Significance of laminar antikeratin antibodies to rat oesophagus in rheumatoid arthritis

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SUMMARY  Antikeratin antibodies reacting in a laminar distribution with keratinised rat oesophagus were found in the sera of a proportion of patients with rheumatoid arthritis but not in healthy controls. In rheumatoid arthritis (RA) the proportion of sera exhibiting this reactivity varied with the site tested in the rat's upper alimentary tract. There were 36·4% of 99 patients with RA who gave positive reactivity to the middle third of the rat oesophagus. This antikeratin reactivity was related to the occurrence of other antitissue antibodies (to reticulin, gastric parietal cells, smooth muscle, mitochondria, or nuclear components) in the same patients with rheumatoid arthritis. It was not related to the duration of early morning stiffness, the Ritchie index, the erythrocyte sedimentation rate, cerain acute phase proteins (haptoglobin and C-reactive protein) nor to the levels of haemoglobin or immunoglobulins. Antikeratin antibodies were not specific for rheumatoid arthritis and also occurred in 50% of 16 patients with progressive systemic sclerosis.

The keratins are a group of fibrous insoluble proteins. Their inter-relationships are complex and they form a markedly heterogeneous group. Experimental evidence for the antigenicity of these proteins was first reported in 1939 by Pillémer el al. In 1979 Young et al. described the presence of antikeratin antibodies (AKA) in human sera reactive with the keratinised tissue of rat oesophagus. They showed that AKA were present in a significant proportion of patients with rheumatoid arthritis (RA) and they suggested AKA may be of diagnostic value in RA. We have investigated the relationship of AKA positivity in patients with RA to the clinical, haematological, and immunological changes of the disease and have evaluated the clinical relevance of tests for this anti-tissue antibody.

Patients and methods

PATIENT STUDIED
Ninety-nine consecutive patients attending a rheumatology clinic with classical or definite RA were studied. They had a mean age of 57·3 years (range 23–82 years), a male to female ratio of 38:61, and a mean disease duration of 10·1 years (range 1–39 years). Their duration of early morning stiffness (in minutes), the Ritchie index, and whether rheumatoid nodules were present were recorded.

Two further groups were studied. Fifty healthy controls attending a blood transfusion centre were one group, and the other consisted of 16 patients with progressive systemic sclerosis (PSS).

IMMUNOHISTOLOGICAL TECHNIQUES
Antitissue antibodies were detected by indirect immunofluorescence. Fresh rat oesophagus (middle third), stomach (with gastro-oesophageal junction), salivary gland, and kidney and human skin were snap frozen in liquid nitrogen and 5 μm frozen sections mounted on glass slides and fixed in absolute acetone at 4°C for 60 seconds. Human serum was used diluted 1:5 with pH 6·8 phosphate buffered saline (PBS) and fluorescein conjugated sheep antihuman immunoglobulin (IgG) antiserum at a dilution of 1:20 with PBS. Sections were examined on a Zeiss microscope, with an HBO 50 W mercury lamp and the following filters: 2 KP 490 blue interference filters, an LP418 ultraviolet barrier, and LP520 orange barrier, an FT510 chromatic beam splitter, and a KP 560 minus-red interference filter.

Accepted for publication 5 September 1980.
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PARAMETERS OF DISEASE ACTIVITY MEASURED

In the patients with RA blood was taken for estimation of haemoglobin, platelet count, and erythrocyte sedimentation rate (Westergren). Serum was stored (at −70°C) until analysed. The sheep cell agglutination titre was measured, serum immunoglobulins (IgG, IgA, and IgM) and acute phase proteins (C-reactive protein and haptoglobin) were estimated by single radial immunodiffusion.7

Results

Sera giving laminar staining in sections of the middle third of the rat oesophagus, in a pattern similar to that in Fig. 1, were considered positive for the presence of AKA. This pattern was distinctive and easily recognised. It was also seen at the gastro-oesophageal junction (Fig. 2). Occasional sera from the patients with RA also showed diffuse or speckled staining of the superficial layers of the rat oesophagus. Diffuse staining of this kind was inconsistently related to the laminar pattern and varied markedly in intensity, sometimes being so weak and diffuse as to be difficult to distinguish from nonspecific background fluorescence. Since this pattern was easily distinguished, when it occurred, from the laminar pattern it was disregarded for the purposes of the present study.

AKA reactivity to keratin in the middle third of the rat oesophagus was present in 36 of the 99 patients with RA but none of the healthy controls (Table 1). This is a highly significant finding (P < 0.001). AKA reactivity at this site was titred in 6 randomly selected positive sera. Reactivity was detected to a titre of 1/80 in 3 cases, 1/320 in 1 case, and 1/640 in 2 cases.

