Isotype switching and titer variation of anti-Ro/SSA antibodies over time in 100 patients with undifferentiated connective tissue disease (UCTD)

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ABSTRACT

Objective. To correlate the clinical course of the disease with the titer, the isotype profile and the switch of the anti-Ro/SSA antibodies in a cohort of patients affected by UCTD.

Methods. One hundred selected patients with anti-Ro/SSA antibodies detected by counterimmunoelectrophoresis (CIE), and affected by UCTD with a mean follow-up of 7.6 years (SD 4.8 yrs.), were studied. The titer of IgA, IgG and IgM anti-Ro/SSA antibodies was determined in two different sera, obtained at the time of diagnosis and at the last visit, by ELISA with Ro/SSA recombinant proteins as substrate.

Results. Thirty-five patients evolved from UCTD to a different connective tissue disease, while 65 showed a stable disease. Anti-Ro/SSA antibodies were detected in 91% and 97% of the patients, at baseline and during follow-up, respectively. IgG dominates the anti-Ro response. The titer of IgA, IgM and IgG anti-Ro/SSA did not differ significantly between the two groups of patients with UCTD. An increasing trend of IgG and IgA anti-Ro/SSA titer could be detected in patients evolving in primary Sjögren’s Syndrome (pSS), but only the increase of IgG anti-Ro/SSA was significant (p = 0.0235).

Conclusion. IgG dominates the anti-Ro/SSA response in patients with UCTD. No substantial change of the antibody isotype against Ro/SSA peptides could be observed during follow-up. The titer of IgG anti-Ro/SSA significantly raised in the group of patients evolving in pSS.

Introduction

Undifferentiated connective tissue disease (UCTD) is characterized by clinical and immunological features suggestive but not diagnostic for a defined connective tissue disease (CTD) (1, 2). Anti-U1RNP and anti-Ro/SSA, found in 15-30% of patients, represent the antinuclear specificities most frequently detected in UCTD (3-5). The profile of the autoantibodies isotype is not usually known. In fact, neither the assays (double immunodiffusion, counterimmunoelectrophoresis) or the antisera commonly employed (directed to human IgG or to total immunoglobulins) allow the identification of the Ig class. Few data are reported about distribution or predictive value of different anti-Ro/SSA antibodies’ isotypes in CTD. According to some authors, IgM anti-Ro/SSA appear shortly before IgG in Systemic Lupus Erythematosus (SLE) (6). Anti-Ro/SSA antibodies are known to be present at least three years before SLE onset, earlier than anti-Sm or anti-dsDNA antibodies (7). Additional autoantibody specificities represent one of the risk factors for the evolution of UCTD (4, 5), but the maturation of the immune response to the single autoantigen has never been studied, so far. Basing on these data, we analysed the distribution of different anti-Ro/SSA isotypes in patients affected by UCTD, to assess if a maturation of immune response to a specific (auto)antigen occurs, and if it could represent a predictor of the evolution to a defined CTD.

Patients and methods

Patients

The present study retrospectively evaluated 100 selected patients, affected by UCTD, attending our outpatient Clinic. All subjects showed anti-Ro/SSA antibodies detected in the serum at least on two different occasions, using counterimmunoelectrophoresis (CIE). The clinical diagnosis of UCTD was done according to the preliminary classification criteria recently proposed (1, 2).

Methods

Antibodies to Ro/SSA were determined by CIE (8), using human spleen extract as substrate (9, 10). We tested two different sera for each one of all 100 patients, the first taken at the onset of UCTD and the second either at the moment of evolution to other CTD or the last obtained during follow-up. Every serum was anti-Ro/SSA positive by CIE. To analyze the presence of IgG, IgM or IgA anti-Ro/SSA antibodies, sera were tested by ELISA (Pharmacia, Freiburg, Germany). The results were considered positive for IgG anti-Ro/SSA, when higher than the cut-off indicated by the manufacturer at 8 units. The same procedure was followed to
evaluate the IgM anti-Ro/SSA isotype. The value of IgM anti-Ro/SSA antibodies was assigned comparing every serum to our reference serum. We used 10 ANA negative sera and 20 ENA negative sera for the definition of the cut-off, resulting in 11.25 Units, calculated as the mean value + 2 SD.

IgA anti-Ro/SSA were searched only in 95 sera, with the following procedure: 100 μl of 1:50 diluted serum were added to the plate coupled with Ro recombinant proteins, then incubated for 4 hours at room temperature (RT). After three washes with PBS (pH 7.2), 100 μl of anti-human IgA, conjugated with alkaline phosphatase (Jackson Immunoresearch, West Grove, PA, USA) diluted up to 1:1000, was added and incubated for 4 hours at RT. The plates were then washed as above. 100 μl of substrate (PNPP; Sigma Aldrich, Italy) dissolved in diethanolamine buffer (1 M, pH 9.8) was added and incubated at 37°C for 50 minutes. The reaction was stopped adding NaOH (2 M) and read at 450 nm. The titration curve was performed with doubling dilution of a strongly positive serum: the cut-off value was 25.9 Units calculated as the mean value + 2 SD of 10 ENA negative sera.

