Association between Anticardiolipin Antibody Positivity and Increased 17-Beta-Estradiol Levels in Premenopausal Women with Rheumatoid Arthritis[a]

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Autoimmune rheumatic diseases, such as rheumatoid arthritis (RA), are characterized by an increased and heterogeneous antibody production. Among these autoantibodies, antiphospholipid (aPL) antibodies, in particular anticardiolipin (aCL) antibodies and lupus anticoagulant (LAC), have received increasing attention due to their possible involvement in extraarticular manifestations of RA, including thrombotic complications. Accumulating evidence shows that a close interaction exists between the immune and the endocrine systems.[1]

Recent epidemiological, clinical, and laboratory evidence has suggested that female sex hormones play a central role in the immune response and in immune-mediated diseases.[2] Evidence suggests that physiological levels of estrogens stimulate, whereas male hormones (androgens) tend to suppress, the immune response.[3] Overall, females have higher levels of immunoglobulins and mount stronger humoral and cellular immune responses to a variety of antigens than do males.[4] In addition, pharmacological doses of estrogens have been demonstrated to exert *in vitro* two main effects: (a) to stimulate the B-cell response and (b) to suppress the T-cell reactivity.[5]

These observations led us to investigate plasma levels of estrogens in aCL-positive versus aCL-negative female patients with RA in order to evaluate possible relationships.

MATERIALS AND METHODS

Patients

Seventy-two female patients, who fulfilled the 1987 American Rheumatism Association Criteria for adult RA, were selected from our Rheumatological Centre. The mean age of the patients was 56 ± 9 years (±SD) (range, 25–72 years). All the patients were tested for aPL antibodies, including three tests for LAC detection and

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aCL antibody levels. RA patients were then grouped as "aCL antibody positive" (aCL+, n = 16, 22%) and "aCL antibody negative" (aCL-, n = 56, 78%), depending on serum aPL antibody positivity.

None of the patients had never been treated with corticosteroids or oral contraceptive pills. All patients analyzed showed a normal variation of gonadal hormone and gonadotrophin concentrations during the menstrual cycle.

Detection of aPL Antibodies

All patients were tested for aCL at least three times during a six-month period of observation. Serum levels of IgG-type aCL (aCL-IgG) and IgM-type aCL (aCL-IgM) were measured by an enzyme-linked immunosorbent assay (ELISA), as previously described.[6] Values are expressed in GPL and MPL units/mL.

Three different tests were employed to detect the presence of LAC; namely, (a) the dilute Russel viper venom time;[7] (b) the dilute one-stage prothrombin time;[8] and (c) the activated partial thromboplastin time.[9] The patients were identified as LAC positive when all three tests were found altered.

Determination of Estrogens

17-β-estradiol (E_2) levels were measured by a solid-phase, chemiluminescent enzyme immunoassay on whole sera and obtained during the first 10 days of the menstrual cycle and stored at –20 °C.[10] The interassay coefficient of variation of the method was 6% (Diagnostic System Laboratories Inc., Webster, TX).

Statistical Analysis

Statistical analysis of the differences between groups (RA patients and controls) was done by Student's unpaired t test. Student's paired t test was used to determine the significance of differences between aCL-positive or -negative patients in the premenopausal or postmenopausal group.

RESULTS

Characteristics of the patients with RA and controls are reported in TABLE 1. Our evaluation of 72 female patients with RA revealed that 16 (22%) of these presented with at least one type of the different aPL antibodies studied. LAC was present in 7 patients, together with aCL antibody positivity. The prevalence in the three classes of aCL antibodies was as follows: IgG aCL, 10 cases (62%; mean values ± SEM, 45 ± 8 GPL units/mL); IgM aCL; 3 cases (19%; mean values ± SEM, 60 ± 9 MPL units/mL); and both IgG-IgM aCL, 3 cases (19%; mean values ± SEM, 36 ± 4 GPL units/mL and 40 ± 6 MPL units/mL).

