact directly with distinct glomerular and vascular cell surface antigens. *Kidney Int* 1996, 49:1214–1221
28. Chan TM, Frampton G, Staines NA, Hobby P, Perry GJ, Cameron JS: Different mechanisms by which anti-DNA MoAbs bind to human endothelial cells and glomerular mesangial cells. *Clin Exp Immunol* 1992, 88:68–74
29. Chen M, Wang YY, Zhao MH, Zhang YK, Wang HY: Autoantibodies against glomerular mesangial cells and their target antigens in lupus nephritis. *Ren Fail* 2005, 27:507–513
30. Termaat RM, Assmann KJ, van Son JP, Dijkman HB, Koene RA, Berden JH: Antigen-specificity of antibodies bound to glomeruli of mice with systemic lupus erythematosus-like syndromes. *Lab Invest* 1993, 68:164–173
31. Liang Z, Xie C, Chen C, Kreska D, Hsu K, Li L, Zhou XJ, Mohan C: Pathogenic profiles and molecular signatures of antinuclear autoantibodies rescued from NZM2410 lupus mice. *J Exp Med* 2004, 199:381–398
32. Berden JH: Lupus nephritis. *Kidney Int* 1997, 52:538–558
33. Kramers C, Hylkema MN, Van Bruggen MC, van de Lagemaat R, Dijkman HB, Assmann KJ, Smeenk RJ, Berden JH: Anti-nucleosome antibodies complexed to nucleosomal antigens show anti-DNA reactivity and bind to rat glomerular basement membrane *in vivo*. *J Clin Invest* 1994, 94:568–577
34. Madaio MP, Hodder S, Schwartz RS, Stollar BD: Responsiveness of autoimmune and normal mice to nucleic acid antigens. *J Immunol* 1984, 132:872–876
35. André-Schwartz J, Datta SK, Shoenfeld Y, Isenberg DA, Stollar BD, Schwartz RS: Binding of cytoskeletal proteins by monoclonal anti-DNA lupus autoantibodies. *Clin Immunol Immunopathol* 1984, 31:261–271
36. Lafer EM, Rauch J, Andrzejewski C Jr, Mudd D, Furie B, Furie B, Schwartz RS, Stollar BD: Polyspecific monoclonal lupus autoantibodies reactive with both polynucleotides and phospholipids. *J Exp Med* 1981, 153:897–909
37. Ray SK, Putterman C, Diamond B: Pathogenic autoantibodies are routinely generated during the response to foreign antigen: a paradigm for autoimmune disease. *Proc Natl Acad Sci USA* 1996, 93:2019–2024
38. Shoenfeld Y, Rauch J, Massicotte H, Datta SK, Andre Schwartz J, Stollar BD, Schwartz RS: Polyspecificity of monoclonal lupus autoantibodies produced by human-human hybridomas. *N Engl J Med* 1983, 308:414–420
39. Wellmann U, Letz M, Herrmann M, Angermuller S, Kalden JR, Winkler TH: The evolution of human anti-double-stranded DNA autoantibodies. *Proc Natl Acad Sci USA* 2005, 102:9258–9263
40. Li H, Jiang Y, Cao H, Radic M, Prak EL, Weigert M: Regulation of anti-phosphatidylserine antibodies. *Immunity* 2003, 18:185–192
41. Cocco BA, Cline AM, Radic MZ: Blebs and apoptotic bodies are B cell autoantigens. *J Immunol* 2002, 169:159–166
42. Cocco BA, Seal SN, D'Agnillo P, Mueller YM, Katsikis PD, Rauch J, Weigert M, Radic MZ: Structural basis for autoantibody recognition of phosphatidylserine-beta 2 glycoprotein I and apoptotic cells. *Proc Natl Acad Sci USA* 2001, 98:13826–13831
43. Schlegel RA, Williamson P: Phosphatidylserine, a death knell. *Cell Death Differ* 2001, 8:551–563
