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ORIGINAL ARTICLE

Anti-SS-A/Ro antibody determination by indirect immunofluorescence and comparison of different methods of anti-nuclear antibody screening

Evaluation of the utility of HEp-2 cells transfected with the 60 kDa SS-A/Ro as a substrate

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Abstract We evaluated the utility of HEp-2 cells transfected with the 60 kDa SS-A/Ro as a substrate for indirect immunofluorescence (IIF-HEp-2000) to compare several methods for screening Japanese serum samples for anti-SS-A/Ro antibodies. Serum samples from 243 Japanese patients were analyzed by IIF for anti-nuclear antibodies (ANAs), using HEp-2 cells (IIF-HEp-2), and for anti-SS-A/Ro 60 kDa antibodies, using IIF-HEp-2000 and enzyme-linked immunosorbent assay (ELISA). We performed double immunodiffusion and immunoprecipitation experiments, using the products of in vitro transcription and translation, to analyze sera for which there were discrepancies in the results of the IIF-HEp-2000 assay and ELISA. A total of 93 of the 243 serum samples showed findings positive for anti-SS-A/Ro antibodies. Notably, eight of the 93 sera gave positive findings for anti-SS-A/Ro antibodies by IIF-HEp-2000 but ANA-negative by IIF-HEp-2 analysis. Seven sera possessing anti-SS-A/Ro antibodies gave false negative results by IIF-HEp-2000; however, those samples were all ANA positive. ELISA for anti-SS-A/Ro antibodies showed that five and two samples gave false positive and negative results, respectively. Analysis by IIF-HEp-2000 was useful for primary screening of patients for ANAs, especially for anti-SS-A/Ro antibodies; the test could detect anti-SS-A/Ro antibodies not identified on standard substrates in samples obtained from the Japanese population, as reported for the Caucasian population.

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Introduction

Analysis of anti-nuclear antibodies (ANAs) is an important tool for the diagnosis of a variety of autoimmune rheumatic diseases [1, 2]. Anti-SS-A/Ro antibodies are detected with a high frequency in patients with systemic autoimmune diseases, including Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), subacute cutaneous lupus, and neonatal lupus [1]. The SS-A/Ro proteins coexist as part of an RNA–protein complex in which several polypeptides are not covalently associated with a family of small RNAs, designated Y1–Y5 [3]. At least two immunologically distinct proteins of 52 kDa and 60 kDa are present in SS-A/Ro ribonucleoprotein (RNP) particles [3, 4]. Based on experiments using monospecific autoantibodies against the 52 kDa and 60 kDa SS-A/Ro proteins, both polypeptides are localized to the nucleus and are associated with the same spectrum of Y-RNAs. Thus, the 52 kDa and 60 kDa polypeptides are components of a single class of intracellular RNP particle [3, 4].

Multiple techniques have been used to detect anti-SS-A/Ro antibodies, including indirect immunofluorescence (IIF) [5–7], immunodiffusion (ID) [8, 9], immunoprecipitation (IP) [3, 10], immunoblotting (IB) [4, 11–13], enzyme-linked immunosorbent assay (ELISA) [14, 15], and counter-immunoelectrophoresis [14–16]. IIF of HEp–2 cells is the classically used technique for the detection of ANAs; this method is an inexpensive and rapid screening technique [17]. Positive fluorescence staining indicates the presence of ANAs in examined sera. The use of IIF of tissue culture cells to detect anti-SS-A/Ro antibodies,

however, is limited by a low specificity and sensitivity, due to the low levels of endogenous SS-A/Ro proteins [18] and the diffusion of the antigen from the nucleus during sample preparation [6].

