Review

Fifty years of anti-ds DNA antibodies: are we approaching journey’s end?

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The year 2007 marks the 50th anniversary of the identification of antibodies to double-stranded (ds) DNA. Whilst widely regarded as synonymous with patients who have systemic lupus erythematosus (SLE), doubts have been raised about their significance and the extent to which they are genuinely part of the pathogenesis of the disease rather than being mere bystanders. Problems with assays used to detect them are still evident but they remain widely utilized both to help establish the diagnosis of SLE and to monitor the progress of the disease. This review explores each of these aspects and concludes that whilst some way short of ideal, their measurement remains a useful criterion for the disease and some of these antibodies do appear to be genuinely pathogenic. However, further research is needed to establish beyond ‘reasonable doubt’ whether they are merely part of the spectrum of anti-nucleosome antibodies, the precise mechanisms by which they ‘exert’ their pathogenic effects and to what extent blocking them would be a useful therapeutic goal.

Key Words: DNA antibodies, SLE, Lupus nephritis.

Introduction

In the 1920s, the playwright R.C. Sheriff (who was later to write the Dam Busters) wrote a powerful play, ‘Journey’s End’, encapsulating his own experience of survival under fire in the trenches during World War I. By analogy, in 2007 we celebrate the 50th birthday of anti-dsDNA antibodies which have also been under fire for many years. Anti-dsDNA antibodies, together with rheumatoid factor, are the best studied of all autoantibodies found in rheumatological diseases. Although other assays, such as anti-nucleosome enzyme-linked immunosorbent assays (ELISAs), have been suggested to be as good as or better than anti-dsDNA antibodies in predicting disease activity in lupus, measurement of anti-dsDNA is still used ubiquitously to help in the diagnosis of systemic lupus erythematosus (SLE), to monitor the progress of patients and to study the pathogenesis of the disease.

In this review, which focuses mainly on human studies, we ask whether these antibodies still have a part to play in diagnosing lupus and managing it, what we know of their structure and origin and if they really contribute to the pathogenesis of the disease? Are we in fact approaching journey’s end in our quest to determine the importance of anti-dsDNA antibodies?

Historical note

Coincident with George Friou’s description of anti-nuclear antibodies (ANA) [1], four separate laboratories identified the presence of antibodies to dsDNA in the sera of patients with lupus [2–5]. The suspicion that these antibodies might genuinely be linked to the pathogenesis of lupus was enhanced by a report in 1967 [6] showing that they could be detected in the eluates of kidney biopsies from patients with lupus nephritis (as well as from the renal tissue of lupus mouse models).

Techniques for measuring anti-dsDNA antibodies

A variety of techniques (reviewed in detail elsewhere [7]) have been used to identify the presence of antibodies to DNA. Accurate determinations can be made using radioimmunoassays—notably the Farr assay, in which bound and free DNA are separated by precipitating immunoglobulins with 50% saturated ammonium sulphate. Bound radioactive DNA precipitates with the immunoglobulins whereas the free DNA remains in the supernatant. This method is regarded as highly reproducible, but may miss low-avidity anti-dsDNA antibodies. The nitrocellulose filter method allows free DNA to pass through the filter but dsDNA protein complexes do not. Retained radioactivity on the filter is proportional to the serum anti-dsDNA antibody concentration. Although accurate, radioimmunoassays remain somewhat time-consuming and have the disadvantage that laboratory staff are exposed to radioactivity. The most commonly used non-radioactive assays are the ELISAs and those using immuno-fluorescent techniques, notably the haemoflagellate Crithidia lucilae. The Crithidia immuno-fluorescent test is simple, reasonably sensitive and has high disease specificity because the DNA in the kinetoplast of this micro-organism appears to be pure circular dsDNA. The method does not however allow easy quantitation. In ELISA systems, the target DNA is coated to a plastic (ELISA) plate. In practice, the sensitivity is enhanced by coating with intermediate components such as poly L lysine before adding dsDNA, although these ‘pre-coats’ may introduce problems relating to the non-specific binding of immune complexes and/or immunoglobulins to the pre-coat rather than to the dsDNA on the plates. Nevertheless, most clinical laboratories use ELISAs to measure anti-dsDNA antibodies as they are easy to perform, safe, rapid and can be used to give a quantitative result.