**Statistical analysis.**

All the parameters were studied by χ² test with Yates’ correction, when indicated. Student t- and Mann-Whitney tests were used to perform comparisons between the two groups. Statistical significance was accepted at p < 0.05.

**Results**

Demographic data of 100 patients affected by UCTD are shown in Table I. Thirty-five patients evolved to different CTD: 19 patients developed pSS (54%), while SLE, Systemic Sclerosis (SSc) and Rheumatoid arthritis (RA) were diagnosed in 10 (28.6%), 4 (11.5%) and 2 subjects (5.5%), respectively.

Anti-Ro/SSA antibodies’ isotype: at the onset of the disease, 91% of sera contained IgG anti-Ro/SSA antibodies, while IgA and IgM isotypes were detected in 26.3% and 19% of sera respectively, without significant difference between stable and evolved patients (Table II). The evaluation of anti-Ro/SSA isotypes, in sera taken during follow-up, did not show significant changes of that profile. Most patients express anti-Ro/SSA antibodies of isolated IgG class or in association with IgM or IgA, without a significant difference between the first and the second serum tested, or between evolved and stable UCTD groups (Table III). None of the patients showed isolated IgA or IgM anti-Ro/SSA antibodies. No significant change of isotype distribution was observed during the time in both groups.

Anti-Ro/SSA antibodies titer: in the entire cohort of patients, IgG anti-Ro/SSA antibodies are normally distributed, showing a mean value of 48 units (SD: 38) and 46 units (SD: 37), in the first and second serum, respectively. IgM and IgA anti-Ro/SSA antibodies were detected at low titer with a non-gaussian distribution. IgG, IgA and IgM anti-Ro/SSA titer did not significantly vary during follow-up. Comparing the two groups of evolved and stable UCTD, we observed: 1) a higher concentration of IgG (51.21 vs. 42.24 U/ml), IgA (15.14 vs. 11.78 U/ml) and IgM (3.33 vs. 2.28 U/ml) anti-Ro/SSA in stable versus evolved UCTD at baseline; 2) a slight increase of IgG anti-Ro/SSA antibodies between the first and the second serum of evolved patients (from 44.24 to 49.86 U/ml), and a slight decrease of IgG anti-Ro/SSA titer in the stable UCTD (from 51.21 to 44.53 U/ml); 3) IgA anti-Ro/SSA increased (from 11.78 to 16.34 U/ml in evolved and from 15.14 to 16.56 U/ml in stable UCTD), while IgM decreased in both groups (from 2.28 to 1.13 U/ml in evolved and from 3.33 to 2.56 U/ml in stable UCTD). The anti-Ro/SSA titre of the first and second serum in patients evolved in pSS showed a significant increase of

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### Table I. demographic data of 100 patients affected by UCTD.

<table>
<thead>
<tr>
<th></th>
<th>Total (100)</th>
<th>Evolved (35)</th>
<th>Stable (65)</th>
<th>p (&lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/M ratio</td>
<td>93/7</td>
<td>33/2</td>
<td>60/5</td>
<td>ns</td>
</tr>
<tr>
<td>Mean age, years (SD)</td>
<td>51.1 (14.4)</td>
<td>53.5 (13.1)</td>
<td>49.8 (14.9)</td>
<td>ns</td>
</tr>
<tr>
<td>Mean follow up, years (SD)</td>
<td>7.6 (4.8)</td>
<td>9.6 (4.2)</td>
<td>6.48(4.8)</td>
<td>0.018</td>
</tr>
<tr>
<td>Mean disease duration, years (SD)</td>
<td>8.5 (5.5)</td>
<td>11.8 (5.3)</td>
<td>6.76(4.8)</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

### Table II. Isotype distribution of anti-Ro/SSA antibodies, at onset and during follow-up, in 100 patients with UCTD.

<table>
<thead>
<tr>
<th>Isotype</th>
<th>1st serum</th>
<th>2nd serum</th>
<th>1st serum</th>
<th>2nd serum</th>
<th>1st serum</th>
<th>2nd serum</th>
<th>p (&lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG anti-Ro/SSA</td>
<td>91</td>
<td>32</td>
<td>97</td>
<td>33</td>
<td>59</td>
<td>64</td>
<td>ns</td>
</tr>
<tr>
<td>IgA anti-Ro/SSA</td>
<td>25/95</td>
<td>7/34</td>
<td>31/95</td>
<td>10/34</td>
<td>18/61</td>
<td>21/61</td>
<td>ns</td>
</tr>
<tr>
<td>IgM anti-Ro/SSA</td>
<td>19</td>
<td>5</td>
<td>20</td>
<td>6</td>
<td>14</td>
<td>14</td>
<td>ns</td>
</tr>
</tbody>
</table>

