CENP-O, a Protein Localized at the Centromere Throughout the Cell Cycle, Is a Novel Target Antigen in Systemic Sclerosis

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ABSTRACT. ***Objective.*** CENP-A, -B, and -C are major centromere components and the main targets of anticentromere antibodies (ACA). Many other proteins are also assembled around CENP-A nucleosomes in interphase nuclei to form the interphase centromere complex (ICEN). The CENP-O protein is a component of the ICEN that localizes at the centromere throughout the cell cycle. We investigated whether CENP-O is also targeted by sera from patients with systemic autoimmune diseases.

Methods. Sera from 114 patients with ACA and 142 patients without ACA were analyzed. Western blotting and an ELISA with bacterially expressed recombinant CENP-O protein were performed to screen for the presence of anti-CENP-O antibodies. In addition, anti-CENP-O antibody-positive sera were tested by Western blotting HeLa cell extracts to examine reactivity with the major centromere antigens.

Results. Four female patients with ACA had anti-CENP-O antibodies. There was no correlation of anti-CENP-O antibodies with specific clinical features or other serological features. However, one of the 4 patients, who showed a unique clinical course of scleroderma, had sera with markedly high reactivity to CENP-O.

Conclusion. CENP-O protein is a novel centromere antigen that is recognized by a very minor population of ACA-positive patients with scleroderma. Because CENP-O is an ICEN component, ICEN may be a large antigenic structure in systemic autoimmunity. (First Release March 15 2009; J Rheumatol 2009;36:781–6; doi:10.3899/jrheum.080726)

Key Indexing Terms:
CENTROMERE AUTOANTIBODY SYSTEMIC SCLEROSIS

Anticentromere antibodies (ACA) have been found in patients with autoimmune diseases, especially in limited cutaneous systemic sclerosis (lSSc), formerly called CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia). The strong association of these antibodies with lSSc has provided a potent diagnostic marker to clinically evaluate patients[1]. However, recent studies have emphasized that ACA are also present in patients with other autoimmune diseases, including systemic lupus erythematosus (SLE)[2] and primary Sjögren's syndrome (SS)[3].

The indirect immunofluorescence (IIF) pattern of ACA in HEp-2 cells is discrete speckled nucleoplasmic staining in interphase cells and distinct centromeric dots that are visible for each chromosome pair in the metaphase plate. ACA target mainly CENP-A, CENP-B, and CENP-C, which are the major components of the centromere proteins[4,5]. ACA have been used as indispensable probes in the field of cell biology to analyze the centromere structure. A centromere is the constricted region of a mitotic chromosome that holds sister chromatids together, and also the site on DNA where the kinetochore forms and then captures microtubules from the mitotic spindle. In interphase nuclei, many proteins are assembled around nucleosomes containing CENP-A to form a centromere chromatin complex. In a recent study, approximately 40 proteins, including the previously reported centromere proteins and proteins of unknown function, were identified by a proteomic analysis of the interphase centromere complex (ICEN) with a CENP-A monoclonal anti-

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body[6]. In a subsequent study, 7 types of ICEN proteins were identified as novel constitutive components of the centromere chromatin complex with a role in kinetochore function[7]. Similar results have been obtained in other studies[8,9]. CENP-O, corresponding to ICEN 36, is one of the novel constitutive centromeric proteins that localize at the centromere throughout the cell cycle. CENP-O forms a complex with 4 other centromere proteins that helps prevent premature sister chromatid separation during recovery from spindle damage[10].

We hypothesized that ACA could target not only CENP-A, -B, and -C but also newly identified ICEN proteins localized at the centromere throughout the cell cycle, and that these autoantibodies could be a new serological marker for a certain disease subset. We investigated the presence of anti-CENP-O antibodies in sera from patients with systemic autoimmune disease, especially ACA-positive sera, by Western blotting and enzyme-linked immunosorbent assay (ELISA). Among 114 ACA-positive patients, we identified 4 patients with anti-CENP-O antibodies, in addition to some or all of anti-CENP-A, -B and -C antibodies. One of these patients showed a markedly strong reactivity to CENP-O and had a rare clinical course of SSc. These findings on CENP-O autoantibodies may identify a further discrete subset of patients with SSc.

