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ORIGINAL ARTICLE

Prevalence of disease-specific antinuclear antibodies in general population: estimates from annual physical examinations of residents of a small town over a 5-year period

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Abstract The aim of this study was to investigate the types and prevalence of disease-specific antinuclear antibodies (ANAs) and their relationship to rheumatic diseases in the general Japanese population. An immunofluorescence (IF) method was used for the first screening of ANA levels in serum samples obtained from 2181 residents of a small Japanese town. Individuals positive for IF-ANA were then further tested for disease-specific ANAs using eight enzyme immunoassays. Physical status and the presence of illness were determined by means of questionnaires and medical examinations. Based on the result of the IF-ANA assay, the rates of positive samples at 1:40 and 1:160 dilutions were 26.0 and 9.5%, respectively, with females have significantly higher positivity rates than males ($P < 0.0001$). Among 566 IF-ANA-positive individuals, 100 individuals were found to have 114 disease-specific ANAs. Anti-SSA/Ro, anti-centromere, and anti-U1RNP antibodies were detected in 58, 30, and 11 individuals, respectively, but anti-Sm, anti-Scl-70, and anti-Jo-1 antibodies were undetectable. Questionnaires and medical examinations revealed that among 60 disease-specific ANA-positive individuals that were available for testing, six had Sjögren's syndrome (SS), five were suspected of having SS, and five had rheumatoid arthritis. Surprisingly, 34 (57%) of the disease-specific ANA-positive individuals were clinically healthy. Anti-SSA/Ro, anti-centromere, and anti-U1RNP antibodies were quite frequent among clinically healthy Japanese subjects, although anti-Sm, anti-Scl-70, and anti-Jo-1 antibodies were not. Of the 60 individuals who tested positive for disease-specific ANAs, 30% (18/60) actually manifested systemic rheumatic diseases, while 50% showed no detectable signs or symptoms of rheumatic diseases.

Keywords Antinuclear antibodies · Centromere · Prevalence · Residents · Ro/SSA

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Introduction

Antinuclear antibodies (ANAs) are frequently detected in several systemic rheumatic diseases, such as systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and Sjögren's syndrome (SS) [1, 2]. An immunofluorescence (IF) method using HEp-2 cells has been widely applied for ANA screening because this method can detect most ANAs associated with human diseases [3, 4]. However, specificity for IF-ANA is poor, as evidenced by the high false–positive rates among healthy individuals, regardless of the high sensitivity among patients with systemic rheumatic

diseases [5]. In 1997, Tan et al. investigated the reference range for IF-ANAs in healthy individuals at 15 institutions worldwide and reported positive IF-ANA results in 31.7 and 5.0% of healthy individuals at dilutions of 1:40 and 1:160, respectively [5]. We also found that IF-ANA positivity rates in healthy Japanese subjects were 26.8 and 8.1% at cutoff dilutions of 1:40 and 1:160, respectively [6]. The detection of disease-specific ANAs is useful for both a diagnosis of systemic rheumatic diseases and for therapeutic evaluation [1, 7]. However, little is known whether disease-specific ANAs occur among healthy individuals [8–12]. The association between the appearance of ANAs and the onset of rheumatic disease has not been fully evaluated.

In the study reported here, we assayed for ANAs in serum samples from individuals who participated in annual physical checkups. Levels of ANA in more than 2000 residents of a small Japanese town were checked by IF, since approximately one-quarter of the residents tested positive for ANAs at a dilution of 1:40. The samples that tested positive in the IF-ANA assay were then subjected to eight individual disease-specific ANA assays, and the physical status and presence of illness in the subjects were also determined by means of a questionnaire and medical examination. This study determined the prevalence of disease-specific ANAs in the general population and their relationship to rheumatic diseases.

