Anti-DNA antibodies — quintessential biomarkers of SLE

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Abstract | Antibodies that recognize and bind to DNA (anti-DNA antibodies) are serological hallmarks of systemic lupus erythematosus (SLE) and key markers for diagnosis and disease activity. In addition to common use in the clinic, anti-DNA antibody testing now also determines eligibility for clinical trials, raising important questions about the nature of the antibody–antigen interaction. At present, no 'gold standard' for serological assessment exists, and anti-DNA antibody binding can be measured with a variety of assay formats, which differ in the nature of the DNA substrates and in the conditions for binding and detection of antibodies. A mechanism called monogamous bivalency — in which high avidity results from simultaneous interaction of IgG Fab sites with a single polynucleotide chain — determines anti-DNA antibody binding; this mechanism might affect antibody detection in different assay formats. Although anti-DNA antibodies can promote pathogenesis by depositing in the kidney or driving cytokine production, they are not all alike, pathologically, and anti-DNA antibody expression does not necessarily correlate with active disease. Levels of anti-DNA antibodies in patients with SLE can vary over time, distinguishing anti-DNA antibodies from other pathogenic antinuclear antibodies. Elucidation of the binding specificities and the pathogenic roles of anti-DNA antibodies in SLE should enable improvements in the design of informative assays for both clinical and research purposes.

Antibodies that recognize and bind to DNA (anti-DNA antibodies) are the serological hallmark of systemic lupus erythematosus (SLE) and quintessential biomarkers to probe the mechanisms of autoimmunity[1–3]. These antibodies have been characterized with numerous state-of-the-art technologies, and more is known about their molecular properties than those of any other autoantibody. Indeed, the molecular analyses of anti-DNA antibodies rival in size and detail those of antibodies to HIV[4]. The extent of these efforts demonstrates the importance of anti-DNA antibodies as a paradigm to enable understanding of the basis of aberrant B-cell expression in autoimmune disease[5].

The importance of anti-DNA antibodies extends beyond the laboratory to the clinical arena, wherein the assumption that they equate with SLE is a foundation of serological diagnosis. Further interest in testing results from the utilization of anti-DNA antibody positivity as a measure to determine eligibility of individuals for inclusion in clinical trials or to guide the therapeutic use of certain agents in routine clinical practice[6,7]. Because the interpretation of test results relating to anti-DNA antibodies can be nuanced and even perplexing[8,9], in this Review I consider the salient features of anti-DNA antibody binding and the value of testing, especially as it moves from a diagnostic to a theranostic setting.

Antigenic properties of DNA

The unexpected ability of antibodies to bind DNA was discovered in the 1950s following efforts to delineate the basis of antinuclear antibody reactivity[10–13]. Prior to this discovery, little evidence existed for recognition of nucleic acids by the immune system. By contrast, evidence for the antigenicity of proteins and carbohydrates was strong, reflecting the focus of immunological research on host defense mechanisms and the development of vaccines to prevent bacterial and viral disease. Subsequent results demonstrated the importance of anti-DNA antibodies in the diagnosis of SLE, and the immune role of DNA seemed to be exclusive to the setting of immunological disease[1,2,9].

The double helix is synonymous with DNA, and double-stranded DNA (dsDNA) consists of two polynucleotide chains wound around each other, with base pairs keeping the chains tightly bound. However, the DNA strands can come apart to form single-stranded DNA (ssDNA). Although dsDNA and ssDNA can be

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Key points

- Antibodies that recognize DNA (anti-DNA antibodies) can bind to sites on the phosphodiester backbone of single-stranded DNA and double-stranded DNA, to nucleotide sequences or to higher-order structures such as nucleosomes
- The molecular properties of anti-DNA antibodies, as well as the associated genetic properties, including variable-region somatic mutations, point to a role for antigen selection in anti-DNA antibody generation
- In the absence of a 'gold standard', various assay formats exist for anti-DNA antibody testing, differing in the nature of DNA substrates and the conditions for binding and detection of antibodies
- High-affinity binding by an anti-DNA antibody depends on monogamous bivalency, in which both Fab sites of an IgG molecule contact the same polynucleotide chain
- The use of anti-DNA antibody testing as a measure of disease activity to determine clinical trial eligibility depends on clear understanding of assay differences and the role of anti-DNA antibodies in pathogenesis

considered as distinct forms, in fact, they comprise a continuum, both biochemically and antigenically[14]. Because all naturally occurring dsDNA can exist as the classic helical B conformation, anti-DNA-antibody assays have used DNA from many sources as the test antigen, including mammalian, bacterial, viral and even protozoal DNA[15]. However, DNA from the protozoan *Crithidia luciliae* can have a bent structure, which has similarities to the conformation of nucleosomal DNA, and might only enable binding of a subgroup of anti-DNA antibodies.

