EXTENDED REPORT

Coexistence of antitopoisomerase I and anticentromere antibodies in patients with systemic sclerosis

T Dick, R Mierau, P Bartz-Bazzanella, M Alavi, M Stoyanova-Scholz, J Kindler, E Genth

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Background: Antibodies targeting DNA topoisomerase I (ATA) or centromere proteins (ACA) are associated with clinical subsets of patients with systemic sclerosis (SSc). The occurrence of those autoantibodies is considered to be mutually exclusive.
Objective: To describe the clinical and immunogenetic data of three patients who are co-expressing both antibodies, and then review previous publications.
Methods: Both antibodies were detected by different methods, including indirect immunofluorescence technique, enzyme linked immunosorbent assay, immunodiffusion, and immunoblot. Patients were HLA typed by serological and molecular genetic methods. Data were extracted from published reports for comparison. The search for published studies was through Medline and other database research programmes.
Results: During routine laboratory diagnostics over several years three patients with scleroderma and coincidence of ATA and ACA were identified: patient 1 with diffuse SSc, Raynaud's phenomenon, puffy fingers and fingertip necrosis, contractures, and calcinosis; patient 2 with diffuse SSc, Raynaud's phenomenon, oedema of the hands, and interstitial calcinosis of hands, knees, and shoulders, and pulmonary fibrosis; patient 3 with scleroderma of hands, forearms, and face, Raynaud's phenomenon, puffy fingers, finger contractures, fingertip necrosis, and calcinosis. All three patients studied were carriers of HLA alleles known to be associated with these autoantibodies. In serial measurements the concentrations of the two antibodies showed independent or even reverse fluctuations. Screening of 100 patients with ACA for ATA and vice versa disclosed no further patients with coincidence of these antibodies. Twenty eight cases of ACA/ATA coexistence in 5423 patients (0.52%) with SSc or SSc associated symptoms were found in an analysis of published studies.
Conclusion: The expression of ATA and ACA is not totally mutually exclusive, but coincidence is rare (<1% of patients with SSc). Patients with both autoantibodies often have diffuse scleroderma and show immunogenetic features of both antibody defined subsets of SSc.

See end of article for authors' affiliations

Correspondence to: Dr T Dick, Rheumaklinik und Rheumaforschungsinstitut, Burtscheider Markt 24, D 52066 Aachen, Germany; mail@thomas-dick.de

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Systemic sclerosis (SSc) is characterised by inflammatory and fibrotic changes of skin, blood vessels, and various internal organs. Disease manifestations differ among patients. On the one hand, there is a limited form of the disease with scleroderma of the fingers, hands, or face and no involvement or delayed involvement of internal organs, mostly identical with the CREST syndrome (calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasias). On the other hand, a subgroup of patients has diffuse scleroderma with widespread skin changes affecting the distal and proximal extremities as well as the trunk. In addition to this, involvement of internal organs is more common in these patients, which tends to be serious.[1]

Antitopoisomerase I antibodies (ATA) and anticentromere antibodies (ACA) are important diagnostic markers of SSc. Each of these autoantibodies can be found in about 25% of patients with SSc.[2 3] In large series of patients with SSc these two antibodies almost never occur together in an individual patient.[3–5] This mutual exclusivity fits the well known association of these two autoantibodies with the different subsets of the disease mentioned above: ACA occur in about 50–90% of patients with CREST syndrome or limited cutaneous SSc,[6–8] although they are not specific for this disease and have been described in patients with other diseases like primary biliary cirrhosis and systemic lupus erythematosus.[7 9–11] ATA, on the other hand, can be found in about 40–90% of patients with diffuse scleroderma.[12–14]

In addition, the HLA class II alleles which are closely associated with these disease subsets are different depending on the autoantibody produced: based on an early report on the association of ACA with HLA-DR1, DR4, and DR8,[15] the HLA association of ACA has been narrowed down to a non-leucin residue at position 26 of the HLA-DQB1 molecule.[16] Likewise, the HLA-DR11 association of ATA[15] has been tracked down to a tyrosine residue at position 30 of HLA-DQB1.[17]

Thus the different clinical, serological, and immunogenetic features of these two disease subsets may lead to the hypothesis that two different and independent kinds of aetiology and pathogenesis are involved. However, in recent years exceptional cases of coexistence of these two antibodies have been reported. In the study presented here, we describe three additional cases and estimate the incidence and clinical significance of the coincidence of these two antibodies.

