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Quantitation of anti-RNP and anti-Sm antibodies in MCTD and SLE patients by immunoblotting

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SUMMARY

A quantitative immunoblotting assay (QIBA) for the determination of specific antibody titres in human autoimmune sera is described. In this assay, a total HeLa nuclear protein fraction, immobilized on nitrocellulose blot strips, was used as source of antigens and immunoreactive species of autoantibodies were quantitated by an enzyme linked second antibody procedure. Besides being more discriminative, QIBA appeared to be up to 500 times more sensitive than immunodiffusion or immunoelectrophoresis. In this study we used 21 sera from patients with SLE or MCTD for a quantitative analysis of their specific autoantibody content. Within this group, a very diverse spectrum of antibody populations was observed; anti-RNP sera appeared to contain, among others, high titred antibody versus 70K and 31K polypeptides while all ($n=6$) anti-Sm sera recognized a 25kD protein doublet. In a follow-up study of two MCTD patients significant flares in specific antibody content could be observed.

Keywords immunoblotting Sm/RNP SLE/MCTD quantitation

INTRODUCTION

Autoantibodies directed against intracellular (mostly nuclear) antigens are frequently found in association with rheumatic diseases (Tan, 1982). Some of these antibodies are characteristic for certain syndromes and therefore of diagnostic value. For example, anti-Sm and high titres of anti-RNP antibodies are found to be associated with systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD), respectively (Sharp *et al.*, 1972, 1976). Much effort has been made to elucidate the clinical significance of the presence of these anti-Sm or anti-RNP antibodies (Williamson, Pennebaker & Boyle, 1983, Field Munves & Schur 1983, Barada *et al.*, 1981, Sharp *et al.*, 1972, 1976) but until now no clear correlations have been found.

Attempts to elucidate the molecular composition of the Sm and RNP antigens were more successful. Both appeared to be a ribonucleoprotein complex consisting of one or more small nuclear RNA species, termed U1, U2, U4, U5 and U6, and a set of five to eight nuclear proteins (Lerner & Steitz 1979). The target for the anti-RNP antibody reaction has been shown by non-immunological isolation procedures to consist of one single RNA species (U1 snRNA) and a set of eight different nuclear polypeptides varying in mol. wt from 70,000 to 9,000 daltons (D) (Kinlaw, Robberson & Berget, 1983, Hinterberger, Pettersson & Steitz, 1983). Polypeptides 70K, A and C (of 70, 31 and 19 kD, respectively) are unique for the U1 snRNA particle and therefore it can be assumed that the U1-RNP specific epitopes are formed by at least one of these proteins. The

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other five proteins B,D,E,F and G (mol. wt 25, 13, 11, 10 and 9 kD, respectively) are also found to be associated with the U2, U4, U5 and U6 snRNA which are among the targets of the anti-Sm reaction. It is not clear yet which of these five proteins carry the Sm specific determinant(s) but the 25 kD B-B′ doublet and the 13 kD D protein are common targets when anti-Sm sera are tested in immunoblotting (White, Billings & Hoch, 1982, Billings *et al.*, 1982).

Quantitation of these antibodies has, until now, only been performed using conventional semi-quantitative techniques as counter-immunoelectrophoresis (CIE) (Field Munves & Schur, 1983; Barada *et al.*, 1981) or haemagglutination (HA) (Sharp *et al.*, 1976, 1972) which both are only capable of measuring the total amount of reactive antibody. The assay we present here has a higher discriminative power in that it measures the amount of antibody directed against each antigenic polypeptide separately. This assay makes it possible to investigate whether there are correlations between the quantity of these specific antibodies and distinct clinical features. We tested 21 MCTD and SLE sera and followed two MCTD patients in a longitudinal study for a period of 2 years.

MATERIALS AND METHODS

Patients. Most patients with MCTD or SLE were seen in the St Radboud hospital or the St Maartensclinic in Nijmegen, and diagnoses were reached as described (Habets *et al.*, 1983a, 1983b). Clinical characteristics of these patients are presented in Table 1.

