# CHARACTERIZATION OF ANTIGENIC POLYPEPTIDES OF THE RNP, Sm AND SS-B NUCLEAR ANTIGENS FROM CALF THYMUS

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**Abstract**—Antinuclear autoantibodies are a hallmark of autoimmune diseases. The RNP, Sm and SS-B nuclear antigens from calf thymus in whole tissue, nuclear extracts and fractions have been studied by using different techniques including immunodiffusion, counterimmunoelectrophoresis and protein blotting. Such studies were done in order to obtain a precise characterization of the polypeptide components of those antigens. From our results it can be established that: (a) one 69.8 Kd polypeptide (for whole tissue and nuclei) and a number of well-defined 32–38-Kd polypeptides (for nuclear extracts and ammonium sulfate fractions) show an antigenic character against anti-RNP sera; (b) anti-Sm sera from different patients show in all cases a variable component of antigenic polypeptides, including one 28.8, 29.7 Kd doublet and two singlets of 14.8 and 11.0 Kd; and (c) a 52.0-Kd SS-B antigenic polypeptide is found for whole tissue, which is gradually degraded in nuclei and nuclear extracts to a more stable 47.1-Kd polypeptide.

## INTRODUCTION

Sera from patients suffering from a number of rheumatic autoimmune diseases are characterized by the presence of antibodies which react with nuclear antigens [for a review see Reichlin (1981)]. Antibody activities against soluble nuclear protein containing antigens such as RNP, Sm and SS-B have been used as markers for mixed connective tissue disease, systemic lupus erythematosus and Sjögren's syndrome, respectively (Sharp *et al.*, 1972; Gaudreau *et al.*, 1978; Leibfarth and Persellin, 1976; Martínez-Lavín *et al.*, 1979). The study of these antigens at the molecular level has been undertaken by different authors giving rise to controversy regarding which polypeptides constitute the snRNP particles and which polypeptides carry the epitopes reacting with the antibodies found in rheumatic patients.

In the case of RNP, Takano *et al.* (1981) reported for calf thymus one antigenic band of 65 Kd, two of 30 Kd and one of 13 Kd and White *et al.* (1982) showed for calf thymus two antigenic bands of 30 Kd. For rabbit thymus extracts, White *et al.* (1982) and Billings *et al.* (1982) reported two antigenic bands of 70 Kd and one of 40 Kd. Gibbons *et al.* (1982) isolated by immunoaffinity chromatography particles with RNP activity, composed of five proteins of 10–15 Kd and Conner *et al.* (1982) immunoprecipitated a 19-Kd RNP antigenic polypeptide from nuclear extracts of different sources.

Using rabbit and calf thymus extracts, White *et al.* (1981) and Douvas (1982) reported the presence of an Sm antigenic band of 13 Kd and an additional 29-Kd Sm doublet using HeLa cell extracts. Eisenberg *et al.* (1983) have also reported in human spleen and rabbit thymus two polypeptides of 17 and 15 Kd with Sm activity and Conner *et al.* (1982) immunoprecipitated from human lymphocytic leukemia cell extracts two bands of 25 and 16 Kd, while, by blot analysis of rabbit thymus extracts, they detected Sm antigenic activity against a 70- and a 16-Kd protein.

In relation to the antigenic polypeptides corresponding to SS-B antigen, Teppo *et al.* (1982) reported for rabbit thymus extracts one band of 68 Kd, Venables *et al.* (1983) have isolated by immunoaffinity chromatography two, 40- and 29-Kd, polypeptides, whereas Akizuki *et al.* (1977) detected a 53-Kd reacting polypeptide that could be degraded into two peptides of 33 and 20 Kd and Matter *et al.* (1982) detected in HeLa cells two, 53- and 45-Kd, antigenic polypeptides.

Such divergent results indicate the need for a systematic clarification of the subject. The present paper deals with the study of RNP, Sm and SS-B nuclear antigens from calf thymus by using different techniques including immunodiffusion (ID), counterimmunoelectrophoresis (CIE) and protein blotting. The main aim of our contribution is to characterize the polypeptide components of these nuclear antigens and to determine those peptides which display an antigenic character. Our studies permit us to obtain valuable information on: (a) the stability of the antigenic protein moiety during the extraction procedures normally used for the preparation of the nuclear antigens and (b) the polydispersity of antigenic polypeptides for the same antigen.

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## MATERIALS AND METHODS

### Tissue and nuclei preparation

Whole calf thymus tissue was grinded at liquid $N_2$ temp in a mortar and the homogenized powder sonicated in 10 m$M$ phosphate buffer, pH 7.5, 0.15 $M$ NaCl (NaCl/Pi) 1% SDS. Nuclei were isolated from calf thymus by the method of Sharp et al. (1972) and sonicated in 1% SDS at different times. Samples were loaded immediately onto polyacrylamide/SDS slab gels prepared according to Laemmli's (1970) procedure.

### Preparation of antigenic extracts.

When preparing nuclear extracts all solutions were made 1 m$M$ phenylmethyl sulfonyl fluoride (PMSF), unless otherwise stated. Isolation and disruption of calf thymus nuclei by homogenization in a hypotonic medium was accomplished following the procedure of Sharp et al. (1972). The nuclear material was extracted with NaCl/Pi and centrifuged at 12,000 $g$, producing a low-speed pellet (Lsp). The recovered supernatant (Lss) was ultracentrifuged for 90 min at 113,000 $g$ and the new supernatant obtained, coded as the high-speed supernatant (Hss), dialyzed once against water and lyophilized, was used as a source of antigen. The pellet was resuspended in 10 m$M$ Tris–HCl, pH 8.0, extracted during 1 hr of agitation and finally clarified by centrifugation at 1000 rpm for 10 min. The clarified resuspension, coded as the high-speed pellet (Hsp), was dialyzed against water and lyophilized.

