The association of lupus anticoagulant and anti-DNA binding in patients with systemic lupus erythematosus[1]

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A study was undertaken to ascertain the association of lupus anticoagulant and anti-DNA binding in patients with systemic lupus erythematosus (SLE). The sera of 67 patients who had positive antinuclear factor were studied to determine whether antibodies to DNA are associated with lupus anticoagulant and if so whether anticoagulant and anti-DNA properties reside on the same immunoglobulin. Binding to native DNA was determined by a modified Farr's assay, and lupus anticoagulant activity was determined by modified kaolin-activated partial thromboplastin time. Of 67 patients, 14 had positive findings for circulating anticoagulant, 37 had antibodies to DNA, and 30 did not. All 14 patients who had circulating anticoagulants had anti-DNA, and conversely, no patient without DNA binding had a lupus anticoagulant. Furthermore, absorption of the test sera with the phospholipid phosphatidyl serine was capable of removing DNA binding in every sera demonstrating lupus anticoagulant and anti-DNA activity. Although it is not clear whether anti-DNA binding and lupus anticoagulant activity coexist in the same immunoglobulin in SLE, lupus anticoagulant and anti-DNA showed a strong positive association in patients with SLE.

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One of the numerous antibodies expressed in systemic lupus erythematosus (SLE) is an inhibitor of phospholipid-dependent coagulation commonly known as *lupus anticoagulant.* This antibody is found in approximately 10% of patients with SLE,[1] though prevalence rates as high as 70% have been reported using more sensitive tests.[2] It is also

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Table. Findings in patients with systemic lupus erythematosus

No. of patients	67
CAC+*	14/67
DNA+†	37/67
DNA+/CAC+	14/14
DNA−/CAC+	0/14

* *CAC* = circulating anticoagulant.
† *DNA* = anti-DNA.

found in patients with other autoimmune disorders, women with recurrent spontaneous abortion, and individuals with no apparent disease. Paradoxically, the antibody is associated with an increased risk of venous and arterial thrombosis rather than bleeding.[3] Although the exact nature of the circulating anticoagulant is not known, the fact that it exhibits in vitro cross-reactivity with cardiolipin (diphosphatidyl glycerol) may explain its association with false-positive serologic tests for syphilis. Using hybridomas, Lafer et al[4] have demonstrated that monoclonal antibodies to native DNA not only react against n-DNA but may cross-react with a variety of phospholipids and also act as anticoagulants in vitro. In addition, they demonstrated that some anti-DNA monoclonal antibodies produced from MRL/1 mice prolong the activated partial thromboplastin time (APTT) in a manner analogous to lupus anticoagulant. Phospholipids used to evaluate the APTT appeared to interfere with interaction between these monoclonal antibodies and native DNA. However, the clinical relevance of these observations is unclear. Antibodies to native DNA found in the blood of lupus patients are a heterogeneous group,[5] with various subpopulations having been characterized by various methods, including immunoglobulin class, complement-fixing ability, avidity, and rates of association with DNA. The site of DNA association could be either particular nucleotides or the phosphodiester backbone of the DNA helix. It has been suggested that the phosphodiester linkage of DNA is structurally similar to the glycerolphosphate component of phospholipids and that antibodies might cross-react with these two compounds.[4] This antibody cross-reactivity to a variety of phospholipids could explain the association between anti-DNA, a circulating anticoagulant, and a false-positive serologic test for syphilis. Evidence for the association of DNA binding and anticoagulant activity in clinical blood samples would therefore be of great interest in understanding the pathogenesis of complications in SLE. This study was designed to ascertain whether these heterogeneous antibodies to DNA are associated with a lupus anticoagulant, and if so, whether the anticoagulant and anti-DNA properties reside on the same immunoglobulin.

Materials and methods

Between January and March 1983, all sera with a positive antinuclear factor submitted for routine clinical determination of anti-DNA at the Cleveland Clinic were selected for study. If enough was available, the charts were reviewed to see whether the diagnosis of SLE was made by an attending rheumatologist in accord with the criteria of the American Rheumatism Association.[6] We did not use sera from patients receiving an anticoagulant, such as those undergoing plasmapheresis at the time of collection.

