Lupus nephritis: A nucleosome waste disposal defect?

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ABSTRACT: Formation of anti-nuclear autoantibodies is a cardinal characteristic of systemic lupus erythematosus (SLE). In recent years the nucleosome has been identified as the major autoantigen, since nucleosome specific T cells have been identified, which also drive the formation of anti-dsDNA and anti-histone antibodies. Nucleosome specific autoantibodies are present in a large majority of SLE patients and lupus mice. Nucleosomes are formed during apoptosis by organized cleavage of chromatin. These nucleosomes together with other lupus autoantigens cluster in apoptotic bodies at the surface of apoptotic cells. Systemic release of these autoantigens is normally prevented by swift removal of apoptotic cells. However, if the rate of apoptosis overflows the removal capacity and/or the cleaning machinery is reduced, nucleosomes are released. Furthermore, during apoptosis autoantigens can be modified, which makes them more immunogenic. Nucleosomes also play a pivotal role in the evolution of tissue lesions, especially glomerulonephritis. In lupus nephritis nucleosomes, anti-nucleosome autoantibodies and nucleosome/Ig complexes have been identified in the glomerular immune deposits. Via their cationic histone part nucleosomes can bind to heparan sulfate, a strong anionic constituent of the glomerular basement membrane.

Key words: *Systemic lupus erythematosus, Nephritis, Apoptosis, Nucleosomes, Heparan sulfate*

INTRODUCTION

Formation of anti-nuclear autoantibodies, especially against double-stranded (ds)DNA, is a hallmark of systemic lupus erythematosus (SLE). Until recently, it was rather curious why these multi-molecular complexes, normally hidden in the nucleus, become immunogenic and targets for autoimmune responses. Especially, in view of the fact that naked dsDNA is hardly immunogenic, and most likely in this form can not be considered as a major autoantigen in SLE. In the last decade several pieces of evidence have shed new light on the mechanisms leading to autoimmune responses and the pathogenesis of SLE (1, 2). Lupus nephritis is one of the most serious complications in SLE, occurring in up to 60% of patients with SLE. Traditionally, it was thought that lupus nephritis resulted from the glomerular deposition of DNA/anti-DNA complexes. However, DNA/anti-DNA complexes are hardly nephritogenic (3). Also with regard to the pathogenesis of lupus nephritis several observations have provided new clues for the events that lead to glomerular inflammation in SLE.

In 1995 we presented a new hypothesis for the development of lupus nephritis (4) shown in Figure 1. Disturbed apoptosis (either too much, at the wrong place or delayed) leads to the persistence of autoreactive T and B cells. If the rate of apoptosis overflows the phagocytic removal of apoptotic cells or phagocytosis is impaired, apoptotic cell derived material will be released. This includes nucleosomes and other nuclear autoantigens. During apoptosis these autoantigens can be modified, which can make them more immunogenic. This (increased) exposure of (modified) nucleosomes to the immune system leads to the formation of autoantibodies to nucleosomes and dsDNA. Nucleosomes are the basic units of chromatin, the structure which enables dense packing of 2 meters of DNA in the nucleus. Nucleosomes are linked to each other with protein free DNA. During apoptosis this linker DNA is cleaved by endonucleases, which leads to the formation of free nucleosomes. Each nucleosome consists of pairs of the four core histones H2A, H2B, H3 and H4 forming the histone octamer.

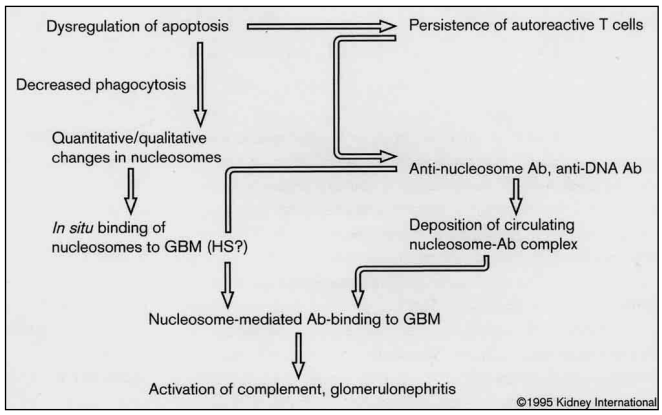

Fig. 1 - Hypothesis for the immune dysregulation in SLE and the development of lupus nephritis (*reproduced with permission from reference (4)*).