### Table III. Different isotype combinations of anti-Ro/SSA antibodies in 95 patients affected by UCTD.

<table>
<thead>
<tr>
<th>Isotype</th>
<th>1st serum</th>
<th>2nd serum</th>
<th>1st serum</th>
<th>2nd serum</th>
<th>1st serum</th>
<th>2nd serum</th>
<th>p (&lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated IgG</td>
<td>52 (55)</td>
<td>50 (52.6)</td>
<td>21 (62)</td>
<td>18 (53)</td>
<td>31 (51)</td>
<td>32 (52.4)</td>
<td>ns</td>
</tr>
<tr>
<td>IgG+IgA</td>
<td>16 (16.8)</td>
<td>22 (23)</td>
<td>5 (15)</td>
<td>8 (23.5)</td>
<td>11 (18)</td>
<td>14 (23)</td>
<td>ns</td>
</tr>
<tr>
<td>IgG+IgM</td>
<td>10 (10.5)</td>
<td>11 (11.6)</td>
<td>3 (8.8)</td>
<td>4 (12)</td>
<td>7 (11.5)</td>
<td>7 (11.5)</td>
<td>ns</td>
</tr>
<tr>
<td>IgG+IgA+IgM</td>
<td>9 (9.4)</td>
<td>9 (9.4)</td>
<td>2 (5.8)</td>
<td>2 (5.8)</td>
<td>7 (11.5)</td>
<td>7 (11.5)</td>
<td>ns</td>
</tr>
<tr>
<td>Negative</td>
<td>8 (8.4)</td>
<td>3 (3.1)</td>
<td>3 (8.8)</td>
<td>2 (5.8)</td>
<td>5 (8.2)</td>
<td>1 (1.6)</td>
<td>ns</td>
</tr>
</tbody>
</table>
antibody concentration of IgG class (p = 0.023) (Fig. 1), while IgM and IgA anti-Ro/SSA antibodies did not significantly change.

Discussion

The choice of anti-Ro/SSA antibodies as a model for the study of the evolution of the antibody response is due to their frequency in UCTD, and to the behaviour of the Ro/SSA antigen in autoimmunity. The immune response to Ro antigen in SLE shows a maturation in terms of isotypical switch, increase of IgG titer and affinity in the antibody link. IgM anti-Ro/SSA are produced during the initial phase of the immune response and their titer declines with the increase of IgG titer and affinity (8).

In our study, we found that IgG dominates the immune response to Ro/SSA from the beginning of the illness, and no patient had anti-Ro/SSA antibodies of IgM class isolated in the serum. Probably, the phase of IgM production preceded or it was coincidental with the onset of the initial symptoms of the disease, and it was already completed when the patients went to our first observation. Furthermore, Arbuckle et al. (7) demonstrated that anti-Ro/SSA antibodies are detectable in the serum years before the clinical onset of SLE.

No substantial modification of the Ig classes was observed during follow-up for the isotype switch of anti-Ro/SSA antibodies. The variation of antibody classes was minimal, and mostly represented by the addition of IgA and by the loss of IgM in the anti-Ro/SSA reactivity. Few reports analysed the isotype distribution and switching of anti-Ro/SSA in CTD: IgM anti-Ro/SSA can be detected in about half of SLE patients together with IgG anti-Ro/SSA (11), while other authors showed a preferential response of IgA anti-Ro/SSA directed to 52 kD Ro, in about 80% of patients with pSS and 73% of SLE (12).

In our study, only a minority of patients presented IgM and IgA anti-Ro/SSA both in stable and evolved UCTD, at onset and during follow-up. These discrepancies could be ascribed either to the different diseases studied, or to different assays employed. In the present study, IgA anti-Ro/SSA are detected in 25-33% of the sera. This frequency is lower than what found with semiquantitative immunoblot (12), but we can confirm that IgA anti-Ro/SSA were not detected in patients also negative for IgM and IgG. The antibody titer of IgA, IgM and IgG anti-Ro/SSA did not differ between the groups of patients with UCTD. A trend to increase of the IgG and IgA isotypes in the evolved group of patients could be noted. These changes seem to reflect a state of immune activation and maturation in patients with UCTD and clinical evolution. In fact, in patients evolved in pSS a significant increase of the IgG anti-Ro/SSA titer was detected (p = 0.0235).

In conclusion, the present study shows that IgG anti-Ro/SSA dominate the immune response in patients with UCTD, and no substantial modification of the antibody isotype against Ro/SSA peptides was observed. During follow-up, only IgG anti-Ro/SSA significantly raised in patients evolving in pSS. This finding represents, with other clinical and immunological features, a risk factor for the evolution from UCTD to other connective tissue diseases.

References

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