Although Hss and Hsp extracts show a similar RNP activity, the total protein recovery per gram of DNA measured by the method of Hutchison et al. (1962) is 4 times higher for Hss than for Hsp. Hss also has a higher content (twice) of SS-B compared to Hsp and the Sm content is the same in both, as measured by CIE.

The lyophilized Hss extract is more easily soluble than the Hsp one. However, during dialysis against water a precipitate is formed. The water-soluble part represents about 50% of the total protein and contains SS-B and Sm activity, but it is devoid of RNP activity. Protein content was measured by the method of Lowry et al. (1951) with bovine serum albumin (BSA) as standard.

### Ammonium sulfate fractionation

Ammonium sulfate fractionation of Hss extracts was done at 4°C, at a pH maintained in the neutral range by adding 0.8 mg of sodium carbonate per gram of solid ammonium sulfate. This fractionation was carried out from both recently prepared and dialyzed and lyophilized Hss extracts and no difference was observed in terms of antigen recovery. After 30 min of stirring, precipitates were recovered by centrifugation at 10,000 $g$ for 1 hr. The pellets were resuspended in NaCl/Pi and either dialyzed against NaCl/Pi (if they were to be used immediately)

or dialyzed against water and lyophilized. Fractions were obtained by successive precipitation at 25, 60, 70 and 90% saturation of ammonium sulfate, with yields of 3, 55, 6 and 14% of the total protein content, respectively. The total protein recovery was 78%.

### Sera and immunological assays

Sera were supplied by the Instituto Nacional de Lucha contra las Enfermedades Reumáticas of Barcelona, and the Hospital Clínico Provincial de Barcelona and they were tested by ID and CIE, using the methods described by Northway and Tan (1972) and Kurata and Tan (1976), respectively. The immunologic specificity of sera was determined according to the sensitivity of the antigens to RNAse and temp treatment (Kurata and Tan, 1976). RNAse treatment was carried out with bovine pancrease 80 U/mg RNAse A (Sigma) at a protein/enzyme ratio of 10, for 1 hr at 37°C. The temp treatment was carried out at 56°C for 1 hr. Serum specificity was confirmed by demonstration of identity or non-identity with prototype sera of different specificities obtained from other laboratories.

CIE proved to be about 10 times more sensitive than ID at detecting antigens and made it easier to discern different bands in sera with polyspecificities. Therefore, CIE was used to screen fractions and to establish the limit of precipitation for each fraction by serial dilution of sera, and as a procedure to compare antigenic activities. CIE plates were stained with amido black, because otherwise minor bands were not detectable.

### Electrophoresis and protein blotting

Either 15% polyacrylamide or 5–20% gradient polyacrylamide slab gel electrophoresis in SDS was performed according to Laemmli's (1970) procedure. BSA (Sigma) (66 Kd), ovalbumin (Sigma) (45 Kd), soybean trypsin inhibitor (Sigma) (21 Kd) and lysozyme (Sigma) (14.3 Kd) were used as mol. wt markers. Gels were stained with Coomassie brilliant blue or silver stained by the method of Guevara et al. (1982). Duplicated samples were transferred electrophoretically as described by Renart et al. (1979) at 70 V for 8 hr to nitrocellulose paper [BA85 (Schleicher and Schüll)] in a Bio-Rad Transblot device.

Nitrocellulose strips were either stained with amido black in order to check the efficiency of the transfer to the filter or soaked in incubation buffer (NaCl/Pi containing 3% BSA min and 0.05% Nonidet P40) and incubated with serum diluted 1/50 in the same buffer. After washing with 10 m$M$ phosphate buffer, pH 7.5, 1 $M$ NaCl, strips were incubated with ^{125}I-labelled protein A [37 $\mu$Ci/mg (Amersham)] at 0.25 $\mu$Ci and 2.5 ml of incubation buffer per slot, washed, dried and autoradiographed. The determination of the mol. wt of antigenic bands was done by scanning the gel tracks containing the markers and the autoradiograms in a Chromoscan 3 densitometer. The

interpolation was carried out by linear regression, after correcting the mobilities for variation due to the transfer, except in the case of bands of slower mobility than BSA where the lack of linearity was corrected by taking the value for BSA as a reference for extrapolation. Between 10 and 20 determinations were done for each band.

*Elution of specific antibodies from nitrocellulose filters*

Antibodies reacting against the 69.8-Kd band were extracted from nitrocellulose filters by using a modification of the method of Olmsted (1981). Polypeptides from total calf thymus tissue were separated by electrophoresis and transferred to nitrocellulose filters as described before.

Nitrocellulose strips (0.5 cm width), corresponding to five lanes (300 μg of total protein per lane), were cut horizontally at the position of the 69.8-Kd polypeptide band. The position of the band was detected by reacting a parallel lane with anti-RNP sera and confirmed by staining the rest of the nitrocellulose paper. After the horizontal strips had been incubated with anti-RNP serum and washed as described previously, the specific antibodies were eluted by treating the nitrocellulose strip with 3 ml of 0.2 M glycine–HCl, pH 2.8, at room temp for 5 min with agitation. The glycine–HCl solution was immediately

neutralized by the addition of 0.2 N NaOH, and diluted with an equal vol of 6% BSA, 0.10% Nonidet P40, 0.3 M NaCl, 10 mM phosphate buffer, pH 7.5. These solutions containing the specific antibodies were used to incubate nitrocellulose papers to which the Hss nuclear extract and whole thymus samples had been transferred. It was observed that horizontal nitrocellulose strips can be used several times.

