Anti-Zo antibodies in Japanese myositis patients detected by a newly developed ELISA

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Abstract

Objective

The myositis-specific autoantibodies that characterise certain forms of idiopathic inflammatory myopathy (IIM) are useful for diagnosing dermatomyositis (DM) / polymyositis (PM) and predicting its prognosis. The autoantibody to phenylalanyl-tRNA synthetase (anti-Zo) has been identified as a disease marker antibody for anti-synthetase syndrome only in a UK cohort. Here we aim to establish an ELISA for the measurement of anti-Zo and to characterise the clinical features of Japanese patients who have this autoantibody.

Methods

Anti-Zo was investigated by immunoprecipitation with recombinant phenylalanyl-tRNA synthetase α/β proteins. The results were confirmed by immunoprecipitation-Western blotting with cell extract. Sera from patients with DM/PM (n=224) were screened by an ELISA with the recombinant proteins. Medical records were retrospectively reviewed to obtain detailed information on the clinical phenotypes of the anti-Zo-positive patients.

Results

Only two male patients were confirmed to have anti-Zo. Both patients had fever, myopathy, interstitial lung disease, and mechanic's hands, and these clinical features are consistent with those of anti-synthetase syndrome. Another patient's serum showed a higher level than the cut-off value for anti-phenylalanyl-tRNA synthetase α by our in-house ELISA, but was judged to be negative for anti-Zo by immunoprecipitation-Western blotting.

Conclusion

This is the first report of anti-Zo-positive IIM patients from Asia. Although Japanese patients with anti-Zo have a clinical phenotype similar to that of Caucasian patients, further large cohort studies are necessary to confirm the frequency of anti-Zo in Japanese IIM patients. Our newly developed ELISA should be validated for sensitivity and specificity in large cohorts.

Key words

anti-tRNA synthetase antibody, biomarker, ELISA, myositis, respiratory

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Introduction

Idiopathic inflammatory myopathies (IIMs) are a group of systemic autoimmune diseases that include polymyositis (PM), dermatomyositis (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 of IIMs (2-4).

Anti-aminoacyl-tRNA synthetase antibodies have been found to be specific for PM and DM and to correlate strongly with complicating interstitial lung disease (ILD) (5). Twenty different tRNA synthetases exist, corresponding to 20 different amino acids, and eight kinds of autoantibodies targeting different tRNA synthetases have been shown in patients with IIM (6). Anti-Zo autoantibodies, which target phenylalanyl-tRNA synthetase (FARS), were reported by Betteridge *et al*. in a single case in 2007 (6), and the same group recently showed an anti-Zo-positive case series of 9 patients in a nationwide UK cohort (7). Patients with anti-Zo antibodies had features of "anti-synthetase syndrome" consisting of myositis, ILD, arthritis, Raynaud's phenomenon (RP), fever and characteristic skin symptoms such as 'mechanic's hands' (5). Anti-Zo antibodies are generally detected by radiolabelled protein immunoprecipitation, in which they appear as two reactive bands corresponding to FARSα (55kDa) and FARSβ (60kDa) proteins (6, 7). Line immunoassays are also commercially available for the detection of anti-Zo (6), but there have been few validation studies on anti-Zo.

This study explores the anti-Zo antibody in Japanese patients with IIM, in order to investigate the clinical features of anti-Zo-positive IIM patients and to establish an ELISA for the measurement of anti-Zo antibodies.

Methods

Patients

Two hundred and twenty-four Japanese patients with IIM (68 males, 156 females) were enrolled in the study. Demographic and medical information was collected from chart reviews. The sera were utilised in our previous study (8). One hundred fand ifty-nine patients fulfilled the criteria of Bohan and Peter for DM/PM, and the remaining 65 met the Sontheimer criteria for clinically amyopathic DM (8). Of the 224 patients, 90 patients had classical DM, 65 had clinically amyopathic DM, 25 had cancer-associated DM, 17 had juvenile DM, 20 had PM and 7 had myositis overlap syndrome. Age-appropriate cancer screening and computed tomography of the chest for the evaluation of ILD were performed. Serum samples had been screened for antibodies against Mi-2, TIF1γ, MDA-5, NXP-2, HMG-CoA, Ku70/80, SRP54, PM/Scl-75/100 and anti-SAE1/2 in the serum samples that were tested by ELISA in our previous study (8). In the patients with positive results by anti-aminoacyl-transfer RNA synthetase (anti-ARS) ELISA kit (MBL, Nagoya, Japan), the individual anti-ARS antibodies of EJ, Jo-1, KS, PL-7 and PL-12 were tested by our in-house ELISA (8). The present study was approved by the ethics committee of Nagoya University Graduate School of Medicine.

