arthritis research&therapy

REVIEW

Chromatin as a target antigen in human and murine lupus nephritis

Annica Hedberg, Elin Synnøve Mortensen and Ole Petter Rekvig*

Abstract

The present review focuses on pathogenic molecular and transcriptional events in patients with lupus nephritis. These factors are renal DNaseI, exposed chromatin fragments and the corresponding chromatin-reactive autoantibodies. Lupus nephritis is the most serious complication in human systemic lupus erythematosus, and is characterised by deposition of chromatin fragment–IgG complexes in the mesangial matrix and glomerular basement membranes. The latter deposition defines end-stage disease. This event is stringently linked to a renal-restricted shutdown of expression of the DNaseI gene, as determined by loss of DNaseI mRNA level and DNaseI enzyme activity. The major aim of the present review is to generate new therapeutic strategies based on new insight into the disease pathogenesis.

Background

Shortly after their discovery in 1957 [1-3], antibodies to dsDNA were associated with renal manifestation of systemic lupus erythematosus (SLE). A prominent observation was that anti-dsDNA antibodies were eluted from affected glomeruli in the context of lupus nephritis [4-8]. At the time when the nephritogenic potential of antibodies to dsDNA was revealed, their binding in glomeruli was logically claimed to depend on exposed 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 [9,10], and the antibodies were specific for DNA [11,12].

One problem was linked with this model. Not all individuals with anti-dsDNA antibodies in their circulation developed nephritis. A convenient model to understand nephritogenicity of anti-dsDNA antibodies proposes that only those antibodies which cross-reacted with inherent renal antigens induced the organ disease. A nephritogenic potential of antibodies against DNA (or nucleosomes) is thus today critically challenged by alternative models implying that antibodies cross-react with glomerular antigens such as α-actinin, laminin, or cell surface structures [13-19]. Conflicting data from different types of analytical strategies have resulted in different models explaining how anti-DNA antibodies induce nephritis. Even though these models are attractive, none have been validated beyond any doubt, although the dominant specificity of nephritogenic antibodies for dsDNA may point to the most obvious target structures in nephritic kidneys – nucleosomes released from dead cells. An alternative model that may explain whether an anti-dsDNA antibody executes a nephritogenic potential might therefore be the availability of exposed chromatin particles within glomeruli. This hypothesis means that anti-dsDNA antibodies execute their pathogenic potential only in situations where chromatin fragments are exposed in glomeruli. In the absence of this target structure, the antibodies remain nonpathogenic epiphenomena despite their diagnostic potential.

The origin of renally exposed chromatin fragments has been difficult to assess. One general idea has been that they reach glomeruli through circulation. Taking into consideration that the target antigens for anti-dsDNA and anti-nucleosome antibodies appear by immune electron microscopy as large chromatin fragments [20], however, it is difficult to explain how these may reach and deposit in glomeruli.

A notable change in thinking entailed by our studies is rather that chromatin fragments exposed in glomeruli are released from dying renal cells, and that these fragments are not degraded during the cell death process because of an acquired loss of the dominant renal nuclease DNaseI [21]. This model is the focus of the present review, and will be discussed in detail below.

Nephritis in systemic lupus erythematosus

SLE, as we understand the disease today, is linked to B-cell and T-cell autoimmunity to nucleosomes, and particularly to the individual components of nucleosomes – native

*Correspondence: olepr@fagmed.uit.no
Molecular Pathology Research Group, Institute of Medical Biology, Faculty of Health Science, University of Tromsø, N-9037 Tromsø, Norway

(ds)DNA and histones. These are important diagnostic parameters for SLE [12,22]. Furthermore, sets of these autoantibodies possess the potential to induce nephritis, the most serious complication in SLE [23,24].

The aetiology of SLE is not fully understood, but there are recent advances in its understanding. For example, there is growing interest in regulatory RNA molecules in SLE. miRNAs belong to a family of short noncoding RNAs. These have been shown to play important roles in gene regulation. Recent data suggest that miR-126 regulates DNA methylation in $CD4^+$ T cells and contributes to T-cell autoreactivity in SLE by directly targeting DNMT1 [25]. Similarly, a recently published comprehensive analysis of miRNA expression patterns in renal biopsies of lupus nephritis patients further demonstrates that miRNAs are probable factors involved in the pathogenesis of lupus nephritis. We see now the contour of a new scientific field to understand elements of lupus nephritis; study of regulatory RNA in autoimmune syndromes such as SLE and lupus nephritis is a new and fast-growing field to analyse transcriptomics in SLE [26], and miRNA may have a strong impact on progressive kidney diseases as discussed by Kato and colleagues [27].

Another cascade of events that may relate to pathogenesis of SLE and lupus nephritis is linked to engagement of Toll-like receptors (TLRs) by exposed chromatin. Activation of TLRs induces upregulation of proinflammatory cytokines (TNFα, IFNγ) and interleukins [28]. For example, IFNγ contributes directly to the progression of lupus nephritis [29]. Furthermore, Rönnblom and colleagues discussed recently the increasing evidence that activated type I interferons in lupus are critical in the aetiopathogenesis of the disease and an important therapeutic target [30]. Kidney sections from patients with SLE glomerulonephritis contain high amounts of TNFα, and expression levels correlated with local (histological) disease activity [31].

TNFα and IFNγ are important inducers of the matrix metalloproteases (MMPs) MMP2 and MMP9. These are collagenases that, when overexpressed, have the potential to disintegrate membranes [32,33]. Membrane disintegration may be the factor that promotes deposition of immune complexes in glomerular basement membranes (GBMs), as discussed recently [34]. The engagement of TLRs is thus an event central in the pathogenesis and progression of SLE and lupus nephritis.

In the next sections, the current insight into murine forms of lupus nephritis will be discussed, with potential implications of data on the human form of this organ disease.

Murine lupus nephritis

Substantial data have been provided during recent years related to why and how anti-dsDNA antibodies are produced (see, for example, [35-39]); to how they exert their clinical impact, whether through interaction with DNA or nucleosomes [5,40-42], or through cross-reaction with inherent renal antigens [13,15,17,43]; and to analyse whether the nature of their glomerular target structures are reflected by their specificity or cross-reactivity [12,40,44].

Glomerular target structures for nephritogenic autoantibodies

In recent studies, we and other workers have developed high-resolution techniques providing evidence that nephritogenic anti-dsDNA/nucleosome antibodies recognise selectively intraglomerular, extracellular chromatin structures *in vivo* [20,40,41,44]. These structures appear as electron-dense structures by transmission electron microscopy, and have been shown to be composed of chromatin fragments and IgG molecules by different forms of immune electron microscopy and by co-localisation terminal deoxynucleotidyltransferase biotin-dUTP nicked end-labelled immune electron microscopy assay [20]. Autoantibody deposits *in vivo* are strictly localised to these structures, and co-localise with antibodies to DNA and histones added to the sections *in vitro* [20].

