Commentary

Is closer linkage between systemic lupus erythematosus and anti-double-stranded DNA antibodies a desirable and attainable goal?

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Published: 10 February 2005

Arthritis Res Ther 2005, **7**:85-87 (DOI 10.1186/ar1707)

Abstract

The anti-double-stranded DNA (anti-dsDNA) antibody test incorporated in the 1982 revised American College of Rheumatology criteria for the classification of systemic lupus erythematosus needs updating to reflect current insights and technical achievements, including allowance for the presence of nonpathogenic anti-dsDNA antibodies. As we need to develop at least some measure of pathogenicity of anti-dsDNA antibodies, we propose that initial anti-dsDNA antibody screening is done by sensitive ELISA and supplemented by more stringent assays. Simultaneously the relevance of anti-dsDNA antibody presence needs to be restricted to clinical manifestations, thought to be caused by anti-dsDNA antibody and within an appropriate time frame.

Introduction

After descriptions of organ involvement in patients with archetypal lupus erythematosus skin lesions, and development of the concept of systemic lupus erythematosus (SLE) as a collagen vascular disease [1,2], a collaborative effort in the USA developed preliminary SLE classification criteria for interseries and epidemiologic evaluation. Retrospectively, the cumulative presence of four or more criteria over a 7-year period correctly classified patients with 88% sensitivity and 95% specificity [3]. In 1982 the earlier autoimmune features (lupus erythematosus cells or false positive test for syphilis) were expanded with fluorescent antinuclear antibodies and serum antibodies against DNA and/or Smith (Sm) antigen in a revised set of criteria [4]. This revision, again based on retrospective statistical associations, has had a huge impact on practical and theoretical considerations of SLE. Although reappraisal of the whole criteria set for SLE is currently being discussed [5], we focus here on the flawed relationship between serum anti-double-stranded DNA (anti-dsDNA) antibodies and SLE. Both B cell and T cell autoimmunity to dsDNA and/or nucleosomes are not confined to SLE, as shown by the specificity of anti-dsDNA antibody-inducing T cells for autologous (that is, non-infectious self) or infectious non-self DNA-binding proteins [6,7] as well as the finding that the potential of DNA-specific B cells to expand clonally and affinity mature towards dsDNA is an inherent property of the immune system of non-diseased individuals [8]. The initiation of IgG anti-DNA antibody production is not itself unique to SLE, as shown by findings especially in single-gene-aberration infections and also drug treatments (which generally induce only transient antibodies of the IgM isotype with tumor necrosis factor-α-blocking agents as the most recent example), as discussed recently [9]. To obtain a closer link between SLE pathophysiology and autoimmunity to DNA and/or nucleosomes, we need to make allowances for pathophysiologically irrelevant anti-dsDNA antibodies in our current empirical approach to anti-dsDNA testing.

The 1982 revised American College of Rheumatology (ACR) classification criteria and anti-dsDNA antibody detection

After a cluster analysis of 30 disease variables in 177 patients from 18 US clinics, 96% of SLE patients fulfilled – over time – at least four criteria, compared with 4% of 166 patients with predominantly chronic polyarthritis [4]. This ability to discriminate retrospectively between SLE and polyarthritis patients has evolved into the understanding that the criteria are useful for diagnosing SLE in general, although this has never been substantiated. Half of the patients in the ACR cohort did not fulfill the criteria at clinical diagnosis and would nowadays be classified (and probably treated) as undifferentiated/lupus-like autoimmune disease. Anti-dsDNA antibodies were detected (by local laboratories at undisclosed time points) in 113 of 166

ACR = American College of Rheumatology; ANA = antinuclear antibodies; dsDNA = double-stranded DNA; ELISA = enzyme-linked immunosorbent assay; SLE = systemic lupus erythematosus; Sm = Smith.

