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REVIEW

The pathogenesis and diagnosis of systemic lupus erythematosus: still not resolved

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Abstract Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with various clinical manifestations affecting different tissues. A characteristic feature of SLE is the presence of autoantibodies against double-stranded (ds)DNA, histones and nucleosomes, and other chromatin components. SLE is a prototype type III hypersensitivity reaction. Local deposition of anti-nuclear antibodies in complex with released chromatin induces serious inflammatory conditions by activation of the complement system. The severe renal manifestation, lupus nephritis, is classified based on histological findings in renal biopsies. Apoptotic debris, including chromatin, is present in the extracellular matrix and circulation of patients with SLE. This may be due to an aberrant process of apoptosis and/or insufficient clearance of apoptotic cells/chromatin. The non-cleared apoptotic debris may lead to activation of both the innate and adaptive immune systems. In addition, an aberrant presentation of peptides by antigen-presenting cells, disturbed selection processes for lymphocytes, and deregulated lymphocyte responses may be involved in the development of autoimmunity. In the present review, we briefly will summarize current knowledge on the pathogenesis of SLE. We will also critically discuss and challenge central issues that need to be addressed in order to fully understand the pathogenic mechanisms involved in the development of SLE and in order to have an improved diagnosis for SLE. Disappointingly, in our opinion, there are still more questions than answers for the pathogenesis, diagnosis, and treatment of SLE.

Keywords Systemic lupus erythematosus · Lupus nephritis · Chromatin · Apoptosis · NETs · DNaseI · Renal

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Background

Systemic lupus erythematosus (SLE) causes a heavy burden for affected patients and their families, for the society, and for the economy [79]. Like other chronic inflammatory diseases, SLE is characterized by protracted morbidity and significant mortality. Substantial efforts are needed in order to develop a better insight into the complex molecular disease mechanisms, diagnostic procedures, and in particular development of biomarkers that indicate progression of the disease and also may reflect response to therapy. The research on SLE is intense, and since 1946, ~56,000 papers have been published (search term in Pubmed: Systemic lupus erythematosus), and ~140 genes have been associated with SLE (search term in OMIM: Systemic lupus erythematosus). Since 1959, ~34,000 papers have been published on the origin and role of anti-DNA antibodies, which are central in the pathogenesis and diagnosis of SLE (search term in Pubmed: Anti-DNA antibodies). This enormous number of scientific papers, which is still growing, reflects a strong international effort to understand the complex pathogenesis of SLE but indicates also that we still do not fully understand the pathogenesis of the disease. In other words, we do not understand the pathogenesis of autoimmunity in SLE and of its clinical manifestations in various affected tissues. This lack of knowledge and some controversies preclude the development of precise diagnostic tools and in addition precise and causal therapeutic interventions.

Introducing remaining questions on the role of anti-dsDNA antibodies in SLE

Anti-double-stranded (ds)DNA autoantibodies are the most studied anti-chromatin autoantibodies in SLE. There is still no international consensus that precisely explains why and how the assumed pathogenic anti-dsDNA antibodies are generated in vivo (origin). In particular, what is the source of DNA/chromatin and how is autoimmunity against DNA/chromatin initiated and developing? There is still debate on the disease mechanisms that causally involve anti-dsDNA antibodies (impact) in the various tissues that can be affected in SLE. Thus, despite tremendous international efforts to understand the nature of SLE and its pathogenic mechanisms, still, our insight is fragmental and elusive [4, 18, 47, 73, 74, 78, 84, 92, 96, 98, 101]. Additional unanswered questions include the following: what are the characteristics of a pathogenic anti-dsDNA antibody, are these characteristics related to the mechanisms responsible for their production, and what is the origin and characteristics of the chromatin fragments retained and targeted by anti-dsDNA antibodies in, e.g., the glomeruli in the context of lupus nephritis? In our opinion, answers to these questions will lead to a better insight into the origin and impact of anti-dsDNA antibodies on disease manifestations in SLE and lupus nephritis, which may in a broader sense be translated into fundamental aspects of causal treatment strategies.

In summary, the pathogenic and diagnostic value of anti-dsDNA antibodies may be dictated by the mechanism from which they originate (transient or sustained) and, secondly, by the mechanism from which the target antigen originates (transient or sustained). In subsequent paragraphs, we will discuss some of the biochemical processes that may induce autoimmunity and ultimately transform a well functional kidney into a destroyed organ.