Table 1. Clinical and laboratory features of patients with MCTD or SLE

Characteristics	Code	%
Polyarthralgia/polyarthritis	A	100
Raynaud's phenomenon	B	89
Swollen hands	C	58
Myalgia/myopathy	D	52
Skin rash (e.g. butterfly, sunlight sensitivity)	E	37
Fever	F	32
Myositis (elevated CPK, abnormal EMG or muscle biopsy)	G	26
Sclerodermatous changes	H	21
Vasculitis	I	21
Pleuro/pericarditis	J	16
Sjögren's syndrome	K	11
Lymphadenopathy	L	11
Renal disease	M	5
CNS involvement	N	5
Decreased pulmonary diffusion capacity	O	38 (16)*
Decreased oesophageal motility	P	35 (17)
Laboratory features		
Positive for ANA, speckled pattern		100
Positive for RNP (CIE)		68
Positive for Sm (CIE)		21
Positive for Sm+RNP (CIE)		11
Hypergammaglobulinaemia	Q	58
Leuco/thrombopenia	R	47
Positive for rheumatoid factor (Waaler–Rose, Latex)	S	42
Hypocomplementaemia	T	32
Positive for anti-dsDNA (Farr assay)	U	32

* Figures in parentheses denote number of patients tested.

Reference sera. Sera containing anti-nuclear antibodies (ANA) were selected essentially as described (Habets *et al.*, 1983b, Kurata & Tan, 1976). Reference anti-RNP, anti-Sm and anti-SS-B sera were a gift of the Centers for Disease Control, Atlanta, Georgia, USA. Anti-Scl-70 reference sera (Douvas, Achten & Tan, 1979) were a kind gift of Dr E. Penner (Vienna).

Culturing and labeling of HeLa S3 cells. HeLa S3 cells were grown in suspension cultures as described (van Eekelen & van Venrooij, 1981). They were shown to contain no Epstein–Barr or Adenovirus and regularly performed mycoplasma tests were always negative.

RNA was labelled for 48 h with 3 μCi/ml ^{3}H-uridine and 1 μCi/ml ^{3}H-cytidine at a density of $0{\cdot}5 \times 10^6$ cells/ml.

Preparation of antigenic protein fractions. Extractable nuclear antigen was prepared by a 4 h extraction at 4°C of rabbit thymus powder (Pel-Freez, Arkansas, USA) with PBS (3mM NaH_2PO_4, 7 mM Na_2HPO_4, 0·9% NaCl, pH 7·6) containing 0·5 mM PMSC (phenyl-methyl-sulphonyl chloride) to inhibit proteolytic activity followed by centrifugation for 30 min at 20,000*g* (Kurata & Tan, 1976).

Hela nuclear protein fraction for blotting analyses was prepared as described earlier (Habets *et al.*, 1983b).

Qualitative immunoblotting. Transfer of proteins from 13 or 15% polyacrylamide gels onto nitrocellulose sheets was performed as described (Habets *et al.*, 1983b). After transfer the blots were dried, cut into strips and stored at room temperature. Immediately before use additional protein

