ANTI-JO-1 ANTIBODIES IN POLYMYOSITIS OR DERMATOMYOSITIS: EVALUATION BY ELISA USING RECOMBINANT FUSION PROTEIN JO-1 AS ANTIGEN

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SUMMARY
We evaluated an enzyme-linked immunosorbent assay (ELISA) for detecting anti-Jo-1 antibodies in patients with polymyositis (PM) or dermatomyositis (DM) by use of the recombinant fusion protein Jo-1. Sera from 64 patients with PM or DM, from 80 patients with other connective tissue diseases, and from 64 healthy subjects matched for age, sex and race, were studied by the ELISA and by the double immunodiffusion (DID) method. Eight patients with myositis (six PM, one DM and one DM with malignancy) with positive anti-Jo-1 by DID also showed positive results by the ELISA method, whereas five patients with positive anti-Jo-1 by this ELISA showed negative results on DID. One of the five had non-specific results. The incidence of positive results for anti-Jo-1 with the ELISA (18.8%) was greater than that for DID (12.5%), but the difference was not statistically significant. All patients with positive results for anti-Jo-1 by DID were also positive by the ELISA. The ELISA system with the recombinant Jo-1 antigen was useful in the detection of anti-Jo-1 antibodies in patients with PM/DM.

KEY WORDS: Jo-1 antibodies, Polymyositis, Dermatomyositis, Recombinant protein antigen, ELISA, Double immunodiffusion, Jo-1 antibody syndrome, Anti-synthetase syndrome.

VARIABLES autoantibodies to nuclear and cytoplasmic antigens are characteristic of patients with polymyositis (PM) and dermatomyositis (DM). Among the antigens to which these autoantibodies are targeted are aminoacyl-tRNA synthetases (histidyl-tRNA synthetase (Jo-1), threonyl-tRNA synthetase (PL-7), alanyl-tRNA synthetase (PL-12), isoleucyl-tRNA synthetase (OJ) and glycyl-tRNA synthetase (EJ)), signal recognition particle (SRP), Mi-2, PM-Scl and Ku [1]. The anti-Jo-1 antibody [2] is most common, and is clinically the most important, as a marker antibody for PM, especially in those patients with interstitial lung disease [3].

Anti-Jo-1 antibodies had been estimated variously by double immunodiffusion (DID) [2, 3], counter-immunoelectrophoresis [4, 5], Western blotting [6], enzyme-linked immunosorbent assay (ELISA) using the column-purified Jo-1 antigen [6, 7], or the enzyme inhibitory activity method [6].

Recently, an ELISA with recombinant fusion proteins has been substituted for DID in the detection of various autoantibodies, such as antibodies to Sm [8], nRNP [9], Ro(SSA) [10], La(SSB) [11], topoisomerase-1 [12] and centromere (CEMP-B) [13]. Anti-Mi-2 antibody [14, 15], a DM-specific autoantibody [14], is also useful in an ELISA system using recombinant antigen [16].

There are no previous published reports of an ELISA system with recombinant fusion Jo-1 antigen for the detection of anti-Jo-1 antibodies; this is the first such report.

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ELISA

For the ELISA, 10 µg protein/ml of the GST–Jo-1 antigen dissolved in 10 mM Tris–HCl (pH 7.6), 6 M urea and 0.01% β-octyl-glucoside was prepared. Polystyrene microtitre plates with a flat bottom (Nunc Maxisorp; Intermed, Co., Roskilde, Denmark) were coated with 100 µl of the GST–Jo-1 antigen solution overnight at 4°C. Blocking of the sensitized wells was carried out with phosphate-buffered saline (PBS), 1% bovine serum albumin (BSA) (Intergen Co., New York, USA) and 5% sucrose for 2 h at room temperature (20–25°C). The plates were dried. Then 100 µl/well of the sera to be tested were diluted 1:200 in PBS (pH 7.4), and 1% BSA was added. This was incubated for 1 h at room temperature. After four washings with PBS (pH 7.4) and 0.1% Tween 20, 100 µl/well of horseradish peroxidase-conjugated mouse anti-human γ-chain (Medical and Biological Laboratories, Co., Nagoya, Japan) diluted 1:5000 were added and incubated for 1 h at room temperature. After four washings with the same buffer, 100 µl/well of 3,3′,5,5′-tetramethylbenzidine as substrate were added and incubated for 30 min at room temperature. The reaction was stopped by 0.1 M phosphate.