PATIENTS AND METHODS

Patients and sera

Clinical data of patients with ACA or with ATA were obtained by chart review, using a standardised documentation protocol. Clinical features were defined as described earlier.[15] Altogether, data from 173 ACA and 118 ATA positive patients could be evaluated, 44 (25%) patients with ACA and 95 (81%) patients with ATA fulfilling the ACR criteria for SSc.[18] For a

Abbreviations: ACA, anticentromere antibodies; ATA, antitopoisomerase I antibodies; IIFT, indirect immunofluorescence test; SSc, systemic sclerosis; SSP-PCR, sequence-specific primer-polymerase chain reaction

detailed serological analysis, 100 sera known to be positive for ACA and 100 sera with ATA were selected, regardless of the diagnosis.

Indirect immunofluorescence test (IIFT)

The test was performed by a standardised method.[19] Briefly, methanol/acetone fixed preparations of HEp-2 cells were incubated with diluted patient serum. The patient serum was removed and the slides were washed, and FITC labelled secondary antibody directed against total human immunoglobulin was added. Afterwards the preparations were washed again, covered, and examined under the fluorescence microscope. Titres of 80 or more were regarded as positive.

Immunodiffusion

ATA were detected by immunodiffusion according to a standard Ouchterlony test protocol as described.[15] A preparation of rabbit thymus extract (Pel-Freez, Rogers, via Paesel and Lorei, Frankfurt, Germany) served as antigen source.

Enzyme linked immunosorbent assay (ELISA)

Commercial ELISA systems (Pharmacia and Upjohn, Freiburg, Germany) were used for the quantitative determination of ATA and ACA. Both tests, using recombinant human antigens and detecting human IgG, were performed according to the manufacturer's instructions. The centromere ELISA detected antibodies against the CENP-B protein. The cut off value for both tests was defined by the supplier as 5 U/ml.

Immunoblot

Results obtained by other methods of detecting ATA were confirmed by immunoblot, as described earlier.[19] In short, an antigen preparation of 5×10^8 HeLa cells was separated on a 5–20% sodium dodecyl sulphate gradient gel and electrophoretically transferred to a nitrocellulose membrane (Biorad, Munich, Germany). Strips of the membrane were incubated with patient sera and, after a washing step, a biotin labelled goat antihuman IgG antibody (Sigma) was added. An avidin-peroxidase substrate reaction was used as read out.

HLA typing

The HLA-A, -B, -C loci of the patients were typed serologically according to the standard NIH microcytotoxicity test, using serum samples from One Lambda (BmT, Krefeld), Biotest (Dreieich), Fresenius (Bad Homburg), BAG (Lich), Bio-Mérieux (Nürtingen), and Behringwerke (Marburg, all Germany). The HLA class II alleles were determined with a multiplex-sequence-specific primer-polymerase chain reaction (multiplex-SSP-PCR; UCLA amplification mixtures, ULCA Tissue typing Laboratory, Los Angeles, USA) including allele-specific primers for DRB1*0101, 02, 03; *0301, 02; *0401–0411; *0701; *0801–05; *0901; *1001; *1101–04; *1201, 02; *1301–05; *1401, 02, 06; *1501–03; *1601, 02; DRB3*0101; *0201, 02; *0301; DRB4*0101; DRB5*0101, 02; *02; DQB1*0201;*0301–03; *04; *0501–03; *0601–04. DQA locus alleles were determined using SSP-PCR kits DQA1 from Dynal (Oslo, Norway).