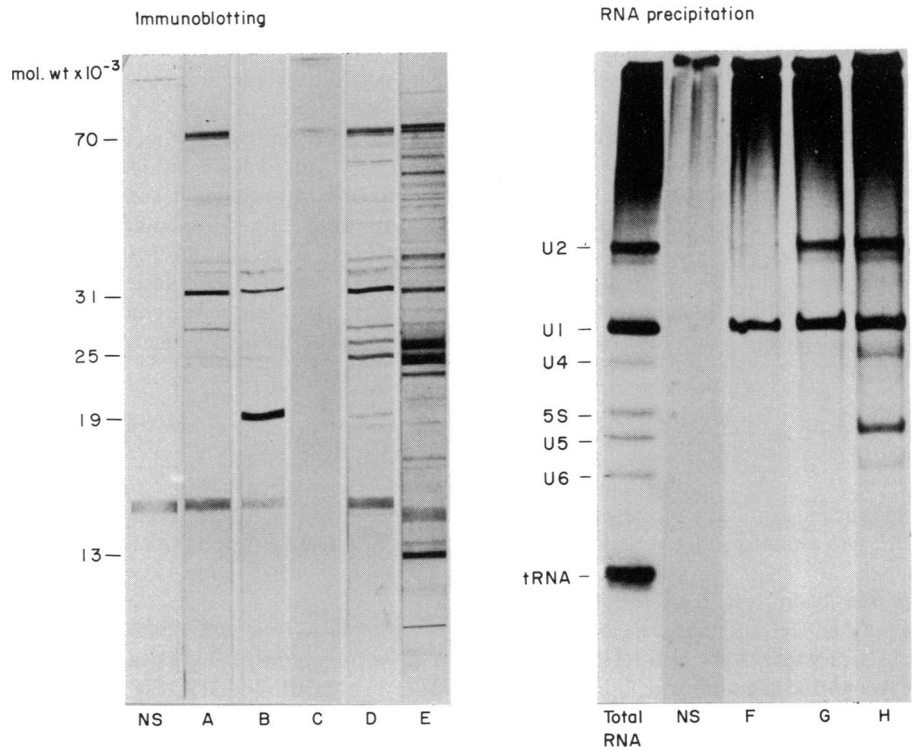

Fig. 1. Characterization of sera by qualitative immunoblotting and RNA precipitation analysis. Immunoblotting: identical strips from one HeLa nuclear protein blot were incubated with 1:100 diluted normal human serum (NS), three different 1:100 diluted human anti-RNP sera (lanes A, B and D), a 1:50 diluted monoclonal anti-70K RNP culture supernatant (White *et al.*, 1982, lane C) and a human anti-Sm serum, 1:100 diluted (lane E). RNA precipitation: polyacrylamide gel analysis of RNA precipitated from a nuclear supernatant of HeLa cells prepared as described by Hinterberger *et al.* (1983) either directly extracted with phenol (lane total RNA) or immunoprecipitated with normal human serum (lane NS), anti-(U1)RNP specific serum (lane F), anti-RNP, U1 plus U2 specific serum (lane G) and anti-Sm serum (lane H). Identity of snRNA was verified by precipitation with anti-2,2,7 trimethylguanosine specific antibody (Bringmann *et al.*, 1983).

binding sites on the strips were blocked by incubation in buffer A (3% bovine serum albumin [BSA], 350 mM NaCl, 10 mM Tris-HCl, pH 7·6) for 1 h followed by a 2 h incubation in 1 : 100 diluted serum in buffer B (0·3% BSA, 150 mM NaCl, 10 mM Tris-HCl, pH 7·6, 1% Triton X-100, 0·5% DOC and 0·1% SDS). After extensive washing (3 × 10 min) in PBS containing 0·5%. (Triton X-100, the strips were incubated for 1 h in 1 : 1,000 diluted horseradish peroxidase labelled human IgG heavy and light chain specific second antibody (Nordic, Tilburg, The Netherlands) in PBS containing 0·5% Triton X-100 and 0·5% BSA (referred to as PTB buffer) and washed again. Immune complexes on the strips were visualized by incubation in substrate solution 1 (PBS containing 0·5 mg/ml 4-chloro-1-naphtol and 0·012% H_2O_2).