MATERIALS AND METHODS

Serum samples. The serum bank in the Department of Dermatology, Nagoya University Hospital, had sera collected from 947 Japanese patients. One hundred fourteen of these sera samples were positive for ACA by IIF using HEp-2 cells (Fluoro Hepana Test; MBL, Nagoya, Japan) as described[11], and these samples were used in this study. The samples included 75 patients with SSc [67 were classified as having lSSc and 8 as diffuse cutaneous SSc (dSSc)], 9 with SS, 8 with SLE, 2 with dermatomyositis (DM), and 20 other patients with various disease conditions (Table 1). SSc was diagnosed according to the SSc diagnostic criteria[12] established by the Ministry of Health, Labour and Welfare of Japan, which were modified from the American College of Rheumatology (ACR) criteria[13]. The Japanese SSc criteria recommend diagnosing SSc in the presence of either the major criterion of proximal scleroderma or the minor criteria of sclerodactyly based on one or more the following criteria: (1) digital pitting scars on the fingertips or loss of the distal finger pads, (2) bilateral basilar pulmonary fibrosis, or (3) positive for anti-Scl-70 antibodies or ACA. SSc patients were subcategorized into lSSc and dSSc according to the classification system proposed by LeRoy, *et al*[14]. SS was defined by the Japanese Ministry of Health and Welfare Diagnostic Criteria[15]. SLE was diagnosed by the ACR criteria for SLE[16]. The diagnosis of probable or definite DM was based on the criteria of Bohan and Peter[17,18]. Other ACA-positive patients consisted of 5 patients with any SS or SSc symptoms who did not fully meet the criteria, 1 with rheumatoid arthritis, 1 with Raynaud's disease, 1 with atopic dermatitis, and 1 with hyper-eosinophilic syndrome, and 11 patients with no available records of their symptoms. Sera from 142 patients without ACA were obtained from the serum bank and analyzed as disease control subjects, and these consisted of samples from 77 patients with SSc (40 classified as lSSc and 37 as dSSc), 24 with SLE, 21 with DM, and 20 with SS. Sera from 20 healthy individuals without antinuclear antibodies (ANA) were used as negative controls.

Written informed consent was obtained from each subject, and this study was approved by the local ethics committee.

Plasmid construction. The CENP-O coding region was amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) from HT1080 total RNA using the following primers: forward, 5'-GAA TTC TAT GGA GCA GGC GAA CCC TTT ACG-3' and reverse, 5'-GTC GAC TTA GGA GAC CAG ACT CAT ATC CAA-3'. The CENP-O PCR products were cloned into the pDrive vector (Qiagen GmbH, Hilden, Germany) and then subcloned into the pEGFP-C1 vector (Clontech, Mountain View, CA, USA) by EcoRI and SalI digestion. The CENP-O coding sequence was PCR-amplified with the following primers: forward, 5'-CAC CAT GGA GCA GGC GAA C-3' and reverse, 5'-TTA GGA GAC CAG ACT C-3'. These CENP-O PCR products were cloned into the pENTR/SD/D-TOPO vector (Invitrogen, Carlsbad, CA, USA) and subcloned into pDEST17 (Invitrogen) following the manufacturer's protocol. The expression protein was fused to a 35-amino acid sequence containing a 6-amino acid histidine tag at the N-terminus, which was derived from the pENTR/SD/D-TOPO and pDEST17 vectors. The pDEST17 insert was verified by sequencing. Compared to the GenBank reference sequence (no. BC002870 and NM_024322), a point mutation was identified that likely occurred due to a PCR error. This mutation caused a single amino acid change from isoleucine to threonine at amino acid position N150.

Expression and purification of recombinant protein. Plasmid DNA was transformed into competent *Escherichia coli* Rosetta2 (DE3) Competent Cells (Novagen, Madison, WI, USA), and the CENP-O protein was induced according to the manufacturer's protocol. The histidine-tagged recombinant protein was purified using Ni Sepharose 6 Fast Flow (GE Healthcare, Buckinghamshire, UK) under denaturing conditions according to the manufacturer's protocol.