To improve the sensitivity and specificity of IIF detecting SS-A/Ro antigens, HEp-2 cells were transfected with a full-length human 60 kDa SS-A/Ro cDNA. Approximately 10–15% of transfected cells successfully overexpressed the 60 kDa SS-A/Ro proteins [19, 20]. Several clinical laboratories have evaluated this commercially available substrate and reported high sensitivities and specificities for the detection of anti-SS-A/Ro antibodies [16, 20–24]. There are several studies about racial differences concerning the prevalence of anti-SS-A/Ro antibody in SLE [25], and the human leukocyte antigen (HLA) alleles and clinical features associated with anti-SS-A/Ro responses [26], between Asian and Caucasian patients. Therefore, it is significant that the laboratory kit be evaluated also for Asian patients. Here, we report the results for 243 Japanese serum samples from patients with a variety of autoimmune diseases and healthy volunteers. We examined the ability and utility of transfected HEp-2 cells to serve as a substrate in assays detecting anti-SS-A/Ro antibodies. We compared the IIF results using transfected and non-transfected HEp-2 cells and evaluated the relative efficacies of ELISA, double immunodiffusion (DID), and IP to detect anti-SS-A/Ro antibodies.

Materials and methods

Sera

We obtained serum samples from 181 patients in the Department of Dermatology at Nagoya University Hospital. Sixty-two sera were obtained from healthy volunteers from the Social Insurance Chukyo Hospital, described in a previous study [27]. All samples were obtained from Japanese individuals. Informed consent was obtained from every participant before the study. Thirty-seven of these patients had been diagnosed with primary SS, 38 with SLE, 25 with systemic sclerosis (SSc), 11 with dermatomyositis (DM), and 70 with a variety of other diseases or symptoms. Diagnoses of SS, SLE, SSc, and DM, were made according to established criteria for each disease [28–31]. The correct numbers of patients with secondary SS were unclear, because not all of the patients enrolled in this study had been examined for possible concurrent secondary SS.

Almost all the serum samples were pre-screened by IIF, using HEp-2 cells (Fluoro HEPANA Test; MBL, Nagoya, Japan) and/or ELISA (described below). Samples were selected predominantly according to the existence of anti-SS-A/Ro antibodies and clinical diagnosis to assess the appropriateness of the IIF technique developed using HEp-2 cells transfected with 60 kDa SS-A/Ro (HEp-2000^{R} fluorescent ANA-Ro test system; Immuno Concepts, Sacramento, CA, USA).

Immunofluorescence

We performed immunofluorescence studies using HEp-2 cell substrate slides according to the manufacturer's protocol. Serum samples diluted to 1/40 in phosphate-buffered saline (PBS) were incubated with the slides for 30 min at 37°C. After they had been washed in PBS, we added a fluorescein isothiocyanate (FITC)-conjugated goat polyclonal antibody against human immunoglobulin (IgG + IgA + IgM heavy and light chain) (fluorescence/protein ratio 1.8) (MBL) for 30 min at 37°C to detect bound antibodies.

Substrate slides for SS-A/Ro-60 kDa-transfected HEp-2 cell (HEp-2000) were used in immunofluorescence studies according to the manufacturer's instructions. Briefly, serum samples diluted 1/40 in PBS were incubated with substrate slides for 30 min at room temperature. After being washed twice with PBS for 5 min, the slides were incubated with FITC-conjugated goat anti-human IgG (heavy and light chains) for an additional 30 min at room temperature. After they had been washed twice in PBS, we placed a coverslip over the slide and examined the samples under ultraviolet light with a fluorescence microscope at ×40 power. Approximately 10–15% of interphase cells overexpress SS-A/Ro antigen, which is localized in a nuclear and nucleolar distribution. These cells can be easily distinguished from those endogenously expressing SS-A/Ro antigen at the levels seen in HEp-2 cells [32]. We defined those serum samples positive for anti-SS-A/Ro antibodies using HEp-2000 cells, which revealed both the characteristic nuclear and nucleolar staining pattern in transfected interphase cells [1]. Specimens that exhibited fluorescence were titrated by two-fold serial dilutions to endpoint, then classified into several ANA staining patterns, such as nuclear, nucleolar, and speckled patterns.