An important characteristic — and possible source of bias — of immunological research relates to the use of biochemically well-defined molecules to probe the molecular basis of B-cell and T-cell recognition. Studies have typically involved highly purified preparations of DNA to characterize its antigenicity and immunogenicity[16]. However, the biochemical properties of DNA pose immediate challenges to this seemingly reasonable approach. Structurally, DNA has a simple aspect in the phosphodiester backbone, but it also has complex elements in the variable nucleotide sequence and the association with nuclear proteins — in particular, histones — to form the nucleosome, the fundamental building block of chromosomal structure[17].

Nucleosomes and chromatin

The use of purified DNA for the assay substrate conforms to paradigms of antigenicity, but could actually impede understanding of the immune recognition of this molecule. In the cell, DNA is rarely — if ever — pure. Essentially, DNA is a protein-binding molecule that exists in the eukaryotic cell in intimate association with histones to form structural units known as nucleosomes[17–19]. In a nucleosome, a stretch of DNA of around 147 bp is wrapped around a core protein octamer comprising two molecules each of histones H2a, H2b, H3 and H4, which neutralize the negative charge of the DNA phosphodiester groups. Many other proteins, such as transcription factors, bind to DNA, and chromatin is the ensemble of nuclear macromolecules in the cell and includes DNA, histones, nonhistone proteins and even nascent RNA transcripts, depending on the preparation[15].

The structure of chromatin means that DNA can be thought of as part of the nucleosome, and anti-DNA antibodies as part of a family of anti-nucleosome or anti-chromatin antibodies[20–24]. In this model, DNA, histones and DNA–histone complexes represent epitopes of the nucleosome[25,26]. The frequent coexistence of anti-DNA antibodies with antibodies to histones supports the idea that the nucleosome, rather than isolated DNA, is the relevant target of autoreactivity. The presence of anti-nucleosome antibodies can be inferred from positive results in assays that use chromatin as the antigen.

In vivo release of DNA from cells

DNA and nucleosomes can be released into the blood by cell death[27–32]. Although apoptotic cells have generally been considered the source of extracellular nuclear material, cell death can occur by various pathways that include apoptosis, necrosis, necroptosis, pyroptosis and NETosis (a form of induced cell death that involves the release of neutrophil extracellular traps (NETs))[33]. These pathways differ in terms of stimuli, molecular events that cause cellular demise, structure of the DNA released (FIG. 1) and the immunological properties of the deceased cells. Events during cell death can contribute to the immunogenicity of DNA by altering the extent of exposure of DNA to the extracellular environment, the molecular context and the presence of other immunoactive molecules. These molecules include IL-1, ATP and high mobility group protein B1 (HMG-1, also known as HMGB1). The DNA-binding protein HMG-1 is a prototypical alarmin and can act as an adjuvant to stimulate both innate and adaptive immune responses[34–36].

All cell-death processes can lead to the release of DNA, but the size of the DNA and the nature of associated molecules vary depending on the mechanism involved. In particular, DNA exiting from apoptotic cells is cleaved to form the classic ladder wherein the fragment sizes are multiples of the unit length of nucleosomal DNA[29,31]. By contrast, DNA released during NETosis can be present in a large mesh studded with proteins following mixing with the contents of cytoplasmic granules (FIG. 1). NETs could have a direct role in the pathogenesis of autoimmune inflammatory disease, including SLE, by stimulating immune cells or endothelium[37,38]. Although assays are available to measure the size and the amount of DNA in blood, few studies have been conducted to determine the origin of cell-free DNA in SLE and its effect on antigenicity and immunogenicity[39,40].

DNA released during apoptotic cell death can also exist in the form of microparticles, which are small, membrane-bound vesicles. Antibodies in sera from patients with SLE and murine models, as well as murine monoclonal anti-DNA antibodies, have been shown to bind DNA in microparticles[41,42]. Microparticles have been identified in immune complexes in the plasma of patients with SLE[41,42]. Whereas serological studies on SLE have utilized purified DNA as the antigenic substrate, DNA is evidently present *in vivo* in alternative antigenic moieties, including complexes of DNA with other macromolecules — as in nucleosomes — as well as higher-order structures such as microparticles.