Material and methods

Study population

Serum samples were obtained from 2181 residents of a town in Hyogo Prefecture, Japan, who underwent an annual physical examination from 1996 to 2000. The median ages (range) of 1409 females and 772 males were 53 (20–91) and 59 (20–93) years, respectively. Initial samples were obtained from individuals who underwent more than one annual physical examination. If a sample was positive for disease-specific ANAs, annual samples from the same individual were further examined for disease-specific ANAs. Sera were separated by centrifugation and stored below −40°C. In accordance with the guidelines of the Japan Epidemiological Association, individual informed consent was obtained for this study, and official permission to use each sample was obtained from the city hall.

Measurement of ANAs

We analyzed ANA using the IF-based kit FANAwell (Mitsubishi Kagaku Iatron, Tokyo, Japan), with HEp-2 cells as substrates and a mixture of fluorescein isothiocyanate (FITC)-labeled mouse anti-human immunoglobulins (IgG, IgA and IgM) as secondary antibodies. The ANA tests were performed using serum samples at 1:40 dilutions. All slides were microscopically examined by two laboratory technologists. All samples positive at the 1:40 dilution were further tested in eight individual disease-specific ANA assays [anti-U1RNP, Sm, SSA/Ro, SSB/La, Scl-70, centromere, Jo-1, and double-stranded DNA (dsDNA)]. We analyzed eight disease-specific ANAs using enzyme immunoassay (EIA) kits with the indicated antigens and cutoff values: anti-U1RNP antibody [MESACUP-2 TEST RNP, Medical & Biological Laboratories (MBL), Nagoya, Japan; antigens were recombinant 70-kDa, A-peptide, and C-peptide, 22], anti-Sm antibody (MESACUP-2 TEST Sm, MBL; purified Sm antigens, 30), anti-SSA/Ro antibody (MESACUP-2 TEST SS-A, MBL; purified SSA/Ro protein, 30), anti-SSB/La antibody (MESACUP-2 TEST SS-B, MBL; recombinant SSB/La protein, 25), anti-Scl-70 antibody (MESACUP-2 TEST Scl-70, MBL; recombinant Scl-70 protein, 24), anti-centromere antibody (ACA: MESACUP-2 TEST CENP-B, MBL; recombinant CENP-B protein, 16), anti-Jo-1 antibody (MESACUP-2 TEST Jo-1, MBL; recombinant Jo-1 protein, 18), and anti-dsDNA antibody (MESACUP DNA-II TEST dsDNA, MBL; lambda-phage-derived purified double-stranded DNA, 12) according to the manufacturer's instructions [13].

To confirm the presence of disease-specific ANAs, we performed double immunodiffusion (DID) assays (ENA-1 and ENA-2, MBL) for anti-U1RNP, -SSA/Ro, and -SSB/La antibodies in disease-specific ANA-positive samples using an EIA [14]. Radioimmunoassays (DPC Anti-DNA, DIA-IATRON) were applied to confirm that the samples were anti-dsDNA-positive according to the EIA [15]. In addition, samples positive for ACA based on the EIA results were identified as having discrete speckled patterns by IF-ANA.

Measurement of viral antibodies

Because many investigators have reported a correlation between viral infection and the appearance of disease-specific ANAs [16, 17], we analyzed anti-HBc (hepatitis B core antigen) antibodies using the EIA-based kit, Enzygnost-Anti-HBc monoclonal (Dade Behring, Marburg, Germany), anti-HCV (hepititis C virus) antibodies by passive hemagglutination, HCV·PHA (Abbott Japan, Tokyo, Japan) and anti-HTLV-I (human T-cell lymphotropic virus type 1) antibodies by passive particle agglutination, SERODIA HTLV-I (Fujirebio, Tokyo, Japan).

Follow-up survey of individuals positive for disease-specific ANAs

Annual changes in antibody levels in individuals positive for disease-specific ANA were studied for 5 years along with their physical status, which was determined from medical examinations and questionnaires.