Literature analysis

Research of published reports was done through Medline, ISI Current Contents, and databases accessible through the internet. Databases were at first screened for publications describing patients with SSc and mentioning ATA or ACA, or both. Articles were selected if the patients enclosed in the studies had SSc or SSc associated symptoms and if antibody testing for both antibodies had been carried out by standardised methods (counterimmunoelectrophoresis, ELISA, IIFT, immunoblot, immunodiffusion, immunoprecipitation). Patient groups which obviously appeared in more than one publication were counted only once.

RESULTS

During routine diagnostic examinations over several years we identified three patients with coincidence of ATA and ACA: both antibodies could be detected by indirect immunofluorescence on HEp-2 cells and ELISA with recombinant antigens. In addition, the presence of ATA was confirmed by immunodiffusion and immunoblot.

To investigate whether these three patients were really exceptional cases or whether ATA/ACA coincidence can be detected more frequently by sensitive autoantibody detection methods, 100 ATA positive sera (immunodiffusion assay) were tested for ACA in an ELISA. Vice versa, 100 sera with a positive ACA IIFT result were tested for ATA by ELISA. None of the ATA positive sera contained ACA against the CENP-B protein. Ninety five of the sera positive for ACA by IIFT were negative in the ATA ELISA, five sera were marginally positive (5.3; 8.8; 11.3; 16.4; 22.5 U/ml). The positive results could not be confirmed by immunodiffusion and immunoblot, suggesting false positive ELISA results. Thus we confirmed that the coincidence of ATA and ACA is rare (<1%). The coexistence of ATA or ACA with other antibodies was not investigated.

In all available sera from different blood samples of the three patients, both antibodies were quantified (fig 1). Whereas for patient No 2 only two samples were available, antibody concentrations of patients No 1 and No 3 have been observed for 12 or 10 years, respectively. Antibody concentrations independently fluctuated over the years; in fact most of the time they varied in opposite directions. In patient No 1, ACA appeared later than ATA and were detected for the first time six years after disease onset.

The HLA typing of the three patients showed that all of them had at least one DQB1 allele with a non-leucine residue

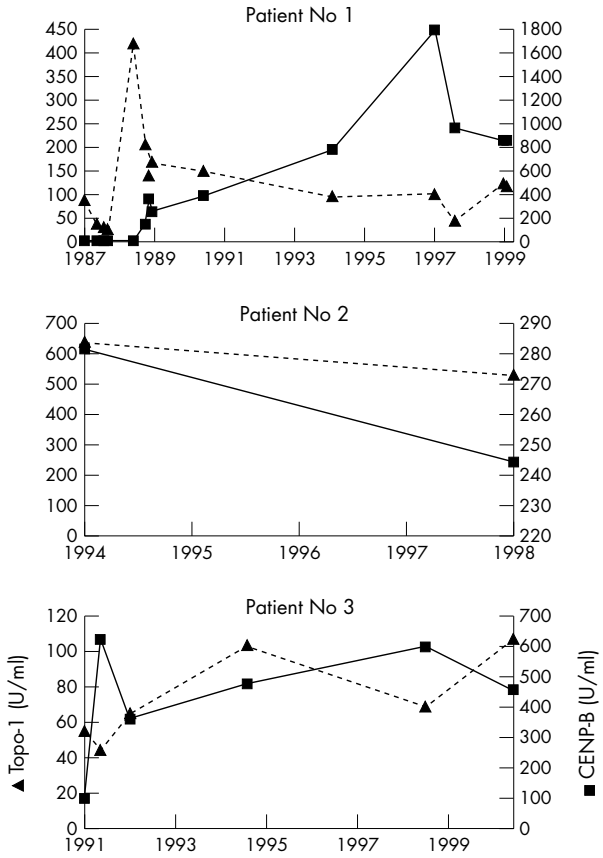

Figure 1 Time courses of autoantibody concentrations. Serial measurements for the three patients. Fresh sera or samples from our serum bank (after storage at –30°C) were tested by ELISA, as outlined in "Patients and methods", for ATA (triangles) and anti-CENP-B (squares).

