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Autoantibodies to ribonucleoprotein particles containing U2 small nuclear RNA

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Autoantibodies exclusively precipitating U1 and U2 small nuclear ribonucleoprotein (snRNP) particles [anti-(U1,U2)RNP] were detected in sera from four patients with autoimmune disorders. When tested by immunoblotting, these sera recognized up to four different protein antigens in purified mixtures of U1–U6 RNP particles. With purified antibody fractions eluted from individual antigen bands on nitrocellulose blots, each anti-(U1,U2)RNP serum precipitated U2 RNP by virtue of the recognition of a U2 RNP-specific B'' antigen (mol. wt. 28 500). Antibodies to the U2 RNP-specific A' protein (mol. wt. 31 000) were found in only one serum. The B'' antigen differs slightly in mol. wt. from the U1–U6 RNA-associated B/B' antigens and can be separated from this doublet by two-dimensional gel electrophoresis, due to its more acidic pI. In immunoprecipitation assays, the purified anti-B'' antibody specificity also reacts with U1 RNPs which is due to cross-reactivity of the antibody with the U1 RNA-specific A protein, as demonstrated by immunoblotting using proteins from isolated U1 RNPs as antigenic material. Thus the A antigen not only bears unique antigenic sites for anti-A antibodies contained in anti-(U1)RNP sera, it also shares epitopes with the U2 RNP-specific B'' antigen.

Key words: autoantibodies/snRNP/antibody-elution/immunoblotting/ U2 snRNA

Introduction

Sera from patients with connective tissue diseases often contain antibodies against nuclear RNA-protein complexes (Tan, 1982; Lerner and Steitz, 1981). The RNA moiety of some of these antigenic complexes has been identified as U snRNAs, a discrete class of stable and highly conserved small nuclear RNAs (for reviews, see Steitz *et al.*, 1983; Reddy and Busch, 1983). U1 RNPs have been shown to function in the splicing of pre-messenger RNAs in *in vitro* systems (Yang *et al.*, 1981; Padgett *et al.*, 1983; Krämer *et al.*, 1984). A similar function has been postulated for U2 RNA (Ohshima *et al.*, 1981), but experimental evidence is still lacking. U4 RNA has been proposed to be involved in the 3' processing of pre-mRNA (Berget, 1984). All of the snRNAs are commonly found to be associated with hnRNP particles (Sekeris and Niessing, 1975; Gallinaro and Jacob, 1979). Some of the snRNAs (U1, U2 and U4) could even be cross-linked to hnRNA by a psoralen derivative (Calvet and Pederson, 1981; Calvet *et al.*, 1982). All of these considerations suggest that the nucleoplasmic U-snRNAs play closely related functions in mRNA processing or transport.

Anti-Sm antibodies react with the complete set of U1, U2, U4, U5 and U6 (U1–U6) RNP particles whereas anti-(U1)RNP sera specifically precipitate particles containing U1 RNA, the most abundant species of snRNAs (Lerner and Steitz, 1979). Anti-(U2)RNP activity has recently been found in a patient with a scleroderma-polymyositis overlap syndrome (Mimori *et al.*, 1984). In contrast, with anti-Sm and anti-(U1)RNP antibodies which are rather common in sera from patients with SLE and MCTD, respectively, exclusive anti-(U2)RNP specificity is very rarely found (Pettersson *et al.*, 1984; Mimori *et al.*, 1984).

At least 10 polypeptides are associated with snRNAs from which U1 RNP particles contain at least nine, with mol. wts. of 70 000 (70 K), 33 000 (A), 29 000 (B'), 28 000 (B), 22 000 (C), 16 000 (D) and a triplet around mol. wt. 12 000 (E, F and G, Lerner and Steitz, 1979; Brunel *et al.*, 1984; Billings and Hoch, 1984; Pettersson *et al.*, 1984). Polypeptides 70 K, A and C have recently been reported to be present only in U1 RNPs (Pettersson *et al.*, 1984; Billings and Hoch, 1984) whereas B/B', D, E, F and G are associated with U1–U6 RNAs. Immunopurified U2 RNPs contain at least one additional polypeptide call-