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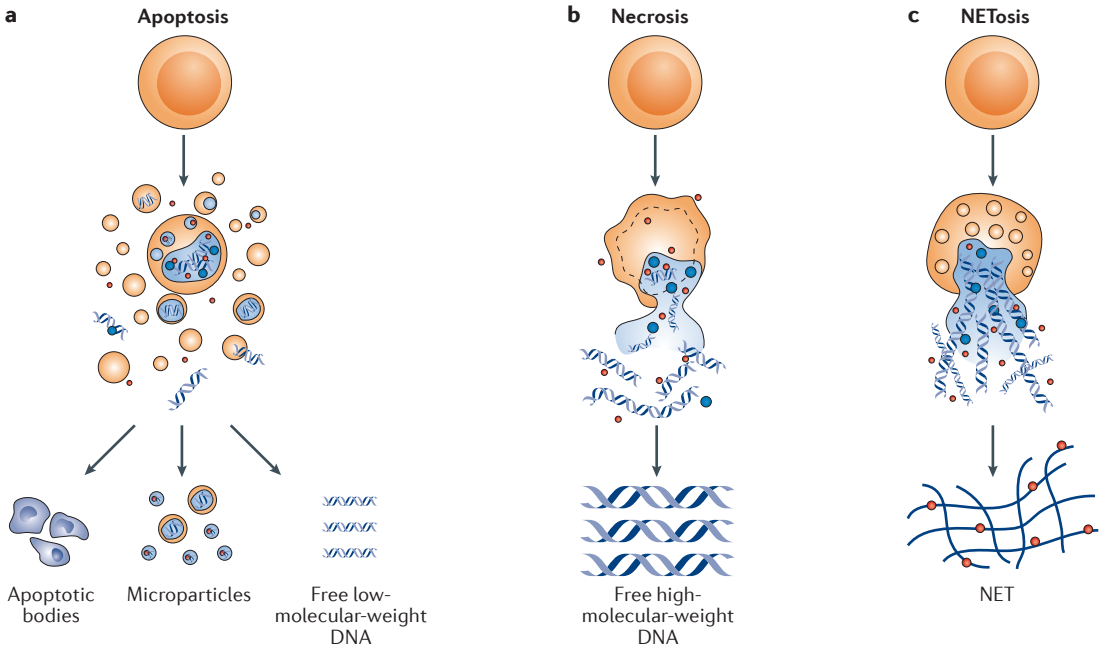

Figure 1 | **The release of DNA from dead and dying cells.** Any cell type can undergo apoptosis or necrosis, but NETosis involves only immune cells, most prominently neutrophils. **a** | In apoptosis, the cell shrinks and the nucleus condenses. DNA undergoes cleavage by nucleases, resulting in a ladder of low-molecular-weight fragments corresponding to multiples of the length of DNA in the nucleosome. Apoptosis results in extracellular DNA in the form of apoptotic bodies (collapsed remains of cells or large fragments thereof), membrane-bound microparticles or free DNA. **b** | In necrosis, as the cell lyses, high-molecular-weight DNA is released in the absence of intracellular cleavage. **c** | In NETosis of neutrophils, DNA mixes with enzymes in neutrophil granules and is then extruded in the form of a mesh or neutrophil extracellular trap (NET) in which DNA is coated with anti-bacterial proteins such as myeloperoxidase and neutrophil elastase.

Assays for anti-DNA antibodies

Anti-DNA-antibody assays vary in the source of DNA for the antigenic substrate and in the principle that is employed to assess binding. In essence, all anti-DNA-antibody assays measure the formation of immune complexes of anti-DNA antibodies and DNA. The physical principle (such as radioimmunoassay, fluorescence immunoassay and ELISA) as well as assay conditions (such as salt concentration) for detection of such complexes vary between assays, influencing the type of anti-DNA antibodies measured and the levels that are considered significant[15] (BOX 1). Notably, in the criteria of the Systemic Lupus International Collaborating Clinics (SLICC) group for disease classification, anti-dsDNA-antibody positivity is defined as an assay result above the laboratory reference range, except for ELISA, where a value of twice the laboratory reference range is considered positive[18]. However, these criteria do not specify which assay should be used, nor the required levels of sensitivity and specificity[43,44].

Given the differences in the various testing platforms, the serum of a patient can give positive results in one assay and negative results in another[15,45–47]. This situation can create confusion in the clinic and raise questions about the use of anti-DNA antibodies as a theranostic measure. In the presence of so many different assays, a fair question is whether a gold standard exists, especially for clinical decision-making. To some investigators, the Farr assay has that status because it detects high-affinity antibodies, which are the indication of a mature antibody response. Detection is limited to high-affinity antibodies because of the high-salt conditions (saturated ammonium sulphate) that are used to precipitate the immune complex of DNA and anti-DNA antibodies[48,49]. Although this assay has high specificity for SLE, it does not have the highest sensitivity of the available assays. Compared with the Farr assay, an ELISA has higher sensitivity, because it detects both high-affinity and low-affinity anti-DNA antibodies[15]. Depending on the concentration of DNA and the manner in which it is attached to plates (for example, using positively charged molecules as a 'pre-coat'), an ELISA can produce a false-positive detection of anti-DNA antibodies. Because an ELISA is a sensitive assay, positive results can sometimes occur in patients with conditions that are not SLE, or in patients who have features of SLE, but who do not yet meet the criteria for this disease; this situation can also occur with other assays, but the importance of these apparently false-positive results is not known. Furthermore, the relative contribution of high-affinity and low-affinity antibodies to the pathogenesis in SLE has not yet been determined.

