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Differential isotype recognition of two centromere associated polypeptides by immunoblotting in connective tissue disease

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SUMMARY

On investigating the immunoblotting profile of 65 systemic sclerosis patients, a 140 kD polypeptide was recognised by sera from 16, when immunoblotted against a nuclear-enriched K562 cell sonicate. All 16 sera contained anticentromere antibodies (ACA) detected by immunofluorescence (IF) and 15 of 16 also recognized a 19 kD polypeptide on immunoblotting. Two ACA positive sera failed to recognize the 140 kD polypeptide but one of these recognized the 19 kD polypeptide. The 140 kD polypeptide identified a group with more limited skin involvement ($P < 0.05$) and all 16 had Raynaud's phenomenon. The sera from three of 100 systemic lupus erythematosus (SLE) patients also recognized both polypeptides. On investigating the isotype specificity, the 140 kD polypeptide was strongly detected by an IgM autoantibody and the 19 kD polypeptide by an IgG autoantibody.

Keywords anticentromere antibodies systemic sclerosis immunoblotting

INTRODUCTION

Anticentromere antibodies (ACA) detected by immunofluorescence (IF) are found in up to 55% of patients with systemic sclerosis (McNeilage *et al.*, 1986) and are more common in the CREST variant (Calcinosis, Raynaud's phenomenon, Oesophageal involvement, Sclerodactyly, Telangiectasia) which has more limited sclerodermatous skin and visceral involvement and a better prognosis (McNeilage *et al.*, 1986; Tan *et al.*, 1980; Catoggio *et al.*, 1983; Moroi *et al.*, 1980; Fritzler, Kinsella & Garbutt, 1980; McCarty *et al.*, 1983; Catoggio, Skinner & Maddison, 1983). ACA are highly specific for systemic sclerosis and have been reported in primary biliary cirrhosis patients with scleroderma (Bernstein *et al.*, 1982a; Makinen *et al.*, 1983), very infrequently in RA (3·3%) (Garcia-de la Torre & Miranda-Mendez, 1982) and SLE (2·5%) (Catoggio, Skinner & Maddison, 1983) and in a minority of patients with idiopathic Raynaud's phenomenon (19–21%) (Fritzler, Kinsella & Garbutt, 1980; Earnshaw *et al.*, 1986), some of whom later develop systemic sclerosis (Fritzler, Kinsella & Garbutt, 1980; Earnshaw *et al.*, 1986; Tramposch *et al.*, 1984). As well as helping in disease catagorization this specificity implies a role for ACA in mechanisms of disease expression.

The kinetochore, a structure that nucleates the assembly of chromosomes during mitosis (Reider, 1982), has been shown to be stained by an ACA serum using an immunoperoxidase technique with electron microscopy (Brenner *et al.*, 1981). In attempts to identify the antigenic determinants, several polypeptides extracted from human cell lines and chromosomal preparations are detected by ACA sera using immunoblotting techniques. A family of three inter-related human centromeric antigens recognized by ACA sera have been termed CENP-A, CENP-B and CENP-C (Earnshaw *et al.*, 1986), with CENP-B an 80 kD polypeptide recognized by all 39 ACA positive sera. The cDNA clones for the mRNA encoding for this polypeptide have been isolated (Earnshaw *et al.*, 1987). Other studies have all detected CENP-A (McNeilage *et al.*, 1986; Cox, Schenk & Olmsted, 1983; Guldner, Lakomek & Bautz, 1984; Valdivia & Brinkley 1985; Palmer *et al.*, 1987), some probably also CENP-B (McNeilage *et al.*, 1986; Valdivia & Brinkley, 1985; Palmer *et al.*, 1987) but none CENP-C. In one of these studies a 72 kD polypeptide recognized by 42 ACA positive sera was found to be highly conserved, being present in human cells and *Leishmania tropica* (McNeilage *et al.*, 1986). The variation in the apparent molecular weights of the polypeptides reported is probably due to variation in extraction and immunoblotting methods and sensitivity of the assays most of which have focused on IgG autoantibody detection alone.

We have extended these findings by studying the incidence, disease specificity and clinical associations of ACA in 65 patients with systemic sclerosis using a combination of IF and immunoblotting techniques and with 100 systemic lupus erythematosus (SLE) and 35 Felty's syndrome patients included in the controls. The results suggest that the IgM response in IgG ACA positive sera is against an epitope on a 140 kD polypeptide.

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MATERIALS AND METHODS

Patients and controls

Sixty-five patients with systemic sclerosis fulfilling proposed ARA criteria (Masi *et al.*, 1980) were studied. All were attending a connective tissue disease clinic where comprehensive clinical and serological documentation was made using a predefined protocol, and adequate serum had been stored. The protocol included full history and physical examination by at least one of the authors, radiographs of chest and hands, barium swallow examination (48 patients), lung function tests including carbon monoxide transfer (TLCO) (55 patients), estimations of serum creatine phosphokinase (CPK), creatinine clearance and 24 h urine protein excretion, and examination of the urine sediment.

