Clinical & Experimental Immunology
The Journal of Translational Immunology
British Society for immunology
doi:10.1111/cei.12296
FOCUS ON DYING AUTOLOGOUS CELLS AS INSTRUCTORS OF THE IMMUNE SYSTEM
Clinical and Experimental Immunology
Series Originators and Editors: Christian Berens and Martin Herrmann

Review Article

Anti-dsDNA antibodies as a classification criterion and a diagnostic marker for systemic lupus erythematosus: critical remarks

O. P. Rekvig
RNA and Molecular Pathology Research Group, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway

Accepted for publication 7 February 2014
Correspondence: O. P. Rekvig, RNA and Molecular Pathology Research Group, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, N-9037 Tromsø, Norway.
E-mail: olepr@fagmed.uit.no

Summary

Antibodies to mammalian dsDNA have, for decades, been linked to systemic lupus erythematosus (SLE) and particularly to its most serious complication, lupus nephritis. This canonical view derives from studies on its strong association with disease. The dogma was particularly settled when the antibody was included in the classification criteria for SLE that developed during the 1970s, most prominently in the 1982 American College of Rheumatology (ACR), and recently in The Systemic Lupus International Collaborating Clinics (SLICC) classification criteria. There are several problems to be discussed before the anti-dsDNA antibody can be accepted without further distinction as a criterion to classify SLE. Old and contemporary knowledge make it clear that an anti-dsDNA antibody is not a unifying term. It embraces antibodies with a wide spectrum of fine molecular specificities, antibodies that are produced transiently in context of infections and persistently in the context of true autoimmunity, and also includes anti-dsDNA antibodies that have the potential to bind chromatin (accessible DNA structures) and not (specificity for DNA structures that are embedded in chromatin and therefore unaccessible for the antibodies). This critical review summarizes this knowledge and questions whether or not an anti-dsDNA antibody, as simply that, can be used to classify SLE.

Keywords: autoimmunity, autoinflammatory disease, inflammation, systemic lupus erythematosus

Table 1. Immunology in the 1982 ACR classification set.

- Positive lupus erythematosus cell preparation
- Anti-DNA: antibody to native DNA in abnormal titre
- Anti-Sm: presence of antibody to Sm nuclear antigen
- False positive serological test for syphilis known to be positive for at least 6 months and confirmed by *Treponema pallidum* immobilization or fluorescent treponema antibody absorption test

Anti-DNA antibodies and systemic lupus erythematosus (SLE) – defining the problem

One canonical parameter to diagnose and classify systemic lupus erythematosus (SLE) is the presence of the 'anti-dsDNA antibody'. This is described in The 1982 American College of Rheumatology (ACR) criteria for classification of SLE [1] as defined in criterion no. 10, immunological aberrancies (Table 1). Furthermore, this criterion is stated valid when: 'An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay (occurred) *at any point in time* and in the absence of drugs known to be associated with "drug-induced lupus syndrome" '. This means that the criterion is valid if anti-nuclear antibody (ANA) or an equivalent antibody occurred at a time-point when there is no clinical manifestation believed, or proved, to be caused by that given antibody. The 1997 update of this set of immunological criteria [2] did not change this idiom, and the criterion remained with the statement 'anti-DNA antibody to native DNA in abnormal titer'.

Recently, the Systemic Lupus International Collaborating Clinics (SLICC) group revised and validated the ACR classification criteria for SLE [3]. This was performed to improve sets of clinically relevant manifestations, meet stringent methodology requirements and to incorporate new knowledge regarding the immunology of SLE [3]. Whether they succeeded with this attempt is questionable, and remains to be discussed and eventually settled.

In the revised SLICC criteria for classification of SLE, several immunological parameters were included (Table 2). Also defined by the SLICC criteria, the criterion on anti-dsDNA antibodies is fulfilled if the patients produce the antibody at abnormal titres (what in fact may that mean?) in any assay (meaning no restriction in fine polynucleotide specificity or affinity?) at any time-point (i.e. linked or unlinked from any immunopathological organ manifestation?). The SLICC criterion simply states an anti-dsDNA antibody level two times the reference value (but do not recommend any assay stringency or quality). This means that in the context of the official classification criteria for SLE, an anti-dsDNA antibody is only that, and nothing else.