Data analysis

The prevalence of disease-specific ANAs in females and males was tested by comparing two proportions or by the Fisher's exact probability test. We conducted multiple logistic regression analysis to determine the prevalence of disease-specific ANAs to adjust age, gender and other known confounding factors using STAT FLEX ver. 5.0 (ARTEC, Tokyo, Japan).

Results

Frequency of ANA in sampled population

Serum samples positive for ANA were initially identified by the IF assay at a 1:40 dilution in 566 of the 2181 subjects (26.0%). This ANA-positive group consisted of 446 of the 1409 females (31.7%) and 120 of the 772 males (15.5%) that were tested. Positivity for IF-ANA at a 1:160 dilution was 9.5% (12.4% in females and 4.3% in males). Table 1 compares IF-ANA positivity at the 1:40 and 1:160 dilutions by gender and age. The proportion of ANA-positivity was significantly higher among females than among males at these dilutions (comparison of two proportions).

Table 1 Comparison of positivity for IF-ANA at 1:40 and 1:160 dilutions by gender and age

Age of participants	*n*	Dilution					
		1:40			1:160		
		Females	Males	Total	Females	Males	Total
20–30	258	39.9**	21.3	34.1	16.3	8.8	14.0
31–40	335	33.1***	11.1	27.2	13.1**	1.1	9.9
41–50	369	28.4***	8.8	21.1	9.5*	2.9	7.0
51–60	301	31.2***	10.1	24.3	11.9	5.1	9.6
61–70	516	30.8*	21.6	27.1	14.9***	5.3	11.1
71–80	343	30.1**	17.2	25.1	10.5*	3.0	7.6
81–93	59	20.0	12.5	16.9	0.0	4.2	1.7
Total	2181	31.7[a]	15.5	26.0	12.4[a]	4.3	9.5

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, females versus males

Expressed values are positivity ratios (%)

Frequency of disease-specific ANAs

Disease-specific ANAs were identified in 100 of the 566 IF-ANA-positive persons (1:40 dilution or higher) using eight disease-specific ANA EIAs. Figure 1 shows the prevalence of disease-specific ANAs according to gender and age. Eleven samples were positive for anti-U1RNP (ten females and one male), 58 for anti-SSA/Ro (50 females and eight males), five for anti-SSB/La (four females and one male), 30 for ACA (26 females and four males), and ten for anti-dsDNA (six females and four males). None of the sample were positive for anti-Sm, anti-Scl-70, or anti-Jo-1. Antibody titers by EIAs (mean ± SD, index) were as high as 48.6 ± 27.1 for anti-U1RNP ($n = 11$), 83.9 ± 33.9 for anti-SSA/Ro ($n = 58$), 61.0 ± 33.3 for anti-SSB/La ($n = 5$), 83.7 ± 61.6 for ACA ($n = 30$), and 26.2 ± 15.2 for anti-dsDNA antibody ($n = 10$). In terms of the prevalence of disease-specific ANAs and IF-ANA staining profiles, the chi-square test showed that anti-SSA/Ro antibody correlated with a speckled pattern [odds ratio (OR) 12.1], anti-SSA/Ro antibody correlated with a cytoplasmic pattern (OR 2.1) and ACA correlated with a discrete speckled pattern (OR 348.9).

Positive results were confirmed by further analyses in seven of the 11 anti-U1RNP-positive samples, 50 of the 58 anti-SSA/Ro-positive samples, one of the five anti-SSB/La-positive samples, 23 of the 30 ACA-positive samples, and three of the ten anti-dsDNA-positive samples.

Assuming that all disease-specific ANAs were detected by IF-ANA screening, the prevalence of anti-SSA/Ro in the general population was 2.7% (3.5% in females and 1.0% in males) and that of ACA was 1.4% (1.8% in females and 0.5% in males), and both were significantly higher in females than in males ($P < 0.0001$ for anti-SSA/Ro according to the comparison of two proportions; $P = 0.011$ for ACA by Fisher's exact probability test). Prevalences of anti-U1RNP, anti-SSB/La, or anti-dsDNA between males and females did not significantly differ ($P = 0.057$, $P = 0.31$ and $P = 0.50$, respectively, by comparison of two proportions). The frequency of anti-SSA/Ro in females tended to significantly increase with age until 80 years ($P = 0.01$; chi-square test). However, the frequency did not significantly differ among the seven age groups for females with ACA ($P = 0.43$).

