Autoimmunity Reviews

Letter to the Editor

Two novel anti-aminoacyl tRNA synthetase antibodies: autoantibodies against cysteinyl-tRNA synthetase and valyl-tRNA synthetase

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Dear Editor,

Anti-aminoacyl-tRNA synthetase (ARS) antibodies have been found to be specific for polymyositis (PM) and dermatomyositis (DM) and to correlate strongly with the complication of interstitial lung disease (ILD) [1]. The 20 distinct ARS correspond to 20 different amino acids, and eight autoantibodies targeting different ARSs have been found in patients with the above diseases (Fig. 1) [1]. We recently reported 13 patients with anti-OJ antibodies from 279 Japanese patients with PM/DM and 22 with idiopathic ILD using an in-house sandwich ELISA with biotinylated *in vitro* translated recombinant isoleucyl ARS as well as lysyl ARS [2]. In the present study, we searched for novel autoantibodies against four different ARSs—cysteinyl (CARS), valyl (VARS), seryl (SARS), and tryptophanyl (WARS) (Fig. 2A)—that are not in the OJ multi-synthetase complex [3].

Using our previously reported cohort, we found two sera through screening with our in-house ELISA and *in vitro* translated recombinants according to our established protocols [2,4]. The local Ethical Committees approved the study protocol, which was carried out in accordance with the Declaration of Helsinki. One serum, from a 56-year-old male (patient 1), specifically reacted to CARS; the other, from a 43-year-old female (patient 2), reacted to VARS. To confirm these reactivities, we performed immunoprecipitation using recombinant proteins and immunoprecipitation–Western blotting with cultured cell extracts [5]. Both sera immunoprecipitated the corresponding recombinant proteins (Fig. 2B) and cellular polypeptides (Fig. 2C), but did not react to noncorresponding proteins, including phenylalanyl-ARS (FARS) α targeted by the anti-

Zo antibody [5]. Both sera carried anti-cytoplasmic antibodies showing a fine cytoplasmic speckled pattern by indirect immunofluorescence (Fig. 2D, E) (4). The serum of patient 2 also exhibited a nuclear speckled pattern.

Patient 1, with anti-CARS antibodies, was diagnosed with DM from muscle weakness, myalgia, Gottron's papule/sign, elevated creatine kinase (2507 U/l) and inflammatory findings from a muscle biopsy. He also had clinical features of anti-synthetase syndrome [1]: ILD, mechanic's hands, non-erosive arthritis, fever, and Raynaud's phenomenon. Patient 2, with anti-VARS antibodies, had neither ILD nor myositis, but did have Gottron's sign, heliotrope rash and mechanic's hands, leading to the diagnosis of clinically amyopathic DM. Interestingly, she also had concomitant autoantibodies against TIF1γ shown by ELISA (MBL, Nagoya, Japan).

Since our cohort included biased samples from collaborating hospitals [2], we were unable to evaluate the frequency of each anti-ARS antibody. A future work using consecutive patients is needed to investigate the clinical characteristics of patients with various anti-ARS antibodies.

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Author contribution

The first and the last authors contributed to the general design of the study and of the questionnaire. All authors contributed to case collection, the critical analysis of the results and to revise the manuscript draft.

Declaration of Competing Interest

The authors declare that there is no conflict of interest for this submission paper.

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Figure legends

Fig. 1. Aminoacyl-tRNA synthetases and autoantibodies. Each amino acid is shown by a one-letter code. Aminoacyl-tRNA synthetases are divided into two classes, class I and class II, by structural similarities [6]. Autoantibodies to four synthetases in red were investigated in this study. MSC is a multi-synthetase complex targeted by the anti-OJ antibody. Glutamyl-prolyl-aminoacyl-tRNA synthetase (EPRS) is a bifunctional aminoacyl-tRNA synthetase of EARS and PARS.