Table 1 HLA typing results

Patient	HLA-A	HLA-B	HLA-C
(a) HLA class I			
1	26, –	35, –	4, –
2	1, 3	7, 35	7, –
3	10, 19	13, 15	5, –

Patient	HLA allele	DQB1 allele with non-Leu-26 typical for ACA	DQB1 allele with Tyr-30 typical for ATA
(b) HLA class II			
1	DRB1*1102, –		
	DRB3*0202		
	DQA1*0101, *0501		
	DQB1*0301	Yes	Yes
	DQB1*0501	Yes	No
2	DRB1*0801, *1104		
	DRB3*0202		
	DQA1*0401, *0501		
	DQB1*0301	Yes	Yes
	DQB1*04	Yes	Yes
3	DRB1*0101, *1102		
	DRB3*0202		
	DQA1*0101, *0501		
	DQB1*0301	Yes	Yes
	DQB1*0501	Yes	No

at position 26, which is described as being associated with ACA, and a DQB1 allele with a tyrosine at position 30 known to be associated with ATA. In most cases alleles carrying these features were present on both chromosomes (table 1).

Table 2 lists the clinical data of the three patients with ATA/ACA coincidence. All three patients were female. In patient No 1, now 38 years old, the disease started at 20 years of age with Raynaud's phenomenon, followed by oedema of the hands, feet, and face. She developed early diffuse SSc with repeated fingertip necrosis, contractures, calcinosis, and telangiectasias. Patient No 2, aged 71 years, with a disease duration of 10 years, had Raynaud's phenomenon, oedema of the hands, rapidly progressing diffuse scleroderma, severe interstitial calcinosis of the hands (fig 2), knees, and shoulder, and pulmonary fibrosis. In patient No 3, 67 years old, the disease started 10 years ago with Raynaud's phenomenon, puffy fingers, and sclerodactyly. During the disease course she developed finger contractures, fingertip necrosis, calcinosis, and scleroderma of forearm and face; there is no serious internal organ involvement so far.

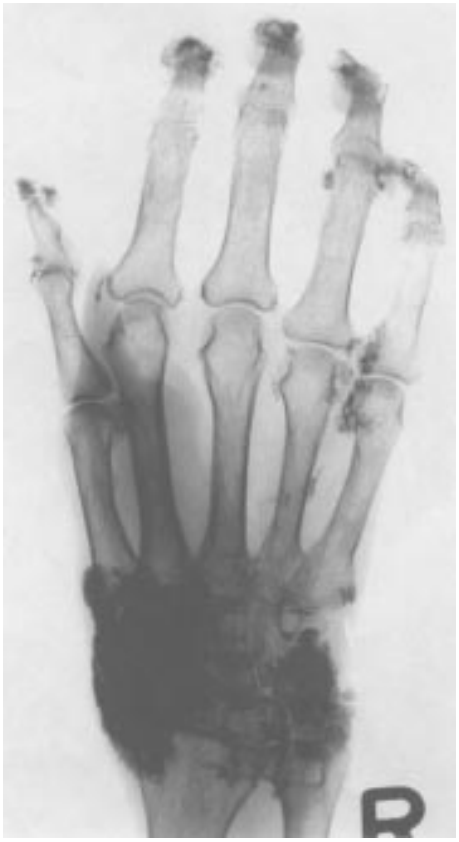

Figure 2 Radiograph of the right hand of patient No 2, eight years after disease onset.