These data confirm the historical hypothesis that anti-dsDNA antibodies form complexes with nucleosomes and these immune complexes deposit in glomerular membranes (reviewed in [44]). This deposition does not exclude the involvement of other autoantibodies that may participate in the progression of lupus nephritis, such as antibodies specific for the membrane and matrix component [6], α-actinin [13,43], C1q [45] and, for example, renal cell membranes [46]. The role of these latter antibodies in lupus nephritis, however, remains to be determined.

Central role of renal DNaseI, chromatin fragments, anti-dsDNA antibodies, and matrix metalloproteases in evolution of murine lupus nephritis

Recently, we demonstrated that anti-DNA antibodies, renal DNaseI and matrix MMP mRNA levels and enzyme activities are cooperative and instrumental in early and late events in murine lupus nephritis, as determined in (NZBxNZW)F1 mice [47]. Early phases of nephritis were associated with chromatin–IgG complex deposition in the mesangial matrix, which correlated with appearance of anti-dsDNA antibodies. Subsequent to this event, we observed a dramatic downregulation of renal DNaseI mRNA level and enzyme activity, while MMP2 and MMP9 mRNA levels and enzyme activities increased. Reduced levels of renal DNaseI correlated with deficient renal fragmentation of chromatin from dead cells, and with accumulation of large chromatin fragments in GBMs. A similar downregulation of DNaseI was not

observed in mesangial nephritis [47], or in nephritis in the context of Wegener's granulomatosis [48]. *In situ* deposition of chromatin fragments has been described in several experimental nuclease deficiencies on nonautoimmune backgrounds (reviewed in [49]).

In contrast to the correlation of renal DNaseI shutdown, Martinez-Valle and colleagues did not observe any statistical relationship between serum DNaseI activity and disease evolution time, clinical and laboratory parameters including proteinuria and autoantibodies, or the treatment pattern received by the patients [50,51]. In agreement with this observation, increasing DNaseI activity *in vivo* by injecting recombinant human DNaseI intravenously and subcutaneously in patients with SLE failed to show any effect on serum markers of disease activity [52]. Furthermore, mutations causing reduced DNaseI in lupus patients did not correlate with unique clinical symptoms [53]. This lack of correlation may mean that extracellular DNaseI enzyme activity is not important in the context of lupus nephritis pathogenesis. Rather, DNaseI is important in the context of cell death, where DNaseI is in fact the initiator of chromatin fragmentation in order to fascilitate a silent removal to avoid, for example, inflammation [54,55]. Renal DNaseI gene shutdown may therefore impose chromatin exposure *in situ* because of inefficient enzymatic degradation. In this model, serum DNaseI may play an inferior role in extracellular chromatin degradation. It is questionable whether extracellular chromatin, when bound to membranes and covered by IgGs, will be degraded at all by DNaseI.

Recent data in murine lupus nephritis thus demonstrate that acquired loss of renal DNaseI enzyme activity is a dominant event responsible for the progression of mesangial nephritis into end-stage organ disease [47]. However, exposed chromatin may not be pathogenic in the absence of antibodies to dsDNA or to nucleosomes [56]. The principal cellular and molecular requirements needed to produce these autoantibodies have been explained experimentally [35-38], but the mechanism(s) accounting for them *in vivo* in the context of SLE and lupus nephritis has not yet been determined. Published data, however, indicate that defects in nucleases linked to apoptotic or necrotic cell death are not associated with induction of anti-dsDNA or anti-nucleosome autoantibodies (for review, see [49]). The data discussed here, nevertheless, explain how an unusual exposure of chromatin may be a central factor in the evolution of lupus nephritis, but not in promoting nephritogenic chromatin-specific autoimmunity.

Since chromatin fragments stimulate TLRs in, for example, dendritic cells [57], this may also explain increased expression of MMPs in lupus nephritic kidneys [58].

With loss of renal DNaseI, the signalling pathway from chromatin fragment-stimulation of TLR to MMP expression has been described [58,59]. MMPs are collagenases with potential to disintegrate membranes [32,33]. Membrane disintegration may promote deposition of immune complexes in GBMs.

Chromatin in murine lupus nephritis: inducer and target for anti-DNA antibodies

In murine lupus nephritis, anti-DNA antibodies gain their pathogenic potential when chromatin fragments are exposed in glomeruli. Chromatin fragments thus represent the axis in a *circulus vitiosus*, where chromatin – the inducer of nephritogenic autoimmunity – is the glomerular target for the autoantibodies, and thereby accounts for the organ disease (discussed in [60,61]).

Chromatin fragments exposed in the kidneys may derive from either increased apoptosis or deficient clearance of apoptotic or secondary necrotic material [61-63]. How tolerance against chromatin components is terminated is not fully understood. Chromatin undergoes alterations during apoptosis and is normally not exposed for the immune system. In the case of increased apoptosis or deficient clearance, however, these components may be exposed as secondary necrotic chromatin with the potential to induce an antigen-selective immune response [64-66]. For example, plasma chromatin found in SLE patients is hypomethylated [66], and hypomethylated DNA is more immunogenic and can induce maturation of dendritic cells and potentially activate autoimmune T cells and B cells [67]. Furthermore, sera of SLE patients contain circulating chromatin fragments complexed with the DNA-binding protein HMGB1 [61]. This protein is a proinflammatory mediator that binds chromatin of apoptotic cells. The HMGB1–nucleosome complexes may activate antigen-presenting cells, which have the potential to promote activation of relevant T-helper cells and then DNA-specific B cells, with production of chromatin-specific autoantibodies as a net result [61]. Exposed and retained chromatin may therefore promote production of chromatin-specific autoantibodies.

In an infectious context, viruses such as polyomavirus BK may induce cell death as a consequence of virus replication. This process may be relevant to lupus nephritis, since there are several reports that demonstrate productive polyomavirus infection in human SLE (see [35] and references therein). Productive renal polyoma virus activation may be imposed by treatment of the disease with immunosuppressive drugs, and may not be specifically linked to the lupus pathogenesis [68,69]. This expression pattern is similar to what is seen in renal transplants during immunosuppresion [70,71].

