Concise report

Clinical features of anti-TIF1-α antibody-positive dermatomyositis patients are closely associated with coexistent dermatomyositis-specific autoantibodies and anti-TIF1-γ or anti-Mi-2 autoantibodies

Yoshinao Muro¹, Asuka Ishikawa¹, Kazumitsu Sugiura¹ and Masashi Akiyama¹

Abstract

Objective. Myositis-specific autoantibodies (MSAs), which characterize certain forms of inflammatory myopathy, are useful in the diagnosis and prediction of prognosis in DM/PM. Anti-transcriptional intermediary factor 1-α (TIF1-α) antibodies were recently reported to be associated with cancer-associated DM in conjunction with anti-TIF1-γ antibodies. This study aimed to identify a subset of DM patients who have anti-TIF1-α antibodies by using biotinylated recombinant proteins and to clarify the clinical and other serological features of DM patients with these antibodies.

Methods. Sera from 202 Japanese patients with CTDs, including 108 with DM and 20 healthy controls, were screened for anti-TIF1-α antibodies by our novel ELISAs. Positive sera were further examined by immunoprecipitation and also investigated for the detection of anti-TIF1-γ and anti-Mi-2 antibodies.

Results. Sera from 12 patients with DM were confirmed to be positive for anti-TIF1-α antibodies. None of the patients with other CTDs and none of the healthy controls had the antibodies. Seven anti-TIF1-α-positive patients simultaneously had anti-TIF1-γ antibodies and the other five had anti-Mi-2 antibodies, both of which are well known to be MSAs. These double-positive patients with anti-TIF1-α and anti-γ antibodies included three JDM and two cancer-associated adult DM patients, whereas all the double-positive patients with anti-TIF1-α and anti-Mi-2 antibodies were classical adult DM.

Conclusion. Although MSAs have been regarded as mutually exclusive, anti-Mi-2 antibody-positive patients simultaneously have anti-TIF1-α antibodies. Anti-Mi-2 antibody-positive patients are associated with classical DM without cancer even with the simultaneous presence of anti-TIF1-α antibodies.

Key words: autoantibodies, dermatomyositis, Mi-2, TIF1-α, TIF1-γ.

Introduction

The idiopathic inflammatory myopathies (IIMs) are a group of systemic autoimmune diseases that include PM, DM and inclusion body myopathies [1]. Several myositis-specific autoantibodies (MSAs) are associated with certain clinical forms of IIMs, and they are useful tools for predicting the prognosis. For example, anti-MDA5-antibody-positive patients demonstrate rapid progressive interstitial lung disease (ILD) and anti-transcriptional intermediary factor 1-γ (TIF1-γ) antibody-positive patients are often complicated with cancer [2]. Very recently, anti-p155/140 antibodies, which are serological markers of cancer-associated DM [3, 4], were analysed by Fujimoto et al. [5]. They determined that p140 is identical to TIF1-α, whereas p155 is known as TIF1-γ [6]. Their study showed that TIF1-β was also targeted in DM patients, but infrequently, although anti-TIF1-γ antibodies alone were frequently detected. Interestingly, anti-TIF1-α antibodies were always associated with anti-TIF1-γ antibodies.

MSAs have been regarded as mutually exclusive [1, 7]. Fujimoto et al. [5] showed that autoantibodies against the
TIF1 protein family occurred in various combinations, often in patients with cancer-associated DM. However, they concentrated on analysing anti-p155/140-positive sera in a large cohort to find autoantibodies against the TIF1 protein family. In this study, we investigated sera from patients with various CTDs for the presence of anti-TIF1-α antibodies by using our developed ELISAs and immunoprecipitation (IPP) with biotinylated recombinant protein. We confirmed that anti-TIF1-α antibodies are specific to DM and are often associated with anti-TIF1-γ antibodies. Surprisingly, the other anti-TIF1-α antibody-positive sera also had anti-Mi-2 antibodies.