Breaking of tolerance: apoptotic blebs and apoptotic chromatin in SLE

The source of chromatin, the main autoantigen, in SLE is most likely apoptotic and/or necrotic cells, including the neutrophilic extracellular traps (NETs). Apoptosis is involved in normal tissue homeostasis and is essential in the regulation of the immune response by central deletion of autoreactive B cells and T cells. Apoptosis can be induced by intrinsic and extrinsic factors, for example by DNA damage and by Fas ligand binding to the Fas receptor. Characteristic for apoptosis is the fragmentation of chromatin and the segregation of apoptotic blebs [37]. Notably, except for the NETs, we do not know the exact origin of apoptotic blebs and chromatin. Importantly, apoptotic blebs contain autoantigens targeted in SLE [17]. Many factors involved in apoptosis have been associated to SLE. Autoantigens can be modified during apoptosis, whereby these autoantigen modifications may facilitate the initial breaching of tolerance [26, 37, 88]. Autoantigens in SLE can be cleaved by caspases and endonucleases, like DNaseI as will be detailed below [16, 122]. In addition, autoantigens, including chromatin, may be modified through the addition of acetyl, phosphoryl, methyl, ubiquitin, citruline, ADP, or glutamine moieties. Autoantibodies against aforementioned modifications are present in patients with SLE [27, 88, 121]. Notably, we have identified specific hyperacetylation patterns on histone H2A, H2B, and H4, as well as a specific methylation pattern on H3 to be associated with apoptosis and SLE [24, 95, 123–125]. In summary, several factors related to apoptosis, including apoptosis-induced chromatin modification, have been associated with SLE.

Breaking of tolerance: clearance defects in SLE

Apoptosis and necrosis explain how normally inaccessible autoantigens can be released and subsequently become exposed to the immune system. In addition, an impaired removal may lead to further accumulation of apoptotic cells and debris. Normally, apoptotic cells are swiftly removed by professional phagocytes, including macrophages, B cells, and dendritic cells. Removal of apoptotic cells can occur in a non-inflammatory and anti-inflammatory manner. However, exposure of apoptotic debris, including chromatin, to dendritic cells may also result in a pro-inflammatory response. As mentioned, apoptotic blebs contain clustered SLE autoantigens, like (modified) chromatin [17]. In addition to apoptotic blebs, NETs can be considered as apoptotic chromatin. Released apoptotic chromatin autoantigens have a dual function: they may lead not only to the induction of autoimmunity but also to the formation of immune complexes in tissues affected by SLE. In the kidney for example, these immune complexes can deposit in the glomerular capillary filter, thereby inciting a severe glomerulonephritis by activation of the complement system. There is convincing evidence for clearance defects of apoptotic cells and debris in SLE, and actually, defects in many factors required for a proper clearance have been described [25, 37, 45, 87, 88, 103]. Actually, the clearance of apoptotic material by phagocytes is impaired in both lupus mice and patients [51, 72]. Downregulation of the expression/activity of the endonuclease DNaseI in the kidney further contributes to the persistence of extracellular apoptotic chromatin and to the development of lupus nephritis as will be further detailed below [105].

Chromatin fragments play a central role in the pathogenesis of lupus nephritis and persist due to silencing of the renal DNaseI gene

We described that early phases of lupus nephritis in lupus-prone (NZBxNZW)F1 mice were characterized by chromatin-IgG complex deposition in the mesangial matrix. A striking observation was that this phenomenon correlated with the early detection of serum antibodies to dsDNA and with mild or clinically silent nephritis [30]. In analogous experiments, injection of monoclonal anti-dsDNA antibodies into BALB/c mice imposed mesangial nephritis [31]. These spontaneous and experimental events were restricted to mesangial nephritis, and for the spontaneously occurring process, this was always preceded by progression of lupus nephritis into end-stage organ disease [30, 105, 107]. Apparently, as a consequence of this early lupus nephritis, renal DNaseI mRNA expression levels and enzyme activity were severely (>80 %) reduced. Reduced levels of renal DNaseI were associated in time with deficient fragmentation of chromatin from dead cells. Large chromatin fragments were retained and accumulated in the glomerular basement membrane (GBM). These observations may in fact explain the basis for deposition of chromatin-IgG complexes in glomeruli in early and late stages of nephritis, leading to complement activation and ultimately loss of glomerular integrity and renal failure.

