Coexistence of antitopoisomerase I and anticentromere antibodies in patients with systemic sclerosis

T Dick, R Mierau, P Bartz-Bazzanella, M Alavi, M Stoyanova-Scholz, J Kindler, E Genth

Background: Antibodies targeting DNA topoisomerase I (ATA) or centromere proteins (ACA) are associated with clinical subsets of patients with systemic sclerosis (SSc). The occurrence of these autoantibodies is considered to be mutually exclusive.

Objective: To describe the clinical and immunogenetic data of three patients who are co-expressing both antibodies, and then review previous publications.

Methods: Both antibodies were detected by different methods, including indirect immunofluorescence technique, enzyme linked immunosorbent assay, immunodiffusion, and immunoblot. Patients were HLA typed by serological and molecular genetic methods. Data were extracted from published reports for comparison. The search for published studies was through Medline and other database research programmes.

Results: During routine laboratory diagnostics over several years three patients with scleroderma and coincidence of ATA and ACA were identified: patient 1 with diffuse SSc, Raynaud’s phenomenon, puffy fingers and fingertip necrosis, contractures, and calcinosis; patient 2 with diffuse SSc, Raynaud’s phenomenon, oedema of the hands, and interstitial calcinosis of hands, knees, and shoulders, and pulmonary fibrosis; patient 3 with scleroderma of hands, forearms, and face, Raynaud’s phenomenon, puffy fingers, finger contractures, fingertip necrosis, and calcinosis. All three patients studied were carriers of HLA alleles known to be associated with these autoantibodies. In serial measurements the concentrations of the two antibodies showed independent or even reverse fluctuations. Screening of 100 patients with ACA for ATA and vice versa disclosed no further patients with coincidence of these antibodies. Twenty eight cases of ACA/ATA coexistence in 5423 patients (0.52%) with SSc or SSc associated symptoms were found in an analysis of published studies.

Conclusion: The expression of ATA and ACA is not totally mutually exclusive, but coincidence is rare (<1% of patients with SSc). Patients with both autoantibodies often have diffuse scleroderma and show immunogenetic features of both antibody defined subsets of SSc.

Patients and sera

Clinical data of patients with ACA or with ATA were obtained by chart review, using a standardised documentation protocol. Clinical features were defined as described earlier.13 Altogether, data from 173 ACA and 118 ATA positive patients could be evaluated. 44 (25%) patients with ACA and 95 (81%) patients with ATA fulfilling the ACR criteria for SSc.29 For a

Abbreviations: ACA, anticentromere antibodies; ATA, antitopoisomerase I antibodies; IIFT, indirect immunofluorescence test; SSc, systemic sclerosis; SSP-PCR, sequence-specific primer-polymerase chain reaction

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detailed serological analysis, 100 sera known to be positive for ACA and 100 sera with ATA were selected, regardless of the diagnosis.

**Indirect immunofluorescence test (IIFT)**

The test was performed by a standardised method. Briefly, methanol/acetone fixed preparations of HEP-2 cells were incubated with diluted patient serum. The patient serum was removed and the slides were washed, and FITC labelled secondary antibody directed against total human immunoglobulin was added. Afterwards the preparations were washed again, covered, and examined under the fluorescence microscope. Titres of 80 or more were regarded as positive.

**Immunodiffusion**

ATA were detected by immunodiffusion according to a standard Ouchterlony test protocol as described. A preparation of rabbit thymus extract (Pel-Freez, Rogers, via Paesel and Lorei, Frankfurt, Germany) served as antigen source.

**Enzyme linked immunosorbent assay (ELISA)**

Commercial ELISA systems (Pharmacia and Upjohn, Freiburg, Germany) were used for the quantitative determination of ATA and ACA. Both tests, using recombinant human antigens and detecting human IgG, were performed according to the manufacturer’s instructions. The centromere ELISA detected antibodies against the CENP-B protein. The cut off value for both tests was defined by the supplier as 5 U/ml.

**Immunoblot**

Results obtained by other methods of detecting ATA were confirmed by immunoblot, as described earlier. In short, an antigen preparation of 5x10⁵ HeLa cells was separated on a 5–20% sodium dodecyl sulphate gradient gel and electrophoretically transferred to a nitrocellulose membrane (Biorad, Munich, Germany). Strips of the membrane were incubated with patient sera and, after a washing step, a biotin labelled goat antihuman IgG antibody (Sigma) was added. An avidin-peroxidase substrate reaction was used as read out.

**HLA typing**

The HLA-A, -B, -C loci of the patients were typed serologically according to the standard NIH microcytotoxicity test, using serum samples from one Lambda (BmT, Krefeld), Biostest (Dreieich), Fresenius (Bad Homburg), BAG (Lich), Bio-Mérieux (Nürtingen), and Behringwerke (Marburg, all Germany). The HLA class II alleles were determined with a multiplex-sequence-specific primer-polymerase chain reaction (multiplex-SSP-PCR; UCLA amplification mixtures, ULCA Tissue typing Laboratory, Los Angeles, USA) including allele-specific primers for DRB1*0101, 02, 03; *0301, 02; *0401–0411; *0701; *0801–05; *0901; *1001; *1101–04; *1201, 02; *1301–05; *1401, 02, 06; *1501–03; *1601, 02; DBR3*0101; *02; *0301; *0601; *0801–05; *0901; *1001; *1101–04; DRB4*0101; DRB5*0101. DQA, DQB loci alleles were determined using SSP-PCR kits DQA1 from Dynal (Oslo, Norway).

