



Anticardiolipin antibodies in systemic lupus erythematosus

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■ Forty-seven patients with systemic lupus erythematosus were studied to determine the presence of IgM and IgG anticardiolipin antibodies and their association with clinical syndromes. Eleven (23.4%) of the patients had IgG anticardiolipin antibodies; four of these also had IgM anticardiolipin antibodies. The cardiolipin-positive group had significantly lower platelet counts, but no increased history of thrombosis was observed. The anticardiolipin activity of seven of the 11 sera could be decreased by preincubation with DNA, providing confirmatory evidence that anticardiolipin activity actually resides in some populations of anti-DNA.

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ANTIBODIES against negatively charged phospholipids (cardiolipin, phosphatidylserine, and phosphatidylinositol) have been reported in systemic lupus erythematosus (SLE) and other autoimmune disorders.^{1,2} These antibodies have been found with a frequency of 23% to 42% in SLE, 33% in rheumatoid arthritis, 28% in psoriatic arthritis, and 18% in Behçet's disease.^{1,3,4} In the setting of autoimmune disease, anticardiolipin (ACL) antibodies have been reported to correlate with venous and arterial thrombosis, thrombocytopenia, recurrent fetal loss, chorea, and livedo reticularis.⁵⁻¹¹ ACL antibodies have also been detected in essentially healthy women with unexplained habitual abortions,¹² in patients experiencing late occlusion of coronary artery bypass grafts,¹³ and transiently in the setting of streptococcal pharyngitis.

The presence of ACL antibodies has also been reported in a patient with a thymoma and recurrent strokes¹⁴ and in Degos' disease, an affliction characterized by thrombotic infarctions of the skin, the central nervous system, and the gastrointestinal tract.¹⁵

The exact nature of ACL antibodies is unknown. They are associated with false-positive syphilis serology, most likely through interaction with the cardiolipin-lecithin-cholesterol substrate of commonly used assays for this.⁴

The structure of cardiolipin bears a striking resemblance to the diphosphoglyceride backbone of polynucleotides. Not unexpectedly, monoclonal mouse and human-human hybridomas produce antibodies that react with native DNA, left-handed Z DNA, polyI, and cardiolipin.¹⁶⁻¹⁸ BALB/c mice immunized with cardiolipin produce both ACL antibodies and anti-DNA. Hybridomas from these mice produce monoclonal ACL antibodies that share idiotypic determinants with the anti-DNA of lupus-prone MRL-lpr/lpr mice.¹⁷

We studied 47 SLE patients to determine the prevalence of IgM and IgG ACL antibodies and their association with clinical syndromes. Absorption studies were

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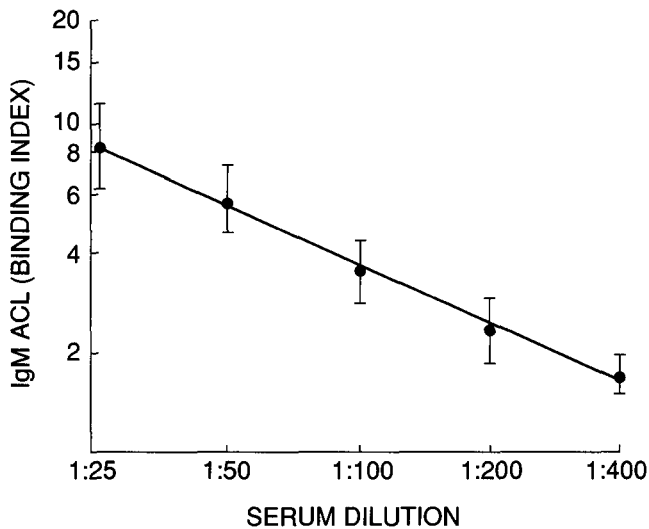


FIGURE 1. Regression curve for serial dilutions of IgM anticardiolipin standard serum.

performed to delineate the relationship between anti-DNA and ACL antibodies.

MATERIALS AND METHODS

Between July and October 1987, all sera submitted for the LE monitor (a battery of tests including a complete blood count, serum creatinine, C4, Westergren sedimentation rate, anti-DNA, and urinalysis) at the Cleveland Clinic were studied. 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 Rheumatology Association.¹⁹ Control sera were obtained from laboratory personnel.