Acquired error of renal chromatin metabolism—a conditio sine qua non for progressive lupus nephritis

As mentioned, lupus nephritis is a prototype immune complex disease where antibodies to dsDNA play a central role [47, 60]. Deposition of chromatin-anti-dsDNA antibody complexes is the core factor that imposes renal inflammation in both murine [9, 30, 62, 63, 127, 128] and human SLE [50, 61, 117, 126]. This is in harmony with observations that most antibodies eluted from nephritic kidneys contain IgG antibodies reactive with components of chromatin, like nucleosomes, dsDNA, and histones [19, 63, 130, 138, 139, 141]. However, the picture is not quite clear, as eluted antibodies aside from targeting components of chromatin also have the potential to recognize non-chromatin antigens [1, 21, 66, 130, 141].

Role of chromatin fragments in lupus nephritis

The origin of chromatin bound to glomerular matrices and membranes has for a long time been unknown, as has been the factors that account for progression of lupus nephritis. Recent data have contributed significantly to new insight into these problems. Current results from our studies on the etiology of murine and human lupus nephritis demonstrate that renal DNaseI, representing >80 % of total endonuclease activity in the kidneys [7], is profoundly downregulated when mild or clinically silent mesangial nephritis is transformed into end-stage organ disease in human SLE [105, 107, 142]. With low DNaseI enzyme activity, apoptotic chromatin is not appropriately fragmented and is instead transformed into secondary necrotic chromatin unmasked from apoptotic blebs (reviewed in [83, 84], see also [8, 126, 127]). Secondary to this, chromatin is exposed to the environment where it binds the GBM and the mesangial matrix at high affinity as is demonstrated in vitro by plasmon surface resonance analyses [81]. In those experiments, we demonstrated that chromatin fragments bound collagen IV and laminins at robust affinities, while the proteoglycan perlecan did not [49, 81]. This has been observed in both murine and human lupus nephritis [61, 62, 80].

Transcriptional interference—a possible explanation for the silencing of renal DNaseI gene expression

The term "transcriptional interference" is widely used but poorly defined in the literature [108]. Transcriptional interference usually refers to the direct negative impact of transcription of one gene on transcription of another gene provided the genes are transcribed in opposite directions and that the two genes overlap with each other. Transcriptional interference is potentially widespread throughout biology; therefore, it is timely to assess exactly its nature, significance, and operative mechanisms especially in clinical medicine. A bioinformatics analysis using the UCSC browser has led to a working hypothesis based on transcriptional interference between the DNaseI gene and a convergently transcribed gene—Trap 1. We hypothesize that this mechanism affects inversely DNaseI and Trap 1 gene expression in vivo.

This model also explains what happens if transcription of one of the gene pairs is initiated. In that situation, transcription of the convergent gene is blocked by the transcriptional activity of the first gene [52]. These results provide insight into fundamental mechanisms of gene expression control and point to an unexplored effect of antisense transcription on gene regulation via polymerase collision. This model is also valid if the genes overlap in the untranslated 3′ regions (UTR) since the primary transcript is elongated far beyond UTR [109, 115].

In summary, both experimental and descriptive data in clinical diseases are consistent and demonstrate that transcriptional interference between convergent and overlapping pair of genes may be a new principle for gene regulation. To establish that the Trap 1 and DNaseI gene are mutually regulated by this mechanism may provide important information on impact of the two genes on prognosis and therapy response

and how to control their expression. Details on studies on transcriptional interference between DNaseI and Trap 1 have recently been reviewed [33, 106].