Binding to native DNA was determined by a modified Farr's assay.[7] Determination of lupus anticoagulant activity was based on a modified kaolin-activated partial thromboplastin time (k-APTT) using a mixture of 0.2 mL of patient serum and 0.2 mL of pooled normal plasma. The mixture was incubated for 30 minutes at 37 °C with 0.4 mL of Thrombofax (Ortho) diluted 1:64 in 0.1 M tris buffer (pH, 7.6), after which a 0.2-mL aliquot was incubated with 0.1 mL of kaolin (20 mg/mL) for 25 minutes at 37 °C and recalcified with 0.1 mL of 0.02 M $CaCl_2$. The interval between addition of $CaCl_2$ and clot formation was compared with the mean of 46.1 seconds ± 1.8 S.D. obtained from 40 normal volunteers, and any values at least 10 seconds greater than the mean (> 4 S.D.) were considered evidence of lupus anticoagulant activity. Absorption of native DNA was promoted by incubating the sera together with formalin-treated human erythrocytes coated with either gelatin (controls) or native DNA. Aqueous suspensions of phosphatidyl serine (Sigma, supplied in $CHCl_3$) were prepared by drying the lipid under nitrogen and resuspending it in distilled water to a final concentration of 1.7 M.

Results

Of 67 patients tested, 14 were positive for circulating anticoagulant; 37 had antibodies to DNA and 30 did not (*Table*). All 14 patients who had circulating anticoagulant also had anti-

DNA, and conversely, no patient without DNA binding had lupus anticoagulant. Thus lupus anticoagulant had a strong positive association with antibodies to native DNA ($P < 0.002$). Bound DNA was absorbed from the sera along with formalin-treated erythrocytes coated with native DNA obtained from a calf thymus. Of the 10 patients studied, binding activity was reduced by an average of 86%, while the average decrease in partial thromboplastin time was only 3%. When phosphatidyl serine was added to sera from 8 patients with positive DNA binding, the binding capacity was eliminated in all 5 patients who had circulating anticoagulant; in the other 3 patients, DNA binding was not significantly reduced after addition of phospholipid. This difference was statistically significant according to Fisher's exact test ($P < 0.05$).

A number of potential clinical and laboratory associations with lupus anticoagulant were examined by chi-square analysis. None attained the preestablished goal of $P < 0.05$ for association with lupus anticoagulant; however, false-positive tests for syphilis, vasculitis, and renal disease did come close.

Discussion

Using the k-APTT, we found that the prevalence of lupus anticoagulant was just over 20% in sera obtained from 67 SLE patients. Although this is slightly higher than accepted levels of 10% to 15%,[1] it is lower than the 70% found by Exner et al[2] using the more sensitive diluted kaolin clotting time. Lupus anticoagulant was found to have a strong positive association with antibodies to native DNA.

The fact that bound DNA was successfully absorbed whereas there was no reduction in partial thromboplastin time suggests that DNA binding and anticoagulant activity reside on different immunoglobulins. Another possible explanation is that the k-APTT is relatively more sensitive as a functional assay for anticoagulant activity than the modified Farr method is as a quantitative assay for DNA antibody. It was of interest that only patients with lupus anticoagulant activity demonstrated a reaction between anti-DNA and the phospholipid phosphatidyl serine. This is believed to be one of the major phospholipids in the platelet membrane, which contributes to platelet factor 3 activity.[8,9]

Koike et al[10] have demonstrated that monoclonal hybridoma antibodies to DNA can be divided into three groups: (*a*) those that react exclusively with single-stranded DNA, (*b*) those that react primarily with double-stranded DNA, and (*c*) those that react with both. They further demonstrated that the polyspecific DNA antibodies in SLE sera which react with both single- and double-stranded DNA also cross-react with the phospholipid cardiolipin.

Inasmuch as other studies[11,12] have failed to show an association between DNA binding and anticardiolipin antibodies, our finding of a strong positive association between DNA binding and lupus anticoagulant activity in SLE requires some explanation. There are several possibilities:

1. There is some controversy as to which technique is most sensitive to dilution of lupus anticoagulant. The diluted kaolin clotting time described by Exner et al[2] may actually be more sensitive, though it is unclear how its use might have altered the results of our study.

2. Though the crithidial technique for detecting anti-DNA antibodies has been used, the Farr's assay used in our study is a more sensitive indicator of titers against both single- and double-stranded DNA.

3. Phosphatidyl serine is more important than cardiolipin, both in the expression of platelet factor 3 activity in vivo[9,10] and in the reagents used for assay of partial thomboplastin time. Shapiro et al[13] have clearly demonstrated the importance of phosphatidyl serine in the behavior of lupus anticoagulant in a patient with macroglobulinemia. Thus it is possible that studies which fail to show an association between DNA binding and either anticardiolipin antibodies or lupus anticoagulant activity may not have used the optimum assays for DNA binding or anticoagulant activity. In addition, they may have focused on phospholipids which are inappropriate to coagulation.

Conclusion

Our data demonstrate a strong association between lupus anticoagulant and antibodies to DNA in SLE sera. While they do not answer the question of whether DNA binding and lupus anticoagulant activity coexist in the same immunoglobulin in SLE, they do suggest a close association that deserves further investigation.

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