ACL antibodies were measured by a modification of the method of Loizou et al.²⁰ Briefly, each well of a polystyrene plate (Dynatech Laboratories, Alexandria, VA) was coated with cardiolipin in ethanol (Sigma, St. Louis, MO) 45 µg/mL, 30 µL/well, by evaporation under a laminar air flow hood. Then 200 µL of 1% gelatin in FTA was added to each well, and the preparation was incubated for two hours at room temperature to block nonspecific binding. After four washes in 0.1% gelatin-FTA solution, 100 µL of test serum diluted 1:10 in gelatin-FTA solution was pipetted into each well. Each sample was tested in triplicate. After four more washes, 100 µL of goat antihuman IgG or IgM was added, and

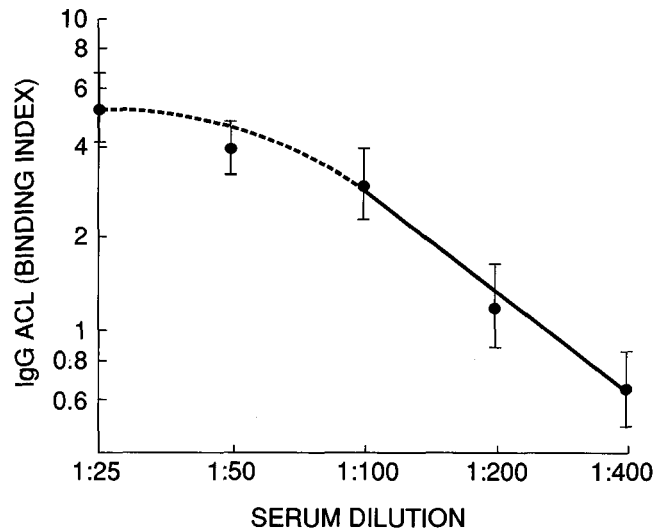


FIGURE 2. Regression curve for serial dilutions of IgG anticardiolipin standard serum.

this was incubated for one hour. This was followed by 100 µL of rabbit anti-goat Ig-alkaline phosphatase conjugate (Sigma). After another hour, the wells were washed and 100 µL of p-nitrophenyl phosphate (Sigma), 1 mg/mL in diethanolamine buffer, pH 9.8, was added. The color reaction was stopped by the addition of 50 µL 10M NaOH. The optical absorbance (OA) at 405 nm was read using a Microplate Reader (Biotek Instruments). Results were expressed as a binding index, defined as: $OA_{\text{sample}} - OA_{\text{blank}} / OA_{\text{normal}} - OA_{\text{blank}}$.

The upper limits of normal for the binding indices were defined as mean plus two standard deviations of a group of 34 normal sera. The upper limit was 2.09 for IgM ACL antibodies, and 1.83 for IgG ACL antibodies. The regression curves for serial dilutions of IgG and IgM standards are shown in Figures 1 and 2. The correlation coefficient for the IgM curve is 0.99; that for the IgG curve is 0.99 between serum dilutions of 1:100 to 1:400.

Anti-DNA antibodies were measured by a modified Farr assay, detailed elsewhere by Clough et al.²¹

For the inhibition studies, test and control sera with 45 µg/mL and 90 µg/mL of inhibitor (either DNA or cardiolipin) were incubated for one hour at 37°C, then overnight at 4°C the day before performance of the respective assays.

Clinical data were obtained by chart review. Thrombotic events were defined as pulmonary embolism docu-

TABLE 1
CORRELATION OF ANTICARDIOLIPIN ANTIBODIES WITH SEROLOGIC AND CLINICAL FINDINGS IN 47 PATIENTS WITH SLE

| | ACL-positive patients (n=11) | ACL-negative patients (n=36) | P |
|-----------------------------------|------------------------------|------------------------------|--------|
| Age (yr) | 38.33 + 11.62 | 35.50 + 17.19 | NS |
| Duration of disease (yr) | 6.89 + 4.88 | 6.79 + 6.88 | NS |
| History of thrombosis | 3 (37%) | 7 (19%) | NS |
| Positive antiDNA | 5 (45%) | 12 (33%) | NS |
| Hemoglobin (g/dL) | 12.71 + 1.61 | 12.93 + 1.99 | NS |
| WBC (x 10 ³ /μL) | 7.05 + 4.86 | 6.77 + 2.64 | NS |
| Platelets (x 10 ³ /μL) | 212.11 + 107.27 | 276.08 + 81.44 | 0.0428 |
| Serum creatinine (mg/dL) | 0.89 + 0.29 | 1.30 + 1.87 | NS |
| LACC | 2.30 + 1.16 | 2.91 + 1.06 | NS |

History of thrombosis and antiDNA are expressed as number of patients (percent of group); other values are expressed as mean + standard deviation. NS= not statistically significant.