Fig. 2. Detection of anti-cysteinyl-tRNA synthetase antibodies and anti-valyl-tRNA synthetase antibodies. **A**, The *in vitro* translation/transcription products used in this study. cDNAs of CARS (NM_139273.4) and VARS (NM_0062925.3) were purchased from GenScript Japan (Tokyo, Japan) and cDNAs of SARS (NM_006513.4), WARS (NM_004184.4), and FARSα (NM_004461.3) from Promega (Madison, WI, USA). **B**, Immunoprecipitation of *in vitro* translation/ transcription products. Patient 1 is a 56-year-old male. Patient 2 is a 43-year-old female. Patient 3 has a prototype of anti-Zo antibody. **C**, Immunoprecipitation–Western blotting of HepG2 cytoplasmic extract (Active Motif, Carlsbad, CA, USA). VARS, CARS, and FARSα were detected with anti-VARS monoclonal antibody (Proteintech, Rosemont, IL, USA), anti-CARS monoclonal antibody (Santa Cruz Biotechnology, Dallas, TX, USA), and anti-FARSα polyclonal antibody (Proteintech), respectively. **D** and **E**, Indirect immunofluorescence staining of HEp-2 cells by patients' sera. HC, healthy control; CARS, cysteinyl-tRNA synthetase; WARS, cysteinyl-tRNA synthetase; SARS, seryl-tRNA synthetase; VARS, valyl-tRNA

synthetase; FARSα, tryptophanyl-tRNA synthetase α; M.W., molecular weight; Pt., patient.

Figure

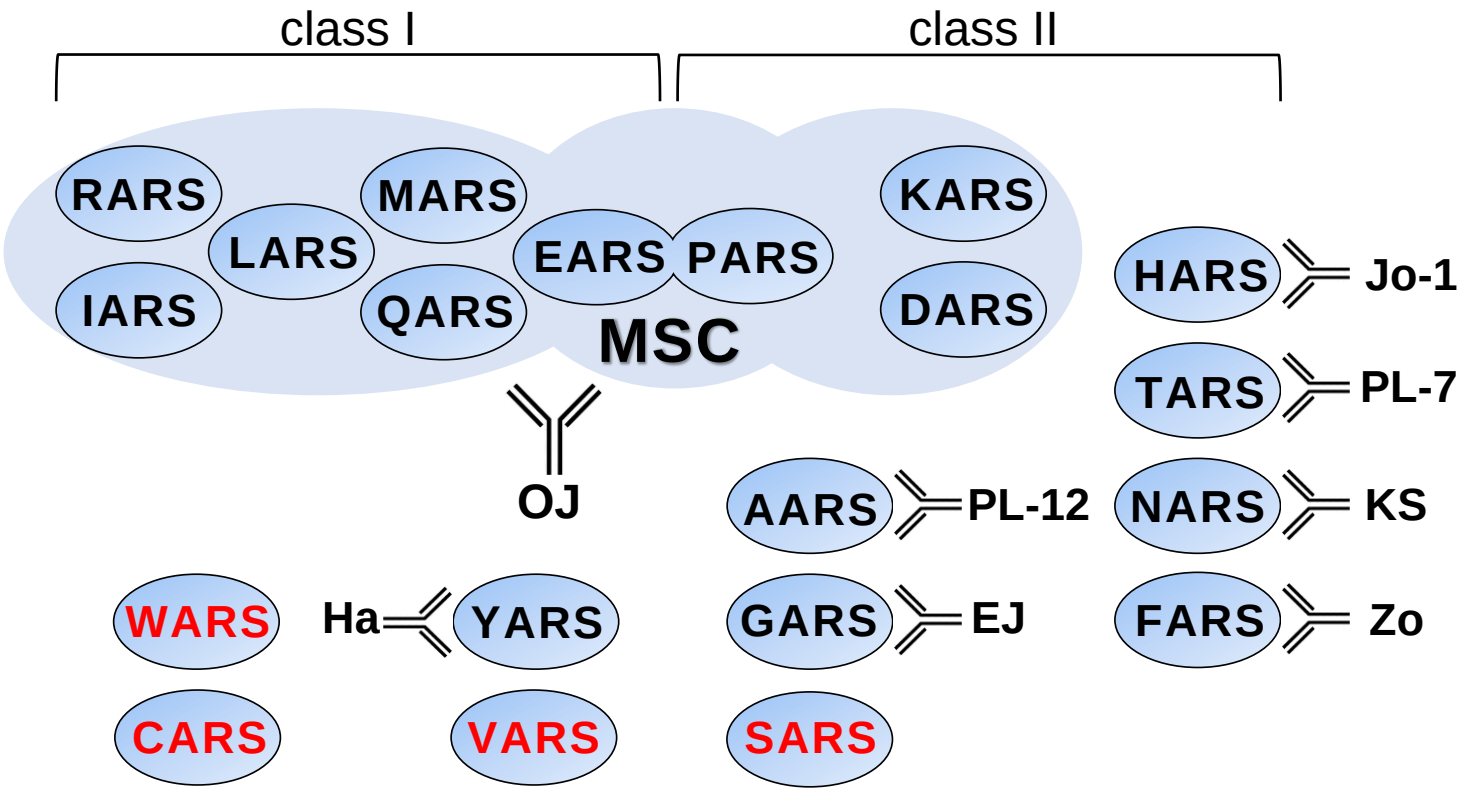

Figure 1. Muro Y, et al.