44. Fadok VA, de Cathelineau A, Daleke DL, Henson PM, Bratton DL: Loss of phospholipid asymmetry and surface exposure of phosphatidylserine is required for phagocytosis of apoptotic cells by macrophages and fibroblasts. *J Biol Chem* 2001, 276:1071–1077
45. Desai DD, Krishnan MR, Swindle JT, Marion TN: Antigen-specific induction of antibodies against native mammalian DNA in nonautoimmune mice. *J Immunol* 1993, 151:1614–1626
46. Krishnan MR, Marion TN: Structural similarity of antibody variable regions from immune and autoimmune anti-DNA antibodies. *J Immunol* 1993, 150:4948–4957
47. Pisetsky DS: Specificity and immunochemical properties of antibodies to bacterial DNA. *Methods* 1997, 11:55–61
48. Gilkeson GS, Ruiz P, Howell D, Lefkowitz JB, Pisetsky DS: Induction of immune-mediated glomerulonephritis in normal mice immunized with bacterial DNA. *Clin Immunol Immunopathol* 1993, 68:283–292
49. Moens U, Seternes OM, Hey AW, Silsand Y, Traavik T, Johansen B, Rekvig OP: *In vivo* expression of a single viral DNA-binding protein generates systemic lupus erythematosus-related autoimmunity to double-stranded DNA and histones. *Proc Natl Acad Sci USA* 1995, 92:12393–12397
50. Bendiksen S, Van Ghelue M, Winkler T, Moens U, Rekvig OP: Autoimmunity to DNA and nucleosomes in binary tetracycline-regulated polyomavirus T-Ag transgenic mice. *J Immunol* 2004, 173:7630–7640
51. Mohan C, Adams S, Stanik V, Datta SK: Nucleosome: a major immunogen for pathogenic autoantibody-inducing T cells of lupus. *J Exp Med* 1993, 177:1367–1381
52. Kaliyaperumal A, Mohan C, Wu W, Datta SK: Nucleosomal peptide epitopes for nephritis-inducing T helper cells of murine lupus. *J Exp Med* 1996, 183:2459–2469
53. Voll R, Roth E, Girkontaite I, Fehr H, Herrmann M, Lorenz H, Kalden J: Histone-specific Th0 and Th1 clones derived from systemic lupus erythematosus patients induce double-stranded DNA antibody production. *Arthritis Rheum* 1997, 40:2162–2171
54. Andreassen K, Moens U, Nossent H, Marion TN, Rekvig OP: Termination of human T cell tolerance to histones by presentation of histones and polyomavirus T antigen provided that T antigen is complexed with nucleosomes. *Arthritis Rheum* 1999, 42:2449–2460
55. Andreassen K, Bendiksen S, Kjeldsen E, Van Ghelue M, Moens U, Arnesen E, Rekvig OP: T cell autoimmunity to histones and nucleosomes is a latent property of the normal immune system. *Arthritis Rheum* 2002, 46:1270–1281
56. Andreassen K, Bredholt G, Moens U, Bendiksen S, Kauric G, Rekvig OP: T cell lines specific for polyomavirus T-antigen recognize T-antigen complexed with nucleosomes: a molecular basis for anti-DNA antibody production. *Eur J Immunol* 1999, 29:2715–2728
57. Gilkeson GS, Pritchard AJ, Pisetsky DS: Cellular requirements for anti-DNA production induced in mice by immunization with bacterial DNA. *Eur J Immunol* 1990, 20:1789–1794
58. Fredriksen K, Osei A, Sundsfjord A, Traavik T, Rekvig OP: On the biological origin of anti-double-stranded (ds) DNA antibodies: systemic lupus erythematosus-related anti-dsDNA antibodies are induced by polyomavirus BK in lupus-prone (NZBxNZW) F1 hybrids, but not in normal mice. *Eur J Immunol* 1994, 24:66–70
