Anti-α-Fodrin Autoantibody Is an Early Diagnostic Marker for Childhood Primary Sjögren's Syndrome

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ABSTRACT. ***Objective.*** α-fodrin is a recently identified autoantigen associated with adult primary Sjögren's syndrome (SS). We tested whether anti-α-fodrin antibody could also be used as a diagnostic marker for childhood SS.

Methods. We performed immunoblot analysis of sera from 7 patients with childhood primary SS using glutathione-S-transferase α-fodrin fusion protein as an antigen.

Results. Anti-α-fodrin antibody was detected in sera from all 7 patients with childhood primary SS, 2 of 4 with secondary SS, and one of 7 with systemic lupus erythematosus, but in no other healthy controls.

Conclusion. The anti-α-fodrin autoantibody was detected before anti-SSA or SSB antibody became positive; thus anti-α-fodrin antibody could be a useful marker for the early diagnosis of SS. (J Rheumatol 2001;28:363–5)

Key Indexing Terms:
α-FODRIN
AUTOANTIBODY
CHILDHOOD SJÖGREN'S SYNDROME
EARLY DIAGNOSIS

Sjögren's syndrome (SS) is an autoimmune exocrinopathy predominantly affecting the salivary and lacrimal gland[1]. SS is clinically characterized by xerostomia and xerophthalmia, and occurs alone (primary SS) or in association with other collagen diseases (secondary SS). Although SS is prevalent in women of the fourth and fifth decades, recent studies have revealed that childhood SS is not so rare as has been estimated[2-5]. About 75% of childhood primary SS lacks sicca symptoms, but yields pathological and laboratory findings similar to those in adulthood cases[3-5]. Indeed, anti-Ro/SSA and La/SSB antibodies are generally used as disease markers in both childhood and adult SS[2-5]. However, 26% of childhood SS cases are negative for both anti-SSA and SSB antibodies[3], indicating limited diagnostic values of these markers. Recently, an autoantibody against the N-terminal 120 kDa form of α-fodrin was identified as highly specific and sensitive to adulthood primary SS[6]. We tested its clinical significance in childhood SS.

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MATERIALS AND METHODS

Sera were obtained from 7 Japanese girls with primary SS, aged 9 to 14 years, and 3 girls and one boy with secondary SS, aged 7 to 15 years; sera were stored at –20°C until use. Clinical and laboratory findings of the cases are summarized in Tables 1 and 2. Some cases have been described[4,7,8]. All the cases were positive for both sialography and lip biopsy and were finally classified as definite or probable SS according to the European criteria for SS[9]. Although all of the primary cases and 3 secondary cases lacked sicca symptoms when the sera were obtained, xerophthalmia developed in 2 primary cases (Patients 2 and 3) 5 and 6 years after the onset, respectively. Control sera were obtained from 7 patients with systemic lupus erythematosus (SLE) alone, 7 with juvenile rheumatoid arthritis, 7 with juvenile dermatomyositis, and 20 with no collagen disease.

Anti-α-fodrin antibody was detected by immunoblot analysis using a fusion protein with α-fodrin[6,10]. *Escherichia coli*, DH5α, was transfected with a glutathione-S-transferase (GST) fusion protein expression vector containing nucleotide 1-1784 of α-fodrin cDNA, which encodes N-terminal 594 amino acids (pGEX-JS-1). Accordingly, the molecular weight of the fusion protein is calculated as roughly 96 kDa. The fusion protein, GST-JS-1, was expressed in the presence of 1 mM isopropylthiogalactoside and was purified with glutathione-sepharose (Amersham Pharmacia, Buckinghamshire, UK) according to the manufacturer's protocol. Two hundred nanograms of the fusion protein were separated on 6% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and was then electrically transferred onto polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). After blocking with 5% nonfat milk at 4°C for 16 h, membranes were incubated with 160-fold diluted sera at 4°C for 16 h. The membrane was washed 3 times with Tris buffered saline containing 0.1% Tween-20, and then was incubated with 5000-fold diluted horseradish peroxidase labeled goat anti-human IgG (Biosourse, Camarillo, CA, USA). After washing 3 times with 50 mM Tris-HCl, pH 7.6, the membrane was incubated with peroxidase substrate, 3,3′-diaminobenzidine (Sigma, St. Louis, MO, USA) dissolved in 50 mM Tris-HCl, pH 7.4, 0.03% hydrogen peroxide, and 0.03% $NiCl_2$.

RESULTS

GST-JS-1 fusion protein was detected at about 100 kDa on

Table 1. Clinical features of the patients.