(67%) SLE patients and 7 of 91 (8%) control patients, also resulting in low positive (8) and negative (0.4) likelihood ratios for SLE with anti-dsDNA antibody testing, as confirmed by more recent data [10]. A basic flaw with this criteria set is the 'two for the price of one' principle: the presence of either anti-dsDNA or anti-Sm antibody results in the fulfillment of two criteria, namely criteria 10 (anti-DNA/anti-Sm antibody) and 11 (a positive antinuclear antibodies (ANA) test, caused by the anti-DNA/anti-Sm antibody). When classifying patients in the clinic or in studies, the presence of anti-dsDNA (or anti-Sm antibodies) should therefore eliminate the use of ANA as a criterion; this will require more clinical features to be present for SLE classification, and this relatively simple alteration might significantly alter the face of SLE as we know it today.

The broad definition of 'antibody to native DNA in an abnormal titer' [4] reflects technical standards more than 30 years old and has allowed an outgrowth of methods for anti-dsDNA antibody detection. All current assays detect anti-dsDNA antibodies with divergent properties in terms of avidity, structural specificity and clinical associations [11]. If the anti-dsDNA antibody criterion in SLE classification is to remain useful, its definition should represent current trends and insights. Head-to-head comparisons of the various assays in contemporary, unselected, multinational cohorts of patients with new-onset disease are needed to determine the specificity and sensitivity (which seem to be inversely related) of various anti-dsDNA antibody assays for SLE, while focusing on the organ specificity of anti-dsDNA antibody-mediated injury.

Anti-dsDNA antibodies and lupus pathophysiology

Anti-dsDNA antibodies and SLE pathophysiology are currently quite loosely connected in both classification and clinical practice. This hampers the study of the correlation of anti-dsDNA antibodies and effects on organs in SLE, because statistical associations cannot substitute for specific anti-dsDNA antibody-mediated pathophysiological processes. Because anti-dsDNA antibodies can be eluted from diseased experimental and human lupus kidneys and are present in patient sera during proliferative lupus nephritis, they are likely to be involved in the development of lupus nephritis [12,13]. Aside from the weak correlation with skin disease activity in patients with discoid and acute cutaneous lupus erythematosus [14], there is little evidence that anti-dsDNA antibodies are pathophysiologically involved in other clinical manifestations. Recent findings that intrathecal binding of anti-dsDNA antibodies to the NR2 glutamate receptor induces apoptotic neuronal death must be confirmed in patient cohorts [15]. Assuming that antibodies detected in serum truthfully reflect the process *in situ*, we therefore need assays that can measure pathogenicity, avidity or specificity for local DNA structures or substructures. However, anti-dsDNA antibodies might be present in sera for many years before the development of experimental and human lupus [16,17], and serum anti-dsDNA antibodies can also be detected by various techniques for a prolonged period in patients with quiescent established SLE [18]. If one accepts that this autoimmunity nonetheless relates to pathophysiological events, there is an obvious need to determine when and why the switch from nonpathogenic and clinically irrelevant to pathogenic and clinically relevant antibodies occurs. If anti-DNA antibody pathogenicity is determined by the interaction between antibody and tissue-specific antigens, the selection principle and/or the organ-specific binding properties induced by local modifications of anti-DNA antibodies must be determined [19,20].

At present, a timely relationship between the presence of anti-dsDNA antibodies and clinical manifestations is not required in SLE classification and diagnosis. For example, the previous presence of anti-dsDNA antibodies (by any assay) and accordingly a positive ANA test will qualify a patient with a UV-sensitive rash for SLE classification and diagnosis, whereas clinical manifestations do not coincide with anti-dsDNA antibody detection. Monthly increases in anti-dsDNA antibody levels (Farr assay) to guide preventive therapy in European lupus patients significantly reduced the number of relapses as well as the number of patients requiring cytotoxic agents [21]. Thus, restricting the time span for relevant (in the context of their potential to induce pathology) anti-dsDNA antibodies to a defined time period (such as 4 to 6 weeks, as suggested on the basis of clinical evidence [22]) before the occurrence of renal and skin lesions in the study and follow-up of SLE patients will provide a stronger framework for the study of the role of anti-dsDNA antibodies in SLE.