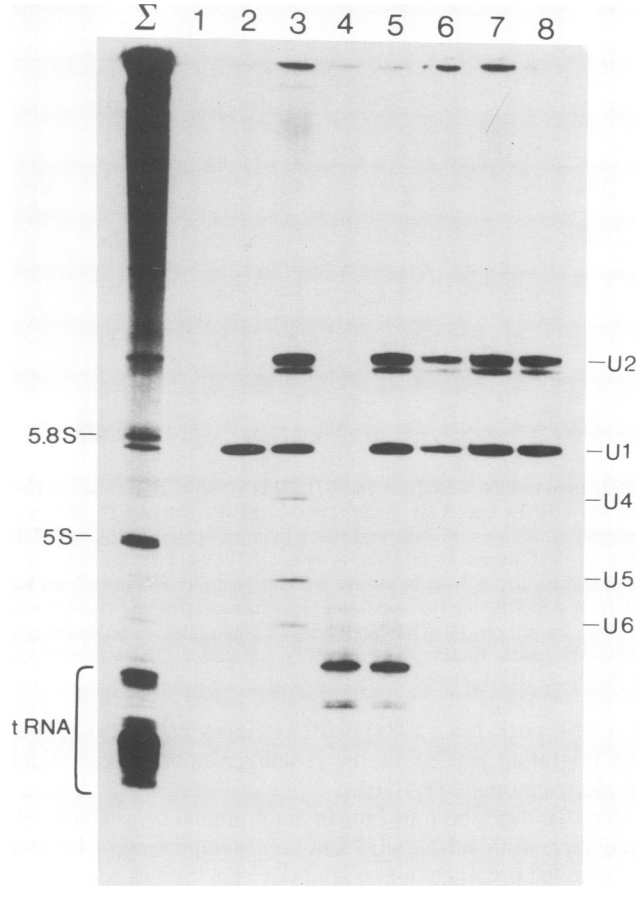

Fig. 1. Polyacrylamide gel fractionation of ^{32}P-labeled immunoprecipitated RNAs. Precipitates were obtained from a total HeLa cell extract (**lane Σ**) using normal human serum (**lane 1**), anti-(U1)RNP serum (**lane 2**), anti-Sm serum (**lane 3**), anti-La serum (**lane 4**) and four different anti-(U1,U2)RNP sera from patients B25, G18, V26 and P21, respectively (**lanes 5–8**).

Table I. Immunodiffusion and immunofluorescence data obtained with the four anti-(U1,U2) RNP sera, and patients' clinical data

Patient	Sex	Age	Diagnosis[a]	Immunofluorescence[b]			Immunodiffusion[c]		
				cyt	nucl	nucleol	Sm	(U1)RNP	La
B25	F	52	RA + SS	−	++	−		+	+
G18	F	19	MCTD	−	++	+/−		+	
V26	F	50	SLE	+	++	+/−	+	+	
P21	F	34	SLE	−	++	+/−		+	

[a]RA = rheumatoid arthritis; SS = Sjögrens syndrome; MCTD = mixed connective tissue disease; SLE = systemic lupus erythematosus.
[b]cyt = cytoplasmic immunofluorescence pattern; nucl = fine speckled nuclear immunofluorescence pattern; nucleol = nucleolar immunofluorescence pattern; +/− denotes a signal at the limit of detection. + denotes a standard signal; ++ denotes a strong signal.
[c]+ Indicates a (partial) identity with a reference serum in immunodiffusion tests.