Around this octamer 146 bp of dsDNA are wound twice. Histone H1 is located on the outside of the nucleosome. The core histones are strong cationic molecules due to the presence of many basic amino acids at the amino terminal portions which protrude outside the nucleosome. Other regions of the nucleosome are anionic due to the negative charges of DNA. Because of the strong positive charges of the histone part of the nucleosome, nucleosomes can bind to negatively charged moieties in basement membranes like heparan sulfate (HS) in the glomerular basement membrane (GBM). This binding to the GBM can occur directly, after which these nucleosomes can act as planted antigens for the binding of anti-nucleosome or anti-dsDNA antibodies. Alternatively, autoantibody binding to the GBM can also proceed via deposition of nucleosome containing immune complexes. In either way, complement activation then incites the glomerular inflammation. Since the proposal of this hypothesis a number of observations has lent additional support for this concept and has fine-tuned it further. In this article we will review the current evidence for each of the different elements of this hypothesis.

Apoptosis and SLE

The first notion that apoptosis was associated with lupus came from the discovery that MRL/lpr lupus mice had a functional Fas deficiency (5). Binding of the Fas ligand to the Fas receptor (CD95), present on activated T and B cells, leads to apoptosis. Also deficiency of the Fas ligand like in gld mice, leads to the same lupus phenotype as in MRL/lpr mice (6). Transgenic correction of these deficiencies prevents the development of autoimmunity (7). Injection of soluble Fas in CD1 female mice, which inhibits Fas-mediated apoptosis, induces autoimmunity (8). Another example of inhibition of apoptosis leading to autoimmunity is transgenic overexpression of Bcl-2. When the Bcl-2 gene was put under the control of an immunoglobulin promotor, the number of autoreactive B cells increased, leading to the generation of anti-nucleosome autoantibodies and glomerulonephritis (9). These observations seem to contrast with the above-mentioned hypothesis in which increased apoptotis was proposed as a driving feature for lupus development, while in these latter animal studies inhibition of apoptosis was associated with lupus. However, this decreased Fas related apoptosis promotes the survival of autoreactive T and B cells, since this mechanism is important for the peripheral elimination of these autoreactive T cells (10). Furthermore, these cells, which escape Fas-mediated apoptosis, are nevertheless primed for apoptosis and become apoptotic via other, non-Fas related mechanisms at other sites. This may lead to apoptosis in micro-environments not properly suited for the swift removal of apoptotic cells. In patients with SLE, Fas-related defects in apoptosis are less clear (11). Patients with a Fas or Fas-ligand deficiency develop an autoimmune lymphoproliferative syndrome (ALPS) (12). This condition is characterized by lymphadenopathy, splenomegaly, hemolytic anemia and thrombopenia. Only a quarter of the patients develop antinuclear antibodies and glomerulonephritis is rarely seen. So, unlike the animal models, in human SLE no clearcut genetic defects in the Fas pathway have been detected so far. However, some other apoptosis related abnormalities have been found: elevated levels of soluble Fas (13, 14), increased Bcl-2 expression (15), increased levels of circulating apoptotic cells (16, 17) and increased "spontaneous" apoptosis *in vitro* (18). However, it has been questioned whether these abnormaliteis are specific for SLE, since they can result from lymphocyte activation (19).