Materials and methods

Patients and sera

From the serum bank of the Department of Dermatology, Nagoya University Hospital, we used sera from 202 Japanese patients with CTDs. They consisted of 108 patients with DM [including 13 with JDM, 38 with clinically amyopathic DM (ADM) and 15 with cancer-associated DM], 9 with PM, 24 with SLE, 20 with SSc, 26 with SS and 15 with myositis overlap syndrome (Table 1). Sera from 21 cancer patients that were used in our previous study [2] were also analysed. Twenty healthy individuals were assessed as normal controls. All the DM patients except those with clinical ADM and all the PM patients fulfilled Bohan and Peter’s criteria [8, 9]. All the clinically ADM patients fulfilled Sontheimer’s criteria [10]. The clinically ADM group included patients who had developed ILD within 6 months after disease onset. Patients were classified as having JDM if they were aged <16 years at the onset of DM [11] and as cancer-associated DM if internal malignancy was diagnosed within 3 years (before or after) of the DM diagnosis [12]. The criteria of other CTDs were based on the established criteria for these diseases used in our previous studies [2, 13]. The ages at disease onset and gender ratios of each clinical group are summarized in Table 1. All the patients and healthy individuals gave fully informed consent for participation. This study was approved by the Ethics Committee of Nagoya University Graduate School of Medicine and conducted in accordance with the Declaration of Helsinki.

ELISAs

Specific binding of serum antibodies to recombinant TIF1-α or Mi-2β was analysed using our recently established sensitive ELISA [14]. This method was based on our previous protocol, which quantitatively measured the antibodies against MDA-5 [15]. Instead of a conventional optical system, this ELISA uses a microplate luminometer for increased sensitivity, thereby reducing the amount of recombinant protein required for the assays. The full-length TIF1-α cDNA clone was purchased from Kazusa DNA Research Institute (Chiba, Japan). The full-length Mi-2β cDNA clone [16] was a kind gift from Kazusa DNA Research Institute.