To judge whether these clinical features were typical for patients with ACA, ATA, or both, we reviewed the clinical data of our 173 patients with ACA and 118 patients with ATA (fig 3), and compared these with those of our three index patients. Most features (for example, Raynaud's phenomenon, telangiectasias, puffy fingers, fingertip necrosis, etc) can be found in both antibody defined patient groups. Of the features common to our three patients with coincident ATA/ACA,

Table 2 Clinical data of patients with ATA and ACA

	Patient 1	Patient 2	Patient 3
Age at onset	20	61	57
Duration (years)	18	10	10
Raynaud's phenomenon	+	+	+
Puffy fingers/ hands	+ (only initially)	+	+ (recurrent)
Scleroderma	Hands, forearms, upper arms, face, trunk	Hands, forearms, upper arms, face, trunk	Hands, forearms, face
Fingertip necrosis	+ (recurrent)	+ (once)	+ (recurrent)
Contractures	Fingers, toes, elbows	Fingers, elbows	Fingers
Calcinosis cutis	+	+	(+)
Telangiectasia	Generalised	Breast	–
Oesophageal hypomotility	+	ND*	+
Pulmonary fibrosis	–	+	–
Sicca symptoms	–	+	–
Renal disease	–	–	+ (proteinuria, decreased GFR)
Arthralgia/arthritis	+/–	+/–	–/–

*No data available.

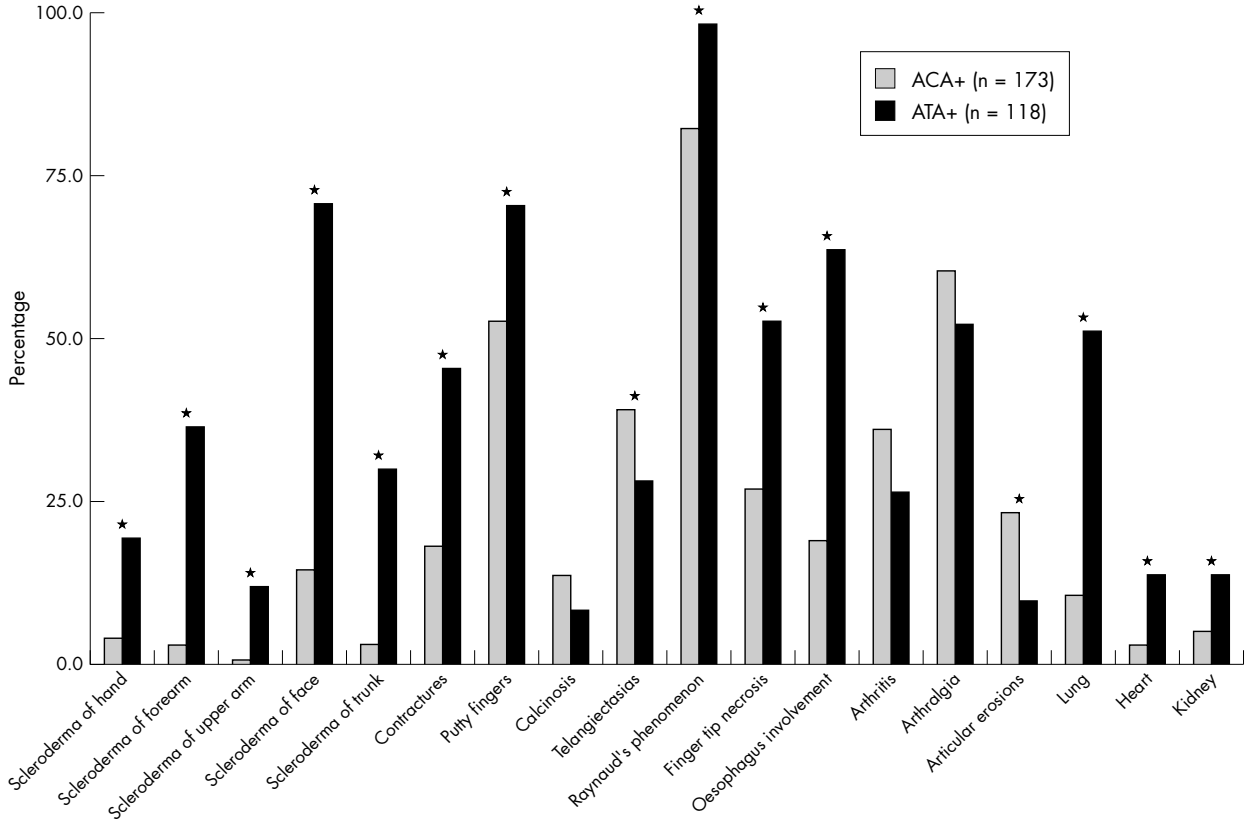

Figure 3 Clinical characteristics of patients with ACA and ATA. Clinical data of 173 patients with ACA (tested with IIFT) and 118 patients with ATA (tested with immunodiffusion) were compared by chart review. Significant differences ($p<0.05$, Fisher's exact test, two sided) between the two groups are marked (*).

