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Recognition of synthetic peptides of Sm-D autoantigen by lupus sera

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SUMMARY

The reactivity of autoantibodies present in the serum of patients with systemic lupus erythematosus (SLE) was investigated by ELISA using seven overlapping synthetic peptides representing the entire sequence of the polypeptide D component of 'Sm antigen'. Of the 165 SLE sera tested, 59% were found to contain IgG antibodies able to bind to peptide 1–20, while 37% of the sera reacted with peptide 44–67. All sera reacting with peptide 44–67 also reacted with peptide 1–20. These two peptides were only seldom recognized by the sera of 187 patients with other rheumatic autoimmune diseases or by 53 sera of normal individuals. In a parallel study using sera that reacted with the D band in immunoblotting, most of the sera recognized peptides 44–67 (89%) and 1–20 (67%), while 33% of them reacted with peptide 97–119. The use of these synthetic peptides in ELISA may be of considerable help for detecting anti Sm autoantibodies.

Keywords Sm-D polypeptide synthetic peptides systemic lupus erythematosus ELISA

INTRODUCTION

The presence of autoantibodies to cell components, especially to nuclear constituents is a general feature of systemic rheumatic diseases such as systemic lupus erythematosus (SLE), scleroderma, polymyositis and mixed-connective tissue disease (MCTD) (Morrow & Isenberg, 1987; Tan *et al.*, 1988). Of the many types of autoantibodies found in these diseases, those reacting with 'Sm antigen(s)' represent one of the most useful markers since they are found in 20–30% of all SLE patients but very rarely in patients with other connective tissue diseases.

Anti-Sm antibody originally described by Tan & Kunkel (1966) is usually found together with other antibodies such as those that bind to RNP. Anti-Sm and anti-RNP antibodies react with the protein moiety of the snRNP particles containing the snRNAs U1, U2, U4, U5 and U6 (Lerner & Steitz, 1979; Brunel, Sri-Widada & Jeantour, 1985). Whereas anti-RNP antibodies precipitate U1 RNPs only, anti-Sm antibodies react with U1, U2, U4, U5 and U6 snRNPs. Seven proteins were found to be present in each of the individual U1, U2, U4, U5 and U6 snRNPs. According to their electrophoretic mobility on polyacrylamide gels, they have been labelled bands B′ (29 kD), B (28 kD), D (16 kD), D′ (15·5 kD), E (12 kD), F (11 kD) and G (9 kD). In addition to these polypeptides that constitute a common core, U1 RNPs contain three unique polypeptides with a size of 70 kD, 34 kD (A) and 22 kD (C). U2 RNPs contain two unique polypeptides denoted A′ (33 kD) and B″ (28·5 kD). Immunoblotting studies have shown that anti-U1 RNP antibodies react with polypeptides A, C and 70 kD while anti-Sm antibodies react with polypeptides B′, B and D. In addition, bands E, F and G are also sometimes recognized by anti-Sm antibodies (Pettersson *et al.*, 1984; Reichlin & Harley, 1987; Hoch, 1989; Combe *et al.*, 1989).

In the cell, the snRNPs appear to play an essential role in the processing of nuclear pre-mRNAs and it is known that anti-RNP and anti-Sm autoantibodies are able to inhibit the *in vitro* splicing reaction. The sequence of several snRNP polypeptides has been established by recombinant DNA technology (Theissen *et al.*, 1986; Habets *et al.*, 1987; Stanford *et al.*, 1987; Sillekens *et al.*, 1987, 1989; Yamamoto *et al.*, 1988; Rokeach *et al.*, 1989). Recently, Rokeach, Haselby & Hoch, (1988) isolated a cDNA clone coding for the Sm-D human nuclear antigen. This gene codes for a polypeptide of 119 residues containing very basic regions and the authors predicted that a repeated motif (Gly-Arg)9 located at the C-terminus was likely to be a potential immunoreactive determinant of Sm-D. The deduced amino acid sequence showed no similarities with other snRNP antigens sequenced so far. However, a good homology to protamines (68% homology and 42% identity) and to a region in the Epstein–Barr nuclear antigen EBNA-1 (79% homology and 63% identity) was found in the domains 89–119 and 96–119 of Sm-D, respectively.

