Clin. exp. Immunol. (1983) **51**, 8–16.

Separation of two molecular species of the Sm antigen by affinity chromatography with murine monoclonal and human anti-nuclear autoantibodies

R. R. C. BUCHANAN,* P. J. W. VENABLES,* A. MORGAN,† N. A. STAINES,† P. R. SMITH* & R. N. MAINI* **Division of Clinical Immunology, Kennedy Institute of Rheumatology, London and †Immunology Section, Chelsea College, University of London, London, UK*

(Accepted for publication 6 August 1982)

SUMMARY

Nuclear ribonuclear protein (nRNP) and Sm were extracted from a 30–60% ammonium sulphate fraction of rabbit thymus extract by affinity chromatography. Immunoadsorbent columns were prepared from IgG extracted from an anti-nRNP serum, an anti-Sm serum and two monoclonal autoantibodies derived from a MRL/lpr mouse. All four immunoadsorbents isolated both nRNP and Sm antigens indicating that they exist as the 'nRNP/Sm complex' A species of Sm which did not bind to the anti-nRNP column and which was subsequently purified with the anti-Sm column was termed 'free Sm'. The Sm in the complex and free Sm were immunologically identical on immunodiffusion and gave similar polypeptide bands on polyacrylamide gel electrophoresis. However, they differed in that complexed Sm was sensitive to heat and RNAase. These studies provide direct evidence of a physical association between the nRNP and Sm antigens and indicate an additional molecular species of Sm whose resistance to RNAase may be due to different associated RNA species.

INTRODUCTION

Antibodies to the non-histone nuclear antigen Sm and nuclear ribonucleoprotein (nRNP) are found in approximately 30% of sera of patients with systemic lupus erythematosus (SLE) (Notman, Kurata & Tan, 1975). In many studies, it has been observed that anti-Sm antibodies are accompanied by antibodies to nRNP in the majority of sera (Notman *et al.*, 1975; Mattioli & Reichlin, 1973; Venables *et al.*, 1982).

In addition to this association of the antibodies, a number of pieces of evidence suggest a relationship between the Sm and nRNP antigens. (1) On indirect immunofluorescence, both gave a similar fine speckled pattern when examined on different cellular substrates (Northway & Tan, 1972). (2) Immunodiffusion experiments suggested that the two antigens may be complexed since the nRNP and the Sm precipitins demonstrated a line of partial identity (Mattioli & Reichlin, 1973). (3) Attempts to isolate nRNP free of Sm using biochemical techniques such as sucrose density gradient centrifugation, ion exchange chromatography or isoelectric focussing have failed although it has been possible to isolate Sm antigen alone (Mattioli & Reichlin, 1973; Peltier *et al.*, 1977; Waelti & Hess, 1980). (4) Immunoprecipitation experiments with internally radiolabelled antigens

Correspondence: P. J. W. Venables, Division of Clinical Immunology, Kennedy Institute of Rheumatology, London W6 7DW, UK.

0009-9104/83/0100-0008$02.00

have shown that sera containing anti-nRNP and anti-Sm antibodies precipitate the same set of polypeptides and some RNA moieties in common (Lerner & Steitz, 1979). (5) The use of immunoadsorbent columns has also failed to separate nRNP from Sm (Takano, Agris & Sharp, 1980) though Douvas *et al.* (1979) have claimed to isolate nRNP from an immunoadsorbent column which did not react with anti-Sm sera.

In this study we have isolated nRNP and Sm from rabbit thymus extract with immunoadsorbent columns prepared from both clinical sera and monoclonal autoantibodies. We have adopted a method whereby the columns were connected in series in a number of combinations. This has enabled us to deplete specific antigens from the extract before purification and enabled us to study the immunochemical relationship of nRNP and Sm.

