Antinuclear antibodies in systemic lupus erythematosus

K.Y. Fong, MBBS, M. Med (Int. Med)
M.L. Boey, MBBS, M. Med (Int. Med), AM
H.S. Howe, MBBS, M. Med (Int. Med)
P.H. Feng, FRCP (E), FRCP (G), MD, AM

Department of Medicine IV
Tan Tock Seng Hospital
Moulmein Road
Singapore 1130

Summary

Autoantibodies to the three extractable nuclear antigens (ENA), Anti-SSA (Ro), Anti-Sm, Anti-RNP and antinuclear antibodies were determined in 150 patients with SLE. Seventy patients (46.7%) had Anti-SSA (Ro), 40 (26.7%) Anti-Sm and 25 (16.7%) Anti-RNP antibodies. Ninety four percent patients had a positive Fluorescent anti-nuclear antibody (FANA) test. The commonest FANA pattern is the speckled pattern. Subclinical keratoconjunctivitis sicca (KCS) was present in 60% patients. No correlation could be demonstrated between the presence of ENA autoantibodies and the clinical features of patients.

Key words: Anti-SSA (Ro) antibodies, Anti-Sm antibodies, Anti-RNP antibodies, Keratoconjunctivitis sicca, Systemic lupus erythematosus.

Introduction

The identification of the LE cells in 1948 led to a series of discoveries of autoantibodies in SLE. In the 1950s, Coon and co-workers developed the indirect immunofluorescence test which was used to demonstrate the presence of antibodies that reacted with various nuclear substrates. This was later used to aid diagnosis of SLE and the Fluorescent anti-nuclear antibody (FANA) test became an important screening test. Some immunofluorescent patterns such as the anticentromere and antinucleolar patterns denotes specific antibodies. Others are less specific and identification of these antibodies require further immunoserologic procedures. Extractable nuclear antigens (ENA) refers to antigens that are saline soluble like the Sm, RNP and Ro antigens. Antibodies to the Sm antigen are highly specific for SLE. Anti-RNP antibodies on the other hand can be found in the sera of patients with other systemic rheumatic diseases such as mixed connective tissue disease (MCTD). Anti-SSA (Ro) antibodies are reported to be associated with several clinical entities. They include photosensitivity, keratoconjunctivitis sicca,¹ anti-nuclear antibody negative lupus, neonatal lupus syndrome, subacute cutaneous lupus and homozygous C2, C4 deficiency.²,³ The frequency of occurrence of these antibodies varies with different SLE populations. This study attempts to determine the frequency of these autoantibodies in our patients with SLE and to identify clinical correlations.

Materials and methods

Patient selection: One hundred and fifty patients who satisfied the revised criteria for classification of SLE⁴ were studied over a six month period from July to December 1987. Patients were
selected from the wards and outpatient clinics. Patients who were on drugs known to cause the lupus syndrome were excluded. Clinical data was obtained retrospectively from hospital records of patients. The consistency of recording and accuracy of these data were good. All patients were under the care of one of the authors. Serological tests were done on fresh sera of all patients.

The study group comprised 124 Chinese (82.6%), 19 Malays (12.7%), six Indians (4%) and one Eurasian (0.7%). These percentages were comparable to the Singapore population composition at the end of 1986.5

Fifty patients (47 females and three males) were randomly selected for opthalmological screening. Keratoconjunctivitis sicca (KCS) was diagnosed if a) Shirmer’s test strips showed <5mm wetting after five minutes, and/or b) positive rose bengal corneal staining.6

Methods: All patients had autoantibodies to ENA and Fluorescent antinuclear antibody tests determined. Autoantibodies to ENA were determined by the counter-immunoelectrophoretic method using calf thymus extract as antigen. FANA was determined using Hep-2 cells as substrate and at serial dilutions of 1:40, 1:160 and 1:640. The immunofluorescence patterns were reported as homogenous, speckled, peripheral (or rim), nucleolar, centromere or a mixed pattern of the above FANA was considered positive at a dilution of 1:40 and above. ANA-negative lupus patients were defined as those having at least two negative FANA tests done three months or more apart. Tests were repeated in cases where the results were equivocal.

Statistical test: Chi-square test was used in assessing statistical significance.

Results

These were 140 (94%) female and ten (6%) male patients, giving a female to male ratio of 14:1. Their ages ranged from 11 to 59 years with a mean age of 23.6 years. Ten (6%) patients had ANA-negative Lupus. Five of them had Anti-Ro/SSA antibodies and one had Anti-Sm antibodies. 70 (46.7%) patients had Anti-Ro/SSA antibodies, 40 (26.7%) Anti-Sm antibodies and 25 (16.7%) Anti-RNP antibodies. Fig. 1 shows the frequency of antoantibodies in 150 SLE patients. The frequency of the FANA patients is shown in Fig. 2. Patients with mixed patterns showed either a Speckled/Peripheral pattern (six patients) or a Homogenous/Peripheral pattern (two patients).