Apart from being a primary abnormality, abnormal apoptosis can also be induced by autoantibodies for instance directed against poly(ADP-ribose) polymerase (PARP). PARP normally repairs DNA breaks, thereby counteracting pro-apoptotic stimuli. Antibodies against PARP (which can be present in SLE) inhibit the inactivation of PARP, which normally occurs during apoptosis (20). From these data it is difficult to draw a clearcut conclusion yet. Both too little apoptotic cell death as well as too much apoptotic cell death might lead to autoimmunity. It seems likely that in SLE the delicate balance regulating apoptosis is lost, resulting in apoptosis occuring at the wrong time and/or in the wrong micro-environment. Abnormal apoptosis in itself is not necessarily to be detrimental if the removal of apoptotic cells is rapid and complete. Therefore, impairment of apoptotic cell disposal is probably more important then disturbances in apoptosis itself.

PHAGOCYTOSIS OF APOPTOTIC CELLS IN SLE

The interest in the body's cleaning machinery for apoptotic cells has increased after the discovery that apoptotic cells are the suppliers of autoantigens. A seminal observation of Rosen et al documented this for the first time. They showed that autoantigens become clustered in surface blebs after induction of apoptosis of keratinocytes with UV light (21). The smaller blebs contain SS-A (52kD), ribosomal P protein, α-fodrin and Jo-1, while the larger apoptotic bodies contain nucleosomes, SS-A (60kD), SS-B, Sm, SnRNP complexes, PARP and other autoantigens (22). Since several mechanisms can alter these autoantigens during apoptosis, it is of utmost importance that these apoptotic cells are removed adequately. It is beyond the scope of this review to describe in detail the process of phagocytosis of apoptotic cells, since excellent reviews are available (23). In brief, induction of the apoptotic process leads, apart from intranucleosomal cleavage of chromatin and nuclear condensation, to a number of surface changes, most notably the expression of phosphatidylserine (PS), which is normally present at the inside of the cell membrane. This and other changes provide "eat-me" signals to neighbouring cells and macrophages. Via a large number of receptors including scavenger receptors, the LPS receptor (CD14), the C1q receptor, the vitronectin receptor, other β-integrins and lectins, the apoptotic cell binds to the macrophage and is subsequently internalised and degraded. For some of these receptors "bridging" molecules are necessary like C1q, β2-glycoprotein I (which binds to PS), thrombospondin, C-reactive protein (CRP) and serum amyloid P protein (SAP). The ligands which bind to these bridging molecules or receptors are for the larger part putative. This complex interaction is graphically depicted in Figure 2 derived from reference (23). An important feature of apoptotic cell removal by macrophages is the induction of an anti-inflammatory reaction (24, 25) instead of release of pro-inflammatory cytokines as observed if phagocytosis occurs via Fc receptors (26).

Because all autoantigens targeted in SLE are either located in small or apoptotic blebs or at the surface of apoptotic cells, we (4) and others (27) have postulated that a defective phagocytosis of apoptotic cells may be a pivotal feature in the generation of the autoimmune response. This impaired phagocytosis may lead to the release of nuclear antigens incuding nucleosomes (28), since the major pathway for the generation of nucleosomes is apoptosis. In fact, circulating nucleosomes have been found in SLE patients (29, 30) and lupus mice (31). Indeed, a defective removal of apoptotic cells has been documented in patients with active SLE (32). However, this defect can be secondary to the disease, since autoantibodies could potentially inhibit binding and/or engulfment of apoptotic cells. Therefore, we analysed phagocytic capacity of apoptotic cells in lupus mouse strains with a sensitive technique (33). In pre-diseased mice no constitutive defect was found (34), while in animals with clinical overt disease phagocytosis of apoptotic cells was impaired. This defect resides in the plasma and is either a shortage of a critical plasma component (complement?) or the presence of an inhibitor (autoantibody?).