MATERIALS AND METHODS

Human antibodies to nRNP and Sm. Sera from two patients were used as sources of IgG for affinity chromatography. Both gave the characteristic fine speckled stain on indirect immunofluorescence on Hep 2 cells. The anti-nRNP serum gave a single precipitin line at all dilutions to a titre of 1:512 on immunodiffusion with rabbit thymus extract (RTE) (Pelfreeze Biologicals, Rogers, Arkansas, USA) and gave a line of identity with a reference of anti-nRNP serum. RNAase digestion of the extract abolished the precipitin and resulted in a fall in the haemagglutination titre from 1:2,048,000 to less than 1:10. The serum had normal DNA binding activity by the Farr assay and did not stain the kinetoplast of *Crithidia lucillae* by indirect immunofluorescence. Rheumatoid factors were detected by the sheep cell agglutination test (SCAT) to a titre of 1:512.

The anti-Sm serum had a titre of 1:64 on immunodiffusion and 1:40,960 by haemagglutination, with no fall in titre after RNAase digestion. Anti-nRNP precipitins were not observed at any dilution of this serum on immunodiffusion or by counter-immunoelectrophoresis. DNA binding activity of 35 units/ml (normal less than 25) was detected by the Farr assay but not by *C. lucillae* and SCAT was negative.

Monoclonal antibodies (MCA). Two MCA, designated KIN 120 and KIN 131, secreted by hybridomas were produced by the fusion of spleen cells from an MRL/lpr strain mouse with X63-AgA.653 myeloma cells (Kearney *et al.*, 1977) according to the general procedures of Galfré *et al.* (1977), and will be described in greater detail elsewhere. Both MCA gave a speckled staining pattern on indirect immunofluorescence and bound to nRNP/Sm in an enzyme linked immunosorbent assay (ELISA). KIN 131 bound to RTE in ELISA but KIN 120 did not. Neither reacted with DNA, RNA, human IgG or affinity purified SS-B antigen in ELISA.

Preparation of immunoadsorbent columns. IgG from two human sera, from KIN 120 ascitic fluid and from KIN 131 supernatant was extracted by staphylococcal protein A (SPA) chromatography. The IgG was adsorbed to Sepharose 4B protein A (Pharmacia Great Britain, Hounslow, Middlesex, UK) and after extensive washing with phosphate-buffered saline pH 7·4 (PBS) was eluted with 0·1 M glycine, 0·5 M NaCl pH 2·5 and 0·1 M bicarbonate, 0·5 M NaCl pH 8·3. Cyanogen bromide activated Sepharose 4B (Pharmacia) was prepared according to the manufacturer's instructions and the SPA purified IgG coupled at 5 mg/ml gel, for 2 hr at room temperature. After blocking for 18 hr at 4°C with 0·2 M glycine pH 8·0, the anti-nRNP and anti-Sm gels (12 ml and 23 ml respectively) were packed into 60 ml syringes and the MCA gels (each 2 ml) into 5 ml syringes. The columns were washed with three cycles of coupling buffer and glycine HCl, and finally with 3 M guanidine hydrochloride.

Preparation of nRNP and Sm antigens. RTE was suspended in PBS at 60 mg/ml and stirred for 18 hr at 4°C. The suspension was centrifuged at 4,000 ***g*** for 30 min, and saturated ammonium sulphate was added to the supernatant to make a 30% saturated solution. The precipitate was separated after 30 min by centrifugation at 4,000 ***g*** for 30 min and discarded. The supernatant was raised to 60% saturation by the addition of solid ammonium sulphate and after standing for 30 min the precipitate was collected by centrifugation at 4,000 ***g*** for 30 min. The precipitate was dissolved in 40 ml PBS and dialysed against PBS over the next 48 hr with three changes of buffer. This 30–60% fraction of RTE (30–60 RTE) (containing 7 mg/ml protein) was passed over the columns at

approximately 10 ml/hr. The columns' ability to extract the nRNP and Sm was monitored by testing sequential samples of non-absorbed material (drop through) by counter-immunoelectrophoresis (CIE). They were extensively washed with PBS and the bound material was eluted with two column volumes of 3 M guanidine HCl (Sigma, Poole, Dorset, UK) directly into a vacuum dialysis flask containing 10 mM Tris, 1 mM EDTA at 4°C. After concentration to approximately 3 ml, dialysis against Tris/EDTA was continued for a further 24 hr with three buffer changes at 4°C.

