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Radioimmunoassay for Antibodies to Cytoplasmic Ribosomes in Human Serum

Abstract. *A radioimmunoassay for the detection of antibodies in human serum to tritium-labeled HeLa cell cytoplasmic ribosomes was developed with the use of Macaloid for the inhibition of endogenous ribonuclease activity. Antibodies were observed in the serum of patients with systemic lupus erythematosus in high incidence and titer. Patients with rheumatoid arthritis and chronic active hepatitis manifested a lower incidence and titer of antibodies to ribosomes, whereas serums from normal individuals and from patients with sarcoidosis, chronic glomerulonephritis, and malignant tumors showed no significant reactivity with cytoplasmic ribosomes. Maximum inhibition of the reaction was achieved with unlabeled HeLa cell ribosomes or rat liver ribosomes and partial inhibition by purified ribosomal RNA.*

Antibodies directed against cytoplasmic ribosomes have been observed in the serums of patients with systemic lupus erythematosus (SLE). A low incidence of antibodies has been detected (13 percent) by immunodiffusion (*1*) and a higher incidence of antibodies has been observed (25 to 50 percent) with bentonite flocculation (*2*) and fluorescent spot assays (*1*), whereas immunofluorescence studies with tissue sections demonstrated ribosomal antibodies in less than 1 percent of SLE serums (*3*). The specificity of the reaction for ribosomes appears to require both RNA and protein although the reactions of ribosomal antibodies demonstrable by immunofluorescence were not affected by treatment with ribonuclease. In addition, a soluble cytoplasmic ribonucleoprotein distinct from ribosomes has been described (*4*).

A radioimmunoassay provides a more sensitive and quantitative test for assaying ribosomal antibodies. Difficulties have been encountered in developing a radioimmunoassay because of the degradation of small quantities of labeled ribonucleoprotein by naturally occurring serum ribonuclease. We now report a radioimmunoassay procedure that has been adapted for the detection of antibodies in human serums reactive with labeled ribosomes derived from the cytoplasm of HeLa cells.

Tritiated ribosomes were obtained from HeLa cells grown for 24 hours in suspension medium containing ^{3}H-labeled uridine (1 μc/ml). Cytoplasm was obtained (*5*) and ribosomes were purified by discontinuous sucrose-gradient centrifugation (*6*). Ribosome preparations showed a specific activity of 12×10^3 to 20×10^3 count/min · μg with more than 97 percent of the radioactive material precipitable by trichloroacetic acid. Ribosomal RNA was prepared from unlabeled ribosomes by the sodium dodecyl sulfate–phenol procedure (*7*). Ribosomal portions retained activity at −70°C for more than 6 months and at 4°C for 10 to 14 days.

When human serum was incubated with labeled ribosomes at 37°C for 1 hour, more than 80 percent of the radioactivity became soluble in trichloroacetic acid. The ability of various inhibitors to inactivate serum ribonuclease activity as measured by trichloroacetic acid precipitability is shown in Table 1. Although rat liver cytoplasmic inhibitor prepared according to the procedure of Roth (*8*) was effective in preventing degradation of ribosomes, it was found to interfere with the Farr assay. Macaloid was an effective inhibitor that did not interfere with the radioimmunoassay procedure. Optimal conditions for the interaction of gamma globulin from human serum with labeled ribosomes were determined. Portions of serums from normal controls and patients were diluted with a buffer consisting of 0.15*M* NaCl and 0.2*M* sodium borate, *p*H 7.8, containing Macaloid (0.1 percent, weight to volume). The mixtures were incubated at 37°C in a gyratory water bath for 90 minutes, then cooled in ice and centrifuged at 8000*g* for 30 minutes. Samples (100 μl) of the supernatant were collected and mixed with 100 μl of borate-saline buffer containing ^{3}H-labeled ribosomes (6×10^3 to 8×10^3 count/min). The mixtures were incubated at 4°C overnight; 2 μl of 70 percent saturated ammonium sulfate were then added. After 1 hour at 4°C, the mixtures were centrifuged at 2000 rev/min for 1 hour, and the pellets were washed once with 2.5 ml of 35 percent saturated ammonium sulfate, dissolved in 1 ml of NCS solubilizer (Amersham/Searle, Arlington, Illinois) and counted in a Beckman scintillation counter. The percentage of precipitation of the ribosomes was calculated by dividing the pellet counts by the input counts. Under these conditions, the radioactivity not present in the pellet was detected in the supernatant and was precipitable by trichloroacetic acid.