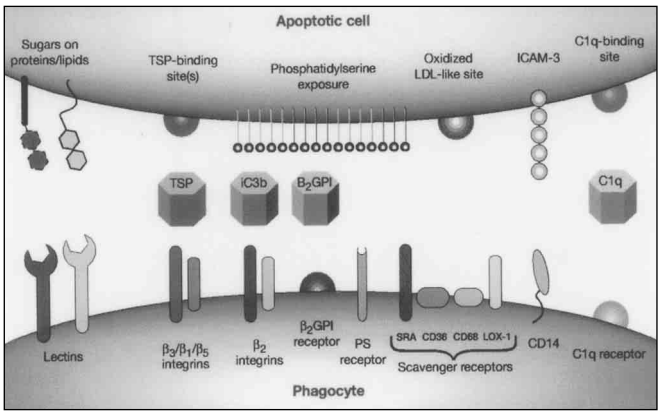

Fig. 2 - The molecular topography of apoptotic cell clearance by macrophages (*reproduced with permission from reference (23)*).

Recently, a number of studies with well defined knock-out mice have been reported which documented the utmost relevance of proper removal of apoptotic cells. These studies are summarised in Table I. In all these knock-out mice three features were observed: i) an impairment of apoptotic cell removal; ii) generation of anti-nuclear autoantibodies most notably against nucleosomes and iii) development of glomerular deposits containing immunoglobulins and complement factors and in some models histological signs of glomerulonephritis. These observations show that inadequate removal of apoptotic cells and/or chromatin may lead to lupus.

MODIFICATION OF AUTOANTIGENS DURING APOPTOSIS IN SLE

The execution of apoptosis involves a number of proteases, the so-called caspases, which cleave either structural proteins necessary for nuclear and cell integrity or enzymes involved in DNA and cell repair (35). Caspase mediated destruction of these cellular components leads to the collapse of the cell. Remarkably, a large number of the proteins that are cleaved during apoptosis are targets for autoimmune responses in SLE. In fact, certain SLE sera react better with the cleaved protein than with the native molecule

TABLE I - PREVALENCE OF ANTI-NUCLEAR AUTOANTIBODIES AND GLOMERULONEPHRITIS IN MICE WITH A TARGETED DISRUPTION OF MOLECULES RELEVANT FOR PHAGOCYTOSIS OF APOPTOTIC CELLS

Deficient molecule[a)]	Anti-nuclear autoantibody formation[b)] (% of positive mice)				Glomerulonephritis[d)] (% of positive mice)	Reference
	ANA[c)]	anti-histone	anti-dsDNA	anti-nucleosome		
C1q	43	43	ND[e)]	ND	25	(71)
SAP	F 82	54	46	74	42	(72)
	M 63	31	25	59	4	
Dnase I	F 73	25	38	88	31	(73)
	M 56	24	12	71	19	
IgM	ND	ND	70	ND	70	(74)
mer^{kd}	ND	ND	58	ND	ND	(75)

a) Except for mer^{kd} mice, deficiency was induced by targeted disruption of the gene coding for the listed molecule. Mer^{kd} mice have a cytoplasmic truncation of the Mer receptor induced by targeted disruption.
b) Several mouse strains were used for these knock-out experiments. In certain wild-type strains a low prevalence of autoantibodies was also present. For details see the respective references.
c) ANA=anti-nuclear antibody test
d) Glomerulonephritis is defined as either histological evidence of glomerulonephritis and/or the presence of immunoglobulin and/or C3 deposits.
e) ND = not determined.

(36). Recently, cleavage by granzyme B has been identified as a major mechanism for autoantigen modification during apoptosis. Granzyme B is the initiator of apoptosis induced by cytotoxic T cells. Granzyme B preferentially cleaves autoantigens in unique fragments, while non-autoantigens are either not cleaved or cleaved similar to other proteases (37). Therefore, it has been proposed that susceptibility for cleavage by granzyme B is predictive for the autoantigen status of a protein. At present a large number of autoantigens is known to be cleaved during apoptosis (38). Apart from cleavage also other modifications of autoantigens have been documented during apoptosis like phosphorylation or dephosphorylation, (de)ubiquitination, methylation and cross-linking by transglutaminase (36).