Correlation between disease-specific ANAs and clinical features

We examined potential correlations between disease-specific ANAs and factors relative to physical status, such as results from urinalyses, routine hematological studies, and blood chemistry tests. We applied multiple logistic

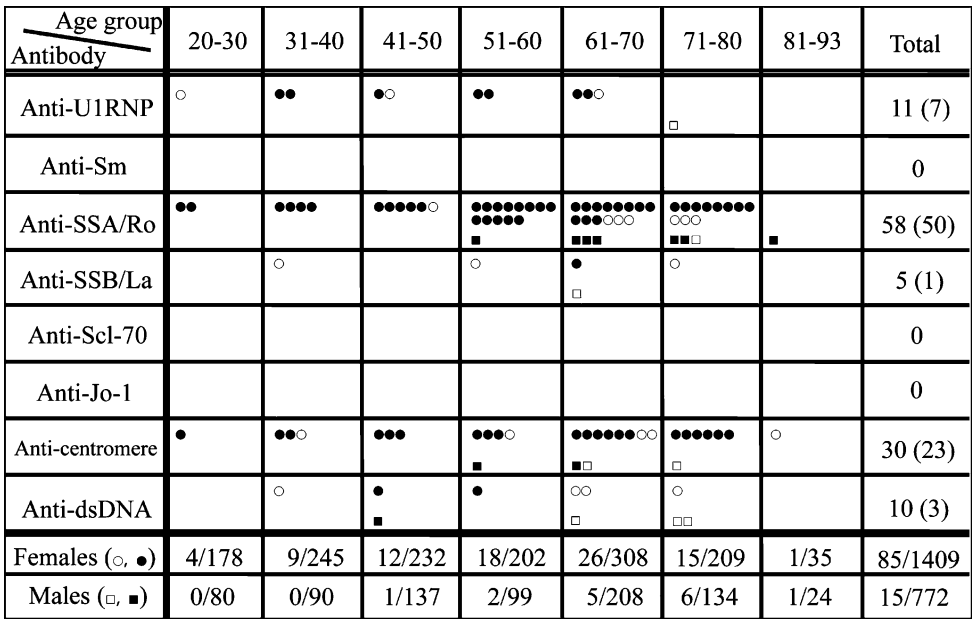

Fig. 1 Frequency of eight disease-specific antinuclear antibodies (*ANAs*) according to gender and age. Enzyme immunoassay (*EIA*) detected 114 disease-specific ANAs in 100 of the 566 immunofluorescence (*IF*)-ANA positive individuals. Samples containing more than one disease-specific ANA included: three samples that were positive for anti-SSA/Ro + anti-SSB/La; two samples positive for anti-SSA/Ro + anti-SSB/La + anti-centromere; four samples positive for anti-SSA/Ro + anti-centromere; three samples positive for anti-SSA/Ro + anti-dsDNA. *Closed circles* and *squares* show samples that were confirmed using methods other than EIA (double immunodiffusion for anti-RNP, anti-SSA/Ro, and anti-SSB/La; radioimmunoassay for anti-dsDNA; discrete speckled patterns by IF-ANA for anti-centromere). *Open circles* and *squares* show samples that were positive only by EIA. Values in the *right-hand column* show numbers of disease-specific ANA-positive subjects according to EIA, and numbers of confirmed samples determined using other methods are shown in *parenthesis*. The *bottom two rows* show the numbers of disease-specific ANA positive/total numbers of females and males in each age group

regression analysis to clarify relationships with hypertension, hypotension, hyperlipidemia, anemia, serum uric acid value, glycosuria, arteriosclerosis, heart disease, liver disease, and kidney disease in four categories (normal, retest required, more precise test required, and medical examination required). In addition to gender and age, anti-SSA/Ro was also associated with liver disease ($P = 0.026$; Table 2). None of the anti-U1RNP antibodies, anti-dsDNA antibodies, and ACA was related to any other factor (data not shown).