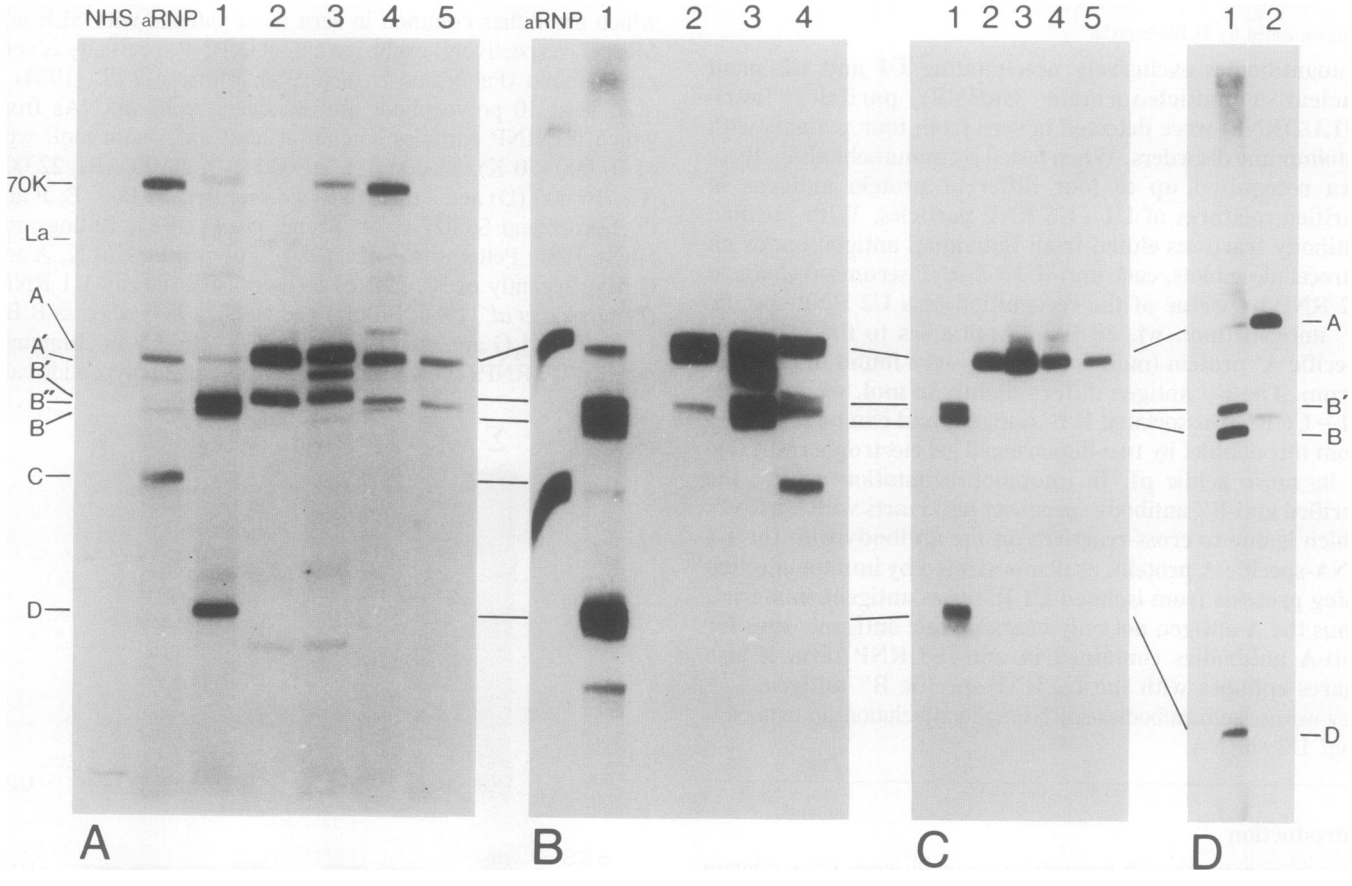

Fig. 2. Immunoblot analysis of anti-(U1,U2)RNP sera. Nitrocellulose blots contained HeLa total nuclear proteins (**panels A** and **D**), anti-m_3G purified U1 − U6 snRNPs (**panel B**) or purified U1 snRNPs (**panel C**). All blot strips presented in one panel originate from the same blot (see Materials and methods). Strips were probed with normal human serum (NHS), anti-(U1)RNP serum (aRNP), anti-Sm serum (**lanes 1**) or the anti-(U1,U2)RNP sera B25 (**lanes 2**), V26 (**lanes 3**), G18 (**lanes 4**) and P21 (**lanes 5**). Immunoreactive proteins were detected using ^{125}I-labeled protein A. The protein blots shown in **panel D** differ from those in **panels A − C** in that they originate from high resolution (40 cm) 13% polyacrylamide gels. The blot strip incubated with serum P21 from **panel B** was lost due to an experimental error and could not be repeated because more serum was not available. Sera V26 and G18 also recognized the 70-K antigen on (U1)RNP blots (**panel C**) as visualized on autoradiograms after a four times longer exposure. However, the C antigen recognized by serum G18 (**panel B, lane 4**) could not be detected in these longer exposures, probably due to some loss of this antigen during the further purification of U1 RNPs on DEAE-Sepharose.

ed A′ (Mimori *et al.*, 1984) or P27 (Kinlaw *et al.*, 1982, 1983), both migrating just below the A antigen upon polyacrylamide gel electrophoresis (PAGE).

Here we describe four human autoimmune sera which selectively react with snRNPs U1 and U2. Precipitation of U2 RNPs is due to the presence of two antibody specificities reactive with U2 RNP-specific polypeptides of mol. wt. 31 000 (A′) in one serum and of mol. wt. 28 500 (B′′) in all four sera. Interestingly this latter antibody specificity cross-reacts with the U1 RNP-specific A protein demonstrating that both antigens share at least one common epitope.

Results

RNA immunoprecipitation

From 400 sera of patients with connective tissue diseases, four sera were selected that precipitated snRNP species U1 and U2 from a ^{32}P-labeled HeLa cell extract (Figure 1, lanes 5 − 8). As a reference, sera with previously described specificities are shown; anti-(U1)RNP (lane 2) and anti-Sm antibodies (lane 3) precipitate U1 RNA and U1 − U6 RNAs, respectively, whereas anti-La antibodies (lane 4) precipitate RNAs of ~95 nucleotides representing tRNA precursors. Immunoprecipitates obtained from

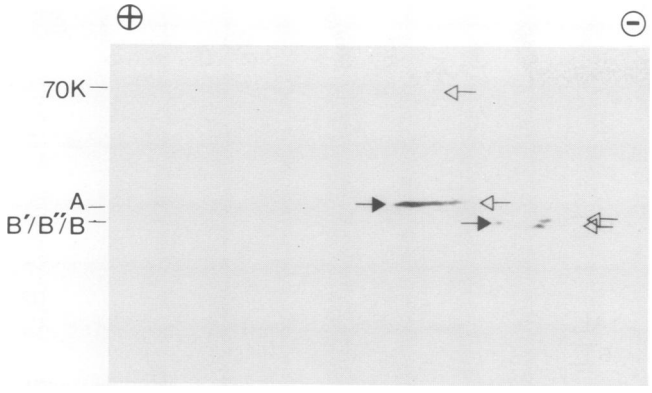

Fig. 3. Two-dimensional protein blots. Total proteins from nuclear extracts of HeLa cells were separated in the first dimension by non-equilibrated-pH-gradient electrophoresis (NEPHGE, + = acidic, − = basic side) which was followed by separation in the second dimension by SDS-PAGE (15%) (O'Farrell *et al.*, 1977). Immunostaining was performed with anti-(U1,U2)RNP serum B25 and peroxidase-conjugated anti-human IgG (filled arrow). Subsequently the same blot was incubated with equal amounts of anti-(U1)RNP and anti-Sm sera, followed by staining with peroxidase-conjugated anti-human IgG (open arrow).