Skin involvement was classified according to three types (Barnett, 1974). Type I had skin changes limited to the digits (sclerodactyly), Type II had proximal scleroderma extending from sclerodactyly to involve hands, forearms, face and neck and Type III had diffuse skin involvement. Other clinical features were determined as follows; Raynaud's phenomenon: a history or observation of a biphasic or triphasic response to cold. Oesophageal involvement: abnormal motility determined radiologically. Pulmonary involvement: bibasilar fibrosis on chest radiograph or a TLCO of less than 75% of the predicted value. Renal involvement: creatinine clearance less than 60 ml/min, active urinary sediment, proteinuria exceeding 0·5 g/day, or accelerated hypertension, any of these occurring in the absence of other cause. Myositis: weakness plus elevated CPK, abnormal electromyogram or characteristic biopsy appearance. Cardiac involvement: clinical or electrocardiographic abnormalities in the absence of other cause. Arthritis: presence of active synovitis as assessed by a rheumatologist and either erosive or non-erosive according to radiographic assessment.

The controls were 100 patients with SLE attending the same clinic studied with a similar protocol, 32 patients with Felty's syndrome and sera from 35 normal blood donors.

Serology

Immunofluorescence. Sera were screened at a dilution of 1/40 for antinuclear antibody (ANA) by indirect IF using Hep-2 cells (Biodiagnostic Ltd, Worcs., UK) and FITC-conjugated polyvalent goat anti-human immunoglobulin (Ig), goat anti-human IgG (gamma chain specific) and goat anti-human IgM (mu chain specific) (Atlantic Antibodies, Berkshire, UK). Positive sera were subsequently titrated in quadrupling dilutions in 1/640 and were assigned a pattern of fluorescence staining.

Immunodiffusion. Precipitating antibodies against soluble nuclear and cytoplasmic antigens were detected by means of double immunodiffusion in Ouchterlony plates containing 0·6% agarose. Saline extracts of human spleen and calf thymus, prepared as described previously (Clark, Reichlin & Tomasi, 1969; Mattioli & Reichlin, 1971) were used as the source of antigen. Antibody specificity was defined using a variety of prototype sera, including those with antibodies to (U1)RNP, Sm, Ro (-SSA) and La (-SSB) antigens. Fresh extracts of rabbit thymus powder (Pel-Freeze, Arkansas, USA) were used as the source of antigen for detecting antibodies to Scl-70 (Catoggio, Skinner & Maddison, 1983).

Rheumatoid factor and DNA antibodies. Rheumatoid factor was measured by laser-nephelometry (Hyland-Disc), and antibodies to double stranded DNA by radio-immunoassay (Amersham, UK).

Antigen preparation for immunoblotting

A nuclear-enriched sonicate of K562 cells was prepared as previously described with some modifications (McNeilage *et al.*, 1986; Guldner, Lakomek & Bautz, 1983). K562 cells were grown in RPMI 1640 (Gibco UK Ltd) supplemented with heat-inactivated 10% fetal calf serum (Gibco UK Ltd). Penicillin and streptomycin each at 5000 iu/ml were routinely added to the culture media, and all buffers contained 1 mM phenylmethylsulphonyl fluoride (Sigma Chemical Co., St Louis, MO), 10 mM iodoacetamide (Sigma Chemical Co., St Louis, MO), and 10 mM EDTA. Following two washes in cold (4°C) 0·01 M phosphate-buffered 0·15 M saline (PBS), pH 7·4, the cells were solubilized at a concentration of 5×10^7 cells/ml in PBS containing 0·5% of Triton X-100 for 30 min on ice, after which the nuclei were pelleted at 5000 ***g*** for 15 min. The supernatant was retained as the cytoplasmic fraction. The nuclei were resuspended in 1 mM $NaPO_4$, pH 7·0, and sonicated at least 10 times for 10 s until all the nuclei had disintegrated as determined by light microscopy. The insoluble material was removed by centrifugation at 5000 ***g*** for 15 min at 4°C and the supernatant retained as the soluble nuclear fraction.

Immunoblotting

Polyacrylamide gel electrophoresis (PAGE) was performed on 1·0 mm thick slab gels in 0·1% sodium dodecyl sulphate (SDS) with a 3·8% stacking gel and a 12·5% resolving gel (Laemmli, 1970). The soluble nuclear protein was diluted with an equal volume of SDS sample buffer (62 mM Tris- HCl, pH 6·8, 0·2%, SDS, 50 mM dithiothreitol and 10% glycerol), was heated at 100°C for 3 min, and then electrophoresed at 20 mA/gel for 4 to 5 h. Following separation the proteins were transferred to nitrocellulose electrophoretically using a transblot cell (Bio-Rad Lab., Watford, UK) at 150 mA constant current overnight.

