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Subclinical Sjögren's syndrome and anti-Ro/SSA-positive autoimmune fatigue syndrome in children

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Abstract Although Sjögren's syndrome (SS) is quite rare among children, subclinical conditions without any sicca symptoms have been reported. This condition is characterized by nonspecific rheumatic symptoms and histopathological findings in salivary glands which are equivalent to SS. Many children with subclinical SS are positive for anti-Ro/SSA. On the other hand, autoimmune fatigue syndrome (AIFS) is characterized by chronic nonspecific complaints and positive antinuclear antibodies, with or without fulfilling the criteria for chronic fatigue syndrome. Although a novel autoantibody against a 62 kD nuclear protein (anti-Sa) is detected in about 40% of AIFS patients, few marker antibodies for autoimmune diseases, such as anti-DNA, anti-Sm, anti-U1-ribonucleoprotein (RNP), or anticardiolipin, are found in AIFS patients. In this study, however, anti-Ro/SSA was detected in sera from 8 out of 122 AIFS patients. Seven of the 8 anti-Ro/SSA-positive patients were female. All 8 patients had fatigue and low-grade fever, but none complained of xerosis. Western immunoblot analysis revealed that 7 sera reacted with Ro52, and that none was positive for anti-La/SSB or anti-Sa. Two of the 8 patients had histories of recurrent parotitis. Lip biopsies showed mild chronic inflammation compatible with subclinical SS in these 2 patients, although the other 6 patients had no abnormal histopathology. Thus, at least some anti-Ro/SSA-positive patients could be diagnosed as having SS.

Key words Antinuclear antibodies (ANA) · Anti-Ro/SSA · Autoimmune fatigue syndrome (AIFS) · Chronic fatigue syndrome (CFS) · Sjögren's syndrome (SS)

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Introduction

Sjögren's syndrome (SS) is a systemic autoimmune disease characterized by complaints of sicca symptoms (dry eyes and mouth). In children, however, it is quite rare to see patients with symptomatic SS with xerosis. Recent studies have shown that subclinical SS without any dryness has been found in children more frequently than expected.[1] This condition is characterized by vague symptoms such as fatigue, myalgia, and low-grade fever, and by histopathological findings from minor salivary glands compatible with SS. As with clinical SS, most patients with subclinical SS are positive for anti-Ro/SSA and/or anti-La/SSB antibodies.[2]

On the other hand, children commonly suffer from nonspecific symptoms such as headache, abdominal pain, fatigue, and low-grade fever. As these symptoms may lead to absence from school, repeated absenteeism may result in some form of discrimination from the school, even if the child wishes to attend. Recently, we found that more than half of the children with chronic, nonspecific complaints were positive for antinuclear antibodies (ANA). Of the ANA-positive children, 40% also had autoantibodies to a 62 kD nuclear protein (anti-Sa). Given the presence of these autoantibodies, and as fatigue was a major symptom, we proposed a distinct disease entity termed autoimmune fatigue syndrome (AIFS).[3] Since then, we have followed those patients and found that some AIFS patients later developed chronic fatigue syndrome (CFS), which suggested some relationship between AIFS and CFS.[4] Moreover, there appeared to be an immunogenetic background in AIFS patients, as observed in other autoimmune disorders. AIFS was found to be positively associated with the class I antigen HLA-B61 and with the class II antigen HLA-DR9, and a negative association was also found between AIFS and HLA-DR2.[5] The negative association with DR2 suggested that AIFS was distinct from systemic lupus erythematosus (SLE) and not a pre-SLE condition, since HLA-DR2 (DRB1*1501) has been reported to be associated with SLE in the Japanese population.[6]

Although very few patients with AIFS are positive for marker autoantibodies for collagen diseases such as anti-DNA, anti-Sm, anti-U1-ribonucleoprotein (RNP), or anticardiolipin, there are a few such patients with anti-Ro/SSA antibodies. To clarify the relationship between AIFS and subclinical SS, we retrospectively studied the prevalence of anti-Ro/SSA antibodies in AIFS patients, and investigated anti-Ro/SSA-positive patients with AIFS both clinically and immunologically.

