Anti-Endothelial Cell Autoantibodies and Soluble Markers of Endothelial Cell Dysfunction in Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** To determine if anti-endothelial cell antibodies (AECA) and plasma markers of endothelial cell function are related to disease severity in systemic lupus erythematosus (SLE).

Methods. We measured AECA by human umbilical vein endothelial cell binding, endothelial markers von Willebrand factor, soluble thrombomodulin, and soluble E-selectin by ELISA, and disease severity by SLEDAI and SLICC/ACR in 35 patients with SLE.

Results. Despite high levels of IgG AECA ($p = 0.001$) and von Willebrand factor ($p = 0.0007$) compared to 21 healthy controls, we found a positive correlation only between IgG AECA and the SLEDAI index ($r = 0.393$, $p = 0.021$).

Conclusion. IgG AECA seem to be related to disease activity in SLE, possibly in a pathogenic role. Conversely, plasma markers of endothelial cell damage seem to be an epiphenomenon and may simply be related to excess inflammation. (J Rheumatol 2003;30:1963–6)

Key Indexing Terms:
ENDOTHELIUM
SYSTEMIC LUPUS ERYTHEMATOSUS
ANTI-ENDOTHELIAL ANTIBODIES
VON WILLEBRAND FACTOR

Systemic lupus erythematosus (SLE) is a progressive systemic disease that frequently deteriorates clinically to vascular involvement and thrombotic complications. In many cases these complications seem to be related to the presence of antiphospholipid antibodies, high titers of antinuclear and anti-DNA, or anti-soluble nuclear antigen antibodies[1]. Anti-endothelial cell antibodies (AECA), directed towards antigens on human umbilical vein endothelial cells (HUVEC) have also been found in the serum of SLE patients[2,3]. It is unclear whether AECA have a pathogenic role in the clinical manifestations, especially vascular events, or whether they are simply the result of polyclonal stimulation or cross-reactions with non-endothelial antigens.

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SLE is also associated with elevated concentrations of various soluble markers of endothelial cell function, including von Willebrand factor, soluble thrombomodulin, and soluble E-selectin[4-15]. Increased levels of these markers imply damage to and/or activation of the endothelium and can be predictive of adverse outcome such as myocardial infarction and stroke. Despite these findings, the significance of the relationship between circulating markers, potentially cytotoxic autoantibodies, and the clinical activity of SLE remains controversial[14-16].

To help clarify these points, we hypothesized that in patients with SLE, there would be a clear relationship between plasma markers of endothelial damage, autoantibodies to endothelial cells, and the clinical severity of disease.

MATERIALS AND METHODS

Patients. Thirty-five patients were recruited during a one-year period in a University Hospital: all fulfilled the American College of Rheumatology criteria for SLE[17] (32 women, 3 men, mean age 37.8 ± 12.5). Visceral involvement was present in 20 patients [kidney in 9, central nervous system (CNS) in 4, cardiovascular in 4, lung in 3]. Kidney disease was defined as proteinuria above 200 mg/24 h; CNS was defined as clinical CNS disease symptoms and magnetic resonance imaging evidence of CNS vascular disease; cardiovascular involvement was defined as deep venous thrombosis or echographic evidence of segmental abnormal kinetics or valvular involvement; and lung disease was defined as interstitial disease as assessed by chest computer tomography (CT) scan and no other cause of lung disease than SLE. The other 15 patients only had cutaneous or articular manifestations. Six patients were untreated at the time of sampling, 8 were taking immunosuppressive drugs and 29 were taking steroids (> 20 mg/day taken by 12 patients and < 20 mg/day taken by 17). The disease

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activity of SLE at sampling was quantified in 34 of the 35 patients by using the SLE Disease Activity Index (SLEDAI)[18], and the cumulative severity of SLE was quantified by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index in 21 of the 35 patients[19]. To avoid biases in the interpretation of soluble endothelial markers, patients with creatininemia above 150 µM/l were excluded. No patient had diabetes mellitus or infection at sampling but the duration of SLE was variable among patients. The patients were sampled once and clinical status was determined at the time of sampling. Twenty-one age-matched healthy subjects (11 women, 10 men, mean age 38.7 ± 17.6) were used as a control group.