Quantitative immunoblotting. Several (mostly 15) identical strips were cut from nitrocellulose blots prepared as described above. In all quantitative experiments two strips from every new sheet of nitrocellulose were always tested with the same reference serum sample as an internal standard. Specific antibody titres were determined by incubating the strips with appropriate dilutions of the test serum in buffer B for 2 h. Then the strips were washed three times for 10 min in PBS containing 0·5% Triton X-100, followed by incubation with horseradish peroxidase labelled anti-human IgG diluted 1 : 1,000 in PTB buffer. The antigen–antibody complexes formed on the strip with the highest serum concentration were visualized by a 2 min incubation in substrate solution 1. The corresponding regions on the other strips were then cut out and incubated separately in 0·5 ml substrate solution 2 (50 mM PO_4 buffer pH 6·0 containing 0·8 mg/ml 5-aminosalicylic acid and 0·024% H_2O_2) in a 24 well multidish (NUNC). After 30 min the extinction at 492 nm (E492) of this substrate solution was determined in a Titertek multiscan. From the results obtained with the reference anti-RNP serum a standard dose–response curve for each of the specific antibody populations was established (see Results section). Shapes of the dose–response curves of 10 patients sera tested, closely fitted with the standard curves. Therefore, binding values for other sera were read directly from these standard curves and expressed in arbitrary units (AU) that we define here as the product of the multiplication factor (MF) read from the standard curve and the corresponding dilution factor. The multiplication factors in the standard curves are chosen in such a way that the standard anti-RNP serum contains 100 AU/ml of all three of the antibody populations measured (see also Fig. 2).

For the longitudinal studies all the serum samples from one patient were tested with identical blot strips originating from one transfer to ensure optimal reproducibility. As a consequence of this modification, standard deviations never exceeded 15% as was established by incubation of a 1 : 500 diluted serum sample with 10 identical blot strips.

RESULTS

The specific autoantibody composition of 21 anti-Sm and/or anti-RNP sera, as such characterized by CIE, was established by qualitative immunoblotting and RNA precipitation analysis.

Qualitative laboratory parameters

With purified HeLa S3 nuclei as antigen source a remarkable diversity was observed between the various sera tested. Most anti-RNP sera (11 out of 13) recognized a 70,000 D antigen and six of these recognized in addition an antigenic polypeptide of 31 kD (70K and 31K, Fig. 1, lane A). Two sera recognized exclusively the 31K and 19K proteins called A and C in the Steitz nomenclature (Lerner & Steitz, 1979, Fig. 1, lane B). Eight sera showed a (weak) reaction with a 25K antigen doublet identical with the B-B′ protein doublet of the Steitz nomenclature (Fig. 1, lane D). The six anti-Sm sera tested, on the other hand, recognized this doublet as most prominent antigens (Fig. 1, lane E). As a reference also the blot pattern of a monoclonal anti-RNP serum is shown (Fig. 1, lane C, Billings *et al.*, 1982).

The possibility that this multiple band pattern is caused by cross-reaction of one antibody type with more than one of these polypeptides can be ruled out, because antibodies eluted from one polypeptide band do not react with one of the other bands as was shown by Guldner, Lakomek & Bautz (1983) and confirmed by us (data not shown). Moreover, the monoclonal anti-70K antibody

exclusively recognizes the 70,000 D antigen. Therefore it is clear that at least the 70K,A,B and C antigen bands represent distinct polypeptides and are not breakdown products of each other as occasionally has been suggested (White *et al.*, 1982, Wooley, Zukerberg & Chung, 1983).

A comparable heterogeneity as described above for the protein antigens was also observed when the results from RNA precipitation analysis of the 21 anti-Sm and anti-RNP sera were compared. Within the anti-RNP group ($n=13$) three reaction types were observed: exclusively anti-U1 ($n=6$, Fig. 1, lane F, anti-U1 plus U2 ($n=1$, Fig. 1, lane G) and anti-U1,2,4,5,6 ($n=6$, Fig. 1, lane H). Anti-Sm sera ($n=6$) or anti-RNP plus anti-Sm sera ($n=2$) all precipitated U1,2,4,5,6 (see also Table 2).

Sensitivity of immunoblotting

One major advantage of immunoblotting lies in its ability to detect insoluble antigens, as is illustrated by the recognition of the mol. wt 70,000 nuclear matrix associated protein (Salden *et al.*, 1982, Habets *et al.*, 1983b, Fritzler, Ali & Tan, 1984). Antigens from this nuclear substructure cannot be detected in double diffusion assays as has been shown previously (Habets *et al.*, 1983a).

