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RIBOSOMAL ANTIBODIES DETECTED BY IMMUNOFLUORESCENCE IN SYSTEMIC LUPUS ERYTHEMATOSUS AND OTHER COLLAGENOSES

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SUMMARY

A new ribosomal antibody is described in patients with systemic lupus erythematosus which reacts with all tissues from varied species, giving a distinctive immunofluorescence pattern. This can best be distinguished from other known autoantibodies on stomach where it reacts with chief cells and pancreas where exocrine cells are brightly stained. The ribosomal nature of the antigen was demonstrated by absorption of immunofluorescence and complement fixation studies using purified subcellular fractions. The antigen was unaffected by ribonuclease and was destroyed by trypsin, suggesting that it is one of the ribosomal proteins, while previously reported antibodies were mainly directed against ribosomal RNA, RNA–protein complexes, or polynucleotides and hybrids. The present ribosomal antibody is uncommon and occurs in less than 1% of SLE patients; its clinical significance is similar to that of ribosomal precipitins as the patients had renal involvement and several have died.

INTRODUCTION

Ribosomal antibodies have been previously described in systemic lupus erythematosus (SLE) by several authors. The methods used were precipitation by gel diffusion (Schur, Moroz & Kunkel, 1967), bentonite flocculation (Sturgill & Carpenter, 1965), immunofluorescence spot tests (Sturgill & Preble, 1967), indirect immunofluorescence using peripheral white blood cells as a substrate (Watanabe, Fisher & Epstein, 1969), complement fixation tests and radioactive antigen binding (Schur *et al.*, 1971) and haemagglutination (Koffler *et al.*, 1971). By these methods up to 50% of SLE patients had antibodies against constituents of ribosomes. Some of the antibodies appeared to be directed against ribosomal RNA (Lamon & Bennett, 1970), some against an RNA–protein complex (Sturgill & Preble,

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1967; Schur *et al.*, 1967) and some against a variety of polynucleotides and hybrids (Koffler *et al.*, 1971).

The antibody described in the present study is distinct from all those previously reported as it gives a distinctive immunofluorescent pattern in all animal tissues, and is found in less than 1% of SLE patients.

MATERIALS AND METHODS

Patients and sera

The seven cases reported here (Table 1) were collected in Paris and London over a period of 4 years. During this time, the two laboratories each examined at least a hundred cases of SLE and many more of other collagenoses per year, yet no further examples of this immunofluorescent pattern were encountered. The sera were tested when received and subsequently kept at −20°C. The antibodies (Ab) were stable for up to 4 years.

Immunofluorescence (IFL)

Five-micrometre cryostat sections of the human and animal tissues shown in Table 2 were used as substrate for IFL with anti-γ-globulin–FITC conjugates and sera diluted 1:10. Conjugates specific to the three main immunoglobulin (Ig) classes and anti-β_{1C} were also used. Rat pancreas was frozen after fixation with 1% formaldehyde for 40 min in the cold in 0·1 M phosphate buffer at pH 7·4, since unfixed blocks were difficult to cut in the frozen state. IgG isolated by DEAE chromatography from the serum of Case 5 was conjugated with FITC. For comparison a direct rhodamine conjugate was similarly prepared from a primary biliary cirrhosis serum containing mitochondrial antibodies.

Tissue fractionation

Rat liver mitochondria, microsomes and soluble supernatant were prepared as described by Duve *et al.* (1955). Microsomes were fractionated into smooth and rough membranes and polysomes by the method of Rothschild (1963). Purified ribosomes were prepared according to Tashiro & Siekevitz (1965): membranes were solubilized with 0·5% sodium deoxycholate and the ribosomes were precipitated by $MgCl_2$.

Mitochondria and microsomes were assessed for contamination with each other by their respective marker enzymes, succinic dehydrogenase and glucose 6-phosphatase; subfractions of the endoplasmic reticulum were checked by electron microscopy (model AEI 6B). Protein content in subcellular fractions was measured by the method of Lowry *et al.* (1951).