Serums from normal individuals and from patients with active SLE and renal disease, active SLE but without renal disease, inactive SLE, rheumatoid arthritis, chronic active hepatitis, sarcoidosis, chronic glomerulonephritis, and malignant tumors were tested at 1:10 and 1:20 dilutions. The patients studied were receiving medical therapy at the Rockefeller University Hospital or at the University of Virginia Hospital. Patient selection was based on (i) fulfillment of the preliminary criteria of the American Rheumatism Association for classification as SLE (*9*) and (ii) presence of anti-

Table 1. Effect of ribonuclease inhibitors on interaction of serum ribonuclease and ^{3}H-labeled ribosomes. The result is expressed as the percent of radioactivity present in the fraction precipitated by trichloroacetic acid.

HeLa cell [^{3}H]ribosomes	Radioactivity precipitable (%)
Alone	> 97
+ serum	20
+ serum + heparin	60
+ serum + RLCI*	> 97
+ serum + Macaloid	95

*Rat liver cytoplasmic inhibitor.

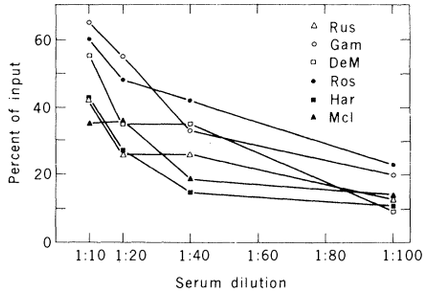

Fig. 1. Inhibition of precipitation of labeled ribosomes; 100 μl of a dilution of serum is added to 100 μl of ^{3}H-labeled HeLa cell cytoplasmic ribosomes.

Table 2. Radioimmunoassay for antibodies to cytoplasmic ribosomes.

Serums	Patients (No.)	Positive (%)	Mean binding of positives
SLE-active (renal disease)	40	63*	38.8 ± 14.0†
SLE-active (no renal disease)	39	77	38.1 ± 15.2
SLE-inactive	32	78	32.8 ± 11.6
Rheumatoid arthritis	47	30	24.1 ± 8.4
Chronic active hepatitis	25	28	26.7 ± 13.0
Chronic glomerulonephritis	21	0	0
Sarcoidosis	20	0	0
Malignant tumors	45	0	0
Normals			
Males	24	4	‡
Females	37	0	0

*Binding greater than 15 percent at 1:10 dilution of serum. †Percentage of precipitation. ‡One serum showed 32 percent precipitation.

Table 3. Comparison of inhibitory activity of rat liver and HeLa cell ribosomes.

Micrograms added*	Rat liver†	HeLa cell†
16	56‡	46
8	48§	40§
4	51§	34
2	44	28
1	26	22

*Serum Rus plus unlabeled ribosomes. †Inhibition with unlabeled ribosomes. ‡Percentage of inhibition of precipitation of labeled ribosomes. §Ribonuclease treatment eliminated inhibitory activity.

body to DNA in their serums. Each patient was placed in one of three clinical groups detemined by clinical criteria alone: (i) active SLE with glomerulonephritis; (ii) active SLE without evidence for glomerulonephritis; and (iii) inactive SLE. The presence of glomerulonephritis was assessed by examination of urinary sediment, blood urea nitrogen, and serum creatinine determinations. Persistent proteinuria (2+ qualitative and 350 mg of total urinary protein per 24 hours), microscopic hematuria and cylindruria, and progressive decline in creatinine clearance rates were considered indicative of active glomerulonephritis. Patients were classified as having active SLE when history and physical examination revealed evidence of at least two of the following signs and symptoms on two consecutive examinations: rash, fever, arthralgia or arthritis, serositis, alopecia, stomatitis, or evidence of central nervous system involvement. Serums were obtained from clotted specimens of peripheral blood; complement was inactivated by heating at 56° for 30 minutes prior to use.

The mean binding of labeled ribosomes by serums of 37 normal females was 7.2 ± 2.4 percent at 1 : 10 dilution and 6.6 ± 3.2 percent at 1 : 20 dilution. No significant difference in binding was observed in the serums of 24 normal males −6.8 ± 1.8 percent at 1 : 10 dilution and 8.8 ± 3.1 percent at 1 : 20 dilution. Serums precipitating more than 15 percent of labeled ribosomes were considered positive.