Plates were read by a microplate reader MPR-A4i (Tohsoh, Co., Tokyo, Japan) at 450 nm. Each sample was estimated in duplicate.

For controls, antibodies to recombinant Sm antigen (Inmunovision, Co., Springdale, AR, USA) and to GST were estimated using the same ELISA system.

Inhibition test

Inhibition of ELISA activity of the anti-Jo-1-positive sera was carried out simultaneously by the recombinant GST–Jo-1 antigen and native column-purified Sm antigen (Inmunovision, Springdale, AR, USA) as control. If the binding of these sera was not inhibited significantly (> 50% of optical density (OD)) by the recombinant GST–Jo-1 antigen, it was considered a sign of non-specific binding or of binding to a contaminant.

Statistical methods

χ² tests with correction were used for 2 × 2 tables.

RESULTS

Purity and antigenic specificity of the recombinant GST–Jo-1 antigen

SDS–PAGE analysis of the recombinant GST–Jo-1 fusion protein showed a 76 kDa main band, which is compatible with the molecular weight (MW) of a complex of two proteins: GST (MW = 25 kDa) and Jo-1 protein (MW ~ 50 kDa) [5, 23]. The main band had ~ 90% homogeneity, as determined by Coomassie blue staining on SDS–PAGE (Fig. 1). The main band and the thinner band located just beneath it, which was possibly a degradation product of the main band, had Jo-1 antigenic activity, as confirmed by the immunoblotting procedure (data not shown).
Anti-Jo-1 ELISA
Sera from the 64 healthy control subjects produced a mean ± 3 s.d. \( \text{OD}_{450} \) for Jo-1 binding of 1.29 ± 3.70 absorbance units (AU). The assay result was considered positive if the \( \text{OD}_{450} \) value at a serum dilution of 1:100 was ≥ 5.0 AU.

The incidence of anti-Jo-1 antibodies in the ELISA and DID, and the actual AU values in the ELISA, are shown in Table I and Fig. 2, respectively. In the ELISA system, 13 patients showed positive results (≥ 5 AU). The positive sera of 12 of the 13 patients were specifically absorbed by the GST–Jo-1 antigen. However, the anti-GST ELISA of all 13 anti-Jo-1-positive sera yielded negative results. Representative results of the absorption tests are shown in Fig. 3.

In one PM patient, the positive anti-Jo-1 activity was absorbed not only by the GST–Jo-1 antigen, but also by the Sm antigen used as the control. This patient’s serum contained two precipitin lines in DID, but their antigenic specificities differed from the Jo-1 and the Sm antibody system. In the other 12 patients positive for anti-GST-Jo-1, four had negative DID results: one had DM alone, one had PM–SLE overlap, one had DM with malignancy and one had Sjögren’s syndrome with polymyositis and elevated serum creatine kinase levels but no proximal muscle weakness or interstitial lung disease. The incidence of the anti-Jo-1 by the ELISA system (18.8%) vs DID (12.5%) did not differ significantly (\( \chi^2 = 0.535, P = 0.4646 \)). Seven (58.3%) of the 12 anti-Jo-1-positive patients had interstitial lung disease. On the other hand, the anti-Jo-1 antibodies were lacking in three (37%) of eight PM patients with interstitial lung disease. All patients who were positive for anti-Jo-1 by DID also had positive results in the ELISA system. No patients tested positive for anti-Jo-1 in DID but not in the ELISA system.