Enzyme linked immunosorbent assay (ELISA). The eluate from the anti-nRNP column was used as antigen in ELISA. Dilutions of the antigen in 0·1 M $NaHCO_3$, pH 8·3 were applied to 96 well microELISA plates (Dynatech, Billingshurst, UK) and 50 μl/well were incubated for at least 18 hr at 4°C. Some plates were stored in this form for periods of up to 3 months without detectable loss of antigenic activity. The optimum antigen concentration was established to be 10 μg/ml by chequerboard with dilutions of standard reference sera (anti-Sm or anti-nRNP). Incubation and washing steps were performed as described by Staines *et al.* (1981) except that either rabbit anti-mouse or rabbit anti-human immunoglobulin linked to horse radish peroxidase (Miles, Slough, UK) was used as the developing antibody.

Other serological techniques. The characterization of nRNP, Sm and their antibodies, by haemagglutination, CIE, immunodiffusion and enzyme digestion was based on previously published methods (Venables *et al.*, 1980), except that with purified antigens the enzyme/substrate ratio was 1:1. Protein estimations were by the Biorad assay (Biorad, Watford, Hertfordshire, UK) with bovine gammaglobulin as a standard.

Polyacrylamide gel electrophoresis (PAGE). Ten and 15% polyacrylamide gels containing 0·1% sodium dodecyl sulphate (SDS) were run as vertical slabs 170 mm × 150 mm and 1 mm thick in 40 mM Tris, 20 mM sodium acetate, 2 mM EDTA, pH 7·4. Samples for electrophoresis were boiled for 1 min in the loading buffer (10 mM Tris, 1 mM EDTA) with 1% SDS and 10% sucrose and run for 5 hr at 40 mA using bromophenol blue as a marker. Protein in the gels was identified by staining in 0·02% Coomassie blue in 50% methanol, 5% acetic acid. Gels were destained in 7% acetic acid, 5% methanol and dried on a GSD4 gel slab drier (Pharmacia).

RESULTS

The human anti-nRNP and anti-Sm and the two monoclonal columns all isolated both nRNP and Sm activities from 30–60 RTE (Expts 1–4, Table 1). Both could be detected as precipitates on

Table 1. Presence of nRNP and Sm antigens in non-adsorbed material (drop through) and eluates from affinity columns

Expt. No.	Affinity column	Antigen source applied (from 10G RTE)	Antigens detected in	
			Drop through (DT)	Eluate
1	anti-nRNP	30–60 RTE	Sm	nRNP Sm
2	anti-Sm	30–60 RTE	−ve	nRNP Sm
3	KIN 120	30–60 RTE	*	nRNP Sm
4	KIN 131	30–60 RTE	*	nRNP Sm
5	anti-nRNP	DT anti-nRNP	Sm	−ve
6	anti-Sm	DT anti-nRNP	−ve	Sm
7	KIN 120	DT anti-nRNP	n.d.	Sm
8	KIN 131	DT anti-nRNP	n.d.	Sm
9	anti-nRNP	DT anti-Sm	−ve	−ve
10	KIN 120	30–60 RTE + RNAase	n.d.	Sm
11	KIN 131	30–60 RTE + RNAase	n.d.	Sm
12	KIN 120	Eluate anti-nRNP	n.d.	nRNP Sm

* The drop through from the monoclonal columns contained nRNP and Sm (see text).

n.d. = not done.