The ability of synthetic peptides encompassing the entire Sm-D sequence to be recognized by sera of autoimmune patients was investigated in the present study. Sera from 165 patients with SLE and 187 patients with other rheumatic autoimmune

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diseases were tested in ELISA against seven overlapping peptides corresponding to the sequence established by Rokeach *et al.* (1988). Of the 165 SLE sera tested, 59% contained IgG antibodies that reacted with peptide 1–20, and 37% of the sera reacted with peptide 44–67. These two fragments were only rarely recognized by the sera from patients with other rheumatic autoimmune diseases ($< 6\%$). The other five peptides of Sm-D were not recognized by any of the patient sera. Using a panel of sera reacting in immunoblotting with the D-band we showed that 89% of them also reacted in ELISA with peptide 44–67 while 67% of the sera reacted with peptide 1–20.

MATERIALS AND METHODS

Synthetic peptides

Assembly of the protected peptide chains was carried out using the step-wise solid-phase method of Merrifield (1963) on a NPS 4000 multi-channel peptide synthesizer (Neosystem Laboratoires, Strasbourg, France). All amino groups were protected at the α amino position with the Boc group. The following side chain protecting groups were used: cyclohexyl ester (Asp, Glu), benzyl ether (Ser, Thr), 2-chlorobenzyloxycarbonyl (Lys), 2,6-dichlorobenzyl (Tyr), *p*-toluenesulfonyl (Arg, His). Methionine was introduced in the form of Boc Met sulfoxide (*o*) which was reduced to methionine at the final cleavage of the peptide from the resin by the Low-High HF procedure (Tam, Heath & Merrifield, 1983). Boc–AA–PAM resins (0·2 mmol) were placed in each reaction vessel. The cycle used for incorporation of Boc amino acids has been described elsewhere (Plaué & Briand, 1988). Boc amino acids were coupled in the form of their benzotriazole ester, except Boc His (Tos) which was introduced as a symmetrical anhydride. The total coupling time was 45 min. Monitoring with ninhydrin test (Kaiser *et al.*, 1970) showed that most of the couplings were complete within this time. If necessary a double coupling was carried out.

At the end of the synthesis and after the last deprotection step, the resins were washed with ethyl ether and dried under vacuum. Depending on the presence of Met (*o*) in the sequence, high or low-high HF procedures were used for deprotection and cleavage from the resin. After lyophilization each crude synthetic peptide was dissolved in 10% acetic acid and purified using a middle pressure chromatography apparatus (Kronwald Separation Technology, Sinsheim, FRG) on a glass column filled up with RP100 material (C18, 20–45 μm). The final purity of each peptide was controlled by an analytical run on a reverse phase aquapure RP300 C8 column, 5 μm (4·6 × 250 mm) using programmed gradient elution (2 ml/min^{-1}) with the following solvents: A, 0·1 M triethylammonium phosphate (TEAP) pH 2·5; B, acetonitrile. Amino acid composition and net peptide content were determined with a Waters Picotag system (Waters Corporation, Milford, MA).

Antisera to synthetic peptides of Sm-D

Antisera to the seven unconjugated peptides of Sm-D were raised in rabbits as previously described for histone peptides (Muller *et al.*, 1986). For each injection, rabbits received 100 μg of peptide in saline solution in the presence of Freund's complete adjuvant (v/v). A series of bi-weekly subcutaneous injections were administered (two rabbits/peptide) during 4 months. After three injections, the rabbits were bled 1 week after each injection and the antibody level was measured in ELISA.