^{32}P-labeled Ehrlich ascites mouse cells revealed identical patterns (not shown), supporting the observations that the structure of the U snRNA-associated proteins are highly conserved during evolution. Serum from patient B25 also contains anti-La antibodies (Figure 1, lane 5), whereas the other three anti-(U1,U2)RNP sera (sera G18, V26 and P21) exclusively precipitate U1 and U2 RNA. Even upon a six times longer exposure of autoradiograms, no other discrete low mol. wt. RNA species could be detected in immunoprecipitates obtained with these sera.

Immunodiffusion and immunofluorescence data obtained with the four anti-(U1,U2)RNP sera, together with some clinical data of the patients are listed in Table I.

Immunoblotting

Identical nitrocellulose strips from electroblots of proteins from a total nuclear extract of HeLa cells (Habets *et al.*, 1983a) were probed with the four anti-(U1,U2) sera. As is shown in Figure 2A, these sera share reactivity with two polypeptides, one of which is located in the region of the B/B′ antigens (and which will be termed B″ hereafter), the other very heavily stained antigen being identical with the U1 RNA-associated A antigen (see below). Sera G18 and V26 also contain antibodies against the 70 K U1 RNA-specific antigen while serum V26 reacts with a single polypeptide of mol. wt. 31 000 (A′) as well. Serum B25 reacts with the La antigen [(mol. wt. 50 000) Habets *et al.*, (1983b)] consistent with the finding that B25 precipitated LaRNAs from ^{32}P-labeled HeLa extracts (see Figure 1, lane 5).

To investigate which of the antigens recognized by these anti-(U1,U2)RNP sera actually were associated with snRNAs, purified (U1 − U6) snRNP particles, isolated by immuno-affinity chromatography with anti-2,2,7-trimethylguanosine (m_3G) IgGs, were used as a source of antigen for immunoblotting. These experiments demonstrated that the B″, A′ and A antigens identified by anti-(U1,U2)RNP sera indeed represent proteins of snRNP particles (Figure 2B). Intensities of individual bands on the autoradiograms vary among the blots obtained with total nuclear extract and purified snRNPs. This is probably due to differences in the concentration of the respective proteins in these two sources of antigenic material. Figure 2C shows the immunoblotting patterns of the same sera when purified U1 RNP particles were used as source of antigen. In this case the four sera only recognized the A antigen and no reaction with the proteins A′ or B″ could be observed.

These results demonstrated that the B″ antigen is not contained in purified U1 RNP particles and suggested further that the B″ protein is distinct from the proteins B and B′, a notion which is corroborated by the data shown in Figure 2D. In a high resolution gel the B″ protein can be shown to have an electrophoretic mobility in between the U1 − U6 RNA-associated proteins B and B′.

Two-dimensional protein blots

Two-dimensional (2D) gels from a total nuclear HeLa extract were blotted onto nitrocellulose sheets, probed with serum B25 and immunostained with peroxidase-conjugated anti-human IgG. Due to the limited amount of antigen that can be applied to the first dimension polyacrylamide gel (NEPHGE), only the dominant antigens A and B″ could be visualized on 2D blots (Figure 3, filled arrow). The same blot was subsequently reacted with an anti-Sm and an anti-(U1)RNP serum. Antigens recognized by these sera are marked by an open arrow in Figure 3. The A antigen recognized by serum B25 appeared as a heterogeneous smear of slightly basic pI and no extra spots showed up in this region after incubation with an anti-(U1)RNP serum. The same result was obtained when the order of incubation of the 2D blots with the sera was reversed, indicating that the same A polypeptide(s) are recognized by anti-(U1)RNP and the anti-(U1,U2)RNP sera. When probed with an anti-Sm serum, the B/B′ antigens always appeared as a quadruplet of spots on 2D blots. Subsequent incubation of the blot with an anti-(U1,U2)RNP serum showed that the B″ antigen had a more acidic pI, corroborating the idea that the B″ antigen is distinct from the B/B′ proteins.

Reactivity of antibody specificities eluted from individual protein bands of immunoblots

To trace the antibodies that contribute to the precipitation of U2 snRNA, antibodies bound to individual bands on nitrocellulose blots were eluted and directly used for immunoblotting and immunoprecipitation of snRNPs from extracts of ^{32}P-labeled HeLa cells.

As a control, we purified antibodies from a blot incubated with a serum containing anti-Sm as well as anti-(U1)RNP antibodies. In agreement with earlier observations (Habets *et al.*, in preparation), purified anti-70 K, anti-A and anti-C antibodies precipitated exclusively U1 RNP, whereas anti-B/B′ and anti-D antibodies recognized U1 − U6 RNPs (Figure 4A, lanes 8 − 14). When protein blot strips were probed with each of the above purified antibody fractions, antibodies eluted from protein bands 70 K, A and C reacted selectively with the corresponding protein antigen from which they had been eluted. Antibodies eluted from protein bands B, B′ or D, however, reacted with all of the three antigens on immunoblots demonstrating that these antigens share common epitopes (Figure 4A, lanes 1 − 7).