The role of dendritic cells in SLE

In SLE, two main subsets of dendritic cells have been implicated to contribute to autoimmunity, i.e., myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC). pDC and mDC differ in their lineage and expression of receptors, including the Toll-like receptors. Macrophages can ingest apoptotic cells, blebs, and debris in an anti-inflammatory manner, which is characterized by the production of TGF-β and interleukin (IL)-10. In addition, dendritic cells encountering autoantigens without being activated will induce immunological tolerance. However, the immunological balance is skewed towards autoimmunity in SLE. We and other researchers demonstrated that mDC can be activated by apoptotic blebs and modified chromatin, by showing an increased expression of co-stimulatory molecules (CD86 and CD40) and increased secretion of pro-inflammatory cytokines (IL-1β, IL-6, and tumor necrosis factor (TNF)-α [12, 13, 35, 36]. Other studies showed that high-mobility group protein B1 (HMGB1) which is attached to apoptotic chromatin leads to the activation of mDC via TLR2 [120]. Presentation by activated mDC of the ingested modified chromatin to autoreactive T cells may be an early step in breaking the immunological tolerance that may occur in patients with SLE. Co-cultures of mDC matured with apoptotic blebs and T cells produce IL-2, interferon (IFN)-γ, and IL-17, which suggests a mixed Th1/Th17 response, as has been shown for patients with SLE. Importantly, IL-6 that is produced by activated mDC is increased in patients with SLE, which inhibits the development of regulatory T cells (T_{REG}) and stimulates the development of Th17 cells. Th17 cells activate autoreactive B cells and recruit inflammatory cells to specific organs [40]. Activated autoreactive T cells, specific for apoptosis-modified histone peptides, can also activate B cells which recognize either modified or unmodified parts of chromatin with their receptor, which results in the production of autoantibodies directed to modified and unmodified chromatin (DNA, histones, nucleosomes) via epitope spreading. After the formation of autoantibodies, immune complexes with circulating chromatin are formed that can activate mDC, thereby creating an amplification loop in the immune response against apoptotic chromatin. Immune complexes also can activate pDC via ligation of TLR7 and TLR9, thereby initiating the production of type I IFN, with IFN-α as the central cytokine. In addition to nucleic acid containing immune complexes, NETs also specifically trigger pDC to produce IFN-α [13, 39, 65, 69]. In patients with SLE, a type I IFN response is frequently observed, suggesting that pDC activation is a central event in the pathogenesis of SLE. IFN-α has several effector functions, such as facilitation of mDC maturation, B cell activation, T cell activation, and stimulation of NETosis, thereby amplifying the autoimmune response against chromatin [102]. In summary, both mDC and pDC play central roles in early events and amplifying events leading to autoimmunity (see Fig. 1 for an integrated hypothesis). The two pathways of immune activation via mDC and pDC are central events; however, additional tolerance-breaching mechanisms in SLE include (i) direct activation of autoreactive B cells by (apoptotic) chromatin; (ii) aberrant presentation of self-peptides by antigen-presenting cells; (iii) defects in the central selection processes for B and T cells; and (iv) defects in the regulatory processes of B and T cell responses, including cytokine regulation [46, 73, 119].

Anti-dsDNA antibodies as diagnostic criterion in SLE: critical remarks

As outlined above, SLE is regarded as a systemic autoimmune syndrome [78, 91, 98, 135]. B cell and T cell autoimmunity to chromatin and particularly to the dominant individual components of nucleosomes that is native dsDNA and histones are important in establishing a diagnosis [34, 47, 116]. The importance of anti-chromatin antibodies is further underscored by the fact that anti-chromatin, including anti-dsDNA, antibodies have the potential to induce lupus nephritis in SLE [28, 54, 55, 126, 131], while the etiology of other clinical manifestations in SLE is largely unknown [56]. Related to the latter remark, it can be theoretically questioned whether SLE represents one disease entity or is represented by a continuous overlap of etiologically unrelated organ manifestations. The American College of Rheumatology (ACR) classification criteria for SLE [116] do not answer this question, neither do the newly defined Systemic Lupus International Collaborating Clinics Classification Criteria for Systemic Lupus Erythematosus (SLICC) criteria [91, 135]. In both the ACR and SLICC classification criteria, the acceptance and inclusion of each criterion are set partly by consensus among experienced clinicians and scientists, partly by statistics, based on statistical co-appearance of organ and laboratory manifestations but also by insight into disease mechanisms for individual organ manifestations. The term "SLE" may, therefore, still theoretically represent an artificial common denominator for a wide variety of intrinsically unrelated disease manifestations. Are we not in fact directing treatments towards major manifestations of the disease? In a provocative way, one can state that there are no established diagnostic criteria for SLE, although the SLICC criteria may be used also to settle the diagnosis SLE. Consequently, two problems concerning the validity of the diagnosis and treatment of SLE can be defined. First, considering the different and complex faces of SLE, it seems not logic to

