

Anti-DNA and Anti-Nucleosome Antibodies: an Update

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Abstract

Among the large spectrum of autoantibodies (Ab) detected in systemic lupus erythematosus (SLE), anti-dsDNA Ab have been used to diagnose and assess disease activity for more than 60 years. Anti-dsDNA Ab are often associated with anti-nucleosome Ab, and in clinical practice combining both is helpful for diagnosis, prognosis, and as biomarkers to monitor response to treatment, especially when using drugs that target B cells and Ab production. In general, the association of anti-dsDNA Ab plus anti-nucleosome Ab is linked with disease flares and can indicate lupus nephritis. Moreover and since anti-nucleosome Ab have higher sensitivity and specificity than anti-dsDNA Ab, the former is a useful marker in SLE patients negative for anti-dsDNA Ab and in the diagnosis of certain types of drug-induced lupus.

Keywords: Lupus; Anti-DNA; Anti-nucleosome; Autoantibodies

Historical Points

In 1957, Cappellini et al. first identified auto-antibodies (Ab) to deoxyribose nucleic acid (DNA) in sera from systemic lupus erythematosus (SLE) patients [1]. Since then, anti-DNA Ab have become an important biomarker explaining its inclusion in the American College of Rheumatology (ACR) criteria and later in the Systemic Lupus International Collaborating Clinics (SLICC) criteria for SLE classification [2,3]. Subsequent observations in the late 1980s further support the idea that anti-DNA Ab development in SLE results, not from a direct immune reaction to DNA, but from a reaction to nucleosomes. In fact, it's suspected that dysregulation of apoptosis in SLE contributes to chromatin fragmentation into nucleosomes and subsequently to DNA and histone exposition. Anti-nucleosome Ab, frequently referred to as anti-chromatin Ab, have further demonstrated their clinical utility when used alone, or better in association with anti-dsDNA Ab, for SLE diagnosis and follow-up.

Autoantigen and Epitopes

The basic chromatin subunit or nucleosome, is a nucleoprotein complex that consists of dsDNA (B-form) wrapped twice around an octameric core of histones (two each of histones: H2A, H2B, H3 and H4). The related anti-chromatin/nucleosome Ab have been characterized and they are subdivided in two groups. First, and restricted to SLE patients, specific anti-nucleosome Ab are encountered and they are directed towards conformational epitopes created by the interaction between dsDNA and the core histones (Figure 1). Second and apart from the specific nucleosome recognition, non-specific anti-nucleosome Ab are usually present and they recognize the basic elements of the nucleosome: histones and DNA. As a consequence, and to detect specific anti-nucleosome Ab, the non-specific anti-dsDNA Ab and anti-histone Ab have to be removed by an absorption step, which is typically not performed in routine.

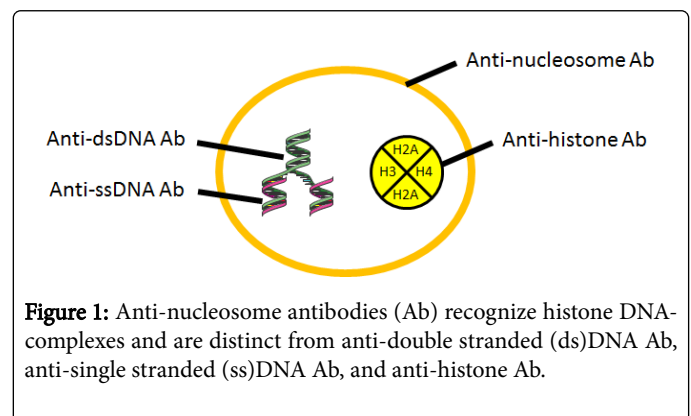


Figure 1: Anti-nucleosome antibodies (Ab) recognize histone DNA-complexes and are distinct from anti-double stranded (ds)DNA Ab, anti-single stranded (ss)DNA Ab, and anti-histone Ab.

DNA as an antigen may be either single-stranded (ss) DNA or double stranded (ds) DNA. On the one hand, specific high avidity (hydrogen interaction binding) anti-dsDNA Ab target epitopes present within the deoxyribose phosphate backbone of the double helix of DNA and a preferential binding to Z-DNA over B-DNA is observed. On the other hand, low avidity anti-dsDNA Ab and anti-ssDNA Ab recognize epitopes present either on short regions of the DNA helix, or on short nucleotide sequences. These low avidity interactions appear to be predominantly based on electrostatic interactions and are sensitive to molarity and pH. The binding to defined purine and pyrimidine sequences is observed in the case of specific anti-ssDNA Ab.