When the results of IIF-HEp-2 were compared with those of IIF-HEp-2000, all ANA-positive sera exhibited various staining patterns of other antibodies that were unaffected by overexpression of the 60 kDa SS-A/Ro proteins (data not shown).

Immunoprecipitation

We used an in vitro transcription/translation (TnT) system (TnT Quick Coupled Transcription/Translation Systems; Promega, Madison, WI, USA) with biotin–lysil–tRNA (Promega) to produce full-length 60 kDa or 52 kDa SS-A/Ro proteins. Full-length 60 kDa and 52 kDa SS-A/Ro

cDNAs were the kind gift of Dr. Edward Chan [33]. TnT products (10 µl) were solubilized in 50 µl IP solution (10 mM PBS and 0.5% NP-40 for the analysis of 60 kDa SS-A/Ro or 10 mM PBS, 0.5% NP-40, 0.1% sodium dodecyl sulfate (SDS), and 0.5% deoxycholic acid for the analysis of 52 kDa SS-A/Ro). After addition of 10 µl serum and 0.5 mg/ml bovine serum albumin to the protein solution, we adjusted sample volumes to 200 µl with IP solution. After incubation with gentle shaking at room temperature for 1 h, immune complexes were precipitated by the addition of 40 µl 50% protein G-Sepharose (Pharmacia Biotech, Wikströms, Sweden) to a total volume of 400 µl, with gentle stirring for 1 h at room temperature. After washing five times in a two-fold volume of IP solution, IgG-bound proteins retained by the Sepharose were resuspended in SDS sample buffer (4% SDS, 10% 2-mercaptoethanol, 20% glycerol, 0.01% bromophenol blue, and 125 mM Tris-HCl, pH 6.8) and heated to 60°C for 5 min. Supernatants were electrophoresed on 7.5% SDS gels and transferred onto polyvinylidene difluoride (PVDF) membranes [27]. To detect immunoprecipitated biotinylated SS-A/Ro 52 kDa or 60 kDa proteins, we stained the membranes using a Transcend colorimetric translation detection system (Promega) according to the manufacturer's instructions. The results for the IP analysis for 60 kDa anti-SS-A/Ro antibodies were the same as those determined with the IP solution for 52 kDa SS-A/Ro (data not shown).

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed according to the manufacturer's instructions using commercial plates coated with 60 kDa-SS-A/Ro proteins (purified from animal tissue samples), 50 kDa-SS-B/La (purified recombinant protein of SS-B/La), or RNP (purified recombinant proteins of 70 kDa-U_1-PNP, U_1-RNP-A, and U_1-RNP-C) (MESACUP-2 test, MBL). Serum samples were added for 1 h at room temperature at a dilution of 1:100 in attachment buffer. The absorbance of duplicate samples was determined at 450 nm. Cutoff values for ELISA have been established by the manufacturer: for the anti-SS-A/Ro antibody; values under 10 U/ml are negative, while those greater than or equal to 30 U/ml are positive. When the results produced values between positive and negative cutoffs, those samples were re-examined. If the redetermined values were below the positive cutoff value (<30 U/ml), they were deemed to be negative.

Double immunodiffusion (DID)

DID was performed using mammalian spleen cell extracts (anti-SS-A antibody "SRL", SRL, Tokyo, Japan) as the antigen source. Patients' serum samples were applied to agarose gels; the precipitin line was then determined.

Statistical analysis

The significances of the frequency of false negative findings of IIF-HEp-2000 for sera from patients with each disease were determined by Fisher's exact test. P values <0.05 were considered to be significant.

Results

All serum samples were analyzed by IIF-HEp-2, regardless of ANAs' positivity. IIF-HEp-2000 and ELISA were then performed to detect anti-SS-A/Ro antibodies. Serum samples were defined as containing anti-SS-A/Ro antibodies when they possessed both the characteristic nuclear and nucleolar staining of HEp-2000 cells and a positive value by ELISA. Serum samples exhibiting inconsistent results between IIF-HEp-2000 staining and ELISA were further examined by DID and IP. Detection of anti-SS-A/Ro antibodies by DID was defined as positive for the presence of antibodies (Fig. 1).