## RESULTS

Immunological techniques such as CIE and ID are currently used for clinical purposes to compare different sera and to define their specificity. In the present work, 360 sera from patients suffering different types of rheumatic diseases were subjected to these techniques to detect their specificity, using Hss nuclear extracts (as defined in Materials and Methods) as the source of antigen. The tissue selected for the study was calf thymus, which is a tissue rich in nuclear antigens, with a low level of protease activity, and it has been used by other authors for similar studies. One hundred and fifty-five sera gave a positive reaction against Hss nuclear extract as detected by CIE. Among these, 49 sera showed an anti-RNP reaction, 20 sera an anti-Sm reaction and 33 an anti-SS-B reaction compared to control sera.
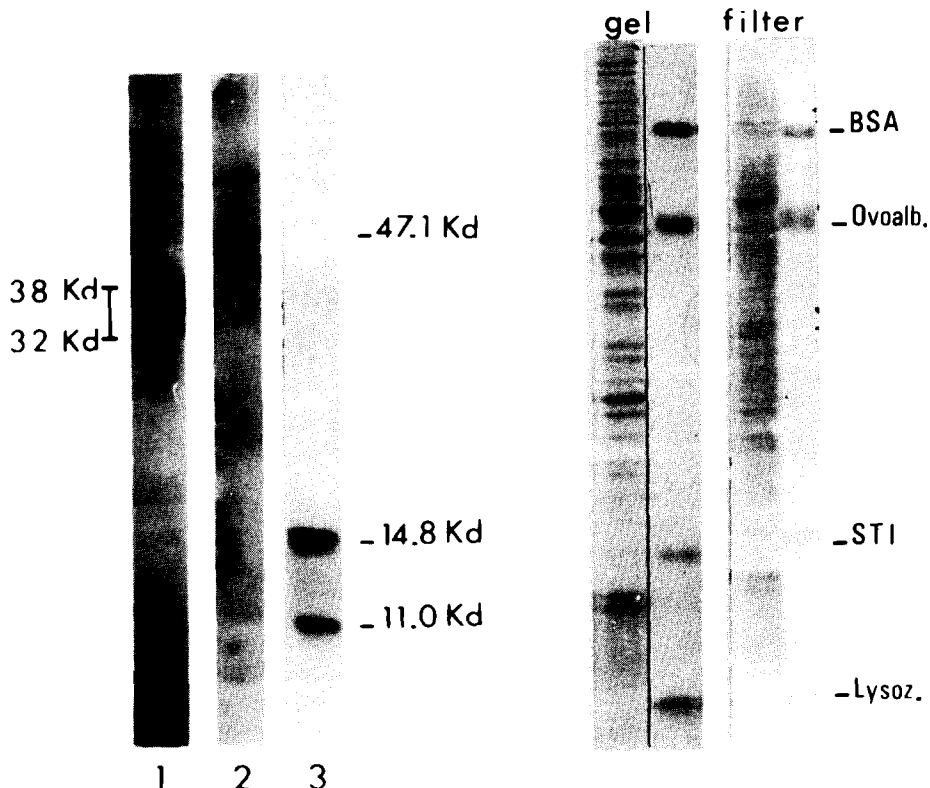


Fig. 1. Left side: autoradiograms of the immunoblots showing the antigenic bands detected for Hss nuclear extracts with anti-RNP (lane 1), anti-SS-B (lane 2) and anti-Sm (lane 3) sera as determined by CIE. Right side: Coomassie blue stained gel with Hss and mol. wt markers (gel), and amido black stained nitrocellulose after transfer (filter).

Table 1. Antigenic polypeptides detected by blot in Hss nuclear extracts with 12 different sera having anti-Sm
activity

| Serum specificity | Antigenic polypeptides | | | | |
|---|---|---|---|---|---|
|  | 11.0 Kd | 14.8 Kd | 28.8, 29.7 Kd | 47.1 Kd (SS-B) | 32–38 Kd (RNP) |
| Sm | + |  | + |  |  |
| Sm | + | + |  |  |  |
| Sm | + | + | + |  |  |
| Sm/RNP |  | + | + |  | + |
| Sm/RNP (two cases) | + | + |  |  | + |
| Sm/RNP (three cases) |  | + |  |  | + |
| Sm/SS-B |  |  | + | + |  |
| Sm/SS-B (two cases) | + |  | + | + |  |

To study the antigenic polypeptides corresponding to each specificity detected, each serum was assayed by protein blotting using the same Hss extract. The patterns obtained for three sera with anti-RNP, anti-SS-B and anti-Sm antibodies are shown in Fig. 1. In the same figure a control for the efficiency of the transfer to the nitrocellulose filter is shown by comparing a stained gel with a filter stained with amido black; the gel was also stained after transfer and no electrophoretic band was observed (not shown).

The blotting assay for a number of sera gave the following results. All the 23 anti-RNP sera detected by CIE showed the same pattern. A constant pattern was also found for all the 28 anti-SS-B sera assayed. However, the pattern obtained with 12 anti-Sm sera was neither unique nor repetitive. Table 1 shows the

reactive polypeptides detected with the 12 different sera with anti-Sm activity on Hss nuclear extracts assayed by blot.

As a control, six sera from healthy donors and 18 sera from patients who were determined to be ANA-negative against nuclear antigens, as assayed by CIE, were also assayed by blot. Twenty-six sera of patients who did show a positive reaction by CIE, but with different specificities to RNP, Sm or SS-B were also assayed by blot, in order to study possible new specificities. None of those 50 sera showed the pattern described in this work for RNP, Sm and SS-B activities.