Antibody specificities contained in serum B25 were also purified from the several reactive regions on a total nuclear protein blot. Figure 4B shows that antibodies eluted from the mol. wt. 50 000 region precipitate La RNAs (lane 7) and that anti-A antibodies from this particular serum selectively react with U1 RNP (lane 8). Surprisingly, anti-B″ antibodies precipitated both U1 and U2 RNPs (lane 9). This was unexpected in view of our finding that the B″ protein is not contained in purified U1 snRNP particles (see Figure 2C). On inspection of the immunoblots obtained with purified anti-B″ antibodies, however, the co-precipitation of both U1 and U2 RNPs by this antibody specificity can be explained: anti-B″ antibodies cross-react with the U1 RNP-specific A protein (Figure 4B, lane 4). Thus these two pro-

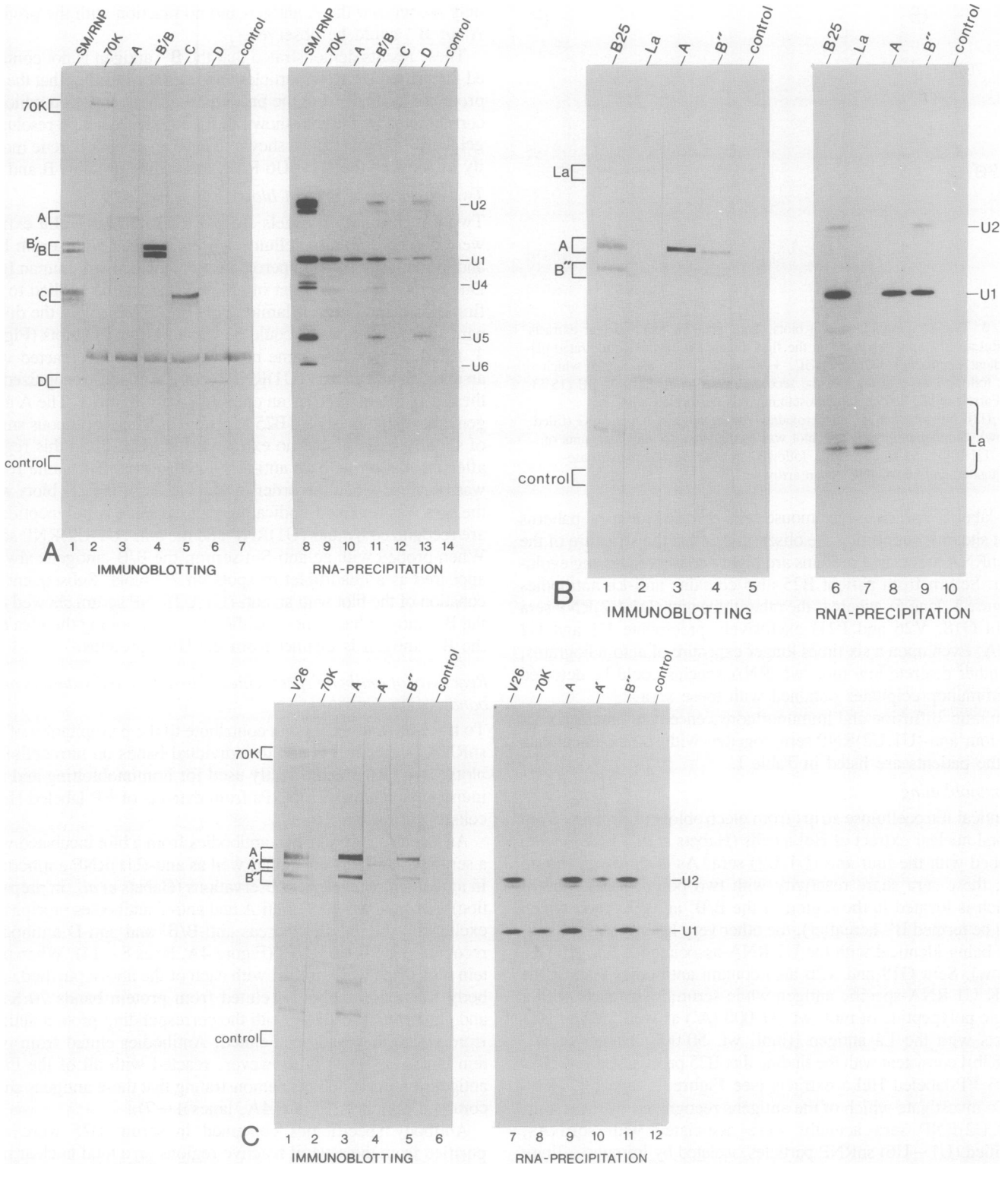

Fig. 4. Purification of antibodies from nitrocellulose blots. HeLa total nuclear protein blots were incubated with an anti-Sm/RNP serum (**panel A**), anti-(U1,U2)RNP sera B25 (**panel B**) or V26 (**panel C**). The reactive regions marked on the left of each panel were excised and bound antibodies were eluted (Smith and Fisher, 1984). These fractions were re-used for immunoblotting or RNA precipitation as indicated on the top of each lane. Immunoblots of purified antibodies were stained for 15 min whereas strips incubated with whole serum (**lanes 1**) were stained for 5 min.

teins must share at least one common epitope. Purified anti-A antibodies react exclusively with the A antigen (Figure 4B, lane 3) and eluted anti-La antibodies show a monospecific reaction with the La antigen of mol. wt. 50 000 (lane 2). No reaction was found when a control region of the nitrocellulose blots was extracted and the eluted fraction was used for immunoblotting and RNA immunoprecipitation (lanes 5 and 10).