The ACR and SLICC criteria to classify SLE do not consider past and current knowledge related to the origin and nature of the anti-dsDNA antibody

In the context of this critical commentary, I will discuss the nature and specificity of the highly diverse anti-dsDNA antibody family, and whether such antibodies must appear in a pathogenic context to be validated as a real classification criterion for SLE, or – even worse – if their pure existence is a clear indicator of SLE as stated by both classification sets [1,3]. As is obvious from these two classification criteria sets, anti-dsDNA antibodies do not need to co-exist with clinical manifestations, as stated originally in the 1982 criteria. Ultimately, this leaves an anti-dsDNA antibody as an autoimmune phenomenon linked to SLE without, however, necessarily having a pathogenic impact upon SLE. Whether this is true remains to be discussed.

From a basic scientific viewpoint, the ACR and the SLICC criteria to classify SLE focus upon the anti-dsDNA antibody as a phenomenon that basically represents a single unifying specificity. The anti-dsDNA antibody is simply that, and nothing else, according to the criterion. In a deeper scientific context it is clear that the criterion does not include reflections upon (i) the molecular specificity of an anti-dsDNA antibody, (ii) whether the antibody is produced transiently or persistently (in other words, in the context of the mechanisms that account for their production, see below) and (iii) whether or not the presence of the antibody (again as a single unspecified phenomenon) is linked to pathological processes.

The following three problems can be defined from these reflections. For the first, antibody specificity for any synthetic or natural duplex polynucleotides (reviewed in [4,5]) elongated or bent dsDNA [6], B helical DNA structures or Z-DNA or cruciform structures [5] fulfil the criteria on anti-DNA antibodies as defined by the ACR and the SLICC

Table 2. The immunological parameters included in the SLICC criteria.

- ANA level above laboratory reference range
- Anti-dsDNA antibody level above laboratory reference range (or twofold the reference range if tested by ELISA)
- Anti-Sm: presence of antibody to Sm nuclear antigen
- Anti-phospholipid antibody positivity, as determined by
  - Positive test for lupus anti-coagulant
  - False-positive test result for rapid plasma reagin
  - Medium- or high-titre anti-cardiolipin antibody level (IgA, IgG or IgM)
  - Positive test result for anti-2-glycoprotein I (IgA, IgG or IgM)
- Low complement (C3, C4 or CH50)
- Direct Coombs' test (in the absence of haemolytic anaemia)

ACR = American College of Rheumatology; ANA = anti-nuclear antibody; ELISA = enzyme-linked immunosorbent assay; Ig = immunoglobulin; SLICC = Systemic Lupus International Collaborating Clinics.

criteria. Secondly, as discussed in more detail below, a transient anti-dsDNA antibody produced in the context of an infection [7–12] has the same value as a SLE-related criterion as persistently produced anti-dsDNA antibodies in the context of true autoimmunity, as they appear in SLE. Thirdly, if a patient fulfils too few criteria (fewer than four of 11 criteria by the ACR) at a given time-point and therefore, by definition, cannot be classified as SLE, a transient anti-dsDNA antibody appearance years after the clinical signs may, again per definition, add the missing criterion. In that situation, the patient may finally (after years) be classified as having SLE, with the socio-economic consequences this may have for the patient. The SLICC criteria do not challenge this definition, as is also defined in the ACR criteria. This practical view on the link between the anti-dsDNA antibody and SLE unlink, in fact, the antibody from SLE as an autoimmune disease. Clinical manifestations may occur without the presence of an autoimmune pathogenic factor, and vice versa. In fact, these aspects imply complex considerations that, in the end, may force us to dissect the anti-dsDNA antibody into different families characterized by specificity, production profiles and pathogenic impact. Some of these aspects may point to clinically important antibodies; some will inevitably classify spectra of anti-dsDNA antibodies as clinical epiphenomena.

Witebsky criteria for autoimmune diseases and Koch's postulate to define a pathogenic factor – something to learn?

In this context, it can be relevant to consider the modern revisions of the Witebsky criteria [13] to accept a disease as being truly autoimmune. These criteria consider three types of evidence: (i) direct evidence from transfer of a pathogenic antibody and/or pathogenic T cells; (ii) indirect evidence based on reproduction of the autoimmune disease in experimental animals; and (iii) circumstantial evidence from clinical parameters.