*Stability of antigenic polypeptides*

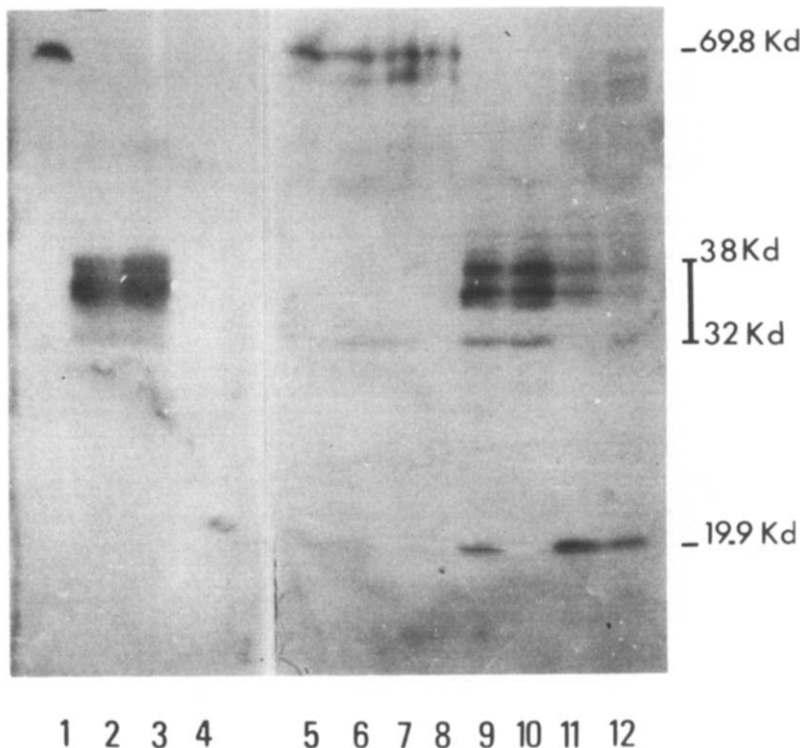Taking into account that the antigenic poly-



Fig. 2. Protein blotting immunodetection with anti-RNP monospecific sera on whole protein from calf thymus tissue (lane 1); freshly prepared nuclei (lane 5); after 3, 6 and 9 hr of incubation of the nuclei at room temp (lanes 6–8); and different nuclear fractions: Lss (lane 9), Hss (lanes 2 and 10), Hsp (lane 11), residue obtained after clarifying Hsp (lane 12) and 60 and 90% ammonium sulfate fractions (lanes 3 and 4).

peptides detected in the Hss might correspond to degradation products of the original material, tissue, nuclei and samples at different stages of the extraction procedure were also assayed by protein blotting. A unique antigenic band of 69.8 ± 1.5 Kd reacting with anti-RNP sera was detected in whole calf thymus tissue and fresh nuclei (Fig. 2, lanes 1 and 5). Another 67.4 ± 0.4 Kd RNP band is detected in nuclei after 3 hr at room temp, and its intensity is increased after 6 and 9 hr treatment (Fig. 2, lanes 6–8).

Hss nuclear extracts reacted with all anti-RNP sera showing a typical electrophoretic pattern corresponding to a group of antigenic bands within the range of 32–38 Kd, two of them (35.8 ± 0.7 and 38.1 ± 0.7 Kd) having an intensity clearly higher than others [Fig. 1 (lane 1) and Fig 2 (lanes 2 and 10)]. A second minor band of 19.9 ± 1.5 Kd reactive with the majority of RNP sera is also detected in some fractions.

It is worthwhile remarking that this 32–38-Kd RNP antigenic banding pattern present in nuclear extracts was never detected in whole tissue and only became apparent concomitantly with the disappearance of the 69.8-Kd band, once nuclei had been disrupted and extracted (Fig. 2). The 69.8-Kd polypeptide was shown to be stable in nuclei, in the absence of PMSF, for at least 3 hr at room temp, only starting to slowly degrade after that time (Fig. 2, lanes 5–8). One possible explanation of these results is that the 32–38-Kd pattern might originate from the 69.8-Kd polypeptide by a degradation process during the preparation of the extracts.

Further experiments were carried out in order to demonstrate such a possibility: sets of samples of the nuclear extracts coded as Lsp and Lss were prepared at different nuclei extraction times and these samples were assayed for RNP activity by protein blotting. The results are presented in Fig. 3. They show that most of the extractable 69.8-Kd antigenic polypeptide is detected in the Lsp moiety, although the amount recovered decreases after longer extraction times. It is interesting to note that for all Lsp extracts a number of faint bands are apparent, the most characteristic being one at 67.4 ± 0.4 Kd and a doublet of 35.8, 38.1 Kd. For the Lss moiety the 69.8-Kd polypeptide is detected only in small amounts in all cases, but the most characteristic feature is the presence of the doublet of 35.8, 38.1 Kd, whose intensity increases with the increase in the extraction time. The absence of PMSF in the extraction solution (not shown) gives rise to the appearance of a great number of antigenic bands intermediate between the 69.8-Kd one and the 35.8, 38.1 Kd doublet.