Indirect immunofluorescence

Indirect immunofluorescence (IIF) was performed by standard methods (9), using HEp-2 cells (Fluoro HEPANA Test; MBL).

Immunoprecipitation and ELISA with biotinylated recombinant proteins

Full-length cDNA clones of FARSα (No. FXC09887) and FARSβ (No. FHC16549) were purchased from Flexi® ORF Clone (Promega, Madison, WI, USA). Biotinylated recombinant proteins were produced from the cDNA using the T7 Quick Coupled Transcription/Translation System (Promega) according to our published protocol (10). Due to the clone's availability, biotinylated recombinant FARSβ was tagged with HaloTag® at the amino-terminus. Antibodies against FARSα and FARSβ were tested by antigen-capture ELISA according to our published protocols (10).

Immunoprecipitation-Western blotting

Imuunoprecipitation-Western blotting (IP-Western) was performed using

K562 cell extracts with chemical cross-linking, as described previously (11, 12). Rabbit affinity-purified polyclonal antibody to human FARSα (18121-1-AP) and mouse monoclonal antibody to human FARSβ (OT14B3) were purchased from Proteintech (Rosemont, IL, USA) and from OriGene (Rockville, MD, USA), respectively.

Other autoantibodies

Anti-U1-RNP, -Sm, -SS-A (Ro52 and Ro60), -SS-B, -Scl-70 and -dsDNA autoantibodies were measured by fluorescence enzyme immunoassay, and anti-DNA antibody was measured by radioimmunoassay (Thermo Fisher Scientific, Tokyo, Japan). Anti-Ro52 antibody was also measured by a commercial ELISA kit (Orgentec, Mainz, Germany).

Results

Detection of anti-Zo-positive sera

We first checked 8 sera by immunoprecipitation with biotinylated recombinant FARSα/β. With reference to the results of Tansley *et al*. (7), these sera were from different patients who had ILD/myopathy and carried anti-cytoplasmic antibodies with the fine cytoplasmic speckled pattern by IIF studies (Fig. 1A-B) without any concomitant MSAs, denoted in *Methods*. Two sera (Cases 1 and 2) immunoprecipitated both FARSα/β proteins (Fig. 1C-D). To confirm specific reactivity against FARSα/β, we also performed IP–Western blots for these sera. Sera from Cases 1 and 2 immunoprecipitated both FARSα and FARSβ in cell extract (Fig. 1E-F). Neither Case 1 nor Case 2 had any other concomitant MSA.

ELISA for anti-Zo antibody

We screened a total of 224 serum samples utilised in our previous study (9) in addition to 20 sera from healthy individuals with antigen-capture ELISA using biotinylated recombinant FARSα and FARSβ independently. Sera from Cases 1 and 2 reacted to both recombinants in ELISA with higher reactivities than 5 standard deviations (SDs) above the mean value for healthy individuals. Except for the 3 sera from Cases 1 to 3, 221 sera did not show higher reactivities than 2 SDs above the mean value of healthy individuals for either recombinant. The serum from Case 3 reacted to FARSα (higher than 5 SDs above the mean value of healthy individuals) but not to FARSβ (lower than 2 SDs above the mean value of healthy individuals). This serum immunoprecipitated only FARSα very faintly, and not FARSβ (Fig. 1C-D). IP–Western blots showed that it reacted to neither FARSα nor FARSβ (Fig. 1E-F). According to these results, Case 3 was judged to be negative for anti-Zo antibodies.

