Review Letter

# THE COMPOSITION OF THE NUCLEAR ANTIGENS Sm AND RNP OF HUMAN RHEUMATIC AND CONNECTIVE TISSUE DISEASES AND THE RELEVANCE OF THEIR AUTOANTIBODIES AS PROBES FOR RNA PROCESSING MECHANISMS

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## 1. Introduction

The human rheumatic and connective tissue diseases are a group of conditions which display autoimmune properties, many of the serum antibody activities being directed against components of the cell nucleus [1,2]. Although antibodies to DNA are characteristic of these conditions, it is of clinical importance that activities against the so-called soluble nuclear protein-containing antigens have been found to act as markers for specific diseases [1,2]. The demonstration [3–6] that several of these nuclear antigens are ribonucleoprotein particles containing small nuclear RNA species (snRNA) has also led to an interest in the use of their specific antibodies due to the proposal that snRNAs may be involved in mechanisms for 'splicing' mRNA precursors in the cell nucleus [4,5,7].

Specifically, Lerner et al. ([3,4,6,8,10], reviewed in [7]) have investigated the composition of the nuclear antigens known as Sm, RNP and La (or Ha), antibodies to which are characteristic of systemic lupus erythematosus, mixed connective tissue disease and Sjogren's syndrome, respectively. These workers found that immune complexes containing Sm or RNP antigens which had been prepared in vitro possessed the same set of seven proteins ($M_r$ 12 000–35 000), but differing types of snRNAs, viz. $U_{1,2,4,5,6}$(Sm) and $U_1$(RNP). La antigen was found to be associated with a heterogeneous group of small RNAs, including several coded by infecting DNA viruses [6,8]. However, several other laboratories have found the composition of the Sm and RNP antigens to differ significantly from those in [3,7] (see [9]). We review here the current evidence which suggests a situation that is more complex than that envisaged in [7].

## 2. Analysis of Sm and RNP antigens as RNA–protein complexes

Details of the reports of the composition of Sm and RNP antigens are summarised in table 1. Analyses have been carried out on antigens obtained by procedures which range from various forms of preparative immunoaffinity chromatography to the precipitation of immune complexes using *Staphylococcus* protein A. Our own approach has been to form immunoprecipitates under conditions of equivalence for each antigen, followed by analysis of their protein and RNA contents by polyacrylamide gel electrophoresis.

### 2.1. *Sm*

Table 1 shows that several laboratories have been able to confirm that the proteins associated with this antigen are within $M_r$ 12 000–14 000, but exceptions of qualitative [17–19] and quantitative natures exist, e.g., the lower $M_r$ polypeptides are common to most studies but they are reported as representing either minor [4] or major [13,19] components. By analysing specific immunoprecipitates we have found that the major proteins associated with Sm are of $M_r \sim 10\,000$ (4 components) and ~12 000 (1 component) [9]. Several authors have reported the presence of a protein of $M_r$ 26 000 in Sm (and RNP) but this would not be seen in the presence of IgG light chains in SDS–polyacrylamide gels [9]. Although there is not complete qualitative agreement amongst the investigations of Sm, components similar to the 5 proteins

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Table 1
Composition of Sm and RNP antigens

