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Antibodies to Ribosomal Ribonucleic Acid (rRNA) in Patients with Systemic Lupus Erythematosus (SLE)*

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INTRODUCTION

Patients with systemic lupus erythematosus have antibodies with specificity for a variety of subcellular constituents (Asherson, 1958) including ribosomes (Sturgill and Carpenter, 1965; Schur, Moroz and Kunkel, 1967). The antigenic component of the ribosome to which these antibodies are directed has not been determined previously. The purpose of this investigation was to characterize the antigen involved in this system.

MATERIALS AND METHODS

Whole ribosomes were isolated essentially as described by Wettstein, Staehelin and Noll (1963) from fresh liver of New Zealand White male rabbits and from human liver obtained at autopsy less than 6 hours after death. Ribosomes were purified by precipitation with 0·05 M Mg^{++} as described by Takanami (1960). Analysis of the ribosomes by the orcinol and biuret procedures showed them to be 40 per cent RNA and 60 per cent protein.

Ribosomal protein separated from the rRNA by urea and LiCl (Spitnik-Elson, 1965) contained 1·5 per cent RNA as estimated by the orcinol procedure.

Ribosomal RNA was isolated from rabbit liver by 4-amino salicylate and phenolic solvents essentially as described by Kirby (1968). Ultracentrifugal analysis of the preparation (Fig. 1) in 0·1 M phosphate buffer, pH 7·5, at 44,770 rev/min and 20° revealed sedimentation rates of 28S and 16S using standard methods of calculation (Schachman, 1957; Boedtker, 1960). Protein contamination was determined on the model 120C Beckman Amino Acid Analyzer after hydrolysis of the sample in constant boiling HCl at 105° for 20 hours. Less than 0·5 per cent protein was found in the rRNA sample. The sample was found to be free of DNA by the diphenylamine method (Giles and Myers, 1965).

Sera from fifty patients with an established diagnosis of SLE and fifty controls were examined. The control sera were from twenty-one normal individuals and twenty-nine patients with other rheumatic diseases. The sera found to have antiribosomal antibodies were subsequently tested against rRNA and ribosomal protein.

Double diffusion against test sera was carried out in 1·0 per cent agar (Difco) which was made up in veronal buffer, pH 8·6, containing 0·21 per cent saline, 7 per cent glycine and 0·1 per cent sodium azide.

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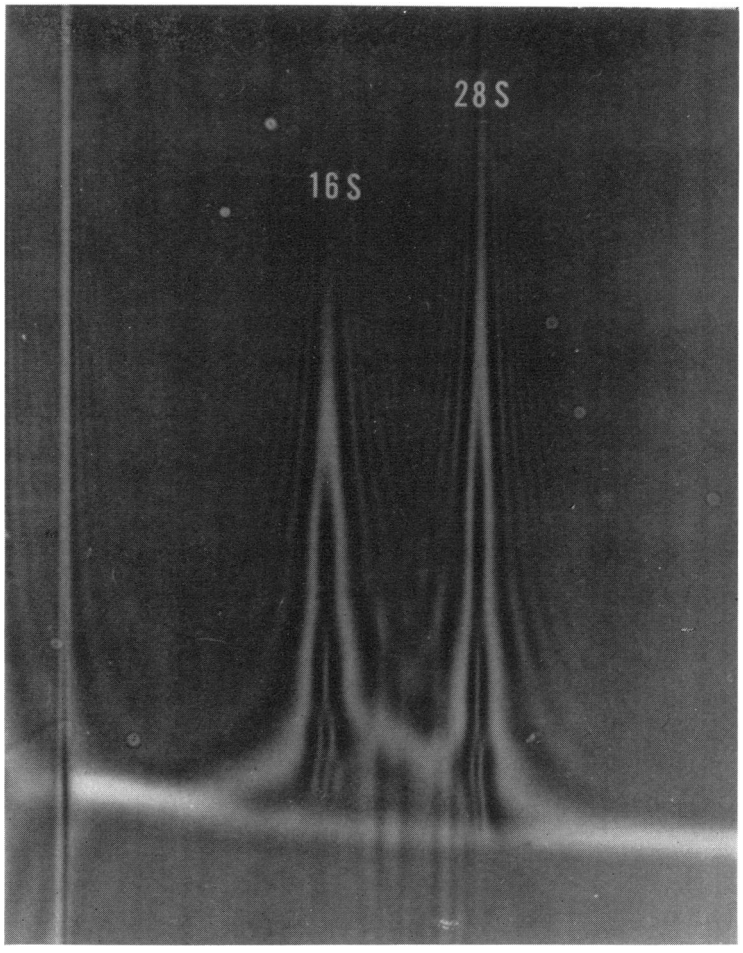