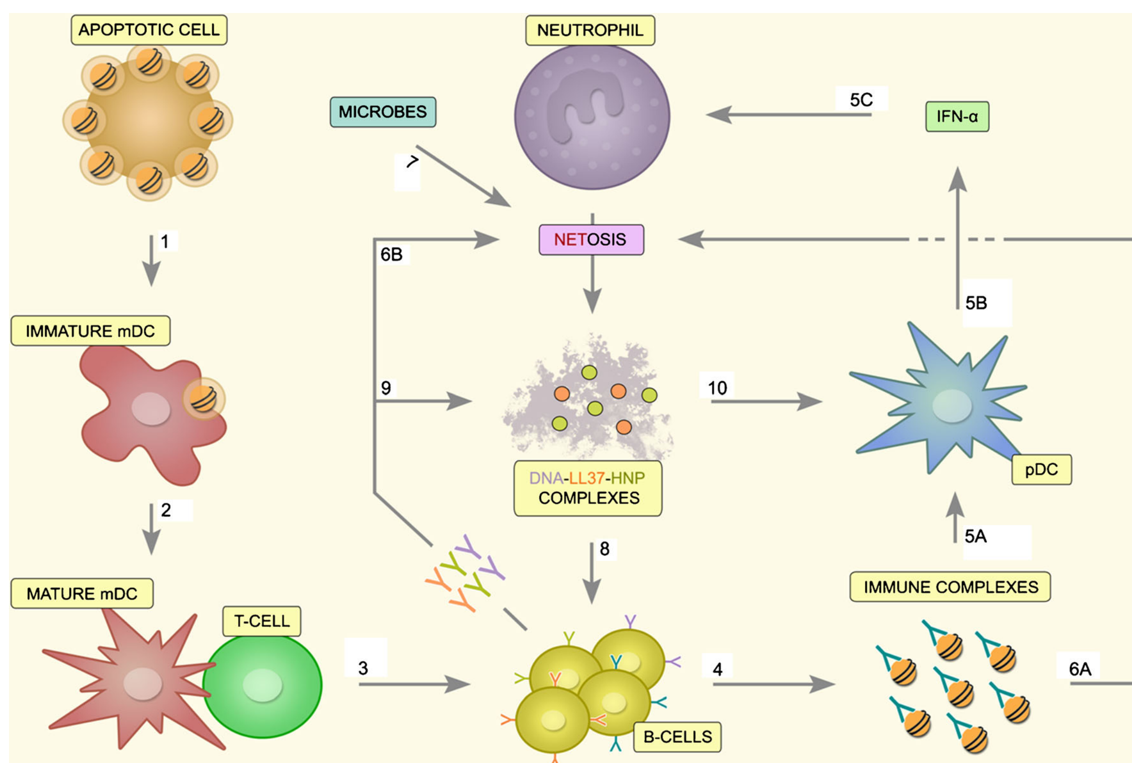

Fig. 1 Integrated hypothesis for the pathogenesis of SLE and lupus nephritis. (*1*) Apoptotic blebs and chromatin are ingested by immature myeloid dendritic cells (*mDC*), which, thereby, (*2*) are matured and present (apoptosis-modified) chromatin in their MHC to autoreactive T cells. (*3*) Activated autoreactive T cells assist autoreactive B cells to produce autoantibodies directed against chromatin. (*4*) Immune complexes between autoantibodies and chromatin are formed. (*5A*) Immune complexes are ingested by plasmacytoid dendritic cells (*pDC*), which thereby are activated and (*5B*) start to produce type I interferons, including IFN-α. (*5C*) IFN-α primes neutrophils, (*6A*, *6B*) autoantibodies against chromatin, and NET-associated proteins LL37 and HNP induce NETosis, which is normally triggered by microbes (*7*). (*8*) Chromatin and NET-associated proteins (*LL37*, *HNP*) spewed into the extracellular space function as autoantigens for the B cell, which leads to anti-LL37, anti-HNP, and anti-chromatin autoantibodies that may form immune complexes with NET (*9*), thereby facilitating their uptake by pDC (*10*). This establishes a loop between pDC and neutrophils that chronify and/or exacerbate the autoimmune response and the inflammatory condition in SLE. Adapted from [106]

correlate single distinct biomarkers, like anti-dsDNA, with the whole syndrome instead of correlating them with individual clinical manifestations within the syndrome. Secondly, for treatment, the opposite holds true, namely treating defined manifestations and not the syndrome as that seems not logic. In the context of the previous reasoning, it is particularly a problem when "biomarkers" in SLE are analyzed and tried to be associated with SLE per se. Principally, it is not possible to link a given "biomarker" or single genes or several sets of genes to a syndrome that is regarded a heterogeneous collection of organ manifestations or to panels of laboratory-determined biological parameters. In our opinion, this should only apply to single manifestations.

To illustrate our reasoning, we will now further focus on antibodies to dsDNA. According to current knowledge, antibodies to dsDNA are directly involved in lupus nephritis [5, 30, 47, 106, 132] and lupus dermatitis [32, 45] and possibly also involved in certain aspects of cerebral lupus ([53], discussed in [4, 23]). This makes up 3 criteria of a total of 11 that classify SLE (ACR criteria). In the remaining eight criteria, the (pathogenic) role of anti-dsDNA antibodies is not demonstrated. This simple reasoning may suggest that SLE classified, and diagnosed, by combinations of these remaining eight criteria may in fact reduce the impact of anti-dsDNA antibodies as marker antibodies for SLE as a syndrome. Thus, to correlate the anti-dsDNA antibody population with SLE per se may at the best be a biased approach since the impact of the antibody in most of these eight criteria is elusive. How then can this antibody remain a criterion for SLE with no further distinction?