In routine testing, the choice of assay is usually made by the clinical or reference laboratory on the basis of considerations that can include cost and ease-of-performance. However, if suspicion is high for the presence of anti-DNA antibodies, or a serological marker is needed to confirm diagnosis, the attending physician can consider requesting another type of

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Box 1 | **Assays for anti-DNA antibodies**[15]

Immunodiffusion assay
Classic Ouchterlony assay leading to visible precipitate of immune complexes

Complement-fixation assay
Fixation of complement by immune complexes formed by combination of DNA with anti-DNA antibodies

Farr assay
Incubation of radiolabelled DNA antigen with serum followed by addition of saturated ammonium sulphate to precipitate immune complexes; the proportion of the initial radiolabel that is precipitated gives an indication of the amount of anti-DNA antibody in the serum sample

PEG-precipitation assay
Similar to the Farr assay, but with precipitation by polyethylene glycol (PEG), which is less stringent than precipitation by ammonium sulphate; the proportion of the initial radiolabel that is precipitated gives an indication of the amount of anti-DNA antibody in the serum sample

Filter-binding assay
Incubation of radiolabelled DNA with serum followed by filtration through nitrocellulose to retain immune complexes; assessment of the proportion of the radiolabel that is retained on the filter gives an indication of the amount of anti-DNA antibody in the serum sample

***Crithidia luciliae* immunofluorescence test (CLIFT)**
Semi-quantitative indirect immunofluorescence assay with the kinetoplast of *C. luciliae* as the source of naked double-stranded DNA; serial dilution of serum samples enables determination of the maximum dilution that gives a positive immunofluorescence reaction

ELISA
Indirect assay in which DNA is bound to a solid-phase support, exposed to serum, and anti-DNA antibody binding detected with enzyme-labelled antibodies to human IgG; quantitation is achieved by comparison of colour development in sample wells with that in wells treated with dilutions of a known standard

Multiplex assay
Immunofluorescence assay in which multiple antigens are bound to solid-phase supports (beads); after incubation with serum, followed by fluorescence-labelled secondary antibody, antigen binding is detected by measurement of fluorescence associated with individual beads

anti-DNA assay. In this regard, the ability of the ELISA to detect a broad range of antibodies might make it a good choice for screening.

Multiplex assays are increasingly being used for routine serological testing, including quantitative determination of levels of anti-DNA antibodies. These assays employ antigen-coated beads, and are a form of solid-phase assay, with the DNA bound to a surface. Such assays are convenient and have the advantage of simultaneously providing data on multiple antibody specificities. The performance of multiplex assays, with regard to the detection of anti-DNA, is comparable to that of other assay formats. Because the diagnosis of SLE depends upon the clinical context and the fulfillment of other clinical and laboratory criteria, no one test will suffice, and a sensitive assay for anti-DNA antibodies will help to ensure that the diagnosis of SLE is not missed.

Box 2 | **Monogamous bivalency**[50–52]

Monogamous bivalency is the term for a mode of antibody binding in which both Fab sites of an IgG molecule interact with contiguous sites on the same antigen. The binding affinity of each Fab site is low, and high functional affinity (or avidity) depends upon the binding of both sites. In this process, interaction of the first Fab site with a determinant on a multivalent antigen increases the likelihood of the second Fab site binding; similarly, dissociation of one site from the antigen is likely to lead to re-association if the antigen remains bound by the other site. In essence, the local concentration of antigen rises. Monogamous bivalency can be indicated by discrepancies in the affinities measured in different assay formats, including the magnitude of binding to antigens in solution or attached to solid-phase supports, or to intact antigens compared with smaller antigenic fragments, or of proteolytic Fab fragments relative to whole IgG. The prevalence of monogamous bivalency either with or without autoimmune responses is unknown, because of the use of single assay formats with intact antibody, so its influence on inter-assay variability has not yet been determined.

Immune recognition of DNA

Monogamous bivalency

Although anti-DNA antibodies can display high affinity (befitting the products of antigen selection), high affinity in this context reflects functional affinity, or avidity, which involves a form of antigen binding known as monogamous bivalency[50–52]. This binding is a type of cross-linking in which the two Fab sites on each antibody contact antigenic determinants on the same DNA molecule, resulting in a stable interaction (BOX 2).

The binding site of each Fab region could accommodate 3–5 nucleotides. In general, oligonucleotides are not effective antigens, and longer DNA fragments are necessary for stable interactions. Depending on the isotype and segmental flexibility, the distance between the Fab sites of an intact IgG molecule is around 100 Å[53]. The minimum size of a helical DNA antigen for antigenicity should be around 30 bp, although longer molecules are more-effective antigens[54–56] (FIG. 2).

Monogamous bivalent interaction means that immune complexes are not likely to form as a result of one antibody binding simultaneously to two different polynucleotide chains. Instead, complex formation results from many antibodies binding to the same piece of DNA, so that the length of the DNA that is bound will influence lattice formation[57,58]. Various lengths of DNA are found in blood, but they are generally <200 bp, reflecting origins in apoptosis or in cleavage by serum nucleases.