The Witebsky set of criteria is a derivative from, and has its infectious counterpart in, Koch's postulate to classify an infectious disease [14]. Koch's postulates are as follows: (i) the microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms; (ii) the microorganism must be isolated from a diseased organism and grown in pure culture; (iii) the cultured microorganism should cause disease when introduced into a healthy organism; and (iv) the microorganism must be re-isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Clearly, confronting the anti-dsDNA antibody as defined by ACR and SLICC criteria with Witebsky criteria and Koch's postulate, both defining a causal pathogenic effect of a factor, will make it difficult to understand the role of the anti-dsDNA antibody as a diagnostic and classification parameter; in particular, by ignoring its pathogenic effect, as the anti-dsDNA antibody has its classification value when detected at any time-point, this states clearly that it may be unlinked from any organ manifestation. Timely detection of this antibody independently from disease processes that are believed to be caused by the antibody is, at best, unclear.

Thus, the 1997 revised ACR criteria, and the recent SLICC criteria, still deal with the anti-dsDNA antibody as that, without taking into account all the growing information on the highly divergent repertoire of molecular polynucleotide specificities, the divergent origins of the antibodies, whether they are true autoantibodies or anti-dsDNA antibodies produced in the context of, e.g. infections, or whether their production is transient or maintained over time. Antibody affinity or avidity is, although difficult to establish, not considered in this strict context.

What is an anti-dsDNA antibody?

An anti-dsDNA antibody produced in a natural context *in vivo* has (most probably) the native (i.e. unmanipulated) DNA as both inducer and target. This form of DNA is, however, presented to the immune system as part of chromatin, where DNA is a major constituent. Testing for anti-dsDNA antibodies using pure dsDNA as target antigen is therefore, by definition, an artificial analytical approach. This leaves us with an uncertainty. Is an antibody that binds dsDNA by assays such as Farr or enzyme-linked immunosorbent assay (ELISA) able to interact with DNA as presented by chromatin? In other words, can such antibodies bind chromatin *in vivo*? If not, the antibody may be regarded as an autoimmune epiphenomenon – at least in a pathogenic context. My perception is that we have not implemented this problem in our contemporary analytical approaches.

In a wider sense, this problem deals with the nature and specificity of an anti-dsDNA antibody. This is based on considerations of experimental approaches to induce antibodies that bind duplex polynucleotides. These experiments have taught us that the specificity of an induced antibody mainly reflects the polynucleotide structure that was used for their production (e.g. reviewed in [4,5]). These specificities include diverse synthetic single-stranded and double-stranded polynucleotides, cruciform DNA structures or mammalian Z-DNA, reflecting specificity for the polynucleotide structures used to elicit such antibodies [5]. One important exception for this is mammalian native B-helical dsDNA that, for a long time, was regarded as non-immunogenic [5,15–17]. Why Z-DNA, when mixed with methylated bovine serum albumin (mBSA), was able to elicit a strong anti-Z-DNA in all mice that were immunized [18], while B DNA in complex with mBSA did not elicit a response to mammalian B DNA, is still a mystery. One explanation, that B cells specific for mammalian B DNA are

anergic or deleted, or that their receptors are edited [19,20], is not very likely, for two reasons. One reason is that anti-dsDNA antibodies can be induced by single-stranded DNA. The specificity of the induced anti-ssDNA can affinity-maturate towards dsDNA [21,22]. The other reason is simply that anti-dsDNA antibodies can be induced easily by, e.g. DNA–viral protein complexes [7,11,12]. Why dsDNA–mBSA complexes are inefficient in inducing anti-dsDNA antibodies is therefore still difficult to explain.

Antibodies against mammalian B helical dsDNA, such as those produced spontaneously and observed in SLE, are specific for (i) the elongated nucleosome linker (B helical) DNA, including specificity for phosphodeoxy ribose backbone [23]; (ii) for higher-order bent DNA structures such as those in the nucleosome [6,24,25] or in *Crithidia luciliae* kinetoplast DNA [26]; (iii) for ssDNA regions that may be present within (nucleosomal) dsDNA; or (iv) for Z-DNA and cruciform DNA structures (reviewed in [4]).

Thus, from a broad and sound series of experiments, it was concluded during the 1980s that mammalian B helical dsDNA was non-immunogenic, and that for theoretical reasons this antibody had its origin in immune responses to cross-reacting antigens such as, e.g. phospholipids (as discussed in, e.g. [23,27,28]), or more recently by alpha-actinin [29,30], laminin [31,32] or entactin [33].

The genesis of anti-dsDNA antibodies *in vivo*: an infectious or a true autoimmune context?