Western blotting with recombinant CENP-O protein and HeLa cell extracts. Whole HeLa cell extracts were obtained as described[19]. CENP-O recombinant protein and HeLa cell extracts were subjected to 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Electro-phoresis and protein transfer were performed as described[5]. To detect the histidine-tagged protein, anti-6×His antibody was used at 1/1000 dilution as a primary antibody (Dako, Glostrup, Denmark) followed by a horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG (Dako) secondary antibody at 1/1000 dilution. Human serum samples were used at

Table 1. Clinical diagnosis of patients with or without anticentromere antibodies (ACA).

Disease	ACA-positive		ACA-negative	
	No. (male/female)	Mean age ± SD, yrs	No. (male/female)	Mean age ± SD, yrs
Limited cutaneous SSc	67 (2/65)	60 ± 9	40 (3/37)	56 ± 12
Diffuse cutaneous SSc	8 (1/7)	54 ± 13	37 (5/32)	55 ± 12
Sjögren's syndrome	9 (0/9)	59 ± 16	20 (2/18)	49 ± 19
Systemic lupus erythematosus	8 (0/8)	45 ± 12	24 (0/24)	48 ± 12
Dermatomyositis	2 (0/2)	58 ± 24	21 (4/17)	54 ± 14
Other	20 (6/14)	57 ± 11	0	
Total	114 (9/105)	58 ± 11	142 (14/128)	53 ± 14

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1/100 dilution as primary antibodies and HRP-conjugated rabbit anti-human IgG (Dako) was used at 1/1000 dilution as secondary antibody. Colorimetric detection was performed with Konica immunostain (Konica, Tokyo, Japan).

ELISA with recombinant CENP-O protein. ELISA was performed as described with slight modifications[11]. Briefly, wells of microtiter plates (MediSorp; Nunc, Roskilde, Denmark) were coated with purified recombinant CENP-O protein (1 µg/100 µl/well). The wells were blocked with 200 µl of fetal bovine serum diluted 1/10 in T-PBS (phosphate buffered saline containing 0.05% Tween 20) for 2 h at room temperature. *E. coli* extract was prepared from *Escherichia coli* [Rosetta2 (DE3) Competent Cells] that did not harbor plasmid DNA. Sample sera diluted 1/100 in reaction buffer (T-PBS containing 0.1 mg/ml of *E. coli* extract) were incubated 1 h at room temperature followed by anti-human IgG antibody conjugated with HRP as a secondary antibody. 1-Step ABTS (Pierce, Rockford, IL, USA) was used as a substrate solution according to the manufacturer's protocol. Each serum sample from patients was tested in duplicate, and the mean optical density (OD) at 405 nm was used for data analysis. Twenty sera samples from healthy individuals were used as negative controls on every plate. In addition, the OD of each serum sample in uncoated wells was measured as the background level. To compare results from different plates, all ELISA results were transformed into ELISA units using the following formula: ELISA units = 100 × (corrected OD of serum from each patient) / the cut-off OD value. The corrected OD value was the OD value of each sample minus the background level. The cutoff value was determined for every plate as the mean of the corrected OD values obtained from 20 control sera + 5 standard deviations (SD).

RESULTS

Western blot analysis to screen anti-CENP-O antibody-positive sera. Using Western blots of recombinant CENP-O protein, we screened serum samples from 114 ACA-positive patients. Sera from 6 ACA-negative patients (3 with lSSc, 1 dSSc, 1 SLE, and 1 SS) and 3 ANA-negative healthy individuals were also examined as control samples. We identified sample no. 607a as a distinctly positive serum, as shown in Figure 1. Western blots showed a dense band at the predicted size (38 kDa) for recombinant CENP-O protein (a total of 335 amino acids) that was fused to 35 amino acids containing a histidine tag at the N-terminus (Figure 1). Next, we tested other serum samples (no. 607b and 607c) from the same patient that were taken 2 and 6 years after sample 607a, respectively. Samples 607b and 607c reacted with CENP-O as strongly as 607a. We considered these sera as positive controls for the anti-CENP-O antibody in further studies. Faint bands of the same size were also detected with sera from several other ACA-positive patients, but not by sera from ACA-negative patients or healthy individuals.

