Letter to the Editor

Clinical and serological features of dermatomyositis and systemic lupus erythematosus patients with autoantibodies to ADAR1

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Running head: Autoimmunity to ADAR1 in dermatomyositis and lupus

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Conflicts of interest

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Abbreviations: ANoA, anti-nucleolar antibody; DM, dermatomyositis; ELISA, enzyme-linked immunosorbent assay; IIF, indirect immunofluorescence; ILD, interstitial lung disease; SARD, systemic autoimmune rheumatic disease; SD, standard deviation; SLE, systemic lupus erythematosus; SSc, systemic scleroderma

Adenosine deaminase acting on RNA-1 (ADAR1) is an RNA-editing enzyme which catalyzes the deamination of adenosine to inosine in double-stranded RNA substrates during post-transcription processing [1]. ADAR1 has two isoforms from alternative spliced transcripts. The long form of human ADAR1, ADAR1-p150 (150 kDa), is interferon-inducible and is mainly expressed in the cytoplasm, while the shorter form, ADAR1-p110 (110 kDa), is constitutively expressed in the nucleus, especially the nucleolus (Figure 1A) [2, 3]. The entire amino acid sequence of ADAR1-p110 is included in ADAR1-p150. Although both forms are enzymatically active, ADAR1-p150 is thought to play a role in antiviral response mechanisms [1]. We previously reported *ADAR1* as the causative gene of dyschromatosis symmetrica hereditaria, an inherited pigmentary disorder, which we determined by positional cloning [4]. *ADAR1* mutations also cause Aicardi–Goutières syndrome, which is a fatal auto-inflammatory disease with an aberrant type I interferon response [4]. Although ADAR1 has been considered in the context of human autoimmunity, the autoimmune response to ADAR1 has not been reported. In this study, we found serum autoantibodies to ADAR1 in patients with dermatomyositis (DM) (Case 1) and systemic lupus erythematosus (SLE) (Case 2), in which a pathogenetic role for type I interferon is implicated [5]. The present study was approved by the ethics committee of the Nagoya University Graduate School of Medicine.

Case 1 is a 41-year-old Japanese man who presented to our hospital with a 2-month history of arthralgia, palmar erythema, and puffy fingers. During the 3 months after the initial visit, Gottron's sign on his fingers with ulceration, and heliotrope rash appeared. Chest high-resolution computed tomography showed interstitial lung disease (ILD) in the bilateral lower lobes. The maximum levels of ferritin and KL-6 were 2730 ng/ml and 811 U/ml, respectively. Other myositis-specific autoantibodies, including anti-MDA5, and systemic autoimmune rheumatic disease (SARD)-specific autoantibodies were not found. Since he had neither myalgia nor muscle weakness and his serum creatine kinase was not elevated, we diagnosed him with clinically amyopathic DM. To treat the acute but not rapidly progressive ILD, oral prednisolone at 30 mg/day was started; however, the ILD activity did not decrease. With the introduction of tacrolimus at 3 mg/day in combination with prednisolone at 10 mg/day, the disease activity stabilized. One year after the initial visit, he moved to another hospital due to having moved homes.

Case 2 was a 28-year-old Japanese woman who visited our hospital with fever, arthralgia, periungual erythema, and puffy fingers. Laboratory examinations showed leucopenia, positive anti-SS-A and anti-centromere antibodies (MESACUP-Test; MBL, Nagoya, Japan) and anti-β2GPI antibody (YAMASA, Tokyo, Japan). No ILD was found. According to these findings, she was diagnosed with SLE and received oral prednisolone

at 20 mg/day. During our 26 years of observation, she showed no lupus involvement in her internal organs, but she sometimes showed malar rash and discoid lupus even when treated with low-dose prednisolone.