The detection level of the immunoblotting assay was determined as follows. Varying amounts of human IgG were spotted directly onto small nitrocellulose squares. The total amount of protein was immediately bound as was verified by control experiments with ^{125}I-labelled human IgG. After incubation with goat anti-human IgG, bound IgG was quantitated as described in the Materials and Methods section using 5-amino salicylic acid as substrate. When performed in this way, amounts of 8 ng IgG could easily be detected.

Standard serum curves

We have chosen for the quantitation of antibodies versus the 70K and 31K (U1)RNP associated antigens and the 25K doublet, the most prominent target for the anti-Sm antibodies. These antibodies are of IgG class, and no reaction could be detected when peroxidase linked anti-IgA, IgM or IgD was used instead of anti-IgG as the second antibody.

Three separate dose–response curves were produced for the antibodies versus the 70K, 31K and 25K antigens by incubating eight identical nitrocellulose strips, prepared as described in the Materials and Methods section, with serial dilutions of a standard serum. The amount of antibody bound to one distinct polypeptide band was then quantitated by an incubation with peroxidase

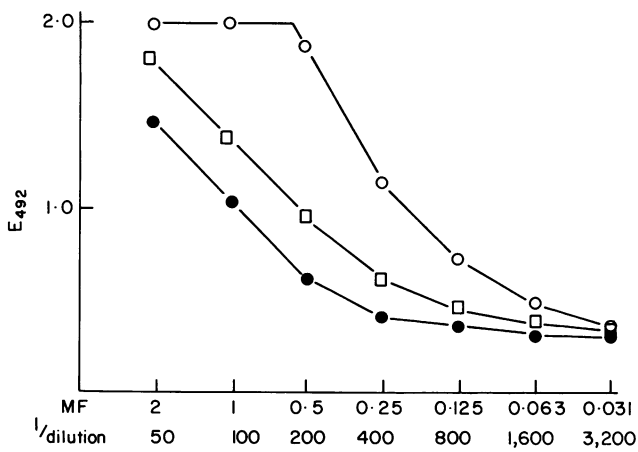

Fig. 2. Standard dose–response curves of three different antibody populations (anti-70K = ○; anti-31K = ●; anti-25K = □) in an anti-RNP reference serum. Seven identical protein blot strips were probed with serial dilutions of an anti-RNP reference serum, followed by excision of the regions at mol. wt 70,000, 31,000 and 28,000 D. Bound IgG on these fragments were quantitated as described in the Materials and Methods section. MF = multiplication factor. See text for details.

Table 2. Clinical and immunoblotting data from individual patients

Patient	Diagnosis	Clinical features*																			CIE			Quantitative immunoblotting†		
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	RNP	Sm	RNA-Prec.	Anti-70K	Anti-31K	Anti-25K
A1	MCTD	A	B	C					H	I						O	P	Q					+		U1	48	200	36
D1	MCTD	A	B	C	D	E	F	G		I		K				O		Q	R		T	U	+		U1	100	150	120
G15	MCTD	A	B	C													P						+		U1	8	50	10
K3	MCTD	A	B	C								K						Q	R	S			+		U1	60	160	40
K4	MCTD	A	B	C	D																		+		U1	16	36	24
T2	MCTD	A	B	C	D			G	H							O							+		U1	32	100	20
S2	MCTD	A	B	C					H										R	S			+		U1+U2	95	84	48
C3	MCTD	A	B		D	E														S			+		U1–U6‡	48	500	150
D4	MCTD	A	B			E	F								N					S			+		U1–U6	60	230	50
H13	MCTD	A	B	C	D			G										Q	R	S	T		+		U1–U6	120	90	60
S3	MCTD	A	B		D	E	F											Q		S			+		U1–U6	97	110	240
W1	MCTD	A	B	C	D											O	P	Q	R	S			+		U1–U6	400	72	46
W2	MCTD	A	B		D		F	G									P	Q					+		U1–U6	28	100	40
B16	SLE/MCTD	A	B		D		F				J						P	Q	R		T	U	+	+	U1–U6	450	1,000	400
D2	MCTD	A	B	C	D			G	H	I	J		L					Q				U	+	+	U1–U6	300	500	300
C12	SLE	A	B			E	F			I								Q	R	S	T	U		+	U1–U6	82	200	520
H11	SLE	A				E										O					T	U		+	U1–U6	14	120	600
M13	SLE	A	B	C												O	P		R					+	U1–U6	17	280	108
R11	SLE	A				E					J		L	M				Q	R		T	U		+	U1–U6	14	104	100
CDC REF Sm§																								+	U1–U6	70	350	> 1,600
A'DAM REF Sm§																								+	U1–U6	30	300	600
Control sera																												
NS ($n=10$)																							NEG	NEG	NEG	< 11	< 35	< 20
SS-B ($n=10$)																							NEG	NEG	ND¶	< 19	< 40	< 20
Scl-70 ($n=10$)																							NEG	NEG	ND	< 20	< 30	< 20