Anti-dsDNA antibodies assays

We currently lack a clear strategy for evaluating the development of pathogenic anti-dsDNA antibodies. Although we recognize the limits of our knowledge on the structural specificities and avidities, affinity maturation and clinical associations of anti-dsDNA antibodies, the following provisional two-step strategy for both diagnosis and follow-up seems reasonable. Screening with the sensitive ELISA assay detects most anti-dsDNA antibodies irrespective of pathogenic impact [23], and following-up positive ELISA results by more stringent assays (*Crithidia luciliae* immunofluorescence, Farr assay with circular dsDNA as antigen, EliA anti-dsDNA assays or solution-phase ELISA) will determine the presence of potentially more pathogenic anti-dsDNA antibodies [11,24]. Limitations notwithstanding, this test strategy might especially aid clinicians to determine whether SLE patients suffer from cool ('benign') lupus, with mainly nonpathogenic anti-dsDNA antibodies present, or hot ('malignant') lupus, in which high-avidity anti-dsDNA

antibodies may mediate end-organ dysfunction. This strategy follows practical developments in which economic considerations have forced the replacement of other anti-dsDNA assays with ELISA testing. Unlike the consensus-based 1997 update of ACR criteria for SLE classification [25], officially redefining the methodology and clinical relevance of anti-dsDNA antibody profiles in SLE classification and diagnosis will require formal testing in unselected cohorts. Such a practically and intellectually challenging undertaking should provide an answer to the contemporary dilemma of whether the presence of anti-dsDNA antibodies in any given assay should be considered relevant only when concomitant with nephritis or dermatitis.

Conclusions

The anti-dsDNA antibody test incorporated in the 1982 revised ACR criteria for SLE classification needs updating. ELISA screening (for lower avidity anti-dsDNA antibodies) followed by risk stratification through the use of more stringent assays (Farr, EliA, *Crithidia*) and restricting the relevance of anti-dsDNA antibody presence to specific clinical manifestations might provide us with a better index of the pathogenicity of anti-dsDNA antibodies. Furthermore, in the presence of anti-dsDNA antibodies ANA should no longer be considered a valid classification criterion. This strategy might ultimately facilitate the differentiation between SLE patients with benign disease variants and those with the classical syndrome of severe skin and renal disease in the presence of pathogenic anti-dsDNA autoantibodies.

Competing interests

Both HCN and OPR have received speaker/travel fees from Pharmacia Norway AS, which produces an ELISA assay for the detection of anti-dsDNA Ab.