TABLE 2
ABSORPTION STUDIES WITH DNA AND CARDIOLIPIN

| Patient Number | ACL | ACL after DNA incubation | AntiDNA | AntiDNA after CL incubation |
|----------------|------|--------------------------|---------|-----------------------------|
| 1 | 3.45 | 3.36 | 0 | 0 |
| 2 | 5.18 | 5.05 | 4.7 | 0 |
| 3 | 4.67 | 4.61 | 0 | 0 |
| 4 | 3.42 | 3.43 | 2.7 | 0 |
| 5 | 2.26 | 1.16 | 0 | 0 |
| 6 | 1.84 | 1.09 | 0 | 0 |
| 7 | 2.83 | 0.46 | 0 | 0 |
| 8 | 2.39 | 0.84 | 0 | 0 |
| 9 | 2.32 | 1.04 | 45.4 | 37.9 |
| 10 | 2.59 | 1.72 | 0 | 0 |
| 11 | 2.62 | 1.76 | 0 | 0 |

ACL is expressed as binding index (see text); AntiDNA is expressed as percent binding of radiolabelled DNA.

mented by ventilation/perfusion scanning or angiography, deep-vein thrombosis documented by peripheral venous recordings or angiography, or cerebral infarction. The lupus activity criteria count (LACC) was determined by the method of Urowitz et al.²² This system scores one point for each of the following: (a) arthritis, (b) lab abnormalities, namely LE cells, anti-DNA, leukopenia, low CH₅₀ or C3 levels, (c) rash, mucosal ulcers, or alopecia, (d) serositis, (e) seizures, psychosis, organic brain syndrome, or lupus headache, (f) vasculitis, and (g) hematuria. A score of 2 or greater represents active lupus, with the maximum achievable score being 7.

RESULTS

Anticardiolipin antibodies were present in 11 of 47 sera (23.4%). All 11 positive sera had IgG ACL antibodies, and four of these also had IgM ACL antibodies. No sample had IgM ACL antibodies alone.

The ACL-positive group did not differ from the

patients without ACL antibodies in age, sex, duration of disease, hemoglobin, leukocyte count, serum creatinine level, or disease activity as measured by the LACC (Table 1). The platelet count in the ACL-positive group was significantly lower (P=.043). History of thrombotic events was the same in both groups.

When incubated with DNA, the anticardiolipin activity of seven of the 11 positive sera decreased by greater than one standard deviation. When assayed for anti-DNA, however, only one of these sera had anti-DNA activity. This activity could be decreased by preincubation with cardiolipin (Table 2).

DISCUSSION

This study confirms the relative thrombocytopenia in the ACL-positive subset of SLE that has been reported by others.⁷ It has been suggested that anticardiolipin binds to the acidic phospholipids on platelet membranes, promoting aggregation, activation, and degrana-

lation, eventually leading to thrombocytopenia.²³

In this small group of patients, we did not find the reported increase in thrombotic events in the ACL-positive subgroup. It is reasonably likely that a significant difference between groups will be missed in the comparison of 3/11 (ACL-positive) v 7/36 (ACL-negative) subjects. Our results, however, are in accord with those of Sturfelt et al,²⁴ who failed to find a correlation between increased amounts of ACL antibodies and thrombosis in a group of 59 unselected SLE patients, 32 of whom were ACL-positive.

Harris et al²⁵ found little cross-reactivity between anti-DNA and ACL antibodies. On the other hand, Koike et al²⁶ demonstrated virtual elimination of anti-DNA activity by preincubating sera with increasing concentrations of cardiolipin. We observed ACL inhibition by DNA in 63% of our sera, suggesting that the

assay was detecting ACL antibodies of broad specificity. This inhibition was greatest when no anti-DNA activity could be detected by the Farr assay.

Smeenk et al²⁷ suggest that this divergence occurs because of the presence of populations of anti-DNA of differing substrate avidity. High-avidity anti-DNA (detected by the Farr assay) does not cross-react with cardiolipin; low-avidity anti-DNA (not detected by the Farr assay) shows marked cross-reactivity. Accordingly, the Farr-negative sera showed more anticardiolipin inhibition when preincubated with DNA.

Anticardiolipin antibodies define a relatively thrombocytopenic subset of SLE. We did not find an increase in the incidence of thrombotic events in the ACL-positive subgroup. Certain populations of anti-DNA cross-react with cardiolipin; these are mostly those that are undetectable by the routinely used Farr assay.