* In code, see Table 1.
† In arbitrary units.
‡ U1–U6 denotes the precipitation of U1, U2, U4, U5 and U6 snRNPs.
§ No clinical data available.
¶ ND = not determined.

linked human IgG specific second antibody, followed by excision of the antigen band concerned, and incubation of this fragment in a substrate solution (see Materials and Methods). The colorimetric change at 492 nm of this substrate solution after 30 min appeared to be a measure for the amount of anti-70K, 31K and 25K antibody in the diluted serum sample (Fig. 2). As a consequence standard curves can be used for the quantitation of these antibodies in various patients sera.

Patients sera

We quantitated the specific antibody content of 51 different sera (21 anti-RNP or anti-Sm, 10 anti-SS-B, 10 anti-Scl-70 and 10 normal human sera). From triplicate analysis of every serum sample the average anti-70K, 31K and 25K antibody content was calculated and expressed in AU. Significant differences in antibody content could be observed between the sera tested; for the antibodies against the 70K protein, none of the SS-B, Scl-70 or normal human sera showed binding values above 20 AU/ml, while from the anti-RNP group only two sera contained less than 20 AU/ml of anti-70K antibody. These two sera decorated exclusively the 31K and the 19K band in the qualitative immunoblotting procedure (see Fig. 1, lane D). Normal binding values for the 31K protein are somewhat higher than for the 70K protein, but the group of anti-Sm and anti-RNP sera had much higher antibody titres against this antigen when compared to the anti-SS-B/anti-Scl-70/ normal control group. High anti-25K activity was mainly found in the anti-Sm group.

Other laboratory parameters such as RNA precipitation analysis from the anti-Sm/RNP group are presented in Table 2 together with some clinical features.

Longitudinal studies

In a follow-up study we determined the titres of three distinct antibody populations in sera from 2 MCTD patients collected during a period of 2 years. Therefore every serum sample was analysed in duplicate with identical protein blot strips at three different dilutions chosen in an appropriate range. Binding values were read directly from the standard curves (see Materials and Methods). The fluctuations of the separate antibody populations, expressed in arbitrary units, are shown in Figs 3 & 4.

Patient K3, a 77 year old woman had since 1974 episodes of Raynauds phenomenon, diffuse swelling of the hands, polyarthralgia, leucocytopenia and thrombocytopenia. During follow-up most symptoms subsided, Raynauds phenomenon and arthralgia remained. All three antibody populations were present in low titres and showed a rather stable course during follow-up as is shown in Fig. 3.

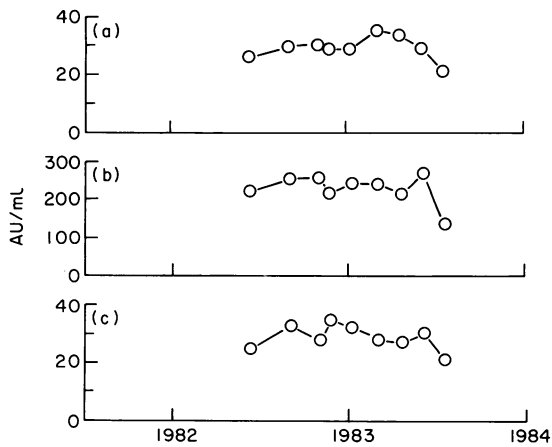

Fig. 3. Longitudinal anti-70K (a), 31K (b) and 25K (c) antibody profiles of MCTD patient K3. AU = arbitrary units.