| Antigen source | Precursor | | Antigen preparation | Sm | | RNP | | Ref. |
|---|---|---|---|---|---|---|---|---|
| | Protein | RNA | | Protein ($M_r \times 10^{-3}$) | snRNA species | Protein ($M_r \times 10^{-3}$) | snRNA species | |
| Hela<br>Ehrlich<br>Ascites<br>Friend cells | [$^{35}$S]Met | $^{32}$P | IC + *Staphylococcus* protein A | 12 (×3, minor),<br>16 (minor),<br>20, 26, 35 | $U_{1a/b}$, 2, 4, 5, 6 | 12 (×3, minor),<br>16 (minor)<br>20, 26, 35 | $U_{1a/b}$ | [3,4, 7,10] |
| HeLa | [$^{35}$S] | $^{32}$P | IP | | | 45, 48, 52, 57, 60 (+ >60, <40) | $U_1$ (+ minor 4 S–5 S RNAs) | [12] |
| Friend cells | [$^3$H]amino acids | [$^3$H]uridine | IP + *Staphylococcus* protein A | <10, 12–14 (×2), 26, 30 | $U_{1,6}$, 5 S, >4 S, <4 S (×3) | <10, 12–14 (×2), 26, 30 | $U_1$, 2, 6, 5 S, <4 S | [13] |
| Rabbit thymus | | | IP | 40 | | | | [14] |
| Pig thymus | | | IP | 10 (×4), 12 | <5 S–>4 S (×10) | 10 (×3), 12, 30 | <5 S–>4 S (×10) | [9] |
| Rabbit thymus | | | IAC | 7 species (11 000–70 000) | n.d. | 7 species (11 000–70 000) | n.d. | [15] |
| Rabbit thymus | | | IAC | 11 species (10 000–42 000) | n.d. | 5 species (10 000–15 000) | n.d. | [16] |
| Rabbit thymus | | | IAC | 50 | | | | [17] |
| Calf thymus | | | IAC | | | 12–13 (×4), 30, 65 | >4 S (×2), <4 S (×2) | [18] |
| Calf thymus | | | IAC | 13 (×4) | Ω4 S (3 minor species) | 13 (×4), 30, 65 | Ω4 S (1 major and 4 minor) | [19] |
| Rat liver | | | IAC | | | 13, 30 | n.d. | [20] |
| Rabbit thymus | | | IAC | 9, 10, 11, 13, 26, 30, 34, 38, 44 | Preparation contains both Sm and RNP | | $U_1$, 4 S (?) <4 S + $U_2$ (minor) | [21] |

*Abbreviations:* IAC, = immuno affinity chromatography; IC, = immune complex; IP, = immunoprecipitate; n.d., not determined

above have been reported by others, including Lerner and Steitz [4], provided allowance is made for differences in estimates of $M_r$-value.

However, the greatest discrepancy in analysis concerns the RNA content of Sm since various combinations of known and unidentified small RNA species have been reported (table 1). Our own results show that Sm antigen from pig thymus is associated with some 10 small RNAs migrating in polyacrylamide gel electrophoresis between 4 S and 5 S RNA [9]. Of the other laboratories which have also found similar species, two have used thymus as a source of antigen [18,19,21].

2.2. *RNP*

As indicated in table 1, a general qualitative similarity has been found amongst the analysis of the proteins of RNP antigens prepared in various laboratories and with those of Sm antigen. The striking exception to this overall view is the difference in analyses of HeLa RNP found in [4,12]. Using preparations and procedures apparently identical to those in [4], the latter group identified 5 proteins ($M_r$ 45 000–60 000) as being associated with RNP antigen (although significant amounts of proteins with $M_r > 60\,000$ and $<40\,000$, including a 10 000 $M_r$ group, were also present). The low $M_r$ group (10 000–12 000) is common to most studies and in some cases [19] the RNP antigen is reported to have at least one extra polypeptide. The latter aspect is confirmed in our own investigations in which we found the RNP antigen of pig thymus to be associated with a protein of $M_r$ 30 000 which was not found in similar analysis of Sm antigen [9]. Where qualitative differences have been reported, these tend to refer to high $M_r$ proteins (cf. [4] and [12,18–20]).

Lerner et al. [3,4,6,7] and Lenk et al. [12] have shown that RNP antigen from several cell sources contains only $U_1$ as the dominant snRNA species, yet other laboratories find that in their preparations either the $U_1$ is accompanied by identified plus unidentified snRNAs [13] or the RNA consists only of very small species [9,18,19]. As in the case of Sm, it is significant that the very small RNA molecules are obtained when RNP is prepared from thymus nuclei [9,18,19,21]. However, it should be noted that extended exposure of autoradiograms of RNA gels containing RNP-immunoprecipitates showed the presence of at least 2 additional small RNA components with mobilities between 4 S and 5 S RNA [12].