**Literature analysis**

Research of published reports was done through Medline, ISI Current Contents, and databases accessible through the internet. Databases were at first screened for publications describing patients with SSc and mentioning ATA or ACA, or both. Articles were selected if the patients enclosed in the studies had SSc or SSc associated symptoms and if antibody testing for both antibodies had been carried out by standardised methods (counterimmunoelectrophoresis, ELISA, IIFT, immunoblot, immunodiffusion, immunoprecipitation). Patient groups which obviously appeared in more than one publication were counted only once.

**RESULTS**

During routine diagnostic examinations over several years we identified three patients with coincidence of ATA and ACA: both antibodies could be detected by indirect immunofluorescence on HEP-2 cells and ELISA with recombinant antigens. In addition, the presence of ATA was confirmed by immunodiffusion and immunoblot.

To investigate whether these three patients were really exceptional cases or whether ATA/ACA coincidence can be detected more frequently by sensitive autoantibody detection methods, 100 ATA positive sera (immunodiffusion assay) were tested for ACA in an ELISA. Vice versa, 100 sera with a positive ACA IIFT result were tested for ATA by ELISA. None of the ATA positive sera contained ACA against the CENP-B protein. Ninety five of the sera positive for ACA by IIFT were negative in the ATA ELISA, five sera were marginally positive (5.3; 8.8; 11.3; 16.4; 22.5 U/ml). The positive results could not be confirmed by immunodiffusion and immunoblot, suggesting false positive ELISA results. Thus we confirmed that the coincidence of ATA and ACA is rare (<1%). The coexistence of ATA or ACA with other antibodies was not investigated.

In all available sera from different blood samples of the three patients, both antibodies were quantified (fig 1). Whereas for patient No 2 only two samples were available, antibody concentrations of patients No 1 and No 3 have been observed for 12 or 10 years, respectively. Antibody concentrations independently fluctuated over the years; in fact most of the time they varied in opposite directions. In patient No 1, ACA appeared later than ATA and were detected for the first time six years after disease onset.

The HLA typing of the three patients showed that all of them had at least one DQB1 allele with a non-leucine residue...
at position 26, which is described as being associated with ACA, and a DQB1 allele with a tyrosine at position 30 known to be associated with ATA. In most cases alleles carrying these features were present on both chromosomes (table 1).

Table 1  HLA typing results

<table>
<thead>
<tr>
<th>Patient</th>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-C</th>
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<tr>
<td>(a) HLA class I</td>
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<td></td>
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<tr>
<td>1</td>
<td>26, –</td>
<td>35, –</td>
<td>4, –</td>
</tr>
<tr>
<td>2</td>
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<td>10, 19</td>
<td>13, 15</td>
<td>5, –</td>
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<table>
<thead>
<tr>
<th>Patient</th>
<th>HLA allele with non-Leu-26 typical for ACA</th>
<th>DQB1 allele with Tyr-30 typical for ATA</th>
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<tr>
<td>(b) HLA class II</td>
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<tr>
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<td>DRB3*0202</td>
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<td>2</td>
<td>DRB1*0801, *1104</td>
<td>DRB3*0202</td>
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<tr>
<td>3</td>
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<td>DRB3*0202</td>
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Table 2  Clinical data of patients with ATA and ACA

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<tr>
<th>Patient</th>
<th>Age at onset</th>
<th>Duration (years)</th>
<th>Raynaud's phenomenon</th>
<th>Puffy fingers/hands</th>
<th>Scleroderma</th>
<th>Fingertip necrosis</th>
<th>Contractures</th>
<th>Calcinosis cutis</th>
<th>Telangiectasia</th>
<th>Oesophageal hypomotility</th>
<th>Pulmonary fibrosis</th>
<th>Sicca symptoms</th>
<th>Renal disease</th>
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<td>18</td>
<td>+</td>
<td>+</td>
<td>Hands, forearms, upper arms, face, trunk</td>
<td>+ (only initially)</td>
<td>Fingers, toes, elbows</td>
<td>+</td>
<td>Generalised</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>Hands, forearms, upper arms, face, trunk</td>
<td>+ (recurrent)</td>
<td>Fingers, elbows</td>
<td>+</td>
<td>ND*</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>Hands, forearms, upper arms, face, trunk</td>
<td>+ (once)</td>
<td>Fingers, elbows</td>
<td>+</td>
<td>Breast</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+/-</td>
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</tr>
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</table>

Figure 2  Radiograph of the right hand of patient No 2, eight years after disease onset.

contractures, fingertip necrosis, calcinosis, and scleroderma of forearm and face; there is no serious internal organ involvement so far.