Immune recognition of DNA in relation to SLE

Anti-DNA antibodies are almost exclusively observed in SLE, and a role for B-cell-specific antigen selection in this disease is suggested by the high affinity and clonal expression of these antibodies, and the association with variable-region somatic mutations[59–64]. Because some of these mutations result in the generation of arginine residues in anti-DNA antibodies, which promote binding to the negative charges of the phosphodiester backbone, DNA has emerged as the selecting antigen for B cells in SLE. To the extent that the generation of anti-DNA antibodies is dependent on T cells, protein recognition by these T cells is likely to involve histones or another chromatin component[65,66]. Delineation of the role of antigen selection is important in determining possible steps at which immunomodulatory agents can be most effective in reducing this response.

Evidence suggests that DNA in the blood of patients with SLE differs from bulk chromosomal DNA in both sequence and methylation, which might reflect its

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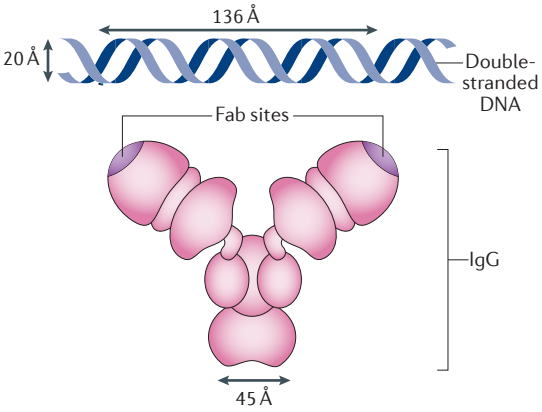

Figure 2 | **The relationship between IgG antibodies and double-stranded DNA.** Binding of IgG antibodies to DNA can occur by monogamous bivalency, wherein both Fab sites of an IgG molecule make contact with a single stretch of DNA. The distance of 136 Å is equivalent to 40 bp in B-form DNA. Permission obtained from the American Society for Clinical Investigation © Papalian, M. *et al.* Reaction of systemic lupus erythematosus antinative DNA antibodies with native DNA fragments from 20 to 1,200 base pairs. *J. Clin. Invest.* 65, 469–477 (1980).

origin in specific cell populations[67–69], and which could also affect its antigenicity. Similarly, if anti-DNA antibodies bind selectively to certain regions of DNA, the nucleic acid content in immune complexes could differ in sequence or conformation from DNA in the extracellular pool[70,71].

For a long time, SLE was thought to be the only setting in humans resulting in an antibody response to DNA. The question of whether anti-DNA antibodies could exist outside of SLE remained unanswered. Furthermore, attempts to replicate immune responses to DNA similar to those seen in SLE by immunization of animals have been largely unsuccessful, and SLE has long lacked an induced animal model. The inherent difficulty in generating B cells with antigenic specificity for dsDNA indicates the value of anti-DNA antibodies as a marker for immune disturbances in SLE, whatever their clinical association.

Although, in animal studies, DNA is at best a poor immunogen, antibody responses to nonmammalian DNA have been demonstrated in humans. The existence of these antibodies was discovered fortuitously in experiments to determine whether anti-DNA from patients with SLE can distinguish between structural variants of DNA[71]. The test antigens consisted of a panel of mammalian and bacterial DNA, and whereas sera from patients with SLE bound similarly to the different antigens, sera from individuals without SLE only bound to DNA from two bacterial species: *Staphyloccus epidermidis* and *Micrococcal lysodeikticus*[71]. Indeed, levels of these antibodies to bacterial DNA in control sera were similar to those in the blood of patients with SLE when measured with bacterial DNA as the test antigen, indicating a robust response to the bacterial DNA[72–74].

The antibodies to DNA in individuals without SLE, however, are clearly distinguishable from those in patients with SLE. The former bind selectively to species-specific bacterial DNA without cross-reactivity with other bacterial DNA, whereas the latter bind non-selectively to all DNA tested[72]. Bacteria are not the only foreign organisms that can stimulate the production of antibodies to DNA, as individuals without SLE can also produce highly selective antibodies to the dsDNA of the BK virus[75,76]. The selectivity suggests that the antigens for these antibodies are not shared with mammalian DNA, and as the phosphodiester backbone is highly conserved, the antigens are probably viral nucleotide sequences.