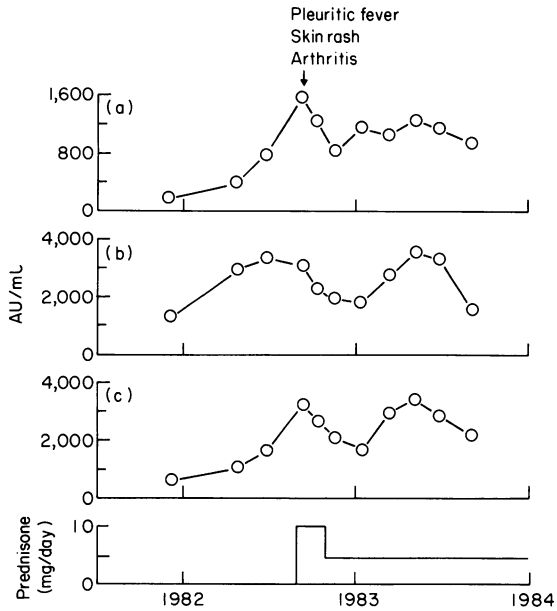

Fig. 4. Longitudinal anti-70K (a), 31K (b) and 25K (c) antibody profiles of MCTD patient D2.

Patient D2, a 32 year old woman developed in 1980 Raynauds phenomenon, polyarthritis, swollen hands and severe polymyositis treated with high doses of prednisone. At the start of follow-up myositis symptoms subsided; mid-1982 she had a flare in disease activity with arthritis, pleuritis, fever, Raynauds phenomenon and cutaneous vasculitis. Antibody populations were present in rather high titres and profiles showed a fluctuating course with two peaks during the period of follow-up (Fig. 4), from which the first coincided with a flare in disease activity. Treatment with low dose of prednisone had a stabilizing effect on her symptoms and all three antibody profiles showed a decline after start of medication. While the patient was still under treatment with prednisone, the second peak in the antibody profile was not accompanied by a clinical flare. Fluctuations in the antibody profiles were not reflected in total serum IgG patterns and could not be detected by CIE (data not shown).

DISCUSSION

The purpose of our investigation is to establish a possible correlation between the presence and titre of autoantibodies and clinical features of patients with rheumatic diseases. We therefore developed a QIBA as described in this study. From a group of 300 anti-nuclear antibody containing sera we selected 21 with antibodies to Sm, RNP or both, using CIE and immunodiffusion (ID). Almost all patients from which these sera were obtained were diagnosed by one of us (DJdeR) and their files were screened for the clinical features shown in Table 1.

It has recently become clear that the anti-Sm and anti-RNP reaction is directed against several distinct nuclear polypeptides, all complexed *in vivo* in RNA–protein particles (Hinterberger *et al.*, 1983; Kinlaw *et al.*, 1983). When tested by qualitative immunoblotting, all anti-RNP sera recognized two to four different antigenic polypeptides with mol. wt of 70, 31, 25 and 19 kD. All anti-Sm sera ($n=6$) contained antibodies against a 25kD doublet and five recognized also a 13K single polypeptide. With the QIBA we were able to quantitate antibody populations against each of these antigenic polypeptides separately. Other laboratory parameters were determined as well using

conventional techniques as CIE or ID (for a review see Tan, 1982) and the more recently described RNA precipitation technique (Lerner & Steitz, 1979, Fisher *et al.*, 1983).