FIG. 1. Ultracentrifugal analysis of rabbit liver rRNA at a concentration of 4·5 mg/ml in 0·1 M phosphate buffer, pH 7·5, after 40 minutes at 44,770 rev/min and 20°.

A modified latex fixation test (Singer and Plotz, 1956) was used to test the sera (heat treated at 56° for 30 minutes) using polystyrene latex particles (Difco) coated with 200 μg/ml of ribosomes, rRNA or ribosomal protein. The two latter were allowed to coat the latex particles by standing overnight at 3°. The tubes were examined for agglutination after incubation overnight at 3° and centrifugation at 1000 ***g*** for 3 minutes.

The antigenicity of rabbit liver rRNA (2 mg/ml) was studied after treatment with trypsin, bovine pancreatic ribonuclease (Sigma) or spleen phosphodiesterase (Worthington) using standard methods. Each of these preparations was tested by double diffusion against the serum which had previously produced a precipitin line with untreated rRNA.

RESULTS AND DISCUSSION

Nine (18 per cent) of the sera from the patients with SLE were found to react with ribosome coated latex particles. No positive reactions were found with the control sera. By double

diffusion in agar, four (8 per cent) of the SLE sera were found to precipitate ribosomes. These sera precipitated both rabbit and human ribosomes, confirming the findings of previous investigators (Sturgill and Carpenter, 1965; Schur *et al.*, 1967). Of the nine sera shown to be reactive with ribosomes, six were available for further testing. Two sera were found which reacted with purified rRNA-coated latex particles and one of these sera precipitated rRNA in agar (Fig. 2). No sera reacted with ribosomal protein alone. The antigenicity of this rRNA preparation was not changed significantly by treatment with either trypsin or bovine pancreatic ribonuclease, but it was destroyed by treatment with spleen phosphodiesterase. The maintenance of antigenicity after treatment with pancreatic ribonuclease seems to indicate that the antigenic site rests in the 'core' RNA oligonucleotides, which are rich in purine bases with few if any adjacent pyrimidine bases (Schmidt, Cubiles, Zollner, Hecht, Strickler, Seraidarian, Seraidarian and Thannhauser, 1951).

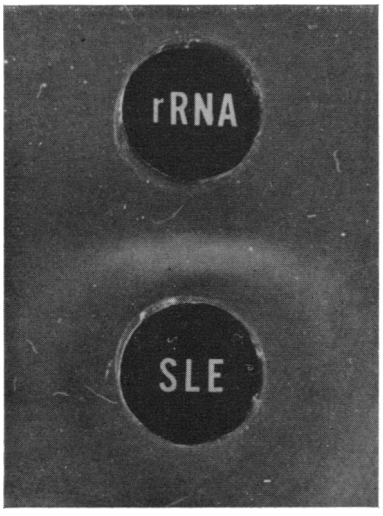

FIG. 2. Precipitation of rRNA by SLE serum (SLE). Double diffusion in 1·0 per cent agar, pH 8·6, was allowed to take place at room temperature.

Antigenic cross-reactivity of ribosomes from both rabbit and human is compatible with earlier work in rabbits which demonstrated common antigenic determinants on ribosomes from varied sources (Panijel and Barbu, 1960; Bigley, Dodd and Geyer, 1963). The antigen involved in this cross-reactivity is RNA (Barbu and Panijel, 1960; Lacour, Havel, Havel and Nohon, 1962; Bigley *et al.*, 1963; Lamon and Bennett, unpublished). It is not surprising, therefore, to find that rRNA is one of the antigenic determinants to which antiribosomal antibody from SLE sera is directed. This finding is supported by the immunofluorescence studies of Watanabe, Fisher and Epstein (1969), which demonstrated inhibition with yeast RNA or rat liver rRNA of cytoplasmic and nucleolar staining by sera from selected patients with SLE.