Irrespective of the cause for polyomavirus replication, the viral transcription factor large T antigen forms

complexes with the host cell chromatin. This complex may affect the immune system in analogy with a hapten–carrier complex, where B cells bind nucleosomal DNA (the hapten) through the DNA-specific antigen receptor and process and present T-antigen-derived peptides (the carrier) to nontolerant T cells (this model is extensively reviewed in [35]). Chromatin may thus generate autoimmunity by quite different pathways linked to modification of chromatin and various infections [36,72].

We recently performed *in vitro* studies demonstrating that nucleosomes and nucleosomes in complex with anti-DNA antibodies have high affinity for glomerular and epidermal basement membrane components such as laminin and collagen [73]. This affinity may be a major factor that explains why chromatin-containing immune complexes associate with membrane and matrix structures in human nephritis [47].

One factor that may contribute to deviation in chromatin composition and size is DNaseI, which is the major nuclease in kidneys [21] but also in serum, where it may participate in chromatin degradation in context of necrosis [55,74]. Several studies have demonstrated reduced levels of serum DNaseI in SLE patients [50,51, 74-76]. This reduction could provisionally explain why the chromatin concentration in circulation of lupus patients with nephritis is reported to be higher than in control individuals [77,78]. Another reason for this reduction could be that immune complexes in SLE patients are protected against nuclease attacks by DNA-binding proteins and immunoglobulins, present in sera. A problem that needs to be solved is therefore whether circulating chromatin-containing immune complexes in lupus nephritis patients are less sensitive to DNaseI than chromatin fragments in anti-chromatin antibody-negative healthy donors.

Most of the data on lupus nephritis so far relate to studies of murine models of the disease. In the next section, we will translate available basic data into a detailed evidence-based model for human lupus nephritis. We shall subsequently convert this information into new and rational treatment modalities.

Human lupus nephritis

Irrespective of the complexity of potentially nephritogenic autoantibodies associated with SLE, a consensus has evolved that antibodies to dsDNA and nucleosomes are central pathogenic factors involved in development of human lupus nephritis [12,23,24,79-81]. The divergent models to explain the basic processes in human lupus nephritis may have evolved simply because we still lack data that provide definitive insight into the nephritic process(es).

In a pilot study, data demonstrate that human nephritogenic anti-DNA antibodies bind chromatin-like structures in GBMs and the mesangial matrix [82], similar to what we have observed in murine lupus nephritis [47]. In that pilot study it became evident that *in-vivo*-bound GBM-associated autoantibodies co-localised in electron-dense structures with experimental antibodies to histone H1, histone H3 and transcription factor TBP, and with nicked DNA [82]; that is, results identical to those observed in murine lupus nephritis. In a recent study, we also demonstrated in advanced stages of human lupus nephritis that the DNaseI protein was nearly absent in the nephritic kidneys compared with non-nephritic kidneys and nonaffected tissue of kidneys extirpated due to cancer [48]. The nephritic processes such as those determined in murine lupus nephritis thus seem highly relevant to understand the process in the human form of the disease. The disease process as outlined in Figure 1 is therefore most probably relevant to understand both forms of lupus nephritis.

Based on the results discussed above, we propose the following model to understand initiation and progression of lupus nephritis in both mice and humans. The data from murine lupus nephritis are summarised as follows (see Figure 1 for details). The impact of antibodies to dsDNA is crucial for early deposition of chromatin fragments in the mesangial matrix. Linked to progression of the disease, secondary necrotic chromatin fragments are generated and retained in kidneys when the renal nuclease DNaseI mRNA level and DNaseI enzyme activity are downregulated. Secondary to this process, chromatin is not degraded appropriately, and instead large chromatin fragments are retained in glomerular capillary membranes in association with chromatin-reactive IgG autoantibodies. In this situation, chromatin fragments are also exposed to macrophages and dendritic cells in which they stimulate TLRs, which may explain the increased expression of MMPs. The increase of MMPs may further impose deposition of chromatin fragments in glomerular membranes because of capillary membrane disintegration [32-34]. Our conclusion is that human lupus nephritis is dependent on the same distinct processes.

Acquired loss of DNaseI in both murine and human lupus nephritis may be controlled at different levels, including activation of convergently encoded genes using sequence elements from the DNaseI gene, methylation of DNaseI encoding elements and the promoter, or interference with miRNA targeting DNaseI mRNA or other mRNAs involved in expression of DNaseI. This hypothesis is currently under investigation in our laboratory.

Potential implications of data on murine lupus nephritis for human lupus nephritis with respect to new treatment strategies

Despite improvements in outcomes of immunosuppressive treatment of patients with lupus nephritis, renal

remission is obtained in less than 50% of cases within 2 years; approximately 10% may progress to end-stage renal disease [83,84], which is associated with significantly increased rates of cardiovascular mortality [85]. In general, patients with SLE and renal involvement have more cardiovascular disease than the remaining SLE patients [86,87]. These findings call for improved treatment regimes for patients with lupus nephritis, not only in terms of improved renal outcome but also with regard to cardiovascular outcome.

The results discussed above on the aetiology of lupus nephritis demonstrate that DNaseI, the major renal nuclease, is profoundly downregulated during development of severe membrano-proliferative nephritis. Considering this single information, it may be sound to conclude that lupus nephritis is a disease entity that depends on processes that are unique to the kidney, and that an acquired shutdown of renal DNaseI is the factor that determines the disease process and clinical outcome, as outlined in Figure 1. This opens the way for new therapeutic directions

Causal therapy of lupus nephritis: are there contours of new tracks in this landscape?

There are strong data allowing us to assume that the two-stepped process accounting for murine lupus nephritis is also relevant in human lupus nephritis. If this assumption is correct then we can introduce new types of treatment focusing on disruption of chromatin structures *in vivo* by chaperone molecules that open the compact and nuclease/protease-resistant chromatin structure. Such molecules may make the chromatin structure more susceptible for proteases and nucleases [88-91]. On the contrary, certain chaperone molecules may prevent binding of nucleosomes to glomerular membranes by altering the net charge of chromatin fragments, as demonstrated *in vitro* by surface plasmon resonance [92], and also potentially *in vivo* in the context of permanent infusion of such chaperone molecules [92]. Therefore, it is important to determine whether processes that account for the potentially fatal human lupus nephritis can be avoided without influence on the immune system. In the next section, a possible strategy and relevant experiments will be discussed.

An approach to new therapeutic principals applied to lupus nephritis

One possible approach is to use molecules that are involved in chromatin assembly, disassembly or remodelling. Such molecules have the ability to alter the conformation of the chromatin structure, which may result in increased sensitivity for both nucleases and proteases. This could lead to increased degradation of the potentially immunogenic chromatin fragments [61,93] – fragments that otherwise would be presented to the immune system – thereby inducing pathogenic anti-dsDNA/anti-nucleosome antibody responses.