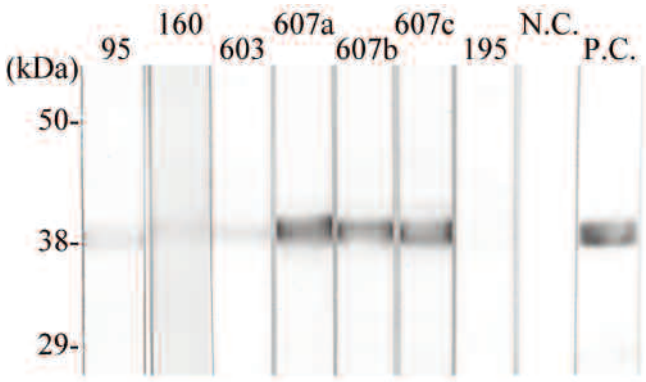

Figure 1. Western blotting of recombinant CENP-O with patient sera. Serum sample numbers are indicated above the lanes. NC: negative control, in which serum from a healthy individual was used. PC: a positive control, in which an anti-6×His antibody was used. The band in this lane migrated at roughly 38 kDa, corresponding to the predicted size of CENP-O. Samples 95, 160, 603, 607a, 607b, and 607c are sera from patients with anticentromere antibodies (ACA); samples 607a, 607b, and 607c are sera from the same patient. Sample 195 was from a patient without ACA, and a 38-kDa band was not detected.

To confirm that these sera did not react with the polypeptide fused to the N-terminus of CENP-O but with the CENP-O protein itself, we examined the ability of these sera to detect another recombinant protein with a molecular weight of 75 kDa that was cloned into the pDEST17 vector and fused with the same polypeptide. None of these sera reacted with this recombinant protein (data not shown).

Measurement of anti-CENP-O antibodies by ELISA. In order to screen multiple serum samples, we established an ELISA using recombinant CENP-O protein as an antigen. Using this system, we measured ELISA units of anti-CENP-O antibodies in sera from 256 patients (114 ACA-positive and 142 ACA-negative patients). Twenty sera from healthy individuals were used as negative controls on every plate. Based on the results of the initial ELISA screening, 37 patients were selected for a more accurate evaluation; we selected sera from all 21 patients (16 ACA-positive and 5 ACA-negative patients) that had OD values higher than the mean OD value of the control sera + 2 SD in the original screening, and sera from 16 (3 ACA-positive and 13 ACA-negative) of 235 patients with OD values under the mean + 2 SD. We reexamined the sera from these 37 patients along with 20 control sera from healthy individuals on the same ELISA plate.

The cutoff value was calculated from the mean OD values of these 20 control sera + 5 SD based on the compatibility between the Western blot and ELISA results. As shown in Figure 2, sera from 4 ACA-positive patients were anti-CENP-O antibody-positive. Sample no. 607a was the highest with 406.0 units, and serum samples 607b and 607c from the same patient also had high units of 369.4 and 338.6, respectively (data not shown). Sera from the 3 other patients (Figure 1, samples 95, 160, and 603) had low anti-CENP-O antibody units (116.0 to 200.7) by ELISA and also showed weak signals by Western blotting (Figure 1). We categorized these 4 patients into the anti-CENP-O antibody-positive group. Only one serum sample (sample 195 from the SLE patient) among 142 ACA-negative samples was positive by ELISA, with 129.5 units (Figure 2). However, because the serum did not react with CENP-O by Western blot (Figure 1), we considered this a false-positive reaction in the ELISA. All healthy individuals were anti-CENP-O antibody-negative by ELISA, and all anti-CENP-O positive-sera were ACA-positive.

