
Central role for nucleosomes in lupus

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the formation of antinuclear autoantibodies especially against double-stranded (ds) DNA [1,2]. Although initially it was thought that the formation of these autoantibodies was due to polyclonal B cell activation, it is now clear that the disease arises as a result of an (auto-) antigen driven response, since somatic mutations, clonal expansion and IgG class switches occur. There is fair evidence that anti-DNA antibodies are important in the initiation of tissue lesions especially lupus glomerulonephritis. Because of this pathogenic role, numerous attempts have been made to induce these anti-dsDNA antibodies by immunization of dsDNA from various sources, either alone or complexed to proteins. These experiments have revealed that DNA is poorly immunogenic. But if the formation of autoantibodies in SLE is antigen driven and DNA is not the driving agent, are there other candidates?

In recent years the nucleosome has been put forward as a major candidate for the autoantigen that drives the autoimmune response in SLE [3,4]. In this editorial comment we will review these data and also discuss the role of nucleosomes in the pathogenesis of lupus nephritis.

Immunogenicity of nucleosomes

Nucleosomes consist of a 146 bp segment of DNA wrapped, in two superhelical turns, around an octameric core complex containing four pairs of the histone proteins H2A, H2B, H3, and H4. DNA within the nucleosome is responsible for clustered negative charges. On the other hand, the amino-terminal 20-30 amino acids of H2A, H2B, H3, and H4, which are exposed on the surface, are strongly positively charged. Nucleosomes can act as polyclonal B cell activators, which might be relevant in the initiation of disease. But more importantly, since lupus is an antigen-driven
Nucleosome-specific autoantibodies

In spontaneous SLE nucleosome-specific reactivity was first described for monoclonal autoantibodies derived from lupus mice. Later, these nucleosome-specific autoantibodies were also detected in serum/plasma of SLE patients and SLE mice. In fact these nucleosome-specific autoantibodies are present in about 80% of both lupus mice and SLE patients [6,7]. Retrospectively, antibodies to nucleosomes are probably the first autoantibodies described in association with SLE, in the so-called 'LE-cell phenomenon' which is due to the phagocytosis of 'LE-cell factors'. These 'LE cell factors' were subsequently identified as autoantibodies and it was shown that nucleosomes, in contrast to free dsDNA or histones, were able to inhibit the formation of the 'LE cell phenomenon'. This suggests that the autoantibodies which are responsible for the 'LE cell phenomenon' are nucleosome-specific antibodies. When analysing polyclonal serum/plasma for anti-nucleosome reactivity one should realize that anti dsDNA and anti-histone antibodies will also give a positive result. These antibodies have to be removed by absorption on a DNA-cellulose and a histone-Sepharose column [6,8,9]. From such absorption studies it appeared that in the presence of anti-DNA antibodies, 25–65% of the anti-nucleosome reactivity in non-absorbed plasma was due to anti-dsDNA antibodies, while this was much lower for anti-histone antibodies (10–20%). Most data on epitope specificity of nucleosome-specific antibodies have been obtained with monoclonal antibodies. Most of these monoclonal antibodies recognize epitopes within the H2A-H2B/DNA and to a lesser extent within the (H3-H4)/DNA complex, but for some monoclonal antibodies the reactivity with the intact nucleosomes is higher than with these subnucleosomal structures, suggesting that additional epitopes are involved. Interestingly, the V_H regions of these nucleosome-specific mAbs contained within the CDR3 region an increased amount of cationic charged residues (like anti-DNA antibodies) and an increase in anionic residues in CDR2 region (as in anti-histone antibodies) [10]. Therefore, these antibodies seem to harbour antigen-binding characteristics for both anionic DNA and cationic histone epitopes.