This idea derives from the described effects of chaperone molecules such as nucleosome assembly protein 1, a histone chaperone molecule that modulates binding of the linker DNA-associated histone H1 to chromatin and induces an extended and open chromatin fibre conformation [94,95]. Nucleoplasmin is also a histone chaperone that binds and exchanges histones to re-establish the chromatin structure, and is involved in opening and relaxation of the chromatin structures [91,96]. The heat shock protein HSP90 has a similar effect on chromatin structure [97,98].

These are examples of molecules that induce changes in chromatin conformation which may result in increased accessibility for proteases and nucleases and in increased degradation of nucleosomes. Whether chaperone molecules are tolerated *in vivo* in doses necessary for therapeutic effect has not been determined. One chaperone molecule that may be used in a therapeutic context is heparin, a negatively charged molecule that is well tolerated *in vivo* and has similar effects on chromatin structure as the molecules mentioned above. Heparin derivatives have been evaluated for their effect on nucleosome and chromatin structure. Common for these studies are data demonstrating that heparin makes nucleosomes more accessible to nucleases [74,99] by binding the trypsin-sensitive solvent-phase tails of the core histones [88]. Heparin also increases enhancer–promoter communication [100] by disassembling the chromatin structure [101,102]. Typical for heparin-induced structural changes is increased fragmentation of the nucleosomal structure by nucleases ubiquitously present in biological fluids [99]. As anionic heparin binds tightly to histone tails, and potentially changes the net charge of the nucleosome, heparin may in fact also inhibit binding of nucleosome-containing immune complexes to components of the GBM, like laminins and collagens. Heparin may thus have a two-sided effect on the role of chromatin fragments in lupus nephritis; increased enzymatic degradation of chromatin fragments, and inhibition of their binding to glomerular membranes.

Heparin derivatives inhibit binding of chromatin to glomerular basement membranes and increase their enzyme-mediated degradation

Interfering with chromatin–IgG complex binding to glomerular extracellular membranes could be a new treatment strategy. Negatively charged heparin binds to positively charged histones in the nucleosome complex and opens their architecture [88].

A pilot study has demonstrated that chromatin is more sensitive to both DNaseI and proteases in the presence of