To reach further insight into this problem, it may be wise to consider what we know about the genesis of the anti-dsDNA antibody (in as wide as possible a definition of the antibody). There is a principally dichotomous process leading to anti-dsDNA antibody production. One has an element of foreign antigen involvement, such as infectious-related DNA or infectious-related immunogenic DNA-binding proteins, while the alternative involves stimulation of the immune system by true autologous chromatin. In the former, the anti-dsDNA antibody response will last predictively for the time of active infection [16,34], and therefore is predicted to be transient in its nature. Conversely, in the situation where both B cell and T cell tolerance for chromatin is terminated, true autoimmune anti-dsDNA antibody production may be similar to a sustained affinity-maturated immune response [35,36].

When an antibody binds a non-immunogenic antigen such as DNA, the antibody may have been induced by a process that is equivalent to a hapten-carrier model [34,37]. This model implies that a non-immunogenic hapten is rendered immunogenic when bound to an immunogenic carrier protein. This means that although the hapten can be recognized by a B cell antigenic receptor, the hapten cannot be processed by the B cell and presented to a T cell in an immunogenic form. In the context of DNA, the DNA differs from a hapten in that a hapten is a small molecule, while DNA is a large duplex structure. However, hapten and DNA have one functional defect in common; they are not presented to a T cell in the context of human leucocyte antigen (HLA) class II molecules. A DNA-bound immunogenic carrier protein can substitute for this defect. This has been proved as follows.

In a prospective set of studies, the first experimental evidence demonstrated that antibodies against mammalian dsDNA antibodies were, in fact, driven by DNA itself, but only when in complex with an immunogenic carrier protein [7,8,10]. The difference between earlier experiments and the more recent ones was a renewed focus on the nature and origin of the immunogenic DNA-binding proteins. Marion's laboratory in Memphis, USA, and my laboratory in Tromsø, Norway, demonstrated that polypeptides from infectious agents, such as a peptide (Fus 1) derived from *Trypanozoma cruzii* [10], or the DNA-binding T antigen from polyomavirus BK [7] when bound to DNA, provided the necessary T helper cell stimulus for dsDNA-specific B cells, provided that the B cells processed and presented such peptides to the relevant T helper cells [16]. The simple conclusion of these experiments was that an anti-dsDNA antibody could be induced *in vivo* if presented in a certain context: bound to an immunogenic polypeptide.

In two different experimental systems, the role of DNA-bound polypeptides were further validated. Immunologically normal mice inoculated with plasmids encoding wild-type, DNA-binding T antigen produced antibodies to dsDNA, histones and to certain transcription factors. The production of antibodies to these sets of antigens was in accordance with the basic idea of the model: all autologous ligands present in the macromolecular complex (chromatin fragments) that were linked physically to polyomavirus T antigen could theoretically be rendered immunogenic if provided with the presence of a (functional) repertoire of B cells [7].

In the second experimental approach, we directly demonstrated that (i) SLE patients were highly susceptible to persistent productive polyomavirus infections and that (ii) linked to virus expression, antibodies to T antigen, DNA and to transcription factors such as TATA-binding protein (TBP) and cAMP response element-binding protein (CREB), but not to other non-nucleosomal autoantigens, were produced [7].

Collectively, the data generated so far demonstrated that *in-vivo* expression of the polyomavirus DNA-binding T antigen or DNA-binding peptides encoded by other viruses (e.g. see [11,12]) resulted in a hapten-carrier-like model to generate anti-dsDNA and other anti-nucleosome antibodies. This model may not be relevant to understanding of the production of anti-dsDNA antibodies in SLE; by definition, the T antigen model, as well as the Fus1 peptide model [10] explains a transient production of anti-dsDNA antibody in contrast to sustained production in SLE. The anti-dsDNA antibody production is terminated upon termination of the

productive infection. Most important in this context is that, in principal, these transient infectious-driven antibodies fulfil the ACR and SLICC criteria to classify SLE, although they may be produced in a completely different, non-autoimmune, clinical context.

Regarding sustained production of anti-dsDNA antibodies in SLE, the mechanism(s) remain elusive. There are published experimental systems that describe activation of autoimmune T cells against histones that may serve as a carrier-protein for anti-dsDNA antibodies in analogy to the role of, e.g. the Fus1 or the T antigen polypeptides [38–40]. Such T cells have been demonstrated as responsive in SLE patients. However, how these cells are activated *in vivo*, and how they are able to exert a cognate interaction with DNA-specific B cells, is still not fully understood. One central question to be answered is why such nucleosome-specific T cells, once activated by a dendritic cell presenting relevant chromatin-derived peptides, are not tolerized when recirculated in the periphery (as discussed in [16]). As for the transient production of anti-dsDNA antibodies in context of infection, the sustained production of these anti-dsDNA antibodies in context of true autoimmunity induced by pure autologous nucleosomes fulfils the ACR and SLICC criteria to classify SLE; but do antibodies from these different origins have the same clinical impact? This is not settled by the data.