2.3. *Further investigations*

In view of the apparent discrepancies between our results [9] and those of others listed in table 1, we have carried out the following checks (A. R. C., M. R. S., unpublished):

(1) We were able to show that, relative to those of rat liver nuclei, the snRNAs of pig thymus differed both quantitatively and qualitatively, $U_1$ and only trace amounts of the other species being present;
(2) Saline extracts containing Sm/RNP from rat liver nuclei showed $U_1$ as the dominant snRNA but this was not the case in similar preparations from pig thymus in which $U_2$ was also virtually absent;
(3) When such liver and thymus extracts were used to form immune complexes containing Sm/RNP according to [3], a wide range of RNA species was observed, that from liver containing $U_{1-6}$ snRNAs as well as large and very small RNAs. However, when complexes were formed from thymus extracts virtually no traces of known snRNAs were seen, the large and very small molecules predominating. The latter are probably those seen in immunoprecipitates of thymus Sm and RNP [9], whereas large components of unknown identity are a feature of most of the analysis quoted in table 1;
(4) We have found (in collaboration with Dr G. E. Blair, University of Leeds) that sera used in [9] will yield immune complexes from radioactively labelled HeLa cell extracts with compositions identical to those found in [7].

We conclude that, given the presence of only certain of the U-snRNAs in pig thymus, Sm and RNP antigens prepared from such cells are not associated with any significant amounts of these RNAs. Yet, if HeLa cell extracts are used, the same sera will precipitate snRNA–protein complexes similar to those reported by Lerner and Steitz [7].

## 3. Analysis of Sm and RNP antigens as determinants on isolated protein or RNA molecules

Table 2 summarises the results of investigations in which anti-Sm-RNP antibodies have been reacted with individual protein or RNA molecules. Two approaches have been used:

(1) Those using 'blotting' techniques whereby proteins from purified antigens are separated by gel

Table 2
Protein and RNA components of Sm and RNP showing antigenic properties

| Antibody | Antigen sources | Detection method | Antigen | | Ref. |
|---|---|---|---|---|---|
| | | | Protein ($M_r \times 10^{-3}$) | RNA | |
| Human *vs* Sm | HeLa | Protein blot | 26 | | [22] |
| Mouse MC *vs* Sm | | | 26 | | |
| Human *vs* RNP | Calf thymus | Haemagglutination Inhibition | (×2) 13 | 40, 60 nucleosides | [18] |
| Human *vs* RNP | Calf thymus | Haemagglutination Inhibition | | (RNP–RNA –ve) | [19] |
| | | Solid phase RIA | 30, 65 | | |
| Human *vs* Sm | | Solid phase RIA | 13 | | |
| Human *vs* RNP | Rabbit thymus | Protein blot | (13), 40, (×2) 70 | (Rat liver nuclear –ve) | [23] |
| | Calf thymus | | <6.5, 12, 13, (×2) 30 | | |
| Human *vs* Sm | Rabbit thymus | | 13 | | |
| | Calf thymus | | 13, ?30 | | |
| Mouse MC *vs* RNP | ? | Protein blot | 40, (×2) 70 | | [11] |
| Human *vs* RNP | Ehrlich Ascites cells | Immune complex | | (snRNAs –ve) | [3] |
| Human *vs* Sm | | Precipitation | | | |

*Abbreviations:* MC, monoclonal; RIA, radioimmunoassay

electrophoresis prior to transfer to a second matrix on which antigens are detected by double antibody or similar techniques;

(2) Those in which RNA or protein is recovered from electrophoresis gels and the presence of antigen determined by conventional immunoassays.

Once more there is little overall consistency in the results obtained by different laboratories, e.g., anti-Sm antibodies are found to react with either a 13 000 $M_r$ [19,23] or a 26 000 $M_r$ [22] protein. Of the reports in which RNA molecules were tested only one [18] showed evidence of reaction with antibody.