Absorption experiments

Two sera containing ribosomal Ab to a titre of 320 (Case 5, SL) and 40 (Case 6, Rou) were absorbed with microsomes, smooth endoplasmic reticulum (ER), rough ER, polysomes, DOC-purified ribosomes and soluble supernatant. 0·2-ml aliquots of the antigen in serial dilutions were incubated overnight in the cold with equal volumes of serum SL diluted 1:40 and serum Rou diluted 1:10 Rat liver, pancreas and kidney were used as substrates for indirect IFL.

Complement fixation (CFT)

CFT was carried out in microtitre trays as described in the WHO manual (Roitt &

TABLE 1 Clinical and immunological features in patients with ribosomal IFL antibodies

Case number	S/A	Age at onset of symptoms	Diagnosis	LE cells	Presenting symptoms	Clinical follow-up	Treatment	Autoantibody titres						
								Ribosomal		ANA		CFT		SMA, AMA, TRC, GPC
								Fluorescence	CFT	Fluorescence	Type	Calf thymus DNA	Yeast RNA	
1	F25	18	SLE	++	Polyarthralgia, Raynaud's, facial erythema	Nephrotic syndrome. Died renal failure 7 years after onset	Steroids & anti-malarials	100	32	20	Diffuse	16	32	–
2	F22	17	SLE	Negative	Polyarthralgia, fever, rash	Proteinuria, sicca syndrome. Died CVA, ITP & granulopenia, aged 23	Steroids	100	256	100	Speckled	±	–	–
3	F46	45	SLE	++	Epilepsy	Died of nephritis 2 years after onset	Steroids & imuran	200	8	20	Diffuse	–	–	–
4	F50	33	SLE	++	Pleural effusion, polyarthritis	Nephrotic syndrome. Alive, with RA-like deformities & mitral insufficiency	Steroids	20	n.t.	50	Diffuse	n.t.	n.t.	–
5	F23	23	SLE	Negative	Fever, malaise, polyarthralgia	Decreasing creatinine clearance. Mesangial proliferation and Ig deposits on biopsy	Steroids	320	256	200	Nucleolar	16	–	–
6	F67	65	Systemic sclerosis	Negative	Raynaud's, digital ulcerations	Scleroderma teleangiectasia. Alive, 2 year follow-up	No specific treatment	40	16	500	Nucleolar	–	–	–
7	M59	59	Collagenosis	Negative	Progessive muscle wasting	Previous discoid LE. Died cachexia	Steroids	80	—	40	Nucleolar	–	–	–

ANA = anti-nuclear Ab; SMA = smooth muscle Ab; AMA = mitochondrial Ab; TRC = tanned red cell agglutination for thyroglobulin Ab; GPC = gastric parietal cell fluorescence; n.t. = not tested, serum not available; – = negative results.

Doniach, 1969), using rat liver mitochondria, microsomes, purified ribosomes, commercial rat liver RNA (Sigma) and calf thymus DNA (Worthington) as antigens. After preliminary chessboard titration, serum SL was selected as a standard and used at a dilution of 1:32 for comparison of subcellular fractions.

Enzyme treatment

Equal volumes of ribosomal suspension incubated for 2 hr with 0·5 and 1·0 mg/ml of ribonuclease A (Sigma Type 1) dissolved in PBS, pH 7, or with crystalline trypsin (Boehringer Ltd), 0·1 and 0·2 mg/ml for 1 and 2 hr at 37°C, digestion in the latter case being terminated by the addition of 0·2 mg of soya bean trypsin inhibitor.

RESULTS

Clinical features

The clinical and immunological features, follow-up and treatment of the seven patients described in this study are summarized in Table 1. These patients were selected from a large number of cases with collagen disorders: there were six females and one male, of whom five had classical SLE, one had systemic sclerosis and one an ill-defined collagen disorder.