In our previous study [6], we found 8 anti-nucleolar antibody (ANoA)-positive patients among 126 DM patients. One of these patients is Case 1 mentioned above. Serum from Case 1 showed predominantly ANoA staining by indirect immunofluorescence (IIF) (Fluoro HEPANA Test; MBL, Nagoya, Japan) (Fig. 1B). To identify the autoantigen targeted by the serum, concerning the six representative autoantigens targeted by ANoA, we performed immunoprecipitation with recombinant proteins derived from the corresponding cDNA clones [6]. The serum reacted with none of the following: Pm/Scl-75/100, Th/To antigens (hPop1 and Rpp25), U3-RNP, and NOR90 (Supplementary Figure). Next, we examined this serum for reactivity to ADAR1 by immunoprecipitation with biotinylated recombinant ADAR1-p150 derived from a plasmid harboring human *ADAR1* full-length cDNA [7]. The serum from Case 1 immunoprecipitated with recombinant ADAR1-p150 (Fig. 1D). Immunoprecipitation-Western blotting analysis with K562 cell extracts [7] and a monoclonal antibody to ADAR1 (sc-73408; Santa Cruz Biotechnology, Dallas, USA), which was raised by the immunization of the amino-terminal portion of ADAR1-p110, showed the serum to react specifically to constitutively

expressed ADAR1-p110, to which the monoclonal antibody also reacted (Fig. 1E).

Using this serum from Case 1 as a positive control, we surveyed anti-ADAR1 autoantibodies in ANoA-positive sera from patients with SARD utilized in our previous study [6]. Twenty-two sera from SSc patients, 10 from SLE patients, and 7 from DM patients were tested by ELISA (Supplementary file) with the standard curve estimation using sera from Case 1. Of these, one serum from a patient with SLE (Case 2) reacted to the recombinant ADAR1-p150 with higher reactivity than five standard deviations (SDs) above the mean value of 20 healthy individuals. Of the 39 sera, 38 sera (i.e., not the serum from Case 2) did not show higher reactivity than two SDs above the mean value of 20 healthy individuals. The serum from Case 2 was confirmed to have the anti-ADAR1 antibody by immunoprecipitation with recombinant ADAR1-p150 (Fig. 1C) and immunoprecipitation-Western blotting (Fig. 1D). Serum from Case 2 also exhibited the ANoA pattern in IIF analysis (Figure 1C).

In this study, we identified autoantibodies against ADAR1 in patients with DM and SLE. Among SARDs, SLE and DM are known to be strongly associated with the type I interferon signature [5]. Autoantibodies in SARD are thought to be generated by the dysregulation and aberrant expression of autoantigens [8]. Further study using a large cohort should be necessary to elucidate the clinical significance of the anti-ADAR1

antibody, which could be a biomarker of type I interferon signature, especially in DM and SLE, or whether autoimmune responses to ADAR1 can develop merely by chance.

This study has a limitation in that we investigated only ANoA-positive patients with SARD. The two alternately spliced forms of ADAR1 are considered to shuttle in the cytoplasm, nucleus, and nucleolus in response to inflammatory conditions [9]. We need to survey not only ANoA-positive sera, but also anti-nuclear and anti-cytoplasmic antibody-positive sera from SARD patients, which can mask the ANoA pattern in IIF analysis. The autoimmune system via ADAR1 might be critically involved in the pathogenesis of type I interferonopathies in some patients with SARD.

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Figure legends

Fig. 1. Anti-ADAR1 autoantibodies detected in immunological assays.