AKA reactivity was found to vary with the anatomical site from which rat tissue was taken. The sera from some patients with RA reacted only with the keratin in the middle third of the oesophagus. Other sera gave positive reactions only at the gastro-oesophageal junction, and other sera were reactive at both sites. These differences are summarised in Table 2. Five sera from patients with RA with AKA reactivity to rat oesophagus were also examined for reactivity with homologous tissue, with sections of human skin being used as the test substrate. All 5 sera showed binding of IgG to human stratum corneum. The pattern of staining was broadly similar to that given by the same sera with rat oesophagus (Fig. 3).

Table 1  Incidence AKA to rat oesophagus in RA

<table>
<thead>
<tr>
<th></th>
<th>RA (n = 99)</th>
<th>Controls (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKA +ve</td>
<td>36.4%</td>
<td>0%</td>
</tr>
<tr>
<td>AKA -ve</td>
<td>63.6%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2  Relationship of AKA reactivity to anatomical site in RA

<table>
<thead>
<tr>
<th>Anatomical site</th>
<th>AKA reactivity per cent patients with RA (n = 99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive midoesophagus</td>
<td>36.4%</td>
</tr>
<tr>
<td>Positive gastro-oesophageal junction</td>
<td>39.4%</td>
</tr>
<tr>
<td>Positive mideoesophagus and gastro-oesophageal junction</td>
<td>20.2%</td>
</tr>
<tr>
<td>Positive mideoesophagus and negative gastro-oesophageal junction</td>
<td>16.2%</td>
</tr>
<tr>
<td>Negative mideoesophagus and positive gastro-oesophageal junction</td>
<td>17.2%</td>
</tr>
</tbody>
</table>
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Table 4 Relationship of AKA to rat oesophagus to the presence of IgM rheumatoid factor and rheumatoid nodules

<table>
<thead>
<tr>
<th>AKA presence</th>
<th>AKA negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCAT negative (&lt;1:32)</td>
<td>38.8%</td>
</tr>
<tr>
<td>SCAT positive (1:32 or more)</td>
<td>61.2%</td>
</tr>
<tr>
<td>Nodules absent</td>
<td>50.0%</td>
</tr>
<tr>
<td>Nodules present</td>
<td>50.0%</td>
</tr>
</tbody>
</table>

SCAT = Sheep cell agglutination test.

Table 5 Relationship of AKA to rat oesophagus to clinical haematological and immunological parameters in RA (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Antikeratin antibodies positive (n=36)</th>
<th>Antikeratin antibodies negative (n=63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early morning stiffness (minutes)</td>
<td>57.0 ± 56.1</td>
<td>72.5 ± 56.3</td>
</tr>
<tr>
<td>Ritchie index</td>
<td>12.1 ± 14.0</td>
<td>11.3 ± 11.7</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>44.5 ± 52.5</td>
<td>41.6 ± 35.5</td>
</tr>
<tr>
<td>Haptoglobin (g/l)</td>
<td>2.56 ± 1.24</td>
<td>2.27 ± 0.93</td>
</tr>
<tr>
<td>ESR (mm/1 h)</td>
<td>41.2 ± 25.6</td>
<td>45.3 ± 22.9</td>
</tr>
<tr>
<td>IgG (g/l)</td>
<td>17.5 ± 3.6</td>
<td>11.7 ± 4.2</td>
</tr>
<tr>
<td>IgA (g/l)</td>
<td>3.49 ± 2.13</td>
<td>2.73 ± 1.42</td>
</tr>
<tr>
<td>IgM (g/l)</td>
<td>1.59 ± 0.85</td>
<td>1.89 ± 1.00</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>12.9 ± 1.5</td>
<td>12.3 ± 1.8</td>
</tr>
<tr>
<td>Platelets (&lt;10^6/l)</td>
<td>371 ± 154</td>
<td>280 ± 174</td>
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</tbody>
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index was marginally higher in AKA-positive patients but the duration of morning stiffness marginally less; AKA-positive patients had slightly higher C-reactive protein and haptoglobin levels but slightly lower ESR values.

Of the 16 sera from patients with PSS 8 (50%) had AKA reactivity to rat oesophagus. These patients also showed a relationship between AKA reactivity and antitissue antibodies. Antinuclear antibodies were present in 7 cases, and 5 of these also had AKA reactivity. Other antitissue antibodies were too infrequent to evaluate whether their presence was correlated with AKA reactivity in sera from this disease.