diffuse scleroderma or at least involvement affecting the forearms is found almost exclusively, and contractures predominantly, in the ATA group, whereas calcinosis is more common in the ACA group (although the latter difference did not reach statistical significance).

To assess further the prevalence of ATA/ACA coincidence in patients with SSc, we carried out a literature search using different sources as outlined in "Patients and methods". From more than 865 publications initially screened, we found 56 publications in which patients with SSc or SSc symptoms were tested for both autoantibodies with standard methods. Altogether, in 5423 patients, antibodies coexisted in 28 (0.52%) (table 3). Furthermore, six additionally reported cases with both antibodies were excluded because they did not fulfil our criteria for selection. Taken together, we found 34 cases documenting the coexistence of ACA/ATA.

DISCUSSION

Autoantibodies are known as useful markers for the diagnosis of SSc. It is established that they occur in about 95% of patients with SSc. Some of them (like ACA and ATA) are associated with subgroups that have distinctive clinical symptoms and prognosis so that the concept of antibody defined syndromes emerged. In systemic lupus erythematosus (as opposed to SSc) the occurrence of more than one antibody is usual.[20] In SSc, coexistence of ACA or ATA with antihistone antibodies,[21] ACA with antimitochondrial antibodies,[22] ATA and anticardiolipin antibodies,[23] ACA/ATA and anti-SS-A[24] or anti-RNP and Th/To antibodies[25] has been reported. However, although co-occurrences with antihistone or antimitochondrial antibodies are relatively common, the detection of more than one subset defining autoantibody in the serum of a patient with SSc is rare. Many authors have regarded the marker antibodies ACA and ATA as mutually exclusive,[3 26 27] and it might be argued that these antibody defined syndromes represent distinct, independent clinical entities with separate aetiologies.

However, from the present report of three patients, as well as from several reports published earlier (table 3), it is known that the coincidence of ATA and ACA in individual patients does occur. The reported prevalence of ATA/ACA coexistence in SSc varies widely: whereas Jarzabek-Chorzelska *et al* claimed a frequency of 5.6% in their 180 patients,[28] Bunn and Black reported that they had seen only one example in over 2000 cases—that is, a prevalence of less than 0.05%.[29] Spencer-Green *et al* reviewed nine publications with 670 patients which revealed three cases of coexistence (0.45%).[12] We extended the literature search to 56 reports with 5423 patients, in which the compiled frequency of ATA/ACA co-occurrence was 0.52%, remarkably consistent with the analysis of Spencer-Green *et al*.[12] Our own results, with three coincidence patients and no further case in 200 serum samples preselected for either antibody, are compatible with these numbers.

Thus, if one regards "ATA associated SSc" and "ACA associated SSc" as separate, independent clinical entities, the question arises: do those rare patients with both antibodies have both diseases independently? One clue towards answering this question is obtained by looking at the signs and symptoms of our three patients and asking whether these can be regarded as typical for both diseases. This seems to be the case because our patients have extended scleroderma and contractures as well as calcinosis and joint involvement. Almost all clinical features of SSc, however, can occur in both antibody defined subsets (fig 3). Therefore, from clinical data of only a few patients alone it is not possible to decide if the two SSc subsets occur independently. Only the analysis of a large number of patients with coincident ATA/ACA would

Table 3 Publications with data of patients tested for antitopoisomerase I and anticentromere antibodies