Table 1 Patient groups and anti-TIF1-α antibody frequencies

<table>
<thead>
<tr>
<th>Clinical group</th>
<th>Age at onset, mean (range), years</th>
<th>Gender, M:F</th>
<th>Total, n</th>
<th>Anti-TIF1-α (n = 12)</th>
<th>Anti-TIF1-γ</th>
<th>Anti-Mi-2</th>
<th>Anti-TIF1-α ELISA unita, mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total DM</td>
<td>47 (1–80)</td>
<td>33:75</td>
<td>108</td>
<td>7</td>
<td>5</td>
<td></td>
<td>41.9 (–12.8 to 472.5)</td>
</tr>
<tr>
<td>Clinically ADM</td>
<td>45 (1–73)</td>
<td>9:29</td>
<td>38</td>
<td>5</td>
<td>5</td>
<td></td>
<td>42.1 (–4.3 to 366.4)</td>
</tr>
<tr>
<td>Cancer-associated DM</td>
<td>65 (48–80)</td>
<td>6:9</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td></td>
<td>45.8 (–6.3 to 279.0)</td>
</tr>
<tr>
<td>Classical DM</td>
<td>44 (1–80)</td>
<td>18:37</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td></td>
<td>40.7 (–12.8 to 472.5)</td>
</tr>
<tr>
<td>Adult DM</td>
<td>52 (19–80)</td>
<td>26:69</td>
<td>95</td>
<td>4</td>
<td>5</td>
<td></td>
<td>38.3 (–12.8 to 472.5)</td>
</tr>
<tr>
<td>Clinically ADM</td>
<td>51 (20–73)</td>
<td>5:27</td>
<td>32</td>
<td>2</td>
<td>0</td>
<td></td>
<td>23.7 (–4.3 to 268.4)</td>
</tr>
<tr>
<td>Cancer-associated DM</td>
<td>65 (48–80)</td>
<td>6:9</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td></td>
<td>45.8 (–6.3 to 279.0)</td>
</tr>
<tr>
<td>Classical DM</td>
<td>49 (19–80)</td>
<td>15:33</td>
<td>48</td>
<td>0</td>
<td>5</td>
<td></td>
<td>45.8 (–12.8 to 472.5)</td>
</tr>
<tr>
<td>Juvenile DM</td>
<td>9 (1–16)</td>
<td>7:6</td>
<td>13</td>
<td>3</td>
<td>0</td>
<td></td>
<td>68.0 (13.8 to 366.4)</td>
</tr>
<tr>
<td>Clinically ADM</td>
<td>6 (1–11)</td>
<td>4:2</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td></td>
<td>140.0 (13.8 to 366.4)</td>
</tr>
<tr>
<td>Classical DM</td>
<td>11 (1–16)</td>
<td>3:4</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td></td>
<td>6.2 (–6.4 to 16.3)</td>
</tr>
<tr>
<td>Total of other CTDs</td>
<td>42 (15–81)</td>
<td>11:83</td>
<td>94</td>
<td>0</td>
<td>15.2 (–30.5 to 63.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>52 (32–67)</td>
<td>1:8</td>
<td>9</td>
<td>0</td>
<td>27.9 (13.1 to 63.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLE</td>
<td>30 (15–51)</td>
<td>4:20</td>
<td>24</td>
<td>0</td>
<td>11.1 (–30.5 to 43.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSc</td>
<td>40 (22–55)</td>
<td>0:20</td>
<td>20</td>
<td>0</td>
<td>16.1 (–23.2 to 58.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overlap syndrome</td>
<td>49 (23–69)</td>
<td>2:13</td>
<td>15</td>
<td>0</td>
<td>16.9 (7.8 to 62.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>47 (21–81)</td>
<td>4:22</td>
<td>26</td>
<td>0</td>
<td>13.0 (10.2 to 39.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>58 (48–78)b</td>
<td>5:15b</td>
<td>21</td>
<td>0c</td>
<td>Not done</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The cut-off level is 121.3 and 83.0 U based on 5 S.D.s and 3 S.D.s above the mean value obtained from 20 healthy control sera, respectively. No patient had an ELISA in the range of 83.0–121.3 U. M:F = male:female. Information on the age and gender of one patient was not available. All sera from cancer patients were examined for anti-TIF1-α antibodies by IPP.
from Drs Kato and Takahashi at Nagoya University. Biotinylated recombinant protein was produced from the cDNA, using the transcription and translation (TnT) T7 Quick Coupled Transcription/Translation System (Promega, Madison, WI, USA) according to our protocol(Fig. 1A) [2, 15]. Nunc Immobilizer Streptavidin Plates (Thermo Scientific Nunc, Roskilde, Denmark) were pre-washed three times with PBS containing 0.05% Tween-20 (T-PBS) and were coated with 1 μl of TnT product diluted with T-PBS (50 μl/well) and incubated for 1 h at room temperature. After three washes with T-PBS, the wells were blocked with 200 μl of a blocking buffer of 0.5% BSA (Wako, Osaka, Japan) in T-PBS for 1 h. Uncoated wells were used to measure the background levels for each sample. Sample sera diluted with blocking buffer (50 μl/well) were incubated for 1 h at room temperature, followed by incubation with anti-human IgG antibody conjugated with horseradish peroxidase (HRP) (Dako, Glostrup, Denmark) (50 μl/well) at 1:30,000 dilution. After incubation for 1 h at room temperature, the plates were washed and incubated with SuperSignal ELISA Femto Maximum Sensitivity Substrate (Thermo Scientific Pierce, Rockford, IL, USA) (50 μl/well) as the substrate. Relative luminescence unit (RLU) number was then determined using the GloMax-Multi Detection System (Promega). Each serum sample was tested in duplicate, and the mean RLU subtracted background was used for data analysis.