The frequency of Anti-Ro/SSA antibodies in the various ethnic groups is shown in Table I. Chinese patients had the highest percentage (50%). Table II shows the correlation between clinical features and ENA autoantibodies. Thirty-one (62%) of the 50 patients had KCS. All except one were asymptomatic. Seventeen (54.8%) of 31 patients had Anti-Ro/SSA antibodies.

Discussion

Anti-SSA (Ro) antibody was the commonest antibody to ENA in our SLE patients. Among Chinese lupus patients, 50% have this autoantibody. A separate study showed a higher prevalence of 63%.15 This is comparable to Japanese figures of 51% to 58%.10,11 Anti-SSA (Ro) often accompanied by Anti-SSB (La) is found in SLE in a frequency varying between 20% to 30%.1,7,8,9 These antibodies are found in up to 70% of patients with primary Sjogren’s Syndrome and in the sicca complex associated with SLA.2 Keratoconjunctivitis sicca was present in 62% of our
Fig. 1  Frequency of autoantibodies in 150 SLE patients

![Bar graph showing frequency of autoantibodies](image)

No. of patients

<table>
<thead>
<tr>
<th>No. of ENA autoantibodies present</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Fig. 2  Frequency of the various FANA patterns

![Pie chart showing frequency of various FANA patterns](image)

- **Homogenous (29%)**
- **Speckled (51.6%)**
- **Mixed (8.6%)**
- **Nucleolar (7.5%)**
- **Peripheral (2.2%)**
- **Centromere (1%)**
Table I
Frequency of Anti-Ro/SSA antibodies according to ethnic groups

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>Chinese</th>
<th>Malay</th>
<th>Indian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with Anti-Ro/SSA</td>
<td>62/124</td>
<td>6/9</td>
<td>2/6</td>
</tr>
<tr>
<td>Percentage (%)</td>
<td>50</td>
<td>31.6</td>
<td>33.3</td>
</tr>
</tbody>
</table>

Table II
Correlation between clinical features and presence of ENA autoantibodies

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Autoantibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ro(+)</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>11</td>
</tr>
<tr>
<td>Serositis</td>
<td>11</td>
</tr>
<tr>
<td>Renal Involvement</td>
<td>25</td>
</tr>
<tr>
<td>Neurological Involvement</td>
<td>20</td>
</tr>
<tr>
<td>Malar Rash</td>
<td>39</td>
</tr>
<tr>
<td>Discoid Rash</td>
<td>3</td>
</tr>
</tbody>
</table>

N.S. = Not Significant
N.S.* = Not Significant except for * (p 0.05)
X^2 T = Chi-square Test

patients. There is a likelihood that sicca symptoms may develop in this subset of patients. The higher frequency of Anti-SSA (Ro) in our population compared to Western series may be linked to genetic factors. HLA-DR2 have been found to be associated with presence of Anti-SSA (Ro).16 A significant excess of HLA-DR2 in Southern Chinese lupus patients have been recently demonstrated.17 The possible involvement of genetic factor in this situation awaits further study and confirmation.

Anti-Sm antibodies were present in 25% of our patients and this corresponds closely with Western series.7,12 However, the prevalence of Anti-RNP antibodies was much lower.7,13 Anti-RNP was associated with less renal disease. This is likely to be due to the association of Anti-RNP with milder lupus disease.13

There was no correlation of clinical features such as malar rash, photosensitivity, serositis and neurological involvement with the three antibodies assayed. This is not surprising as SLE is a disease marked by a proliferation of autoantibodies. It is therefore difficult to associate clinical features with single autoantibodies.

Keratoconjunctivitis sicca was present in 62% of patients. This high prevalence of KCS is noteworthy. The occurrence of KCS has been reported to be 1.3 – 28%.14,18 Subclinical Sjogren's
Syndrome is definitely common and all lupus patients should undergo ophthalmological screening on diagnosis.

In conclusion, our study shows differences in antibody profile between Oriental and Caucasian lupus patients. These differences could undoubtedly be accounted for by patient selection bias and referral pattern. However, the role of genetic factors, hormones and environment in disease manifestations and antibody production deserve further study. The elucidation of these factors will contribute greatly to our understanding of the autoimmune process in SLE.

Acknowledgement
The authors wish to thank Dr. E. Y. Yap for assistance in ophthalmological assessment of patients, Miss F. Rajadurai for excellent secretarial assistance, Mr. E.S. Koh and his staff for assistance in tracing medical records.

References