In contrast to the several protein autoantigens, relatively little is known about modification of nucleosomes during apoptosis. The modifications so far described are mainly located on histones and include (de)phosphorylation, (de)methylation, hyperactylation or deacetylation, ubiquitination (H2A) and transglutaminase cross-linking (H2B). In addition to protein modifications, DNA can become more immunogenic by abnormal methylation, formation of double strand breaks, or cleavage. Some of these modifications are induced by reactive oxygen species induced during apoptosis (39). Although some of the modifications are recognised by polyclonal autoantibodies from SLE patients, the impact of nucleosome modifications for the autoimmune response in SLE remains to be determined.

IMMUNOGENICITY OF NUCLEOSOMES

Since naked dsDNA has long been regarded as the major autoantigen in SLE, many attempts have been made to immunise with dsDNA in all sorts of forms and conditions. However, these procedures failed to induce anti-dsDNA antibodies with lupus specific characteristics. The first positive result has been obtained after immunisation with dsDNA complexed to histone-like DNA binding proteins from either viral or protozoal origin. The antibodies formed were directed against dsDNA and nucleosomes (40). Seminal studies by Datta et al showed that in the SNF1 murine lupus model 50% of the pathogenic T helper cells were directed against nucleosomes. These T helper cells did not only provide help for the production of nucleosome specific antibodies, but also for anti-dsDNA and anti-histone antibodies, a phenomenon known as antigen spreading (41). This observation shed new light on the initiation of the anti-dsDNA antibody response: not dsDNA but the nucleosome is the driving autoantigen in SLE. Subsequently, similar observations have been reported in human SLE (42, 43). These nucleosome specific T cells respond to histone epitopes on MHC class II molecules presented after

TABLE II - PREVALENCE OF ANTI-NUCLEOSOME REACTIVITY IN SLE

Reference	Analysed population	Prevalence (%)	Characteristics
Fisher et al (1988) (76)	MRL/lpr mice (n>500)	100	Increasing titer with age 50% IgG2a, 30% IgG2b, 10% IgG1, 10% IgG3
	MRL/+ mice	72	
	(NZBxNZW)F1	61	Subclass distribution similar and titers lower
	BxSB	69	than in MRL/lpr mice
	control strains	0	
Burlingame et al (1994) (51)	SLE patients (n=40)	88	Anti-chromatin and anti-H2A-H2B/DNA correlated with lupus nephritis
Suenaga et al (1996) (77)	SLE patients with nephritis (n=6)	60	
Amoura et al (2000) (54)	systemic autoimmune disease (n=496) chronic hepatitis C infection (n=100) healthy controls (n=406)	SLE: 72 SSC: 46 MCTD: 45 Controls: 0	IgG3 antinucleosome antibodies exclusively present in SLE and related to lupus nephritis and disease activity (SLEDAI)
Bruns et al (2000) (43)	SLE patients (n=106) other systemic autoimmune diseases (n=26) healthy controls (n=105)	SLE: 56 All others: 3	- Better sensitivity and specificity than measurement of anti-dsDNA (diagnostic confidence anti-nucleosome: 90%. anti-dsDNA: 69%) - anti-nucleosome correlated significantly with disease activity (ECLAM), lupus nephritis and psychosis

Abbreviations: SSC=systemic sclerosis; MCTD=mixed connective tissue disease; SLEDAI=SLE disease activity index; ECLAM=European Consensus Lupus Activity Measurement Index.

