*ANTIBODIES TO RIBONUCLEIC ACID IN SYSTEMIC LUPUS ERYTHEMATOSUS**

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Abstract.—Sera of 16 of 89 patients with systemic lupus erythematosus contained precipitating antibodies to RNA. These antibodies were found infrequently in patients with rheumatoid arthritis and Sjögren's syndrome, and not at all in patients with juvenile rheumatoid arthritis, myeloma, nor in normal individuals. These studies present evidence for the presence of antibodies to double-stranded RNA (both synthetic and viral). The fact that mammalian RNA's are probably single-stranded nucleotides suggests that these anti-RNA antibodies are directed primarily to a foreign, double-stranded RNA antigen.

Introduction.—Sera from patients with systemic lupus erythematosus often contain antibodies not only to deoxyribonucleic acids but to ribosomes, which suggests that antibodies to ribonucleic acid might also be present. The failure to detect antibodies to RNA in previous investigations may be due to the nature of the RNA used, i.e., yeast RNA. The availability of synthetic polynucleotides, which represent both single-stranded RNA and double-stranded RNA, has provided an opportunity to investigate further the problem of anti-RNA antibodies. In the present study, precipitating antibodies have been found in systemic lupus erythematosus sera to both single- and double-stranded polynucleotides (RNA) as well as to double-stranded viral RNA. These findings coincide with those of Steinberg *et al.*[1] who found such antibodies in NZB/NZW mice.

Materials and Methods.—Serum specimens were collected from normal individuals and from patients with systemic lupus erythematosus, rheumatoid arthritis, juvenile rheumatoid arthritis, Sjögren's syndrome, and myeloma.

Sera were tested for precipitating antibodies against various types of RNA by the Ouchterlony immunodiffusion method using Petri dishes. The agar "(Agar-Agar," Baltimore Biological Co., Baltimore, Maryland) was in phosphate (0.01 *M*) buffered saline pH 7.4, with 0.1% added sodium azide.[2] Holes were cut in the agar 8 mm in diameter and 4 mm apart. Precipitin patterns were read daily for 7 days with lines generally appearing in 24–72 hr.

Types of RNA tested were: polyinosinic-cytidylic acid (poly I-C) (Miles Laboratories, Inc., Elkhart, Illinois); polyadenylic-uridylic acid (poly A-U) (Miles Laboratories, Inc.); polyguanylic-cytidylic acid (poly G-C); single-stranded F2 phage RNA and single-stranded polio RNA (the kind gifts of Dr. David Baltimore, M.I.T., Cambridge, Mass.); rat liver sRNA (the kind gifts of Dr. Edgar C. Henshaw, Beth Israel Hospital, Boston, Mass.); yeast RNA (Worthington Lab., Freehold, N. J.); *E. coli* sRNA (Miles Laboratories, Inc.); rat and rabbit liver ribosomal RNA (rRNA); statalon viral double-stranded RNA (the kind gift of Dr. W. J. Kleinschmidt, Eli Lilly Lab., Indianapolis, Indiana); and polyadenylic acid (poly A), polyinosinic acid (poly I), polycytidylic acid (poly C), polyuridylic acid (poly U), and polyguanylic acid (poly G) (from Miles Laboratories, Inc., and Schwarz BioResearch, Inc., Orangeburg, N. Y.). Antibodies to poly I-C were prepared in rabbits by immunizing with poly I-C coupled to methylated bovine serum albumin, according to the method of Plescia *et al.* (8).

Those sera with antinuclear factors, as determined by immunofluorescence, were also examined for precipitating antibodies to calf thymus deoxyribonucleic acid, heat-denatured DNA, calf thymus soluble nuclear extract,[3] calf thymus nuclear protein, and rabbit liver ribosomes.[4] Serum hemolytic complement was determined by the method of Kent and Fife;[5] normals had levels of 200 CH_{50} units/ml.