**Fig. 1** Anti-TIF1-α antibodies coexist with anti-TIF1-γ or anti-Mi-2 antibodies.

(A) Biotinylated recombinant proteins used in our laboratory were subjected to 7.5% SDS-PAGE and analysed by immunoblotting. Lane 1: Mi-2β; lane 2: TIF1-γ; lane 3: TIF1-α; lane 4: NXP-2; lane 5: MDA-5. Mi-2β was shown at the larger size of 250 kDa, probably due to the addition of N-terminal haemagglutinin tag and C-terminal V6His tag indicated by an asterisk [16]. (B) IPP of recombinant TIF1-α. In.: the input was a full dose (10 μl) of biotinylated TIF1-α protein that was used for the IPP assay. Lanes 1–8 contain the TIF1-α immunoprecipitated by the sera of different DM patients. In lanes 9–11, anti-TIF1-γ-positive sera from DM patients, which immunoprecipitated TIF1-γ, did not immunoprecipitate TIF1-α. N: normal control. (C) IPP of recombinant TIF1-γ. In.: the input was half the dose (5 μl) of the biotinylated TIF1-γ protein used for the IPP assay. Lanes 1–6 contain the TIF1-γ immunoprecipitated by the sera of different DM patients. In lanes 7–11, anti-TIF1-α/γ-positive sera from DM patients, which immunoprecipitated TIF1-α and TIF1-γ, did not immunoprecipitate TIF1-γ. N: normal control. (D) IPP of recombinant Mi-2β. In.: the input was half the dose (5 μl) of the biotinylated Mi-2β protein used for the IPP assay. Lanes 1–5 contain the Mi-2β immunoprecipitated by the sera of different DM patients. In lanes 6–9, anti-TIF1-α/γ-positive sera from DM patients, which immunoprecipitated TIF1-α and TIF1-γ, did not immunoprecipitate Mi-2β. N: normal control. (E) Immunoprecipitates from cell extracts with anti-TIF1-α-positive sera were probed with anti-Mi-2α MoAb (upper panel) and with anti-TIF1-α PolyclAb (lower panel). Lane In. contains a half dose of the input of K562 cell extracts. Lane N: normal control; lanes 1–5: anti-TIF1-α/Mi-2-positive sera; lane 6, anti-TIF1-α/TIF1-γ-positive serum.
A standard curve was obtained from serial concentrations of a serum sample containing a high titre of the autoantibody against each antigen.

**Detection of autoantibodies using IPP**

IPP was performed using TnT products of TIF1-α, Mi-2β or TIF1-γ as previously described [2]. Briefly, 10 μl of patient sera was mixed and incubated with 20 μl of a 50% slurry of Protein G Sepharose (GE Healthcare, Buckinghamshire, UK) and 270 μl IPP buffer (PBS containing 1% Nonidet P-40) at 4°C for 1 h. Sepharose beads were mixed and incubated with 270 μl binding buffer (IPP buffer containing 0.5% BSA) and 10 μl of the TnT products, which was pre-cleared using the sepharose beads, at 4°C for 1 h. The beads were washed five times with IPP buffer and suspended in Laemmli sample buffer, and the IgG-bound proteins were electrophoresed on 7.5% SDS-PAGE gel. Immunoblot was performed as previously described [2].

**IPP—western blotting**

IPP—western blotting was performed using K562 cell extracts without chemical cross-linking [17]. Monoclonal anti-Mi-2α and polyclonal anti-TIF1-α antibodies were purchased from Sigma-Aldrich (St Louis, MO, USA) and MBL (Nagoya, Japan), respectively. HRP-conjugated anti-mouse immunoglobulin and anti-rabbit IgG antibodies were purchased from DAKO (Glostrup, Denmark).

**Statistical analysis**

The frequency of antibodies between disease groups was analysed using chi-square test with Yates’ correction. P < 0.05 was considered statistically significant.

