Assessment of anti-endothelial cell antibodies in systemic sclerosis and Sjögren’s syndrome

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Abstract

Objectives—Anti-endothelial cell antibodies (AECA) have been detected in 19 to 30% of patients with systemic sclerosis (SSc). The objective of this study was first to assess the role of a secondary Sjögren’s syndrome (SS) in the occurrence of AECA in SSc. Secondly, we researched AECA in patients with primary SS, and investigated whether AECA were associated with vascular manifestations (Raynaud’s phenomenon and vasculitis).

Methods—IgG-AECA were tested by an ELISA method in serum samples from 50 patients with SSc (16 of them had also a secondary SS), 50 patients with primary SS, and 50 healthy controls.

Results—AECA levels were significantly higher in patients with SSc or primary SS than in healthy controls (p < 0.01 and p < 0.01, respectively). In patients with SSc, AECA values were significantly higher in patients with secondary SS (p < 10⁻⁴). In patients with primary SS, AECA levels were significantly higher in patients with Raynaud’s phenomenon (p < 0.01), but not in patients with vasculitis.

Conclusion—In patients with SSc, AECA are associated with a secondary SS. In patients with primary SS, AECA are associated with Raynaud’s phenomenon, but not with vasculitis.

Systemic sclerosis (SSc) is a connective tissue disease characterised by collagen accumulation and vascular lesions in the skin and internal organs. Selectin could result from the resident mast cell derived tumour necrosis factor α.

Other studies have suggested the participation of anti-endothelial cell antibodies (AECA) in the occurrence of the endothelial injury in patients with SSc. AECA have been detected in 19 to 30% of patients with SSc, and their potential role was suggested by the demonstration of a mechanism of antibody dependent cell cytotoxicity. AECA have also been detected in several other systemic diseases such as rheumatoid arthritis, Kawasaki syndrome, and systemic lupus erythematosus. In all these diseases, AECA are associated with inflammatory vascular lesions.

The specific vascular injury of SSc is different, because it is characterised by a subendothelial sclerosis of capillary walls. In salivary glands of patients with SSc, the detection of an inflammatory infiltrate is usually related to an associated Sjögren’s syndrome (SS).13–15 SS is frequently associated with a B cell polyclonal activation and several auto-antibodies. Consequently, the presence of AECA in patients with SSc could be in fact secondary to an associated SS, rather than a specific marker of SSc.

SS can also be isolated (primary SS). Fifteen to 30% of the patients with primary SS have vascular manifestations, mainly Raynaud’s phenomenon and vasculitis.16 These vascular complications are significantly more frequent in primary SS with cryoglobulinaemia, anti-SS-A or anti-SS-B antibodies, or both, but to our knowledge, the incidence of AECA in patients with primary SS has not been previously studied.

The aim of this study was first to assess the role of a secondary SS in the occurrence of AECA in patients with SSc. Secondly, we researched AECA in patients with primary SS, and investigated whether these antibodies were associated with Raynaud’s phenomenon or vasculitis.

Methods

PATIENTS

We used in this study the preliminary American College of Rheumatology (ACR) criteria for the diagnosis of SSc14 We used the Copenhagen criteria for the diagnosis of SS15: two positive tests of the following three were required for keratoconjunctivitis sicca:
Schirmer, Rose Bengal, and break-up time tests. A positive salivary scintigraphy, and a Chisholm’s focus score ≥3 on lip biopsy were required for xerostomia. All these tests were performed in patients with SSc to detect a secondary SS. We selected frozen serum samples obtained from consecutive patients seen in our departments. The samples had been generally obtained immediately after diagnosis, and none of the patients received any specific treatment (specially corticosteroid therapy) before serum collection.

According to these diagnostic criteria: 34 patients had an isolated SSc (sex ratio: 7/27, median (SD) age 51.3 (14.1) years, range 18-73, median disease duration 5.3 (4.2) years); 16 patients had a SSc associated with a secondary SS (sex ratio: 2/16; median age 54.8 (9.8) years, range 29-75, median disease duration: 6.5 (4.7) years); 50 patients had a primary SS (sex ratio: 0/50; median age 52.2 (12.5) years, range 29-75; median disease duration: 4.7 (4.7) years).

We also studied serum samples from 50 healthy controls who gave their informed consent (sex ratio: 7/43; median age 49 (7) years, range 21-72).

All patients with SSc, SSc and secondary SS, or primary SS had a clinical evaluation including research of a Raynaud’s phenomenon. The diagnosis of Raynaud’s phenomenon was based on history, or the observation of bilateral, episodic, cold induced pallor and/or cyanosis of the fingers, or both the fingers and the toes. A nailfold capillaroscopy was performed in all patients.

Findings suggestive of vasculitis were clinically researched in all patients. Cutaneous lesions suggestive of vasculitis were biopsied.