Methods of Detection

Anti-dsDNA Ab and anti-nucleosome Ab detection is challenging since a large panel of assays has been developed but the assays are difficult to transpose for several reasons: (i) DNA used in the assays may be obtained from tissues, eukaryotic cells, prokaryotes, bacteriophages or plasmids; (ii) DNA size is not always normalized as well as its global methylation status; (iii) spatial DNA conformations vary based on whether the DNA is coated on plastic (enzyme linked immunosorbent assay, ELISA), in solution (Farr assay, a radio-

immunological method), or present in cells (*Crithidia luciliae* immunofluorescent test, CLIFT); (iv) the nature of the buffers (salt, pH) for low avidity Ab; (v) the choice of the conjugated Ab selected for detection if using IgG, IgA, IgM or total immunoglobulins; and (vi) the presence of contaminant such as ss-DNA (higher avidity to ELISA plates), the linker protein histone H1, and topoisomerase I (Scl70). The latter two were present in the first generation of anti-nucleosome ELISA and have produced false positive results in patients with systemic sclerosis [4,5].

Described by Hargraves in 1948, the lupus erythematosus (LE) cell formation can be produced by combining IgG from sera with damaged granulocytes and complement [6]. The nucleosome was subsequently identified as the nuclear antigens recognized by the "LE cell factor" [7]. Indeed, purified nucleosomes, but not free dsDNA or histone alones, are able to inhibit the opsonization of nuclear material in the presence of sera from patients with "LE cell factor". Now this method is not used as much as those assays based on complement fixation and hemagglutination. For the same reasons (time consuming assay and lack of sensitivity or reproducibility), the radio-immunological method developed by Farr or its PEG variant are progressively less frequently used.

The CLIFT assay specifically detects anti-dsDNA Ab present in the kinetoplast, a giant mitochondrion, of *Crithidia luciliae* that contains dsDNA but no ssDNA or other common anti-nuclear autoantigens. In order to eliminate trace amounts of histones present in CLIFT preparations, *Crithidia luciliae* substrates are treated with hydrogen chloride (HCl) and the use of long term cultures is recommended since histones are detected mostly after the second day of culture and decrease after that [8]. The CLIFT assay detects medium to high avidity anti-dsDNA Ab.

In ELISA assays, and to fix dsDNA, plates have to be pre-coated with intermediates (e.g. protamine, poly-L-Lysine, methylated BSA). In contrast, nucleosomes (and ssDNA) have a strong ability for direct coating. This allows one to avoid using intermediates for coating, which can prevent false positives and can even outperform the Farr assay [9]. In order to remove ssDNA, treatment with nuclease S1 after coating is highly recommended. More recently alternatives to ELISA have been developed using (i) biotinylated DNA which is able to affix to streptavidin pre-coated on plates, (ii) strips blotted with dsDNA (immunoblotting), (iii) laser bead immunoassays (ALBIA), and (iv) chemiluminescent immunoassays (CLIA). A different approach is to specifically detect high avidity anti-dsDNA Ab by using stringent buffers [10].

Sensitivity and Specificity

The sensitivity and specificity of anti-dsDNA, CLIFT and anti-nucleosome Ab assays vary according to the test and to the characteristics of the clinical population which is influenced by factors like ethnicity, disease activity, disease duration and treatment (Table 1). In addition and to reduce the risk of misinterpretation, it is recommended to test anti-dsDNA Ab and anti-nucleosome Ab only on those patients with antinuclear Ab detected on HEp-2 cells. Therefore, there is sound evidence that anti-nucleosome Ab sensitivity is higher than that of anti-dsDNA Ab detected by ELISA and even more when using CLIFT. The combined detection of anti-nucleosome Ab with high avidity anti-dsDNA Ab detected by CLIFT and/or low avidity anti-dsDNA Ab assay (e.g. ELISA) reflects patients with active SLE (Figure 2). Moreover, and with regards to the results obtained

during the course and evolution of the disease, many findings support a positive test restricted to anti-nucleosome Ab, but not to anti-dsDNA Ab detected by CLIFT and/or ELISA, in the related conditions: at early stages of SLE, in SLE disease with low activity, and in certain cases of drug-induced lupus but not in cutaneous lupus. An isolated CLIFT assay is rarely detected, usually at low levels, and indicates in most cases an inactive mature disease. Finally, an isolated positivity of the anti-dsDNA Ab by ELISA does not always support the diagnosis of SLE.

| Assay | Sensitivity | Specificity | Antibody avidity |
|------------|-------------|-------------|------------------|
| ELISA | 44-79% | 71-97% | + |
| ELIA/CLIA | 40-73% | 84-94% | + |
| Farr | 32-85% | 95-99% | ++++ |
| CLIFT | 13-47% | 99-100% | +++ |
| Nucleosome | 50-100% | 97-100% | ++ |

Table 1: Specificity and sensitivity of different detection methods to detect anti-double strand (ds)- DNA and anti-nucleosome antibodies.