Classification of samples without ANAs

Of the total 243 samples, 58 (24%) were ANA-negative (≥1:40) by IIF-HEp-2 analysis; 50 of these samples (86%) were negative for both the anti-SS-A/Ro antibodies staining pattern by IIF-HEp-2000 analysis and anti-SS-A/Ro antibodies by ELISA. The eight remaining samples (14%), however, revealed the characteristic positive staining pattern by IIF-HEp-2000 and also positive results by ELISA (Fig. 1 and Fig. 2a, b). These sera were from three patients with SS, one with SLE, one with the overlap syndrome of SLE and SS, and three healthy volunteers.

Classification of samples with ANAs

Of 185 sera (76% of 243 sera) with ANAs (≥1:40) by IIF-HEp-2, 76 samples (41% of 185 sera) were also positive for anti-SS-A/Ro antibodies by both IIF-HEp-2000 and ELISA (Fig. 2c, d), while 95 (51%) were negative by both assays (Fig. 1). Of the remaining 14 serum samples, 12 (from patients 1–12 in Table 1) were negative for anti-SS-A/Ro antibodies by IIF-HEp-2000, but positive by ELISA, and two samples (from patients 13 and 14 in Table 1) were positive by IIF-HEp-2000, but negative by ELISA. These samples were further investigated for the presence of anti-SS-A/Ro antibodies by DID and IP (Figs. 1 and 3).

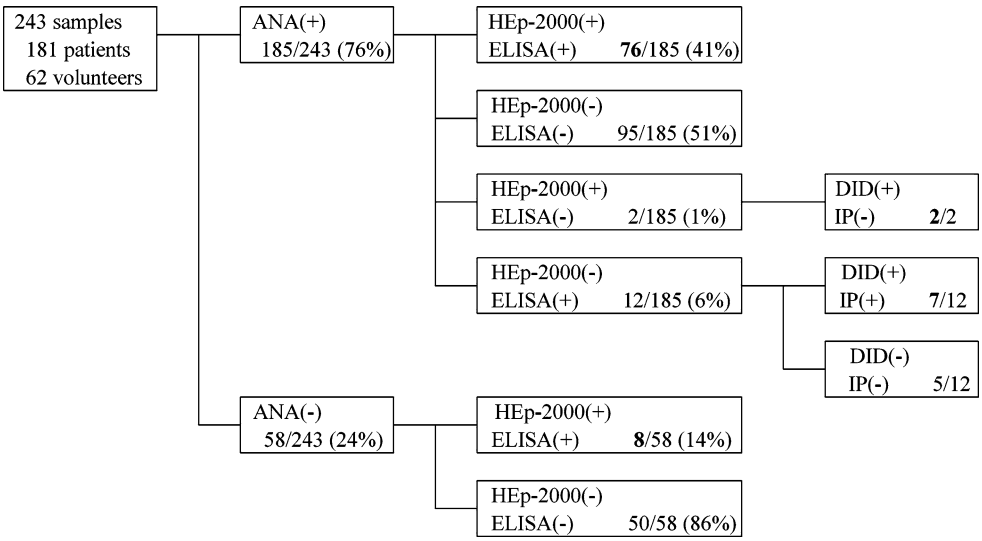

Fig. 1 Schema of the study protocol and results of various assays for the detection of anti-SS-A/Ro antibodies in 243 serum samples. *Bold letters* denote the number of anti-SS-A/Ro antibody-positive sera. ANA (+) or (−) ANA-positive or -negative by IIF-HEp-2, HEp-2000 (+) or (−) positive or negative for the characteristic nuclear and nucleolar staining patterns by IIF-HEp-2000, ELISA (+) or (−) anti-SS-A/Ro antibody-positive or -negative by ELISA, IP (+) or (−) 60 kDa anti-SS-A/Ro antibody-positive or -negative by IP, DID (+) or (−) anti-SS-A/Ro antibody-positive or -negative by DID