Table 2 Correlation between anti-SSA/Ro antibody and test results at physical examination

Factor	Estimate	*Z* value	*P*
Intercept	−6.36	10.0	–
Age	0.03	3.5	0.0005
Gender	1.52	4.1	<0.0001
Hypertension	0.09	0.7	0.469
Hypotension	0.17	0.5	0.646
Hyperlipidemia	−0.21	1.2	0.213
Heart disease	−0.23	1.1	0.286
Arteriosclerosis	−0.34	0.7	0.457
Anemia	−0.18	0.9	0.353
Liver disease	0.36	2.2	0.026
Glycosuria	0.21	1.3	0.195
Kidney disease	−0.02	0.1	0.904
Hyperuricemia	−0.24	0.8	0.421

Data were analyzed by multiple logistic regression

Correlation between the presence of ANA and viral antibodies

To evaluate the correlation between ANA presence and viral infection, we compared the prevalence of anti-HBc, anti-HCV and anti-HTLV-I antibodies between 566 ANA-positive and 370 ANA-negative individuals randomly selected from corresponding gender- and age-matched groups. The two groups did not significantly differ with respect to the prevalence of these antibodies ($P = 0.86$, $P = 0.86$ by comparison of two proportions, and $P = 0.69$ by Fisher's exact probability test). Six of 40 (15%) anti-SSA/Ro-positive individuals and one of 17 (6%) ACA-positive individuals had anti-HBc antibody, and each one of 40 (3%) anti-SSA/Ro-positive persons and 17 (6%) ACA-positive individuals had anti-HCV antibody.

Table 3 Physical conditions of individuals positive for disease-specific ANA

	SS confirmed	SS suspected	SSc	RA	SCLE	Hepatitis including history	Asymptomatic for rheumatic diseases
Anti-U1RNP ($n = 4$)	2 (50)	1 (25)	0	0	0	0	1 (25)
Anti-SSA/Ro ($n = 40$)	4 (10)[a]	4 (10)[a]	0	3 (8)	1 (3)[a]	7 (18)	24 (60)
ACA (CENP-B; $n = 17$)	1 (6)[b]	0	1 (6)	2 (12)	0	4 (24)	9 (53)[d]
Anti-dsDNA ($n = 6$)	0	0	0	1 (17)[b]	0	0	5 (83)[c]
Total ($n = 60$)	6 (10)[a]	5 (8)[a]	1 (2)	5 (8)	1 (2)[a]	11 (18)	34 (57)

Rheumatic diseases were identified from medical examinations and questionnaires among samples from 60 individuals that were positive for U1RNP, SSA/Ro, anti-centromere (ACA), or anti-dsDNA antibodies. Expressed values *in parenthesis* are the ratios (%) of the total number of samples

SS, Sjögren's syndrome; SSc, systemic sclerosis; RA, rheumatoid arthritis; SCLE, subacute cutaneous lupus erythematosus