The SLE patients were all females, and the age at onset of symptoms varied from 17 to 45 years. Abundant LE cells were found in three cases, two of whom died of lupus nephritis after 2 and 7 years of illness. Evidence of renal involvement was present in all five cases. A patient who had proteinuria died in coma 5 years after onset of her illness, following a cerebrovascular accident due to idiopathic thrombocytopenic purpura. One patients with nephrotic syndrome, rheumatoid joint deformities and mitral insufficiency is alive on steroids 17 years after onset of symptoms. The fifth SLE patient is a young woman of 23 who presented with fever and arthralgia, and after 6 months follow-up shows deteriorating creatinine clearance and mesangial proliferation with glomerular deposits of complement and Ig on renal biopsy. Of the two patients with allied disorders, one is a female aged 67 years who has scleroderma with severe Raynaud's phenomenon, digital ulcerations and telangiectasia, followed up for 2 years so far. The second was a male of 59 who died after a 6 months illness, with cachexia and muscle wasting.

Serologically, in addition to the ribosomal IFL which was present in a titre of 20–320, all the patients had nuclear fluorescence (ANA) in titre of 20–500, the ANA pattern being mainly of the diffuse variety in three, speckled in one and nucleolar in three cases. All patients gave negative results for smooth muscle, mitochondrial and gastric parietal cell antibody, and there was no anti-thyroglobulin by the tanned red cell test.

Ribosomal immunofluorescence pattern

These antibodies are completely non-organ and non-species-specific. Fluorescence was seen in every tissue tested and was proportional to the known content of ribosomes in each cell type. The distribution within organs and relative intensities of staining are shown in Table 2, where ribosomal and mitochondrial (AMA) immunofluorescence patterns are compared. The organs which allow the best distinction from other known tissue reactions are the stomach, the salivary glands and the pancreas. In gastric glands, the ribosomal antibody stains only the chief cells (Fig. 1) and this contrasts well with the organ-specific antibody of pernicious anaemia, which is negative on chief cells and with the mitochondrial

Ab which stains gastric parietal cells more brightly than chief cells (Fig. 2). Pancreatic exocrine cells show strong and diffuse fluorescence with ribosomal Ab (Fig. 3) and only a faint reaction with AMA. On rat liver sections, the ribosomal Ab has a particular appearance, showing a coarsely granular perinuclear fluorescence (Fig. 4). Thyroid cells are diffusely stained (Fig. 5), and this can easily be confused with the organ-specific microsomal Ab of thyroiditis, except for the fact that the latter antigen is concentrated in the microvilli, so that the staining with thyroiditis sera is brightest near the colloid edge.

TABLE 2. Tissue distribution of immunofluorescence obtained with ribosomal antibodies in comparison with mitochondrial patterns

	Antibody	
Tissue*	Ribosomal	Mitochondrial
Stomach		
Chief cells	++	Weak
Parietal cells	−	++
Submaxillary		
Ducts	−	++
Exocrine	+	−
Pancreas		
Ducts	−	−
Exocrine	++	+
Islets	−	+
Kidney		
Cortex	+	+
Medulla	+	++
Thyroid		
Normal epithelium	++	Weak
Askenazy cells	+	++
Liver	++	+
Brain, Betz cells	++	++
Adrenal	Trace	+
Bronchial mucosa	++	Trace
Intestinal mucosa	+	+
Striated muscle	+	++

* Comparable results were obtained with tissues from human, rat and mouse, and beef, lamb and pig liver.

IFL on lymphocytes

To compare this ribosomal Ab with those reported by other authors, sera from six of the cases were tested on a cytocentrifuged preparation of human tonsillar lymphocytes. With this substrate, strong cytoplasmic IFL was obtained with cases 2 and 6 and weaker reactions with cases, 1, 5 and 7. ANA was also seen in these preparations.