The nitrocellulose was then cut into strips and sequentially probed with serum at a 1/100 dilution, and then immunoperoxidase-labelled polyvalent goat anti-human immunoglobulin (Ig) conjugate (Sigma Chemical Co., St Louis, MO) at a 1/1000 dilution. In some experiments goat anti-human IgG (Sigma Chemical Co., St Louis, MO) and goat anti-human IgM conjugates (Sigma Chemical Co., St Louis, MO) were also used. Peroxidase activity was detected by incubating the strips in 0·01% 3 amino-9-ethylcarbazole in dimethylformamide diluted 1/20 in 0·5 M sodium acetate and adding 1 μl hydrogen peroxide per ml of solution immediately before use. Autoantibody recognition of a polypeptide was graded as either 0 (absent), + (weak) or + + (strong). The molecular weight of a polypeptide recognised by a serum was estimated relative to low molecular weight standards (Pharmacia AB, Uppsala, Sweden) which were detected using a Biotin-blot protein stain (Bio-Rad Lab., Watford, UK). This also served as a control for efficient transfer of protein.

RESULTS

Immunofluorescence

The sera from 60 of 65 patients with systemic sclerosis were

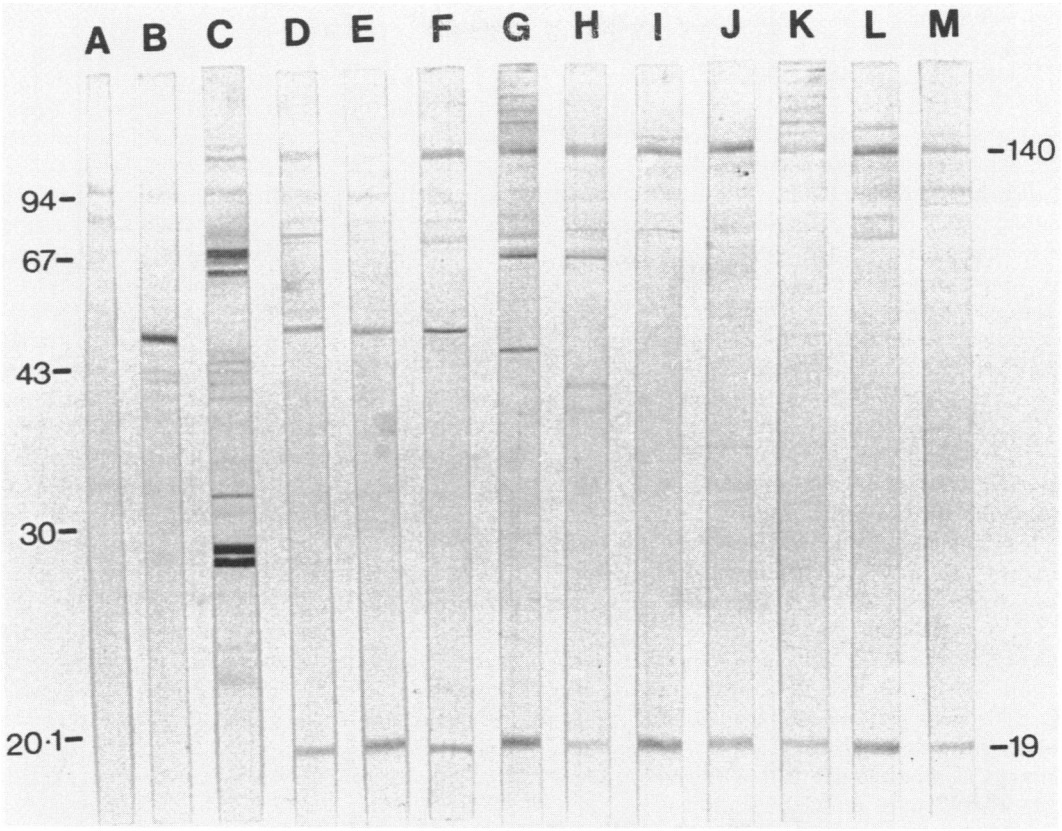


Fig. 1. An immunoblot showing reactivity of ACA positive sera (lanes D–M) against a nuclear sonicate of K562 cells. All ACA positive sera bind to a 19 kD polypeptide and all but one (lane E) bind to a 140 kD polypeptide. Control sera are: lane A, serum from a healthy subject; lane B, anti-La containing serum binding to the 48kD La protein; lane C, anti-(U1) RNP serum binding to 68 kD, A (32 kD) and B/B′ (29/28 kD) polypeptides.

ANA positive using HEp-2 cells as the substrate for IF. Eighteen (28%) gave an anticentromere pattern seen as discrete large speckles in interphase nuclei with characteristic clustering during mitosis and in all but two present in a titre of greater than 1/640. A combination of other patterns was also observed. Eighteen had diffuse, fine speckling, three of which had in addition nuclear dots (MND), and four had a grainy appearance with perinucleolar accentuation of staining, a pattern reported as specific for anti-Scl-70 (Bernstein, Steigerwald & Tan, 1982). Nine had discrete coarse speckling, one of which also had MND. Eleven had nucleolar staining this being homogeneous in ten and speckled in one. Four sera had homogeneous nuclear staining with one of these having MND. MND alone was present in two sera, and multiple cytoplasmic dots alone in one serum. Prominent cytoplasmic staining was present in 12.

Eleven ACA positive sera were examined for IgG and IgM antibodies in addition to broad spectrum antibodies with IF using the appropriate conjugate. All gave the characteristic ACA pattern with anti-IgG conjugate and were negative for ACA with anti-IgM conjugate.

