Positivity for antinuclear antibody in patients with advanced rheumatoid arthritis.

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Abstract

Some patients with rheumatoid arthritis (RA) as well as those with other collagen diseases are positive for antinuclear antibody (ANA). We investigated the frequency of positivity for ANA in 104 patients with RA and evaluated the clinical features and laboratory data in the ANA-positive and -negative groups. The presence of ANA in sera was studied by indirect immunofluorescence using HEp-2 cells as the antigen substrate. Sera with a positive fluorescence at a dilution of 1:20 were considered to be positive for ANA. Of the 104 patients, 39 (37.5%) were positive for ANA. The staining pattern in the positive cases varied, but most were speckled (64.1%) and homogeneous (48.7%). A small number showed a nucleolar (20.5%) or a centromere (10.3%) pattern. None showed a shaggy pattern. The ANA titer was lower in RA patients compared with those with other collagen-related diseases such as systemic lupus erythematosus or progressive systemic sclerosis. None of the patients positive for ANA with either a nucleolar or centromere staining pattern had progressive systemic sclerosis or the CREST syndrome. One patient each had Raynaud’s phenomenon and pulmonary fibrosis. There was no correlation between ANA positivity and indicators of joint inflammation. The prevalence of ANA positivity in patients with advanced or prolonged disease was higher than those with early stages or short durations. There was no correlation with drug therapy.

KEYWORDS: joint inflammation, autoantibody, immunofluorescence, HEp-2 cells, d-penicillamine

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Positivity for Antinuclear Antibody in Patients with Advanced Rheumatoid Arthritis

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Some patients with rheumatoid arthritis (RA) as well as those with other collagen diseases are positive for antinuclear antibody (ANA). We investigated the frequency of positivity for ANA in 104 patients with RA and evaluated the clinical features and laboratory data in the ANA-positive and -negative groups. The presence of ANA in sera was studied by indirect immunofluorescence using HEP-2 cells as the antigen substrate. Sera with a positive fluorescence at a dilution of 1:20 were considered to be positive for ANA. Of the 104 patients, 39 (37.5%) were positive for ANA. The staining pattern in the positive cases varied, but most were speckled (64.1%) and homogeneous (48.7%). A small number showed a nucleolar (20.5%) or a centromere (10.3%) pattern. None showed a shaggy pattern. The ANA titer was lower in RA patients compared with those with other collagen-related diseases such as systemic lupus erythematosus or progressive systemic sclerosis. None of the patients positive for ANA with either a nucleolar or centromere staining pattern had progressive systemic sclerosis or the CREST syndrome. One patient each had Raynaud's phenomenon and pulmonary fibrosis. There was no correlation between ANA positivity and indicators of joint inflammation. The prevalence of ANA positivity in patients with advanced or prolonged disease was higher than those with early stages or short durations. There was no correlation with drug therapy.

Key words: joint inflammation, autoantibody, immunofluorescence, HEP-2 cells, d-penicillamine

The detection of serum antinuclear antibody (ANA) by indirect immunofluorescence in patients with collagen disease allows one to differentially diagnose such disorders without the need for more advanced techniques of molecular biology (1, 2). Interpretation of the sensitivity and specificity (3, 4) of ANA results is influenced by the use of HEP-2 cells, mouse kidney, or rat liver in such testing. While the sensitivity of mouse kidney is superior to that of HEP-2 cells, nucleolar and centromere staining patterns can be detected only by using HEP-2 cells (3). As a result, HEP-2 cells are widely used as an antigen substrate for detecting ANA (5, 6).

ANA have been detected in patients with various connective tissue diseases, autoimmune thyroiditis, infection and cancer (6). Nearly 100 % of patients with systemic lupus erythematosus (SLE) are reportedly positive for ANA (2). ANA positivity in patients with rheumatoid arthritis (RA) ranges from 42 % to 74 % (2). Quismorio et al. (7) reported that the frequency of ANA positivity in RA did not differ in response to the presence of extraarticular manifestations or of systemic vasculitis. Camus et al. (8) reported that patients with RA who were treated with d-penicillamine showed a higher incidence of positivity for ANA than an untreated group.