In summary, how are we going to determine the clinical impact of an anti-dsDNA antibody in context of specificity and origin?

Considering the ACR and SLICC criteria on the impact of anti-dsDNA antibodies, this relates to a historical view that states that anti-dsDNA antibodies appear in SLE, cannot be induced by experimental immunization of mice with normal genetic backgrounds and that they possess a unique specificity for mammalian dsDNA. The problem with this view today is that the data rely upon early observations stating that DNA is non-immunogenic, and does not consider data demonstrating that DNA is not only immunogenic, but immunogenic in quite different contexts.

Conclusion

The immunogenicity of dsDNA depends upon its context, either as part of nucleosomes in patients having functional nucleosome-specific T cells (sustained anti-dsDNA autoimmunity) or in patients suffering from viral infections that create a hapten (nucleosome/DNA)-carrier-like (viral DNA binding protein) complex (transient anti-DNA antibody). In the first situation, sustained anti-dsDNA antibody production may appear that may relate to SLE. In the other situation, a transient antibody profile may appear that may not relate at all to SLE.

Disclosure

O. P. R. declares that he has no competing interests as defined by this journal, or other interests that might be perceived to influence the results and discussion reported in this paper; and has not received any financial support or other benefits from commercial sources for the work reported on in the manuscript.

Acknowledgements

This study was supported by the Northern Norway Regional Health Authority Medical Research Program (grant numbers SFP-100-04, SFP-101-04), and the University of Tromsø as Milieu support given to O. P. R.

References

1 Tan EM, Cohen AS, Fries JF *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982; **25**:1271–7.
2 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997; **40**:1725.
3 Petri M, Orbai AM, Alarcon GS *et al*. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum 2012; **64**:2677–86.
4 Stollar BD. Antibodies to DNA. CRC Crit Rev Biochem 1986; **20**:1–36.
5 Stollar BD. Immunochemistry of DNA. Int Rev Immunol 1989; **5**:1–22.
6 Widom J. A relationship between the helical twist of DNA and the ordered positioning of nucleosomes in all eukaryotic cells. Proc Natl Acad Sci USA 1992; **89**:1095–9.
7 Rekvig OP, Moens U, Sundsfjord A *et al*. Experimental expression in mice and spontaneous expression in human SLE of polyomavirus T-antigen. A molecular basis for induction of antibodies to DNA and eukaryotic transcription factors. J Clin Invest 1997; **99**:2045–54.
8 Gilkeson GS, Grudier JP, Karounos DG, Pisetsky DS. Induction of anti-double stranded DNA antibodies in normal mice by immunization with bacterial DNA. J Immunol 1989; **142**: 1482–6.
9 Gilkeson GS, Ruiz P, Howell D, Lefkowith JB, Pisetsky DS. Induction of immune-mediated glomerulonephritis in normal mice immunized with bacterial DNA. Clin Immunol Immunopathol 1993; **68**:283–92.
10 Desai DD, Krishnan MR, Swindle JT, Marion TN. Antigen-specific induction of antibodies against native mammalian DNA in nonautoimmune mice. J Immunol 1993; **151**:1614–26.
11 Cerutti ML, Zarebski LM, de Prat GG, Goldbaum FA. A viral DNA-binding domain elicits anti-DNA antibodies of different specificities. Mol Immunol 2005; **42**:327–33.
12 Sundar K, Jacques S, Gottlieb P *et al*. Expression of the Epstein–Barr virus nuclear antigen-1 (EBNA-1) in the mouse can elicit the production of anti-dsDNA and anti-Sm antibodies. J Autoimmun 2004; **23**:127–40.