Immunodiffusion

Precipitating antibodies were present in 33 of 65 SS sera. Eight of these were to Scl-70, five to (U1)RNP, three to Ro (-SSA) of which one was also to La(-SSB), and 18 were unidentified precipitins against calf thymus or rabbit thymus extract. Five sera that were anticentromere positive on IF had unidentified precipitins against calf thymus, with a line of immunological identity shared by three of them. The eight anti Scl-70 sera all had fine speckled staining on IF (with one also having speckled nucleolar staining), and accounted for three of the four that had in addition a grainy appearance.

Table 1. Polypeptides recognized by ACA positive systemic sclerosis sera

	No	140 kD	19 kD	26+23 kD	70 kD	52 kD
ACA positive	18	16	16	2	4	2
ACA negative	47	0	0	0	5	4

Control sera

Three SLE patients had sera containing ACA on IF. One of these also had precipitating antibodies to (U1)RNP. No patients with SLE or Felty's syndrome had precipitating antibodies to Scl-70.

Immunoblotting

Centromere associated polypeptides. A polypeptide of 140 kD was detected by 16 systemic sclerosis sera when immunoblotted using a polyvalent anti-Ig conjugate (Fig. 1). All 16 sera

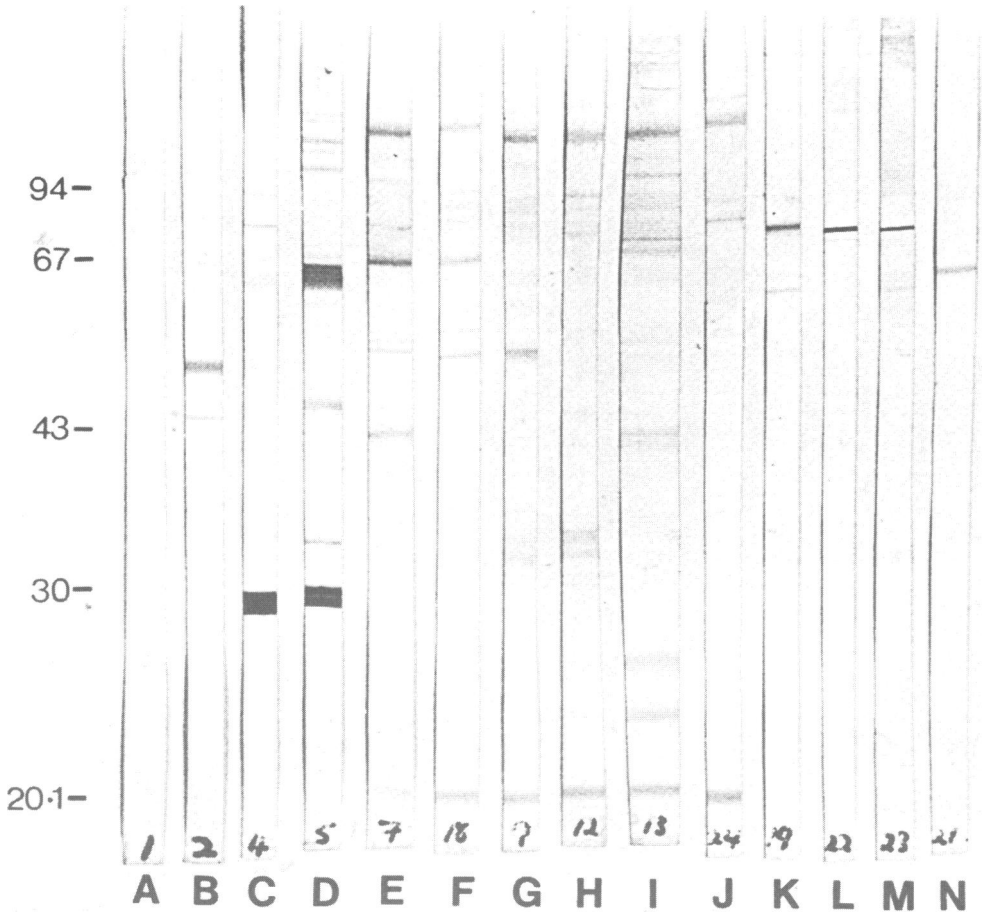


Fig. 2. An immunoblot showing a 78 kD polypeptide bound by sera containing anti-Scl-70 (lane M) as well as sera negative for anti-Scl-70 (lanes K and L). ACA positive sera (lanes E–J) bind 140 kD and 19 kD polypeptides with lane I also binding 26 kD and 23 kD polypeptides. Control sera are: lane A, normal healthy subject; lane B; serum containing anti-La; Lane C, serum containing anti-Sm; lane D, serum containing anti-(U1) RNP.

contained ACA on IF and 15 of the 16 also reacted with a 19 kD polypeptide. Two ACA positive sera failed to detect the 140 kD polypeptide although one of these detected the 19 kD polypeptide and the other had relatively low titre ACA (1/160) on IF. Other polypeptides recognized by ACA positive sera are shown in Table 1, and included a 26 kD and 23 kD pair of polypeptides detected by two. Two other polypeptides 70 kD and 52 kD were detected by both ACA positive sera as well as by ACA negative sera.