Anti-RNP and anti-Sm specificity as established by CIE correlated with the diagnosis of MCTD and SLE, respectively. Among the 13 anti-RNP sera however, only six appeared to be exclusively anti-(U1)RNP, while one showed anti-U1 plus U2 activity, a finding not reported earlier in MCTD. The remaining six anti-RNP sera also contained some anti-U2,4,5,6 activity which correlated in five cases with elevated (> 40 AU/ml) anti-25K levels in QIBA. These samples were negative for anti-Sm antibodies in CIE and immunodiffusion, using RNAase digested extractable nuclear antigen from rabbit thymus. Presence of low titres of anti-Sm antibodies in MCTD has been reported earlier (Williamson *et al.*, 1982) using haemagglutination (HA) techniques. In this respect one should realize that the association of anti-RNP and anti-Sm antibodies with MCTD and SLE, respectively, has been established using conventional techniques as CIE or ID, and that this concept might need some reconsideration now that more sophisticated and more sensitive techniques have become available.

The antigen of 70,000 D (70K) seems to be a triplet of bands (see Fig. 1) reactive with patients sera as well as with the monoclonal anti-70K antibody. Whether this represents a genuine *in vivo* situation or is a result of *in vitro* modification of the antigen is subject of present investigations. Considerable variations in anti-70K activity (from 8 to 400 AU/ml) were observed within our population of 21 patients, but high levels were mainly found in MCTD patients (see Habets *et al.*, 1983b). High anti-25K activity (> 100 AU/ml) was always found in sera exhibiting anti-Sm activity in CIE, while high anti-31K activity was found in SLE as well as in MCTD patients. Anti-(U1)RNP was found equally distributed in mild and severe cases of MCTD, this in contrast with the findings of Assens *et al.* (1982) who reported a correlation between presence of these antibodies and a mild course of disease.

Fisher *et al.* (1983) have suggested that some antibodies in anti-Sm and anti-RNP sera could be directed against SDS sensitive epitopes. Since immunoblotting is based on SDS-PAGE, at least some of these sites are destroyed during preparation of the blot strips. As a consequence it might be possible that the lack of any apparent correlation between distinct clinical features and levels of antibodies measured in QIBA is caused by this phenomenon.

To investigate whether such a correlation would exist within the same patient we followed two patients with MCTD in a longitudinal study. One of them, having a stable course of disease showed almost no significant fluctuations (see Fig. 3) with respect to the three antibody populations measured. Antibody profiles of the other, clinically unstable patient, showed two peaks from which one coincided with a flare in disease activity that was treated with low dose of prednisone. Thereafter, while still on medication, the second peak in the antibody profiles did not correlate with a recognizable change in clinical symptoms. Most interesting was the observation that profiles of the anti-70K, anti-31K as well as anti-25K antibodies appeared to be of an identical shape which could point towards a coupled mechanism of autoantibody production.

The unique aspect of our study is the separate quantitative determination of anti-70K, 31K and 25K antibodies in anti-RNP and anti-Sm sera and the possibility of measuring titres of these antibodies in longitudinal studies. Our results with a group of 21 patients with anti-Sm and/or anti-RNP antibodies show a great quantitative diversity in the subpopulations of these antibodies which together are responsible for the Sm or the RNP precipitation reaction. The three subpopulations of antibodies we investigated showed no apparent correlation with diagnosis or clinical parameters but peaks in longitudinal antibody profiles were found to correlate with a flare in disease activity in one patient. This finding might lend support to the concept that anti-Sm and anti-RNP antibodies play a pathophysiological role in systemic diseases. In this respect clinically useful data might be obtained by quantitating anti-Sm and anti-RNP antibody subpopulations. The quantitative immunoblotting described here is to our knowledge the only technique sufficiently sensitive for such an analysis. Performed on a large scale, QIBA therefore might be an aid in the management of systemic diseases.

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Vienna, Austria, for 10 anti-Scl-70 sera, Dr J. Festen, Hospital Stadsmaten, Enschede and Dr P. Houtman, University Hospital, Groningen, The Netherlands for patients sera. We also thank Dr R. de Waal for the quantitative determination of total serum IgG, A. Groeneveld for excellent technical assistance, Dr P. Faaber for helpful advice and Dr J. Leunissen for the development of computer programs for statistical analyses. This work was supported in part by grants from the Netherlands League against Rheumatism; WJH was a recipient of a grant from the 'Praeventiefonds', The Hague.

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