Human sera and monoclonal antibodies

Sera from 165 patients with active and quiescent SLE were tested. Many of these sera had previously been analysed with other antigens such as histone and histone peptides, native DNA and ubiquitin (Muller, Briand & van Regenmortel, 1988; Muller *et al.*, 1989; Plaué, Muller & van Regenmortel, 1989). Their activity was compared with that of sera from 32 patients with scleroderma, 14 patients with MCTD, two with polymyositis, 13 with sarcoidosis, five with Sjögren's syndrome, 35 with rheumatoid arthritis (RA) and 86 with juvenile chronic arthritis (JCA). As control, 53 sera from normal healthy volunteers were used. Eighteen human sera and five mouse monoclonal antibodies known to recognize specific Sm bands in immunoblotting were also used.

ELISA

The following ELISA procedure was used to measure the binding of antibodies from human sera: microtitre plates (Falcon, Ref. 3912) were coated at 37°C with 1–2 μM of each peptide in solution in 0·05 M carbonate buffer, pH 9·6. Blocking of remaining sites on the plastic was achieved by incubation for 1 h at 37°C with 10 mg/ml bovine serum albumin (BSA) in phosphate-buffered saline (PBS), pH 7·4, containing 0·05% Tween 20 (PBS-T-BSA). After three washings of microtitre plates with PBS-T, patient sera diluted 1/1000 in PBS-T-BSA were added for 1 h at 37°C. After repeated washings, positive reactions were detected by successive incubations with a biotin conjugate specific for human IgG (Amersham, UK, Ref. RPN 1186) for 1 h at 37°C and with a streptavidin peroxidase conjugate (Amersham, Ref. RPN 1231) also for 1 h at 37°C. The final reaction was visualized by addition of the peroxidase substrate 2,2′-azinobis (3-ethylbenzthiazoline sulfonate) (ABTS) for 1 h at 37°C. OD values were measured at 405 nm. All reagents contained 0·01% thimerosal. Antibodies to DNA were detected as previously described (Muller *et al.*, 1988), using patient sera diluted 1/500 in PBS-T-BSA.

In order to determine the cut-off line of the assay, a series of 53 sera from normal individuals was tested with peptides 1–20 and 44–67 (see below). Using sera diluted 1/1000 and plates coated with respectively 1 μM and 2 μM of the two peptides, the average absorbance was 0·10 (s.d. 0·084). Sera were considered positive when the OD values were higher than this average OD value + 2 s.d., i.e. about 0·3 OD unit. When this threshold value was used, 1·8% of normal sera were found positive with both peptides 1–20 and 44–67 (OD range 0·30–0·33 and 0·30–0·53, respectively).

The ELISA procedure used to measure the binding of rabbit antibodies to Sm-D peptides was similar to that described above except that the reaction was revealed by addition of goat anti-rabbit immunoglobulins conjugated to horseradish peroxidase (Jackson, Ref. 111-035-003) for 1 h followed by peroxidase substrate ABTS. For testing monoclonal antibodies, culture supernatants of hybridomas were diluted 1/50 in PBS-T-BSA and allowed to react with the various peptides. The reaction was revealed by successive addition of rabbit anti-mouse immunoglobulins (Nordic, Tilburg, The Netherlands) and goat anti-rabbit immunoglobulins conjugated to peroxidase as described above.