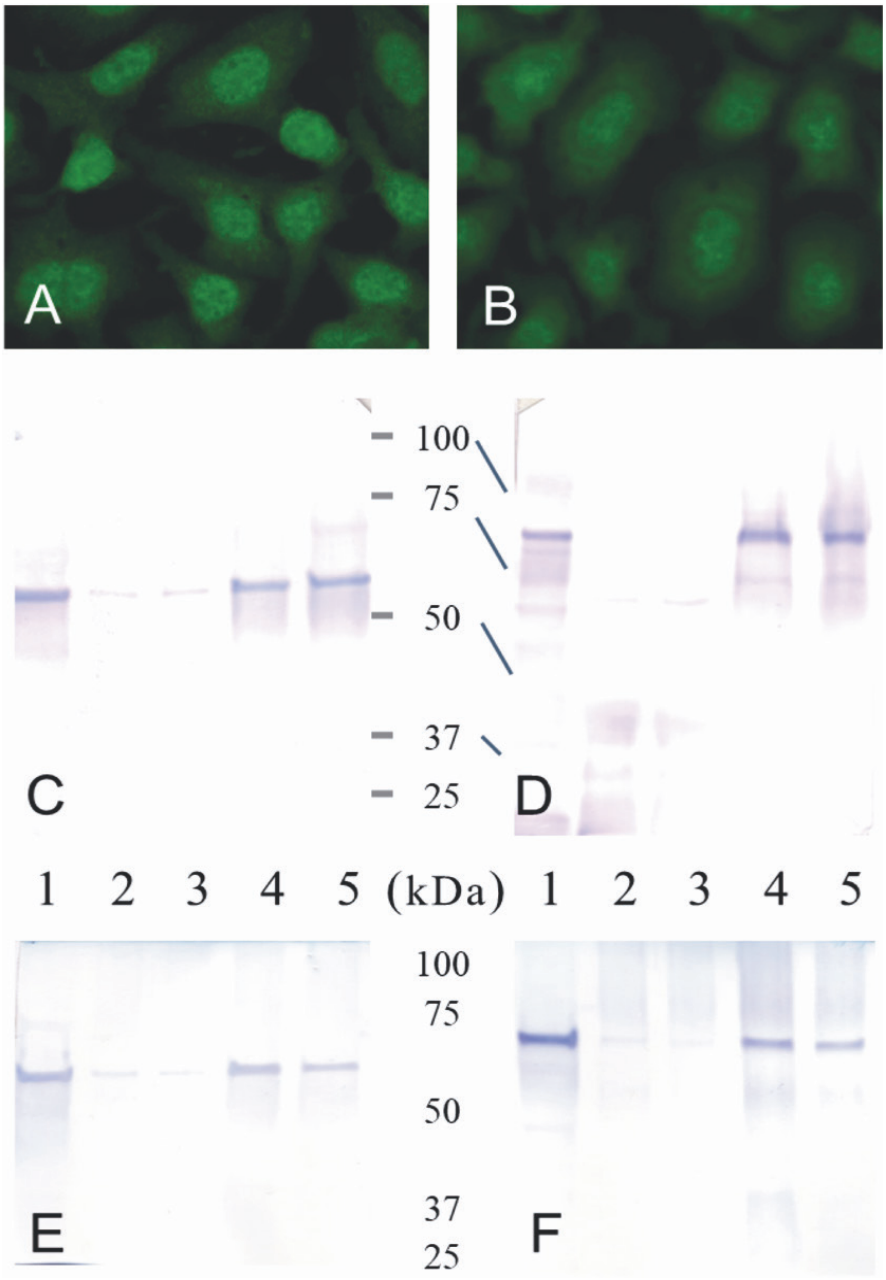

Fig. 1. Detection of anti-Zo antibodies by indirect immunofluorescence, ELISA and Immunoprecipitation-Western blotting. Indirect immunofluorescence staining of HEp-2 cells by patients' sera (**A**; Case 1 and **B**; Case 2). Immunoprecipitation of biotinylated recombinant FARSα (**C**) and FARSβ (**D**). Recombinant proteins were subjected to 4% to 20% SDS-PAGE and analysed by immunoblotting with streptavidin-alkaline phosphatase and substrate. Immunoprecipitates from cell extracts with patient's serum were probed with anti-FARSα polyclonal antibody (**E**) and with anti-FARSβ monoclonal antibody (**F**). Lane 1: input for immunoprecipitation, lane 2: healthy control, lane 3: Case 3, lane 4: Case 1, lane 5: Case 2.

Clinical and laboratory profiles of patients with anti-Zo antibodies

The clinical and laboratory profiles of the two patients with anti-Zo antibodies (Cases 1 and 2) are summarised in Table I. Both patients were male. They

Table I. Clinical and laboratory findings in Japanese IIM patients with anti-Zo autoantibodies.