**Results**

**ELISA and IPP with biotinylated recombinant TIF1-α**

For the screening of anti-TIF1-α antibodies in a large number of serum samples we used an ELISA system with biotinylated recombinant TIF1-α. We examined a total of 202 serum samples from patients with various CTDs. When the cut-off level was set at 121.3 U, based on 5 s.d.s above the mean value obtained from 20 healthy control sera, the 12 sera from patients with DM were positive for anti-TIF1-α antibodies, whereas none of the sera from patients with other CTDs was positive for those antibodies (12 positive sera/108 total DM sera vs 0/94 total CTD sera, P = 0.0012) (Table 1). Even based on the cut-off level (83.0) at 3 s.d.s above the mean value, the results were not different.

After the initial screening by ELISA, we investigated antibodies against TIF1-α in sera from 12 anti-TIF1-α ELISA-positive patients and several anti-TIF1-α ELISA-negative patients in order to confirm their ability to immunoprecipitate biotinylated recombinant TIF1-α. All 12 anti-TIF1-α-positive sera in ELISA showed a distinct protein band with a molecular weight of 140 kDa in IPP (Fig. 1B). Twelve anti-TIF1-α-negative sera in ELISA and six healthy control sera did not immunoprecipitate the recombinant. Moreover, none of the 21 serum samples from cancer patients immunoprecipitated (data not shown).

**Coexistence of anti-TIF1-α and anti-TIF1-γ antibodies**

According to the previous report [5], anti-TIF1-α antibodies always coexist with anti-TIF1-γ antibodies. Our anti-TIF1-α-positive sera were examined for anti-TIF1-γ antibodies by IPP (Fig. 1C). Unexpectedly, only 7 of the 12 sera were also positive for anti-TIF1-γ antibodies. Since IPP for anti-TIF1-γ antibodies had been performed on 81 sera from DM patients in our previous study [2], we examined the antibodies by IPP for the remaining 27 sera. We found a total of 16 sera with anti-TIF1-γ antibodies among the 108 patients with DM. Summarizing the results, in the present DM cohort of 108 patients, 12 and 16 patients had anti-TIF1-α antibodies and anti-TIF1-γ antibodies, respectively. Only 7 patients had both anti-TIF1-α and anti-TIF1-γ antibodies (anti-TIF1-α/γ-positive), and 87 patients had neither anti-TIF1-α antibodies nor anti-TIF1-γ antibodies.

**Coexistence of anti-TIF1-α and anti-Mi-2 antibodies**

As mentioned above, we found five anti-TIF1-α-positive, anti-TIF1-γ-negative sera. Previously, two of them had been examined and identified as positive for anti-Mi-2 antibodies by IPP with cell extract (data not shown). Thus, in the present study, we investigated anti-Mi-2 antibodies by IPP with the recombinant Mi-2β in the 12 anti-TIF1-α-positive sera (Fig. 1D). All five of the anti-TIF1-α-positive, anti-TIF1-γ-negative sera were positive for anti-Mi-2 antibodies, whereas none of the seven anti-TIF1-α/γ-positive sera was positive for anti-Mi-2 antibodies. In addition, we investigated anti-Mi-2 antibodies for all the sera from the present DM cohort by ELISA with recombinant Mi-2β, although we detected no additional anti-Mi-2-positive sera except for the five sera described above (data not shown). The IPP—western blotting results showed that each protein precipitated by the five sera reacted to mAb against Mi-2α (Fig. 1E, upper panel) and to polyclonal antibody against TIF1-α (Fig. 1E, lower panel). These sera were confirmed to have anti-Mi-2 antibodies, which, in general, react to both Mi-2α and Mi-2β isoforms [7] and to have anti-TIF1-α antibodies.