A laboratory analysis was performed to determine levels of serum γ globulin and creatinine, and to test for antinuclear antibodies (indirect immunofluorescence technique), anti-SS-A and anti-SS-B antibodies (Ouchterlony double diffusion in agarose gel), anti-native DNA antibodies (radio-immunology), monoclonal dysglobulinemia, cryoglobulinemia, and hypocomplementemia.

**AECA DETECTION**

Endothelial cells from human umbilical vein were cultured on a matrix of 1% gelatin. The detached cells were pipetted into a 96 well plates for detection of IgG AECA by an ELISA method. The endothelial cell ELISA was performed as previously described. All serum samples were tested twice at a dilution of 1/500. In this study, results are not presented as concentrations. The term ‘level’ is used and corresponds to the serum sample related optical density (OD). An AECA level was recorded as ‘increased’ if the OD was greater than the mean seen in the healthy control group + 3 standard deviations (SD).

**STATISTICAL ANALYSIS**

Results of the ELISA assay are reported as mean (SD). Differences between groups were compared using the Mann-Whitney U test. Correlation between detection of AECA and clinical manifestations was assessed using the χ² test with Yates’s correction.

**Results**

**CLINICAL FEATURES**

Four of 50 patients (8%) with SSc had a proximal sclerosis (major ACR criteria), and the others had an acrosclerosis associated with digital pitting scars or pulmonary fibrosis, or both. Seven patients had the CREST variant of SSc.

All patients with isolated SSc had a Raynaud’s phenomenon, and none had clinical manifestations suggestive of vasculitis. Cryoglobulinemia was detected in five of 34 cases (14%), antinuclear antibodies in 13 of 34 cases (38%) (table 1).

All patients with SSc and associated SS had Raynaud’s phenomenon, and none had clinical manifestations suggestive of vasculitis. Cryoglobulinemia was detected in one of 16 cases (6%), antinuclear antibodies in seven of 16 cases (43%), anti-SS-A and anti-SS-B antibodies in two of 16 (12%) and 0 cases, respectively.

Nineteen of 50 patients (38%) with primary SS presented with a Raynaud’s phenomenon. Nailfold capillaroscopy was abnormal in six of these patients. Eleven patients (22%) had clinical manifestations of vasculitis: 10 had cutaneous involvement, three had peripheral neuropathy, one had central neuropathy, and one had ocular involvement. Vasculitis was confirmed histologically in all patients with clinical skin lesions. Cryoglobulinemia was detected in nine cases (18%), antinuclear antibodies in 31 cases (62%), anti-SS-A and anti-SS-B antibodies in 23 (46%) and 19 cases (38%), respectively. Four patients (8%) with primary SS had hypocomplementemia.

None of the patients had evidence of renal disease.

**AECA DETECTION**

The mean (SD) AECA level found in the group of 50 healthy controls was 0.31 (0.09). It was significantly higher in the group of 50 patients with SSc (0.44 (0.21), p < 0.01), and in the group of 50 patients with primary SS (0.43 (0.20), p < 0.01). Moreover it was significantly higher in the 16 patients with SSc and secondary SS, than in the 34 patients with isolated SSc (0.67 (0.21) v. 0.34 (0.10), p < 10⁻³) (fig 1). AECA levels were considered increased if they were higher than the healthy
control mean +3 SD (0.57). In the SSc patients, levels were increased in 11 of 50 patients (22%), but nine of 16 patients (56%) with SSc and secondary SSc had increased levels, when only two of 34 patients (6%) with isolated SSc had increased levels (p < 0.05).

AECA levels were increased in 16 of 50 patients with primary SS (32%), and in one of 50 healthy controls (2%).

CORRELATIONS
As hypergammaglobulinaemia is common in SS, we investigated whether a relation existed between IgG levels and AECA levels. In each group of patients, we compared the mean IgG levels found in patients with increased AECA levels with the mean IgG levels found in patients with AECA levels that were not increased. In all groups, no significant difference was found for mean IgG levels (respectively 15.4 (7.5) g/l v 14.0 (6.3) g/l in SSc group; respectively 22.4 (14.1) g/l v 20.8 (11.6) g/l in primary SS group).

As all patients with SS had Raynaud’s phenomenon, and none had vasculitis, there was no correlation between these vascular manifestations and AECA levels.

In the group of primary SS, AECA levels were significantly higher in patients with Raynaud’s phenomenon than in patients without Raynaud’s phenomenon (0.55 (0.21) v 0.37 (0.17), p < 0.01) (fig 2). AECA levels were more frequently increased (≥0.57) in patients with Raynaud’s phenomenon (10 of 19) than in patients without Raynaud’s phenomenon (six of 31) (p < 0.05). Raynaud’s phenomenon was correlated with AECA, but not with cryoglobulinaemia (table 2). Manifestations of vasculitis were not correlated with the presence of AECA, but were correlated with the presence of cryoglobulinaemia.