Reference†	Reference No	Number of patients‡	ACA and ATA positive	ACA detection	ACA positive No (%)	ATA detection	ATA positive No (%)	Remarks
Behr, König, *et al* 1990	30	74	0	IFT	12 (16)		21 (28)	
Bunn, Denton, *et al* 1998	31	735	0	IFT	184 (25)	CIE, IP	157 (21)	
Cassani, Tosti, *et al* 1987	32	35	0	IFT	7 (20)	ID, CIE	11 (31)	
Catoggio, Skinner, *et al* 1983	6	40	0	IFT	11 (28)	ID	8 (20)	
Chang, Wang, *et al* 1998	33	89	0	IFT	26 (29)	ID	11 (12)	
Conrad, Stahnke, *et al* 1995	34	228	2	IFT, ELISA (CENP-B)	50 (22)	ID, IB, ELISA	28 (12)	
De Rooij, van de Putte, *et al* 1988, 1989	35,36	77	0	IFT, IB (CENP-A)	10 (13)	IB	11 (14)	
Falkner, Wilson, *et al* 1998, 2000	37, 38	292	0	IFT	56 (19)	ID	71 (24)	
Fanning,Welsh, *et al* 1998	39	130	0	IFT	35 (27)	CIE	32 (25)	
Ferri, Bernini, *et al* 1991	40	151	1	IFT	32 (21)	CIE	61 (40)	
Gabay and Kahn 1992	41	12	0	IFT	0	ID	4 (33)	
Grigolo,Mazzetti, *et al* 2000	42	92	1	IFT	25 (27)	ELISA	46 (50)	Personal communication
Harvey, Butts, *et al* 1999	43	122	0	IFT	42 (34)	ID, IP	36 (30)	
Haustein, Ziegler, *et al* 1990	45	12	0	IFT	5 (42)	ID	3 (25)	
Herrmann, Schulze, *et al* 1990	44							
Hietarinta, Tertti, *et al* 1993	46	35	1	IFT	4 (11)	ID, IB	12 (34)	
Hietarinta, Lassila, *et al* 1994	47							
Igarashi, Takehara, *et al* 1990	48	65	0	IFT	13 (20)	ID	30 (46)	
Jakobsen, Halberg, *et al* 1998	49	230	0	IFT	79 (34)	ID	31 (13)	
Jarzabek-Chorzelska, Blaszczyk, *et al* 1986	50	107	0	IFT	20 (19)	ID	60 (56)	
Jarzabek-Chorzelska, Blaszczyk, *et al* 1990	28	180	10	IFT, IB		IIFT, IB, ID		
Jablonska, Blaszczyk, *et al* 1991	14							
Jarzabek-Chorzelska, Blaszczyk, *et al* 1990	51							
Johanet, Agostini, *et al* 1989	52	183	0	IFT	43 (23)	ID, IB	48 (26)	
Kallenberg, Wouda, *et al* 1988	53	85	0	IFT, IB	6 (7)	IB	11 (13)	
Kipnis, Craft, *et al* 1990	54	112	1	IFT	32 (29)	IP	15 (13)	
Kuwana, Kaburaki, *et al* 1999	55	117	0	IFT	0	ID, IP	117 (100)	Topo-1 preselected
Lakomek, Guldner, *et al* 1987	56	36	0	IFT, IB	8 (22)	ID	13 (36)	
Maekawa, Yano, *et al* 1992	57	1	1					Case report
McCarty, Rice, *et al* 1983	58	52	0	IFT	27 (52)*	ID	5 (10)	*Some patients ACA preselected
McHugh, Whyte, *et al* 1994	59	58	0	IFT, IB	11 (19)	ID	6 (10)	
McNeilage, Whittingham, *et al* 1986	60	77	1	IB, IFT	42 (55)	CIE	18 (23)	
McNeilage, Youngchaiyud, *et al* 1989	61	49	1	IFT, IB (CENP-A)	1 (2)	CIE, IB	37 (76)	
Meurer, Scharf, *et al* 1985	62	104	0	IFT	18 (17)	ID	21 (20)	
Mora, Rivero, *et al* 2000	63	1	1	IFT		ID		Case report
Parodi, Puiatti, *et al* 1991	64	91	0	IFT	30 (33)	ID	27 (30)	
Picillo, Migliaresi, *et al* 1997	65	105	0	IFT	18 (17)	ID	70 (67)	
Renier, Le Normand, *et al* 1992	66	67	0	IFT	67 (100)*	CIE	0 (0)	*ACA preselected
Reveille, Durban, *et al* 1992	17	161	1	IFT	21 (13)	ID, IB	45 (28)	
Riboldi, Asero, *et al* 1985	13	84	0	IFT	15 (18)	ID	42 (50)	
Ruffatti, Calligaro, *et al* 1985	67	121	2	IFT	?	ID	?	
Russo, K, Hoch, *et al* 2000	68	45	0	IFT/ELISA	45 (100)*	ELISA	0	*ACA preselected
Sato, Ihn, *et al* 1993	69	236	2	IFT, IB (CENP-A)	72 (31)	IB	71 (30)	
Sato and Takehara 1991	70							
Stahnke, Meier, *et al* 1994	71	80	1§	ELISA (CENP-B)	80 (100)*	IB, ELISA	?	*ACA preselected
Steen, Powell, *et al* 1988	2	397	0	IFT	86 (22)	ID	102 (26)	
Tan, Rodnan, *et al* 1980	72	45	0	IFT	14 (31)	ID	9 (20)	
van Venrooij, Stapel, *et al* 1985	73	33	1§	IFT	7 (21)	IB,ID	13 (39)	
Vlachoyiannopoulos, Drosos, *et al* 1993	74	47	3§	IFT	47 (100)*	CIE	3 (6)	*ACA preselected
Wade, Sack, *et al* 1988	75	20	0	IFT	20 (100)*	CIE	0	*ACA preselected
Weiner, Earnshaw, *et al* 1988	76	297	2	IFT, IB	83 (28)	ID, IB	IB 37 (12)	
Zuber, Gotzen, *et al* 1994	11	13	1* + 1§	IFT, IB (CENP-A)	13 (100)*	ELISA	2 (15)	*ACA preselected
Total		5423	28 (34)					