To further test that the 32–38-Kd banding pattern corresponds to degradation products of the 69.8-Kd antigenic polypeptide, specific antibodies recognizing this polypeptide were obtained by eluting the antibodies bound to the 69.8-Kd band onto nitrocellulose filters (Fig. 4). Section A in Fig. 4 corresponds to the controls run to test that the elution procedure of the antibodies from the nitrocellulose paper did not coelute with the antigenic polypeptide. The antibodies eluted from the 69.8-Kd region were used to incubate new nitrocellulose filters to which the Hss nuclear extract and whole thymus samples had been
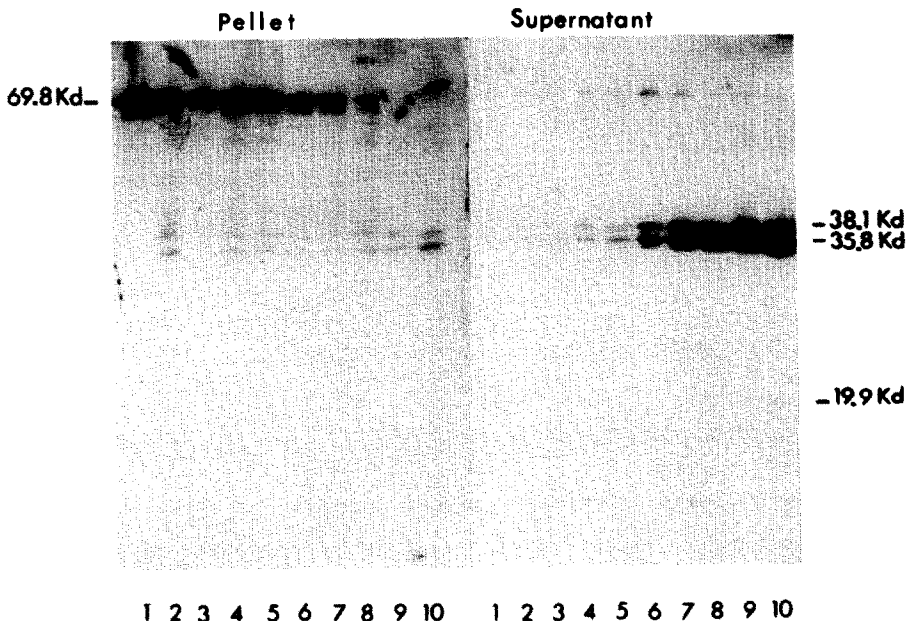


Fig. 3. Immunodetection of antigenic polypeptides reacting with anti-RNP serum in Lsp (pellet) and Lss (supernatant) samples after 0, 30, 60, 90 and 120 min of extraction at 4°C (lanes 1–5) and 120 min of extraction at 4°C plus 30, 60, 90, 120 and 180 min of extraction at room temp (lanes 6–10).

transferred. Lane 2 in section C (Fig. 4) shows that the eluted 69.8-Kd specific antibodies keep their specificity as they still recognize the same polypeptide on another whole-tissue sample. Those antibodies also recognize the same 32–38-Kd banding pattern (Fig. 4, section C, lane 1) that is reactive with whole anti-RNP sera (Fig. 4, section B) on Hss nuclear extract samples.

The 69.8 and 19.9-Kd bands were also detected in the insoluble residue obtained after resuspension and clarification by centrifugation of the Hsp (Fig. 2, lane 12).

Monospecific anti-SS-B sera recognize a single band of $52.0 \pm 1.6$ Kd in whole-tissue samples (Fig. 5, lane 1) and one main band of $47.1 \pm 1.8$ Kd in Hss extracts (Fig. 1, lane 2). Nuclei and samples at different stages of extraction, assayed with all sera having anti-SS-B activity, show several bands in the range of 47–52 Kd. Figure 5 (lanes 2–10) shows assays using a bispecific sera (anti-SS-B/anti-Sm). In the region of 47–52 Kd the coexistence of at least three bands in nuclei [freshly prepared or incubated at room temp for different times (Fig. 5, lanes 3–6)] and at all stages of extraction (Fig. 5, lanes 7–10) is apparent. A predominance of the 47.1-Kd band for Lss and Hss extracts can also be observed (Fig. 5, lanes 7 and 8).

When whole tissue and nuclei were assayed by protein blotting with different anti-Sm sera, as defined by immunological criteria, qualitative and quantitative differences in the reactive polypeptide bands were detected. Some anti-Sm sera reacted with one band of $11.0 \pm 0.4$ Kd and another of $14.8 \pm 0.6$ Kd (Fig. 1, lane 3), whereas other sera reacted with the 11.0-Kd band and a $28.8 \pm 0.5$, $29.7 + 0.5$ Kd doublet [see Fig. 5 (lane 2) as an example]. The distribution of antigenic polypeptides in the different sera studied is presented in Table 1.

As Fig. 5 shows, the antigenic Sm pattern detected with anti-SS-B/anti-Sm sera in nuclear extracts (lane 8) was the same as the pattern obtained in whole tissue and nuclei (lanes 2 and 3), indicating that no degradation of the Sm peptides had occurred during preparation of nuclear extracts. The same figure shows that, in terms of antigenic character, the 11.0-Kd band is insensitive either to the incubation of nuclei at room temp for different periods of time (lanes 4–6) or to the progress of the extraction (lanes 7–10). This is not the case for the 28.8, 29.7 Kd doublet: the 29.7-Kd band tends to disappear with prolonged incubation of nuclei (lanes 4–6) and the relative amount of the two bands varies according to the extract (lanes 7–10).
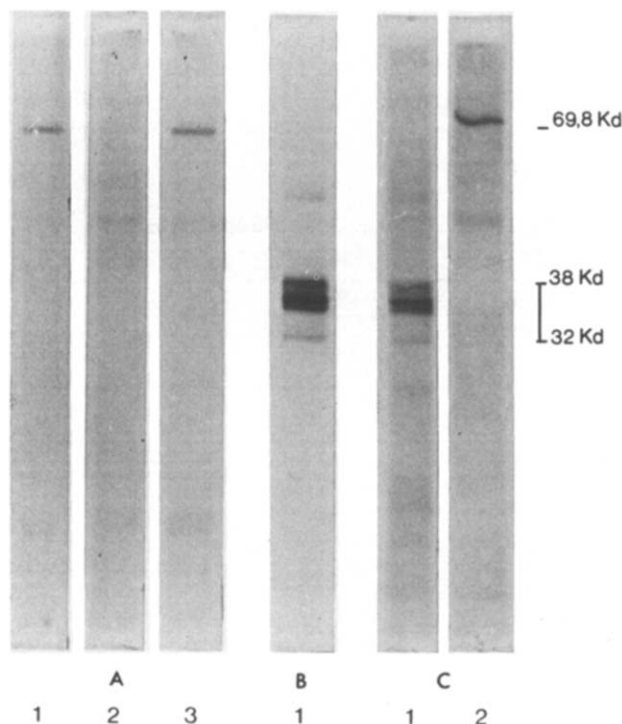