There is no unifying definition of the term anti-dsDNA antibodies in SLE

In the context of the discussion in the previous paragraph, it is important to note that anti-dsDNA antibodies are not representing a homogenous antibody population [48, 59, 64, 113], both with respect to their molecular and structural specificities. Since nucleosomal DNA is tightly wrapped around

the histone core, DNA in nucleosomes possesses a highly bent structure, in addition to a more extended (linear-shaped) structure in the linker region. The overall twist of nucleosomal DNA is only 10.2 bp/turn, varying from a value of 9.4 to 10.9 bp/turn [137]. This implies that DNA may be targeted by B cells and consequently by antibodies in two ways, either by binding an elongated DNA structure or by binding the (conformational) bent DNA structure. This may explain the fact that many antibodies bind DNA in ELISA, while fewer bind in the *Crithidia luciliae* assay (see, e.g., [48]). This is in harmony with the fact that the *Crithidia* kinetoplast DNA has one of the greatest known degrees of stable curvature [44]. Other specificities of anti-DNA antibodies include nucleotide sequences, synthetic polynucleotides, and Z DNA [14, 112, 113].

Of particular interest in this context is the fact that the mechanisms accounting for production of anti-dsDNA antibodies is highly diverse; some are sustained, and some are transient (see below). Even though, the anti-dsDNA antibodies are regarded specific (as a biomarker) for SLE [47, 59, 75, 112], and the ACR [116] and SLICC [91] criteria for the disease include this antibody population as a diagnostic criterion by stating that this criterion is validated by detection of the antibodies by *any* assay at abnormal titers. Important variables are not included in diagnostic testing for anti-dsDNA, including antibody characteristics like avidity, whether produced transiently or sustained, whether cross-reactive or specific for defined chromatin structures. The SLICC criteria ask for a stringent cutoff value in ELISA to link anti-dsDNA antibodies to SLE by stating: "The new anti-dsDNA antibody criterion, however, requires a stricter cut-off for ELISA assays." Both the ACR and the SLICC recommendations for testing for anti-dsDNA antibodies may be poor approaches, as they do not consider basic knowledge on the highly diverse origins of anti-dsDNA antibodies. Thus, the validation of anti-dsDNA antibodies in both classification systems may represent an oversimplification and an overestimation of the classification, and pathogenic and diagnostic impact of these antibodies.

With the development of new insight into their factual and plural genesis, it is even tentatively hard to accept a strong relationship between the anti-dsDNA antibody and SLE as a syndrome. Antibodies that phenomenologically bind dsDNA as demonstrated by any assay (ACR criteria) or at a certain magnitude (SLICC criteria) may be produced in the context of several quite different mechanisms, some driven by non-self DNA antigens like bacterial DNA, some by cross-reactive antigens, and some by pure autologous autoantigens. Published mechanisms involve processes linked to (i) acquired, infection-related, or true autoimmune mechanisms similar to hapten-carrier systems, where, e.g., dsDNA or pure nucleosomes represent the principally non-immunogenic hapten-like ligand and the DNA-binding protein (like a DNA-binding viral protein) represents the carrier protein presented to non-tolerant T cells [6, 15, 22, 41, 42, 71, 76, 82, 100, 114, 133, 134] (Fig. 2a); (ii) molecular mimicry [2, 20, 68, 85, 89, 90, 97, 99]; (iii) single gene defects or mutations [10, 11, 29, 43, 57, 58, 136]; (iv) translocation of a cluster of X-linked genes onto the Y chromosome creating the genetic lesion underlying Yaa [29, 94]; (v) the stimulation by non-self DNA like bacterial DNA [42, 93, 140]; and finally (vi) the stimulatory effect of apoptotic and secondary necrotic cell debris like nucleosomes on the immune system in vivo [8, 25, 26, 38, 67, 120] (Fig. 2b). In this latter situation, impaired removal of apoptotic cells may account for exposure of secondary necrotic material as outlined in previous sections [8, 67, 86, 120].