Sensitivity and specificity

Although an immunofluorescence test for ANA is the most sensitive test for SLE, being positive in about 95% of cases, it is not specific. A positive ANA is relatively common, even in the healthy population. In contrast, the presence of antibodies to dsDNA, especially IgG antibodies, is more or less specific for lupus but less sensitive. In our cohort of 450 patients with lupus followed up since 1978, 70% have had raised anti dsDNA antibodies at some stage of their disease (data not shown). Occasionally, myeloma proteins are found to have anti-dsDNA
antibodies [8] and rarely patients with chronic active hepatitis or rheumatoid arthritis test positive for anti-dsDNA.

Clinical associations
Assessment of the associations between disease pattern and antibodies to dsDNA, reviewed recently by a European League Against Rheumatism (EULAR) working group [9], was hampered, until the past decade, by the lack of validated and reliable disease activity indices. Serum anti-dsDNA antibody levels as measured by the Farr assay have, however, been correlated with nephritis in some cohorts [10–16], with progression to end-stage renal disease [17–19] and with increased damage or reduced survival [20–25]. In the main, antibodies to dsDNA have been most strongly associated with renal involvement in patients with lupus [26–29]. Using a variety of assays, anti-dsDNA levels often, but not always, correlate with disease activity and at least one study has shown their capacity to predict future flares in longitudinal patient studies [30]. There is a difference of opinion in the literature as to whether a rise in dsDNA antibody levels per se predicts flare, or whether this rise is then followed by a fall before the flare becomes evident [31]. The presumption, in the latter case, is that the high levels of circulating antibodies are lowered as these antibodies are deposited in the tissues, causing pathology. It has also been suggested that an increase in proteinuria, invariably present during a renal flare, leads to a loss of anti-dsDNA antibodies in the urine [32].

In fact, anti-dsDNA antibodies may be present in patients even before they develop clinical features of SLE. Using stored blood samples from American military personnel who had developed SLE years after the samples were taken, Arbuckle and colleagues [33] demonstrated the development of anti-dsDNA antibodies prior to the clinical diagnosis of lupus. It is notable that other antibodies including those binding Ro and Sm antedated the arrival of anti-dsDNA antibodies by several years in many cases. Similarly, it should be noted that many types of antibodies including anti-Ro, La, Sm and chromatin have been identified as forming glomerular deposits in the kidneys of patients with SLE [34].

If anti-dsDNA antibody levels reflect disease activity in SLE, does it follow that treatments designed to reduce these levels would be beneficial in reducing disease activity? Bootsma et al. [35] carried out a trial in which patients were divided into two groups. In one group, patients were treated with increased immunosuppression whenever their anti-dsDNA levels rose by a defined amount, whereas patients in the other group were treated according to their clinical condition and not solely according to level of anti-dsDNA. The group treated according to anti-dsDNA level alone did have reduced mean anti-dsDNA level and fewer flares of disease compared with the other group but only at the cost of unacceptably high level of steroid side effects. Tseng et al. [36] used a 4-week course of reducing prednisone (30 mg for 2 weeks, 20 mg for 1 week, 10 mg for 1 week) to prevent, successfully, severe flares in patients deemed inactive using a modified Systemic Lupus Erythematosus Disease Activity index (SLEDAI) of <4 points or, stable active (SLEDAI score 6–12). These patients were given the steroids if their anti-ds DNA antibody levels increased by >25% and their C3a levels rose concomitantly by >50% (both measured by ELISA).