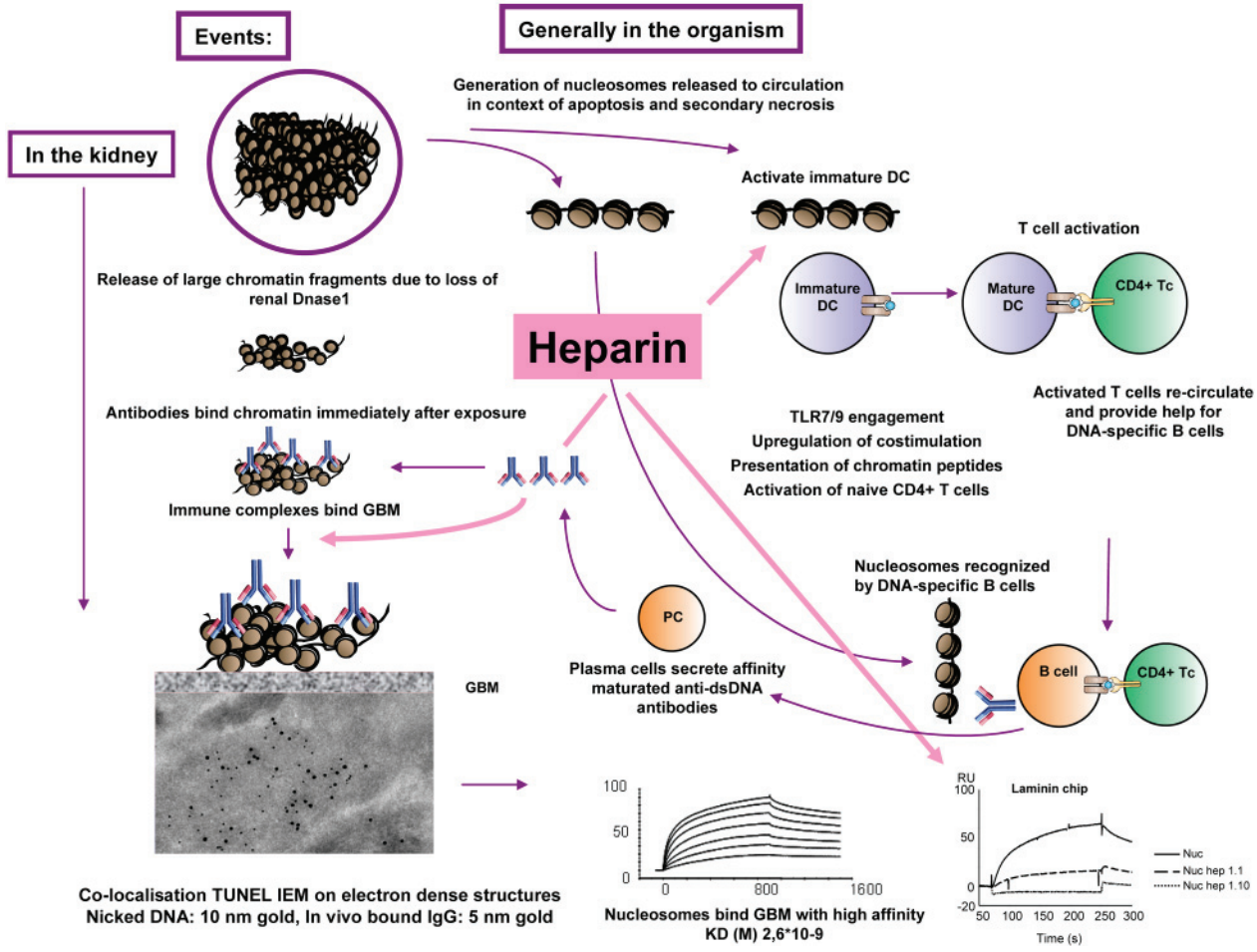

Figure 1. Exposed, extracellular chromatin is a central factor in evolution of lupus nephritis – a model. In normal situations, chromatin is effectively removed in the context of apoptosis. When chromatin fragments are not appropriately cleared, they may be exposed in tissue or in circulation. Exposure of chromatin may have an impact on the immune system. Chromatin may recirculate as oligonucleosomes, and eventually activate dendritic cells (DC). These cells present chromatin-derived peptides and upregulated co-stimulatory molecules to naïve peptide-specific $CD4^+$ T cells. Activated T cells may subsequently recirculate and provide help to DNA-specific or nucleosome-specific B cells to be transformed into antibody-secreting plasma cells. In this situation the antibodies are potentially pathogenic, but to exert this potential they must bind exposed chromatin fragments. This may happen in the kidneys when DNaseI is downregulated, and may have an immense impact on the pathogenic effect of the autoantibodies. Chromatin in cells dying from, for example, apoptosis may, due to loss of DNaseI, not be degraded, and instead of clearance they become exposed as secondary necrotic chromatin in, for example, glomerular basement membranes (GBMs), where they are targeted by induced anti-chromatin antibodies. Chromatin fragments may thus exert two effects with fatal consequences for the kidneys: they may induce autoimmunity (nucleosomes), and they represent targets for the induced autoantibodies (chromatin fragments). This identifies two hot points for therapy with chaperone molecules such as heparin: increased nuclease-mediated digestion of nucleosomal DNA, and thereby reduced load of immunogenic DNA; and prevention of binding of immunocomplexes containing chromatin fragments to GBMs and matrices (arrow to the left, surface plasmon resonance analysis of the effect of heparin, bottom right). Binding of nucleosomes to laminin was reduced by unfractionated heparin reaching approximately 75% and 100% inhibition at a nucleosome:heparin molar ratio of 1:1 and 1:10, respectively. For experimental details, see [73]. One single chaperone molecule may thus have a two-sided beneficial effect on lupus nephritis. KD, equilibrium dissociation constant; PC, plasma cell; TLR, Toll-like receptor; TUNEL IEM, terminal deoxynucleotidyltransferase biotin-dUTP nicked end-labelled immune electron microscopy. Modified with permission from [34], © 2010 Macmillan Publishers Ltd.

low molecular weight heparin, indicating changes in chromatin structure. Highly promising was the observation that heparin inhibited binding of chromatin–IgG complexes to glomerular laminin and collagen *in vitro*, as demonstrated by surface plasmon resonance (Figure 1) [92]. There is thus a strong indication that heparin derivatives (or other nucleosome re-modelling proteins such as nucleoplasmin [91]) indeed exert a two-sided therapeutic effect on lupus nephritis: heparin alters chromatin structures and allows a nearby complete degradation of B-cell-recognising DNA in chromatin, thus preventing production of nephritogenic anti-DNA

antibodies; and heparin prevents binding of chromatin–IgG fragments that escape enzymatic degradation *in vivo* [92]. Continuous infusion of heparin delayed production of anti-dsDNA antibodies and development of lupus nephritis in (NZBxNZW)F1 hybrid mice. Previous studies have revealed that heparin/heparinoid treatment has a therapeutic effect on the activity of lupus nephritis in MRL-lpr/lpr mice [103]. The mechanism(s) for this therapeutic effect, however, was not determined – but the anticoagulant effect does not seem to be essential [103]. In another study, Naparstek and colleagues indicated that the binding of antibodies to dsDNA could be inhibited by heparin [104]. This potentially important observation, however, has not been followed up by further studies.

The aim of current experiments in our laboratory is to determine an epigenetic mechanism(s) for renal DNaseI shutdown, and to analyse whether DNaseI sensitivity of immune complexes purified from patients with lupus nephritis is increased by heparin at concentrations tolerated in a clinical context. Highly relevant also are the planned experiments to analyse whether low molecular weight heparin interferes with processing and presentation of chromatin fragments by antigen-presenting cells.