Investigation with DID and IP analysis

In sera from patients 1–12 (Table 1), SS-A/Ro 60 kDa antigen reactivity could be detected in seven samples by both DID and IP; these samples were judged to be false negatives by IIF-HEp-2000. Five of these samples were obtained from patients with SLE, three of whom also had anti-U_1-RNP antibodies detected by ELISA. The remaining two sera were from anti-U_1-RNP antibody-positive patients, one with DM and the other with SSc (Table 1). The remaining five samples in Fig. 1 (patients 8–12 in Table 1) were negative by both DID and IP, which suggested these were false positive reactions by ELISA for anti-SS-A/Ro antibodies. The values determined by ELISA for these five samples were under 50 U/ml.

The two samples (from patients 13 and 14 in Table 1) that were positive by IIF-HEp-2000 but negative by ELISA were also positive by DID. Therefore, the ELISA result was a false negative reaction. One of the two serum samples (from patient 14 with SLE) was positive for anti-SS-A/Ro 52 kDa antibodies by IP analysis and for anti-SS-B antibodies by ELISA. The other (from patient 13) possessed anti-DFS70 antibodies [27]; the patient lacked any symptoms of autoimmune rheumatic disease.

Relationship between the HEp-2000-false negative samples and clinical diagnosis

As shown in Table 2, 93 of the 243 sera were determined to show positive findings for anti-SS-A/Ro antibodies. The majority of antibody-positive samples were obtained from patients with SLE (30 sera) and SS (35 sera). Only five of the 30 serum samples (17%) obtained from patients with SLE lacked the characteristic anti-SS-A/Ro antibody staining pattern by IIF-HEp-2000; the sera of all 35 patients with SS exhibited this characteristic staining pattern (SLE vs SS, $P = 0.017$). Only two of the 63 non-SLE patients with anti-SS-A/Ro antibodies lacked the characteristic staining pattern (SLE vs non-SLE, $P = 0.034$).

Discussion

Staining of HEp-2000 substrates with anti-SS-A/Ro antibodies labels the nucleus and nucleolus. In ordinary HEp-2 substrates, however, it generates a speckled pattern. These discrepant results may be due to the presence of different epitopes which are specific for overexpressed antigens or structural changes in SS-A/Ro antigen in HEp-2000 cells. Fouraux et al. described the association of the RNA-binding nucleolar protein, nucleolin, with Ro RNPs containing hY1 or hY3 RNA [34], which could explain the intranucleolar localization of 60 kDa SS-A/Ro antigen in HEp-2000. Similar changes or differences in exposed epitopes may also occur during the other techniques. For example, the antigen required for IP might be the target of intracellular modifications during in vitro translation and expression.

In total, 93 sera were concluded to be positive for anti-SS-A/Ro antibodies in our study. Eight of these samples gave negative findings by IIF-HEp-2, but produced positive findings for anti-SS-A/Ro antibodies by IIF-HEp-2000 and