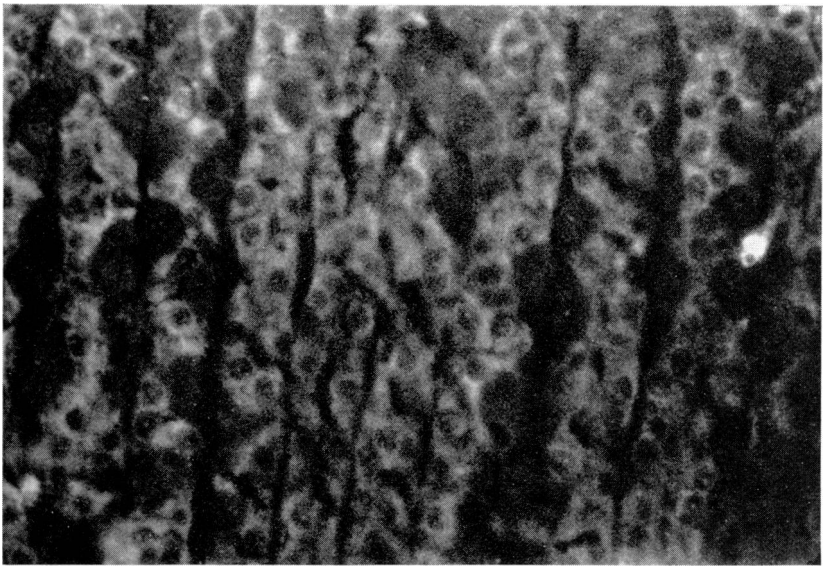

FIG. 1. Unfixed cryostat section of rat stomach treated with SLE serum containing ribosomal antibody followed by anti-human γ-globulin–FITC conjugate, showing fluorescence in cytoplasm of gastric chief cells and no reaction on parietal cells. The serum also contained nucleolar ANA. (Magnification $\times 250$.)

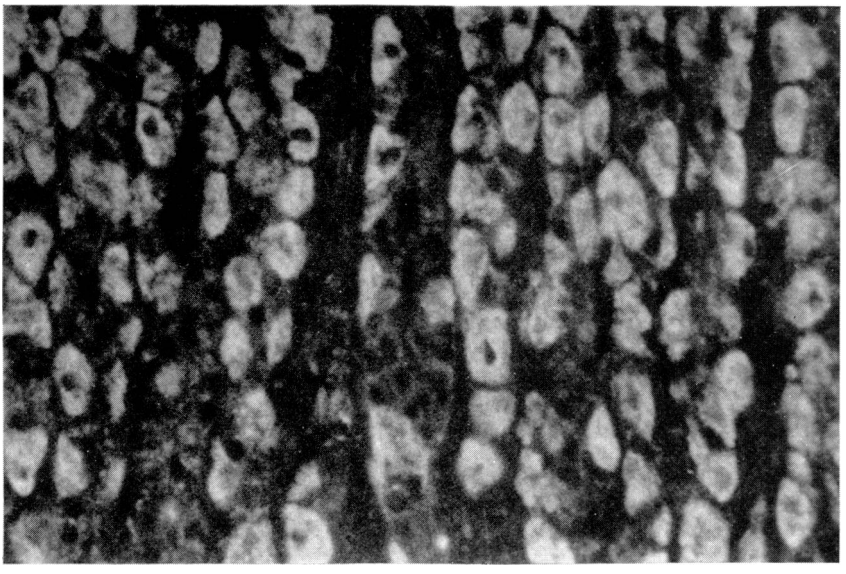

FIG. 2. Rat stomach treated with serum from primary biliary cirrhosis patient with mitochondrial antibodies followed by anti-human γ-globulin–FITC conjugate. The gastric parietal cells are brightly stained while the chief cells show faint granular fluorescence. (Magnification $\times 250$.)