Gamma, beta, and alpha globulin and albumin were isolated from a serum by starch block electrophoresis.[6] Gamma G globulin was isolated from selected sera by DEAE chromatography.[7]

Results.—Incidence of precipitins to poly I-C: Precipitating antibodies to poly I-C were found in the sera of 16 of 89 patients with systemic lupus erythematosus (see Fig. 1). Of 59 rheumatoid arthritis patients studied, two showed precipitins to poly I-C. These two patients had positive lupus cell preparations and some clinical features of systemic lupus erythematosus, including hypocomplementemia. One of 18 patients with Sjögren's syndrome had a barely perceptible precipitin. None of the patients with juvenile rheumatoid arthritis (60 tested) or myeloma (70 tested) and no normal individuals (21 tested) had such antibodies. Pooled normal human serum and Cohn Fraction II (Lederle Laboratories, Pearl River, N. Y.) at 10 mg/ml also did not precipitate with poly I-C.

FIG. 1.—Precipitin reactions between poly I-C (*A*) and systemic lupus erythematosus sera *3*, *4*, and *5*. Sera *1*, *2*, and *6* do not react.

Incidence of precipitins to other RNA's: The sera which precipitated with poly I-C often precipitated with other RNA's. The incidence of these other precipitins is recorded in Table 1. A comparison between the reaction of selected sera with different RNA's, DNA, heat-denatured DNA, and ribosomes is made in Table 2. Different patterns of reactions are seen.

Of 18 selected systemic lupus erythematosus sera, many of which precipitated with DNA and/or ribosomes, but none with poly I-C, only one reacted with another RNA, poly I.

Normal sera also did not react with poly I, F2 phage RNA, statalon viral RNA, polio RNA, poly-A-U, or poly G-C.

Comparison between reactions to different RNA's, DNA, and ribosomes: Of the sera which precipitated with poly I-C, most, but not all, also formed precipitins to DNA and/or ribosomes (Table 2). Optimal reactions occurred with 10 γ RNA/ml and 1 mg/ml of DNA and ribosomes. There was no fusion of lines formed between systemic lupus erythematosus sera and DNA, ribosomes, and poly I-C.

TABLE 1. *Incidence of precipitins to RNA's in patients with systemic lupus erythematosus.*

Nucleic acid	Number of patients tested	Number positive
Poly I-C	89	16
Poly A-U	16	12
Statalon double-stranded RNA	15	4
Poly G-C	15	0
Poly I	15	8
Poly A	16	6
Poly G	15	7
Poly U	15	0
Poly C	15	0
rRNA	15	0
Yeast RNA	15	0
E. coli sRNA	15	0
Rat liver sRNA	15	0
F2 phage	15	0
Polio	15	0

TABLE 2. *Precipitin reactions between selected sera and antigens.*

	Systemic Lupus Erythematosus Patients						Normal individuals	Rabbit anti-poly I-C
	B.A.	E.A.	R.V.	A.B.	W.E.	B.G.		
Poly I-C	+	+	+	+	+	+	0	+
Poly A-U	+	+	+	+	+	0	0	+
Statalon double-stranded RNA	+	0	+	±	0	0	0	+
Poly G-C	0	0	0	0	0	0	0	0
Poly I	+	0	+	+	+	0	0	+
Poly A	+	0	0	+	+	0	0	0
Poly G	+	0	+	+	+	0	0	0
Poly U	0	0	0	0	0	0	0	0
Poly C	0	0	0	0	0	0	0	0
rRNA	0	0	0	0	0	0	0	0
Yeast	0	0	0	0	0	0	0	0
E. coli sRNA	0	0	0	0	0	0	0	0
Rat liver sRNA	0	0	0	0	0	0	0	0
F2 phage	0	0	0	0	0	0	0	0
Polio	0	0	0	0	0	0	0	0
DNA	+	+	+	+	0	0	0	0
Heat-denatured DNA	+	+	+	+	0	0	0	0
Ribosomes	+	+	+	0	0	+	0	0

In comparing the reactions between systemic lupus erythematosus sera and the different types of RNA, lines of fusion or spurring could not always be detected. Where immunological specificity was discernible, poly I-C lines fused with poly A-U lines, and poly I-C lines either spurred over or fused with poly I and/or poly G lines.