Materials and methods

Patients

The patients included in this study had been diagnosed as having autoimmune fatigue syndrome, and their anti-Ro/SSA antibodies had been measured at the pediatric outpatient clinic in Nippon Medical School Chiba-Hokusoh Hospital from 1994 to 1999. The definition of autoimmune fatigue syndrome was that a patient had persistent nonspecific complaints for more than 3 months and was consistently positive for ANAs (1:40 or higher), with no alternate medical explanation for the complaints. One hundred and twenty-two patients met the entrance criteria. All patients gave informed consent to participate in this approved clinical research protocol.

Serum samples

Serum samples were separated from freshly drawn blood samples by centrifugation at 3000 rpm for 5 min, and frozen at −70°C for later use.

Methods

All patients underwent a comprehensive clinical and laboratory assessment, including complete blood counts, blood biochemistry, erythrocyte sedimentation rate, urinalysis, and serological tests which included measurements of each class of immunoglobulin and complement components C3, C4, and CH50. X-ray films of the chest, abdomen, and paranasal sinus were taken for all patients, and in some cases a barium enema, a brain computed tomography (CT), and an EEG were performed to exclude possible organic diseases. ANAs were measured by the indirect immunofluorescent method using HEp2 cells. Autoantibody analysis was performed by the Ouchterlony double-immunodiffusion method (DID) using calf thymus extract, enzyme-linked immunosorbent assay (ELISA) for anti-Ro/SSA, anti-La/SSB, anti-Sm, anti-U_1RNP (using affinity-purified antigens from calf spleen), anti-dsDNA, anti-ssDNA, and anti-cardiolipin β_2GPI, and by Western immunoblotting using HeLa cell extracts, as previously described.[7]

Results

Eight out of 122 (6.6%) patients with AIFS were positive for anti-Ro/SSA antibodies as determined by DID. Other than the anti-Ro/SSA-positive patients, there was one patient among the 122 AIFS patients who was positive for anti-U_1RNP, but none were positive for anti-ssDNA IgG, anti-dsDNA IgG, anti-Sm, anti-La/SSB, or anticardiolipin β_2GPI. Table 1 shows the clinical manifestations, and the ANA, anti-Ro/SSA, and anti-La/SSB results from the 8 anti-Ro/SSA-positive patients at the time of their first visit to our clinic. Their ages ranged from 8 to 17 years (mean 11.9 years), and 7 of them were female. All complained of fatigue and low-grade fever, but none had either dry eyes or a dry mouth. Two patients (cases 1 and 2) had histories of recurrent parotitis, and one had had occasional lymphadenopathy and a sore throat (case 3). Case 3 complained of serious fatigue, and fulfilled the case definition criteria for CFS 15 months later.

ANA titers in the 8 anti-Ro/SSA-positive patients ranged from 1:40 to 1:320. Fluorescent patterns were of the "homogeneous and speckled" type in 6 cases, and of the

Table 1. The eight anti-Ro/SSA-positive patients with AIFS

Patient	Sex	Age (years)	Chief complaints	ANA		Anti-Ro/SSA		Anti-La/SSB	
				Titer	Pattern	DID	ELISA	DID	ELISA
1. TW	F	17	Low-grade fever, fatigue, sore throat, recurrent parotitis	1:320	Homo, spec	1:32	213	–	<7
2. YI	F	14	Low-grade fever, fatigue, recurrent parotitis	1:160	Spec	1:16	87.2	–	<7
3. AU	F	9	Low-grade fever, fatigue, lymphadenopathy, sore throat	1:160	Homo, spec	1:16	21.6	–	<7
4. TI	F	11	Low-grade fever, fatigue, headache	1:80	Homo, spec	1:8	16.4	–	<7
5. MI	F	12	Low-grade fever, fatigue, myalgia	1:80	Spec	1:8	<7	–	<7
6. TH	F	8	Low-grade fever, fatigue, dizziness	1:80	Homo, spec	1:4	17.6	–	<7
7. HK	F	11	Low-grade fever, fatigue, headache	1:80	Homo, spec	1:4	<7	–	<7
8. AS	M	13	Low-grade fever, fatigue, abdominal pain, nausea	1:40	Homo, spec	1:4	<7	–	<7