13 Rose NR, Bona C. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). Immunol Today 1993; **14**:426–30.
14 Fredericks DN, Relman DA. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. Clin Microbiol Rev 1996; **9**:18–33.
15 Madaio MP, Hodder S, Schwartz RS, Stollar BD. Responsiveness of autoimmune and normal mice to nucleic acid antigens. J Immunol 1984; **132**:872–6.
16 Rekvig OP, Nossent JC. Anti-double-stranded DNA antibodies, nucleosomes, and systemic lupus erythematosus: a time for new paradigms? Arthritis Rheum 2003; **48**:300–12.
17 Rekvig OP, Bendiksen S, Moens U. Immunity and autoimmunity induced by polyomaviruses: clinical, experimental and theoretical aspects. Adv Exp Med Biol 2006; **577**:117–47.
18 Edgington SM, Stollar BD. Immunogenicity of Z-DNA depends on the size of polynucleotide presented in complexes with methylated BSA. Mol Immunol 1992; **29**:609–17.
19 Chen C, Prak EL, Weigert M. Editing disease-associated autoantibodies. Immunity 1997; **6**:97–105.
20 Chen C, Nagy Z, Radic MZ *et al*. The site and stage of anti-DNA B-cell deletion. Nature 1995; **373**:252–5.
21 Marion TN, Tillman DM, Jou NT. Interclonal and intraclonal diversity among anti-DNA antibodies from an (NZB × NZW)F1 mouse. J Immunol 1990; **145**:2322–32.
22 Shlomchik M, Mascelli M, Shan H *et al*. Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. J Exp Med 1990; **171**:265–92.
23 Lafer EM, Rauch J, Andrzejewski C Jr *et al*. Polyspecific monoclonal lupus autoantibodies reactive with both polynucleotides and phospholipids. J Exp Med 1981; **153**:897–909.
24 Richmond TJ, Davey CA. The structure of DNA in the nucleosome core. Nature 2003; **423**:145–50.
25 Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 A resolution [see comments]. Nature 1997; **389**:251–60.
26 Griffith J, Bleyman M, Rauch CA, Kitchin PA, Englund PT. Visualization of the bent helix in kinetoplast DNA by electron microscopy. Cell 1986; **46**:717–24.
27 Carroll P, Stafford D, Schwartz RS, Stollar BD. Murine monoclonal anti-DNA autoantibodies bind to endogenous bacteria. J Immunol 1985; **135**:1086–90.
28 Limpanasithikul W, Ray S, Diamond B. Cross-reactive antibodies have both protective and pathogenic potential. J Immunol 1995; **155**:967–73.
29 Mostoslavsky G, Fischel R, Yachimovich N *et al*. Lupus anti-DNA autoantibodies cross-react with a glomerular structural protein: a case for tissue injury by molecular mimicry. Eur J Immunol 2001; **31**:1221–7.
30 Zhao Z, Weinstein E, Tuzova M *et al*. Cross-reactivity of human lupus anti-DNA antibodies with alpha-actinin and nephritogenic potential. Arthritis Rheum 2005; **52**:522–30.
31 Amital H, Heilweil M, Ulmansky R *et al*. Treatment with a laminin-derived peptide suppresses lupus nephritis. J Immunol 2005; **175**:5516–23.
32 Qureshi F, Yang Y, Jaques SM *et al*. Anti-DNA antibodies cross-reacting with laminin inhibit trophoblast attachment and migration: implications for recurrent pregnancy loss in SLE patients. Am J Reprod Immunol 2000; **44**:136–42.
33 Krishnan MR, Wang C, Marion TN. Anti-DNA autoantibodies initiate experimental lupus nephritis by binding directly to the glomerular basement membrane in mice. Kidney Int 2012; **82**:184–92.
34 Van Ghelue M, Moens U, Bendiksen S, Rekvig OP. Autoimmunity to nucleosomes related to viral infection: a focus on hapten-carrier complex formation. J Autoimmun 2003; **20**: 171–82.
35 Dieker JW, van der Vlag J, Berden JH. Triggers for anti-chromatin autoantibody production in SLE. Lupus 2002; **11**:856–64.
36 Munoz LE, Lauber K, Schiller M, Manfredi AA, Herrmann M. The role of defective clearance of apoptotic cells in systemic autoimmunity. Nat Rev Rheumatol 2010; **6**:280–9.
37 Rock KL, Benacerraf B, Abbas AK. Antigen presentation by hapten-specific B lymphocytes. I. Role of surface immunoglobulin receptors. J Exp Med 1984; **160**:1102–13.
38 Voll RE, Roth EA, Girkontaite I *et al*. Histone-specific Th0 and Th1 clones derived from systemic lupus erythematosus patients induce double-stranded DNA antibody production. Arthritis Rheum 1997; **40**:2162–71.
39 Andreassen K, Moens U, Nossent H, Marion TN, Rekvig OP. Termination of human T cell tolerance to histones by presentation of histones and polyomavirus T antigen provided that T antigen is complexed with nucleosomes. Arthritis Rheum 1999; **42**:2449–60.
40 Mohan C, Adams S, Stanik V, Datta SK. Nucleosome: a major immunogen for pathogenic autoantibody-inducing T cells of lupus. J Exp Med 1993; **177**:1367–81.