Whether the insight given above on the multiple and diverse mechanisms accounting for production of anti-dsDNA antibodies fits with the idea that anti-dsDNA antibodies per se are associated with SLE is far from logic. One major argument against the general impact of anti-dsDNA antibodies in SLE and lupus nephritis is the simple perception that in some cases, the stimulus is transient and results in poor antibody affinity maturation, like for example for infectious-related hapten-carrier-like complexes that can be terminated by the normal functioning immune system. In other cases, the stimulus is sustained allowing maturation into high affinity antibodies [110, 111, 118], like for example when there is a sustained reduced clearance of apoptotic cells or in context of gene defects/mutations as outlined above. Thus, the link between anti-dsDNA antibodies as that, without any distinction, with, in our opinion, insufficiently defined syndrome SLE must from theoretical considerations be seriously questioned. Indeed, the assay principle may be decisive for the detection of clinically significant anti-DNA antibodies, as has been indicated by Haugbro et al. [48]. By means of different assays to analyze the presence of various anti-dsDNA antibodies and using an unbiased clinical approach, it may be possible to identify anti-dsDNA antibody subpopulations that may show a stronger association with certain organ manifestations that really are imposed by organ-specific pathogenic anti-dsDNA antibodies. In order to obtain that insight, a wide variety of assays must be performed in a blinded, prospective way, to identify antibody subsets that may be directly involved in organ manifestations but not linked to, e.g., the *syndrome* SLE. One study [3] showed, in a cohort of soldiers, the presence of anti-chromatin antibodies several years before the onset of clinical manifestations. However, these authors in a retrograde approach first identified contemporary SLE patients and then analyzed backwards in time detection of relevant antibodies. In our opinion, it would have been preferred that they screened for early detection of relevant antibodies in unselected serum samples from the whole military biobank. This approach would include serum samples of subjects that may not develop SLE. This would give a more true picture of the predictive role of autoantibodies in clinical