ANA, antinuclear antibodies; DID, double-immunodiffusion method; ELISA, enzyme-linked immunosorbent assay; homo, homogeneous; spec, speckled

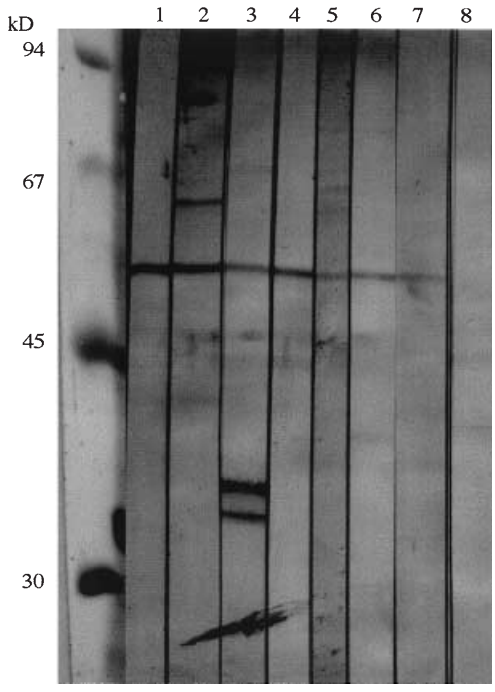

Fig. 1. Western immunoblot analysis of sera from the eight autoimmune fatigue syndrome (AIFS) patients with anti-Ro/SSA using HeLa cell extract. Seven sera (lanes 1–7) reacted with Ro52, and only one serum (lane 2) reacted with Ro60

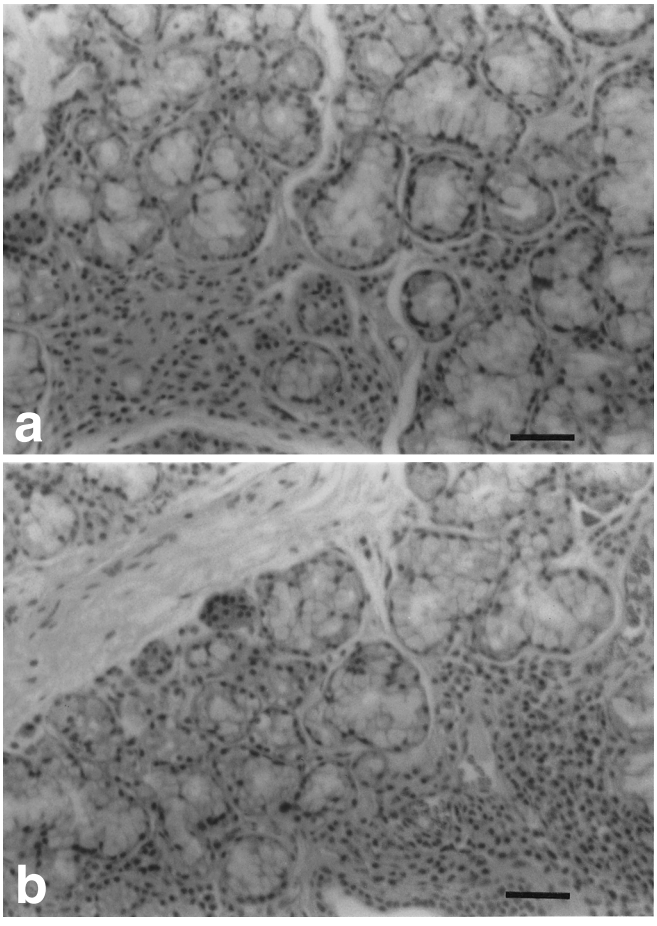

Fig. 2. Histopathological findings from lip biopsies of **a** case 1, and **b** case 2 (hematoxylin and eosin dye). Both specimens showed mild chronic inflammatory cell infiltration around the ducts, suggesting mild Sjögren's syndrome (SS). *Bar* 50 μm