## 4. Correspondence between Sm and RNP antigens

Lerner and Steitz [7] view their data as indicating that anti-Sm/RNP antibodies recognise determinant sites on snRNP particles, each of the U-series of snRNA being complexed with proteins in specific particles. Should such a model be correct, then it implies that in the cells studied by Lerner and Steitz one snRNP particle ($U_1$-snRNP) possesses antigenic sites for both Sm and RNP (other snRNPs containing $U_{2,4,5,\text{ or }6}$ react only with anti-Sm antibodies). Examination of other data in table 1 suggest that, according to the model of Lerner and Steitz, both Sm and RNP determinants are found together in more than one type of snRNP particle in Friend erythroleukaemic and thymus cells. Previous work has also been interpreted as indicating that the antigens are part of the same nuclear complex [16,24], particularly the claims that on purification Sm and RNP can show properties of RNP and Sm, respectively [19,21]. In contrast both Jonsson's laboratory and our own have shown that Sm and RNP can be separated by chromatography of saline extracts of thymus and lymphocyte nuclei ([9,25]; M. R. S., unpublished). Such results can be interpreted as indicating that either hidden antigenic sites remain undisturbed under these conditions or the determinants are on separate complexes. The recent claims (table 2) that the antigenic sites for Sm and RNP have been located on isolated proteins makes the latter proposal feasible. As a result of these data

and those in tables 1 and 2 we feel that models other than that in [7] should be considered and this aspect will be given further consideration below.

## 5. Factors influencing antigen composition

The true identity of nuclear antigens to which patients have antibodies is obviously of importance not only in furthering our understanding of the connective tissue diseases but also in enabling their specific antibodies to be used with certainty as probes in investigations of RNA splicing mechanisms. Curiously, the analyses of Sm and RNP antigens carried out by various laboratories do not show a uniformly consistent pattern. In our opinion there are several major factors which can influence the composition of RNA–protein complexes with which anti-Sm/RNP antibodies will react.

### 5.1. *Detection of antigens*

Several authors have used antigens which have been radioactively labelled in vivo, whereas others use unlabelled extracts (table 1). Thus, the use of labelled material may bias subsequent analyses towards detecting proteins and RNA with a relatively rapid turnover or proteins of a specific amino acid composition. Could the detection of antigen protein by fluorography or staining explain the differences in relative abundance of the low $M_r$ protein species previously referred to?

The prevalence of anti-Sm and anti-RNP antibodies in human disease has been documented elsewhere, e.g., antibody titre and specificity may be determined by techniques such as haemagglutination, complement fixation, counterimmunoelectrophoresis and double diffusion in agar(ose) [26]. Such autoimmune sera are known to contain antibodies of the IgG class which are precipitating in nature. However, most investigators have used a limited number of sera obtained from quite separate, though clinically defined, groups of patients. There appears to have been little, if any, exchange of sera amongst laboratories for comparative purposes and techniques such as those described above show immunological identities that are probably due to properties of a gross nature compared to those seen using analysis of molecular constituents.

It is possible to conclude, therefore, that antibodies from individuals in the same disease class may recognise antigenic sites carried by different groups of proteins and RNAs. Indications of this nature have been shown in recent claims that individual small RNA species or protein components obtained from purified antigens can react with anti-Sm/RNP antibodies (table 2). These studies show little consistency in the type of protein detected as autoantigens and they suggest that other factors are at play. Admittedly the use of hybridoma techniques can allow selection of subsets of antibody, but the fact that apparently conflicting results can also be obtained with human autoimmune sera requires explanation.

In their comprehensive survey Steitz's laboratory (see [7,10]) found that a wide range of RNAs are in fact precipitable by individual sera. In addition, White and Hoch [23], using a 'blotting' technique, have observed significant differences in the proteins reacting with anti-Sm/RNP sera that are both of a species (for antigen) and patient (for antibody) origin. The striking differences found in the proteins associated with HeLa RNP antigen by two laboratories [4,12] have also been interpreted as indicating variations in the detailed specificity of anti-RNP sera from different patients [12]. Hence some degree of antibody specificity for Sm and RNP at a molecular level cannot be dismissed, even in sera from patients with the same clinically defined condition.