[a] One individual with overlapping hepatitis

[b] One individual also positive for anti-SSA/Ro

[c] Two individuals also positive for anti-SSA/Ro

[d] Three individuals also positive for anti-SSA/Ro

Physical condition of individuals positive for disease-specific ANA

The presence of rheumatic or immunological diseases was determined by a medical examination in 60 individuals and by a questionnaire among the 100 disease-specific ANA-positive individuals with anti-U1RNP, anti-SSA/Ro, anti-dsDNA antibodies, or ACA (Table 3). Four of 40 anti-SSA/Ro-positive individuals had SS, while SS was suspected in an additional four individuals, three individuals had rheumatoid arthritis (RA), and one individual had sub-acute cutaneous lupus erythematosus (SCLE). One of four individuals with SS was positive for anti-SSA/Ro and anti-SSB/La, but none of those with suspected SS was positive for anti-SSB/La. Among 17 ACA-positive individuals, one had SS and was also positive for anti-SSA/Ro, two had RA, and one was affected by limited type systemic sclerosis. Two of four anti-U1RNP-positive individuals had SS, while one had suspected SS. Eighteen individuals among the 60 were already affected by or were suspected of having rheumatic diseases. Although the individuals with RA had already been diagnosed at other medical institutions, six were diagnosed with SS during this physical examination and referred to medical institutions where the diagnosis was subsequently confirmed using the criteria of Vitali et al. [18]. The ratio of each age group was almost parallel to that of the average Japanese population, and the prevalence of diseases such as RA was also almost concordant with those of general Japanese population. Therefore, we considered that the influence of self-selection bias was small.

Of the 60 individuals who were disease-specific ANA-positive, 34 (57%) were clinically healthy without symptoms of rheumatic diseases, whereas 11 (18%) who were positive for anti-SSA/Ro or ACA had abnormal liver function tests or a history of liver disease.

Annual changes in levels of anti-SSA/Ro and ACA antibodies

The annual change in antibody titers was evaluated in the 24 individuals who were positive for anti-SSA/Ro and who had undergone three or more physical examinations within 5 years. These 24 individuals comprised seven with rheumatic diseases (three with SS, two with suspected SS, one with suspected RA, and one with SCLE; Fig. 2a) and 17 who were clinically healthy (Fig. 2b). An annual increase in antibody titers was identified in two individuals from the healthy group (cases 1 and 2 in Fig. 2b), but signs or symptoms of rheumatic diseases had not yet manifested at the end of survey. Annual antibody levels did not significantly differ among 14 ACA-positive individuals (data not shown).

Discussion

The type and prevalence of disease-specific ANAs have been investigated among specific cohorts, such as consecutive patients and uranium miners [19, 20], but few large-scale surveys have been conducted within the general population. In our study, the detection of disease-specific ANAs in serum samples from 2181 individuals yielded an anti-SSA/Ro prevalence of 2.7% (3.5% in females; 1.0% in males). These values are somewhat higher than those reported previously. One earlier study showed that one serum sample (0.2%) from among 485 healthy volunteer blood donors (228 females and 257 males; age range 18–70 years) was positive for anti-SSA/Ro antibody [8]. In another study, anti-SSA/Ro antibody was identified in the serum of an 83-year-old female among 64 apparently healthy individuals (32 females and 32 males, mean age