"speckled" type in 2 cases, and none showed the classic "anti-Ro cytoplasmic stain." Anti-Ro/SSA titers were from 1:4 to 1:32 by DID, but 3 sera were negative by ELISA. Western immunoblot analysis using an HeLa cell extract showed that 7 out of the 8 sera reacted with the 52 kD Ro/SSA protein (Ro52), and that only one patient reacted with the 60 kD protein (Ro60) (Fig. 1). No patient was positive for anti-La/SSB by either DID, ELISA, or Western immunoblot. Anti-Sa antibody, which could be detected in about 40% of AIFS patients, was negative in all 8 patients.

Lip biopsies were performed in all 8 patients. Figure 2 shows the histopathological findings of the lip biopsies of the 2 patients with recurrent parotitis (cases 1 and 2). Mild chronic inflammatory cell infiltration, mainly of plasma cells, was observed around the ducts without glandular lobe destruction in both specimens. These findings were compatible with mild SS. On the other hand, the other 6 patients without a history of parotitis did not show any histopathological evidence of SS.

We could not persuade any patients to agree to sialography.

Discussion

AIFS is defined by chronic nonspecific complaints, consistently positive ANA, and an absence of any other medical explanation for the complaints. Although only about 10% of AIFS patients fulfill the criteria for CFS, the most common complaint is fatigue. About 40% of patients have autoantibodies against a 62 kD nuclear protein (anti-Sa) which has not been found in patients with other autoimmune diseases. Of the CFS patients who were initially diagnosed as having AIFS, about 80% were positive for anti-Sa. CFS patients sometimes have heterogeneous pathophysiology, and in such cases ANA-positive CFS patients could be categorized as having a severe form of AIFS.

As Cabral et al.[8] described, to date none of the children with AIFS have been reported to develop any collagen diseases, and in addition, very few patients with AIFS are positive for marker autoantibodies for collagen diseases such as anti-DNA, anti-Sm, anti-U1RNP, or anticardiolipin. In this study, however, it was shown that 6.6% of AIFS patients were positive for anti-Ro/SSA antibodies. Anti-Ro/SSA antibodies are known to be present in most patients with SS, although it can also be detected in patients with some other autoimmune disorders.[9] In recent years, various conditions and diseases associated with anti-Ro/SSA have been collectively categorized as "anti-Ro/SSA syndrome,"[10] including SS, subacute cutaneous lupus, neonatal lupus syndrome, complement C2 or C4 deficiency, ANA-negative lupus, and photosensitive rash. Thus, anti-Ro/SSA antibodies are thought to be pathogenic, in spite of

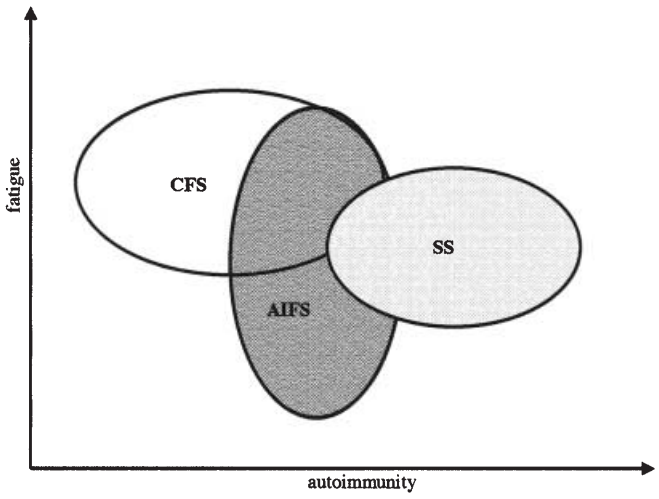

Fig. 3. A schematic diagram describing the relationship among chronic fatigue syndrome (*CFS*), *AIFS*, and *SS*. Some of the CFS patients could be defined as having a severe form of AIFS. On the other hand, some of the AIFS patients could be diagnosed as having SS (even though it was subclinical), and not AIFS