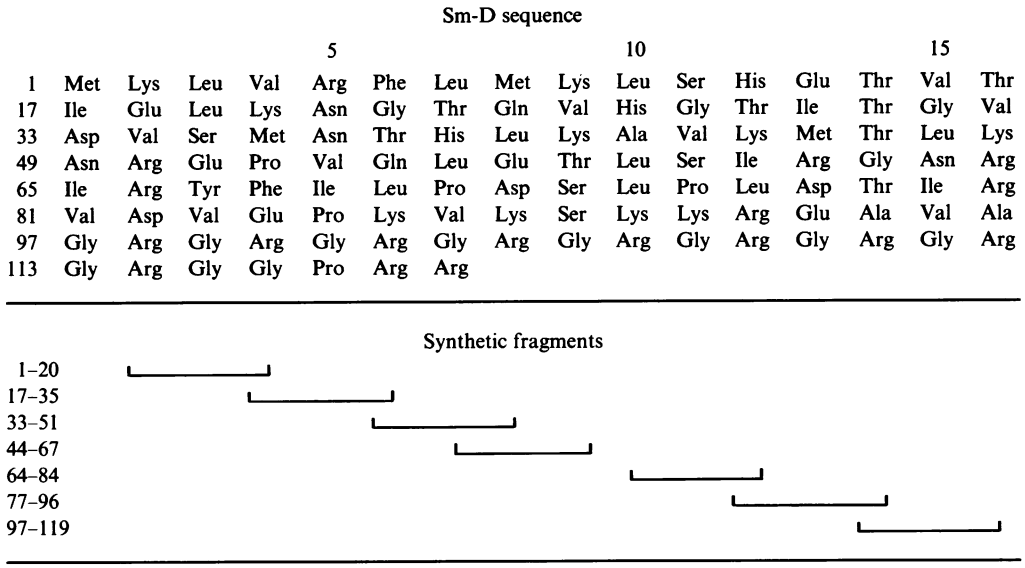

Fig. 1. Predicted sequence of Sm-D (from Rokeach *et al.*, 1988) and sequence of synthetic fragments tested for Sm-D epitope mapping.

RESULTS

Synthetic peptides of Sm-D

From the Sm-D sequence described by Rokeach *et al.* (1988), seven peptides corresponding to residues 1–20, 17–35, 33–51, 44–67, 64–84, 77–96 and 97–119 were selected for synthesis (Fig. 1). These seven peptides of a length of 19–24 residues spanned the entire Sm-D molecule. The peptide 97–119 corresponds to the region predicted by Rokeach *et al.* (1988) to be antigenic. The degree of purity of the peptides assessed by HPLC was at least 85%. The net peptide content was used as a basis for molarity calculation. Amino acid analysis of two samples of each peptide showed that the purified products had the expected composition (data not shown).

Study of SLE sera recognizing the D band in immunoblotting

Eighteen SLE sera that reacted with the D band in immunoblotting were kindly provided by Drs J. Cohen (Reims, France), F. Danon (Paris), R. Lührmann (Marburg, FRG) and E. Penner (Vienna). They were tested in ELISA against the seven Sm-D peptides. The investigation was restricted to the IgG anti-Sm response (Eisenberg *et al.*, 1985). Of these sera, 12 (67%) were found to react with peptide 1–20 ($\overline{OD}$ 0·47, s.d. 0·15; OD range 0·30–0·81), 16 (89%) with peptide 44–67 ($\overline{OD}$ 0·57, s.d. 0·25; OD range 0·30–1·20) and six (33%) with peptide 97–119 ($\overline{OD}$ 0·46, s.d. 0·16; OD range 0·30–0·73). All sera positive with peptide 1–20 and/or peptide 97–119 also reacted with peptide 44–67. None of the sera reacted with peptides 17–35, 33–51, 64–84 and 77–96. It seems therefore that most sera reacting in immunoblotting with the D band can be identified by their reactivity in ELISA with a single peptide, i.e. peptide 44–67.

Five murine monoclonal autoantibodies known to react with certain Sm bands in immunoblotting were tested with Sm-D peptides. These are clone H126 which reacts with bands B′, B and D (Reuter & Lührmann, 1986), clone Y12 which reacts with B′, B, D and E (Pettersson *et al.*, 1984), clone H57 which reacts with B′ and B (R. Lührmann, personal communication) and clones 2–73 (anti-U1 RNP) and 7–13 (anti-Sm) which bind to polypeptide D (Billings *et al.*, 1982; Billings, Barton & Hoch, 1985). Both antibodies 2–73 and 7–13 (with supernatants diluted 1/50) were found to react with peptide 97–119 in ELISA (OD at 405 nm, respectively, 1·5 and 2·0, after 60 min substrate

Table 1. Reactivity in ELISA of autoimmune sera with peptides of Sm-D

Sera source	Patients (*n*)	Peptides 1–20	Peptides 44–67
SLE	165	58·8%	37·0%
Scleroderma	32	6·3	0
MCTD	14	0	0
Polymyositis	2	0	0
Sarcoidosis	13	0	0
Sjögren Syndrome	5	0	0
RA	35	5·7	5·7
JCA	86	1·2	1·2
Normal human	53	1·8	1·8