In the present study, we evaluated the frequency of ANA positivity and staining pattern for ANA in 104 patients with RA using HEP-2 cells as antigen substrate for indirect immunofluorescence, and evaluated the relationship of the findings to the patients' clinical features.

Patients and Methods

Patients. A total of 104 Japanese patients who fulfilled the 1987 criteria of the American Rheumatism

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Association for RA (9) were randomly chosen for study. There were 15 men and 89 women, ranging from 26 to 79 years of age (mean 57.2 years). Those whose symptoms were associated with Sjögren’s syndrome or that overlapped with those of other collagen diseases were excluded, as such patients reportedly have a high prevalence of positivity for ANA (2). The clinical profile of the patients studied appears in Table 1.

The duration of disease ranged from 2 months to 38 years (mean 8.5 years). Disease stage and functional class were assigned according to Steinbrocker et al. (10). Seventeen patients had Stage I, 20 had Stage II, 38 had Stage III, and 29 had Stage IV disease. Considering the class of disease, 12 patients had I, 80 had II, 11 had III, and 1 had IV. Treatment being administered at the inception of study is shown in Table 1.

Methods. ANA was detected by the indirect immunofluorescence technique using HEP-2 cells as the antigen substrate (Fluoro-HEPANA test; MBL, Nagoya, Japan). Sera that showed a positive fluorescence at a dilution of 1:20 were considered as positive for ANA. The erythrocyte sedimentation rate (ESR) was determined by the Westergren method. C-reactive protein (CRP), serum immunoglobulin, and rheumatoid factor were assayed by nephelometry. Anti-SS-A and anti-SS-B antibody were detected by double radial immunodiffusion (DRID). All patients were evaluated for the characteristic clinical features of progressive systemic sclerosis (PSS) or of the calcinosis, Raynaud’s phenomenon, elongation of esophagus, sclerodactyly, telangiectasia (CREST) syndromes such as calcinosis, Raynaud’s phenomenon, scleroderma, pulmonary fibrosis, short frenulum of the tongue, sclerodactyly, telangiectasia, and esophageal hypomotility (11).

Statistics. The data in Figs. 1 and 2 were analyzed by the chi-square test for contingency tables for significant differences. A P value less than 0.05 was considered statistically significant.

Results

Sera of 39 (37.5 %) of the 104 patients were positive for ANA (Table 2). The staining patterns for ANA varied (Table 3). The speckled pattern was the most common, with 25 (64 %) of the 39 sera positive for ANA showing this pattern. Thirteen sera with a speckled

<table>
<thead>
<tr>
<th>Stage</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: 17</td>
<td>I: 12</td>
</tr>
<tr>
<td>II: 20</td>
<td>II: 80</td>
</tr>
<tr>
<td>III: 38</td>
<td>III: 11</td>
</tr>
<tr>
<td>IV: 29</td>
<td>IV: 1</td>
</tr>
</tbody>
</table>

Drug therapy

<table>
<thead>
<tr>
<th>No medication</th>
<th>NSAIDs alone</th>
<th>Gold compounds</th>
<th>Sulfasalazine</th>
<th>Salazosynprin</th>
<th>Immunomodulators</th>
<th>Immunosuppressants</th>
<th>Steroid hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>6</td>
<td>50</td>
<td>56</td>
<td>12</td>
<td>11</td>
<td>25</td>
<td>52</td>
</tr>
</tbody>
</table>

NSAIDs: Non-steroidal anti-inflammatory drugs.

Table 2  Incidence of anti-nuclear antibody (ANA) in sera from 104 patients with rheumatoid arthritis

<table>
<thead>
<tr>
<th>ANAα</th>
<th>Number of cases</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>65</td>
<td>62.5</td>
</tr>
<tr>
<td>Positive</td>
<td>39</td>
<td>37.5</td>
</tr>
</tbody>
</table>

α: ANA was detected by indirect immunofluorescence using HEP-2 cells as the nuclear substrate at a serum dilution of 1:20.