More recently, B-cell depletion with rituximab (anti-CD20) has been used to treat refractory cases of SLE. Impressive clinical responses in these patients have been associated with falls in levels of anti-dsDNA and anti-nucleosome antibodies whereas titres of other lupus associated autoantibodies e.g. anti-Sm and levels of antimicrobial antibodies were unchanged [37]. LJLP 394, a B-cell toleragen, has been developed to target B cells producing anti-dsDNA antibodies. In two clinical trials using this agent to treat 487 patients with lupus, it was shown that a 50% reduction in dsDNA antibodies was associated with a 52% reduction (95% CI 26–68%, nominal P = 0.0007) and a 53% reduction (95% CI 33–69%, nominal P = 0.0001) in the risk of renal flare [38].

What is the evidence that antibodies to dsDNA are pathogenic?
Circumstantial, and more direct evidence, in mouse models and observations of human disease strongly supports the notion that antibodies to dsDNA may indeed be pathogenic.

As mentioned earlier, anti-dsDNA antibodies have been eluted from kidneys affected by lupus [6], which, whilst it does not prove that these antibodies ‘pull the trigger’, does at least place them at the scene of the crime. Okamura and colleagues [27] in a study of ~40 untreated patients with lupus nephritis showed a clear correlation between the presence of IgG antibodies to ds-DNA, but not to single-stranded DNA, and severe disease on renal biopsy. Data have already been discussed linking the serum levels of anti-dsDNA antibodies to lupus activity in general and to flare in particular. A group of lupus patients designated serologically active (i.e. they have high anti-dsDNA antibody levels) but clinically quiescent are worthy of mention. In both an older study [39] and one published recently [40] it has been shown that, if not most, of these patients will go onto flare in due course though it is debatable whether anti-nucleosome antibodies might better differentiate those who will flare sooner.

Amongst the more compelling data from experimental model studies, the work of Eilat and his colleagues [41] is notable. They used an isolated rat kidney perfusion system and showed clearly that some murine anti-dsDNA antibodies were capable of increasing the amount of proteinuria produced by the kidneys very significantly.

The severe combined immuno-deficient (SCID) mouse has been used to demonstrate the potential pathogenicity of human monoclonal anti-dsDNA antibodies [42]. Some, but not all of these antibodies produced by hybridoma cells implanted intra-peritoneally into these mice, showed the ability to induce significant proteinuria. The proteinuria was associated with the ability of the antibodies to bind to the kidney. It seems likely that different mechanisms were utilized by different antibodies, since some bound directly to kidney tissues while others seem to have the capacity to penetrate living cells.

Factors determining whether anti-dsDNA antibodies are pathogenic
How can we explain the apparent paradox that some patients with SLE have persistently high levels of anti-dsDNA antibodies but remain well? The answer is that not all anti-dsDNA antibodies are pathogenic, a fact which has also been noted in mouse models [42]. It is generally agreed that active lupus is associated with a predominance of IgG rather than IgM or IgA anti-dsDNA antibodies [43]. Natural IgM may even be protective [44]. There is also evidence that the subclass of IgG anti-dsDNA antibodies may be important which may be related to their differing capacity to activate complement or engage Fc receptors [45]. Small changes in the amino acids at the antigen-binding site have been shown to have significant effects on the antigen-binding capacity and functional abilities of anti-dsDNA antibodies. For example, Mason and colleagues [46] showed that introducing a single light chain-mutation into the human monoclonal IgG antibody B3, by reverting a positively charged arginine amino acid to the neutral serine, reduced antibody binding to dsDNA and nucleosomes. Katz et al. [47] showed that changes of just one or two amino acids in the sequence of a mouse monoclonal antibody R4A caused dramatic changes in both binding to dsDNA and binding to the kidneys of recipient mice in vitro.

Both murine [48] and human [49] sequence analysis studies have shown a high prevalence of certain amino acids, principally
arginine, asparagine and lysine, in the complementarity determining regions (CDR) of monoclonal anti-dsDNA antibodies. The accumulation of these particular amino acids is almost certainly driven by somatic hyper-mutation, which helps to increase antigen affinity and probably to promote the survival of B-cell clones. It is not merely the presence of these amino acids in the CDR regions that is important but their actual positions within the sequence which is critical. Compare for example the human monoclonal anti-dsDNA antibodies D5 and RT79 [49]. Whilst having similar VH sequences derived from the gene V\(_{\mu}34\), RT79 has five arginine residues in the VH\(_{\mu}3\) region (generally agreed to be important in terms of autoantibody binding) while D5 has only two, but it is the latter that binds dsDNA whereas RT79 only binds single-stranded DNA.