Sera from patients were diluted 1/1000 and allowed to react with 1 μM of peptide 1–20 or 2 μM of peptide 44–67 adsorbed to the plastic plate. Values are expressed as % positive sera among total tested sera (*n*). The sera were considered positive when the OD values were ≥0·3 (mean OD for 53 normal sera +2 s.d.) after 1 h of incubation of the anti-human IgG enzyme conjugate with the substrate. Peptides 17–35, 33–51, 64–84 and 77–96 were not recognized by any of the patient sera. Peptide 97–119 was recognized by 1·2% of SLE sera. Tests were repeated at least twice (some of them up to five times) in independent ELISA experiments.

MCTD, mixed connective tissue disease; RA, rheumatoid arthritis; JCA, juvenile chronic arthritis.

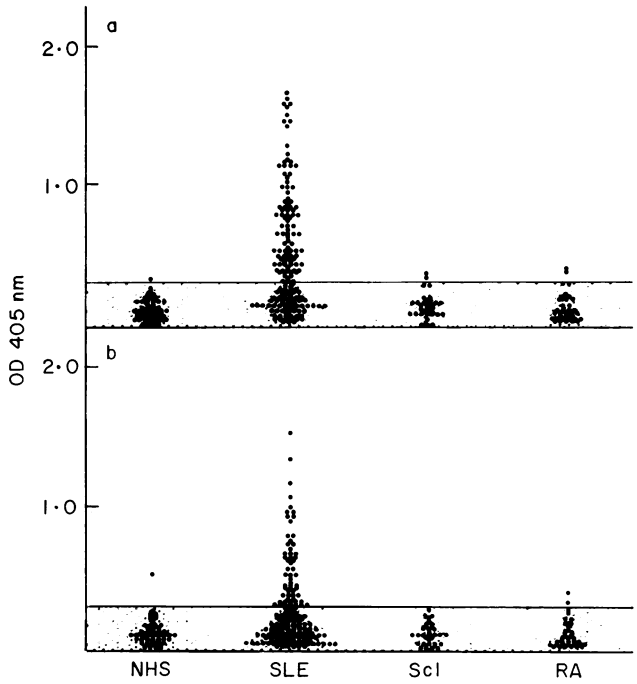

Fig. 2. Binding in ELISA of peptides 1–20 (a) and 44–67 (b) of Sm-D. Anti-peptide IgG activity was measured in 53 normal human sera (NHS), 165 SLE sera, 32 scleroderma (Scl) and 35 RA sera. All sera were diluted 1:1000. Antibody levels are expressed in OD units at 405 nm after substrate hydrolysis time of 60 min. The cut-off line corresponds to the upper limit of a normal population corresponding to the average OD of 53 NHS (0·100) plus 2 s.d.

Table 2. Reactivity of 165 SLE sera in ELISA with dsDNA and peptides 1–20 and 44–67 of Sm-D

	Sera (*n*)	Frequency (%)
DNA^+ peptide $1-20^+$	62	37·6
DNA^+ peptide $1-20^-$	26	15·8
DNA^- peptide $1-20^+$	36	21·8
DNA^- peptide $1-20^-$	41	24·9
DNA^+ peptide $44-67^+$	42	25·5
DNA^+ peptide $44-67^-$	50	30·3
DNA^- peptide $44-67^+$	19	11·5
DNA^- peptide $44-67^-$	54	32·7

incubation). The other three monoclonal antibodies did not react with Sm-D peptides.

Study in ELISA of sera from patients with SLE and other rheumatic diseases

Sera from 165 patients with SLE and 187 patients with other rheumatic diseases were tested in ELISA for the presence of antibodies able to react with the seven synthetic peptides of Sm-D (Table 1, Fig. 2). Of the 165 patients with known SLE, 59% possessed antibodies able to react with peptide 1–20 (OD range 0·30–1·67; OD 0·77) while 37·0% of them possessed antibodies reacting with peptide 44–67 (OD range 0·30–1·55; OD 0·58).