To judge whether these clinical features were typical for patients with ACA, ATA, or both, we reviewed the clinical data of our 173 patients with ACA and 118 patients with ATA (fig 3), and compared these with those of our three index patients.

Most features (for example, Raynaud’s phenomenon, telangiectasias, puffy fingers, fingertip necrosis, etc) can be found in both antibody defined patient groups. Of the features common to our three patients with coincident ATA/ACA,
diffuse scleroderma or at least involvement affecting the forearms is found almost exclusively, and contractures predominantly, in the ATA group, whereas calcinosis is more common in the ACA group (although the latter difference did not reach statistical significance).

To assess further the prevalence of ATA/ACA coincidence in patients with SSc, we carried out a literature search using different sources as outlined in “Patients and methods”. From more than 865 publications initially screened, we found 56 publications in which patients with SSc or SSc symptoms were tested for both autoantibodies with standard methods. Altogether, in 5423 patients, antibodies coexisted in 28 (0.52%) (table 3). Furthermore, six additionally reported cases with both antibodies were excluded because they did not fulfil our criteria for selection. Taken together, we found 34 cases documenting the coexistence of ACA/ATA.

DISCUSSION

Autoantibodies are known as useful markers for the diagnosis of SSc. It is established that they occur in about 95% of patients with SSc. Some of them (like ACA and ATA) are associated with subgroups that have distinctive clinical symptoms and prognosis so that the concept of antibody defined syndromes emerged. In systemic lupus erythematosus (as opposed to SSc) the occurrence of more than one antibody is usual. In SSc, coexistence of ACA or ATA with antihistone antibodies, ACA with antimitochondrial antibodies, ATA and anticardiolipin antibodies, ACA/ATA and anti-SS-A or anti-RNP and Th/To antibodies has been reported. However, although co-occurrences with antihistone or antimitochondrial antibodies are relatively common, the detection of more than one subset defining autoantibody in the serum of a patient with SSc is rare. Many authors have regarded the marker antibodies ACA and ATA as mutually exclusive, and it might be argued that these antibody defined syndromes represent distinct, independent clinical entities with separate aetiologies.

However, from the present report of three patients, as well as from several reports published earlier (table 3), it is known that the coincidence of ATA and ACA in individual patients does occur. The reported prevalence of ATA/ACA coexistence in SSc varies widely: whereas Jarzabek-Chorzelska et al claimed a frequency of 5.6% in their 180 patients, Bunn and Black reported that they had seen only one example in over 2000 cases—that is, a prevalence of less than 0.05%. Spencer-Green et al reviewed nine publications with 670 patients which revealed three cases of coexistence (0.45%). We extended the literature search to 56 reports with 5423 patients, in which the compiled frequency of ATA/ACA co-occurrence was 0.52%, remarkably consistent with the analysis of Spencer-Green et al. Our own results, with three coincidence patients and no further case in 200 serum samples preselected for either antibody, are compatible with these numbers.

Thus, if one regards “ATA associated SSc” and “ACA associated SSc” as separate, independent clinical entities, the question arises: do those rare patients with both antibodies have both diseases independently? One clue towards answering this question is obtained by looking at the signs and symptoms of our three patients and asking whether these can be regarded as typical for both diseases. This seems to be the case because our patients have extended scleroderma and contractures as well as calcinosis and joint involvement. Almost all clinical features of SSc, however, can occur in both antibody defined subsets (fig 3). Therefore, from clinical data of only a few patients alone it is not possible to decide if the two SSc subsets occur independently. Only the analysis of a large number of patients with coincident ATA/ACA would
<table>
<thead>
<tr>
<th>Reference†</th>
<th>Reference No</th>
<th>Number of patients‡</th>
<th>ACA and ATA positive</th>
<th>ACA detection</th>
<th>ACA positive No (%)</th>
<th>ATA detection</th>
<th>ATA positive No (%)</th>
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<td>Behr, König, et al 1990</td>
<td>30</td>
<td>74</td>
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<td>De Rooij, van de Putte, et al 1988, 1989</td>
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<td>ELISA</td>
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<td>ID</td>
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<td>13 (20)</td>
<td>ID</td>
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possibly allow such a conclusion, if the clinical features typical for either disease subset occurred as frequently as in the two single antibody defined diseases. The immunogenetics of our patients are at least compatible with the hypothesis of independence because all three patients carry those HLA alleles known to be closely associated with ATA as well as with ACA. Likewise, the quantitative courses of antibodies in serial measurements, with non-parallel and, of the time, even reverse fluctuations (fig. 1), argue for independence. Of particular interest in this regard is the disease course of patient No 1: at first she developed diffuse scleroderma with ACA. Likewise, the quantitative courses of antibodies in serial samples of both patients with systemic sclerosis, “ATA associated SSC” and “ACA associated SSC”, unless one postulates common risk factors for both diseases that enhance the chance of finding them together in one and the same patient.

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References


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