Fig. 4. Section A: control of elution of antibodies reacting with the antigenic polypeptides of the whole thymus samples transferred to nitrocellulose. Lane 1—nitrocellulose strip incubated with 0.2 M glycine–HCl, pH 2.8, followed by incubation with anti-RNP serum. Lane 2—nitrocellulose strip incubated with anti-RNP serum followed by incubation with 0.2 M glycine–HCl, pH 2.8. Lane 3—nitrocellulose strip incubated only with anti-RNP serum. The strips were incubated further with $^{125}$I-labelled protein A as described. Section B: Hss nuclear extract sample transferred to nitrocellulose and immunostained normally with whole anti-RNP serum. Section C: immunodetection using the specific antibodies eluted from the 69.8-Kd RNP antigenic band, on Hss nuclear extract (lane 1) and whole thymus sample (lane 2).
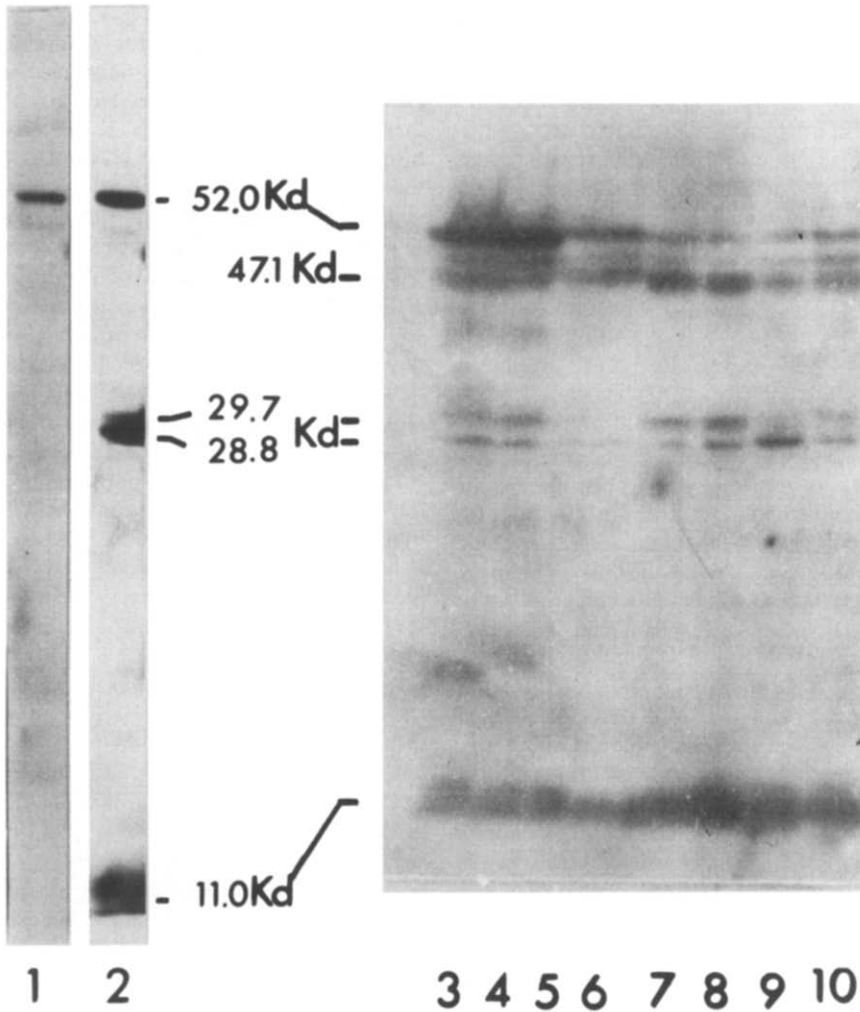
Fig. 5. Immunodetection of antigenic polypeptides reacting with anti-SS-B serum (lane 1) and anti-SS-B/anti-Sm serum (lane 2) on calf thymus tissue and the same anti-SS-B/anti-Sm serum reacting on freshly prepared nuclei (lane 3) and after 3, 6 and 9 hr of incubation of the nuclei at room temp (lanes 4–6) and on different nuclear fractions: Lss (lane 7), Hss (lane 8), Hsp (lane 9) and residue obtained after clarifying Hsp (lane 10).

*Ammonium sulfate fractionation*

Ammonium sulfate fractionation has been used in order to obtain fractions with selected antigenic activities (White *et al.*, 1981; Venables *et al.*, 1983). Our results show that sequential fractionation of the Hss nuclear extract rendered a fraction precipitating at 60% saturation two-fold enriched in RNP and Sm activity but with no SS-B activity, as measured by CIE. The 90% saturation fraction has no detectable RNP or Sm activity but it is six-fold enriched in SS-B activity.

A systematic analysis was carried out by using the protein blotting techniques for all ammonium sulfate fractions (i.e. 25, 60, 70 and 90% saturation). The sera used for immunodetection were monospecific sera (anti-RNP, anti-SS-B and anti-Sm) and bispecific ones (anti-RNP/anti-Sm and anti-SS-B/anti-Sm).