Table 3  Staining pattern of ANA-positive sera from 39 patients with rheumatoid arthritis

<table>
<thead>
<tr>
<th>Staining pattern</th>
<th>Number of cases</th>
<th>% of ANA-positive sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneous (Ho)</td>
<td>19</td>
<td>48.7</td>
</tr>
<tr>
<td>Ho alone</td>
<td>5</td>
<td>12.8</td>
</tr>
<tr>
<td>Ho + Sp</td>
<td>11</td>
<td>28.2</td>
</tr>
<tr>
<td>Ho + Nuc</td>
<td>3</td>
<td>7.7</td>
</tr>
<tr>
<td>Speckled (Sp)</td>
<td>25</td>
<td>64.1</td>
</tr>
<tr>
<td>Sp alone</td>
<td>12</td>
<td>30.8</td>
</tr>
<tr>
<td>Sp + Ho</td>
<td>11</td>
<td>28.2</td>
</tr>
<tr>
<td>Sp + Nuc</td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>Nucleolar (Nuc)</td>
<td>8</td>
<td>20.5</td>
</tr>
<tr>
<td>Nuc alone</td>
<td>3</td>
<td>7.1</td>
</tr>
<tr>
<td>Nuc – Ho</td>
<td>3</td>
<td>7.1</td>
</tr>
<tr>
<td>Nuc + Sp</td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>Centromere</td>
<td>4</td>
<td>10.3</td>
</tr>
<tr>
<td>Shaggy</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

ANA: See Table 1.
pattern showed either a homogeneous or a nucleolar pattern, and 12 specimens showed only a speckled pattern. The pattern was homogeneous in 19 (49%), nucleolar in 8 (21%), and a centromere pattern was seen in 4 (10%). Some specimens showed a combination of these patterns; none of the specimens showed a shaggy pattern. The ANA titer in most of the RA patients positive for ANA was below $\times 80$, except for two patients who showed $\times 160$ and $\times 1280$, respectively.

We evaluated the clinical characteristics of all RA patients studied, and focused on 12 RA patients whose sera were positive for ANA and who showed a nucleolar or centromere pattern. The ANA titer was below $\times 40$ in all but 2 of the 12 cases, and these 2 patients had a titer of $\times 160$ and $\times 1280$, respectively. ANA positivity and staining pattern were evaluated repeatedly in these 12 patients; the centromere pattern became negative in one of 4 patients who had previously shown a positive pattern, while 3 patients continued to show a centromere pattern. Three of the 8 patients with a nucleolar pattern became ANA-negative, with 3 of the 8 changing to a speckled pattern. Two of the 8 patients continued to show the same nucleolar pattern. One of the 4 patients with a centromere pattern had pulmonary fibrosis, and one of the 8 patients with a nucleolar pattern had Raynaud’s phenomenon. None of the remaining 10 patients exhibited clinical symptoms suggestive of either PSS or CREST. In addition, no characteristic disease duration, stage or class and values for ESR, CRP and RF was observed in those 12 patients (data not shown).

The presence of anti-SS-A and anti-SS-B antibody was evaluated in 35 out of 39 ANA-positive sera. Four of 35 sera were positive for SS-A and one was positive for SS-B. None of these SS-A or SS-B positive patients with RA had the clinical features of Sjögren’s syndrome.

Comparison of laboratory findings (WBC, Hb, ESR, CRP levels, immunoglobulins and rheumatoid factor) in the ANA-positive and ANA-negative patients showed no significant differences (data not shown).

The prevalence of ANA positivity was analyzed according to the stage of RA (Fig. 1). The percentage of ANA positivity in 17 RA patients with stage I was 12%, 40% in 20 with stage II, 42% in 38 with stage III, and 45% in 29 with stage IV. ANA positivity was significantly correlated with advanced stage of disease ($P < 0.05$). When patients were divided into three groups according to duration of disease (group 1: $\leq 2$ years, group 2: $2 < \sim \leq 5$ years, and group 3: $5 < \sim$ years), a significant correlation was found between ANA positivity and disease duration (Fig. 2). ANA positivity was signifi-
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Discussion