We have based our identification of the two antigens on immunoprecipitation, i.e., the criteria used in the original designation of the antigens [2]. Techniques which employ other means of immunological identification, e.g., 'protein-blotting' [22,23], immunoaffinity chromatography [15–21] or precipitation of immune complexes with *Staphylococcus* protein A [3,13] will detect or utilise non-precipitating as well as precipitating antibodies. Should the detailed specificities of these 2 categories of antibody differ then different methods of detection could play an important role in the analysis given in tables 1 and 2. It is important to note that no comprehensive investigation has been carried out of such features of antibodies found in these autoimmune diseases.

### 5.2. *Cell and tissue sources*

Sm and RNP are present in the nuclei of numerous (but not all) eukaryotic species [2,4,7,10]. The cell types used in the analyses given in table 1 consist of 3 main groups:

(i) Rapidly dividing tumour cells such as HeLa [4];
(ii) Virus-producing Friend erythroleukaemia mouse

cells undergoing erythropoietic differentiation [13];

(iii) Differentiated tissues undergoing significant (liver, [20]) or little (thymus, [9,18,19]) RNA synthesis.

It may be significant, therefore, that extreme variations in antigen composition are found in HeLa and thymus cells.

5.3. *Extraction and isolation of antigens*

Most studies have involved nuclei from fresh tissue/cells but in the case of rabbit thymus several laboratories [18,19,21] have used commercially available, acetone-dried tissue powders as their source of antigen. As no comparative studies have been carried out it is unknown how the composition of antigens from this source differs from those derived by the more conventional preparative procedure, viz. as soluble extracts obtained by sonication [4] or homogenisation [9] of nuclei in saline solution. The latter procedure yields Sm and RNP antigens largely as forms which sediment at 10 S [9], but, as will be described later, both can be obtained either exclusively [13,20] or in part [4] associated with nuclear material of an $S$-value >30. Three investigations [4,13,20] mentioned in table 1 would appear to have involved such large forms of the antigens. Hence, it is conceivable that in the immunoprecipitates obtained the antigenic determinants were part of a complex containing RNA and protein molecules not seen in the purely 10 S forms of the antigens. Such a proposal is supported by the differences in the composition of Friend cell Sm and RNP antigens when they are studied as part of either >30 S [13] or <30 S complexes [10].

The means of isolation of the antigens has varied from immunoaffinity chromatography [15–21] to purely analytical procedures such as the rapid formation of immune complexes from crude nuclear extracts [3]. The use of chaotropic salts in the former and the potential for non-specific binding of molecules in the latter approach (see controls in [12] and discussion in [13]) illustrate the range of conditions to which these antigens are exposed during their isolation.

5.4. *Nuclear sources of protein associated with Sm/RNP*

Although snRNAs have been highly conserved in sequence, the proteins accompanying them in nuclear particles could be cell specific. Alternatively, these variations could indicate either non-specific binding components or some structural nuclear proteins which are complexed with the antigenic sites in a cell-specific manner. Such possibilities are indicated by the fact that treatment of a nuclear extract with trypsin still yields a precipitable Sm antigen which has lost the 12 000 $M_r$ protein and some of the 10 000 $M_r$ group [9]. Similar results have been found in [21] showing that certain of the lower $M_r$ group of proteins do not appear to be essential for the precipitable antigenic structure.

Apart from the results in [12] it is obvious that the group of proteins associated with most preparations of these antigens bear little resemblance to those of isolated snRNP particles, since in general the latter have $M_r > 40\,000$ [27,28]. It should be noted that Brunel et al. [29] did obtain a preparation of snRNPs, the proteins of which were exclusively between 10 000–14 000 $M_r$, but no reaction occurred when such snRNPs were exposed to anti-RNP antibodies.

