Anti-dense Fine Speckled 70 Autoantibodies in Japanese Children with Dermatomyositis, Localized Scleroderma, and Idiopathic Arthritis with Iridocyclitis

To the Editor:

We read with interest the article by Schmeling, *et al*[1]. They screened a large number of sera from children with antinuclear antibody (ANA)–associated rheumatic disease (AARD) and related conditions, as well as sera from a reference cohort, for anti-dense fine speckled 70 autoantibodies (anti-DFS70ab). They showed that the frequency of anti-DFS70ab in healthy children (3/145, 2.1%) was within the lower range of that reported in adults, but that anti-DFS70ab were found at significantly higher frequencies in children with localized scleroderma (LS; 4/29, 13.8%), juvenile dermatomyositis (JDM; 2/11, 18.2%), and uveitis (3/26, 11.5%). For 331 patients with systemic lupus erythematosus (SLE) and 41 patients with systemic sclerosis, they investigated concomitant AARD-associated autoantibodies and found that only 6 patients with SLE had isolated anti-DFS70ab. This result fits the theory that anti-DFS70ab positivity in AARD is usually associated with additional AARD-related antibodies[2]. However, because their disease cohort was small, except for SLE and juvenile idiopathic arthritis (JIA; 202 patients), the higher frequency of the antibodies in LS (4/29), JDM (2/11), or uveitis (3/26) is inconclusive. In addition, neither several recently identified DM-specific autoantibodies [anti-MDA5, antitranscriptional intermediary factor 1-γ (TIF1-γ), anti-NXP-2, etc.][3] nor anti-ssDNA antibodies that are associated with some patients with LS[4] were examined in their study. We examined anti-DFS70ab and myopathy-associated autoantibodies in Japanese children with JDM, and investigated anti-DFS70ab and anti-ssDNA antibodies in Japanese children with LS. Moreover, we investigated anti-DFS70ab in patients with JIA complicated with iridocyclitis.

Our child cohort consisted of 29 patients with JDM, 14 with LS, and 10 with JIA. The age range for children was set at 18 years and younger, in line with previous publications. This study was approved by the Ethics Committee of each hospital, and it complies with the Declaration of Helsinki guidelines. ANA testing was performed by indirect immunofluorescence (IIF) on HEp-2 cell substrates (MBL)[5], and anti-DFS70ab levels were measured by ELISA (MBL). JDM-specific autoantibodies including anti-Mi-2, anti-TIF1-γ, anti-MDA5, anti-NXP2, anti-SAE, anti-MLH1, and anti-PMS1 antibodies were measured for patients with JDM by the ELISA we developed, which uses *in vitro* transcription and translation biotinylated recombinant protein[6,7,8]. The anti-aminoacyl-tRNA synthetase antibodies anti-Jo1, anti-PL-7, anti-PL-12, anti-EJ, and anti-KS were also investigated by ELISA (MBL) for patients with JDM. Anti-ssDNA antibody was measured by ELISA (MBL) for patients with LS.

The anti-DFS70ab ELISA found 7 (24.1%) positive patients in our JDM cohort (Table 1). Two patients with JDM showed the DFS pattern in IIF studies, but were anti-DFS70ab–negative in the ELISA. Immunoblotting analysis with bacterially expressed DFS70 recombinant protein and HeLa cell extract[5] (data not shown) confirmed that 1 of the 2 was positive for anti-DFS70ab (Table 2, case #8) and the other was negative. One patient with anti-DFS70ab did not show the characteristic DFS pattern in IIF. This patient (Table 2, case #5) was confirmed to be positive for the antibody by the same immunoblotting analysis (discussed below).

The frequency of anti-DFS70ab in JDM was similar to that of the previous study by Schmeling, *et al*[1] (27.6% vs 18.2%). The most important result is that there was only 1 (3.4%) isolated (monospecific) anti-DFS70ab–positive patient with JDM in our present study (Table 2). This datum fits our previous data indicating that the monospecificity of anti-DFS70ab differs between AARD and non-AARD in adults[2]. Although the Schmeling, *et al* study showed a relatively high frequency of anti-DFS70ab in patients with LS (13.8%), none of our patients with LS had that antibody. However, there was no statistical difference ($p < 0.287$ by Fisher's exact test). Interestingly, our patients with LS often had anti-ssDNA antibodies (9/14, 64.3%).