ANA positivity has also been detected in a small number (6.9%) of 290 healthy Swedish adults (aged 20-88 years) at a titer of 1:40 (4) and in 5% of 72 healthy Japanese adults at a titer of 1:20 (12). Though positivity for ANA is a nonspecific finding (6), the presence of ANA is important in the diagnosis of certain connective tissue diseases. Patients with rheumatoid arthritis demonstrated serum ANA (7, 8); the frequency ranges from less than 10% to greater than 70%, depending on the substrate used in testing (7, 13, 14). Human epithelial HEp-2 cells have replaced the rodent liver or kidney as a substrate; several reports have shown that the HEp-2 substrate is more sensitive than such rat sections (15). The present study evaluated the frequency of ANA positivity in patients with RA using HEp-2 cells as the antigen substrate, and investigated the clinical features of the ANA-positive patients. ANA was demonstrated in 39 of the 104 patients (37.5%). The percent ANA positivity in this investigation resembled that in the previous studies in which rodent liver and kidney were used as substrates (16, 17).

ANA titers of 1:20 and 1:40 were common in the RA patients studied. One patient was ANA-positive (nucleolar pattern) with a titer of 1:1280 and another patient (speckled pattern) had a titer of 1:160. In a retrospective study, Shiel and Jason found that a statistically significant proportion of patients with reciprocal titers of greater than 320 had connective tissue or organ-specific autoimmune diseases (6). Regarding the association between ANA staining pattern and the type of collagen disease, Miller et al. (15) reported that the ANA-positive staining pattern in 13 out of 22 RA sera was speckled but not other patterns, and a homogeneous pattern was predominant in patients with SLE. Thus, in the presence of a homogeneous pattern, the possible diagnosis of SLE should not be excluded, even if the ANA titer is low (15). In the present study, a high proportion (48.7%) of RA sera with positivity for ANA showed a homogeneous pattern, but the patients lacked the clinical features of SLE.

ANA has been detected in patients with progressive systemic sclerosis (PSS) by indirect immunofluorescence methods using HEp-2 cells as a substrate with a nucleolar pattern (18). In the present study, 8 cases of a nucleolar staining pattern lacked the clinical features of PSS. A previous report (19) suggested that the centromere staining pattern was highly specific for patients with the CREST syndrome. This pattern was detected with HEp-2 cells but not with rat liver sections, in which mitotic figures are sparse. This pattern is due to an antibody that reacts with the chromosome region of HEp-2 cells in metaphase (15). In the present study, 4 RA patients with a centromere pattern lacked the clinical features of the CREST syndrome.

Sjögren's syndrome is often complicated by RA. Since anti-SS-A and anti-SS-B antibody may be contained in ANA-positive groups and may have increased the positivity for ANA in the present study, we evaluated serum anti-SS-A and anti-SS-B antibodies in the ANA-positive patients. Anti-SS-A antibody was detected in 4 of the 35 patients tested, while anti-SS-B antibody was detected in only one of the patients. None of these anti-SS-A or anti-SS-B-positive patients with RA had the clinical features of Sjögren's syndrome. However, the presence of subclinical Sjögren's syndrome could not be excluded.

While ANA positivity may not have been related to
disease activity, the stage and duration of RA were related to a high ANA positivity. ANA may thus be involved in the late phase of the autoimmune process, or it may be an epiphenomenon in RA as an anti-neutrophil cytoplasmic antibody (20). The present observations suggest that ANA is a marker for patients with chronic severe RA, not for early disease.

SLE is known to be a complication of d-penicillamine administration (21). Camus et al. (8) found a high incidence of ANA positivity in patients who were receiving treatment with d-penicillamine. However, Weinstein and Rothfield (22) demonstrated that the frequency of ANA positivity was not increased in a group of patients administered d-penicillamine compared with a group given placebo. We compared ANA positivity with the type of treatment and found that ANA positivity was not increased in patients receiving sulphydryl compounds, i.e., d-penicillamine or bucillamine. However, only 5 patients were receiving d-penicillamine, whereas 51 were receiving bucillamine in the present study. Positivity for ANA was not related to the drug administered, as shown in Fig. 3.

Thus, the ANA positivity of patients with RA was correlated with an advanced stage of disease and a prolonged duration of disease, and was unrelated to drug therapy.

References


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