The consequences to host defense of antibodies to bacterial and viral DNA in individuals without SLE are not known. However, the existence of these antibodies suggests a possible mechanism for the production of anti-DNA antibodies in SLE. This mechanism could involve a shift in individuals who develop SLE from an antibody response to specific nucleotide sequences to a response to the phosphodiester backbone, rather than the acquisition of responsiveness to an otherwise inert molecule[77–79] (FIG. 3). Notably, some patients with SLE seem to lack antibodies specific for bacterial DNA, and prior to the development of SLE might be unable to clear this material from the system[76]. This deficiency might result from a specific, albeit limited, immunodeficiency in the production of antibodies to bacterial DNA; this state could represent a susceptibility factor for certain patients with SLE. Alternatively, a defect in the production of antibodies to bacterial DNA could be a facet of the overall immune disturbance in SLE. Because bacterial DNA — by virtue of its content of CpG motifs — can stimulate immune responses regulated by Toll-like receptor (TLR) 9, the deficiency might lead to persistent immune stimulation and the emergence of autoantibodies to cross-reactive determinants. In other patients with SLE, antibodies specific for bacterial DNA coexist with autoantibodies that can bind both bacterial and mammalian DNA[77–79]. The basis for the differences between patients in the production of antibodies to bacterial DNA is not known, nor is the effect on clinical features of SLE. However, individuals who display an intact response to bacterial DNA might be protected from the immune stimulation that results from the persistent presence of DNA with CpG motifs, thus having less severe immune activation than individuals without a bacterial DNA response.

Anti-DNA antibodies in pathogenicity

Formation of pathogenic anti-DNA antibodies

Anti-DNA antibodies that are produced in the abnormal setting of disease can be termed pathological (BOX 3). Anti-DNA antibodies that cause a disease manifestation can be termed pathogenic and, as shown in studies involving both human patients and animal models, antibodies to DNA have diverse roles in pathogenesis, including the formation of immune complexes[80]. Antibody–DNA complexes can form whether the antigen is free or exists as particles[81–84]. Once formed, the complexes can fix complement and stimulate

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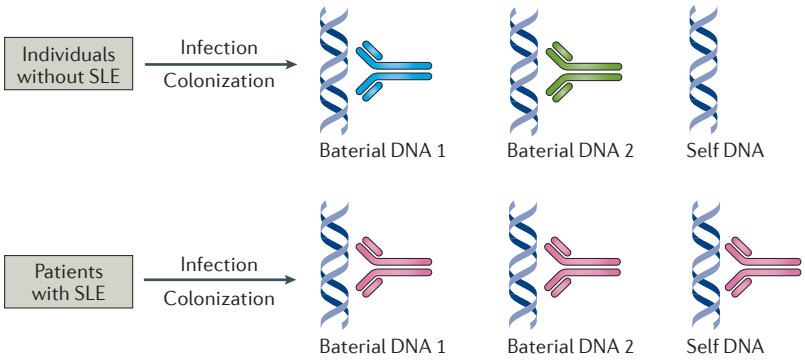

Figure 3 | **The induction of anti-DNA antibodies by bacterial DNA.** Antibody responses to bacterial DNA differ between individuals with and without systemic lupus erythematosus (SLE). In individuals without SLE, antibodies specific for DNA from certain bacteria are generated. Each antibody binds only to DNA from a particular bacterial species, and none of the antibodies binds to self DNA. This binding pattern indicates specificity for the nucleotide sequence (or for backbone structures dependent on sequence). In at least some patients with SLE, anti-DNA antibodies bind to DNA from different sources, including self DNA. These antibodies bind to backbone determinants and are autoantibodies. Antibodies that bind only to mammalian or self DNA might exist, but are difficult to detect. This scheme suggests that the abnormality in SLE relates to the specificity of antibodies to DNA, rather than to a unique ability to produce antibodies that bind to DNA.

inflammation and tissue damage. The kidney is the major site of this involvement. In the generation of renal injury, anti-DNA antibodies are enriched in renal eluates, fluctuate in levels with active nephritis, and are deposited in the kidney when administered to otherwise normal mice, all of which is evidence of the importance of anti-DNA antibodies in immune-complex-mediated renal pathology[80,85].

As shown most clearly with monoclonal products of murine origin, only certain anti-DNA antibodies are nephritogenic and have the potential to induce renal injury[86] (BOX 3). The capacity to form immune complexes with renal localization seems to be related to antibody avidity, fine specificity, charge, isotype and ability to fix complement. These complexes might form in the circulation or, alternatively, in the kidney with nucleosomal DNA released on local cell death. Immune complexes can also form by the binding of antibodies to DNA that has been bound or 'planted' onto the basement membrane as a result of interaction with charged molecules in the membrane[87]. This mechanism is called *in situ* immune-complex formation.