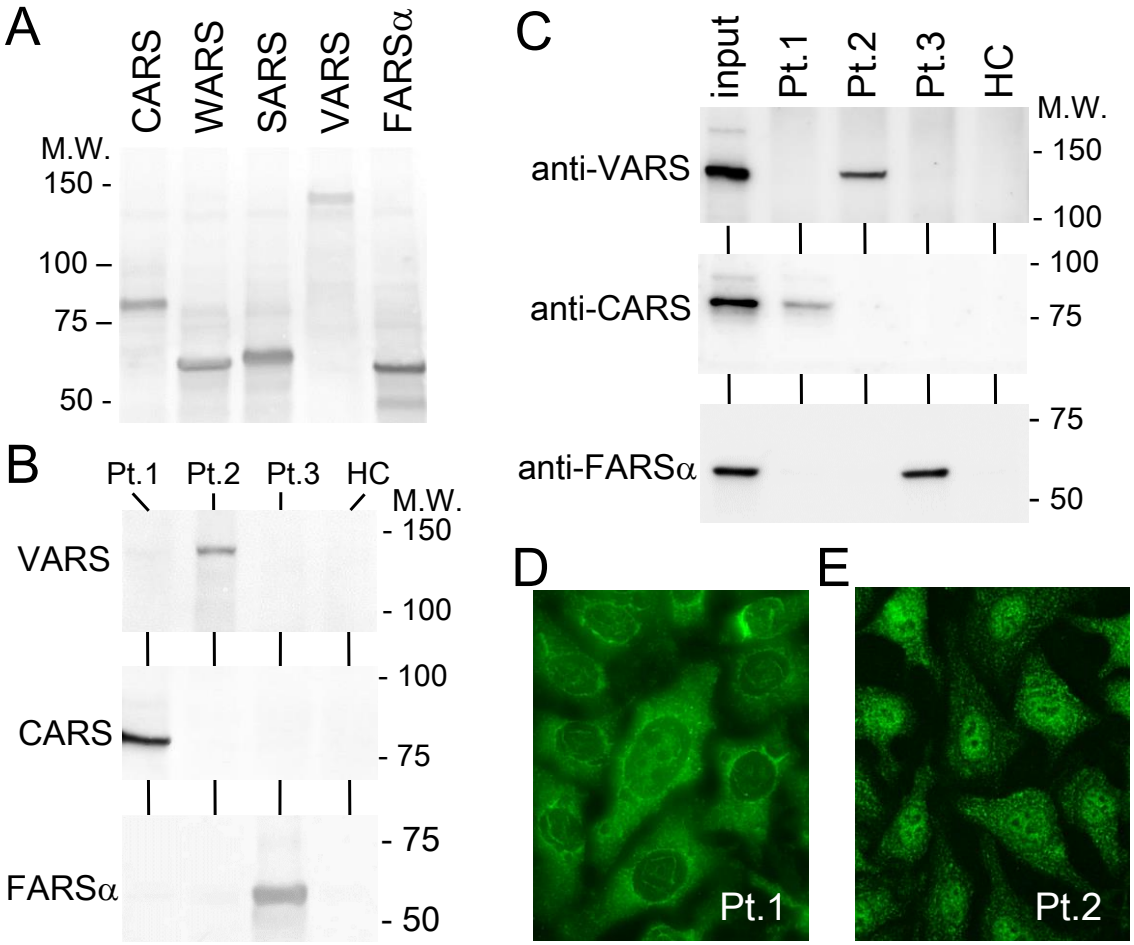

Figure 2. Muro Y, et al.

Highlights:

- We investigated novel anti-aminoacyl-tRNA synthetase autoantibodies.
- We found an anti-cysteinyl-tRNA synthetase antibody-positive dermatomyositis patient.
- We found an anti-valyl-tRNA synthetase antibody-positive dermatomyositis patient.