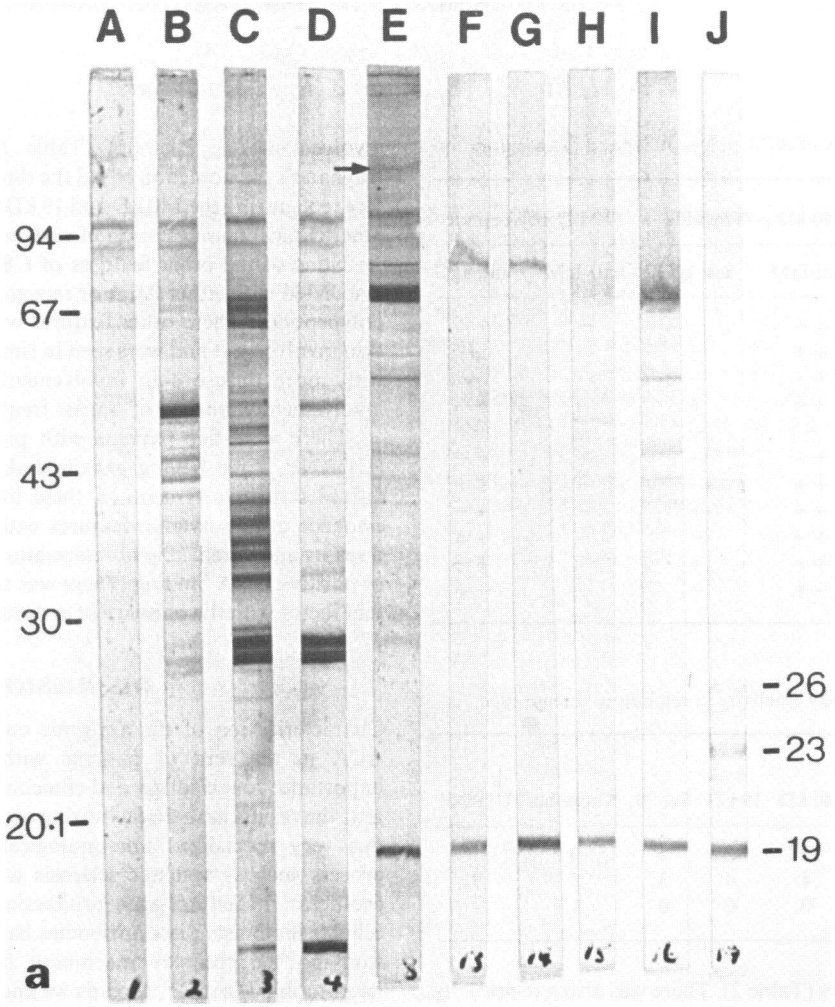
Three SLE sera recognized both 140 kD and 19 kD centromere associated polypeptides. Two of these sera were ACA positive on IF, one contained precipitating antibodies to (UI)RNP and reacted weakly with 68 kD and B′/B polypeptides (Steitz *et al.*, 1982) on immunoblotting. The other serum gave a complex staining pattern on IF.

Other polypeptides recognized by SS sera. A 78 kD polypeptide (Fig. 2) was detected by eight systemic sclerosis sera including three who had precipitating antibodies to Scl-70. Of the other five Scl-70 positive sera, three were negative on immunoblotting, one recognized a 52 kD polypeptide and the other a 49 kD polypeptide. Using freshly prepared rabbit thymus powder as the antigen source for immunoblotting, although La (-SSB) and (U1)RNP associated polypeptides were detected by the appropriate sera, no polypeptides could be detected by anti-Scl-70 positive sera.

Four systemic sclerosis sera had precipitating antibodies to (U1)RNP accompanied by a discrete course speckled pattern on IF, and three of these detected a 68 kD polypeptide with one also detecting the A and B/B′ polypeptides (Steitz *et al.*, 1982). Two sera detected a 48 kD La (-SSB) associated polypeptide on immunoblotting, one that had precipitating antibodies to La (-SSB) and Ro (-SSA) and the other that had precipitating antibodies to Ro (-SSB) alone.

Isotype specificity of ACA associated polypeptides

Eleven ACA positive sera that recognized the 140 kD polypeptide using a polyvalent anti-Ig conjugate were further studied using isotype specific conjugates. The 140 kD polypeptide was recognized strongly by ten of these using an anti-IgM conjugate to probe the bound immune complex, and only weakly by three using an anti-IgG conjugate (Fig. 3) suggesting the predominant reactivity against this 140 kD polypeptide resided in an IgM autoantibody. The reverse applied with the 19 kD polypeptide which was strongly detected by nine and weakly by two sera using an anti-IgG conjugate and weakly by four sera using an anti-IgM conjugate. The disease duration in these 11 patients