The results are presented in Table 2. It can be observed that the RNP 32–38-Kd bands are present mainly in the 60% saturation fraction, whereas the SS-B 47.1-Kd band is located largely in the 90% fraction and in a lesser amount in the 70% fraction. Finally, almost all Sm polypeptides accompany RNP ones in the precipitation at 25 and 60% saturation of

Table 2. Distribution of antigenic bands of different specificities detected by ammonium sulfate fractionation of Hss extracts

| Antigenic activity | | Ammonium sulfate fractions | | | |
|---|---|---|---|---|---|
| | | 25% | 60% | 70% | 90% |
| SS-B | (47.1-Kd) | – | – | + | + + + |
| RNP | (32–38-Kd) | + | + + + | – | – |
| Sm | (29.7-Kd) | + | + + | – | – |
| Sm | (28.8-Kd) | + | + + | – | – |
| Sm | (14.8-Kd) | + | + + | – | – |
| Sm | (11.0-Kd) | + | + + | – | – |

ammonium sulfate. The localization of the Sm 29.7-Kd band is difficult, since it is the most sensitive to degradation [see Fig. 5 (lanes 3–6)] of all Sm antigenic polypeptides and its possible localization in the 70 and 90% fraction is difficult to detect due to the overlapping in this electrophoretic region of an SS-B antigenic band which is a degradation product of the SS-B 47.1-Kd band.

The use of monospecific anti-Sm sera reacting with the 28.8, 29.7 Kd Sm doublet and the 14.8-Kd Sm band shows that the Sm doublet is also found in the 60% fraction but not in the 90% fraction.

## DISCUSSION

The protein blotting technique is a powerful tool which allows characterization of number of antigenic polypeptides present in nuclear extracts reacting with sera from patients suffering rheumatic diseases. Using this technique, several laboratories have reported divergent data concerning the number and mol. wt of the antigenic polypeptides for RNP, Sm and SS-B nuclear antigens.

In the case of RNP the spread of results found in the references, including our finding of a 69.8-Kd band and a 32–38-Kd reproducible set of bands, indicates the possibility that some of the identified proteins are degradation products of larger poly-peptides. Although we cannot rule out the possibility that degradation happens even when whole tissue is sonicated in the presence of SDS, proteolysis, if it occurs, is expected to be negligible. Therefore, the 69.8-Kd band most probably corresponds to the undegraded RNP antigenic polypeptide. The unique relationship between the disappearance of the 69.8-Kd polypeptide in Lsp extracts and the appear-ance of the 35.8, 38.1 Kd doublet in Lss extracts with increasing extraction times is a clear demonstration that the doublet is obtained by degradation of the 69.8-Kd polypeptide. Furthermore, the antibodies that react against the 69.8-Kd polypeptide also react with the 35.8, 38.1 Kd doublet, indicating that they contain common antigenic determinants. These facts strongly suggest that they correspond to the same protein that is degraded during the extraction pro-cedures.

The persistence of the 32–38-Kd pattern indicates that these polypeptides probably correspond to stable structural domains of the 69.8-Kd antigenic moiety. All bands of the 32–38-Kd pattern correspond to approximately half the size of the undegraded 69.8-Kd polypeptide, a fact which is consistent with the presence of a sensitive hinge zone between two stable domains. During the preparation of nuclear extracts the 69.8-Kd molecule would break down producing two polypeptides through the action of proteases on probably more than one site of the hinge region. The 32–38-Kd pattern has a remarkable stability, being preserved in the 60% ammonium sulfate fraction. This finding indicates that the

32–38-Kd pattern corresponds to stable structural protein domains, which can be reversibly coupled with RNA and other non-antigenic polypeptides giving rise to a RNP antigenic complex (Lerner and Steitz, 1979). Our findings are in agreement with those reported by White et al. (1982), who reached the conclusion that the native RNP antigenic poly-peptide has a mol. wt of 70,000 and is readily susceptible to proteolysis, generating smaller poly-peptides that still retain antigenicity.

The 19.9-Kd antigenic polypeptide, detected only with some anti-RNP sera in nuclei, Lss extracts and in the heavier components of the Hsp fractions, correlates with the 19-Kd band immunoprecipitated with anti-RNP sera from Lss-type extracts by Conner et al. (1982) and Zeller et al. (1983).

In regard to the antigenic polypeptides correspond-ing to SS-B antigen, our results consistently indicate that all anti SS-B sera recognize in whole calf thymus a unique 52.0-Kd polypeptide that is rapidly de-graded to 47.1 Kd during preparation of nuclei and the extraction process. We also detected a 52.0-Kd band accompanied by the 47.1-Kd degradation prod-uct in rabbit thymus acetone powder (results not shown). In this case it clearly appears that the dispersity of results is not due to polyspecificity of anti-SS-B sera but to a degradation process.

The published data regarding the Sm antigenic polypeptides, partially reviewed in the Introduction, seen to be confusing. We tested different anti-Sm sera which detected different antigenic polypeptides, despite the fact that sera showed total identity on CIE. The sera react with either a 28.8, 29.7 Kd doublet and a 11.0-Kd component or with an addi-tional 14.8-Kd component. The different Sm poly-peptides have been already detected in whole calf thymus tissue, showing that the multiplicity of bands does not correspond to proteolysis products but to different specificities of anti-Sm sera.

It has been postulated by other authors that the RNP, Sm and SS-B nuclear antigens are probably associated in snRNP complexes (Lerner and Steitz, 1979). In this sense, the polyspecificity of anti-Sm sera for different polypeptides, while total identity is demonstrated by CIE or ID, suggests that the different polypeptides must be associated with the same precipitating particles in agar gel.

The results we present show that the discrepancies between authors, even using the same system to characterize antigenic polypeptides, come, on the one hand, from degradation that takes place during the extraction procedures and, on the other hand, from serum polyspecificity. If results have to be compared by the use of protein blotting some marker bands have to be considered in several fractions. Anti-RNP sera present a 69.8-Kd band in whole tissue but a 32–38-Kd pattern in 60% ammonium sulfate fraction and the Hss nuclear extract. Anti-SS-B sera show a 52.0-Kd band in whole tissue but a 47.1-Kd band in the 90% ammonium sulfate fraction and anti-Sm sera

present a 14.8- and/or a 11.0-Kd band and/or a 28.8, 29.7 Kd doublet in whole tissue, the Hss nuclear extract and the 60% ammonium sulfate fraction.