A number of murine models of lupus have illustrated the occasional lack of association between antibody deposition in the kidney and lupus disease. For example, lupus prone NZM mice engineered to lack Stat4, a transcription factor that drives Th1 responses, display accelerated disease compared with their unmanipulated NZM littermates, despite having greatly reduced anti-dsDNA antibodies [50]. This study not only demonstrates the lack of association of anti-DNA titre and disease, but also that the Th1/Th2 paradigm cannot be used simply to explain the pathogenesis of lupus disease. In particular, cytokines attributed to both Th1 and Th2 responses have been linked to disease pathogenesis in lupus. A dissociation between autoantibody titres and renal pathology has also been observed in B cell-activating factor belonging to the TNF family (BAFF)-deficient NZM mice, where IgG anti-dsDNA antibody titres remain high but disease is significantly diminished [51]. BAFF is known to drive B-cell responses, display accelerated disease compared with their unmanipulated NZM littermates, despite having greatly reduced anti-dsDNA antibodies [50]. This study not only demonstrates the lack of association of anti-DNA titre and disease, but also that the Th1/Th2 paradigm cannot be used simply to explain the pathogenesis of lupus disease. In particular, cytokines attributed to both Th1 and Th2 responses have been linked to disease pathogenesis in lupus. A dissociation between autoantibody titres and renal pathology has also been observed in B cell-activating factor belonging to the TNF family (BAFF)-deficient NZM mice, where IgG anti-dsDNA antibody titres remain high but disease is significantly diminished [51]. BAFF is known to drive B-cell responses, and when overexpressed leads to lupus like autoimmunity [52]. The relative lack of disease in the BAFF-deficient NZM mice despite significant amounts of antibody deposited in the kidney may be due to a switch in the isotype from IgG2a to IgG1. IgG1 anti-DNA antibodies are considered to be less pathogenic than IgG2a, since the former are much less efficient at activating Fc receptors and complement [53].

**Tissue targeting by anti-dsDNA antibodies**

Over the past 40 yrs, various theories have been proposed to explain how antibodies to DNA come to deposit in the kidney. It was thought, for example, that circulating antibody/DNA complexes could become passively trapped within the glomerulus. This theory now receives little support, firstly because concentrations of circulating immune complexes in patients with lupus are relatively low, and secondly as it has been hard to demonstrate the presence of antibodies to DNA in these complexes [54].

More recent suggestions include the possibility that antibodies to DNA are not, in reality, binding naked DNA, but rather to the nucleosome which consists of DNA coiled round an octamer of histone proteins (pairs of H2A, H2B, H3 and H4). The histone H1 protein acts as the link between the coils. In support of this notion nucleosomes, rather than free DNA, have been identified in the circulation of patients with lupus [55, 56]. The work of Anthony Rosen and his colleagues [57] has shown clearly that nucleosomes are released during apoptosis. Herrmann et al. [58] have demonstrated that there is impaired uptake of apoptotic cells into macrophages in patients with lupus providing a mechanism by which anti-nucleosome/DNA antibodies may be produced. Mechanistically, it is proposed that once anti-nucleosome antibodies have formed in the circulation the histone component, being positively charged, binds to the negatively charged components of the glomerular basement membrane so that the nucleosome in effect acts as a bridge linking the antibodies and the renal tissue. This hypothesis was tested using a rat kidney model [59]. Monoclonal antibodies were stringently purified to ensure removal of any nucleosomal antigen. Renal perfusion of antibodies in this non-complexed form demonstrated no glomerular binding. In contrast, perfusion of antibodies complexed to nucleosomal material resulted in the deposition of antibody in the glomerular capillaries and activation of complement. More recently, accurate electron microscopy studies by Kalaaji et al. [60] showed that autoantibodies in lupus-prone NZB/W F1 mice tend to localize in electron dense deposits also containing chromatin.