Patient	Diagnosis	Age of Onset, yrs	Age of Diagnosis, yrs	Duration of Followup, yrs	Sicca Symptom	Initial Symptoms	Complications
1[7]	pSS	5	12	13	No	Parotitis, fever, arthralgia	RTA
2[7]	pSS	9	9	11	No*	Parotitis	
3[7]	pSS	9	9	11	No*	Fever, arthralgia	
4[4]	pSS	10	10	12	No	Parotitis, uveitis	RTA
5[4]	pSS	8	11	10	No	Fever, headache	Meningitis
6	pSS	14	14	2	No	Fever, arthralgia	
7[8]	pSS	14	14	2	No	Fever, arthralgia, neuropathy	Neuropathy
8	SS JDM	11	11	2	No	Fever, headache	Meningitis
9[4]	SS + MCTD	10	12	9	No	Fever, parotitis, arthralgia, goiter, Raynaud's	Thyroiditis
10[4]	SS + SLE	7	10	13	Yes	Arthralgia, xerophthalmia	GN, purpura
11	SS + SLE	12	12	9	No	Fever, heart failure	Pericarditis

*Sicca symptoms appeared later. pSS: primary Sjögren's syndrome, JDM: juvenile dermatomyositis, MCTD: mixed connective tissue disease, SLE: systemic lupus erythematosus, RTA: renal tubular acidosis, GN: glomerulonephritis.

Table 2. Initial laboratory findings of the patients.

Patient	ESR, mm/h	WBC, mm^3	Serum IgG, g/l	Antinuclear Antibody	Rheumatoid Factor	Anti-SSA	Anti-SSB	Other Autoantibodies
1	98	5400	31.0	1:1280	+	+	+	
2	40	6600	18.0	1:320	+	+	+	
3	40	6200	15.0	1:2560	+	+	+	Thyroid
4	73	4500	37.4	1:40	+	–*	–	Thyroid
5	35	3700	22.0	1:1280	+	–*	–*	
6	52	10,200	21.6	1:320	–	+	+	
7	98	7800	26.1	1:160	+	+	+	
8	65	6600	23.5	1:1280	+	+	+	
9	20	4800	27.9	1:1280	+	–*	–	RNP, thyroid
10	112	4200	48.9	1:640	+	+	–	DNA
11	38	16,600	24.9	1:1280	–	+	+	DNA, thyroid

*The indicated antibodies became positive later. ESR: erythrocyte sedimentation rate, WBC: white blood cell count.

SDS-PAGE and by immunoblot analysis with anti-GST antibody, consistent with a previous report[6]. The 100 kDa signal was detected by immunoblotting of GST-JS-1 with the sera from all 7 patients with primary SS and 2 of 4 patients with secondary SS (Figure 1). Neither this band nor the band corresponding to GST was detected in the lysate of *E. coli* transfected with pGEX vector alone, indicating that the signal represents the reaction of recombinant α-fodrin with anti-α-fodrin antibody (data not shown). Anti-α-fodrin antibody was already detectable in the sera before anti-SSA and SSB antibodies became positive (Patients 4, 5, and 9). The autoantibody was also detected in serum from one of the 7 patients with SLE alone, but not in any other control sera (data not shown).

DISCUSSION

We observed that anti-α-fodrin antibody is a sensitive and possibly specific marker for childhood primary SS, as in adult cases[6,11]. α-fodrin is a 240 kDa subunit of fodrin, a component of cytoskeleton, and is proteolytically cleaved to form a 120 kDa fragment[6,10,12]. Although α-fodrin is ubiquitously expressed, the 120 kDa form is abundant in the salivary gland, a major target organ of SS, and is able to stimulate peripheral blood T cells in patients with SS[6]. Thus it is speculated that this molecule is critical for the development of adult primary SS[6]. Our results suggest that childhood SS develops on the same pathological basis as adult SS and is an early onset type of SS. In addition, sicca symptoms later developed in 2 of our primary cases, which is consistent with the previous clinical and pathological observations[5,13,14]. This indicates that SS without sicca symptoms is an early stage of the disease.

We observed that anti-α-fodrin antibody was already detectable before anti-SSA or SSB antibody became positive in 2 of the primary cases. The 2 patients had renal tubular acidosis or aseptic meningitis at the initial examination[4]. Several reports including ours have shown that most of the complications commonly observed in adult SS also

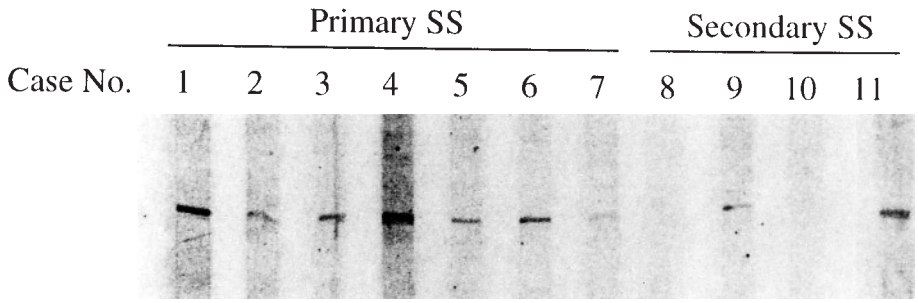

Figure 1. Immunoblot analysis of anti-α-fodrin antibody using fusion protein GST-JS-1. Sera from Patients 4, 5, and 9 were obtained one year before anti-SSA and SSB antibodies became positive.

occur in childhood cases, whether sicca symptoms are present or not[3-5,7,8,15]. Some of the complications are serious, and need early recognition of the underlying SS. We conclude that the detection of anti-α-fodrin antibody could be useful for early diagnosis of primary SS.

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