Laboratory measurements. Blood was sampled after informed consent by venous puncture after a 12 h fast. AECA were measured by enzyme immunoassay on cultured HUVEC according to established criteria[20]. The endothelial cells were extracted according to Jaffe's method then cultured in a GIBCO 199 supplemented with 10% fetal calf serum to confluence in 96-well microplates. Cells were fixed with 0.1% glutaraldehyde for 10 min at 4°C. Then the plates were washed 3 times with phosphate buffer saline (PBS) supplemented with 1% human serum albumin (HSA). To avoid nonspecific fixation, blocking was carried out with 3% HSA for 2 h at 37°C. Then the plates were washed 3 times with PBS/HSA 1%. PBS/HSA 0.5% was used as a negative control. Then the cells were incubated with the serum diluted to 1/800 (IgA), 1/1600 (IgM) and 1/3200 (IgG) in PBS/HSA for 2 h at 37°C. The plates were washed 3 times with PBS/HSA 1%. Then plates were incubated with peroxidase-labeled F(ab')2 diluted to 1/2000 for anti-IgA and anti-IgM and to 1/1000 for IgG in PBS/HSA 0.5%. The plates were washed 3 times with PBS/HSA 1%, then twice in PBS only. Plates were then incubated with the substrate orthophenylene diamine (OPD) 2.2 mg/l in phosphate citrate buffer, pH 5 with hydrogen peroxide 1/2400 for 5 min at room temperature. The reaction was stopped with sulfuric acid 3 mmol/l and the optical densities (OD) were read during the following 10 min to 1 h. Results were expressed as the ratio of the patient's OD: positive control OD. The cut-off value (positivity level) was chosen as mean + 3 standard deviations (SD) of the ratios found in the controls. Sera found to be positive were later used as positive controls on subsequent plates.

Plasma thrombomodulin, von Willebrand factor, and soluble E-selectin were measured in citrated plasma by established commercial ELISA (Asserachrom Stago Diagnostica, France and Dako, Denmark). The intraassay coefficents of variation of these assays are < 5%. Interassay coefficents are < 10%. Antinuclear antibodies were determined by immunofluorescence on HEp2 cells; anti-DNA and anti-ECT (anti-soluble antigens) were determined by ELISA.

Data analysis. Statistical analysis was performed using student's t test (data distributed normally), the Mann-Whitney U test (data distributed non-normally), and Spearman's correlation test (Microsoft Excel 4 software and Minitab Release 12).

RESULTS

Cross-sectional data (Table 1). Compared to controls, SLE patients had a significantly higher IgG AECA ratio. Twenty-one of the 35 SLE patients had positive IgG AECA while none of the controls was positive for IgG AECA. Of the plasma markers, only von Willebrand factor was higher in the patients relative to the controls. The 9 patients with kidney disease (but creatininemia < 150 µmol/l) had higher levels of soluble thrombomodulin than the 26 who were free of hypercreatininemia: 53 ± 16 ng/ml versus 34 ± 10 ng/ml, p = 0.001. The 19 patients with active SLE (SLEDAI > 6) had higher AECA than those 16 whose SLEDAI was < 6: 1.28 ± 0.77 versus 0.58 ± 0.46, p = 0.004. Eighteen percent of patients had a positive result for antiphospholipid antibodies (anti-cardiolipin or anti-ß$_2$-glycoprotein I ELISA).

Correlations within patients with SLE (Table 2). We found positive correlations between IgG AECA and the SLEDAI (r = 0.53, p < 0.0001) (Figure 1), and between soluble thrombomodulin and SLICC (r = 0.465, p = 0.045). However, there were no statistically significant correlations between any of the AECA titers and levels of the plasma markers.

DISCUSSION

Our data confirm the presence of AECA and raised von Willebrand factor in SLE patients. In previous studies, the prevalence of AECA in SLE varied from 0 to 80%[2,3,21-23]. Such differences among the studies may arise from the absence of standardization of the measurement methods and from differences among the study populations. Standardization of AECA measurement is in process[24]. Various indices have also been used to assess SLE activity. We used those (SLEDAI and SLICC/ACR) that seemed to us as the best validated and most currently used differentiating activity and damage scores. However the SLICC/ACR index may not only reflect damage from SLE but also from treatment (for example visual loss may come from SLE but also from steroid-associated cataracts). The only endothelial marker that we found to differ significantly in this sample of patients compared with controls was von Willebrand factor, a marker of endothelial damage or activation[5].

Our results show that IgG AECA are present in SLE patients and correlate with disease activity. Most of our patients were receiving treatment and this may explain the lack of increase in soluble thrombomodulin and soluble E-selectin. However, although levels were not significantly raised relative to controls, we found a correlation between soluble thrombomodulin and SLICC but not SLEDAI index, suggesting a relationship between soluble thrombomodulin and damage but not with activity of the disease. Conversely, we, like others[2,21,25], found a correlation between IgG AECA and the activity of the disease. The epitopes recognized by AECA have not been clearly identified so far although a close cross-reactivity has been shown between AECA and anti-cardiolipin or anti-ß$_2$-glycoprotein I antibodies[26-29]. Hill found anti-endothelial cell antibodies in half of the patients with primary antiphospholipid syndrome (APS) and two-thirds of 32 SLE patients, and also showed that differences exist when using HUVEC or human microvascular endothelial cells for the detection of AECA[30]. A direct pathogenic role has been suggested for AECA in APS, Wegener's granulomatosis, and in scleroderma[27,31] since IgG AECA are able in these conditions to enhance the expression of adhesion molecules (vascular cellular adhesion molecule-1, E-selectin) on endothelial cells. In SLE, as in vasculitides, IgG AECA have been shown to be cytotoxic towards endothelial cells[3,32]. Antiphospholipid antibodies (aPL) may be responsible for vascular lesions in many patients: SLE patients with aPL have been found to have increased thrombin