Box 3 | **Functional classification of anti-DNA antibodies**[80–94]

Pathological
- Abnormally produced
- Biomarker for diagnosis

Pathogenic
- Cause any manifestation of systemic lupus erythematosus (such as nephritis or production of interferon) by immune complex formation or cross-reactive binding

Nephritogenic
- Cause nephritis by forming immune complexes in blood or kidneys
- May bind directly to renal antigens
- May also lead to cytokine induction

Cytokine-inducing
- Form immune complexes that stimulate interferon production
- May also lead to nephritis

Determinants of pathogenicity

Pathogenicity might not be determined solely by the properties of the antibody, as the exposure of a self-antigen can transform a pathological antibody into a pathogenic antibody. Such a change could occur systemically or locally[88,89]. As shown in both human and murine systems, a decrease in renal expression of DNase can be associated with greater exposure of nucleosomal DNA in the kidney[90,91]. These factors, combined with differences in the properties of anti-DNA antibodies deposited in the kidney compared with those in the circulation, mean that a blood-based assessment of nephritogenicity might not be possible.

In addition to promotion of nephritis, anti-DNA antibody pathogenicity can result from the formation of immune complexes that cause generalized immune-cell disturbances by the induction of cytokine release — in particular type 1 interferon — from plasmacytoid dendritic cells[91] (BOX 3). In addition to effects on immune cells, interferon can lead to systemic complaints such as fatigue and malaise that characterize the course of SLE. The induction of interferon results from DNA stimulation of internal nucleic acid receptors including TLR9[92–94]. These receptors, which ordinarily respond to bacterial or viral DNA introduced into the cell during infection, are an internal host-defense system[95,96]. An immune complex with anti-DNA antibodies provides a vehicle to shuttle DNA into the cell, where it can access the receptors. Similarly, antibodies that recognize RNA-binding proteins (RBPs) such as the small nuclear ribonucleoproteins Sm and RNP, can shuttle RNA into cells to activate internal RNA sensors[97,98].

Pathogenicity of anti-DNA antibodies can also result from cross-reactive binding to non-DNA antigens such as the *N*-methyl-D-aspartate (NMDA) receptor in the context of nervous neuropsychiatric manifestations of SLE[99,100] (BOX 3). Cross-reactive binding to an intrinsic renal antigen such as α-actinin has been proposed as a mechanism by which anti-DNA antibodies can mediate nephritis[101–103].

Anti-DNA antibodies as markers of SLE activity

In the context of nephritis, anti-DNA antibody expression does not always correlate with active disease, providing another source of uncertainty about their marker function. Some patients with nephritis lack anti-DNA antibodies and some patients with anti-DNA antibodies do not develop nephritis[104]. One possibility is that anti-DNA antibodies causing nephritis are deposited in the tissue and cannot be detected in the blood. Another explanation for these discrepancies is technical and relates to the fact that most existing assays for anti-DNA antibodies are validated for diagnosis — to distinguish patients with SLE from patients with other diseases — rather than for disease activity. A serologically positive, clinically negative

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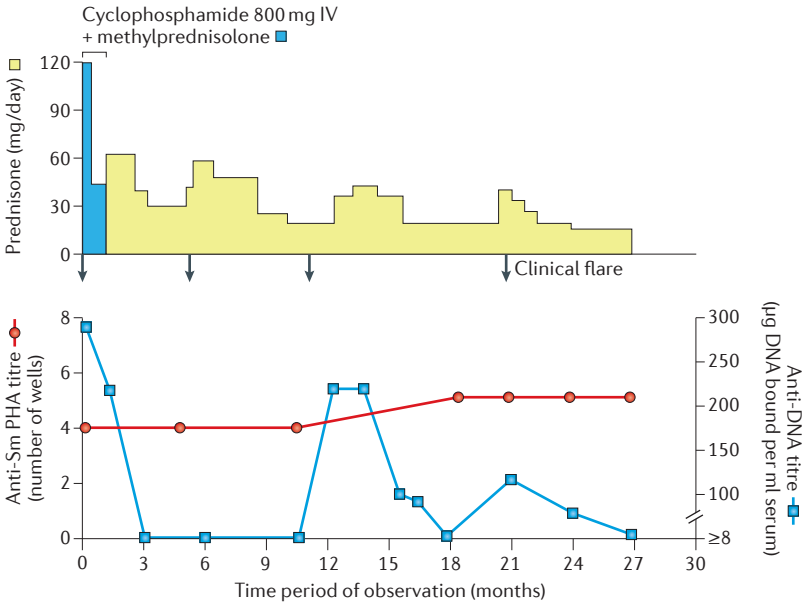

Figure 4 | **Antinuclear antibodies and disease activity in SLE.** Levels of anti-DNA and anti-Sm antibodies were determined in the serum of a patient with systemic lupus erythematosus (SLE). Flares of disease activity resulted initially in treatment with cyclophosphamide and methylprednisolone, and subsequently with prednisone. In these studies, assay of anti-Sm antibodies (which bind to small nuclear riboproteins) was performed by passive haemagglutination (PHA), whereas anti-DNA antibody levels were determined by a filter-binding assay in which the presence of antibodies is detected by the capture of a radiolabelled DNA substrate with a nitrocellulose filter. In the absence of antibody binding, the DNA does not bind to the filter. Permission obtained from *The Journal of Rheumatology* © McCarty, G. A. *et al.* Independent expression of autoantibodies in systemic lupus erythematosus. *J. Rheumatol.* 9, 691–695 (1982).

patient might have non-nephritogenic anti-DNA antibodies, whereas a serologically negative, clinically positive patient might have nephritis caused either by an antibody that is not anti-DNA or by an anti-DNA antibody that is not well-detected by a particular assay. Indeed, analysis of antibodies eluted from the kidneys of patients with lupus nephritis can show different spectra of anti-DNA antibodies to those in the sera as well as the enrichment of antibodies for antigens such as La, Sm and C1q[105,106].