Systemic release of nucleosomes and apoptosis

There is now increasing evidence that apoptosis (a process of programmed cell death leading to the internucleosomal cleavage of chromatin) is disturbed in both murine and human lupus. In several (MRL/lpr and gld) but not all (NZBWF1) lupus mouse strains it was demonstrated that this disturbance in apoptosis was due to a deficient expression of either the Fas receptor (APO-1; CD95) or its ligand. Activation of this Fas-receptor leads to apoptosis. In human SLE the expression of the Fas system is normal, but increased concentrations of soluble Fas, increased expression of the apoptosis inhibitor bcl-2 and an increased rate of spontaneous apoptosis in vitro of lymphocytes from SLE patients point to abnormalities in apoptosis also in human SLE. These disturbances in apoptosis lead not only to persistence of auto-reactive T cells but also to quantitative and qualitative changes in the release and composition of nucleosomes. This process might be enhanced by a decreased phagocytosis of apoptotic cells. This concept is in line with the observation that nuclear material in the circulation of SLE patients is present in the form of (oligo-) nucleosomes, suggesting that it originates from apoptotic cells [11]. Recently, in an experimental set-up, Casciola-Rosen et al. [12] elegantly demonstrated the relationship between apoptosis, nucleosomes and nucleosome-specific autoantibodies. They found that these lupus-derived nucleosome-specific antibodies bound to nucleosomes localized in apoptotic bodies (blebs) at the surface of keratinocytes undergoing apoptosis induced by UV light. Similar evidence is provided by the observation that nucleosome-specific autoantibodies bind to nucleosomes released from apoptotic mononuclear cells. The apoptotic 'blebs', in which the nucleosomes appear, are topologically restricted to selected sites (nuclear membranes, mitochondria and endoplasmatic reticulum) where free radical-induced protein modifications (e.g. fragmentation, amino-acid modification, novel proteolytic cleavages) may generate novel (more immunogenic) nucleosome epitopes, that may then trigger non-tolerant T helper cells.

Role of nucleosomes in the binding of autoantibodies to the GBM

An alternative explanation, other than deposition of DNA/anti-DNA complexes, for the pathogenicity of antinuclear antibodies is that these antibodies cross-react with epitopes present within the glomerulus. Many direct interactions of anti-DNA and other lupus autoantibodies with glomerular antigens (endothelial
and epithelial cell surface antigens, individual basement membrane components, and matrix constituents) have been described [13,14]. Several years ago we found that both monoclonal and polyclonal anti-DNA antibodies could bind to heparan sulphate (HS), an intrinsic constituent of the GBM. (HS has a strong negative charge and is responsible for the net negative charge of the GBM and for its charge-dependent permeability.) Later we found that this HS cross-reactivity was due to nucleosomal material complexed to the antibodies. Subsequent experiments showed that monoclonal antinuclear autoantibodies (mAbs) which are complexed to nucleosomes are able to bind to the GBM in vivo, whereas purified antibodies are not [8]. The positively charged histone part in the nucleosome is responsible for the binding to negatively charged determinants in the GBM. The pathophysiological significance of this binding is underlined by the fact that complement activation occurs after binding. Besides HS, collagen IV has been put forward as a ligand in the GBM [15]. The importance of HS as ligand for this binding was illustrated by the fact that after renal perfusion of heparitinase, which removes HS from the GBM, the binding of the complexed mAbs decreased considerably [8]. The involvement of HS in the pathogenesis of SLE nephritis was further substantiated by the finding that the staining of HS in the GBM was almost completely absent both in human and murine (MRL/l and NZBWF1 mice) SLE. This decrease was due to the binding of nucleosome-containing immune complexes to HS in the GBM. The pathophysiological significance of this binding was illustrated by the inverse correlation between GBM HS staining and proteinuria [16].

Several other observations point also to the in vivo relevance of this nucleosome-mediated binding of autoantibodies to the GBM in SLE nephritis. First, elution of antibodies from glomeruli disclosed specificities towards all components of the nucleosome i.e. DNA, histones and nucleosomes [17,18]. Furthermore, deposition of anti-nucleosome antibodies preceded the deposition of anti-DNA antibodies. Second, histones and nucleosomes are present in glomerular deposits in human SLE. With a panel of histone and nucleosome-specific monoclonal antibodies we found respectively 100% and 45% of human kidney biopsies with diffuse proliferative lupus nephritis deposits along the GBM. This observation confirms and extends the detection of histones in glomerular deposits reported earlier by Schmiedeke and Stöckel [19,20]. The clinical relevance of nucleosome-complexed autoantibodies was substantiated by the finding that anti-HS reactivity (as a method to identify nucleosome-complexed autoantibodies in the circulation) was elevated at the onset or exacerbation of SLE nephritis [21,22].