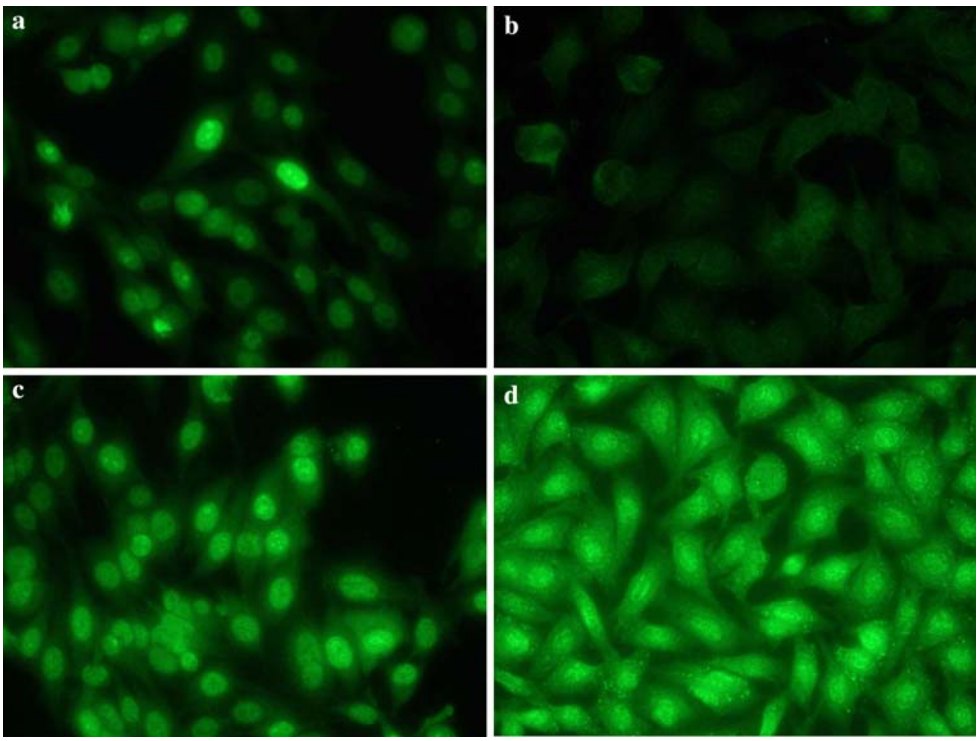

Fig. 2 a–d Representative immunofluorescence staining of HEp-2 cells transfected with full-length human 60 kDa SS-A/Ro cDNA and untransfected HEp-2 cells. **a**, **c** Transfected HEp-2 cells stained with human sera containing anti-SS-A/Ro antibodies reveal the characteristic nuclear and nucleolar staining pattern. The sera used in **a** and **c** were obtained from different patients. **b** Untransfected HEp-2 cells stained with the same sera as used in **a** display no obvious staining. **d** Untransfected HEp-2 cells stained with the same sera as used in **c**. A speckled staining pattern is observed for all interphase cells. ×400

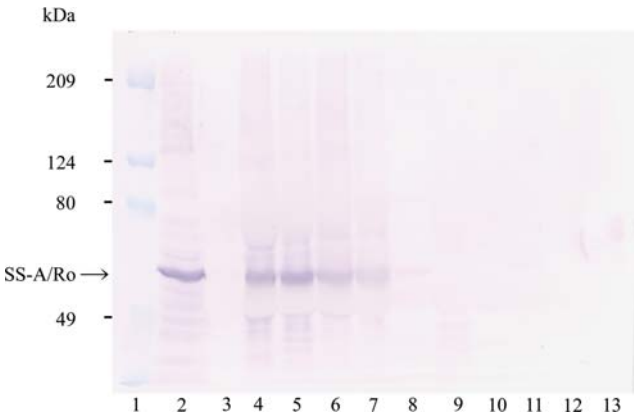

Fig. 3 Immunoprecipitation using in vitro transcription/translation production of full-length 60 kDa SS-A/Ro protein. *Lane 1* molecular mass marker, *lane 2* an aliquot of the raw TnT product, *lane 3* negative control serum, *lane 4* positive control serum, *lanes 5–7* representative positive examined serum samples. The specific signals at 60 kDa could be detected. *Lanes 8–12* representative negative examined serum samples. A specific signal was not detected. *Lane 13* mock control: TnT product was incubated with PBS alone in the absence of sera

ELISA (Fig. 1). Thus, IIF-HEp-2 analysis for ANAs missed the presence of anti-SS-A/Ro antibodies in approximately 9% of antibody-positive samples. As these eight sera were obtained from patients with SS, SLE, or healthy volunteers, there may have been some ligands that were not unique that were cross-reacting with the epitopes of overexpressed SS-A/Ro antigen but not with antigens in HEp-2 cells.