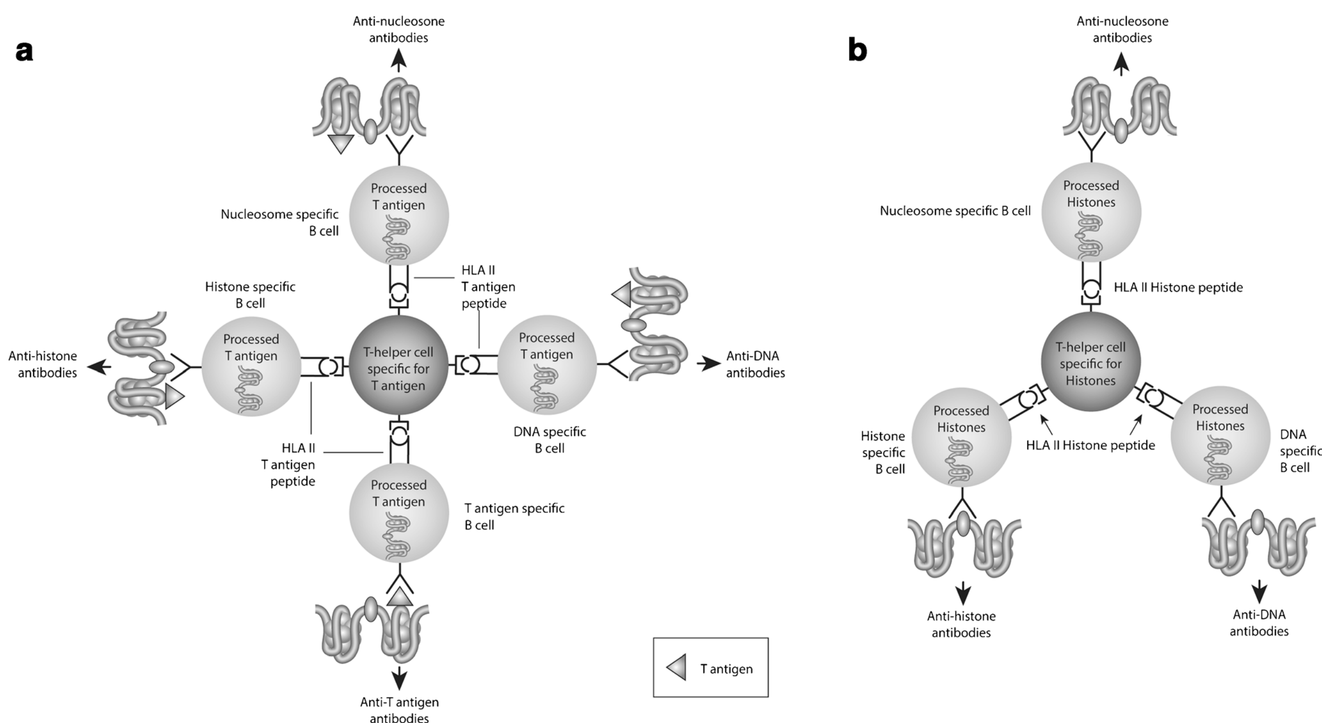

Fig. 2 Cognate interaction of nucleosome-specific B cells and peptide-specific T cells. The figure presents a classical hapten-carrier model to explain sustained production of arrays of anti-nucleosome antibodies. In this model, chromatin constituents play the role as a hapten, while either heterologous (infectious-derived DNA-binding proteins like polyomavirus large T antigen) or homologous (e.g., histone-derived) peptides play the role as carrier proteins. **a** In the *left panel*, T cells are primed by polyomavirus T antigen peptides presented by an antigen-presenting cell (*APC*). Then, these T cells recirculate, and eventually, they bind the same peptides presented by B cells specific for different nucleosome structures. Here, T cell tolerance is intact as T antigen is a non-self viral protein. The immune responses are sustained as long as T antigen is expressed. **b** In the *right panel*, the T cells are primed by histone-derived peptides presented by APC. Subsequently, these T cells encounter the same peptides on nucleosome-specific B cells and provide the required help to transform them into an array of nucleosome-reactive antibody secreting plasma cells. In this situation, T cell tolerance to nucleosomal proteins is terminated, and the immune response is truly autoimmune. The immune responses are sustained as long as histone-specific T cell tolerance is not controlled. Both mechanisms may in fact be operational in vivo and account for a wide variety of nucleosome-reactive antibodies. The principal paradigm for the hapten-carrier models presented in this figure is based on strong experimental evidences (see text for details). Thus, the cognate interaction of chromatin-specific B cells and immune or autoimmune peptide-specific T cells may explain the origin of the comprehensive repertoire of chromatin-reactive IgG antibodies in human patients

medicine. Till now, this approach has not been performed but must be done in our opinion in order to try to select valid diagnostic assays that leave behind antibodies that are purely stochastic epiphenomena, i.e., those that evidently are not involved in disease pathogenesis, nor has any diagnostic impact.

How do the anti-dsDNA antibodies exert their assumed pathogenic impact?

Aside from the problems linked to pure existence of processes that impose anti-dsDNA antibody production, there is also a yet controversial problem whether, and how, these antibodies exert their pathogenic potential. From the simple statement that individuals de facto produce anti-dsDNA antibodies without having organ manifestations like, e.g., nephritis, this may mean that there exists a selection principal that determines their pathogenicity. One possibility is that only those antibodies that bind inherently expressed glomerular antigens are pathogenic [1, 21, 77, 85, 104]. Alternatively, anti-dsDNA antibodies are pathogenic only when chromatin fragments are exposed in glomeruli [62, 70, 83, 84, 128, 129]. This obviously requires that chromatin structures must be retained and *exposed* in the kidney (discussed in [84]). Therefore, not even how anti-dsDNA antibodies exert their pathogenic potential is clearly defined. Recently, it has been indicated that anti-dsDNA antibodies are a nonpathogenic factor in the absence of exposed chromatin, whereas exposed chromatin represents a structural epiphenomenon in the absence of antibodies to dsDNA [30, 81]. In this context, the observation that exposure of chromatin in glomerulus membranes and matrices correlates in lupus nephritis is closely linked to loss of renal DNaseI [106].

Concluding remarks

The pathogenesis of SLE involves apoptotic chromatin, clearance defects including downregulation of renal DNaseI,

mDC, pDC, and B and T lymphocytes. The role of anti-DNA antibodies as a criterion for the diagnosis of SLE as a syndrome is questioned in this review. Two important questions need to be resolved in order to understand the role of anti-dsDNA antibodies as a diagnostic tool and as a pathogenic antibody: Are all mechanisms for production of anti-dsDNA antibodies linked to SLE, and what is the mechanism that accounts for, e.g., glomerular exposure of chromatin that can be targeted by anti-dsDNA antibodies? The latter problem is partly solved for the kidney, as it has been demonstrated that an *acquired* silencing of the renal DNaseI enzyme result in impaired chromatin degradation and a consequent retention in the glomerular tissue [30, 105, 107, 142, 143]. However, whether similar mechanisms are operative in other tissues affected with SLE remains to be established. In conclusion, with respect to the pathogenesis, diagnosis, and treatment of SLE, we still have much more questions than answers.

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