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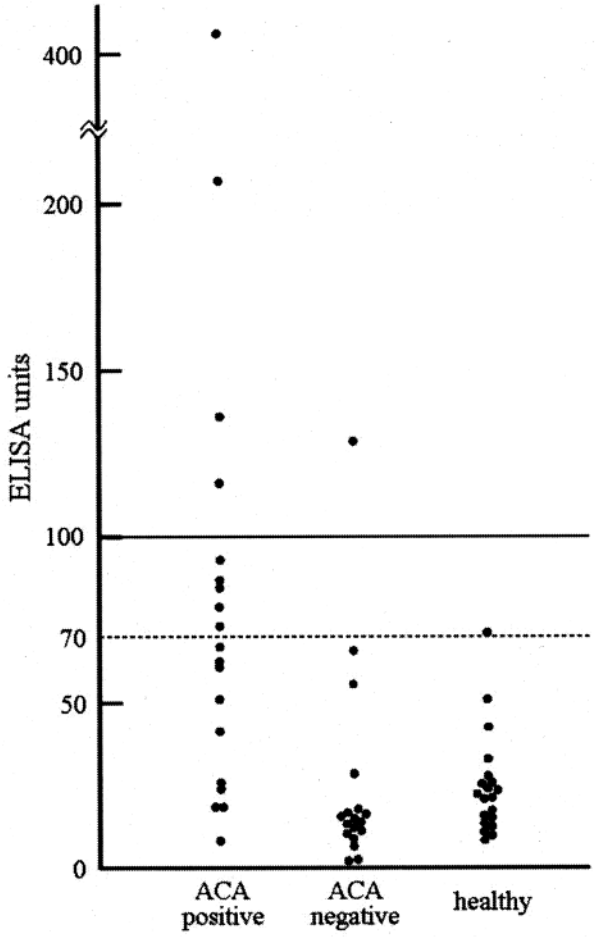

Figure 2. Anti-CENP-O antibody levels measured by ELISA. The anti-CENP-O antibody units of sera from 19 ACA-positive patients, 18 ACA-negative patients, and 20 healthy individuals without ANA were simultaneously measured by ELISA using recombinant CENP-O protein. Solid line indicates the cutoff value (100 units), calculated from the mean OD values of 20 sera samples from healthy individuals + 5 SD (mean + 5 SD). Broken line corresponds to the mean + 3 SD or 70 units.

Five ACA-positive patients and one healthy individual had anti-CENP-O antibody units (70.9 to 93.2) that were between the mean + 3 SD (70 units) and mean + 5 SD (100 units) by ELISA, as shown in Figure 2. All 5 of these ACA-positive sera showed very faint signals by Western blot, while the control sera showed no signals (data not shown). Because these results raised the possibility that these 5 patients were anti-CENP-O antibody-positive, we categorized them into the equivocal group.

Clinical features of patients with anti-CENP-O antibodies and other serological features. We tested sera from the positive group and the equivocal group by Western blots of HeLa cell extracts to examine reactivity with CENP-A, CENP-B, CENP-C, 23-kDa chromo protein (p23), and 25-kDa chromo protein (p25). CENP-A, -B, and -C are major target antigens of ACA. Anti-p23 and anti-p25 antibodies, which have been termed "anti-chromo antibodies," are always produced in patients with ACA[20]. All 9 serum samples in the positive group and the equivocal group also reacted with CENP-A to various degrees, whereas not all of them reacted with CENP-B, CENP-C, p23, and p25 (data not shown). There was no clear relationship between the reactivity of sera to recombinant CENP-O and these 5 other HeLa cell proteins. We were unable to detect any bands corresponding to CENP-O when these 9 sera were used for Western blots of HeLa extracts (data not shown).