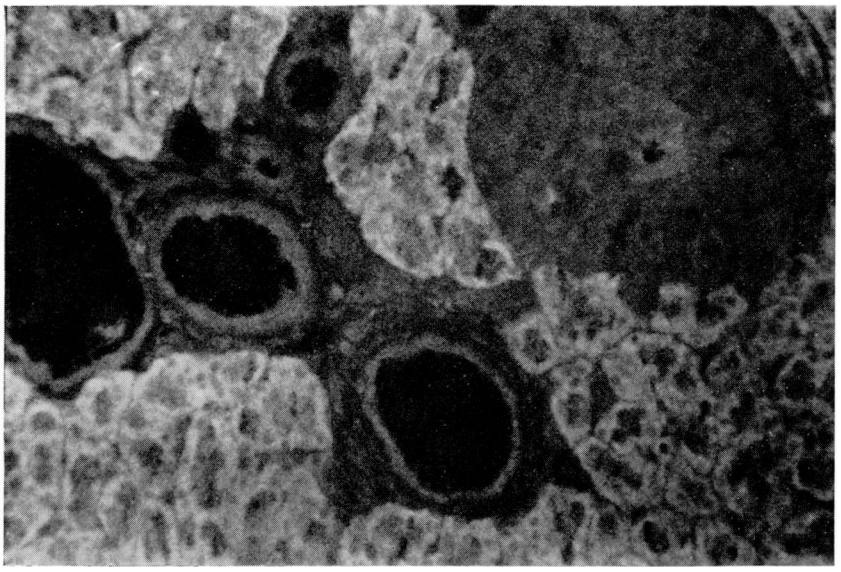

FIG. 3. Rat pancreas treated with SLE serum containing ribosomal antibody followed by anti-human γ-globulin–FITC conjugate. Ribosomal antibody stains cytoplasm of exocrine cells diffusely. Islet of Langerhans (top right) is negative and ducts and vessels are not stained. (Magnification $\times 250$.)

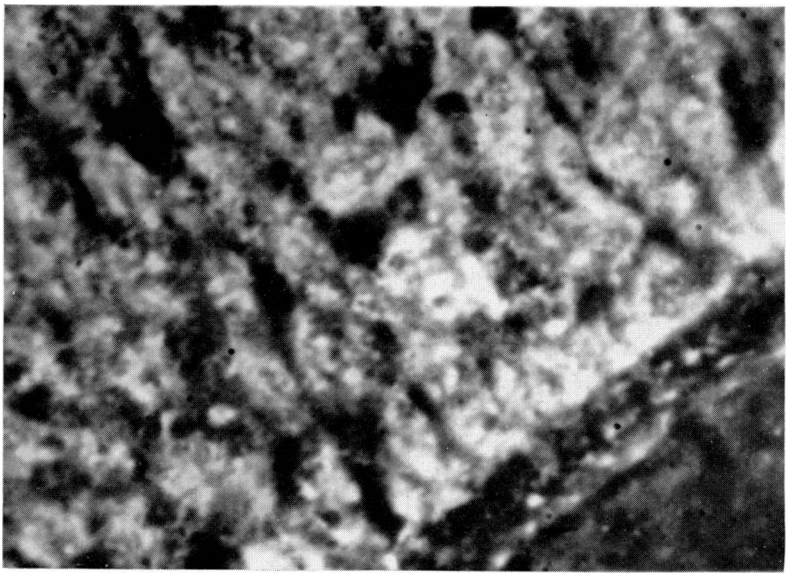

FIG. 4. Rat liver treated with SLE serum containing ribosomal antibody followed by anti-human γ-globulin–FITC conjugate. Ribosomal antibody gives a coarsely granular, mainly perinuclear fluorescence. (Magnification $\times 400$.)

Complement fixation

Sera from six of the patients were available for testing. With isolated rat liver ribosomes, CFT titres varied from 8 to 256 in five cases and the test was negative in the male patient. The same titres were also obtained with whole liver and thyroid microsomes. Two patients (cases 1 and 5) gave positive CFT to 1:16 with calf thymus DNA and case 1 also reacted with purified yeast RNA to a titre of 32. The latter reactions can be attributed to co-existing antibodies to nuclear constituents.