5.5. *Nuclear sources of RNA associated with Sm/RNP*

The presence of unidentified, particularly very small, RNA species in immunoprecipitates is suggestive that these components are degradation products of larger molecules. Admittedly, little attempt has been made to inhibit ribonucleases in many of these studies, but when this is done [9,21] very small RNAs are still observed. Even so, the action of nucleases cannot be ignored since Takano et al. [19] indicated that the very small RNA seen associated with thymus RNP is similar to $U_{1a}$-snRNA and furthermore, Epstein et al. [30] have reported that after cleavage of its $U_1$ at some 5 sites with $T_1$-RNase, Sm antigen is still precipitable by anti-Sm antibodies. Thus, the snRNA does not have to be intact for immunological recognition.

From these compositions Sm and RNP antigens are not necessarily representative of the nuclear snRNP particles of each cell type. They could, for example, be a subset resulting from cellular degradation processes. Alternatively other nuclear sources of RNA should not be dismissed. Nuclear RNP particles, other than snRNPs, have been described which sediment between 5–20 S, e.g., HnRNP degradation products [31] which include a 15 S particle containing the 3′-poly(A) tract [32] or the 7 S particle containing processed HnRNA as proposed in [33]. It is conceivable that all or any of these sources could contribute to a nuclear pool of 'core' RNP particles which possess common antigenic determinant sites recognisable by anti-Sm/RNP antibodies. The significant quantitative

differences in the nuclear content of snRNAs amongst rat tissue [34] and in Friend cells at different stages of growth and differentiation [35] suggest that relative contributions from such sources could be tissue or growth dependent, resulting in different patterns of small RNAs precipitating with the autoimmune antibodies. The presence of sn- and unidentified RNAs in antigens precipitated from HeLa and thymus cells respectively support the existence of such effects.

## 6. Antigens as single protein or RNA molecules

The data given in table 2 suggest that of the isolated components of purified antigens with which anti-Sm/RNP antibodies react, protein, rather than RNA, carries the antigenic determinants. It has been concluded that these sites are the same as those involved in the forms of the antigens described in table 2 and, if so, these results contrast with some of the established properties of Sm and RNP. For example, both Sm and RNP can be found to be sensitive to protease and ribonuclease [9,36], indicating that the antigenic determinant sites contain protein and RNA. An alternative view has now been put that the RNA is simply acting as a carrier for the protein determinant [23]. The wide range of RNA molecules found associated with the antigens (table 1) would thus mean that the antigenic protein species were bound to different RNAs in different cell types. However, the complexity of the situation is shown by both direct [11,23] and indirect comparative studies (table 2) in which the antigenic proteins are found to be species and sera specific.

It has to be stressed that there is no evidence to indicate that the antibodies utilised in analysing RNA–protein complexes (table 1) are the same as those used to detect antigenic determinants on separated protein or RNA molecules (table 2). Equally, we do not know at present if the antigenic determinants seen on individual RNAs and proteins (table 2) are detectable in the complexes used in table 1. These matters await clarification.

## 7. Subnuclear location of antigens in vivo

Although there is considerable evidence that Sm and RNP antigens are of nuclear origin [2], their true subnuclear location has only been considered recently. The majority of studies have been carried out using soluble preparations of these components but as described in [9] a significant proportion of Sm and RNP remains in the nuclear residue after extraction with saline. Admittedly conditions of extraction (time, volume, ionic strength) can be devised which will extract virtually all of the antigens from nuclei, but their ease of partial solubility has precluded attempts to identify the subnuclear sites to which they bind [9].

Independent evidence has recently accumulated suggesting that the subnuclear location of the antigens in vivo may be large HnRNP particles (up to 200 S) [13,20]. Such forms of RNP particles are released from nuclear preparations by sonication in either 0.01 M or 0.14 M NaCl [13,20], i.e., the effect does not appear to follow directly the salt-dependent distribution of snRNAs between 10 S and HnRNP particles [37]. Further manipulation (e.g., incubation at 37°C) of these high *S*-value forms of the antigens results in their conversion to a form sedimenting at <20 S, i.e., similar to that obtained by simple extraction of nuclei with saline (T. Y. J., unpublished).

