Concise Report

Anti-synthetase syndrome: a new autoantibody to phenylalanyl transfer RNA synthetase (anti-Zo) associated with polymyositis and interstitial pneumonia

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Objective. Autoantibodies directed against the aminoacyl tRNA synthetases are associated with myositis, arthritis, Raynaud's phenomenon, mechanic's hands, fever and interstitial pneumonia, clinically referred to as the anti-synthetase syndrome (ASS). The aim of this study was to characterize the autoantibody profile in a patient with clinical features of ASS whose routine diagnostic testing was negative for the previously identified anti-synthetase autoantibodies.

Methods. Serum from a patient presenting with interstitial pneumonia followed by proximal myopathy, Raynaud's phenomenon and identified anti-synthetase autoantibodies. The autoantibody specificity was further analysed by RNA and protein immunoprecipitation. Novel autoantigens on protein immunoprecipitation were further characterized using a proteomic approach, combining immunoprecipitation, SDS-PAGE and MALDI-TOF mass spectrometry.

Results. Diagnostic testing on the patient's serum was negative by ELISA and immunodiffusion. Indirect immunofluorescence using Hep-2 cells was ANA negative, although a strong cytoplasmic speckle was seen. Immunoblotting with the patient serum displayed an unknown positive band at approximately 60 kDa. Protein immunoprecipitation revealed the presence of two proteins with molecular weights of approximately 60 and 70 kDa, and RNA immunoprecipitation revealed the presence of a band corresponding to a tRNA synthetase. Using a combination of immunoprecipitation and mass spectrometry, the novel immunoprecipitation targets were identified as phenylalanyl tRNA synthetase alpha and beta chains.

Conclusions. We report the identification of previously uncharacterized autoantibodies to phenylalanyl tRNA synthetase, entitled anti-Zo. This is the eighth anti-synthetase autoantibody in a patient with anti-synthetase syndrome.

KEY WORDS: Autoantibody, Autoantigen, Myositis, Anti-Synthetase syndrome, Interstitial pneumonia.

Introduction

Myositis specific autoantibodies (MSA) are found in up to 50% of patients with polymyositis (PM) and dermatomyositis (DM) [1]. Both are heterogeneous conditions with varying degrees of muscle inflammation and other clinical features including skin changes and lung involvement. There are well-established associations between certain MSA profiles and corresponding clinical patterns [1]. One example is patients who have autoantibodies directed against cytoplasmic autoantigens; the aminoacyl-transfer RNA synthetases (ARS). These are a distinct group of enzymes that catalyse the binding of specific amino acids to their cognate tRNA. To date six anti-synthetase autoantibodies (ASA) have been fully described; anti-Jo-1 (anti-histidyl-tRNA synthetase) [2], anti-PL-7 (anti-threonyl) [3], anti-PL-12 (anti-alanyl) [4], anti-OJ (anti-isoleucyl) [5], anti-EJ (anti-glycyl) [5] and anti-KS (anti-asparaginyl) [6], along with a preliminary report of a seventh ARS (anti-tyrosyl) [7].

The clinical picture of patients with anti-ARS has been termed Anti-Synthetase Syndrome (ASS) [8]. Features of ASS include myositis, Raynaud's phenomenon, arthralgia, fever, skin changes called 'mechanic's hands' and interstitial pneumonia. In our study of patients with idiopathic inflammatory myositis (IIM), we have found a new anti-ARS directed against phenylalanyl tRNA synthetase in a patient with typical features of ASS.

Materials and methods

Index case

A 49-yr-old woman developed shortness of breath; 6 months later, she presented with proximal muscle weakness, Raynaud's phenomenon, puffy fingers and arthralgia. Clinical findings demonstrated a proximal myopathy with a creatinine kinase of 9533 IU/l. Muscle biopsy confirmed a necrotizing myopathy with inflammatory cells. Pulmonary function tests revealed a restrictive pattern and high-resolution computerized tomography (HRCT) showed non-specific interstitial pneumonia (NSIP). The patient was treated with pulsed intravenous methylprednisolone (MP) and cyclophosphamide (CyC), followed by azathioprine and prednisolone. She relapsed two years later and repeat HRCT showed progression with further reticular changes. She was retreated with MP/CyC and switched to mycophenolate mofetil.