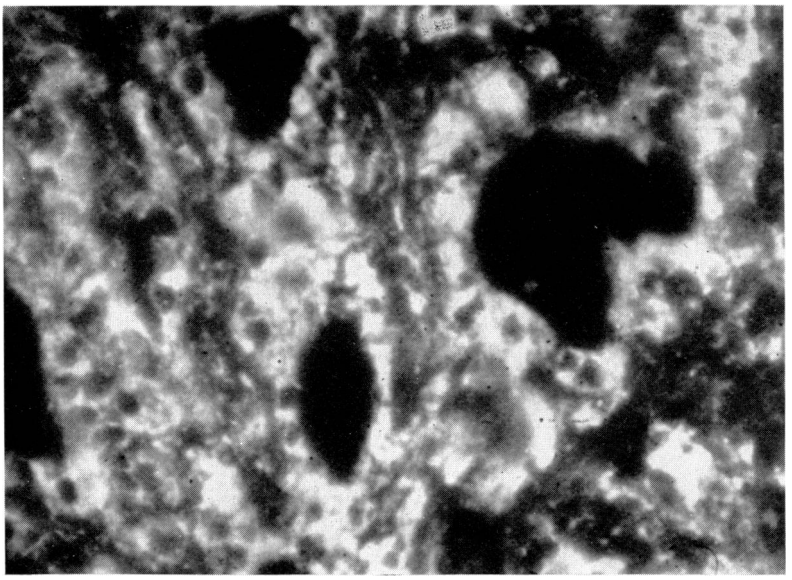

FIG. 5. Human thyrotoxic thyroid treated with SLE serum containing ribosomal antibody followed by anti-human γ-globulin–FITC conjugate. Ribosomal antibody gives diffuse bright cytoplasmic fluorescence maximal around the nuclei. (Magnification $\times 400$.)

Evidence of ribosomal nature of antigen

Results of absorption experiments are shown in Table 3. The IFL in pancreas, liver and kidney was absorbed out with ribosomes and to a lesser extent with rough ER membranes, and with microsomes. Smooth membranes, mitochondria, soluble supernatant and ribosomal RNA had no effect. When CFT was carried out with subcellular fractions using standard serum SL at a dilution of 1:32, DOC-isolated ribosomes had more antigen activity than whole microsomal fraction, while mitochondria gave a trace of fixation due to contamination with microsomes, and ribosomal RNA was inactive (Table 4).

Digestion with RNase and trypsin

CFT results after incubation with the treated ribosomes, are shown in Table 5. RNase led to a small decrease of antigen activity when used in excess, while trypsin digestion destroyed the antigen.

TABLE 3. Absorption of ribosomal fluorescence with rat liver subcellular fractions

Antigen (mg/protein/ml)	Mitochondria		Microsomes		Ribosomes		Smooth ER		Rough ER		Ribosomal RNA		Soluble supernatant	
	Rou	SL	Rou	SL	Rou	SL	Rou	SL	Rou	SL	Rou	SL	Rou	SL
5·0	++	++	−	−	−	−	+	+	−	−	++	++	++	++
2·5	++	++	−	−	−	−	++	+	−	−	++	++	++	++
1·25	++	++	+	−	−	−	++	++	+	−	++	++	++	++
0·6	++	++	+	±	−	−	++	++	+	+	++	++	++	++
0·3	++	++	++	+	−	−	++	++	++	++	++	++	++	++
0·15	++	++	++	++	+	−	++	++	++	++	++	++	++	++

Aliquots of SLE sera Rou (dilution 1:10) and SL dilution (1:40) were absorbed with equal volumes of each antigen dilution. Immunofluorescence on liver, kidney and pancreas were graded by visual assessment.

TABLE 4. Microtitre CFT results with rat liver mitochondria, microsomes, purified ribosomes and ribosomal RNA

Antigen concentration (mg/protein/ml)	Antigen			
	Mitochondria	Microsomes	Ribosomes	RNA
0·5	3*	4	4	0
0·25	2	4	4	0
0·125	0	4	4	0
0·06	0	3	4	0
0·03	0	3	4	0
0·015	0	2	4	0
0·007	0	0	4	0
0·0035	0	0	3	0

* CFT grading: 4 = no haemolysis; 3 = 25% haemolysis; 2 = 50% haemolysis; 0 = 100% haemolysis.
Standard SLE serum (SL) used at 1:32 dilution with all fractions.