Bossuyt et al. noticed that the reactivities of overexpressed SS-A/Ro antigen in transfected cells differed among diseases. In their study, 24 of 25 sera from patients with SS reacted with overexpressed antigens, while only seven of 17 sera from SLE patients did [32]. In our study, seven serum samples (from patients 1–7 in Table 1) with anti-60 kDa-SS-A/Ro antibody reactivity did not reveal the typical staining pattern of anti-SS-A/Ro antibodies by IIF-HEp-2000. Five of these seven were obtained from patients with SLE, and the other two were from non-SLE patients. These results represent a statistically significant difference by Fisher's exact test in the frequency of false negative findings by IIF-HEp-2000 in SLE patients in comparison with SS patients and non-SLE patients. Some epitopes especially associated with SLE might not have been displayed by the effect of overexpression of the antigen in the transfected cells.

Bossuyt et al. also determined that the characteristic anti-SS-A/Ro antibody staining pattern by IIF-HEp-2000 could be detected in almost all samples positive for anti-SS-A/Ro and anti-SS-B antibodies, but not in samples in which anti-U_1-RNP antibodies coexisted with anti-SS-A/Ro antibodies [32]. In our study, the seven samples with anti-SS-A/Ro antibodies demonstrating an absence of the characteristic anti-SS-A/Ro antibody staining pattern by IIF-HEp-2000, that were positive for anti-SS-A/Ro antibodies by ELISA, did not have anti-SS-B antibodies, while four samples also possessed anti-U_1-RNP antibodies. Such anti-U_1-RNP antibodies may have had an inhibitory effect

Table 1 Sera which revealed discrepancy in results between IIF-HEp-2000 and anti-SS-A antibodies for ELISA

Patient number	Diagnosis	IIF-HEp-2[a]	IIF-HEp-2000[b]	ELISA 60 kDa SS-A/Ro	IP-60 kDa SS-A/Ro	IP-52 kDa SS-A/Ro	ELISA anti-RNP	ELISA anti-SS-B	DID
1	SLE	Speckled	−	+	+	−	+	−	+
2	SLE	Homogeneous, nucleolar	−	+	+	+	−	−	+
3	SLE	Homogeneous, speckled	−	+	+	−	+	−	+
4	SLE	Speckled	−	+	+	−	−	−	+
5	SLE	Homogeneous, speckled	−	+	+	+	+	−	+
6	SSc	PCNA, cytoplasmic	−	+	+	+	−	−	+
7	DM	Speckled	−	+	+	−	+	−	+
8	SLE	Speckled	−	+	−	−	−	−	−
9	SLE	Homogeneous, speckled	−	+	−	−	−	+	−
10	SLE	Homogeneous, nucleolar, cytoplasmic	−	+	−	−	−	−	−
11	RA + SSc	Homogeneous, nucleolar	−	+	−	−	−	−	−
12	SSc	Speckled	−	+	−	−	+	−	−
13	ANA (+)	Homogeneous, speckled	+	−	−	−	−	−	+
14	SLE	Speckled	+	−	−	+	−	+	+

IP immunoprecipitation, *DID* double immunodiffusion, *SLE* systemic lupus erythematosus, *SSc* systemic sclerosis, *PCNA* proliferating cell nuclear antigen, *DM* dermatomyositis, *RA* rheumatoid arthritis, *ANA* anti-nuclear antibody

[a] IIF-HEp-2: indirect immunofluorescence using HEp-2 cells

[b] IIF-HEp-2000: anti-SS-A/Ro staining pattern in indirect immunofluorescence using HEp-2000 cells

Table 2 Clinical diagnosis and profile of anti-SS-A/Ro antibodies

Parameter	Total numbers	ANA (+)[a] HEp-2000 (+)[b] ELISA (+)[c]	ANA (+) HEp-2000 (+) ELISA (−)	ANA (+) HEp-2000 (−) ELISA (+)	ANA (−) HEp-2000 (+) ELISA (+)
SLE	30	22	1	5*,**	2
SS	35	32	0	0*,**	3
SSc	14	13	0	1**	0
DM	3	2	0	1**	0
Volunteers	5	2	0	0**	3
Others	6	5	1	0**	0
Total numbers	93	76	2	7	8