†Publications obviously or probably containing the same patient series are grouped together and were counted only once; ‡selection of patients as described in "Patients and methods"; §patients not covered by our selection criteria but described as being both ACA and ATA positive. CIE, counterimmunoelectrophoresis; ELISA, enzyme linked immunosorbent assays; IIFT, indirect immunofluorescence; IB, immunoblot; ID, immunodiffusion; IP, immunoprecipitation.

possibly allow such a conclusion, if the clinical features typical for either disease subset occurred as frequently as in the two single antibody defined diseases. The immunogenetics of our patients are at least compatible with the hypothesis of independence because all three patients carry those HLA alleles known to be closely associated with ATA as well as with ACA. Likewise, the quantitative courses of antibodies in serial measurements, with non-parallel and, most of the time, even reverse fluctuations (fig 1), argue for independence. Of particular interest in this regard is the disease course of patient No 1: at first she developed diffuse scleroderma with ATA. Only after ACA were detected did telangiectasias appear and progress from a facial to a generalised localisation.

On the other hand, the sheer number of reported cases of ATA/ACA coincidence argues against completely independent aetiologies of these two very rare diseases, "ATA associated SSc" and "ACA associated SSc", unless one postulates common risk factors for both diseases that enhance the chance of finding them together in one and the same patient.

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Authors' affiliations
T Dick, R Mierau, E Genth , Rheumaklinik u Rheumaforschungsinstitut, Aachen, Germany
P Bartz-Bazzanella, St Antonius Hospital, Eschweiler, Germany
M Alavi, Hautklinik der RWTH, Aachen, Germany
M Stoyanova-Scholz, Städtische Kliniken, Duisburg, Germany
J Kindler , Kreiskrankenhaus Würselen, Germany

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