In view of the snRNA content of antigens extracted from some cell types it is relevant that snRNAs have been found tightly bound to HnRNP (see [38]). Secondly, the current view that HnRNPs are themselves bound to the nuclear matrix [39] means a possible association of antigen with components of at least 2 nuclear structures. Assuming that the sonication procedure involved retains structures found in vivo, the above experiments support the proposal that Sm and RNP determinants are integral components of the HnRNPs. Hence, the release of antigen from nuclei may be the result of dissociation from or degradation of HnRNP complexes during extraction. Such a model would also suggest that the cell type and the method of preparation would determine the particular (protein) antigenic sites, their range of associated non-antigenic RNA and protein molecules and thus whether the determinants were on single or multiple antigen complexes. The differences in composition of Friend cell Sm and RNP found using complexes sedimenting at >30 S [13] or <30 S [10] confirm the importance of preparative procedures in these analyses. Furthermore, the following forms of Sm and RNP appear to have been used in the studies listed in table 1, Sm and RNP >30 S [13,20], Sm associated with both 30 S HnRNP and 10 S particles [4] and Sm and RNP solely as 10 S particles [9]. In view of the

other variables discussed it is perhaps not surprising to find that these 4 laboratories have produced the range of analyses described in table 1.

## 8. Conclusions

Our view is that Sm and RNP are antigenic determinants on large HnRNP particle complexes and that these determinants may be released either singly or together associated with other proteins and RNA molecules. Variations in the reports of the composition of Sm and RNP antigens can then be attributed to combinations of the aspects reviewed above, e.g., cell type, extraction conditions and method of detection.

Several important issues are unresolved. These include:

(1) The correspondence or otherwise of the antigenic sites found on isolated proteins to those detected in RNA-protein complexes;
(2) The factors behind the presence of different types of RNA associated with antigens precipitated from different cells.

The data reviewed above suggest that we are far from ascertaining the function or even the exact composition of the Sm and RNP antigens. We propose, therefore, that the evidence in this review suggests that caution should be exerted in experiments in which these autoantibodies are used in investigations of RNA splicing mechanisms. Lewin [40] has already suggested that appropriate experimental controls are required in such investigations and we would recommend that for any type of application characterisation of the antigens is a necessity for each cell type and serum to be used. The recent work of Lenk et al. [12] supports such an approach.