Schmeling, *et al*[1] also studied anti-DFS70ab in patients with uveitis that was associated with JIA or that was idiopathic. Two of 19 JIA patients with uveitis (10.5%) and 1 of 7 patients with idiopathic uveitis (14.3%) had anti-DFS70ab. According to the study by Ravelli, *et al*[9], JIA patients with iridocyclitis very frequently had ANA (58/60, 96.7%). Surprisingly, in our present study, 5 of 10 JIA patients with iridocyclitis had anti-DFS70ab. The association of anti-DFS70ab with JIA with iridocyclitis in Japanese children should be confirmed by future studies. Although 1 of these 5 anti-DFS70ab–positive sera did not show a DFS pattern in IIF, the antibody-positive result was confirmed by immunoblotting analysis (Table 2, case #12). In a total of 53 examined sera, 4 sera had discrepant results between the DFS pattern in IIF and the anti-DFS70ab in ELISA. Schmeling, *et al*[1] also showed that, although the concordance rate of IIF patterns and chemiluminescence immunoassay results for anti-DFS70ab was > 90%, IIF patterns do not always correspond to the other immunoassays results for anti-DFS70ab. Because recognizing the DFS pattern in IIF is not easy[10], a specific immunoassay for anti-DFS70ab should be used to confirm the presence of anti-DFS70ab. Moreover, clinicians should not overinterpret isolated ANA results in anti-DFS70ab–positive patients, but should pay attention to detecting concomitant disease-specific autoantibodies.

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Table 1. Clinical profiles and frequency of anti-DFS70 antibodies in the present cohorts.

Cohort	n	Sex, M:F	Age, yrs, Range (Median)	Positive, n (%)	Antinuclear Antibody* Range (Median)	DFS Pattern–positive, n (%)	Anti-DFS70–positive, n (%)	Other Autoantibody–positive, n (%)
Juvenile DM	29	11:18	1–16 (9)	23 (79.3)	1/40–1/320 (× 80)	8 (27.6)	8 (27.6)	Anti-TIF1-γ, 9 (31.0) Anti-MDA5, 8 (27.6) Anti-PMS1, 6 (20.7) Anti-NXP2, 5 (17.2) Anti-MLH1, 3 (10.3)
Localized scleroderma	14	2:12	3–18 (12)	13 (92.9)	1/40–1/640 (× 80)	0 (0)	0 (0)	Anti-ssDNA, 9 (64.3)
JIA with iridocyclitis	10	4:6	1–11 (6)	10 (100)	1/40–1/640 (× 160)	4 (40)	5 (50)	NT

* Antinuclear antibody also includes anticytoplasmic antibody. DFS: dense fine speckled; DM: dermatomyositis; JIA: juvenile idiopathic arthritis; NT: not tested; anti-TIF1-γ: antitranscriptional intermediary factor 1-γ.

Table 2. Profiles of patients with anti-DFS70 antibodies.

Patient No.	Diagnosis	Age, yrs	Sex	DFS70 Reactivity by ELISA, unit/ml*	ANA Pattern/ titer by IIF	Other Autoantibodies
1	JDM	6	Female	22.5	DFS 1/80	Negative
2	JDM	10	Female	111.9	DFS 1/160	Anti-TIF1-γ
3	JDM	1	Male	51.3	DFS 1/80	Anti-TIF1-γ
4	JDM	9	Male	121.7	DFS 1/320	Anti-NXP2, anti-MLH1, anti-PMS1
5	JDM	6	Male	85.4	Speckled 1/160	Anti-NXP2, anti-MLH1, anti-PMS1
6	JDM	9	Female	40.5	DFS 1/80	Anti-MDA5
7	JDM	11	Female	33.3	DFS 1/80	Anti-MDA5
8	JDM	11	Female	12.8	DFS 1/80	Anti-MDA5
9	JIA iridocyclitis	2	Female	252.5	DFS 1/320	NT
10	JIA iridocyclitis	7	Female	68.8	DFS 1/160	NT
11	JIA iridocyclitis	6	Female	62.0	DFS 1/160	NT
12	JIA iridocyclitis	5	Male	52.9	Nucleolar 1/80	NT
13	JIA iridocyclitis	5	Male	23.2	DFS 1/80	NT

* Positive > 15 unit/ml. Sera from all patients were confirmed to have anti-DFS70 antibodies by immunoblotting analysis with DFS70 recombinant protein and with HeLa cell extract. DFS: dense fine speckled; ANA: antinuclear antibody; IIF: indirect immunofluorescence; JDM: juvenile dermatomyositis; JIA: juvenile idiopathic arthritis; NT: not tested; anti-TIF1-γ: antitranscriptional intermediary factor 1-γ.

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