Discussion

Our results confirm the original observations of Young et al. that AKA occur in the sera of a proportion of cases of RA. Similar results have recently also been reported by Johnson et al. The actual percentage of cases showing AKA reactivity in each series has differed. This probably reflects differences in case selection and in the criteria adopted for defining AKA reactivity. But Johnson et al. found, as we have done, that the reaction of this antibody with rat keratin varies with the anatomical site in the upper alimentary tract yielding the sections used for testing. Moreover, all

All the other antitissue antibodies studied occurred with increased frequency in patients with RA who showed AKA reactivity to rat oesophagus, as shown in Table 3. However, in view of the relatively small numbers involved there was no statistical correlation between AKA reactivity and any particular antitissue antibody. But on grouping together the results of tests for reactivity with reticulin, smooth muscle, gastric parietal cells, mitochondria, and nuclear components it was found that the 36 AKA positive sera gave positive reactions in 38 out of 180 tests for these antitissue antibodies as a group, whereas the 63 AKA negative sera contained these antibodies in only 41 out of 315 such tests, which is a significant difference ($\chi^2=5.60; DF=1; p<0.02$). Similarly more AKA positive patients had positive sheep cell agglutination tests for IgM rheumatoid factor and had rheumatoid nodules (Table 4).

There was no relationship between AKA positivity and clinical, haematological, and other parameters of disease activity in RA (Table 5). Not only were none of these parameters statistically related to AKA reactivity but no clear pattern emerged on a detailed assessment of the results. For example, the Ritchie

Fig. 3 Deposition of IgG in the stratum corneum of human skin; the serum with this reactivity (demonstrated by indirect immunofluorescence) also had AKA reactivity with rat oesophagus. (× 533).
3 studies have agreed in finding no reactivity to oesophageal keratin (as opposed to that of the gastro-oesophageal junction) in the sera of healthy subjects.

Another possible technical difference between the foregoing studies and our's lies in the particular pattern of immunofluorescent staining accepted as characteristic of AKA reactivity. In our study we have restricted the designation of a positive reaction for AKA antibodies to those sera showing a distinctive and easily distinguished laminar pattern of staining for reasons discussed above. Our decision to reject weak and diffuse staining of the keratinised layer we would acknowledge to be an arbitrary one which may be open to debate.

It was nevertheless interesting that, even after adopting this restriction, we encountered sera showing variable AKA reactivity between tissue taken from the oesophagus and from the gastrooesophageal junction. This suggested that differences may exist in the antigenicity, or in the access to antigenic determinants, of keratin at different sites. It was also of interest that the limited number of AKA-positive sera so far tested against an homologous tissue (human skin) have all shown reactivity with cells of the stratum corneum. We have not yet attempted the absorption of such sera with human skin homogenates to see whether reactivity to rat keratin is thus removed, but these experiments are planned and the results will be reported in due course.

Antibodies reactive with an undefined antigen of stratum corneum have been reported in a proportion of normal sera and with greatly increased frequency in patients with psoriasis—particularly psoriatics with erythroderma and arthritis. We have not yet had the opportunity to examine many sera from patients with psoriatic arthropathy, but on examining specimens from patients with another connective tissue disease often affecting the skin and upper alimentary tract, progressive systemic sclerosis, we have encountered a high proportion of AKA reactivity. This finding makes it clear that AKA reactivity is not 'disease specific' for RA.

In addition, although AKA reactivity was broadly related to the occurrence, in the same sera from RA patients, of other antitissue antibodies and showed some relation to IgM rheumatoid factor and rheumatoid nodules, yet there was an absence of any relationship to other parameters of disease activity. With regard, therefore, to the original proposition that testing for AKA may be of diagnostic assistance in RA, we offer the following considerations:

(i) Testing for AKA antibodies can indeed be carried out in such a way as to discriminate between RA and controls. We consider this is best done by testing sera against rat oesophagus (rather than against tissue from the gastro-oesophageal junctions) and by accepting only a laminar pattern of fluorescence. This appears to eliminate 'false positive' reactions in the sera of healthy persons.

(ii) However, in our hands, as in those of others, the test thus performed is positive in less than 50% of cases otherwise acceptable as RA, so the test is relatively insensitive (i.e., gives many 'false negatives').

(iii) Even in those instances in which the test is positive in patients with RA, no relation is discernible with various established indices of disease activity. The test is thus unsuitable for monitoring the progress of RA.

(iv) As mentioned above, the test is also not 'disease specific' for RA but is positive with equal or greater frequency in cases of progressive systemic sclerosis.

The application to practical diagnostic and prognostic use in the management of RA of a test which is insensitive, unsuitable for longitudinal studies, and which is not disease specific is not something we would wish to recommend. Our final conclusion is therefore that, while it is of theoretical interest to confirm that yet another immunological aberration (in this case, reactivity with a heterologous antigen, rat keratin) is demonstrable in the sera of some cases of certain connective tissue diseases, we feel the test is of very limited value, even as a screening procedure in rheumatology.

Financial support for this project came from the Endowment Fund of the Medical Research Committee, Central Birmingham Health District.

References

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doi: 10.1136/ard.40.3.267

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