59. Stollar BD: The experimental induction of antibodies to nucleic acids. *Methods Enzymol* 1980, 70:70–85
60. Stollar BD: The antigenic potential and specificity of nucleic acids, nucleoproteins, and their modified derivatives. *Arthritis Rheum* 1981, 24:1010–1018
61. Stollar BD: Antibodies to DNA. *CRC Crit Rev Biochem* 1986, 20:1–36
62. Stollar BD: The origin and pathogenic role of anti-DNA autoantibodies. *Curr Opin Immunol* 1989, 2:607–612
63. Stollar BD: Immunochemistry of DNA. *Int Rev Immunol* 1989, 5:1–22

64. Wang M, Desai D, Marion TN: T cells specific for DNA-binding peptides. *Lupus* 1997, 6:349–350
65. Marion TN, Krishnan MR, Steeves MA, Desai DD: Affinity maturation and autoimmunity to DNA. *Curr Dir Autoimmun* 2003, 6:123–153
66. Flaegstad T, Fredriksen K, Dahl B, Traavik T, Rekvig OP: Inoculation with BK virus may break immunological tolerance to histone and DNA antigens. *Proc Natl Acad Sci USA* 1988, 85:8171–8175
67. Rekvig OP, Fredriksen K, Hokland K, Moens U, Traavik T, Krishnan MR, Marion T: Molecular analyses of anti-DNA antibodies induced by polyomavirus BK in BALB/c mice [published erratum appears in *Scand J Immunol* 1995 Aug;42(2):286]. *Scand J Immunol* 1995, 41:593–602
68. Rekvig OP, Moens U, Sundsfjord A, Bredholt G, Osei A, Haaheim H, Traavik T, Arnesen E, Haga HJ: Experimental expression in mice and spontaneous expression in human SLE of polyomavirus T-antigen. A molecular basis for induction of antibodies to DNA and eukaryotic transcription factors. *J Clin Invest* 1997, 99:2045–2054
69. Lin RH, Mamula MJ, Hardin JA, Janeway CA Jr: Induction of autoreactive B cells allows priming of autoreactive T cells. *J Exp Med* 1991, 173:1433–1439
70. Mamula MJ, Lin RH, Janeway CA Jr, Hardin JA: Breaking T cell tolerance with foreign and self co-immunogens. A study of autoimmune B and T cell epitopes of cytochrome c. *J Immunol* 1992, 149:789–795
71. Fatenejad S, Mamula MJ, Craft J: Role of intermolecular/intrastructural B- and T-cell determinants in the diversification of autoantibodies to ribonucleoprotein particles. *Proc Natl Acad Sci USA* 1993, 90:12010–12014
72. Roth R, Nakamura T, Mamula MJ: B7 costimulation and autoantigen specificity enable B cells to activate autoreactive T cells. *J Immunol* 1996, 157:2924–2931
73. Dong X, Hamilton KJ, Satoh M, Wang J, Reeves WH: Initiation of autoimmunity to the p53 tumor suppressor protein by complexes of p53 and SV40 large T antigen. *J Exp Med* 1994, 179:1243–1252
74. DeSilva DR, Urdahl KB, Jenkins MK: Clonal anergy is induced in vitro by T cell receptor occupancy in the absence of proliferation. *J Immunol* 1991, 147:3261–3267
75. Jenkins MK: The role of cell division in the induction of clonal anergy. *Immunol Today* 1992, 13:69–73
76. Datta SK: Production of pathogenic antibodies: cognate interactions between autoimmune T and B cells. *Lupus* 1998, 7:591–596
77. Datta SK, Kaliyaperumal A: Nucleosome-driven autoimmune response in lupus. Pathogenic T helper cell epitopes and costimulatory signals. *Ann NY Acad Sci* 1997, 815:155–170
78. Matzinger P: Friendly and dangerous signals: is the tissue in control? *Nat Immunol* 2007, 8:11–13