Concluding remarks and perspectives

Detailed studies have offered new insight into molecular and transcriptional events that explain processes contributing to lupus nephritis. This insight has provided us with new therapeutic ideas and possibilities. Analysing chemical compounds that inhibit binding of chromatin–IgG complexes to components of the extracellular matrices and membranes, in combination with alteration of extracellular chromatin structure to make them more sensitive to proteases and nucleases, is the focus for our investigation. In the future it may be possible to prevent both autoimmunity to DNA and chromatin fragments on one side, and to inhibit binding of chromatin fragments to the mesangial matrix and the GBMs on the other side.

Autoimmune Basis of Rheumatic Diseases

This article is part of a series on *Systemic lupus erythematosus*, edited by David Pisetsky, which can be found online at http://arthritis-research.com/series/lupus

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Abbreviations

dsDNA, double-strand DNA; GBM, glomerular basement membrane; IFN, interferon; miRNA, microRNA; MMP, matrix metalloprotease; SLE, systemic lupus erythematosus; TLR, Toll-like receptor; TNF, tumour necrosis factor.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The authors thank Søren Jacobsen for help with the manuscript. The present study was supported by grants from the Northern Norway Regional Health Authority Medical Research Program (grant numbers SFP-100-04 and SFP-101-04), and from the University of Tromsø as Milieu support given to OPR.

Published: 18 April 2011

References

1. Ceppellini R, Polli E, Celada F: **A DNA-reacting factor in serum of a patient with lupus erythematosus diffusus.** *Proc Soc Exp Biol Med* 1957, **96:**572-574.
2. 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.
3. 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.
4. Xie C, Liang Z, Chang S, Mohan C: **Use of a novel elution regimen reveals the dominance of polyreactive antinuclear auto-antibodies in lupus kidneys.** *Arthritis Rheum* 2003, **48:**2343-2352.
5. 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.
6. Van Bruggen MC, Kramers C, Hylkema MN, Smeenk RJ, Berden JH: **Significance of anti-nuclear and anti-extracellular matrix auto-antibodies 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.
7. Winfield JB, Faiferman I, Koffler D: **Avidity of anti-DNA antibodies in serum and IgG glomerular eluates from patients with systemic lupus erythematosus. Association of high avidity antinative DNA antibody with glomerulonephritis.** *J Clin Invest* 1977, **59:**90-96.
8. Dang H, Harbeck RJ: **The in vivo and in vitro glomerular deposition of isolated anti-double-stranded-DNA antibodies in NZB/W mice.** *Clin Immunol Immunopathol* 1984, **30:**265-278.
9. 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.
10. Izui S, Lambert PH, Fournie GJ, Turler H, Miescher PA: **Features of systemic lupus erythematosus in mice injected with bacterial lipopolysaccharides: identificantion of circulating DNA and renal localization of DNA–anti-DNA complexes.** *J Exp Med* 1977, **145:**1115-1130.
11. Khalil M, Spatz L, Diamond B: **Anti-DNA antibodies.** In *Systemic Lupus Erythematosus*. 3rd edition. Edited by Lahita RG. San Diego, CA: Academic Press; 1999:197-217.
12. Hahn BH: **Antibodies to DNA.** *N Engl J Med* 1998, **338:**1359-1368.
13. Mostoslavsky G, Fischel R, Yachimovich N, Yarkoni Y, Rosenmann E, Monestier M, Baniyash M, Eilat D: **Lupus anti-DNA auto-antibodies cross-react with a glomerular structural protein: a case for tissue injury by molecular mimicry.** *Eur J Immunol* 2001, **31:**1221-1227.
14. Deocharan B, Qing X, Lichauco J, Putterman C: **Alpha-actinin is a cross-reactive renal target for pathogenic anti-DNA antibodies.** *J Immunol* 2002, **168:**3072-3078.
15. 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.
16. Mageed RA, Zack DJ: **Cross-reactivity and pathogenicity of anti-DNA auto-antibodies in systemic lupus erythematosus.** *Lupus* 2002, **11:**783-786.
17. 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.
18. D'Andrea DM, Coupaye Gerard B, Kleyman TR, Foster MH, Madaio MP: **Lupus auto-antibodies interact directly with distinct glomerular and vascular cell surface antigens.** *Kidney Int* 1996, **49:**1214-1221.
19. 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.