SLE systemic lupus erythematosus, *SS* Sjögren's syndrome, *SSc* systemic sclerosis, *DM* dermatomyositis

[a] ANA (+) or (−): anti-nuclear antibody positive or negative by IIF using non-transfected HEp-2 cells

[b] HEp-2000 (+) or (−): positive or negative for anti-SS-A/Ro staining pattern by indirect immunofluorescence (IIF) using HEp-2000

[c] ELISA (+) or (−): anti-SS-A/Ro antibody positive or negative by ELISA

*, ** Statistically analyzed by Fisher's exact test. The frequency of false negative reactions by IIF using transfected HEp-2 cells (HEp-2000) for the sera from patients of these diseases were significant (* SLE vs SS, $P = 0.017$; ** SLE vs non-SLE, $P = 0.034$)

on the ability of anti-SS-A/Ro antibodies to recognize the overexpressed epitope produced by IIF-HEp-2000. Alternatively, it is possible that coexisting high-titer anti-U_1-RNP antibodies may have masked the staining pattern of SS-A/Ro. This conclusion was supported by the fact that characteristic staining pattern seen with anti-SS-A/Ro antibodies in HEp-2000 cells could be masked by the addition of high-titer anti-U_1-RNP or other anti-nuclear antibodies during the primary antibody incubation step in IIF experiments (data not shown).

The sera of patients 13 and 14 (Table 1) demonstrated the characteristic anti-SS-A/Ro antibody staining pattern by IIF-HEp-2000. These samples gave positive findings by DID but negative ones for anti-SS-A/Ro 60 kDa reactivity by ELISA and IP. There might be new SS-A/Ro epitopes displayed by protein–protein and/or protein–nucleic acid

interactions, depending on experimental methods; this possibility is supported by previous reports that a few serum samples reacted with the hyRNA component of the small cytoplasmic RNP-SS-A/Ro complex and with the overexpressed SS-A/Ro antigen present in HEp-2000 cells [35].

In 229 samples there were no discrepancies in the results of the analyses of IIF-HEp-2000 and the ELISA; these samples were not analyzed further by IP and DID. However, we cannot deny the possibility of false positive or negative results in these 229 samples by IIF-HEp-2000 or ELISA. The false negative findings by IIF-HEp-2000 did not impair the detection of anti-SS-A/Ro antibodies, as sera were definitely positive for ANAs by IIF-HEp-2 and then confirmed to have anti-SS-A/Ro antibodies by ELISA analyses. Eight sera (9% of the 93 anti-SS-A/Ro antibody-positive sera) were negative for ANAs by IIF-HEp-2 and would have been missed if other analyses, such as IIF-HEp-2000, had not been done to detect anti-SS-A/Ro antibodies.

In this study, concurrent detection of anti-SS-A/Ro antibodies by IIF-HEp-2000 and ELISA was useful. As a first screening step for ANAs in a variety of autoimmune rheumatic diseases, IIF-HEp-2000 was as equally effective at detecting autoreactive antibodies as IIF-HEp-2 was (data not shown). IIF-HEp-2000 has the additional utility over IIF-HEp-2 of being able to detect anti-SS-A/Ro antibodies specifically. IIF-HEp-2 has the disadvantage of reduced specificity and sensitivity for the detection of anti-SS-A/Ro antibodies; ELISA is only able to examine restricted kinds of autoantibodies per experiment. Moreover, IIF-HEp-2000 is more inexpensive than ELISA per well. As seen with IIF-HEp-2, IIF-HEp-2000 was rapid and inexpensive and could detect anti-SS-A antibodies in samples in which further investigation could not be performed due to the negative findings by IIF-HEp-2 at the primary screening for autoimmune rheumatic diseases. Such serum samples comprise an important population of anti-SS-A/Ro antibody-positive individuals that cannot be ignored.

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