TABLE 5. Microtitre CFT results with RNase A- and trypsin-treated rat liver ribosomes*

Antigen (mg/protein/ml)	Untreated ribosomes	RNase (0·5 mg/ml for 2 hr)	RNase (1 mg/ml for 2 hr)	Trypsin (0·1 mg/ml for 1 hr)	Trypsin (0·1 mg/ml for 2 hr)	Trypsin (0·2 mg/ml for 1 hr and for 2 hr)
0·5	4	4	4	3	2	1
0·25	4	4	4	2	Traces	0
0·125	4	4	3	1	0	0
0·06	4	4	2	0	0	0
0·03	4	4	2	0	0	0
0·015	4	2	0	0	0	0
0·007	4	0	0	0	0	0
0·003	3	0	0	0	0	0

* CFT grading: 4 = no haemolysis; 3 = 25% haemolysis; 2 = 50% haemolysis; 0 = 100% haemolysis.
Standard SLE serum (SL) used at 1:32 dilution in all tests.

DISCUSSION

The ribosomal antibody described in this study differs from those observed by other authors in at least three of its main characteristics: (1) the distinct immunofluorescence pattern it produces in a variety of tissues; (2) its reaction with a ribosomal protein rather than with RNA; (3) the rarity of its occurrence in SLE.

None of the previous authors used IFL on multiple tissue blocks, a method which has made it possible to distinguish separate antigens in closely related subcellular structures by the patterns produced in different organs and tissues. As more refined methods are developed for the fragmentation of cytoplasmic organelles, sera giving defined IFL patterns on organ sections can be absorbed with increasingly purified fragments of these complex membrane systems.

Regarding the findings of other authors, Watanabe *et al.* (1969) used IFL on lymphocyte smears and apparently on thyroid epithelial cytoplasm, and obtained 29% positive results in thirty-one SLE sera. This is difficult to correlate with our experience, since we have always found a very low incidence of thyroid cytoplasmic staining when testing large series of sera from patients with collagen disorders, even including mitochondrial antibodies (Doniach, 1972) which are found in a form of SLE (Maas & Schubothe, 1973; Berg, Traunecker & Marker, 1973) and the organ-specific reactions associated with thyroiditis. The ribosomal antigen/antibody system described in the present paper has been further characterized, and details will be reported shortly (Bianchi *et al.*, 1974). The antigen was not affected by RNase and was resistant to certain fixatives, but was destroyed with trypsin, suggesting that it is one of the ribosomal proteins. Previous antigens were identified as ribosomal RNA (Lamon & Bennett, 1970), ribosomal RNA–protein complex (Schur *et al.*, 1967; Sturgill & Preble, 1967), soluble RNA or polynucleotides (Sturgill & Carpenter, 1965; Schur *et al.*, 1972; Koffler *et al.*, 1971). If all methods of detection are considered, it is evident that many different antibodies directed against ribosomal constituents exist in SLE sera. Animals immunized with ribosomes developed a multiplicity of antibodies mainly to RNA and RNA–protein complexes, but also to various ribosomal proteins (Barbu & Panijel, 1960; Bigley, Dodd & Geyer, 1962). None of these were exactly similar to those found in SLE patients.

After extensive searching in two countries, only seven patients out of many hundreds of SLE sera tested by IFL on composite tissue blocks could be shown to contain these unusual antibodies. Their frequency is low in comparison with antibodies related to ribosomal RNA, or with RNA–protein complexes (40% were found by Schur *et al.* (1967), 51% according to Sturgill & Preble (1967) and 8% were reported by Lamon & Bennett (1970)). The clinical implications of this new antibody are similar to those of ribosomal precipitins in that all the SLE patients in the present series had renal involvement which proved fatal within 1–7 years in half the cases. It is not known whether ribosomal antibodies develop as a result of other events in SLE or whether they are instrumental in producing any of the lesions. Animals immunized with ribosomes in Freund's adjuvant developed a Coombs-positive haemolytic anaemia, leucopenia, focal hepatitis, myocarditis and encephalitis, together with histological changes compatible with nephritis (Dodd *et al.*, 1962), and the disease could be transmitted with serum to normal rabbits within 2 days. The relation of this experimental disease to clinical SLE is not apparent. Human autoantibodies are particularly valuable for the investigation of macromolecules as they react with fewer determinants than sera of animals immunized with the help of Freund's adjuvants. The characteristic IFL pattern produced by the ribosomal antibody described presently makes it especially suitable for analytical work on the structure of ribosomes, and hopefully this will contribute to an understanding of systemic lupus erythematosus.