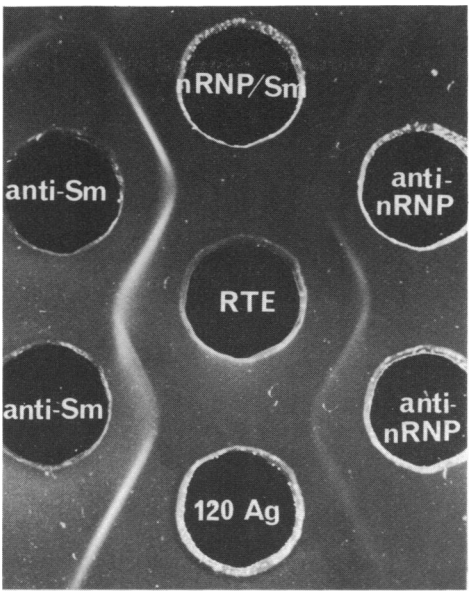

Fig. 1. Immunodiffusion study showing nRNP and Sm precipitins. The three antigens are: nRNP/Sm-antigen activities eluted from the anti-RNP column; RTE–rabbit thymus extract; 120 Ag–antigen activities eluted from MCA 120 column.

immunodiffusion to antigen dilutions of 1:16–1:32 (equivalent to a minimum protein concentration of 20 μg/ml) indicating a 66-fold purification with a yield of 15%. Precipitin lines of complete identity were obtained with antigens eluted from the human antibody columns, the MCA columns and the unpurified extract when tested with anti-Sm and anti-nRNP sera (Fig. 1). These experiments indicate that there is a molecular complex expressing both antigen activities which we have termed the 'nRNP/Sm complex'. The binding of the MCAs and the human sera to the complex could also be demonstrated by ELISA where all four reagents reacted to high dilutions with the eluate from the anti-nRNP column as antigen on the plate (Fig. 2).

The anti-Sm and the anti-nRNP columns could deplete all detectable antigen from up to 20 g (dry powder) of RTE and were therefore used to prepare antigen depleted substrates for further

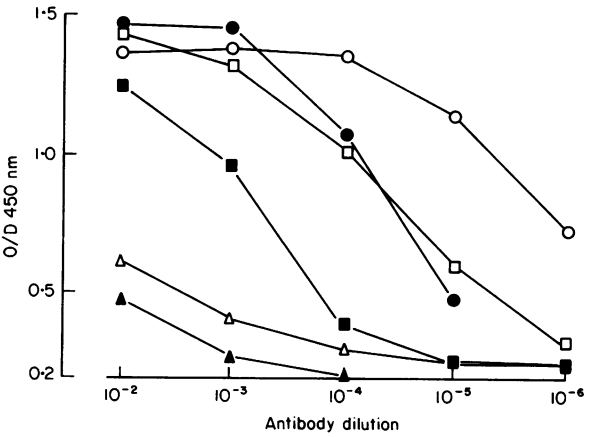

Fig. 2. ELISA with nRNP/Sm as antigen showing dilution curves of the two MCAs, KIN 120 (○——○) and KIN 131 (●——●), and the anti-nRNP (□——□) and anti-SM (■——■) sera. A normal human serum (NHS, △——△) and a MCA anti-DNA (▲——▲) are shown as controls.

separation. To avoid saturation of the human antibody columns, 30–60 RTE derived from 10 g dry powder was used for subsequent experiments. The absorptive capacity of the MCA columns was smaller and with this quantity of antigen applied both RNP and Sm activities were detectable in the drop through fractions.

Analysis of the antigens in these fractions showed an important difference between the anti-nRNP and anti-Sm columns. In Expt. 1, Sm was detected in all drop through fractions from the anti-nRNP column. By contrast the drop through from the anti-Sm column (Expt. 2) contained no detectable nRNP or Sm and neither antigen was present after the material was concentrated by a subsequent passage over, and elution from, the anti-nRNP column (Expt. 9). The drop through fractions from the anti-nRNP column were used as a source of RTE depleted of nRNP (Expts 5–8). When this material was passed down a regenerated washed anti-nRNP column (Expt. 5) the Sm antigen was again detected in all drop through fractions and was not recovered in the eluate. This experiment confirmed that the human anti-nRNP column does not bind isolated Sm. However the Sm antigen in this material was extracted by the anti-Sm column and could be recovered in the eluate (Expt. 6). The two monoclonal columns (Expts 7 & 8) behaved in a similar way providing further evidence of anti-Sm activity similar to the human antibody. This is supported by Expts 10 & 11 where both columns extracted Sm from RNAase digested RTE. Expt 12 indicates that the Sm component of the nRNP/Sm complex purified by the anti-nRNP column also binds to one of the monoclonal columns.