processing of nucleosomal material by antigen presenting cells (APC) (44, 45). Whether modifications on these histone peptides, induced during apoptosis, are present and important for the T cell response is unknown yet, and needs to be elucidated. However, it has been demonstrated that APC can indeed present peptides derived from engulfed apoptotic cells to T cells (46, 47), although surprisingly (in face of the exogenous nature of the antigen) this presentation took place via MHC class I molecules. So, these data indicate that T cells towards nucleosomal epitopes are present in both human and murine lupus. In fact, in murine lupus this T cell reactivity can be demonstrated long before any serological or clinical sign of the disease (41). These obervations posed the question whether anti-nucleosome antibodies are present in SLE. This anti-nucleosome reactivity was first demonstrated for monoclonal antibodies derived from lupus mice (48). Subsequently, they were also detected in the great majority of lupus mice (49, 50) and patients (51, 52). From these studies, it also appeared that the formation of anti-nucleosome antibodies preceded that of other anti-nuclear specificities like anti-dsDNA and anti-histone. The prevalence of anti-nucleosome antibodies in lupus mice and patients are summarised in Table II. It is important to realise that not only nucleosome specific antibodies bind to nucleosomes, but also anti-dsDNA and anti-histone antibodies. Very often SLE patients who have anti-nucleosome antibodies, also have anti-dsDNA and/or anti-histone reactivity. Nucleosome specific antibodies without anti-dsDNA or anti-histone antibodies could be found in 16 to 30% of the patients. Absorption studies on DNA and histone columns revealed that approximately 25% of the anti-nucleosome reactivity was due to anti-dsDNA and/or anti-histone antibodies (52, 53). The studies summarised in Table II reported a strong correlation between anti-nucleosome reactivity and disease activity and lupus nephritis. In one study anti-nucleosome antibodies of the IgG3 subclass were exclusively found in SLE and specifically related to renal flares. A similar correlation was not found for IgG3 anti-dsDNA (54). From these data, it is clear that measurement of anti-nucleosome reactivity is preferable and more specific than anti-dsDNA. Moreover, it was recently demonstrated that anti-dsDNA reactivity, as measured with the gold standard, the Farr-assay, was for a large part due to histone containing immune complexes (55).

NUCLEOSOME MEDIATED AUTOANTIBODY BINDING TO THE GBM

Nucleosomes are not only important for the induction of the autoimmune response, but play also a decisive role in the development of tissue lesions, in particular lupus nephritis. The first clue for this notion came from the observation that anti-dsDNA antibodies could cross react with an intrinsic component of the GBM, namely heparan sulfate (HS) (56). HS is the strongly anionic side-chain of agrin, the major heparan sulfate proteoglycan (HSPG) of the GBM (57). HS determines the charge-dependent permeability of the GBM. Injection of monoclonal anti-HS antibodies instantly induces a massive proteinuria (58). So far, in various proteinuric diseases a number of HS alterations has been identified (59). The binding of antinuclear antibodies to HS was not due to cross-reactivity, as thought initially, but was mediated by nucleosomes (60). If monoclonal anti-dsDNA antibodies, that reacted in ELISA with HS, were treated with DNAse and were subsequently purified under high salt conditions on a protein-A column, all HS reactivity was lost. Addition of the protein A column effluent restored the binding to HS. Subsequent analysis revealed that histone/DNA complexes (i.e. nucleosomes) were responsible for the binding to HS. Also *in vivo*, in renal perfusion studies in the rat, nucleosomes could mediate the binding to the GBM, while non-complexed anti-nucleosome and anti-dsDNA antibodies did not bind (61). This nucleosome mediated binding occurred via binding of the cationic N terminal tails of the core histones to the strong anionic charges of HS. This was deducted from a number of observations. First, removal of HS, by prior intrarenal perfusion of heparanase (which cleaves HS), strongly reduced the binding to the GBM of subsequently perfused nucleosome/autoantibody complexes (61). Second, binding of anti-histone antibodies to the N terminal parts of the core histones to a great extent prevented the binding of nucleosome/anti-histone complexes to the GBM. In contrast to this, complex formation of nucleosomes with either anti-dsDNA or anti-nucleosome autoantibodies created a nephritogenic complex (62). Because the epitopes of anti-histone antibodies are mainly localised on the N terminal regions, their binding masks the positive charges on these histone tails, thereby preventing binding to anionic HS. These positive charges on the N termini of histones can not be neutralised by the binding of anti-dsDNA or anti-nucleosome antibodies. In fact, their binding to the nucleosome has an opposite effect, since they neutralise in part the anionic charges of dsDNA, which makes the complex even more nephritogenic. Third, once we realised the importance of the cationic regions on the core histones for the binding to HS, we argued that neutralisation of these positive charges on the histone tails with a HS 'look a-like' molecule could perhaps prevent binding to HS. Since heparin is such a HS decoy molecule, the effect of heparin was analysed in different ways. *In vitro*, heparin could inhibit in ELISA dose-dependently the binding to HS of nucleosome complexed anti-dsDNA or anti-nucleosome antibodies. In the renal perfusion system addition of heparin to nephritogenic nucleosome/autoantibody complexes completely prevented GBM binding. Based on these observations the protective effect of daily heparin injections on lupus nephritis was analysed. Treatment of MRL/lpr mice from week 8 onwards with heparin or non-coagulant heparinoids prevented the development of proteinuria and 80% of the animals had no histological signs of glomerulonephritis at the age of 22 weeks. In contrast to PBS treated MRL/l mice, which developed proteinuria and glomerulonephritis in 80%. The mechanism behind this renoprotective effect was revealed by immunofluorescence. In PBS treated mice extensive deposits along the glomerular capillary loops and in the mesangium were seen while in heparin(oid) treated mice only mesangial deposits were observed (63). So, neutralisation of the positive charges of the histones within the nucleosome/autoantibody complex by heparin prevented binding of these complexes to HS in the GBM.