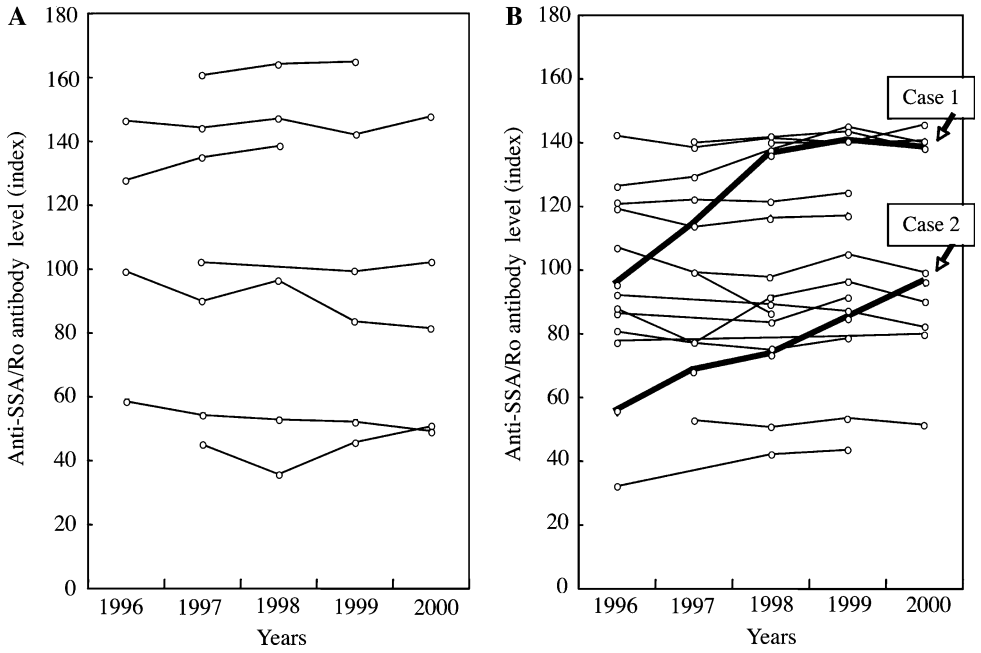

Fig. 2 Yearly changes in anti-SSA/Ro antibody levels. Antibody levels were determined in 24 individuals positive for anti-SSA/Ro who underwent at least three physical examinations within the 5-year follow-up period, including seven individuals with rheumatic diseases (**a**) and 17 clinically healthy individuals (**b**). Annual increases in antibody titers were found in two of the healthy individuals (*cases 1* and *2*)

81.0 years), an incidence of 1.6%, suggesting that this antibody occurs in elderly populations and that it is linked to SS [1, 9]. The median age in our study was relatively high—55 years—which might raise the positivity rates for anti-SSA/Ro antibody.

The prevalence of ACA was also high (1.4%; 1.8% in females and 0.5% in males) in our study. Although few investigators have reported the prevalence of ACA in the general population, Ruffatti et al. [21] reported that ACA was undetectable in serum samples from 82 healthy individuals and from relatives of ACA-positive patients. In contrast, Soma et al. screened 401 Japanese serum samples for ACA and found that 16 were ACA-positive, including eight of 62 patients with SSc and three of seven with primary Raynaud's disease. Although ACA is a useful immunological marker for the CREST variant of SSc, it is also widely distributed among other conditions, although at a lower frequency [22]. In contrast, Spiewak et al. reported that at least one autoantibody was detected in 30% of 90 residents of a rural community (anti-dsDNA, 12.2%; SSA/Ro, 7.8%; U1RNP, 5.6%; Scl-70, 5.6%, Jo-1, 3.3%; Sm, 2.2%; SSB/La, 2.2%; ACA, 2.2%), with a 12% (6/50) occurrence of disease-specific ANAs in control samples from urban blood donors. These researchers speculated that the high incidence of disease-specific ANAs resulted from long-term pesticide exposure [23]. To clarify whether the high incidence of disease-specific ANAs in our study of the residents of a small Japanese town was a specific regional event, we carried out a similar survey of our hospital staff. Among 231 hospital workers (114 females and 117 males; age range 21–60 years), 67 were IF-ANA positive at a 1:40 dilution (29.0%). Among these, seven (3.0%) were positive for disease-specific ANAs, three (1.3%) had anti-SSA/Ro, two (0.9%) had U1RNP, one (0.4%) had SSB/La (this individual also had anti-SSA/Ro), two (0.9%) had ACA, and no one was positive for anti-dsDNA, Sm, Scl-70, or Jo-1. Age- and gender-matched comparisons between data from our hospital staff and from the town residents did not significantly differ in terms of the occurrence of disease-specific ANAs, suggesting that the data presented here are not influenced by regional factors.