Finally, indirect evidence for nucleosome involvement in lupus nephritis comes from our studies in which we used different heparins. Heparin and non-coagulant heparin derivatives are able to inhibit the binding of nucleosome-complexed autoantibodies to HS in vitro (ELISA), but more importantly also, after renal perfusion, to the GBM in vivo. In the MRL/l lupus mouse model we found that heparin and non-coagulant heparinoids prevented the deposition of nucleosome complexed autoantibodies in the GBM and delayed the onset of nephritis.

**Concluding remarks**

These data suggest that nucleosomes may be the primary and major autoantigen in SLE. Besides serving as an immunogen for pathogenic T helper cells, nucleosomes contribute to the development of lupus nephritis by mediating the binding of antinuclear antibodies to the glomerular basement membrane.

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**References**

15. Di Valerio R, Bernstein KA, Varghese E, Lefkowitz JB. Murine lupus glomerulotrophic monoclonal antibodies exhibit differing
specifications but bind via a common mechanism. J Immunol 1995; 155: 2258-2268


Introduction

There is no doubt that during water conservation, kidneys supersaturate urine, especially with respect to calcium salts, and that protein inhibitors of stone formation play the major role in the natural defense against nephrocalcinosis. This editorial will first focus on the recent advances concerning the protein inhibitors of calcium oxalate (CaOx) crystallization and then on some unsolved problems.

Glycosaminoglycans (GAGs)

Urinary GAGs are enzymatic products of proteoglycans. They are excreted in urine and present in the stone matrix. Their concentration in urine is too low to decrease calcium supersaturation significantly. In the crystal growth model and by zeta-potential measurements, GAGs have been shown to act as inhibitors of CaOx crystal growth and aggregation by blocking the growth sites. GAGs prevent crystal adhesion to renal cells, which is likely to be an important step in urolithiasis. Exogenous GAGs can restore the anti-adherence properties of an injured urothelium, thereby preventing crystal adhesion [1].

Oral administration of GAG to a group of stone formers (SF) led to a significant decrease in oxalate self exchange, which is abnormal in the majority of SF. Many investigators have studied urinary GAGs but it remains unclear whether there are qualitative and/or quantitative differences between SF and normal individuals (N).

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Neprocalcin (NC)

From urine, kidney tissue, and CaOx stones, Coe et al. isolated a glycoprotein inhibiting CaOx crystal growth by absorption to crystal surfaces [2]. Neprocalcin inhibits CaOx nucleation and aggregation, and crystal adhesion to renal cells. One mole of NC binds 4 moles of Ca²⁺ and its binding sites differ completely from those in other Ca²⁺-binding proteins [3]. Neprocalcin is produced in human kidney by proximal tubule and the thick ascending limb of Henle’s loop. It contains 2–3 residues of γ-carboxyglutamic acid (Gla) per molecule, therefore NC is a vitamin-K-dependent protein. Abnormal forms of NC, lacking Gla, seem to occur in SF.

In a cloning study of the mouse osteocalcin gene, Desbois et al. found a cluster of three genes. Two of them are expressed only in bone. The third one, named ORG, is expressed in kidney but not in bone. Several lines of evidence suggest that ORG encodes neprocalcin [4].

Neprocalcin has never been sequenced; thus its role in kidney stone disease is unclear.

Uropontin

Uropontin is very closely related, if not identical, to osteopontin, the bone phosphoprotein that binds to the bone matrix. There may be differences between the two molecules with respect to post-translational modifications such as phosphorylation, glycosylation and sulphation, which are tissue specific. Uropontin is rich in non γ-carboxylated aspartic residues, and thus distinct from NC. At concentrations that normally prevail in urine, the urinary form of osteopontin is a potent inhibitor of CaOx nucleation, growth and aggregation.