**Clinical features of DM patients with anti-TIF1-α antibodies**

Of the 12 anti-TIF1-α-positive patients, 5 patients with anti-Mi-2 antibodies were classical DM without cancer/ILD, a subset of DM that is associated with anti-Mi-2 antibodies [1, 7]. In contrast, the seven patients with anti-TIF1-α/γ antibodies consisted of three patients with JDM, two with adult ADM and two with cancer-associated DM (Table 1). Demographic data of nine adult patients with anti-TIF1-α antibodies, including gender (male: female = 2:7) and age at onset of adult DM [mean (s.o.) = 60.0 (13.5)], and clinical features, including the presence of cutaneous signs of DM (heliotrope rash or Gottron’s
papules), RP and elevated creatine kinase, were not statistically different compared with anti-TIF1-α-negative patients (data not shown). However, the presence of ILD in anti-TIF1-α-positive patients was significantly less than that for anti-TIF1-α-negative patients (0/9 vs 40/86, \(P = 0.0098\)).

We investigated other MSA or myositis-associated autoantibodies, e.g. anti-MDA5, -MJ, -PL-7, -PL-12, -EJ and -KS (by IPP with TnT product), -Jo-1, -SS-A and -U1-RNP (by commercial ELISA kits) in the 12 anti-TIF1-α antibody-positive patients. Only two patients had concomitant anti-SS-A antibodies, and no other autoantibodies were found.

**Discussion**

Recently, Fujimoto et al. [5] reported that the TIF1 protein family of TIF1-α, -β and -γ is an autoimmune target in DM, especially in cancer-associated DM and JDM patients. Our study clarified that anti-TIF1-α antibodies were not specific markers of a certain subset of DM and that clinical features in the antibody-positive patients were influenced by the other coexistent antibodies against TIF1-γ or Mi-2. The most surprising result in our study is that all classical DM patients with anti-Mi-2 antibodies simultaneously carried anti-TIF1-α antibodies in our cohort, because MSAs have been generally regarded as mutually exclusive [1, 7].

Another new finding in this study was that anti-TIF1-α antibodies were found only in patients with DM and not in those with other CTDs or cancer. The previous report [5] investigated the prevalence of the autoantibodies only in DM patients.

The different result in our study from the previous study is that anti-TIF1-α/γ-positive sera (7/108) are seen less than anti-TIF1-γ-positive, anti-TIF1-α-negative sera (9/108). Fujimoto et al. [5] showed that anti-TIF1-α/γ-positive sera (\(n = 52\)) were more numerous than anti-TIF1-γ-positive, anti-TIF1-α-negative sera (\(n = 25\)). This discrepancy might be caused by the difference of experimental methods. They evaluated anti-TIF1-α/γ-positive sera using conventional IPP assays, whereas our assays use original recombinant proteins that we have developed. The stringency of our IPP buffer was much higher than that of their methods (the present methods, 1% NP-40 and 150 mM NaCl vs their methods, 0.1% NP-40 and 50 mM NaCl). They noted the possibility that TIF1-γ IPP would be caused by anti-TIF1-α antibodies that have cross-reactivity to a TIF1-γ sequence. Our assay may have failed to find such cross-reactive antibodies with low avidity.

Some sera from myositis patients have shown several positive polypeptide bands around 140–160 kDa on SDS-PAGE by IPP, corresponding to anti-NXP-2, -MDA5, -TIF1-γ and -OJ antibodies [18]. Although anti-Mi-2 antibodies mainly target Mi-2α/β of ~240 kDa, bands immunoprecipitated by the antibodies included polypeptides ~140 kDa [18, 19]. The Mi-2/NuRD complex is involved in multiple transcriptional regulatory processes and contains many components [20]. Although TIF1-β was reported to interact with Mi-2α [21], there have been no reports of the NuRD complex containing TIF1-α.

**Rheumatology key messages**

- Anti-TIF1-α antibodies are detected in DM patients with anti-TIF1-γ or anti-Mi-2 antibodies.
- Anti-Mi-2 antibodies are associated with classical DM even when coexistent with anti-TIF1-α antibodies.

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