The same elution experiments were also performed with serum V26. Anti-70 K antibodies again precipitated exclusively U1 RNP (Figure 4C, lane 8), but in contrast with the findings in serum

B25, anti-A antibodies purified from serum V26 precipitated U1 and U2 RNA (Figure 4C, lane 9) and reacted with A as well as B'' on protein blots (Figure 4C, lane 3). Purified anti-A' antibodies precipitated predominantly U2 RNPs (Figure 4C, lane 10) in accordance with the previous demonstration that the A' protein is unique to U2 RNP particles (Mimori *et al.*, 1984). The co-precipitation of the low amounts of U1 RNPs observed with the anti-A' antibody is most probably due to contamination with anti-A antibodies from the nearby A protein band. In fact some reaction with the A protein is also observed with the A'-specific antibody fraction on immunoblots (Figure 4C, lane 4). Such dual contamination can be avoided if proteins are separated in high resolution gels (data not shown). Anti-B'' antibodies reacted in the same way as those purified from serum B25, i.e., they selectively precipitate snRNPs U1 and U2 (Figure 4C, lane 11) and cross-react with the A antigen on protein blots (Figure 4C, lane 5).

Discussion

This paper describes a novel autoantibody specificity reacting with proteins associated with snRNAs U1 and U2 which we detected in four out of 400 sera of patients with connective tissue diseases. The four sera precipitated selectively the snRNAs U1 and U2 which excluded the presence of anti-Sm antibodies, known to react with proteins common to all nucleoplasmic snRNPs (Lerner and Steitz, 1979; Pettersson *et al.*, 1984). When total proteins of purified U1 – U6 snRNPs were used as antigenic material for immunoblotting studies, all four sera showed reactivity with the U1 RNP-specific A protein and a second polypeptide of mol. wt. 28 500 (B'' protein, Figure 2). This protein could be shown to be distinct from the B and B' antigens and not to be contained in purified U1 RNP particles (Figure 2C), indicating that the B'' protein is unique to U2 RNPs and that precipitation of U2 RNPs was due to B''-specific autoantibodies in the four sera. In this respect it was surprising that purified B''-reactive antibodies not only precipitated U2 RNPs, but also recognized U1 RNPs (Figure 4B, lane 9; 4C, lane 11). This suggested that co-precipitation of U1 RNPs was either due to interaction of U1 with U2 RNPs *in vitro*, or due to cross-reaction of B''-reactive antibodies with one of the U1-specific polypeptides. The latter possibility is the more likely one as we could demonstrate cross-reactivity of anti-B'' antibodies with the A polypeptide (Figure 4B, lane 4; 4C, lane 5). At least with serum V26 the reverse was also found to be true, i.e., antibodies eluted from the A band cross-reacted with the B'' antigen and precipitated both U1 and U2 RNPs (Figure 4C, lane 9). This clearly demonstrates that both polypeptides have at least one antigenic determinant in common.

It should be pointed out that the A antigen also bears antigenic sites which are unique to this protein and which are recognized by anti-A antibodies contained in anti-(U1)RNP sera. The presence of this antibody specificity in serum B25 would explain our finding that the purified A-reactive antibody fraction did not cross-react with the B'' polypeptide though this serum contains antibodies reacting with antigenic sites common to both proteins (Figure 4B, lane 3). It would further suggest that recognition of the respective antigenic sites on the A polypeptide by the two classes of autoantibodies is mutually exclusive.

The observed immunological cross-reactivity between the polypeptides A and B'' is a further example to demonstrate structural relatedness between snRNP proteins encompassed in distinct snRNP particles. It has previously been shown that the proteins B, B' and D react with the same monoclonal antibody, indicating that these antigens also share common epitopes (Mimori *et al.*, 1984; Pettersson *et al.*, 1984).

We characterized the B'' antigen as a 28 500 mol. wt. polypeptide migrating in between the B' and B antigen upon PAGE, while exhibiting a more acidic pI upon 2D gel electrophoresis. This antigen is probably identical with a third B polypeptide, occasionally observed as a very faint band in ^{35}S-labeled immunoprecipitates obtained with anti-Sm sera (Billings and Hoch, 1984; Pettersson *et al.*, 1984). Further fractionation of these U1 – U6 snRNP precipitates indeed revealed that this third B polypeptide co-purified with U2 RNP (Hinterberger *et al.*, 1983; Mimori *et al.*, 1984).

Sequential immunoaffinity chromatography utilizing successively the anti-m_3G antibody, anti-(U1,U2)RNP serum V26 and a patient anti-(U1)RNP serum enabled us to select highly pure U1 and U2 RNP moieties. Protein analysis of these purified fractions support the conclusions drawn above; the A protein is exclusively contained in the U1 RNP fraction while B'' is specific for U2 RNP (P.Bringmann and R.Lührmann, in preparation).

Very recently, the anti-(U1,U2)RNP serum P21 described in this paper was used to determine the binding site of specific snRNP proteins on mutant U2 RNAs (Mattaj and De Robertis, 1985). From their results and the immunoblotting profiles of serum P21 (Figure 2, lanes 5) it can be deduced that it is most probably the B'' antigen which interacts with the two 3' loops of the U2 RNA molecule.