## REFERENCES

Akizuki M., Boehm-Truitt M. J., Kassan S. S., Steinberg A. D. and Chused T. M. (1977) Purification of an acidic nuclear protein antigen and demonstration of its antibodies in subsets of patients with Sicca syndrome. *J. Immun.* **119**, 932–938.

Billings P. B., Allen R. W., Jensen F. C. and Hoch S. O. (1982) Anti-RNP monoclonal antibodies derived from a mouse strain with lupus-like autoimmunity. *J. Immun.* **128**, 1176–1180.

Conner G. E., Nelson D., Wisniewolski R., Lahita R. G., Blobel G. and Kunkel H. G. (1982) Protein antigens of the RNA–protein complexes detected by anti-Sm and anti-RNP antibodies found in serum of patients with systemic lupus erythematosus and related disorders. *J. exp. Med.* **156**, 1475–1485.

Douvas A. S. (1982) Autoantibodies occurring in two different rheumatic diseases react with the same nuclear ribonucleoprotein particle. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5401–5405.

Eisenberg R. A., Klapper D. G. and Cohen P. L. (1983) The polypeptide structure of the Sm and RNP nuclear antigens. *Molec. Immun.* **20**, 187–195.

Gaudreau A., Amor B., Kahn M. F., Ryckewaert A., Sany J. and Peltier A. P. (1978) Clinical significance of antibodies to soluble extractable nuclear antigens (anti-ENA). *Ann. rheum. Dis.* **37**, 321–327.

Gibbons J. J., Jr, Augustynek D., Tsai C. C. and Roodman S. T. (1982) Characterization of RNP and Sm ribonucleoprotein nuclear antigens. *Molec. Immun.* **19**, 765–777.

Guevera J., Jr, Johnston D. A., Ramagali L. S., Martin B. A., Capetillo S. and Rodriguez L. V. (1982) Quantitative aspects of silver deposition in proteins resolved in complex polyacrylamide gels. *Electrophoresis* **3**, 197–205.

Hutchison W. C., Downie E. D. and Munro H. N. (1962) Factors affecting the Schneider procedure for estimation of nucleic acids. *Biochim. biophys. Acta* **55**, 561–570.

Kurata N. and Tan E. M. (1976) Identification of antibodies to nuclear acidic antigens by counterimmunoelectrophoresis. *Arthritis Rheum.* **19**, 574–580.

Laemmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, Lond.* **227**, 680–685.

Leibfarth J. H. and Persellin R. H. (1976) Characteristics of patients with serum antibodies to extractable nuclear antigens. *Arthritis Rheum.* **19**, 851–856.

Lerner M. R. and Steitz J. A. (1979) Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5495–5499.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

Martínez-Lavín M., Vaughan J. H. and Tan E. M. (1979) Autoantibodies and the spectrum of Sjögren's syndrome. *Ann. intern. Med.* **91**, 185–190.

Matter L., Schopfer K., Wilhelm J. A., Nyffenegger T., Parisot R. F. and DeRobertis E. M. (1982) Molecular characterization of ribonucleoprotein antigens bound by antinuclear antibodies: a diagnostic evaluation. *Arthritis Rheum.* **25**, 1278–1283.

Northway J. D. and Tan E. M. (1972) Differentiation of antinuclear antibodies giving speckled patterns in immunofluorescence. *Clin. Immun. Immunopath.* **1**, 140–154.

Olmsted J. B. (1981) Affinity purification of antibodies from diazotized paper blots of heterogeneous protein samples. *J. biol. Chem.* **256**, 11955–11957.

Reichlin M. (1981) Current perspectives on serological reactions in SLE patients. *Clin. exp. Immun.* **44**, 1–10.

Renart J., Reiser J. and Stark G. R. (1979) Transfer of protein from gels to diazobenzyloxymethyl-paper and detection with antisera: a method of studying antibody specificity and antigen structure. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3116–3120.

Sharp G. C., Irvin W. S., Tan E. M., Gould G. R. and Holman H. R. (1972) Mixed connective tissue diseases: an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen. *Am. J. Med.* **52**, 148–159.

Takano M., Golden S. S., Sharp G. C. and Agris P. F. (1981) Molecular relationships between two nuclear antigens, ribonucleoprotein and Sm: purification of active antigens and their biochemical characterization. *Biochemistry* **20**, 5929–5936.

Teppo A. M., Gripenberg M., Kurki P., Baklien K., Helve T. and Wegelius O. (1982) Purification and characterization of a nuclear SS-B antigen. *Scand. J. Immun.* **15**, 1–7.

Venables P. J. W., Charles P. J., Buchanan R. R. C., Yi T., Mumford P. A., Schrieber L., Room G. R. W. and Maini R. N. (1983) Quantitation and detection of isotypes of anti-SS-B antibodies by ELISA and Farr assays using affinity purified antigens. *Arthritis Rheum.* **26**, 146–155.

White P. J., Billings P. B. and Hoch S. O. (1982) Assays for the Sm and RNP autoantigens: the requirement for RNA and influence of the tissue source. *J. Immun.* **128**, 2751–2756.

White P. J., Gardner W. D. and Hoch S. O. (1981) Identification of the immunogenetically active components of the Sm and RNP antigens. *Proc. natn. Acad. Sci. U.S.A.* **78**, 626–630.

Zeller R., Nyffenegger T. and DeRobertis E. M. (1983) Nucleocytoplasmic distribution of snRNPs and stockpiled snRNA binding proteins during oogenesis and early development in *Xenopus laevis. Cell* **32**, 425–434.