Among the 15 patients with a diagnosis of ‘possible’ PM/DM, one tested positive for anti-Jo-1 in the ELISA, but not in DID. This patient exhibited interstitial lung disease, arthritis and Raynaud’s phenomenon.

**DISCUSSION**

An ELISA that uses recombinant antigens has replaced DID in tertiary care hospitals in Japan and

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**Table I**
Incidence of antibodies to the recombinant GST-Jo-1

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of patients</th>
<th>No. positive in ELISA (%)</th>
<th>No. positive in DID* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic inflammatory myopathies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymyositis (PM)</td>
<td>21</td>
<td>7 (33.3)</td>
<td>6 (28.6)</td>
</tr>
<tr>
<td>Dermatomyositis (DM)</td>
<td>19</td>
<td>2 (10.5)</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>Overlap with other</td>
<td>11</td>
<td>1 (9.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>connective tissue diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Childhood DM</td>
<td>3</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>PM/DM with malignancy</td>
<td>10</td>
<td>2 (20.0)</td>
<td>1 (10.0)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>64</td>
<td>12 (18.8)</td>
<td>8 (12.5)</td>
</tr>
<tr>
<td>Other connective tissue diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>20</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Systemic sclerosis</td>
<td>20</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>20</td>
<td>1 (5.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>20</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>64</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

*Double immunodiffusion.
other countries for the detection of various autoantibodies, such as those to Sm, nRNP, Ro(SSA), La(SSB) and topoisomerase-1. However, an ELISA system for the anti-Jo-1 antibody has not been available until now. This report is the first to demonstrate the feasibility of recombinant Jo-1 antigen for the anti-Jo-1 ELISA.

Previous studies [1–3] have shown that the Jo-1 antibody is highly specific for myositis, being much more common in PM than in DM, especially in those patients with interstitial lung disease [3]; it is rare in childhood DM [24]. The present report confirmed these findings.

Compared with DID, ELISA is generally superior, with a greater sensitivity. Since Jo-1 antibodies are found only in a small portion of the general population with myositis [1–3], and since serum anti-Jo-1 antibody levels can fluctuate [3, 25], detection of low titres of Jo-1 antibodies may be useful. Such cases may be false negative by DID and positive by ELISA, as reported by Targoff and Reichlin [6] in one patient. We found five additional patients with the ELISA using the recombinant antigen. The weakly positive activities for anti-Jo-1 in ELISA were genuine in most cases, as evidenced by the absorption tests. One serum sample, however, demonstrated a non-specific or false-positive result. The presence of a non-specific reaction in the ELISA system is a problem that needs to be resolved, whatever the specificity of the antigen. In addition, since our GST–Jo-1 antigen contains ~10% contaminants (components of E. coli), the absorption tests used in the present study were themselves incomplete. In theory, a completely pure GST–Jo-1 antigen should be used in the absorption tests. However, impurity of the antigen does not seem to present a practical problem, since anti-Jo-1 has thus far been found solely in patients affected by myositis.

Anti-Jo-1 antibody was detected in the serum of a patient with possible PM and interstitial lung disease, arthritis and Raynaud’s phenomenon. This patient had the so-called Jo-1 antibody syndrome [26] or anti-
synthetase syndrome [27]. ‘Possible’ PM/DM could be an appropriate diagnosis for this distinct subset of idiopathic inflammatory myopathies.

Recent advances in serology suggest that a disease entity should be established by its serological characteristics as well as by its clinical and pathological characteristics. Revised criteria for SLE [18], RA [20] and Sjögren’s syndrome [28] reflect this concept. For more than 20 yr, however, physicians have used the diagnostic criteria for PM/DM [17] which lack serological factors as elements of diagnosis. The classification criteria for PM/DM as recently reported in Japan [29] have introduced the Jo-1 antibody as a diagnostic criterion for PM/DM.

In conclusion, an ELISA system that employed the recombinant Jo-1 antigen proved to be a useful replacement for DID in the detection of anti-Jo-1 antibodies in patients with PM/DM.

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