All these results strongly suggest that the B'' antigen is a unique polypeptide specifically associated with U2 RNA. The question of whether this protein is a primary translation product or a result of post-translational modification (from one of the other snRNP-associated proteins) can only be answered unequivocally when the genes coding for snRNP proteins have been cloned.

One anti-(U1,U2)RNP serum also decorated a polypeptide on snRNP immunoblots migrating just below the A protein (referred to as A'). Recently, Mimori *et al.* (1984) used a human autoimmune serum (Ya) to identify this A' protein as a methionine-deficient, exclusively U2 RNA-associated polypeptide. We have several reasons to assume that the A' antigen recognized by serum V26 is identical with the polypeptide identified by the Ya serum. Both antigens exhibit identical mol. wts. when sera V26 and Ya were probed on blots (not shown) and, as is the case with the Ya serum, serum V26 recognizes the A' antigen only on protein blots containing U1 – U6 RNPs and not in purified U1 RNP blots. Moreover, anti-A' antibodies purified from serum V26 recognize almost exclusively U2 RNP particles.

In contrast with anti-(U1)RNP and anti-Sm antibodies, the occurrence of anti-(U1,U2)RNP antibodies does not seem to be syndrome-specific (Table I), and is therefore of little diagnostic value. However, these sera might serve as an important tool in the fractionation of snRNP into its individual constituents and so greatly facilitate the investigations on their function and structure.

Materials and methods

Reference sera

Immunological identity of sera was tested by immunodiffusion and counter-immunoelectrophoresis using extractable nuclear antigen (ENA, Kurata and Tan, 1976) and reference sera obtained from the Centers of Disease Control (CDC) in Atlanta, GA. Human sera were further obtained from patients treated at the St. Radboud Hospital in Nijmegen, the Hospital Stadsmaten in Enschede and the Dijkzigt Hospital, Rotterdam, The Netherlands, and from the Centre Hospitalier, Luxembourg. Ya serum was a kind gift of Dr. T.Mimori (New Haven, CT). Clinical data of the patients from which anti-(U1,U2)RNP sera were obtained are listed in Table I. Monoclonal anti-Sm and anti-(U1)RNP antibodies

(Billings and Hoch, 1984; Billings *et al.*, 1982) were used as references in RNA-immunoprecipitation assays (see below).

Preparation of radiolabeled cell extracts

HeLa S3 cells were maintained in suspension culture as described (van Eekelen and van Venrooij, 1981).

For RNA analyses, 1.5 x 10^8 HeLa cells were labeled for 15 h with [^{32}P]-phosphate (30 μCi/ml) at 5 x 10^5 cells/ml in phosphate-free essential medium supplemented with 10% dialysed fetal calf serum. After harvesting, cells were washed twice with PBS (20 mM Na-phosphate pH 7.3, 130 mM NaCl) and resuspended in 3 ml sonication buffer (100 mM NaCl, 1 mM EDTA, 20 mM K-phosphate buffer, pH 7.5). The suspension was sonicated 2 x 60 s at setting 3 of a Branson sonifier and clarified by centrifugation at 10 000 *g* for 30 min. The supernatant was used either immediately for immunoprecipitation assays or stored at −70°C.

RNA immunoprecipitation assay

Analysis of immunoprecipitated RNA was based on the method described by Matter *et al.* (1982). Non-specifically binding material in ^{32}P-labeled cell extracts was pre-adsorbed by incubation with one volume of 10% protein A-Sepharose in IPP and two volumes of IPP (500 mM NaCl, 10 mM Tris pH 7.5, 0.05% Nonidet P40) for 1 h at 4°C. Meanwhile, 10 μl serum was adsorbed on 250 μl 10% protein A-Sepharose in PBS and washed five times with IPP. After centrifugation, the pellet was incubated with 400 μl of the pre-adsorbed ^{32}P-labeled cell extract by end-over-end rotation for 1 h at 4°C, and washed five times with IPP. After the final wash, the pellet was re-suspended in NET-2 (150 mM NaCl, 50 mM Tris-HCl pH 7.4, 0.05% Nonidet P40) and 20 μl of 10% SDS was added. After phenol extraction, RNAs were ethanol-precipitated, separated by electrophoresis on 10% polyacrylamide gels containing 7 M urea, 0.1 M Tris-borate pH 8.3 and 2 mM EDTA and detected by autoradiography.

Affinity purification of snRNPs

Anti-m_3G affinity columns were used to purify U1 − U6 RNPs from nuclear extracts of HeLa cells (Bringmann *et al.*, 1983, 1984). From this fraction U1 RNPs were purified according to Hinterberger *et al.* (1983) using DEAE-Sepharose chromatography.