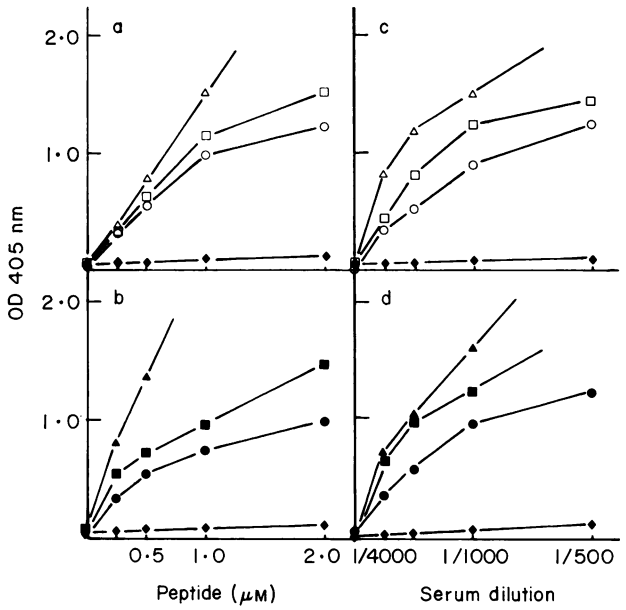

Fig. 3. Binding in ELISA of six SLE sera (●, ■, ▲, ○, □, △) and one normal human serum (◆) to peptides 1–20 (a, c) and 44–67 (b, d) of Sm-D. a, b, patient sera diluted 1/1000 and allowed to react with various concentrations of peptide; c, d, various dilutions of patient sera were used with, respectively, 1 and 2 μM of the peptide 1–20 and 44–67. Absorbance values were measured after hydrolysis time of 60 min.

Two sera out of 165 tested (1·2%) reacted with peptide 97–119 (Table 1). None of the SLE sera showed a reaction with peptides 17–35, 33–51, 64–84 and 77–96. Peptides 1–20 and 44–67 were rarely recognized by antibodies from patient sera with other rheumatic diseases (Table 1, Fig. 2). Six percent of RA sera reacted with one of two peptides (with OD ranges 0·30–0·39 and 0·30–0·41, respectively, for peptides 1–20 and 44–67). Only 6% of scleroderma sera reacted with peptide 1–20 (OD range 0·30–0·36), and none reacted with peptide 44–67. When the threshold for considering a serum positive with peptide 1–20 was a cut-off line corresponding to mean OD for normal human serum +5 s.d. (instead of 2 s.d.) the test was found to be totally specific for SLE. In these conditions 51% of SLE reacted with peptide 1–20.

Since all SLE sera positive with peptide 44–67 were also positive with peptide 1–20, it follows that in using peptide 1–20 in ELISA, close to 60% of SLE sera can be identified. The two sera positive with peptide 97–119 also contained antibodies reacting with peptides 1–20 and 44–67 of Sm-D. Binding of autoantibodies to peptides 1–20 and 44–67 is illustrated in Fig. 3.

The average intraplate coefficient of variation, calculated from 30 wells tested in the same assay, was 5·3% (mean OD 1·44) and 5·7% (mean OD 0·88); the interplate coefficient of variation calculated using 60 wells was 6·0% (mean OD 1·44) and 6·9% (mean OD 0·88).

Antisera to Sm-D peptides

The ability of the anti-peptide sera raised in rabbits to react with the homologous peptides was measured in ELISA using peptide-coated plates. A strong response was obtained for six of the seven peptides (1–20, 17–35, 33–51, 44–67, 64–84, 77–96) after three injections of rabbits (OD at 405 nm > 2·0 after 60 min substrate incubation with antisera diluted 1/5000–1/10 000; data

not shown). These results indicated that the inability of peptides 17–35, 33–51, 64–84 and 77–96 to react with patient sera (Table 1) was not due to the fact that they did not become adsorbed to the ELISA plate. Peptide 97–119 gave only a moderate immune response in the two rabbits (OD 2·0 after 60 min substrate incubation with antiserum diluted 1/250–1/500). This fairly low reactivity of the antisera to peptide 97–119 is not due to inadequate coating of the peptide to the plate since monoclonal antibodies 2–73 and 7–13 reacted strongly with the same peptide (see above).