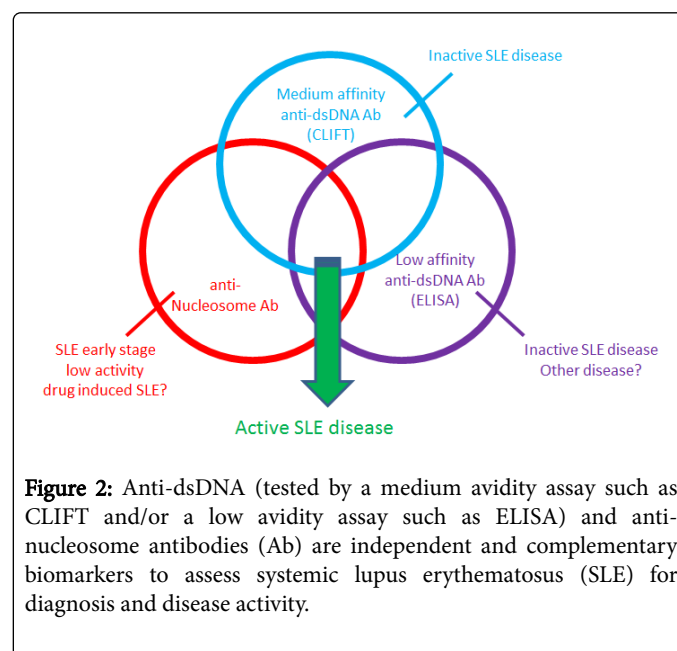


Figure 2: Anti-dsDNA (tested by a medium avidity assay such as CLIFT and/or a low avidity assay such as ELISA) and anti-nucleosome antibodies (Ab) are independent and complementary biomarkers to assess systemic lupus erythematosus (SLE) for diagnosis and disease activity.

Accordingly for the screening and follow-up of defined SLE patients, coupling anti-nucleosome Ab with anti-dsDNA Ab detection (using ELISA and/or CLIFT) is recommended rather than a sequential detection using a suspected high sensitivity technique (e.g. anti-dsDNA Ab ELISA) followed, when positive, by a method with high disease specificity (e.g. CLIFT or anti-nucleosome Ab) [11].

Clinical Utility and Limitations

In humans, occurrence of anti-dsDNA Ab can precede SLE diagnosis by 2 years, and in SLE-prone mice anti-nucleosome Ab are detected before intramolecular epitope spreading to anti-dsDNA Ab and anti-histone Ab [12]. The highest levels of anti-dsDNA Ab and anti-nucleosome Ab are found at the time of diagnosis in untreated

young patients, in patients with high disease activity, and in those with lupus nephritis [13]. Accordingly, anti-dsDNA Ab (and complement deficiency) have been included in the clinical assessment to follow SLE disease activity in the SLEDAI since a clear relation exists between anti-dsDNA Ab levels and disease activity and, in particular, with hematological and organ flares including lupus nephritis [14,15]. Anti-dsDNA Ab levels are usually reduced following treatment (e.g. immunosuppressive therapy, anti-Bly's monoclonal Ab) and a reduction predicts therapeutic benefit as a stable negativity to anti-dsDNA Ab and anti-nucleosome Ab can prevent a subsequent relapse [16,17]. A recent meta analysis further confirmed that anti-nucleosome Ab have higher capacity than anti-dsDNA Ab to correlate with disease activity, but not with kidney involvement [18]. Such an observation is not surprising since the anti-dsDNA/nucleosome Ab contribution to kidney damage is complex and involves several actors [19,20].

Other Associations

Anti-dsDNA Ab have the capacity to cross-react with multiple autoantigens which explains, in part, the role of anti-dsDNA Ab in the development of: (i) lupus nephritis through its cross reactivity with mesangial targets (e.g. alpha-actinin, annexin A1), podocyte targets (e.g. α -enolase) and anti-anti-C1q [21-24]; (ii) psychiatric and/or neurological involvement through cross-reactivity with anti-ribosomal P Ab and anti-cardiolipin Ab [25,26]; and (iii) infections by inhibiting plasmodial growth [27] or by cross reacting with the Herp protein synthesized by *Escherichia Coli* [28] and the EBNA-1 protein from the Epstein Barr virus [29].

Conclusion

The events leading to development of Ab in SLE are multifactorial and poorly understood [30]. With the development of proteomics technologies, novel Ab are still being discovered. However, the list of Ab providing clinical indication specific for SLE is limited and includes anti-dsDNA/nucleosome Ab, anti-Sm/U-RNP Ab [31], anti-C1q Ab [32], anti-ribosomal P protein Ab, and anti-phospholipid Ab [33]. This may reflect separate Ab clusters with distinct clinical manifestations and disease outcome, as recently suggested [34].

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