Immunoblotting

For blotting analyses, either purified snRNPs or a total nuclear protein fraction (Habets *et al.*, 1983a, 1983b) was used. An appropriate amount of protein was dissolved in 2% SDS-containing sample buffer and loaded over the entire width of a 13% polyacrylamide gel (Laemmli, 1970). After electrophoresis, gels were blotted overnight onto nitrocellulose in a Bio-Rad trans-blot cell at 60 V/0.3 A in 192 mM glycine, 25 mM Tris pH 8.3 and 20% methanol. After transfer, the blots were dried and cut from top to bottom into strips of ~7 mm. Individual strips were saturated with 3% BSA and incubated with serum as described (Habbets *et al.*, 1983a, 1983b). Antigen-bound antibody specificities were visualized either with [^{125}I]protein A or with horseradish peroxidase-conjugated second antibody (Habets *et al.*, 1985).

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References

Berget,S.M. (1984) *Nature*, **309**, 179-182.
Billings,P.B. and Hoch,S.O. (1984) *J. Biol. Chem.*, **259**, 12850-12856.
Billings,P.B., Allen,R.W., Jensen,F.C. and Hoch,S.O. (1982) *J. Immunol.*, **128**, 1176-1181.
Bringmann,P., Rinke,J., Appel,B., Reuter,R. and Lührmann,R. (1983) *EMBO J.*, **2**, 1129-1135.
Bringmann,P., Appel,B., Rinke,J., Reuter,R., Theissen,H. and Lührmann,R. (1984) *EMBO J.*, **3**, 1357-1363.
Brunel,C., Sri-Widada,J. and Jeanteur,P. (1984) *Prog. Mol. Subcell. Biol.*, in press.
Calvet,J.P. and Pederson,T. (1981) *Cell*, **26**, 363-370.
Calvet,J.P., Meyer,L.M. and Pederson,T. (1982) *Science (Wash.)*, **217**, 456-458.
Gallinaro,H. and Jacob,M. (1979) *FEBS Lett.*, **104**, 176-182.
Habets,W.J., de Rooij,D.J., Salden,M.H., Verhagen,A.P., van Eekelen,C.A.G., van de Putte,L.B. and van Venrooij,W.J. (1983a) *Clin. Exp. Immunol.*, **54**, 265-276.
Habets,W.J., den Brok,J.H., Boerbooms,A.Th., van de Putte,L.B.A. and van Venrooij,W.J. (1983b) *EMBO J.*, **2**, 1625-1631.
Habets,W.J., de Rooij,D.J., Hoet,M.H., van de Putte,L.B. and van Venrooij,W.J. (1985) *Clin. Exp. Immunol.*, **59**, 457-466.
Hinterberger,M., Pettersson,I. and Steitz,J.A. (1983) *J. Biol. Chem.*, **258**, 2604-2613.
Kinlaw,C.S., Dusing-Swartz,S.K. and Berget,S.M. (1982) *Mol. Cell. Biol.*, **2**, 1159-1166.
Kinlaw,C.S., Robberson,B.L. and Berget,S.M. (1983) *J. Biol. Chem.*, **258**, 7181-7189.
Krämer,A., Keller,W., Appel,B. and Lührmann,R. (1984) *Cell*, **38**, 299-307.
Kurata,N. and Tan,E.M. (1976) *Arthritis Rheum.*, **19**, 574-581.
Laemmli,U.K. (1970) *Nature*, **227**, 680-683.
Lerner,M.R. and Steitz,J.A. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 5495-5499.
Lerner,M.R. and Steitz,J.A. (1981) *Cell*, **25**, 298-301.
Mattaj,I.W. and De Robertis,E.M. (1985) *Cell*, **40**, 111-118.
Matter,L., Schopfer,K., Wilhelm,J.A., Nijffenegger,T., Parisot,R.F. and De Robertis,E.M. (1982) *Arthritis Rheum.*, **25**, 1278-1289.
Mimori,T., Hinterberger,M., Pettersson,I. and Steitz,J.A. (1984) *J. Biol. Chem.*, **259**, 560-565.
O'Farrell,P.Z., Goodman,H.M., and O'Farrell,P.H. (1977) *Cell*, **12**, 1133-1142.
Ohshima,Y., Itoh,M., Okada,N. and Miyata,T. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 4471-4474.
Padgett,R.A., Mount,S.M., Steitz,J.A. and Sharp,P.A. (1983) *Cell*, **35**, 101-107.
Pettersson,I., Hinterberger,M, Mimori,T., Gottlieb,E. and Steitz,J.A. (1984) *J. Biol. Chem.*, **259**, 5907-5914.
Reddy,R. and Busch,H. (1983) *Proc. Nucleic Acid Res. Mol. Biol.*, **30**, 127-162.
Sekeris,C.E. and Niessing,J. (1975) *Biochem. Biophys. Res. Commun.*, **62**, 642-650.
Smith,D.E. and Fisher,P.A. (1984) *J. Cell Biol.*, **99**, 20-28.
Steitz,J.A., Wolin,S.L., Rinke,J., Pettersson,I., Mount,S.M., Lerner,E.A., Hinterberger,M. and Gottlieb,E. (1983) *Cold Spring Harbor Symp. Quant. Biol.*, **47**, 893-900.
Tan,E.M. (1982) *Adv. Immunol.*, **33**, 167-240.
Van Eekelen,C.A.G. and van Venrooij,W.J. (1981) *J. Cell Biol.*, **88**, 554-562.
Yang,V.W., Lerner,M.R., Steitz,J.A. and Flint,S.J. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 1371-1375.

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