the fact that the pathogenicity of many other autoantibodies is still controversial. SS refers to keratoconjunctivitis sicca and xerostomia resulting from immune lymphocytes that infiltrate the lacrimal and salivary glands. In addition, patients with SS often suffer from vague symptoms such as fatigue and myalgia,[11] and it is now recognized as an important health problem in women.[12] In our two AIFS patients who had recurrent parotitis, the histopathological findings from the lip biopsies were equivalent to mild SS, indicating that at least some AIFS patients with anti-Ro/SSA antibodies could be diagnosed as having subclinical SS, and should not be diagnosed as having AIFS. Moreover, one AIFS patient with anti-Ro/SSA antibodies later fulfilled the CFS criteria. This suggests that some CFS patients with anti-Ro/SSA might be diagnosed as having subclinical SS and not CFS. Anti-Ro/SSA must be tested for a differential diagnosis for ANA-positive patients with CFS. None of the eight anti-Ro/SSA-positive patients had anti-Sa antibodies, suggesting a difference in immunological pathophysiology from other ANA-positive and anti-Ro/SSA-negative CFS cases. Figure 3 shows a schematic diagram illustrating the relationship among CFS, AIFS, and SS. We believe that ANA-positive CFS should be recognized as a severe form of AIFS. However, once patients previously diagnosed as having AIFS show evidence of SS, they should not be considered still to have AIFS, but should be diagnosed as having SS.

In terms of anti-Ro/SSA antibody specificity, however, seven of the eight AIFS patients with anti-Ro/SSA antibodies reacted with Ro52, and only one patient with Ro60, suggesting some difference between the condition of these patients and that of patients with SS, because SS sera have been reported predominantly to react with Ro60.[13] This may reflect not only a difference in age, but also pathophysiological differences. It could be that not all AIFS patients with anti-Ro/SSA antibodies would necessarily develop symptomatic SS. For instance, almost half the mothers of children with neonatal lupus erythematosus have neither SS nor SLE, and their sera predominantly react with Ro52.[14]

There had been a suspicion about the pathogenicity of anti-Ro52, because Ro52 could not be recognized by antibodies unless the antigen was denatured. Previously, however, we have showed that only Ro52 was expressed on the cell surface by stress such as heat shock or ultraviolet irradiation, and that Ro52 was antigenic only when it was expressed on the cell surface in the native state.[15] This suggests that anti-Ro52 antibodies could directly attack the cell surface antigen and induce type II allergy and tissue damage. Thus, anti-Ro52 can be pathogenic when the patient is under stressful conditions.

Finally, the method chosen to measure anti-Ro/SSA antibody titers might be crucial, because three of the eight sera were defined as negative by ELISA using affinity-purified antigen from calf spleen. Our previous study on the evolution of the Ro/SSA antigen showed that bovine tissues contain very small amounts of Ro52 protein.[16] Affinity-purification procedures would have let the Ro52 escape, because anti-Ro52 antibodies are mainly directed at the denatured form of the antigen.[17] Moreover, it has been shown that antigens are partially denatured by the coating procedure for ELISA plates.[18] Since the vast majority of anti-Ro60 antibodies are known to be directed to conformational epitopes of native antigen,[19] anti-Ro60 antibodies may lose reactivity in the ELISA. To date, the methods recommended to screen for anti-Ro/SSA antibodies would be a combination of DID using calf thymus extract and ELISA using a mixture of purified Ro60 and recombinant Ro52. Nevertheless, there may still be some antibodies which escape the screening process until recombinant human Ro60 with native structure is available for the screening test.

Thus, it is very important to measure anti-Ro/SSA antibodies in patients with AIFS. At least some of anti-Ro/SSA-positive patients could be excluded from AIFS, and these must be followed carefully because of the risk of a future development of SS.

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