We investigated whether the IF-ANA screening method used reflects the actual prevalence of anti-SSA/Ro and ACA in the population. Because anti-SSA/Ro and anti-Jo-1 antibodies might be frequently overlooked by the IF method [7, 24], we randomly selected 150 serum samples from age- gender-matched IF-ANA-negative individuals and used EIA methods to test for the presence of eight disease-specific ANAs. Among the 150 serum samples assayed, one each was positive for low titers of anti-SSA/Ro, ACA, and anti-dsDNA antibodies. Thus, most disease-specific ANAs can be detected by our IF method, especially those that are present at high titers. In addition, as the performance of the EIA kits for detecting disease-specific ANAs varies [25, 26], we further investigated the presence of anti-SSA/Ro antibodies and ACA using the DID assay and by the discrete speckled pattern of IF-ANA, respectively, and confirmed their presence with few oversights. Many ANAs have been identified in patients with hepatitis B or C [16, 27, 28]. However, the prevalence of viral antibodies, such as anti-HBc, anti-HCV and anti-HTLV-I antibodies, did not significantly differ between IF-ANA-positive and IF-ANA-negative groups in our study. Li et al. [29] reported that anti-SSA/Ro, anti-U1RNP, ACA, anti-

dsDNA, and anti-histone are detectable in 44.4, 41.7, 33.3, 27.7 and 33.3% of AIH patients. Seven of 40 anti-SSA/Ro-positive individuals (18%) and four of 17 ACA-positive individuals (24%) had abnormal liver function tests or a history of liver disease. Five of those 11 individuals (seven anti-SSA/Ro-positive and four ACA-positive ones) may have had autoimmune hepatitis (AIH) as their levels of immunoglobulin G or γ-GTP were high.

We surveyed the general health of the residents who were positive for disease-specific ANAs by performing physical examinations and using questionnaires. The physical examinations of the 60 persons who were positive for anti-U1RNP, anti-SSA/Ro, anti-dsDNA antibodies, or ACA revealed that 18 of these were probably affected by rheumatic diseases, such as SS, SSc, and RA, including six who had been diagnosed with SS. Surprisingly, 34 (57%) of the 60 individuals who were positive for disease-specific ANA were asymptomatic and clinically healthy. These results show that approximately half of individuals who are positive for anti-SSA/Ro antibodies or ACA are clinically healthy. Thus, these antibodies may not be disease-specific at the time of detection.

Another notable finding was the annual changes in the levels of disease-specific ANA and the physical status of ANA-positive individuals. We postulated that if pre-symptomatic individuals could be identified, the onset and progression of rheumatic diseases could be observed at annual physical examinations and by clinical tests. Therefore, we annually screened for disease-specific ANA-positive samples for 5 years and investigated the relationship between changes in antibody titers and health. Anti-SSA/Ro antibody titers remained relatively stable in the seven individuals with rheumatic disease, but they increased annually in two of 17 apparently healthy individuals. As the anti-SSA/Ro IgM antibody titers decreased, IgG and IgA antibody titers increased in both of these individuals (data not shown), but they did not show any clinical signs or symptoms of rheumatic disease until August of 2006.

In contrast, ACA titers remained stable in 14 ACA-positive individuals, including one patient with SS and one patient with SSc. Although ACA is frequently detected in patients with limited type SSc (CREST syndrome), it is seldom found in other systemic rheumatic diseases. Other studies have detected ACA in 25% of patients with Raynaud's phenomena who were at high risk of developing limited type SSc [30], and ACA frequently develops in patients with primary biliary cirrhosis [31]. Although four of our 17 ACA-positive individuals had current or a history of liver disease, most of the others did not develop rheumatic or liver diseases during the 5-year follow-up period.

This is the first report to describe the frequency of anti-SSA/Ro- or ACA-positive antibodies in clinically healthy individuals among the general population who were healthy carriers of these antibodies for at least several years. The results of our study suggest that a sizable population remains healthy while harboring anti-SSA/Ro or ACA antibodies, which are generally regarded as disease-specific. In that respect, other disease-specific autoantibodies, such as anti-Sm or anti-Scl-70 or anti-Jo-1 antibodies, seemed to be rare in healthy individuals. Disease-specific ANAs are detectable in the serum of patients with rheumatic disease before the onset of a particular disease entity [32]. Whether these individuals will maintain their health status or develop systemic rheumatic disease is of great importance, and clarification of this situation requires larger long-term studies.

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