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REFERENCES

BARBU, E. & PANIJEL, J. (1960) Presence d'anticorps anti-acide ribonucleique dans les immunserums anti-ribosomes. *C. R. Acad. Sci. (Paris)*, **251,** 1382.

BERG, P.A., TRAUNECKER, U. & MARKER, A. (1973) Mitochondrial antibodies in non-hepatic disorders. *Germ. Med.* **3,** 58.

BIANCHI, F.B., RIZZETTO, M., PENFOLD, P., SWANA, G.T. & DONIACH, D. (1974) Ultrastructural localization and characterization of a ribosomal antibody detected by immunofluorescence in SLE. *Clin. exp. Immunol.* **17,** 631.

BIGLEY, N.J., DODD, M.C. & GEYER, V.B. (1962) The immunologic specificity of antibodies to liver ribosomes and nuclei. *J. Immunol.* **90,** 416.

DEICHER, H.R.G., HOLMAN, H.R. & KUNKEL, H.G. (1960) Anticytoplasmic factors in the sera of patients with systemic lupus erythematosus and certain other diseases. *Arthr. and Rheum.* **3,** 1.

DODD, M.C., BIGLEY, N.J., GEYER, V.B., MCCOY, F.W. & WILSON, H.E. (1962) Autoimmune response in rabbits injected with rat and rabbit liver ribosomes. *Science*, **137,** 688.

DONIACH, D. (1972) Autoimmunity in liver disease. *Progr. clin. Immunol.* **1,** 45.

DUVE, C. DE, PRESSMAN, B.C., GIANETTO, R., WATTIAUX, R. & APPELMANS, F. (1955) Tissue fractionation studies. VI. Ultracellular distribution patterns of enzymes in rat liver tissue. *Biochem. J.* **60,** 604.

KOFFLER, D., CARR, R., AGNELLO, V., THOBURN, R. & KUNKEL, H.G. (1971) Antibodies to polynucleotides in human sera: antigenic specificity and relation to disease. *J. exp. Med.* **134,** 294.

LAMON, E.W. & BENNETT, J.C. (1970) Antibodies to ribosomal ribonucleic acid (rRNA) in patients with systemic lupus erythematosus (SLE). *Immunology*, **19,** 439.

LOWRY, O.H., ROSENBROUGH, M.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265.

MAAS, D. & SCHUBOTHE, H. (1973) Ein lupus-erythematodes-ahnliches Syndrom mit antimitochondrialem Antikorpern. *Dtsch. med. Wschr.* **98,** 131.

ROITT, I.M. & DONIACH, D. (1969) WHO manual for autoimmune serology. *World Health Organization, Geneva.*

ROTHSCHILD, J. (1963) The isolation of microsomal membranes. *Biochem. Soc. Symp.* **22,** 4.

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

SCHUR, P.H., STOLLAR, B.D., STEINBERG, A.D. & TALAL, N. (1971) Incidence of antibodies to double-stranded RNA systemic lupus erythematosus and related diseases. *Arthr. and Rheum.* **14,** 342.

STURGILL, B.C. & CARPENTER, R.R. (1965) Antibody to ribosomes in SLE. *Arthr. and Rheum.* **8,** 213.

STURGILL, B.C. & PREBLE, M.R. (1967) Antibody to ribosomes in systemic lupus erythematosus: demonstration by immunofluorescence and precipitation in agar. *Arthr. and Rheum.* **10,** 538.

TASHIRO, Y. & SIEKEVITZ, P. (1965) Ultracentrifugal studies on the dissociation of hepatic ribosomes. *J. molec. Biol.* **11,** 149.

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