Table 2 summarizes the clinical and serological information for the 4 patients in the positive group and the 5 patients in the equivocal group. All 9 patients were female, but there were few shared clinical symptoms that distinguished these 9 patients. Next, we examined the presence of concomitant antibodies in these patients. All 9 patients had ACA. In addition, the SS patient was anti-SSA antibody-positive and the SLE patients were anti-dsDNA antibody- or anti-Sm antibody-positive. On the other hand, the dSSc patient (no. 607) was negative for dSSc autoantibodies, such as anti-Scl-70 antibody and anti-RNA polymerase I/III antibodies (data not shown). Further, her sera were strongly anti-CENP-O antibody-positive by Western blot, and had the highest ELISA titer against the CENP-O protein.

Patient "607" had an unusual clinical course of SSc. She had experienced Raynaud's phenomenon since her late teens. At 34 years of age, she first visited our hospital and was initially diagnosed with lSSc based on sclerodactyly and ACA positivity. For 4 years after her first visit, the skin sclerosis was limited to her fingers and the back of her hands. Serum sample 607a was collected at the age of 36 years. When she was 38 years old (sample 607b), she experienced rapid progression of edematous thickening of the bilateral forearm, upper arm, and abdominal skin within 1 month. At that time, her modified Rodnan total skin thickness score (TSS)[21] was elevated from 4 to 15 points. Therefore, we changed her diagnosis from lSSc to dSSc. After taking oral prednisolone at 20 mg/day for 2 weeks, her symptoms improved markedly, and the prednisolone dose was gradually tapered. One year after the rapid progression of skin sclerosis, she presented with dyspnea on exertion and was diagnosed with dilated cardiomyopathy. At 42 years of age (sample 607c), her skin sclerosis was not exacerbated, even without prednisolone administration, but the slight thickening of her skin persisted.

DISCUSSION

The presence of ACA is a strong serological indicator for lSSc, even in patients with only Raynaud's phenomenon, and is closely related to limited cutaneous involvement and less severe internal organ involvement for the long term[1,14]. We focused on an ACA-positive patient (patient 607) with a distinct clinical course because no other patients in our study progressed as rapidly from typical lSSc to dSSc. In Western blot analysis of HeLa extracts, her sera reacted with

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Table 2. Clinical and laboratory data for patients in the anti-CENP-O antibody-positive group and the equivocal group. All patients were female.

				Clinical Symptoms					Laboratory Data				
	Patient	Age, yrs	Diagnosis	Calcinosis	RP	Esophageal Dysmotility	Telangiectasia	ILD	IIF Pattern	IIF Titer	ELISA* units	WB**	Other Positive Autoantibodies
Anti-CENP-O antibody-positive[†]	607	36	dSSc	–	+	+	–	–	DS Diffuse	10,240 160	320.5	++	
	160	45	lSSc	–	+	+	–	–	DS	640	172.6	+	
	603	53	lSSc	–	+	+	–	–	DS Diffuse	1280 80	125.5	+	
	95	63	SS	–	–	–	–	NA	DS Cytoplasmic Diffuse	320 160 80	110.6	+	Anti-SSA
Equivocal[††]	66	73	lSSc	–	+	–	–	–	DS Speckled	1280 1280	93.2	+/–	Anti-SSA
	521	58	lSSc	+	+	+	+	–	DS	320	87.0	+	
	107	37	SLE	–	+	–	–	–	Cytoplasmic DS Diffuse	320 160 80	85.0	+/–	Anti-ds-DNA, anti-SSA
	48	43	SLE	–	+	–	–	NA	DS Diffuse	640 160	79.6	+/–	Anti-Sm
	255	27	SLE	–	–	–	–	–	DS Speckled Cytoplasmic	640 640 80	73.9	+/–	Anti-SSA, anti-Sm

* ELISA units of anti-CENP-O antibodies. ** Reactivity to recombinant CENP-O by Western blotting. [†] Patients with higher anti-CENP-O antibody units than mean + 5 SD (> 100 units); [††] anti-CENP-O antibody units between mean + 3 SD and + 5 SD (> 70 and < 100 units) by ELISA. IIF: indirect immunofluorescence; ELISA, enzyme-linked immunosorbent assay; dSSc: diffuse cutaneous systemic sclerosis; lSSc: limited cutaneous systemic sclerosis; SS: Sjögren's syndrome; SLE: systemic lupus erythematosus; NA: information not available; DS: discrete speckled; RP: Raynaud's phenomenon; ILD: interstitial lung disease.