The rabbit anti-poly I-C also precipitated with statalon RNA, poly A-U, and poly I, but not with poly A, poly C, poly U, poly G, *E. coli* sRNA, polio RNA, phage RNA, rat liver sRNA, DNA, heat-denatured DNA, nuclear protein, calf thymus soluble nuclear extract, or ribosomes (Table 2).

Identification of precipitating factor in serums: The precipitation with poly I-C of one serum (tested) was not affected by heating for 30 minutes at 56°C. When the different electrophoretic fractions of a serum that precipitated with poly I-C

were tested, only the gamma globulin cut precipitated with poly I-C, forming a line which fused with that of whole serum. The β and α globulin and albumin cuts did not react with poly I-C. Gamma G globulin, isolated by DEAE chromatography from a serum that precipitated with poly I-C, also precipitated with the antigen.

Discussion.—The present studies indicate that sera from some individuals with systemic lupus erythematosus have precipitins to RNA. The precipitins have the characteristics of antibodies, in that they are heat-stable, show specificity, migrate as gamma globulins in electrophoresis, and appear with γG globulins on DEAE chromatography. These antibodies are present in low concentration, since only 10 γ/ml of antigen is required for precipitation. This fact may account for failure to detect such antibodies in earlier investigations.

These anti-RNA antibodies are frequently found in the presence of precipitating antibodies to DNA and ribosomes. Such antibodies are not related, however, as shown by Ouchterlony immunodiffusion, and by their marked difference in optimal precipitin concentrations. The antibodies are directed primarily toward double-stranded RNA (poly I-C, poly A-U, and statalon viral RNA), although some reaction with single-stranded polynucleotides was noted. The greater resistance of double-stranded RNA to digestion by ribonucleases in the serum could account for the relative specificity. However, the precipitation of more sera with double-stranded RNA than single-stranded RNA, and the observed spurring by double-stranded RNA over single-stranded RNA on Ouchterlony, supports the hypothesis that systemic lupus erythematosus sera react preferentially with double-stranded RNA.

Lacour *et al.*[9] have shown that rabbit antibodies to poly I-C also react with poly A-U and poly I, but not with poly A, poly G, and poly G-C. Rabbit antibodies to poly A-U also react with poly I-C and poly A, but not with poly U, poly I, poly C, and poly G-C. They postulated, therefore, that these antibodies have specificity for the region of N7 and the 6-amino or 6-keto group of the purine component of the copolymer. The sera from systemic lupus erythematosus patients studied here showed greater heterogeneity as far as reactions with different nucleotides are concerned.

The fact that some systemic lupus erythematosus sera have anti-DNA and antiribosome antibodies has been interpreted to mean that systemic lupus erythematosus patients make these antibodies either to a foreign antigen or to their own nucleotides. Previous studies have suggested that the anti-DNA antibodies are formed to such a patient's own DNA[2] but cross-react with DNA from other species. In the present investigation, antibodies to rRNA and sRNA were not detected in the systemic lupus erythematosus sera. These RNA's are largely single-stranded and make up more than 95 per cent of mammalian RNA. Moreover, neither single-stranded F2 phage RNA or single-stranded polio RNA reacted with the systemic lupus erythematosus sera. These results suggest that the anti-RNA antibodies which form precipitins primarily to double-stranded RNA are directed against a foreign antigen, such as a double-stranded RNA virus. Whether these anti-RNA antibodies represent a genetically controlled hyperimmune response by systemic lupus erythematosus patients

to a particular virus, or whether the presumed viremia and/or the response to it play some role in the pathogenesis of systemic lupus erythematosus, has yet to be determined.

Double-stranded RNA in the form of statalon virus or synthetic polynucleotides (poly I-C, poly A-U) can be used to induce interferon. The administration of these substances has been advocated for therapy in viral infection. Studies in rabbits with experimental herpetic keratoconjunctivitis have suggested that treatment with poly I-C caused improvement, presumably through the induction of interferon.[10] These studies and those of Steinberg *et al.*[1] show that synthetic double-stranded RNA (and perhaps viral double-stranded RNA) induce antibodies to double-stranded RNA. From these results one may speculate that the administration of double-stranded RNA protects against viral infections through the induction of anti-RNA antibodies as well as via interferon.

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