Patients

Serum samples were obtained from patients with IIM (Bohan and Peter criteria [9] (n = 44) and systemic sclerosis (SSc) (criteria described by LeRoy et al. [10]) (n = 150), followed in clinics at the Royal National Hospital for Rheumatic Disease, Bath, UK. Normal control samples (n = 40) were obtained from the National Blood Service. The study was approved by the Bath local ethics committee with informed written consent provided by patients according to the Declaration of Helsinki [11].

Immunoprecipitation (IPP) using [³⁵S] methionine

Ten micro litres of sera was mixed with 2 mg protein-A-Sepharose beads (Sigma, UK) in 500 µl IPP buffer (10 mM Tris-Cl pH 8.0, 500 mM NaCl, 0.1% v/v Igepal) at room temperature for 30 min.
with end-over-end rotation. Beads were washed five times in IPP buffer prior to the addition of 120 μl [35S] methionine-labelled K562 cell extract and 380 μl IPP buffer. Samples were mixed at 4°C for 2 h. Beads were washed four times in IPP buffer and once in TBS (10 mM Tris-Cl pH 7.4, 150 mM NaCl) before being re-suspended in 50 μl SDS sample buffer (Sigma, UK). After heating (95°C for 4 min), proteins were fractionated by 10% SDS-PAGE gels, enhanced in 0.5 M sodium salicylate, fixed being re-suspended in 50 μl SDS sample buffer (Sigma, UK). After heating (95°C for 4 min), proteins were fractionated by 10% SDS-PAGE gels, enhanced in 0.5 M sodium salicylate, fixed and stained with Coomassie blue. The gels were scanned and the bands were quantified using ImageJ software.

Preparative IPP for autoantigen isolation

Forty micro litres of sera was mixed with 2 mg protein-A-Sepharose beads in 500 μl IPP buffer at room temperature for 30 min with end-over-end rotation. Beads were washed twice in 1 ml 0.2 M triethanolamine pH 8.2 (Sigma, UK) and bound antibodies were crosslinked to the beads using 5 mM bis-(sulphosuccinimidyl)-suberate (Perbio, UK) in 1 ml triethanolamine, mixing at room temperature for 30 min. The reaction was stopped with 1 ml 50 mM Tris-Cl pH 7.5, mixing at room temperature for 15 min. The antibody-coated Sepharose beads were washed three times in phosphate-buffered-saline and twice in IPP buffer prior to the addition of 1 ml K562 cell extract, corresponding to approximately 1 x 10⁹ cells. Samples were mixed with end-over-end rotation at 4°C for 1 h. The supernatant was removed and the beads were re-suspended in a further 1 ml K562 cell extract and were mixed for 1 h at 4°C. Beads were washed four times in IPP buffer and once in TBS before being re-suspended in 80 μl SDS sample buffer. After heating (95°C for 4 min), proteins were fractionated by 10% SDS-PAGE. Gels were washed 3 x 5 min in pure water, stained for 60 min using Imperial Protein Stain (Perbio, UK) and de-stained overnight in pure water. Unique bands were removed to a 96-well plate.

Mass Spectrometry (MS)

Samples were prepared for MS at the University of the West of England using an Ettan robotic digester (GE Biosciences). Gel pieces were de-stained in 50% Methanol/50 mM Ammonium Bicarbonate, dehydrated in 70% acetonitrile, air dried and digested overnight at room temperature with 20 ng/μl modified porcine trypsin (Promega) in 20 mM ammonium bicarbonate. Peptides were extracted from gel pieces to a clean plate using 50% Acetonitrile/0.1% TFA (trifluoroacetic acid) that was then dried down. The peptides were re-dissolved in 50% Acetonitrile/0.1% TFA and mixed with an equal amount of 10 mg/ml alpha-cyano-4-hydroxycinnamic acid before being spotted on the MALDI target plate using an Ettan Robotic Spotter (GE Biosciences). Peptide mass fingerprints were acquired using Waters Micromass MALDI-TOF MS. Sera from either the index case or controls and three separate occasions for the index case were digested by trypsin, analysed by MS and SwissProt database matched. Matches required peptide coverage of over 20% and scores of approximately 12. All matches were repeated on at least two separate occasions for the controls and three separate occasions for the index case. The bands were digested by trypsin, analysed by MS and SwissProt database matched. Matches required peptide coverage of over 20% and scores of approximately 12. All matches were repeated on at least two separate occasions for the controls and three separate occasions for the index case.