20. 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.
21. Basnakian AG, Apostolov EO, Yin X, Napirei M, Mannherz HG, Shah SV: **Cisplatin nephrotoxicity is mediated by deoxyribonuclease I.** *J Am Soc Nephrol* 2005, **16:**697-702.
22. 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.
23. Balow JE, Boumpas DT, Ausin HA: **Systemic lupus erythematosus and the kidney.** In *Systemic Lupus Erythematosus*. 3rd edition. Edited by Lahita RG. San Diego, CA: Academic Press; 1999:657-685.
24. Shlomchik MJ, Madaio MP: **The role of antibodies and B cells in the pathogenesis of lupus nephritis.** *Springer Semin Immunopathol* 2003, **24:**363-375.
25. Zhao S, Wang Y, Liang Y, Zhao M, Long H, Ding S, Yin H, Lu Q: **MicroRNA-126 regulates DNA methylation in CD4(+) T cells and contributes to systemic lupus erythematosus by targeting DNA methyltransferase 1.** *Arthritis Rheum* 2011. [Epub ahead of print]
26. Dai Y, Sui W, Lan H, Yan Q, Huang H, Huang Y: **Comprehensive analysis of microRNA expression patterns in renal biopsies of lupus nephritis patients.** *Rheumatol Int* 2009, **29:**749-754.
27. Kato M, Arce L, Natarajan R: **MicroRNAs and their role in progressive kidney diseases.** *Clin J Am Soc Nephrol* 2009, **4:**1255-1266.
28. Smits EL, Ponsaerts P, Berneman ZN, Tendeloo VF: **The use of TLR7 and TLR8 ligands for the enhancement of cancer immunotherapy.** *Oncologist* 2008, **13:**859-875.
29. Miyake K, Nakashima H, Akahoshi M, Inoue Y, Nagano S, Tanaka Y, Masutani K, Hirakata H, Gondo H, Otsuka T, Harada M: **Genetically determined interferon-gamma production influences the histological phenotype of lupus nephritis.** *Rheumatology (Oxford)* 2002, **41:**518-524.
30. Rönnblom L, Alm GV, Eloranta ML: **The type I interferon system in the development of lupus.** *Semin Immunol* 2011, **23:**113-121.
31. Aringer M, Feierl E, Steiner G, Stummvoll GH, Hofler E, Steiner CW, Radda I, Smole JS, Graninger WB: **Increased bioactive TNF in human systemic lupus erythematosus: associations with cell death.** *Lupus* 2002, **11:**102-108.
32. Overall CM, Butler GS: **Protease yoga: extreme flexibility of a matrix metalloproteinase.** *Structure* 2007, **15:**1159-1161.
33. Butler GS, Overall CM: **Updated biological roles for matrix metalloproteinases and new 'intracellular' substrates revealed by degradomics.** *Biochemistry* 2009, **48:**10830-10845.
34. Tveita AA, Rekvig OP, Zykova SN: **Glomerular matrix metalloproteinases and their regulators in the pathogenesis of lupus nephritis.** *Arthr Res Ther* 2008, **10:**229.
35. Rekvig OP, Bendiksen S, Moens U: **Immunity and autoimmunity induced by polyomaviruses: clinical, experimental and theoretical aspects.** *Adv Exp Med Biol* 2006, **577:**117-147.
36. Pisetsky DS, Vrabie IA: **Antibodies to DNA: infection or genetics?** *Lupus* 2009, **18:**1176-1180.
37. Radic MZ, Weigert M: **Genetic and structural evidence for antigen selection of anti-DNA antibodies.** *Annu Rev Immunol* 1994, **12:**487-520.
38. Marion TN, Krishnan MR, Steeves MA, Desai DD: **Affinity maturation and autoimmunity to DNA.** *Curr Dir Autoimmun* 2003, **6:**123-153.
39. Munoz LE, Janko C, Schulze C, Schorn C, Sarter K, Schett G, Herrmann M: **Autoimmunity and chronic inflammation – two clearance-related steps in the etiopathogenesis of SLE.** *Autoimmun Rev* 2010, **10:**38-42.
40. van Bavel CC, van der Vlag J, Berden JH: **Glomerular binding of anti-dsDNA auto-antibodies: the dispute resolved?** *Kidney Int* 2007, **71:**600-601.
41. Berden JH, Licht R, Van Bruggen MC, Tax WJ: **Role of nucleosomes for induction and glomerular binding of auto-antibodies in lupus nephritis.** *Curr Opin Nephrol Hypertens* 1999, **8:**299-306.
42. Pisetsky DS, Ullal AJ: **The blood nucleome in the pathogenesis of SLE.** *Autoimmun Rev* 2010, **10:**35-37.
43. Zhao Z, Weinstein E, Tuzova M, Davidson A, Mundel P, Marambio P, Putterman C: **Cross-reactivity of human lupus anti-DNA antibodies with α-actinin and nephritogenic potential.** *Arthritis Rheum* 2005, **52:**522-530.
44. van Bavel CC, Fenton KA, Rekvig OP, van der Vlag J, Berden JH: **Glomerular targets of nephritogenic auto-antibodies in systemic lupus erythematosus.** *Arthritis Rheum* 2008, **58:**1892-1899.
45. Holers VM: **Anti-C1q auto-antibodies amplify pathogenic complement activation in systemic lupus erythematosus.** *J Clin Invest* 2004, **114:**616-619.
46. Raz E, Ben Bassat H, Davidi T, Shlomai Z, Eilat D: **Cross-reactions of anti-DNA auto-antibodies with cell surface proteins.** *Eur J Immunol* 1993, **23:**383-390.
47. Fenton K, Fismen S, Hedberg A, Seredkina N, Fenton C, Mortensen ES, Rekvig OP: **Anti-dsDNA antibodies promote initiation, and acquired loss of renal DNaseI promotes progression of lupus nephritis in autoimmune (NZBxNZW)F1 mice.** *PLoS One* 2009, **4:**e8474.
48. Zykova SN, Tveita AA, Rekvig OP: **Renal DNaseI enzyme activity and protein expression is selectively shut down in murine and human membranoproliferative lupus nephritis.** *PLoS One* 2010, **5:**e12096.
49. Fismen S, Mortensen ES, Rekvig OP: **Nuclease deficiencies promote end-stage lupus nephritis but not nephritogenic autoimmunity in (NZB x NZW) F1 mice.** *Immunol Cell Biol* 2010, **89:**90-99.
50. Martinez-Valle F, Balada E, Ordi-Ros J, Bujan-Rivas S, Sellas-Fernandez A, Vilardell-Tarres M: **DNaseI activity in systemic lupus erythematosus patients with and without nephropathy.** *Rheumatol Int* 2010, **30:**1601-1604.
51. Martinez-Valle F, Balada E, Ordi-Ros J, Bujan-Rivas S, Sellas-Fernandez A, Vilardell-Tarres M: **DNase 1 activity in patients with systemic lupus erythematosus: relationship with epidemiological, clinical, immunological and therapeutical features.** *Lupus* 2009, **18:**418-423.
52. Davis JC Jr, Manzi S, Yarboro C, Rairie J, Mcinnes I, Averthelyi D, Sinicropi D, Hale VG, Balow J, Austin H, Boumpas DT, Klippel JH: **Recombinant human Dnase I (rhDNase) in patients with lupus nephritis.** *Lupus* 1999, **8:**68-76.
53. Yasutomo K, Horiuchi T, Kagami S, Tsukamoto H, Hashimura C, Urushihara M, Kuroda Y: **Mutation of DNASEI in people with systemic lupus erythematosus.** *Nat Genet* 2001, **28:**313-314.
54. Kawane K, Nagata S: **Nucleases in programmed cell death.** *Methods Enzymol* 2008, **442:**271-287.
55. Samejima K, Earnshaw WC: **Trashing the genome: the role of nucleases during apoptosis.** *Nat Rev Mol Cell Biol* 2005, **6:**677-688.
56. Rekvig OP, Kalaaji M, Nossent H: **Anti-DNA antibody subpopulations and lupus nephritis.** *Autoimmun Rev* 2004, **3:**1-6.
57. Krieg AM, Vollmer J: **Toll-like receptors 7, 8, and 9: linking innate immunity to autoimmunity.** *Immunol Rev* 2007, **220:**251-269.
58. Lim EJ, Lee SH, Lee JG, Kim JR, Yun SS, Baek SH, Lee C: **Toll-like receptor 9 dependent activation of MAPK and NF-kB is required for the CpG ODN-induced matrix metalloproteinase-9 expression.** *Exp Mol Med* 2007, **39:**239-245.
59. Lim EJ, Lee SH, Lee JG, Chin BR, Bae YS, Kim JR, Lee CH, Baek SH: **Activation of toll-like receptor-9 induces matrix metalloproteinase-9 expression through Akt and tumor necrosis factor-alpha signaling.** *FEBS Lett* 2006, **580:**4533-4538.
60. Mortensen ES, Rekvig OP: **Nephritogenic potential of anti-DNA antibodies against necrotic nucleosomes.** *J Am Soc Nephrol* 2009, **20:**696-704.
61. Kruse K, Janko C, Urbonaviciute V, Mierke CT, Winkler TH, Voll RE, Schett G, Munoz LE, Herrmann M: **Inefficient clearance of dying cells in patients with SLE: anti-dsDNA auto-antibodies, MFG-E8, HMGB-1 and other players.** *Apoptosis* 2010, **15:**1098-1013.
62. Seredkina N, Zykova S, Rekvig OP: **Progression of murine lupus nephritis is linked to acquired renal DNaseI deficiency and not to up-regulated apoptosis.** *Am J Pathol* 2009, **175:**97-106.
63. 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.
64. 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.
65. Cline AM, Radic MZ: **Apoptosis, subcellular particles, and autoimmunity.** *Clin Immunol* 2004, **112:**175-182.
66. Sekigawa I, Kawasaki M, Ogasawara H, Kaneda K, Kaneko H, Takasaki Y, Ogawa H: **DNA methylation: its contribution to systemic lupus erythematosus.** *Clin Exp Med* 2006, **6:**99-106.
67. Agrawal A, Tay J, Yang GE, Agrawal S, Gupta S: **Age-associated epigenetic modifications in human DNA increase its immunogenicity.** *Aging (Albany NY)* 2010, **2:**93-100.
68. Lu MC, Yu CL, Yin WY, Tung CH, Huang KY, Liu SQ, Lai NS: **Increased prevalence of polyomavirus BK viruria that correlates with thrombocytopenia in patients with systemic lupus erythematosus on intensive immunosuppressive therapy.** *Autoimmunity* 2009, **42:**216-223.
69. Tolstov YL, Pastrana DV, Feng H, Becker JC, Jenkins FJ, Moschos S, Chang Y, Buck CB, Moore PS: **Human Merkel cell polyomavirus infection II. MCV is a common human infection that can be detected by conformational capsid**