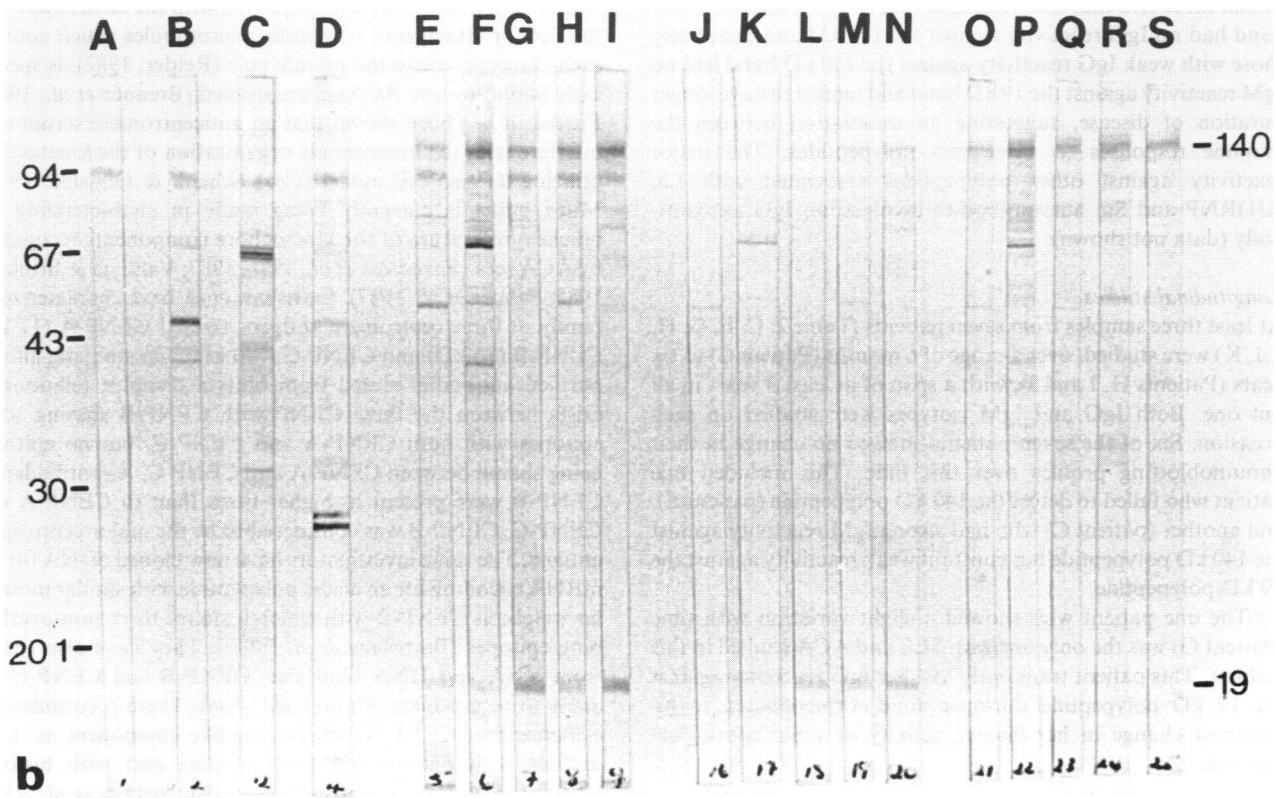


Fig. 3. (a) An immunoblot showing reactivity of ACA positive sera against a 19kD polypeptide (lanes F to J) detected with an anti-IgG conjugate. This contrasts to an ACA positive serum which detects both 140 kD (arrow) and 19 kD polypeptides when probed with polyvalent conjugate. Controls also probed with polyvalent conjugate are: lane A, normal healthy subject; lane B, serum containing anti-La; lane C, serum containing anti-(U1) RNP; lane D, serum containing anti-Sm, anti-La and anti-Ro. (B) In this immunoblot the same five ACA positive sera are probed with a polyvalent conjugate (lanes E to I), an anti-IgG conjugate (lanes J–M) and an anti-IgM conjugate (lanes O–S). The major reactivity against the 140 kD polypeptide resides in an IgM autoantibody (lanes O–S). Controls also probed with polyvalent conjugate are: lane A, normal healthy subject; lane B, serum containing anti-La; lane C, serum containing anti-(U1) RNP; lane D, serum containing anti-Sm.

Table 2. Isotype specificity of ACA polypeptides and disease duration

Patient	Disease duration	140 kD polypeptide		19 kD polypeptide	
		anti-IgM	anti-IgG	anti-IgM	anti-IgG
A	1	++	−	+	++
B	2	++	−	+	+
C	3	++	−	−	+
D	5	++	+	−	++
E	5	−	−	−	++
F	6	++	−	+	++
G	9	++	−	−	++
H	14	++	+	−	++
I	18	++	−	+	++
J	21	++	+	−	++
K	60	++	−	−	++

Table 3. Autoantibody reactivity in relation to disease type

Skin involvement	No. Patients	140 kD	19 kD	Scl-70	Nucleolar	U1RNP
Type 1	35	12	12	5	4	1
Type 2	22	4	4	3	5	3
Type 3	8	0	0	0	1	1

varied from 1 to 60 years (Table 2). There was also a reciprocity in that those sera that had weak IgM reactivity against the 19 kD band had no IgG reactivity against the 140 kD and conversely those with weak IgG reactivity against the 140 kD band had no IgM reactivity against the 19 kD band and tended to have longer duration of disease, suggesting an association between the immune responses to these two polypeptides. The major reactivity against other polypeptides associated with La, (U1)RNP and Sm autoantibodies involved an IgG autoantibody (data not shown).