REFERENCES

- Fort JG, Cowchock FS, Abruzzo JL, Smith JB. Anticardiolipin antibodies in patients with rheumatic diseases. *Arthritis Rheum* 1987; **30**:752-760.
- Harris EN, Gharavi AE, Hughes GRV. Antiphospholipid antibodies. *Clin Rheum Dis* 1985; **11**:591-601.
- Hull RG, Harris EN, Gharavi AE, et al. Anticardiolipin antibodies: occurrence in Behçet's syndrome. *Ann Rheum Dis* 1984; **43**:746-748.
- Koike T, Sueishi M, Funaki H, Tomioka H, Yoshida S. Anti-phospholipid antibodies and biological false positive test for syphilis in patients with systemic lupus erythematosus. *Clin Exp Immunol* 1984; **56**:193-199.
- Harris EN, Gharavi AE, Asherson RA, Boey ML, Hughes GR. Cerebral infarction in systemic lupus: association with anticardiolipin antibodies. *Clin Exp Rheumatol* 1984; **2**:47-51.
- Hatron P-Y, Bouchez B, Wattel A, Arnott G, Devulder B. Chorea, systemic lupus erythematosus, circulating lupus anticoagulant (letter). *J Rheumatol* 1986; **13**:991-993.
- Harris EN, Chan JKH, Asherson RA, Aber VR, Gharavi AE, Hughes GRV. Thrombosis, recurrent fetal loss, and thrombocytopenia. *Arch Intern Med* 1986; **146**:2153-2156.
- Weinstein C, Miller MH, Axtens R, Buchanan R, Littlejohn GO. Livedo reticularis associated with increased titers of anticardiolipin antibodies in systemic lupus erythematosus. *Arch Dermatol* 1987; **123**:596-600.
- Cowchock S, Smith JB, Gocial B. Antibodies to phospholipids and nuclear antigens in patients with repeated abortions. *Am J Obstet Gynecol* 1986; **155**:1002-1010.
- Hedfors E, Lindahl G, Lindblad S. Anticardiolipin antibodies during pregnancy. *J Rheumatol* 1987; **14**:160-161.
- Levine SR, Welch KMA. The spectrum of neurologic disease associated with antiphospholipid antibodies: lupus anticoagulants and anticardiolipin antibodies. *Arch Neurol* 1987; **44**:876-883.
- Unander AM, Norberg R, Hahn L, Årfors L. Anticardiolipin antibodies and complement in ninety-nine women with habitual abortion. *Am J Obstet Gynecol* 1987; **156**:114-119.
- Morton KE, Gavaghan TP, Krilis SA, et al. Coronary artery bypass graft failure—an autoimmune phenomenon? *Lancet* 1986; **2**:1353-1356.
- Levine SR, Diaczok IM, Deegan MJ, et al. Recurrent stroke associated with thymoma and anticardiolipin antibodies. *Arch Neurol* 1987; **44**:678-679.
- Englert HJ, Hawkes CH, Boey ML, et al. Degos' disease: association with anticardiolipin antibodies and the lupus anticoagulant. *Br Med J* 1984; **289**:576.
- Schoenfeld Y, Rauch J, Massicotte H, et al. Polyspecificity of monoclonal lupus autoantibodies produced by human-human hybridomas. *N Engl J Med* 1983; **308**:414-420.
- Lafer EM, Rauch J, Andrzejewski C Jr, et al. Polyspecific monoclonal lupus autoantibodies reactive with both polynucleotides and phospholipids. *J Exp Med* 1981; **153**:897-909.
- Rauch J, Tannenbaum H, Stollar RD, Schwartz RS. Monoclonal anticardiolipin antibodies bind to DNA. *Eur J Immunol* 1984; **14**:529-534.
- Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; **25**:1271-1277.
- Loizou S, McCrea JD, Rudge AC, Reynolds R, Boyle CC, Harris EN. Measurement of anti-cardiolipin antibodies by an enzyme-linked immunosorbent assay (ELISA): standardization and quantitation of results. *Clin Exp Immunol* 1985; **62**:738-745.
- Clough JD, Couri J, Youssoufian H, Gephart GN, Tubbs R. Antibodies against nuclear antigens: association with lupus nephritis. *Cleve Clin Q* 1986; **53**:259-265.
- Urowitz MB, Gladman DD, Tozman ECS, Goldsmith CH. The Lupus Activity Criteria Count (LACC). *J Rheumatol* 1984; **11**:783-787.
- Colaco CB, Male DK. Anti-phospholipid antibodies in syphilis and a thrombotic subset of SLE: distinct profiles of epitope specificity. *Clin Exp Immunol* 1985; **59**:449-456.
- Sturfelt G, Nived O, Norberg R, Thorstenson R, Krook K. Anticardiolipin antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum* 1987; **30**:382-388.
- Harris EN, Gharavi AE, Loizou S, et al. Crossreactivity of antiphospholipid antibodies. *J Clin Lab Immunol* 1985; **16**:1-6.
- Koike T, Tomioka H, Kumagai A. Antibodies cross-reactive with DNA and cardiolipin in patients with systemic lupus erythematosus. *Clin Exp Immunol* 1982; **50**:298-302.
- Smeenk RJT, Lucassen WAM, Swaak TJG. Is anticardiolipin activity a cross-reaction of anti-DNA or a separate entity? *Arthritis Rheum* 1987; **30**:607-617.