The best assay of anti-DNA antibodies for making these determinations is not known, but the choice of assay affects clinical assessment, and should vary depending on whether the goal is to determine the diagnosis, or to assess disease activity or eligibility for inclusion in a clinical trial, or for determining therapy choice in clinical practice. To monitor disease activity, a less specific, more sensitive assay might have greater utility than a more specific assay. Whichever assay is used, quantitative measurement is vitally important for the utility of anti-DNA antibodies as biomarkers for disease activity. For most other antinuclear antibodies, testing is either not quantitative or occurs only at the time of initial evaluation, although repeat testing can occur if a patient seeks care by a new provider or otherwise needs re-evaluation.

Identifying and quantitating anti-DNA immune complexes in blood can be difficult. One approach is to measure the amount of DNA that is bound to antibodies isolated by techniques including purification by Protein A or Protein G[80–83]. The results of these studies suggest that the amount of DNA in the blood in the form of immune complexes can be lower during flares than at times of stable disease activity[83]. A possible explanation for this phenomenon is that the complexes are no longer in the blood because they have been deposited in the tissue. In this circumstance, the presence of decreased levels of complement or increased levels of complement split products might suggest the presence of immune complexes, although the composition of the complexes cannot be inferred from these determinations[107,108].

Many efforts to evaluate the role of DNA-containing immune complexes in SLE disease activity have focused on serum, although plasma could facilitate analysis of microparticles and reduce technical problems that occur with serum as a result of blood clotting. These studies show that disease activity is associated with the presence in plasma of IgG-positive particles containing DNA[41,42,109]. Furthermore, these particles have a distinct molecular profile, including expression of galectin-3-binding protein (G3BP, also known as MAC2BP, encoded by *LGALS3BP*), which can bind to galectin on the surface of apoptotic cells as well as microparticles. Immune electron microscopy has identified co-localization of G3BP with IgG in electron-dense immune deposits in the kidney in SLE, suggesting microparticles are a source of immune complexes that contain DNA bound by anti-DNA antibodies[110].

An important aspect of anti-DNA antibodies that affects their function as biomarkers is their quantitative variation over time. Anti-DNA antibody levels can fluctuate widely, and in some patients they can essentially disappear with treatment[111–113]. By contrast, levels of antibodies to RBPs are often stable over time and seem resistant to the effects of treatment; persistently high levels occur even as anti-DNA antibody levels become undetectable[111] (FIG. 4). This situation probably reflects fundamentally different mechanisms of production of the two types of autoantibody and the contribution of different B-cell populations to the response[114]. In assessing the role of anti-DNA antibodies as biomarkers of SLE with respect to their function in more generalized systemic manifestations of SLE related to the effects of interferon, the context — such as the presence of anti-RBP antibodies — is important[115,116]. Even if anti-DNA immune complexes contribute to pathogenicity related to cytokine production, their contribution could be masked or overwhelmed by that of anti-RBP antibodies, which can drive persistent expression of interferon. As a result, symptoms related to interferon expression — such as fatigue, malaise and possibly arthritis — might persist in some patients, even as levels of anti-DNA antibodies diminish. This situation can lead to discrepancy between objective measures of disease activity and patients' reports of their conditions. The effects of anti-DNA antibodies on disease must be understood in the context of the company that they keep.

Conclusions

Understanding the role of anti-DNA antibodies in the manifestation of SLE requires recognition of the unique features of their interactions with DNA in terms of

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monogamous bivalency and the distinction between pathological, pathogenic and nephritogenic specificities, as well as the diverse origins and structural properties of DNA that can be bound to form immune complexes. Although these immunochemical issues might seem arcane, they are fundamental to the use of one of the most venerable biomarkers in all of medicine and can provide insights into the mechanisms by which these antibodies can both cause autoimmunity in SLE and mirror its activity. Future studies should define the determinants of the pathogenicity of anti-DNA antibodies and lead to the development of assays suitable for theranostic as well as diagnostic use. As the field of personalized or precision medicine evolves, biomarkers to determine the stage and subset of disease in individuals will be essential. The close association with SLE that has so clearly been documented will likely put anti-DNA antibodies near the top of any list of biomarkers.

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Competing interests statement
The author declares no competing interests.

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