Longitudinal studies

At least three samples from seven patients (Table 2: C, E, G, H, I, J, K) were studied, over a range of 6 months (Patient C) to six years (Patients H, I and J), with a span of at least 3 years in all but one. Both IgG and IgM isotypes were studied on each occasion. Six of the seven patients showed no change in their immunoblotting profiles over this time. This included one patient who failed to detect the 140 kD polypeptide (patient E), and another (patient C) who had strong IgM reactivity against the 140 kD polypeptide but constant weak reactivity against the 19 kD polypeptide.

The one patient who showed a slight variation with time (Patient G) was the only one with SLE and ACA studied in this fashion. This patient transiently lost her IgG reactivity against the 19 kD polypeptide during a time corresponding to no apparent change in her disease activity or other serological features.

Clinical associations

Within the systemic sclerosis group of patients reactivity against the 140 kD polypeptide identified a group with more limited skin involvement (χ^2; $P<0{\cdot}05$) (Table 3). All 16 patients had Raynaud's phenomenon as did the three patients with SLE with sera recognizing the 140 kD and 19 kD polypeptides who had no other clinical manifestation of systemic sclerosis.

None of the other features of CREST (i.e. CE or T) was associated with either ACA or recognition of 140 kD or 19 kD polypeptides. These other features were not unique to type 1 skin involvement and were seen in similar frequency in patients with more diffuse skin involvement. Pulmonary and renal involvement were also of similar frequency in all three types.

There were five patients with precipitating antibodies to (U1)RNP, three having proximal skin involvement and one diffuse skin involvement. In these five patients there was no evidence of any overlap features with other connective tissue diseases and specifically no association with myositis, arthritis, or positive DNA binding. There was no association of rheumatoid factor with the presence of arthritis, erosive or non-erosive.

DISCUSSION

Characterization of the antigenic components recognized by ACA in the sera of patients with systemic sclerosis has importance for scientists and clinicians alike. For rheumatologists the results have direct bearing on understanding the role of this very specialized immunological response in a disease process such as systemic sclerosis where increased fibroblast proliferation and collagen production predominate. And for cellular biologists these antibodies have been useful probes for studying the complex machinery for cellular divison and microtubule assembly. Already we know that the kinetochore, a trilaminar structure closely associated with the centromere and the site for attachment of spindle microtubules which connect each chromosome to the spindle pole (Reider, 1982), is specifically bound by one ACA serum studied (Brenner *et al.*, 1981). Further it has been shown that an anticentromere serum may interfere with the microtubule organization of the kinetochore utilizing a lysed cell model (Cox, Schenk & Olmsted, 1983). Much effort is currently being made in characterizing the biochemical nature of the kinetochore components recognized by ACA sera (Earnshaw *et al.*, 1986; 1987; Valdivia & Brinkley, 1985; Palmer *et al.*, 1987). Earnshaw *et al.* have characterized a family of three centromere antigens termed CENP-A (17 kD) CENP-B (80 kD) and CENP-C (140 kD). On studying affinity purified antibodies eluted from blots a complex relationship exists between the three CENP with CENP-B sharing some epitopes with both CENP-A and CENP-C, but no epitopes being shared between CENP-A and CENP-C. As antibodies to CENP-B were present in higher titres than to CENP-A and CENP-C, CENP-B was considered to be the major centromere antigen. The same investigators have now cloned cDNA for the mRNA encoding for an acidic polypeptide with similar molecular weight as CENP-B with which it shares three non-overlapping epitopes (Earnshaw *et al.*, 1987). They have also shown from RNA and DNA blots that CENP-B and CENP-C are likely to be products of unrelated genes. There is considerable evidence that CENP-A is a histone-like component as it co-purifies with nucleosome core particles and with histones (Palmer *et al.*, 1987). Another study (McNeilage *et al.*, 1986) detected a 72 kD polypeptide with 42 ACA positive sera which probably corresponds to CENP-B. This polypeptide was highly conserved being detected in *Leishmania tropica* and had distinct

epitopes from a 19·5 kD polypeptide (CENP-A) recognised by 35 of the same sera.