E_2 levels were found higher in patients with RA compared with those in controls, but the difference was not statistically significant (0.136 ± 0.02 nmol/L vs. 0.103 ± 0.01 nmol/L, respectively; p = 0.08). However, higher E_2 levels were observed in

TABLE 1. Characteristics of Patients with Rheumatoid Arthritis and Controls

	Patients with RA		
	aCL Positive ($n = 16$)	aCL Negative ($n = 56$)	Controls ($n = 68$)
Age (years)	55 ± 11 (25–68)	58 ± 9 (28–72)	53 ± 8 (22–68)
Duration of disease (years)	9.4 ± 7.8 (6–14)	9.8 ± 6.6 (7–16)	Negative
IgM-RF positive (no. of patients)	14 (87%)	50 (89%)	Negative
ANA positive (no. of patients)	11 (69%)	38 (68%)	Negative
ERS (mm/h)	62 ± 24 (36–120)	64 ± 22 (32–110)	Negative
CRP (mg/L)	46 ± 36 (8–128)	48 ± 32 (6–96)	Negative

Values are expressed as the mean ± SD and range (within parentheses). IgM-RF, IgM rheumatoid factor; ANA, antinuclear antibodies; ERS, erythrocyte sedimentation rate; CRP, C-reactive protein.

aCL antibody-positive RA patients than in aCL antibody-negative RA patients (0.253 ± 0.05 nmol/L vs. 0.084 ± 0.01 nmol/L, respectively; $p = 0.001$).

Furthermore, when patients with RA were grouped depending on their pre- or postmenopausal status, significantly higher E_2 levels were found in premenopausal patients with RA and aCL antibody positivity compared with premenopausal patients with RA and identified as aCL antibody negative (0.418 ± 0.07 nmol/L vs. 0.176 ± 0.03 nmol/L, respectively; $p = 0.07$). No significant differences were found within postmenopausal RA patients with or without positivity for aCL antibodies.

DISCUSSION

In the present study we found that in premenopausal patients with RA there is an association between aCL antibody positivity and increased levels of E_2 compared with patients with RA who were aCL antibody negative. Our results seem to agree with previous experimental observations showing that estrogens can stimulate the immune system in normal C57BL/6 mice to allow the expression of IgG and IgM antibodies against cardiolipin.[11] In addition, the induction of these autoantibodies persisted for months after the exposure to exogenous estrogen had been stopped.[11]

The mechanisms responsible for the increased production of aCL antibodies by estrogens are unknown. There are different hypotheses that might explain this issue: (a) estrogens may act directly on B-cell populations: to date, however, receptors for estrogens on B cells have not been definitively identified; (b) estrogens may act through the hypothalamic-pituitary-thymic axis, i.e., by increasing the prolactin production, and altering the neuroimmunoendocrine regulatory circuits;[12] (c) estrogens may affect B cells by down-regulating other immune cells such as suppressor T cells

or NK cells or by changing the production of cytokines (i.e., IL-4, IL-5, IL-6, TGF-β);[13,14] and (d) estrogens might increase the availability of phospholipids to the immune system through direct cellular toxicity or by boosting cell activation.[15] The pathogenetic significance of the association of aCL antibodies and increased levels of E_2 in premenopausal females with RA needs further research. Nevertheless, it is important to confirm that estrogens can potentiate polyclonal B-cell activation, with the production of antibodies, that is, aCL antibodies.

A recent study in female patients with systemic lupus erythematosus (SLE) seems to support our results.[16] In this study SLE patients were classified as either belonging to the "high female sex hormone at onset (HH)" group or to the "low female sex hormone at onset (LH)" group according to age at diagnosis. The Cox regression model revealed that the relative mortality risk of HH patients versus controls was 4.2 times higher than the relative mortality risk of LH patients compared to controls.[16,17] On the other hand, our results suggest that persistent high E_2 levels, as observed at least in premenopausal patients with RA, may predispose to more efficient immune response and therefore may increase some extraarticular manifestations of RA, including the enhanced expression of aCL antibodies.

In conclusion, these data, combined with the higher mortality risk observed in SLE when the disease develops during the reproductive years and the increased risk of developing SLE in past users of oral contraceptives,[16] further support the recognized immunostimulating activities exerted at least by physiological concentrations of the estrogens.[15,16]

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