Results

Identification of a new tRNA related autoantibody

Indirect immunofluorescence on the index case revealed a strong discrete cytoplasmic speckle (supplementary Fig. 1, available as supplementary data at Rheumatology Online) and protein immunoprecipitation produced a novel pattern with two bands at approximately 60 and 70 kDa (Fig. 1A, Lane 5). This pattern was not detected in sera from a further 23 PM, 20 DM, 150 SSc and 40 normal control samples. The molecular weights of the bands immunoprecipitated did not match any of the known tRNA synthetases associated with myositis, as shown for Jo-1, PL-7 and PL-12 (Fig. 1A, Lanes 2–4) and as previously described for KS.

Identification of the phenylalanyl tRNA synthetase autoantigen

In order to further characterize the ASA, the corresponding autoantigen was purified and identified using SDS-PAGE and MALDI-TOF MS. Sera from either the index case or patients with known autoantibodies to Jo-1, PL-7 or PL-12 were used to immunoprecipitate autoantigens from a K562 cell extract. A Coomassie-stained SDS-PAGE of the immunoprecipitates showed bands of expected molecular weight for the Jo-1, PL-7 and PL-12 controls (Fig. 1B) as well as 60 and 70 kDa bands from the index case. The bands were digested by trypsin, analysed by MS and SwissProt database matched. Matches required peptide coverage of over 20% and scores of approximately 12. All matches were repeated on at least two separate occasions for the controls and three separate occasions for the index case. The bands were digested by trypsin, analysed by MS and SwissProt database matched. Matches required peptide coverage of over 20% and scores of approximately 12. All matches were repeated on at least two separate occasions for the controls and three separate occasions for the index case immunoprecipitates. Table 1 demonstrates the correct identification of Jo-1, PL-7 and PL-12 autoantigens in the control sera and the database matching of the autoantigens precipitated by the index case. On each of the three separate occasions, the 60 kDa band was matched to phenylalanyl tRNA synthetase alpha chain (59 kDa protein) and the 70 kDa band was matched to phenylalanyl tRNA synthetase beta chain (66 kDa protein). We therefore report the presence of a novel autoantibody directed against phenylalanyl tRNA synthetase in a patient with myositis and ASS.

Discussion

MSAs are detected in up to 50% of patients with PM and DM, and are directed against both nuclear and cytoplasmic autoantigens [1–8, 12–14]. They have been shown to define specific homogeneous patient groups; for example, autoantibodies to the signal recognition particle ribonucleoprotein complex (SRP) have been demonstrated in patients with severe proximal myopathy and dysphagia that is often poorly responsive to immunosuppression [14, 15]. In addition, anti-MI2 autoantibodies, directed against
Comparison of peptide fragments of antigens precipitated by serum containing anti-Jo-1, PL-7 and PL-12 along with peptides from the 60 kDa and 70 kDa bands precipitated by index case [patient 1] serum. Mass peptide fingerprints matched to histidyl-, threonyl- and alanyl-, phenylalanyl (alpha chain)- and phenylalanyl (beta chain)-tRNA synthetase, respectively. Matches were deemed positive if the peptide coverage was over 20%, the same major theoretical and experimental peaks were present and the maximal MALDI-TOF score (using the software and database combination) was approximately 12.

A novel anti-synthetase autoantibody in IIM

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<th>Accession number</th>
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Rheumatology key messages

- Autoantibodies to phenylalanyl tRNA-synthetase alpha and beta chains are novel myositis-specific autoantibodies.
- Eight different anti-synthetase autoantibodies have now been described in patients with clinical features of anti-synthetase syndrome.

Acknowledgements

The authors would like to thank Mrs Juliet Dunphy and Mrs Pat Owen for their technical assistance with the immunodiffusion, ELISAs, immuno blotting and indirect immunofluorescence. We would also like to thank Miss Charlotte Carmichael for her assistance in collecting informed consent from all the patients in this study. This work was supported by a grant from the Raynaud’s and Scleroderma Association.

The authors have declared no conflicts of interest.

References