To our knowledge we are the only other group apart from Earnshaw *et al.* who have now consistently detected a 140 kD polypeptide (CENP-C). Activity against this polypeptide was present in 16 of 18 ACA positive sera, 15 also recognizing a 19 kD polypeptide (CENP-A). Three of 100 SLE patients had sera which also recognized both polypeptides, two of which were ACA positive on IF, one also having precipitating antibodies to (U1)RNP, and the third was negative for ACA on IF. We have not been able to consistently detect CENP-B but instead have identified 70 kD and 52 kD polypeptides in a minority of ACA positive as well as ACA negative sera. These polypeptides may therefore not correspond to CENP-B which has either been degraded, insufficiently enriched or not degraded from a higher molecular weight species in our extraction procedure. Alternatively autoradiography using protein-A may be more sensitive for detecting CENP-B. These are more likely reasons than differences attributable to the type of cell line used for the source of antigen, as CENP-B is reported to be highly conserved (McNeilage *et al.*, 1986) and we have obtained similar results using HEp-2 cells in place of K562 cells as our source of soluble nuclear antigen. Two sera detected a pair of polypeptides 26 and 23 kD respectively which correspond to 25·5 and 23 kD polypeptides immunoreactive with five of 18 ACA sera in another study (Guldner, Lakomek & Bautz, 1984).

However, more importantly we have shown that the predominant activity against the 140 kD polypeptide (CENP-C) resides in an IgM autoantibody which may explain why other studies which have used methods to specifically detect IgG autoantibodies (McNeilage *et al.*, 1986; Cox, Schenk & Olmsted, 1983; Guldner, Lakomek & Bautz, 1984; Valdivia & Brinkley, 1985; Palmer *et al.*, 1987) have failed to detect CENP-C. Earnshaw *et al.*, however, have detected CENP-B using an anti-IgG conjugate in their method but have used metaphase chromosomes as the substrate for blotting which may enhance the sensitivity of detection. Also we have detected weak IgG reactivity against the 140 kD polypeptide in a few sera from patients who have relatively longer duration of disease (Table 2). Conversely the 19 kD antigen in our study (CENP-A) was strongly detected by an IgG autoantibody and weakly in a few sera by an IgM autoantibody. Moreover none of these eleven ACA positive sera had IgM ACA on IF. This may suggest that patients with ACA mount an on-going IgM response to an epitope on a 140 kD polypeptide which cross-reacts with epitopes present on centromere associated polypeptides, the latter of which are targets for a more finely tuned IgG immune response.

Most reported antinuclear antibodies are of the IgG class, although certain techniques such as immundiffusion may bias against the detection of IgM autoantibodies because of their slower mobility (Pollard & Tan, 1985). However recently, using immunblotting techniques, it has been shown that while antibodies against the 68 kD (U1)RNP polypeptide are predominantly of the IgG isotype, antibodies to the B/B′ polypeptides associated with Sm were found to be more frequently of the IgM isotype (Guldner, Lakomek & Bautz, 1986). The clinical relevance of the isotype specificity has yet to be determined.

Skin involvement limited to the fingers (sclerodactyly) was found in 35 of 65 SS patients in this study which included only those patients fulfilling ARA criteria for SS. The presence of antibody to centromere and to its associated 140 kD and 19 kD polypeptides was associated with limited skin involvement and was seen in none of the eight patients with diffuse skin disease. There was no association with calcinosis, oesophageal involvement and telangiectasia which were not unique to Type I disease and were seen in similar frequency irrespective of extent of skin involvement. Raynaud's phenomenon which was present in 64 of our 65 patients with systemic sclerosis, and 78 of 100 patients with SLE was present in all ACA positive patients regardless of diagnosis.

Combining the results from this study with others (McNeilage *et al.*, 1986; Tan *et al.*, 1980; Catoggio *et al.*, 1983; Fritzler, Kinsella & Garbutt, 1980; Bernstein *et al.*, 1982; Garcia-de la Torre & Miranda-Mendez, 1982; Earnshaw *et al.*, 1986) the incidence of Raynaud's phenomenon in patients with ACA is 98% (186/190). It would seem therefore that Raynaud's phenomenon is either a prerequisite for the generation of ACA or a consequence of this immunological response. ACA may be directly or indirectly involved in mechanisms leading to or potentiating vasospasm, possibly through interference with endothelial cell or vascular smooth muscle cell function. Furthermore the presence of ACA is an immunological marker for those patients with limited skin involvement and arguably a protective factor against more diffuse disease.

Autoantibodies to Scl-70 were found in eight of 65 patients and were not found to correlate with any particular clinical feature. The 95–100 kD DNA topoisomerase I polypeptide recently reported to be the Scl-70 antigen (Guldner *et al.*, 1986; Shero *et al.*, 1986) was not recognized by any anti-Scl-70 sera with our present blotting system, although an 84 kD polypeptide was with three, but the nature of this polypeptide is uncertain and currently being explored. Precipitating antibodies to (U1)RNP were found in five sera, three of which recognized a 68 kD polypeptide on blotting. The presence of this antibody has been accepted as a marker for patients with mixed connective tissue disease (MCTD). The five patients with anti-(U1)RNP and systemic sclerosis in this study had no manifestation of another connective tissue disorder. Within the SLE group the presence of anti-U1RNP was infrequently (15%) a marker for MCTD (manuscript in preparation).

In this study we have shown that antibodies to centromere related polypeptides are immunological markers for a form of scleroderma with limited skin involvement and are intimately linked with the presence of Raynaud's phenomenon. Furthermore there appears to be an ongoing differential autoantibody isotype class response to two major centromere-related antigens, which remains constant with time. This has important implications regarding the generation of ACA, and the role of humoral immunity in the pathogenesis of SS.

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