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Table 1. Anti-endothelial cell antibodies and soluble endothelial cell markers in patients and controls. Data are presented as mean ± standard deviation or median and interquartile range.

	SLE Patients	Controls	p
IgA AECA ratio	0.640 (0.443–0.930)	0.535 (0.391–0.846)	0.3884
IgM AECA ratio	0.373 (0.261–0.709)	0.470 (0.270–627)	0.9541
IgG AECA ratio	0.744 (0.468–1.394)	0.211 (0.133–0.325)	< 0.0001
Von Willebrand factor (IU/dl)	130 ± 23	103 ± 35	0.0007
Soluble thrombomodulin (ng/ml)	42 ± 21	37 ± 11	0.175
Soluble E-selectin (ng/ml)	57 ± 38	51 ± 18	0.383

AECA: anti-endothelial cell antibodies.

Table 2. Spearman correlations and p values between AECA, endothelial cell markers, and indices of disease activity in patients with SLE.

	SLEDAI		SLICC	
	r	p	r	p
IgA AECA	0.18	0.297	–0.301	0.197
IgM AECA	0.03	0.878	0.035	0.88
IgG AECA	0.53	0.001	–0.28	0.23
VWf	–0.17	0.47	0.23	0.26
STM	0.24	0.33	0.035	0.89
Soluble E-selectin	0.15	0.55	0.023	0.92

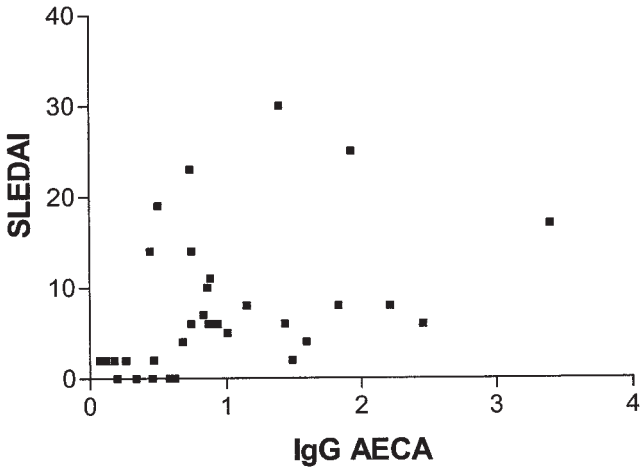

Figure 1. Correlation between anti-endothelial cell IgG and SLEDAI.

generation following microvascular injury *ex vivo*[33] and prothrombic state assessed by both increase in von Willebrand factor and tissue plasminogen activator when they had aPL[34]. However, more data are needed to establish such a pathogenic role that is not suggested from our results. In SLE patients the situation is complicated by the presence of vascular lesions and inflammation, perhaps related to aPL[1]. The interpretation of markers of endothelial dysfunction that can increase in both conditions[35-39] is difficult. We excluded patients with renal insufficiency but not those with nephrotic syndrome. Heavy proteinuria may increase the concentration of von Willebrand factor so that a bias might be present that affects the interpretation of any correlation. Part of vascular dysfunction may be immune related (perhaps due to cytokines) and perhaps caused by AECA or aPL, while part may be related to enhanced atherogenesis, accelerated by dyslipidemia or steroid therapy. Additional experiments are required to clarify these issues.

We found increased levels of AECA and von Willebrand factor, but no correlation between them, and also a correlation between AECA and disease activity. Raised von Willebrand factor may be an epiphenomenon unrelated directly to the disease process, but the small number of patients as well as the heterogeneous nature of SLE vascular injury may also explain this absence of correlation. Serial testing was not available in our study but would provide more potent data to clarify this lack of correlation. Lack of correlation between AECA and von Willebrand factor is reminiscent of a similar study in diabetes. Petty, *et al*[40] found a correlation between raised von Willebrand factor and diabetic retinopathy (a possible index of disease activity) but no correlation with increased levels of IgG or IgM AECA. This therefore underlines the difficulty in interpreting the roles of AECA and von Willebrand factor in inflammatory (e.g., SLE) and non-inflammatory (e.g., diabetes) vascular diseases. Our data do not support the use of anti-endothelial cell antibodies as a measure of disease activity.

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