all major centromere antigens, CENP-A, -B, and -C (data not shown). Therefore, identifying in her sera an autoantibody to a novel ICEN component might identify a further discrete subset of patients with SSc. Therefore, we investigated the presence of autoantibodies against several novel constitutive centromeric proteins[7] in her sera by Western blotting using individual recombinant proteins (data not shown). Her sera showed remarkably high reactivity to CENP-O, as shown in Figure 1. For this reason, we concentrated on analyzing the association of autoantibodies to CENP-O with systemic autoimmune diseases using multiple patient samples. In this study, anti-CENP-O antibodies were detected in sera of 4 (samples 95, 160, 603, and 607) out of 256 patients with autoimmune diseases by both Western blots and ELISA (Table 2). Sera from 3 anti-CENP-O-positive patients, excluding 607, had low reactivity to CENP-O, and they presented with no obviously common clinical features. Additionally, sera from 5 patients (48, 66, 107, 255, and 521) that showed relatively high anti-CENP-O antibody units compared to anti-CENP-O antibody-negative sera by ELISA, but had units below the cutoff value (mean + 5 SD), reacted very faintly with CENP-O by Western blot. These 5 patients also had no distinctive clinical features. We classified these 5 patients into the equivocal group. Western blotting is not a useful method to detect a few autoantibodies, such as anti-PCNA[22] and anti-DFS70[23] antibodies, because of the presence of conformational epitopes. Because of this possibility, we also examined data from patients whose sera had high reactivity to CENP-O by ELISA but no definite reactivity by Western blot. Future studies will examine the clinical courses and possible changes in anti-CENP-O antibody units in these patients.

Characterization of autoantigens that are recognized by sera from patients with autoimmune disease has traditionally been performed by Western blot and immunoprecipitation of cell extracts, while immunological screening of human cDNA expression libraries has been used to clone genes encoding autoantigens. However, it can be difficult to obtain positive signals by Western blot or positive clones by screening an expression library if the target proteins are expressed at limited levels in these conventional assays. In this study, we demonstrated that CENP-O, an ICEN component identified by a proteomics analysis, is a new centromere autoantigen. This innovative approach was based on a proteomic analysis of a macromolecular complex that is predicted to be targeted by autoantibodies, and this technique will be useful to discover autoantigens that were left unidentified by conventional approaches. ICEN, which represents a large CENP-A chromatin complex, contains various functional proteins: kinetochore function, chromatin remodeling, heterochromatin formation, etc. Major centromere autoantigens, CENP-A, -B, and -C, and centromeric chromatin components HP1α (p25) and HP1γ (p23) are mainly recognized by ACA and were all found, except for HP1α, in ICEN com-

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ponents[6]. More interestingly, fibrillarin, a nucleolar protein involved in pre-rRNA processing and ribosome assembly[24], is also an ICEN component[6]. Fibrillarin is a protein targeted by sera from scleroderma patients[25]. Thus, ICEN may contain many functional proteins important for centromeric regions and may be an immunogenic subcellular particle for autoimmune patients. Recently, CENP-O was reported to form a complex with the CENP-O class proteins, CENP-P, -Q, -R, and -U, that helps prevent premature sister chromatid separation during recovery from spindle damage[10]. Further studies are needed to determine if other components of this complex are recognized by autoantibodies. It is even more important to determine what produces differences in autoantigenicity between "major" and "minor" centromere autoantigens, such as expression level, protein conformation, or proteolytic degeneration associated with cell death for antigen presentation[26].

The CENP-O protein, an ICEN component, has been identified as a novel autoantigen in ACA-positive patients with systemic autoimmune diseases. These findings suggest that the centromere autoantigenic complex might form a larger target structure than was originally discovered. We have also described a rare clinical course in a patient with SSc with strong reactivity to CENP-O. We are now examining autoimmune responses against other ICEN components and the biological stimuli that produce biochemical differences to antigenic structures of ICEN members.

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