From these data, it seems that an antigenic determinant in the ribosome–anti-ribosome system of SLE is rRNA. A parallel is implied with the DNA–anti-DNA system in SLE and suggests that these patients may have a broad immunological responsiveness to polynucleotides.

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REFERENCES

ASHERSON, G. L. (1958). 'Antibodies against nuclear and cytoplasmic cell constituents in S.L.E. and other diseases.' *Brit. J. exp. Path.*, **40**, 209.

BARBU, E. and PANIJEL, J. (1960). 'Présence d'antibodies anti-acid ribonucléique dans les immunsérums antiribosomes.' *C. r. Acad. Sci. (Paris)*, **251**, 1382.

BIGLEY, N. J., DODD, M. C. and GEYER, V. G. (1963). 'The immunologic specificity of antibodies to liver ribosomes and nuclei.' *J. Immunol.*, **90**, 416.

BOEDTKER, H. (1960). 'Configurational properties of tobacco mosaic virus ribonucleic acid.' *J. molec. Biol.*, **20**, 171.

GILES, K. W. and MYERS. A. (1965). 'An improved diphenylamine method for the estimation of desoxyribonucleic acid.' *Nature (Lond.)*, **206**, 93.

KIRBY, K. S. (1968). 'Isolation of nucleic acids with phenolic solvents.' *Meth. Enzymol.*, **12**, 87.

LACOUR, F., HAVEL, J., HAVEL, L. and NOHON, E. (1962). 'Étude immunologique de l'acide ribonucléique (RNA) par la microméthode de double diffusion dans l'agar. *C. r. Acad. Sci. (Paris)*, **255**, 2322.

PANIJEL, J. and BARBU, E. (1960). 'Communauté Antigenique entre ribonucleoprotéines d'origines diverse.' *C. r. Acad. Sci. (Paris)*, **250**, 232.

SCHACHMAN, H. K. (1957). 'Ultracentrifugation, diffusion and viscometry.' *Meth. Enzymol.*, **4**, 55.

SCHMIDT, G., CUBILES, R., ZOLLNER, N., HECHT, L., STRICKLER, N., SERAIDARIAN, K., SERAIDARIAN, M. and THANNHAUSER, S. J. (1951). 'On the action of ribonuclease.' *J. biol. Chem.*, **192**, 715.

SCHUR, P. H., MOROZ, L. A. and KUNKEL, H. G. (1967). 'Precipitating antibodies to ribosomes in the serum of patients with SLE.' *Immunochemistry*, **4**, 447.

SINGER, J. M. and PLOTZ, C. M. (1956). 'The latex fixation test. I. Application to the serologic diagnosis of rheumatoid arthritis.' *Amer. J. Med.*, **21**, 888.

SPITNIK-ELSON, P. (1965). 'Preparation of ribosomal protein from *Escherichia coli* with lithium chloride and urea.' *Biochem. biophys. Res. Commun.*, **18**, 557.

STURGILL, B. C. and CARPENTER, R. R. (1965). 'Antibody to ribosomes in S.L.E.' *Arthr. and Rheum.*, **8**, 213.

TAKANAMI, M. (1960). 'A stable ribonucleoprotein for amino acid incorporation.' *Biochim. biophys. Acta*, **39**, 318.

WATANABE, N., FISHER, H. M. and EPSTEIN, W. V. (1969). 'Specificity and reactivity of cytoplasmic and nucleolar antibody in SLE sera.' *Arthr. and Rheum.*, **12**, 173.

WETTSTEIN, F. O., STAEHELIN, T. and NOLL, H. (1963). 'Ribosomal aggregate engaged in protein synthesis, characterization of the ergosome.' *Nature (Lond.)*, **197**, 430.