A more recent, and currently popular, additional hypothesis explores the notion that pathogenicity of anti-dsDNA antibodies might depend on their potential polyreactivity. Amongst the various constituents of the glomerular basement membrane and the glomerular epithelial cell or podocyte. It seems plausible that the precise target for antibodies to DNA within the kidney may help to explain the different histological appearances. For example patients with sub-endothelial antibody deposition may have the proliferative changes associated with a WHO grade IV appearance [61] whilst others with sub-epithelial deposition are linked to the membranous appearances of WHO grade V [62].

There has been much recent interest in cross-reactivity between antibodies to DNA and the actin-associated protein, alpha-actinin (α-actinin). The importance of α-actinin in normal renal function was established following the discovery that point mutations in the gene for α-actinin 4 (the only form of α-actinin expressed in human kidneys) were responsible for some forms of familial focal, segmental glomerulosclerosis [63].

Eilat and colleagues [63] demonstrated in their murine studies that nephritogenicity was determined by the ability of monoclonal anti-dsDNA antibodies to cross react with α-actinin. Mason et al. [64] affinity purified anti-dsDNA antibodies from patients with SLE and showed that cross-reactivity with α-actinin was most commonly observed in patients with lupus nephritis rather than those who lacked renal disease. Zhao et al. [65] strengthened the argument for the importance of the cross reaction by demonstrating an association between antibody affinity for α-actinin and binding to glomeruli in vitro. Renaudineau et al. [66] in a study of 100 SLE patients also reported that high-avidity anti-dsDNA antibodies cross-react with α-actinin, and are associated with renal disease.

**What is the mechanism by which anti-dsDNA antibodies cause pathology?**

As is evident from above, experimental evidence and clinical association strongly support the view that antibodies to dsDNA and/or nucleosomes do have pathogenic capacity. However, the precise mechanism by which the presence of these antibodies actually causes tissue inflammation and damage remains uncertain. In a small number of cases, it has been possible to show that murine and human anti-dsDNA antibodies can penetrate living cells [42, 67, 68]. For some of these antibodies, the in vivo penetrating process appears to be antibody Fab dependent.

Other possible mechanisms of ds-DNA antibody pathogenicity are likely to be dependent upon the recruitment of inflammatory cells via the IgG Fc receptors and the activation of complement. The complexities linked to both of these possible mechanisms have been reviewed recently [69]. The role of complement is particularly intriguing. On the one hand, active disease is clearly associated with complement consumption and complement deposition is a hallmark of lupus nephritis [70]. On the other hand, however, it has been established that genetic deficiencies of the early components of the complement system e.g. Clq, C1r, C1s, C2 are associated with a lupus like disease [71]. Recent analyses linking inherited deficiency of Clq with the development of lupus have focused on the use of a homozygous Clq knockout mouse [72]. Intriguingly, this mouse has a propensity to develop both autoantibodies and...
Fifty years of anti-ds DNA antibodies

glomerulonephritis and shows an impaired clearance of apoptotic debris similar to that noted in the studies of Herrmann and colleagues [8] referred to above.

In conclusion

During the 50 yrs since anti-dsDNA antibodies were identified in lupus, many clinical correlations between their presence and disease activity have been demonstrated and there are clearly strong reasons for believing that these antibodies, or the linked anti-nucleosome antibodies, may in some instances be involved in the pathogenesis of the disease. With the appreciation that apoptosis causes a ‘turning inside out’ of the cell contents, a plausible mechanism, linked to the failure of apoptotic material to be removed efficiently, helps to explain the reasons why antibodies are formed against structures usually buried deep within the cell. Several potential mechanisms have been proposed to explain the binding of anti-dsDNA/nucleosome antibodies to renal antigens, but it is to be hoped that in the next decade that much more work will focus on the precise mechanism by which the deposition of antibodies to DNA/nucleosomes in the kidney is actually associated with the development of disease in that organ. Fifty years after their discovery we have not reached journey’s end with the treatment of SLE.

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References