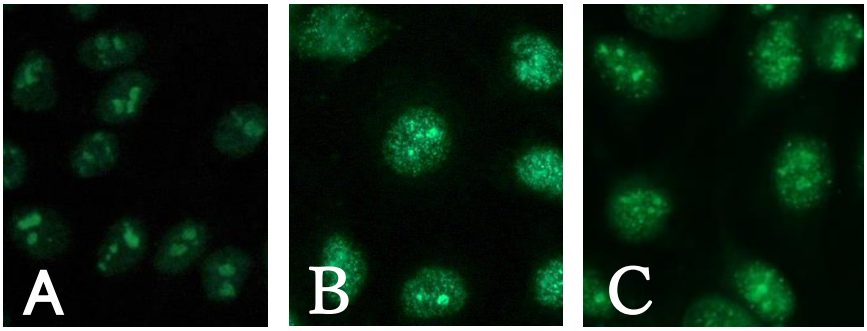
(A, B, C) Indirect immunofluorescence staining of HEp-2 cells by monoclonal antibody against ADAR1 (sc-73408; Santa Cruz Biotechnology, Dallas, USA), which was raised by immunization against the common part of p110 and p150 (A), patients' sera (B, Case 1; C, Case 2). (D) Detection of anti-ADAR1 antibodies by immunoprecipitation with biotinylated recombinant ADAR1-p150 protein. Recombinant proteins were subjected to 4% to 20% SDS-PAGE and analyzed by immunoblotting with streptavidin-alkaline phosphatase and substrates. Lane 1, full amount of input for immunoprecipitation; lane 2, healthy control; lane 3, Case 1; lane 4, Case 2; lane 5, anti-nucleolar-antibody-positive lupus patient without anti-ADAR1. (E) Immunoprecipitation-Western blotting. Immunoprecipitates from cell extracts with patients' serum were probed with anti-ADAR1 monoclonal antibody used in (A). Lane 1, half amount of input for immunoprecipitation; lane 2, healthy control; lane 3, Case 1; lane 4, Case 2; lane 5, anti-nucleolar antibody-positive lupus patient without anti-ADAR1.

Figure 2. Detection of anti-ADAR1 antibodies in ELISA

ELISA units of anti-ADAR1 antibodies in serum samples from 8 patients with

dermatomyositis (DM), 10 patients with systemic lupus erythematosus (SLE), 22 patients with systemic sclerosis, and 20 healthy controls. The upper and lower broken lines (1.56 unit and 0.98 unit) indicate the mean value of 20 healthy individuals plus five standard deviations and two standard deviations, respectively.

Figure 1



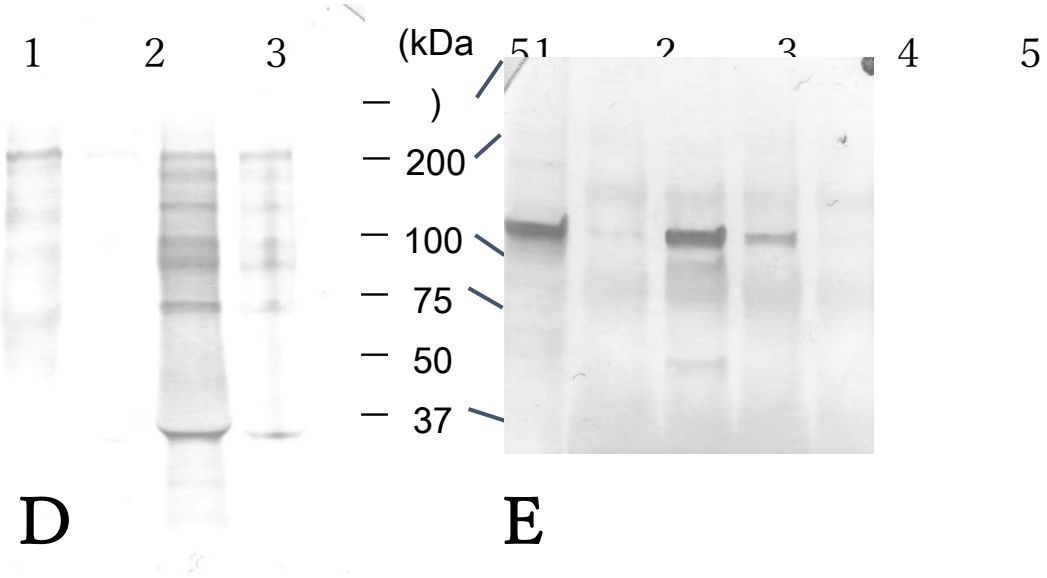


Figure 2