These data indicate that the Sm antigen exists in two forms, firstly as part of the nRNP/Sm complex and secondly as an independent molecule which does not bind to the anti-nRNP column but which was subsequently purified by the anti-Sm column. The second form of Sm we have termed 'free Sm'. The antigens could be conveniently prepared by passing 30–60 RTE down the anti-nRNP column followed by the anti-Sm column which was connected in series. After passage of the extract the columns were disconnected and run separately for washing and elution.

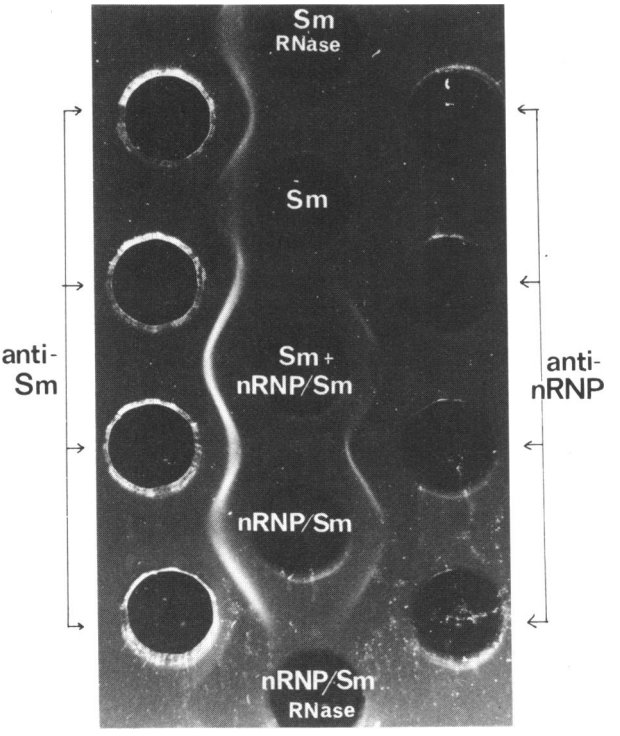

Fig. 3. Immunodiffusion study showing identity of RNP and Sm precipitins with different antigens and the effect of RNAase digestion. The antigens are: Sm-free Sm eluted from anti-Sm column preabsorbed by anti-nRNP; Sm + nRNP/Sm antigens eluted from the anti-Sm column; nRNP/Sm eluate from anti-nRNP column.

Table 2. Preliminary biochemical data

Sensitivities	Free Sm	nRNP/Sm complex	
		nRNP component	Sm component
RNAase (37°C, 1 hr)	Resistant	Sensitive	Sensitive
Trypsin (37°C, 1 hr)	Resistant	Sensitive	Resistant
Heat (56°C, 30 min)	Resistant	Sensitive	Sensitive
Heat (37°C, 1 hr)	Resistant	Resistant	Resistant
Mean* protein yield (mg)	0·98 (±0·02)	0·82 (±0·38)	
Mean* OD 260/280 ratio	1.36 (±0·05)	1·51 (±0·23)	
PAGE	11K, 15K	11K, 15K, 50K, 78K, 85K	

* Mean (± standard deviation) values from four column runs.

Serological and physiochemical properties of free Sm and the RNP/Sm complex

On immunodiffusion, anti-nRNP sera precipitated the nRNP/Sm complex but not the free Sm. Anti-Sm sera precipitated with both antigens and the precipitins obtained with anti-Sm sera showed a line of complete identity with both free Sm and nRNP/Sm (Fig. 3).