Independent occurrence of anti-DNA and anti-Sm-D antibodies

The SLE sera analysed for the presence of antibodies reacting with Sm-D peptides were also tested for the presence of antibodies reacting in ELISA with native, dsDNA. No correlation was found between the presence of anti-DNA antibodies and antibodies reacting with peptides 1–20 or 44–67 of Sm-D (Table 2). McCarty *et al.* (1982) also reported that anti-dsDNA antibodies occurred independently from antibodies to 'Sm antigen.'

DISCUSSION

Anti-Sm antibodies are highly specific markers for the diagnosis of SLE. In immunoblots, anti-Sm antibodies recognize the B, B′ and D polypeptides contained in U1 snRNP particle while anti-U1 RNP antibodies react with the 70 kD, A- and C-unique polypeptides that are also associated with U1 RNA. The physical association of these antigens in the same RNP particle is responsible for the fact that anti-Sm antibodies are usually accompanied by anti-U1 RNP antibodies. The traditional assays for Sm and RNP antibodies include Ouchterlony double immunodiffusion, counter-immunoelectrophoresis and passive haemagglutination using total enriched rabbit thymic extracts as antigen. The sensitivity and specificity of these assays are low especially when both anti-Sm and anti-U1 RNP antibodies are present in the same serum. As a result, it is often necessary to carry out a second assay in order to distinguish between the two reactivities. To overcome these problems, it is possible to use highly purified Sm proteins (Bringmann & Lührmann, 1986; Williams, Charles & Maini, 1988) or recombinant polypeptides. In the present study, we replaced these various sources of Sm antigen by synthetic peptides corresponding to putative epitopes of the Sm-D polypeptide. Seven overlapping synthetic peptides were tested in an attempt to identify regions in the polypeptide D that could be used as probes for the detection of Sm-D specific autoantibodies.

When 165 sera from patients with known SLE were examined, two peptides corresponding to residues 1–20 and 44–67 of Sm-D were recognized in ELISA by, respectively, 59% and 37% of sera while peptide 97–119 was recognized by only 1% of the sera. When sera selected for their ability to react in immunoblotting with the Sm-D band were tested, 89% of them reacted with peptide 44–67; 75% of these sera reacted with peptide 1–20 and 37% with peptide 97–119. These results show that while the two peptides 1–20 and 44–67 were most frequently recognized by the two serum populations, the frequency of recognition was different when sera were selected on the basis of the clinical criterion or after a first screening according to their reactivity in immunoblotting.