### Table 2 Correlations between Raynaud’s phenomenon, vasculitis, and immunological features in patients with primary Sjögren’s syndrome

<table>
<thead>
<tr>
<th></th>
<th>Primary SS with Raynaud’s phenomenon (n=19)</th>
<th>Primary SS without Raynaud’s phenomenon (n=31)</th>
<th>p Value*</th>
<th>Primary SS with vasculitis (n=13)</th>
<th>Primary SS without vasculitis (n=37)</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AECA level (mean (SD) OD)</td>
<td>0.55 (0.21)</td>
<td>0.37 (0.17)</td>
<td>&lt;0.01</td>
<td>0.47 (0.25)</td>
<td>0.42 (0.19)</td>
<td>NS</td>
</tr>
<tr>
<td>Cryoglobulinaemia</td>
<td>3</td>
<td>6</td>
<td>NS</td>
<td>6</td>
<td>3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Antinuclear antibodies</td>
<td>11</td>
<td>20</td>
<td>NS</td>
<td>10</td>
<td>21</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-SS-A antibodies</td>
<td>8</td>
<td>15</td>
<td>NS</td>
<td>11</td>
<td>12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Anti-SS-B antibodies</td>
<td>7</td>
<td>12</td>
<td>NS</td>
<td>10</td>
<td>9</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*The Mann-Whitney U test was used for comparison of AECA levels. The χ² test with Yates’s correction was used for the other parameters. OD: optical density; SS: Sjögren’s syndrome.
anti-SS-A, and anti-SS-B antibodies (p < 0.01, p < 0.01, and p < 0.01, respectively) (table 2).

Discussion

Several studies suggested the role of AECA in the occurrence of vascular lesions in patients with systemic diseases. In rheumatoid arthritis and Kawasaki syndrome, AECA are associated with inflammatory vascular lesions.29 In a recent study of 28 patients with systemic lupus erythematosus, Yosio found a correlation between the presence of AECA and clinical manifestations of vascular injury: Raynaud’s phenomenon, digital vasculitis, and pulmonary hypertension.30 Previous studies performed in patients with systemic lupus erythematosus by D’Cruz suggested a correlation between presence of AECA, vasculitis and nephritis.12 AECA have also been detected in SSc, but their significance in this disease remains unclear. In the present study, AECA were detected in 22% of patients with SSc. This is in accordance with the incidence found in the study of Rosenbaum.7 By the same ELISA method, Rosenbaum found AECA in 30 of 97 (30%) patients with SSc. In another study, Penning found that the serum of nine of 39 (23%) patients with SSc had a cytotoxic effect on cultured endothelial cells.6

In this study, we found that significantly increased levels of AECA were in fact detected in only 6% of patients with isolated SSc (which was similar to the frequency found in the healthy control group), compared with 56% of patients with SSc and associated SS (p < 0.05). This suggests that in patients with SSc, presence of AECA could be the result of a secondary SS, rather than SSc. The pathogenic role of AECA in the development of vascular lesions of SSC remains controversial, and these antibodies could be explained by the non-specific B cell polyclonal activation of SS. Consequently, the presence of a secondary SS should be mentioned in the studies performed on the detection of AECA in patients with SSc.

To define the role of SS in occurrence of AECA, we tested these antibodies in 50 patients with primary SS. AECA were found in 16 of 50 patients (32%). No correlation was found between the presence of AECA and manifestations of vasculitis in patients with primary SS. Yet, manifestations of vasculitis were correlated with the presence of cryoglobulinemia, anti-SS-A, and anti-SS-B antibodies (p < 0.01, p < 0.01, and p < 0.01, respectively). This is consistent with previous studies performed by Alexander.13 In the present study, AECA were significantly more frequent in the presence of a Raynaud’s phenomenon (p < 0.01). Moreover, in the 13 patients with primary SS and Raynaud’s phenomenon, the latter was correlated with AECA, but not with cryoglobulinemia. These data suggest that AECA may be involved in the pathogenesis of Raynaud’s phenomenon in patients with primary SS. AECA have been shown to change the release of prostaglandin I_2 by endothelial cells.21 This AECA mediated mechanism could result in an increased vascular reactivity.

The results of this study together with other previous studies suggest that Raynaud’s phenomenon in conjunction with primary SS or systemic lupus erythematosus could have a different pathogenesis than Raynaud’s phenomenon of SSc. In this study, all the patients with isolated SSc had a Raynaud’s phenomenon, but only 6% of them had AECA. Conversely, in primary SS and in systemic lupus erythematosus, Raynaud’s phenomenon is not constantly observed, and it could result from a specific immunological aggression. This immunological aggression could be in part supported by AECA. Whether this immunological reaction results from a primary lymphocytic defect or is secondary to an abnormal antigenic expression by endothelial cells remains to be clarified, because in systemic diseases the endothelial antigenic targets of AECA are still unknown.

In conclusion, in patients with SSc, the presence of AECA seems to be more associated with a secondary SS than with the SSc. Consequently, the participation of AECA in pathogenesis of SSc remains hypothetical. AECA are frequently present in patients with primary SS, in which they are associated with Raynaud’s phenomenon.