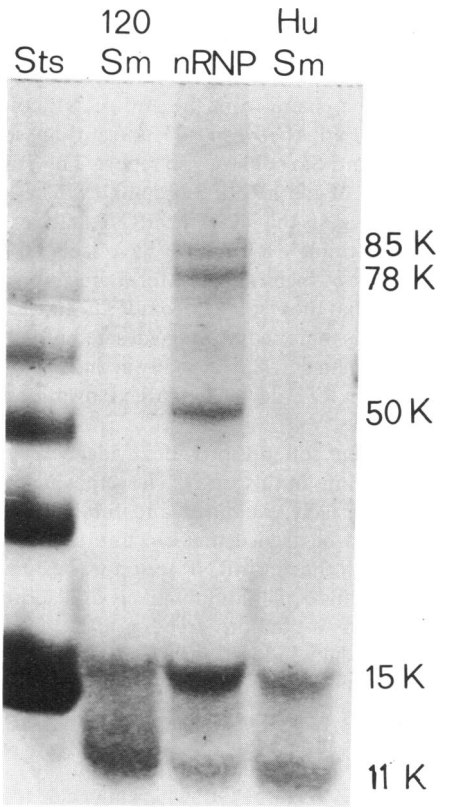

Fig. 4. Ten percent PAGE gel showing the major polypeptide components of nRNP/Sm and free Sm prepared from both human (Hu) and MCA 120 anti-Sm immunoadsorbent columns. The standards (Sts) are 14·6K polymer markers.

Preliminary biochemical data on the two antigens is presented in Table 2. The nRNP/Sm complex had a consistently higher OD 260/280 ratio than free Sm suggesting that nucleic acids formed a relatively large component of the complex. There were differences in the sensitivities to enzymes. Free Sm shared the properties of Sm in unpurified RTE. It was resistant to both RNAase and trypsin and to heating at 56°C for 30 min. By contrast pre-treatment of the nRNP/Sm complex with RNAase (Fig. 3) or heating abolished the Sm as well as the nRNP precipitins. However the Sm component of the complex appeared to be trypsin resistant.

On SDS-PAGE, free Sm immunopurified by both the anti-Sm and MCA columns gave two major bands with apparent molecular weights of 15K and 11K. Electrophoresis of the nRNP/Sm complex revealed the same two bands though the 15K band was more densely staining, with additional higher molecular weight bands at 50K, 78K and 85K (Fig. 4). Similar high molecular weight bands were seen with the nRNP/Sm complex isolated by the anti-Sm column, though this preparation also contained free Sm.

DISCUSSION

Our study has provided further evidence that Sm and nRNP exist as a complex and that, in addition, Sm also exists in free form. Previous attempts to isolate nRNP using biochemical techniques have usually resulted in associated Sm activity (Mattioli & Reichlin, 1973; Peltier *et al.*, 1977). This relationship between the two antigens has been interpreted either as evidence for their molecular association (Mattioli & Reichlin, 1973) or that they exist as independent macromolecules which tend to co-purify because of similarities in their molecular properties (Northway & Tan, 1972).

Affinity chromatography provides a powerful technique for resolving this controversy, and in one report, where purified anti-nRNP antibody was used as the immunoadsorbent both Sm and nRNP were recovered in the eluate (Takano *et al.*, 1980). Our study confirms this observation in that Sm was always recovered in the eluate from the anti-nRNP column and it gave a line of complete identity with reference precipitins between RTE and anti-Sm sera. These findings together with the isolation of nRNP with an anti-Sm column also reported by Takano *et al.* (1981) represent powerful evidence for the existence of the nRNP/Sm complex. The lack of nRNP in the drop through from the anti-Sm column suggests that all of the nRNP in the extract is in complexed form.

From the autoimmune mouse strain MRL/lpr we have isolated two hybridomas secreting MCAs with anti-Sm activity. Because of their failure to form precipitins in gel, their specificity was defined by ELISA and the antigens that they isolated from RTE. In ELISA both MCAs bound the nRNP/Sm complex. This binding was inhibited by anti-Sm sera but not by anti-nRNP (data to be published). They behaved like the human anti-Sm antibody in that they isolated nRNP/Sm complex from the whole RTE and free Sm from RTE depleted of nRNP by prior passage over an anti-nRNP column or by digestion with RNAase.