epitope immunoassays. *Int J Cancer* 2009, **125:**1250-1256.
70. Bonofiglio R, Papalia T, Caroleo MC, Mazzucco G, Merlino C, Bergallo M, Giraldi C, Perugini D, De Stefano R, Iuele R, De Napoli N: **Polyomavirus BK nephropathy in renal transplant: 2 cases with different clinical expressions and review of the literature.** *G Ital Nefrol* 2003, **20:**38-42.
71. Hirsch HH, Suthanthiran M: **The natural history, risk factors and outcomes of polyomavirus BK-associated nephropathy after renal transplantation.** *Nat Clin Pract Nephrol* 2006, **2:**240-241.
72. 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.
73. Mjelle JE, Rekvig OP, Fenton KA: **Nucleosomes possess a high affinity for glomerular laminin and collagen IV and bind nephritogenic antibodies in murine lupus-like nephritis.** *Ann Rheum Dis* 2007, **66:**1661-1668.
74. Napirei M, Wulf S, Mannherz HG: **Chromatin breakdown during necrosis by serum DNaseI and the plasminogen system.** *Arthritis Rheum* 2004, **50:**1873-1883.
75. Lachmann PJ: **Lupus and desoxyribonuclease.** *Lupus* 2003, **12:**202-206.
76. Tsukumo S, Yasutomo K: **DNaseI in pathogenesis of systemic lupus erythematosus.** *Clin Immunol* 2004, **113:**14-18.
77. Amoura Z, Piette JC, Bach JF, Koutouzov S: **The key role of nucleosomes in lupus.** *Arthritis Rheum* 1999, **42:**833-843.
78. Amoura Z, Piette JC, Chabre H, Cacoub P, Papo T, Wechsler B, Bach JF, Koutouzov S: **Circulating plasma levels of nucleosomes in patients with systemic lupus erythematosus: correlation with serum antinucleosome antibody titers and absence of clear association with disease activity.** *Arthritis Rheum* 1997, **40:**2217-2225.
79. Muller S, Dieker J, Tincani A, Meroni PL: **Pathogenic anti-nucleosome antibodies.** *Lupus* 2008, **17:**431-436.
80. Jang YJ, Stollar BD: **Anti-DNA antibodies: aspects of structure and pathogenicity.** *Cell Mol Life Sci* 2003, **60:**309-320.
81. Waldman M, Madaio MP: **Pathogenic auto-antibodies in lupus nephritis.** *Lupus* 2005, **14:**19-24.
82. 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.
83. Jacobsen S, Starklint H, Petersen J, Ullman S, Junker P, Voss A, Rasmussen JM, Tarp U, Poulsen LH, van Overeem Hansen G, Skaarup B, Hansen TM, Pødenphant J, Halberg P: **Prognostic value of renal biopsy and clinical variables in patients with lupus nephritis and normal serum creatinine.** *Scand J Rheumatol* 1999, **28:**288-299.
84. Houssiau FA, Ginzler EM: **Current treatment of lupus nephritis.** *Lupus* 2008, **17:**426-430.
85. Foley RN: **Clinical epidemiology of cardiovascular disease in chronic kidney disease.** *J Renal Care* 2010, **36(Suppl 1):**4-8.
86. Urowitz MB, Ibanez D, Gladman DD: **Atherosclerotic vascular events in a single large lupus cohort: prevalence and risk factors.** *J Rheumatol* 2007, **34:**70-75.
87. Wells DK, Ward MM: **Nephritis and the risk of acute myocardial infarction in patients with systemic lupus erythematosus.** *Clin Exp Rheumatol* 2010, **28:**223-229.
88. Villeponteau B: **Heparin increases chromatin accessibility by binding the trypsin-sensitive basic residues in histones.** *Biochem J* 1992, **288(Pt 3):**953-958.
89. Brotherton TW, Jagannadham MV, Ginder GD: **Heparin binds to intact mononucleosomes and induces a novel unfolded structure.** *Biochemistry* 1989, **28:**3518-3525.
90. Eitoku M, Sato L, Senda T, Horikoshi M: **Histone chaperones: 30 years from isolation to elucidation of the mechanisms of nucleosome assembly and disassembly.** *Cell Mol Life Sci* 2008, **65:**414-444.
91. Laskey RA, Mills AD, Philpott A, Leno GH, Dilworth SM, Dingwall C: **The role of nucleoplasmin in chromatin assembly and disassembly.** *Philos Trans R Soc Lond B Biol Sci* 1993, **339:**263-269.
92. Hedberg A, Fismen S, Fenton KA, Fenton C, Osterud B, Mortensen ES, Rekvig OP: **Heparin exerts a dual effect on lupus nephritis by enhancing enzymatic chromatin degradation and preventing chromatin-binding in glomerular membranes.** *Arthritis Rheum* 2011, **63:**1065-1075.
93. Pisetsky DS: **HMGB1: a dangerous player in lupus pathogenesis.** *J Rheumatol* 2010, **37:**689-691.
94. Kepert JF, Mazurkiewicz J, Heuvelman GL, Toth KF, Rippe K: **NAP1 modulates binding of linker histone H1 to chromatin and induces an extended chromatin fiber conformation.** *J Biol Chem* 2005, **280:**34063-34072.
95. Mazurkiewicz J, Kepert JF, Rippe K: **On the mechanism of nucleosome assembly by histone chaperone NAP1.** *J Biol Chem* 2006, **281:**16462-16472.
96. Prado A, Ramos I, Frehlick LJ, Muga A, Ausio J: **Nucleoplasmin: a nuclear chaperone.** *Biochem Cell Biol* 2004, **82:**437-445.
97. Zhao R, Davey M, Hsu YC, Kaplanek P, Tong A, Parsons AB, Krogan N, Cagney G, Mai D, Greenblatt J, Boone C, Emili A, Houry WA: **Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the hsp90 chaperone.** *Cell* 2005, **120:**715-727.
98. Zhao R, Houry WA: **Hsp90: a chaperone for protein folding and gene regulation.** *Biochem Cell Biol* 2005, **83:**703-710.
99. Napirei M, Ludwig S, Mezrhab J, Klockl T, Mannherz HG: **Murine serum nucleases – contrasting effects of plasmin and heparin on the activities of DNaseI and DNaseI-like 3 (DNaseII3).** *FEBS J* 2009, **276:**1059-1073.
100. Polikanov YS, Rubtsov MA, Studitsky VM: **Biochemical analysis of enhancer-promoter communication in chromatin.** *Methods* 2007, **41:**250-258.
101. Conde e Silva N, Black BE, Sivolob A, Filipski J, Cleveland DW, Prunell A: **CENP-A-containing nucleosomes: easier disassembly versus exclusive centromeric localization.** *J Mol Biol* 2007, **370:**555-573.
102. Simeonova M, Tchacarov E: **Prefixation chromosome banding with heparin.** *Hum Genet* 1980, **56:**63-66.
103. Van Bruggen MC, Walgreen B, Rijke TP, Corsius MJ, Assmann KJ, Smeenk RJ, van Dedem GW, Kramers K, Berden JH: **Heparin and heparinoids prevent the binding of immune complexes containing nucleosomal antigens to the GBM and delay nephritis in MRL/lpr mice.** *Kidney Int* 1996, **50:**1555-1564.
104. Naparstek Y, Ben-Yehuda A, Madaio MP, Bar-Tana R, Schuger L, Pizov G, Neeman ZV, Cohen IR: **Binding of anti-DNA antibodies and inhibition of glomerulonephritis in MRL-lpr/lpr mice by heparin.** *Arthritis Rheum* 1990, **33:**1554-1559.

doi:10.1186/ar3281
Cite this article as: Hedberg A, *et al.*: Chromatin as a target antigen in human and murine lupus nephritis. *Arthritis Research & Therapy* 2011, **13:**214.