Clinical and laboratory features	Case 1	Case 2
Gender	Male	Male
Age at onset	68	36
Symptoms at onset	Skin, Muscle, Respiratory	Fever
Muscle disease		
Clinical	Proximal myopathy, Myalgia	Proximal myopathy
Creatine kinase	>1000	>8000
Electromyogram	Normal	Myopathic
Muscle biopsy	Not done	Inflammatory myopathy
Dysphagia	Not present	Not present
Interstitial lung disease (Patterns on high-resolution CT)	Yes (NSIP)	Yes (NSIP with OP)
KL-6	>3000	>1000
Ferritin	>1000	>1000
Skin		
Raynaud's phenomenon	Not present	Not present
Mechanic's hands	Yes	Yes
Gottron's sign	Not present	Yes (fingers)
Others	Not present	Puffy fingers
Other clinical features		
Fever	Yes	Yes
Arthritis/arthralgia	Yes	Not present
Cardiac involvement	Heart failure	Not present
Pulmonary arterial hypertension	Not present	Not present
Other connective tissue disease	Not present	Sjögren's syndrome
Malignancy	Burkitt lymphoma	Not present
other autoantibodies	DNA (6.3 by RIA*)	SS-A (Ro52/60), SS-B
Prognosis	Alive	Alive

IIM: idiopathic inflammatory myopathies, NSIP: non-specific interstitial pneumonia, OP: organising pneumonia, RIA: radioimmunoassay. *normal level <6.0.

had clinical myopathy (one also had pathological myopathy), ILD, arthralgia and mechanic's hands. These findings are consistent with the clinical features of anti-synthetase syndrome. RP was found in neither patient, while other DM-associated skin changes were varied. Both patients had ILD with a non-specific interstitial pneumonia pattern on high-resolution CT (HRCT), and KL-6 and ferritin were significantly elevated. From the available data, the probability of IIM as the diagnosis was calculated using the published ACR/EULAR classification criteria calculator (http://www.imm.ki.se/biostatistics/calculators/iim/) (13). The IIM probability scores ranged from 62% (Case 1) to 98–100% (Case 2). If anti-Zo antibody were included in items as a surrogate for anti-Jo-1 antibody, both patients would be classified as definite IIM: The IIM probability scores for Case 1 and Case 2 would be 99% and 100%, respectively. Tansley *et al*. showed that anti-Ro52 antibodies were present in 6 of 9 patients with anti-Zo antibodies (7), while one of our patients had anti-Ro52 antibodies.

Case 1 received steroid pulse treatment without other immunosuppressive agents. Although he was complicated with Burkitt lymphoma which was found 3 months after the onset of anti-synthetase syndrome, he has remained stable for 4 years. Case 2 received oral prednisolone and tacrolimus in addition to one course of intravenous cyclophosphamide therapy. He has remained stable for 8 years.

Discussion

Anti-Zo antibody is a rarely found anti-synthetase antibody that was reported as the eighth anti-synthetase antibody (6). Although a recent report on 9 patients with anti-Zo antibody in the UK confirmed that clinical features of anti-Zo-positive patients are consistent with anti-synthetase syndrome (7), the present Japanese patients showed slight differences in clinical characteristics. Six of the 9 patients in the UK were female, whereas both of our patients were male. All but one of the patients in the UK had RP, whereas neither of our patients had RP. Half of the patients in the UK showed mechanic's hands, whereas both of our patients did. The cases are too few to conclude whether these differences might be due to racial differences.

Autoantibodies directed to 8 different ARSs have been reported. Although a few reports have mentioned that anti-synthetase syndrome-associated ILD phenotypes differ depending on the anti-ARS subtype (5, 14), a recent international collaborative study showed that the antibody specificity only partially influences the clinical presentation (15). Although 7 of 9 patients with anti-Zo antibody in the UK had ILD, the HRCT pattern of ILD was heterogeneous. Since ILD is a major cause of death in patients with IIM, the severity and outcome of lung involvement related to different anti-ARS subtypes are expected to be explored.

The present study did not investigate anti-OJ (isoleucyl-tRNA synthetase) and anti-Ha (tyrosyl-tRNA synthetase) autoantibodies. No commercial anti-ARS kit (MBL) is available to measure these autoantibodies. One serum containing anti-EJ (glycyl-tRNA synthetase; GARS) antibody (Case 3) reacted to FARSα in our in-house ELISA. Since the 4 other sera with anti-EJ antibodies (9) showed no reaction to FARSα in our in-house ELISA, there may be a small possibility of cross-reactivity between FARSα and GARS.

In conclusion, anti-Zo antibody-positive cases are also present in Japanese patients with IIM and their clinical characteristics correspond to those of anti-synthetase syndrome. We constructed an in-house ELISA for the measurement of anti-Zo antibodies. Further study in a larger cohort is needed to validate this assay system.

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