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## References

[1] Notman, D. D., Kurata, N. and Tan, E. M. (1975) Ann. Int. Med. 83, 464–469.
[2] Tan, E. M. (1979) in: The Cell Nucleus, Chromatin, part C (Busch, H. ed) vol. 7, pp. 457–477, Academic Press, New York.
[3] Lerner, M. R. and Steitz, J. A. (1979) Proc. Natl. Acad. Sci. USA 76, 5495–5499.
[4] Lerner, M. R., Boyle, J. A., Mount, S. M., Wolin, S. L. and Steitz, J. A. (1980) Nature 283, 220–224.
[5] Yang, V. W., Lerner, M. R., Steitz, J. A. and Flint, S. J. (1981) Proc. Natl. Acad. Sci. USA 78, 1371–1375.
[6] Lerner, M. R., Andrews, N. C., Miller, G. and Steitz, J. A. (1981) Proc. Natl. Acad. Sci. USA 78, 805–809.
[7] Lerner, M. R. and Steitz, J. A. (1981) Cell 25, 298–300.
[8] Lerner, M. R., Boyle, J. A., Hardin, J. A. and Steitz, J. A. (1981) Science 211, 400–402.
[9] MacGillivray, A. J. and Carroll, A. R. (1982) in: Biochemistry and Biology of the Cell Nucleus: Non-histone Proteins (Hnilica, L. S. ed) CRC Press, Boca-Ratori FL.
[10] Mount, S. M. and Steitz, J. A. (1981) Nucleic Acids Res. 9, 6351–6368.
[11] Billings, P. B., White, P. J. and Hoch, S. O. (1981) J. Cell Biol. 91, 138A.
[12] Lenk, R. P., Maizel, J. V. and Croucia, R. J. (1982) Eur. J. Biochem. 121, 475–482.
[13] Barque, J. P., Yeni, P., Peraudeau, L., Danon, F. and Larsen, C. J. (1981) Biochem. Biophys. Res. Commun. 99, 284–291.
[14] Tan, E. M., Reyes, P. and Fritzler, M. J. (1978) in: Biological Markers for Neoplasia: Basic and Applied Aspects (Ruddon, R. E. ed) pp. 399–410, Elsevier Biomedical, Amsterdam, New York.
[15] Gibbons, J. J., Tsai, C. C. and Roodman, S. T. (1980) Fed. Proc. FASEB 39, 1023.
[16] Augustynek, D. A., Gibbons, J. J., Tsai, C. C. and Roodman, S. T. (1982) Arthritis Rheum. in press.
[17] Agelli, M., Bombardieri, S., Ioppolo, S., Siracusano, A. and Natali, P. G. (1980) IRCS Med. Sci. Biochem. 8, 754.
[18] Takano, M., Agris, P. F. and Sharp, G. C. (1980) J. Clin. Invest. 65, 1449–1456.
[19] Takano, M., Golden, S. S., Sharp, G. C. and Agris, P. F. (1981) Biochemistry 20, 5929–5936.
[20] Douvas, A. S., Stumph, W. E., Reyes, P. and Tan, E. M. (1979) J. Biol. Chem. 254, 3608–3616.
[21] White, P. J., Gardner, W. D. and Hoch, S. O. (1981) Proc. Natl. Acad. Sci. USA 78, 626–630.
[22] Lerner, E. A., Lerner, M. R., Janeway, C. A. and Steitz, J. A. (1981) Proc. Natl. Acad. Sci. USA 78, 2737–2741.
[23] White, P. J. and Hoch, S. O. (1981) Biochem. Biophys. Res. Commun. 102, 365–371.
[24] Peltier, A. P., Aussel, C., Haim, T. and Cyna, L. (1977) J. Immunol. Methods 16, 153–163.
[25] Jonsson, J., Schilling, W. and Norberg, R. (1981) Scand. J. Immunol. 13, 175–187.
[26] Akizuki, M., Powers, R. and Holman, H. R. (1977) Arthritis Rheum. 20, 693–701.
[27] Raj, N. B. K., Ro-Choi, T. S. and Busch, H. (1975) Biochemistry 14, 4380–4385.

[28] Maxwell, E. S., Maundrell, K., Puvion-Dutilleul, F. and Scherrer, K. (1981) Eur. J. Biochem. 113, 233–247.
[29] Brunel, C., Widada, J. S., Lelay, M. N., Jeanteur, P. and Liautard, J. P. (1981) Biochem. Biophys. Res. Commun. 9, 815–831.
[30] Epstein, P., Reddy, R. and Busch, H. (1981) Proc. Natl. Acad. Sci. USA 78, 1562–1566.
[31] Howard, E. F. (1978) Biochemistry 17, 3228–3236.
[32] Quinlan, T. J., Billings, P. B. and Martin, T. E. (1974) Proc. Natl. Acad. Sci. USA 71, 2632–2636.
[33] Douvas, A. S. and Tan, E. M. (1981) in: The Cell Nucleus, Nuclear Particles, part A (Busch, H. ed) vol. 8, pp. 369–387, Academic Press, New York.
[34] Gunning, P. W., Shooter, E. M., Austin, L. and Jeffrey, P. C. (1981) J. Biol. Chem. 256, 6663–6669.
[35] Juncio, G. S. and Goldstein, L. (1981) J. Cell Physiol. 109, 235–241.
[36] Jonsson, J., Schilling, W. and Norberg, R. (1981) Scand. J. Immunol. 13, 175–187.
[37] Zieve, G. and Penman, S. (1981) J. Mol. Biol. 145, 501–523.
[38] Calvet, J. P. and Pederson, T. (1981) Cell 26, 363–370.
[39] Van Eekelen, C. A. G. and Van Venrooij, W. J. (1981) J. Cell Biol. 88, 554–563.
[40] Lewin, B. (1980) Cell 22, 324–326.