The use of MCA immunoadsorbent columns provides evidence against one important artefact which could explain some of the findings in this study. The sera which were selected as a source of anti-nRNP and anti-Sm were extensively examined and fulfilled all the available criteria for 'mono-specificity'. However, it could be argued that our anti-nRNP immunoglobulin has some anti-Sm activity. The means by which the anti-nRNP serum was examined (by immunodiffusion, CIE and haemagglutination) makes this explanation unlikely and additional evidence is shown by the failure of the anti-nRNP column to bind any free Sm after regeneration and cleaning. The possibility that the anti-Sm antibody had 'hidden' anti-nRNP activity is more difficult to discount with certainty. Most anti-Sm sera also contain detectable anti-nRNP antibodies (Mattioli & Reichlin, 1973) and these may not be detected by haemagglutination (by a fall in HA titre after RNAase digestion) if the anti-nRNP titre is relatively low. However, it has been suggested that anti-nRNP activity in most sera can be detected on immunodiffusion if the serum is examined at a range of dilutions (Northway & Tan, 1972) or by CIE which may help to separate double precipitins (Venables, Erhardt & Maini, 1980). Our anti-Sm serum was examined by all these techniques and consistently showed only one precipitin line. Even allowing for these precautions the possibility of anti-nRNP activity in our serum cannot be completely excluded (Mattioli & Reichlin, 1973). It is in

answering this potential criticism that the monoclonal anti-Sm antibodies have produced definitive evidence that anti-Sm antibodies of restricted specificity reproducibly isolate both nRNP and Sm antigens.

In addition to the nRNP/Sm complex, our study has confirmed that Sm also exists in a free form equivalent to 'Sm without RNP activity' reported by Takano *et al.* (1981). This explains the findings of previous reports, where Sm was found in the absence of nRNP after preparative isoelectric focussing (Waelti & Hess, 1980) and in relatively low molecular weight fractions from a sucrose density gradient (Mattioli & Reichlin, 1973). On PAGE free Sm contains two major polypeptides 11K and 15K. Identical bands are also present in nRNP/Sm complex and we suggest that these represent the Sm component and that the higher molecular weight bands, 50K, 78K and 85K, constitute the nRNP part of the complex. This is supported by the findings of White & Hoch (1981) who, using a transfer technique, found that anti-Sm sera reacted with a single band at 13K and anti-nRNP sera reacted with a similar band as well as higher molecular weight bands ranging in molecular weights from 30K to 70K. Similar results were obtained by Takano *et al.* (1981) using a radioimmunoassay on gel slices.

In spite of the similarities of the polypeptide components of Sm in the complex and in free Sm, the antigens differ in their sensitivities to RNAase. This implies that like nRNP, complexed Sm requires the presence of RNA to produce precipitins in gel, whereas free Sm is in some way protected from the activity of the enzyme. The reason for this is unclear. Lerner & Steitz (1979) have found that anti-nRNP sera precipitate only U_1 RNA species, which are RNAase sensitive. Anti-Sm sera also precipitate U_1 RNA species, a finding consistent with the hypothesis of nRNP/Sm complex, but in addition, precipitate U_2, U_4, U_5 and U_6. These additional RNA moieties which would not be detectable by SDS-PAGE are presumably present on free Sm and for reasons which remain unclear confer RNAase resistance to the molecule. Another possibility suggested by its sensitivity to periodate (Tan & Kunkel, 1966) is that free Sm is heavily glycosylated, 'protecting' the molecule not only from RNAase but also trypsin.

Lerner & Steitz (1979) have stressed the importance of studying the nRNP and Sm antigens at a molecular level in order to understand the mechanisms by which anti-nRNP and anti-Sm antibodies can cause disease. We propose that the isolation of the nRNP/Sm complex and its separation from free Sm constitutes an important step in resolving some of the confusion which has surrounded the association of the antigens.