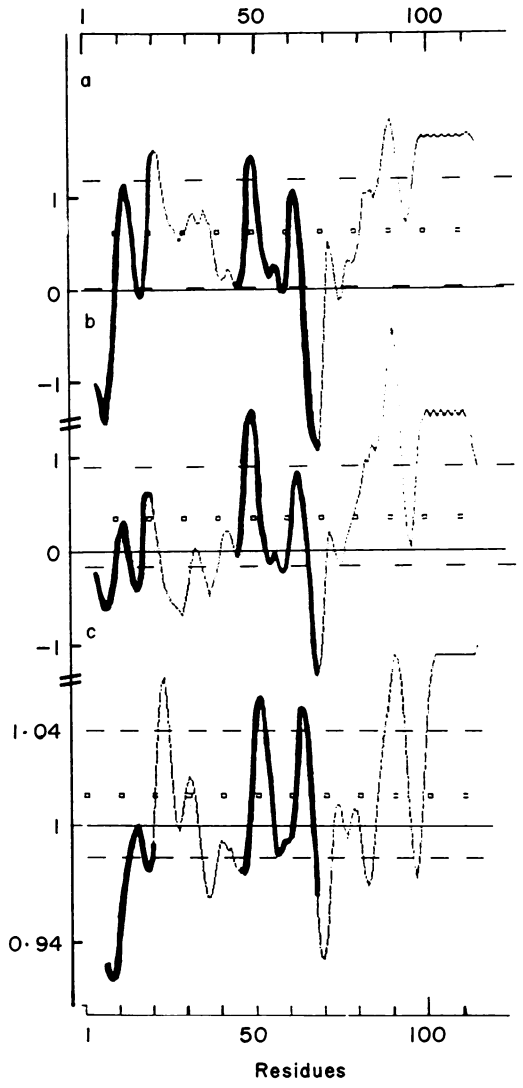

Fig. 4. Antigenicity prediction profiles of Sm-D constructed with the scale of: a, Parker *et al.* (1986); b, Hopp & Woods (1981); c, Karplus & Schultz (1985). The scales were normalized according to Van Regenmortel & Daney de Marcillac (1988). The plots are traced from the fourth residue onward and until the $(n-3)^{th}$ residue. The location of epitopes recognized by antibodies from SLE sera (regions 1–20 and 44–67) is represented by thick lines.

The frequency of anti-'Sm' antibodies in SLE patients has been reported to vary in different studies from 5% to 30%, considerably lower than the frequency of antibodies reacting with Sm-D peptides found in the present study. Although it is difficult to compare both sets of data since different types of antigen and different laboratory methods were used for testing, our results indicate that short synthetic peptides are better probe than whole 'Sm' antigen for detecting anti-'Sm' antibodies. The superiority of short peptides compared with the whole molecule has also been observed in previous studies where patient sera were tested with whole histone molecules and with histone peptides (Muller *et al.*, 1989; Tuaillon *et al.*, 1990). It is possible that different subsets of autoantibodies which preferentially recognize certain conformations of the test Sm antigen are detected in the various assays.

It is also interesting to observe that the two antigenic regions and particularly the epitope localized in the peptide 1–20 of Sm-D, were not predicted to be antigenic by any of the algorithms used (Fig. 4). These methods, based on parameters such as the hydrophilicity and mobility of short segmental regions in the protein primary structure, are known to have only limited predictive value (Van Regenmortel & Daney de Marcillac, 1988). In the particular case of RNP autoantigens, their predictive value may even be lower than usually the case since the exact nature and presentation of the antigen involved in the induction of anti-Sm antibodies are not known. There are strong arguments in favour of an antigenic stimulation of the immune system by a selected set of nucleoprotein particles (Hardin, 1986) in which part of the individual polypeptides is buried. This could explain the lack of antigenic activity observed in the regions of Sm-D covered by peptides 17–35, 33–51, 64–84 and 77–96; these regions are probably not accessible in the RNP particle, in contrast with the N-terminal region of the molecule which contains most of the antigenic reactivity.

There are compelling reasons for studying the fine antigenic structure of Sm-specific polypeptides, one of the most important being that synthetic fragments of these polypeptides may form the basis of sensitive, quantitative diagnostic assays. The observation that 59% of SLE patients possessed IgG antibodies recognizing peptide 1–20 of Sm-D while less than 6% of sera from patients with other rheumatic autoimmune diseases (scleroderma, MCTD, polymyositis, Sjögren's syndrome, RA) and less than 4% of normal sera reacted with the same peptide in ELISA lead us to the conclusion that this peptide (possibly used together with peptide 44–67) could be used as a valuable probe for SLE diagnosis. Further studies are currently in progress in order to determine whether antibodies revealed with Sm-D peptides correlate with particular clinical features of the disease (Muller *et al.*, 1990). In view of the present results, a comparative study of the different methods (immunodiffusion, haemagglutination, immunoblotting and ELISA) used for measuring anti-Sm antibodies may be of considerable value. Together with recent data obtained with other autoantigens (Muller *et al.*, 1988, 1989; Plaué *et al.*, 1989), our results clearly demonstrate the usefulness of synthetic peptides for the diagnosis of autoimmune diseases. In addition, the antibodies raised in this study against Sm-D peptides may help to unravel the structure and function of RNP particles and may be useful for purifying RNP particles.

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