79. Matzinger P: The danger model: a renewed sense of self. *Science* 2002, 296:301–305
80. Matzinger P: Tolerance, danger, and the extended family. *Annu Rev Immunol* 1994, 12:991–1045
81. Medzhitov R, Janeway CA Jr: Decoding the patterns of self and nonself by the innate immune system. *Science* 2002, 296:298–300
82. Medzhitov R, Janeway CA Jr: How does the immune system distinguish self from nonself? *Semin Immunol* 2000, 12:185–188
83. Janeway CA Jr, Medzhitov R: Innate immune recognition. *Annu Rev Immunol* 2002, 20:197–216
84. Kuenkele S, Beyer TD, Voll RE, Kalden JR, Herrmann M: Impaired clearance of apoptotic cells in systemic lupus erythematosus: challenge of T and B cell tolerance. *Curr Rheumatol Rep* 2003, 5:175–177
85. Gaip US, Voll RE, Sheriff A, Franz S, Kalden JR, Herrmann M: Impaired clearance of dying cells in systemic lupus erythematosus. *Autoimmun Rev* 2005, 4:189–194
86. Gaip US, Sheriff A, Franz S, Munoz LE, Voll RE, Kalden JR, Herrmann M: Inefficient clearance of dying cells and autoreactivity. *Curr Top Microbiol Immunol* 2006, 305:161–176
87. Dieker JW, van der Vlag J, Berden JH: Deranged removal of apoptotic cells: its role in the genesis of lupus. *Nephrol Dial Transplant* 2004, 19:282–285
88. Licht R, Dieker JW, Jacobs CW, Tax WJ, Berden JH: Decreased phagocytosis of apoptotic cells in diseased SLE mice. *J Autoimmun* 2004, 22:139–145
89. Dieker JW, Fransen JH, van Bavel CC, Briand JP, Jacobs CW, Muller S, Berden JH, van der Vlag J: Apoptosis-induced acetylation of histones is pathogenic in systemic lupus erythematosus. *Arthritis Rheum* 2007, 56:1921–1933
90. Reeves WH, Dong X, Wang J, Hamilton K: Initiation of autoimmunity to self-proteins complexed with viral antigens. *Ann NY Acad Sci* 1997, 815:139–154
91. Dieker JW, van der Vlag J, Berden JH: Triggers for anti-chromatin autoantibody production in SLE. *Lupus* 2002, 11:856–864
92. Kalaaji M, Mortensen E, Jorgensen L, Olsen R, Rekvig OP: Nephritogenic lupus antibodies recognize glomerular basement membrane-associated chromatin fragments released from apoptotic intraglomerular cells. *Am J Pathol* 2006, 168:1779–1792
93. Kalaaji M, Fenton KA, Mortensen ES, Olsen R, Sturfelt G, Alm P, Rekvig OP: Glomerular apoptotic nucleosomes are central target structures for nephritogenic antibodies in human SLE nephritis. *Kidney Int* 2007, 71:664–672
94. Sabbaga J, Line SR, Potocnjak P, Madaio MP: A murine nephritogenic monoclonal anti-DNA autoantibody binds directly to mouse laminin, the major non-collagenous protein component of the glomerular basement membrane. *Eur J Immunol* 1989, 19:137–143
95. Mjelle JE, Rekvig OP, Fenton KA: Nucleosomes possess high affinity for glomerular laminin and collagen IV and bind nephritogenic antibodies in murine lupus-like nephritis. *Ann Rheum Dis* 2007, 66:1661–1668
96. Herrmann M, Zoller OM, Hagenhofer M, Voll R, Kalden JR: What triggers anti-dsDNA antibodies? *Mol Biol Rep* 1996, 23:265–267
97. Radic MZ, Weigert M: Genetic and structural evidence for antigen selection of anti-DNA antibodies. *Annu Rev Immunol* 1994, 12:487–520