The authors gratefully acknowledge the support of the Arthritis and Rheumatism Council. We would like to thank Jennifer Linfield for her help in preparing the manuscript.

REFERENCES

DOUVAS, A.S., STUMPH, W.E., ROYES, P. & TAN, E.M. (1979) Isolation and characterisation of nuclear ribonucleoprotein complexes using human antinuclear ribonucleoprotein antibodies. *J. biol. Chem.* **254,** 2608.

GALFRÉ, G., HOWE, S.C., MILSTEIN, C., BUTCHER, G.W. & HOWARD, J.C. (1977) Antibodies to major histocompatibility antigens produced by hybrid cell lines. *Nature*, **266,** 550.

KEARNY, J.F., RADBRUCH, A., LIESEGANG, B. & RAJEWSKY, K. (1979) A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody secreting cell lines. *J. Immunol.* **125,** 1548.

LERNER, M.R. & STEITZ, J.A. (1979) Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA.* **76,** 5495.

MATTIOLI, M. & REICHLIN, R. (1973) Physical association of two nuclear antigens and mutual occurrence of their antibodies: the relationship of the Sm and RNA protein (Mo) systems in SLE sera. *J. Immunol.* **110,** 1318.

NORTHWAY, J.D. & TAN, E.M. (1972) Differentiation of antinuclear antibodies giving speckled staining patterns in immunofluorescence. *Clin. Immunol. Immunopathol.* **1,** 140.

NOTMAN, D.D., KURATA, N. & TAN, E.M. (1975) Profiles of antinuclear antibodies in systemic rheumatic diseases. *Ann. Intern. Med.* **83,** 464.

PELTIER, A.P., AUSSEL, C., HAIM, T. & CYNA, L. (1977) Ribonucleoprotein (RNP) soluble nuclear antigen: demonstration of its reaction with serum antibodies by counter immunoelectrophoresis and further partial characterisation. *J. Immunol. Meth.* **16,** 153.

STAINES, N.A., HARDINGHAM, T., SMITH, M. & HENDERSON, B. (1981) Collagen induced arthritis in the rat: Modification of immune and arthritic responses by free collagen and immune anti-collagen antiserum. *Immunology*, **44,** 737.

TAKANO, M., AGRIS, P.F. & SHARP, G.C. (1980) Purification and biochemical characterisation of nuclear ribonucleoprotein antigen using purified antibody from serum of a patient with mixed connective tissue disease. *J. clin. Invest.* **65,** 1449.

TAKANO, M., GOLDEN, S.S., SHARP, G.C. & AGRIS, P.F. (1981) Molecular relationships between two nuclear antigens, ribonucleoprotein and Sm: purification of active antigens and their biochemical characterisation. *Biochemistry*, **20,** 5,929.

TAN, E.M. & KUNKEL, H.G. (1966) Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. *J. Immunol.* **96,** 464.

VENABLES, P.J.W., ERHARDT, C.C. & MAINI, R.N. (1980) Antibodies to extractable nuclear antigens in rheumatoid arthritis: Relationship to vasculitis and circulating immune complexes. *Clin. exp. Immunol.* **39,** 146.

VENABLES, P.J.W., TUNG, Y., WOODROW, D.F., MOSS, J. & MAINI, R.N. (1982) Relationship of antibodies to soluble cellular antigens and histological manifestations of renal disease in SLE. *Ann. rheum. Dis.* (in press.)

WAELTI, E.R. & HESS, M.W. (1980) Nuclear antigens: characterisation of the acidic nuclear ribonucleoprotein-Sm-antigen complex by analytical and preparative isoelectric focussing. *J. Immunol. Meth.* **32,** 177.

WHITE, P.J. & HOCH, S.O. (1981) Definition of the antigenic polypeptides in the Sm and RNP ribonucleoprotein complexes. *Biochem. Biophys. Res. Commun.* **102,** 365.