References

1. Kaposi M: **Neue Beitrage zur Kenntnis des Lupus Erythematosus.** *Arch Dermatol Syphil* 1872, **4:**36-78.
2. Klemperer P, Pollack AD, Baehr G: **The pathology of disseminated lupus erythematosus.** *Arch Pathol* 1941, **32:**569-631.
3. Cohen AS, Reynolds WE, Franklin EC, Knlka JP, Ropes MW, Shulman LE, Wallace SL: **Preliminary criteria for the classification of systemic lupus erythematosus.** *Bull Rheum Dis* 1971, **21:**643-648.
4. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ: **The 1982 revised criteria for the classification of systemic lupus erythematosus.** *Arthritis Rheum* 1982, **25:**1271-1277.
5. Petri M, Magder L: **Classification criteria for systemic lupus erythematosus: a review.** *Lupus* 2004, **13:**829-837.
6. Rekvig OP, Andreassen K, Moens U: **Antibodies to DNA – towards an understanding of their origin and pathophysiological impact in systemic lupus erythematosus.** *Scand J Rheumatol* 1998, **27:**1-6.
7. Kaliyaperumal A, Mohan C, Wu W, Datta SK: **Nucleosomal peptide epitopes for nephritis-inducing T helper cells of murine lupus.** *J Exp Med* 1996, **183:**2459-2469.
8. Chen C, Nagy Z, Radic MZ, Hardy RR, Huszar D, Camper SA, Weigert M: **The site and stage of anti-DNA B-cell deletion.** *Nature* 1995, **373:**252-255.
9. Rekvig OP, Nossent JC: **Anti-double-stranded DNA antibodies, nucleosomes, and systemic lupus erythematosus: a time for new paradigms?** *Arthritis Rheum* 2003, **48:**300-312.
10. Kavanaugh A: **The utility of immunologic laboratory tests in patients with rheumatic diseases.** *Arthritis Rheum* 2001, **44:**2221-2223.
11. Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP: **Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity.** *Ann Rheum Dis* 2004. **63:**386-394.
12. Winfield JB, Faiferman I, Koffler D: **Avidity of anti-DNA antibodies in serum and IgG glomerular eluates from patients with systemic lupus erythematosus. Association of high avidity antinative DNA antibody with glomerulonephritis.** *J Clin Invest* 1977, **59:**90-96.
13. Nossent HC, Henzen-Logmans SC, Vroom TM, Berden JH, Swaak TJ: **Contribution of renal biopsy data in predicting outcome in lupus nephritis. Analysis of 116 patients.** *Arthritis Rheum* 1990, **33:**970-977.
14. Crowson AN, Magro C: **The cutaneous pathology of lupus erythematosus: a review.** *J Cutan Pathol* 2001, **28:**1-23.
15. DeGiorgio LA, Konstantinov KN, Lee SC, Hardin JA, Volpe BT, Diamond B: **A subset of lupus anti-DNA antibodies cross-reacts with the NR2 glutamate receptor in systemic lupus erythematosus.** *Nat Med* 2001, **7:**1189-1193.
16. Hahn BH: **Antibodies to DNA.** *N Engl J Med* 1998, **338:**1359-1368.
17. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, Harley JB: **Development of autoantibodies before the clinical onset of systemic lupus erythematosus.** *N Engl J Med* 2003, **349:**1526-1533.
18. Isenberg DA, Ravirajan CT, Rahman A, Kalsi J: **The role of antibodies to DNA in systemic lupus erythematosus – a review and introduction to an international workshop on DNA antibodies held in London, May 1996.** *Lupus* 1997, **6:**290-304.
19. Li J, Fernandez L, O'Connor KC, Imanishi-Kari T, Stollar BD: **The rearranged V(H) domain of a physiologically selected anti-single-stranded DNA antibody as a precursor for formation of IgM and IgG antibodies to diverse antigens.** *J Immunol* 2001, **167:**3746-3755.
20. Jang YJ, Stollar BD: **Anti-DNA antibodies: aspects of structure and pathogenicity.** *Cell Mol Life Sci* 2003, **60:**309-320.
21. Bootsma H, Spronk P, Derksen R, de Boer G, Wolters-Dicke H, Hermans J, Limburg P, Gmelig-Meyling F, Kater L, Kallenberg C: **Prevention of relapses in systemic lupus erythematosus.** *Lancet* 1995, **345:**1595-1599.
22. Swaak AJ, Smeenk RJ: **Following the disease course in systemic lupus erythematosus: are serologic variables of any use?** *J Rheumatol* 1996, **23:**1842-1844.
23. Fritzler MJ, Wiik A, Tan EM, Smolen JS, McDougal JS, Chan EK, Gordon TP, Hardin JA, Kalden JR, Lahita RG, *et al.*: **A critical evaluation of enzyme immunoassay kits for detection of antinuclear autoantibodies of defined specificities. III. Comparative performance characteristics of academic and manufacturers' laboratories.** *J Rheumatol* 2003, **30:**2374-2381.
24. Derksen RH, Bast EJ, Strooisma T, Jacobs JW: **A comparison between the Farr radioimmunoassay and a new automated fluorescence immunoassay for the detection of antibodies against double stranded DNA in serum.** *Ann Rheum Dis* 2002, **61:**1099-1102.
25. Hochberg MC: **Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus.** *Arthritis Rheum* 1997, **40:**1725.