If nucleosomes are important for the targeting of autoantibodies to the GBM, they should be present in glomerular deposits in murine and human lupus nephritis. Using polyclonal and monoclonal antibodies to the various components of the nucleosomes as probes, nucleosomes were indeed identified in human lupus nephritis, predominantly in the diffuse proliferative form (WHO class IV) (64). With elution studies of isolated glomeruli from MRL/lpr mice anti-nucleosome antibodies were identified (65). A time study revealed that these anti-nucleosome antibodies were deposited first, with subsequent deposition of anti-dsDNA antibodies. This sequence suggests that after lodging of the nucleosome in the GBM, it acts as a planted antigen for subsequent binding of anti-dsDNA. In concordance with the above-mentioned studies that nucleosome/anti-histone complexes are less nephritogenic, the amount of anti-histone antibodies was low and did not increase when the severity of the glomular lesion progressed. An analysis of various glomerular diseases with monoclonal antibodies against HS and the HSPG core protein revealed an almost complete absence of HS staining in the GBM in 90% of the biopsies from patients with proliferative lupus nephritis (66). A similar observation was mode in murine lupus nephritis (67), which allowed a further analysis

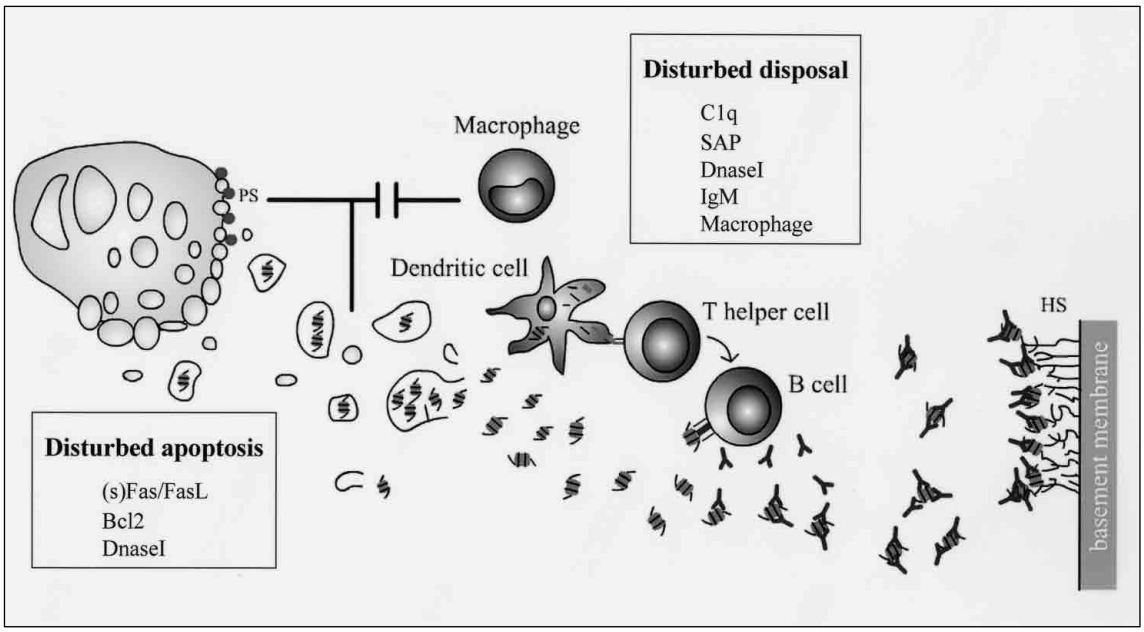

Fig. 3 - Graphical summary of the mechanisms leading to autoimmunity to nucleosomes in SLE and the development of lupus nephritis.

of the responsible mechanism. In a time study in MRL/lpr mice an inverse correlation was found between HS staining and albuminuria and between HS staining and immunoglobulin deposits in the GBM. This suggested masking of HS by immune complexes. Measurement of the HS content in isolated glomeruli from these mice showed a normal amount of HS, which indicated that the decrease in HS staining was not due to a reduction of the HS content in the glomerulus. Further proof for the masking of HS by deposited glomerular immune complexes came from three observations: i) *in vitro* pre-incubation of HS coated ELISA plates with nucleosome/autoantibody complexes inhibited the binding of the anti-HS monoclonal to HS dose-dependently (67); ii) heparin treatment of MRL/lpr mice not only prevented glomerular deposition as mentioned before, but also prevented the loss of HS staining (63); iii) in human lupus nephritis an inverse correlation was seen between GBM-HS staining and the amount of nucleosome deposits (64). So, although indirectly, this decreased HS staining in lupus nephritis indicates the presence of nucleosome/Ig complexes in the GBM. Taken together, these results document the presence of nucleosomes, anti-nucleosome antibodies and nucleosome/autoantibody complexes in glomerular deposits in lupus nephritis. They underline the relevance of nucleosome mediated targeting of autoantibodies to the GBM, as identified in experimental animal studies.

Because of their nephritogenic potential, it could be helpful to identify nucleosome/autoantibody complexes in the circulation of SLE patients. Using an ELISA, plasma samples of SLE patients were screened for anti-HS reactivity. Onset (68) or exacerbation (69) of lupus nephritis was indeed associated with higher anti-HS reactivity. Using a GBM based ELISA similar results were found (70). With a more direct approach, by measuring nucleosome/Ig complexes, an association was found in MRL/lpr mice between these nucleosome/autoantibody complexes and development of proteinuria (31).

Conclusion

In the last decade it has become clear that SLE is an auto-antigen driven T cell dependent autoimmune disease. The nucleosome has been identified as a major autoantigen. Nucleosomes are generated during apoptosis. Either an increased or delayed apoptosis or a reduced clearance of apoptotic cells (which are not mutually exclusive) leads to an increased exposure of (modified, more immunogenic) nucleosomes to the immune system. This generates the formation of nucleosome specific T cells and anti-nucleosome autoantibodies. After complex formation of anti-nucleosome or anti-dsDNA antibodies with nucleosomes, these autoantibodies are targeted to basement membranes, especially the GBM. This nephritogenic potential is due to the binding of the positively charged histone components of the nucleosome to the negatively charged HS within the GBM. This incites the glomerular inflammation. The sequence of these events is graphically depicted in Figure 3.

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