A high incidence of antibodies reactive with ribosomes was found in all groups of patients with SLE (Table 2). The slightly lower incidence in patients with renal disease ($P = .09$ compared by the chi-square test for nonrenal SLE patients) raises the possibility that these antibodies may be a component of an immune complex system deposited in the kidneys of patients with glomerulonephritis. In contrast to antibodies to native DNA, no correlation between disease activity and ribosomal antibodies was noted. Patients with rheumatoid arthritis and chronic active hepatitis manifested a lower incidence and titer of ribosomal antibodies, whereas serums from patients with chronic glomerulonephritis, sarcoidosis, and malignant tumors and serums from normal individuals did not manifest a significant incidence of ribosomal antibodies. SLE serums precipitated ribosomes at dilutions ranging from 1 : 5 to 1 : 100 (Fig. 1). A higher incidence of positive reactions and increased precipitation of ribosomes for SLE serums was observed at 1:10 dilution (36.8 ± 13 percent) when compared to a 1 : 20 dilution (30.5 ± 9.7 percent). When gamma globulin was isolated by DEAE chromatography from an SLE serum and reconstituted to a comparable concentration of gamma globulin in serum, a similar reactivity compared to serum was observed (78 percent precipitation of ribosomes by serum Rus at 1 : 5 dilution and 80 percent precipitation by isolated gamma globulin). Unlabeled HeLa ribosomes and rat liver ribosomes (*8*) were found to have comparable activity as inhibitors of antibody interaction (Table 3). The specificity of the interaction was in part attributable to RNA, as indicated by the ability of purified rat liver ribosomal RNA to partially inhibit the reaction in selective SLE serums

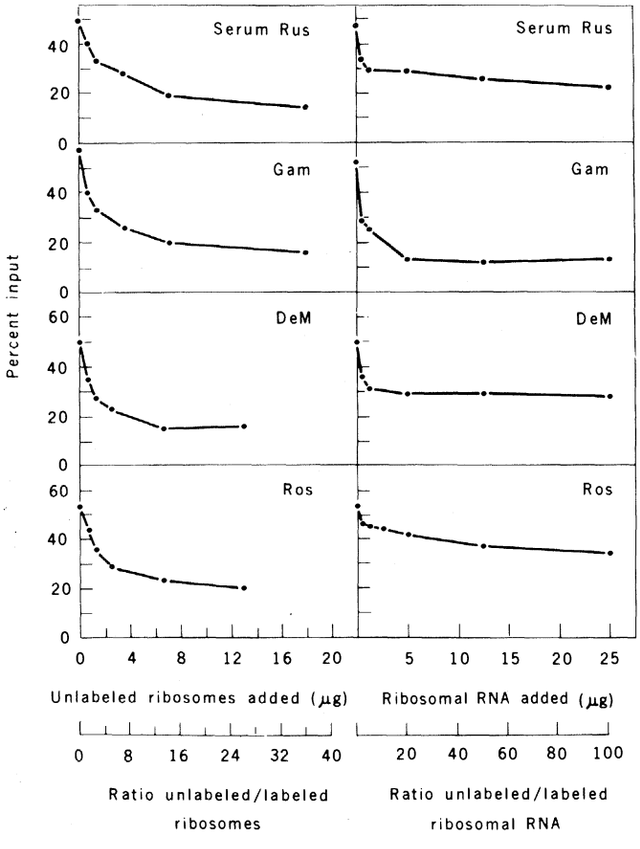

Fig. 2. Effect of serum dilution on precipitation of labeled ribosomes; 50 μl of ribosomal RNA or rat liver ribosomes is added to 100 μl of a 1:5 dilution of test serum followed by 50 μl of ^{3}H-labeled HeLa cell cytoplasmic ribosomes.

(Fig. 2) and the loss of inhibition achieved after treatment of ribosomes with Sepharose linked ribonuclease.

The radioimmunoassay procedure is capable of detecting ribosomal antibodies with considerably greater sensitivity than previously utilized assays. The specificity of the antibody appears to be similar, that is, related partially to RNA, although the relationship of ribosomal antibodies to nuclear ribonucleoprotein and soluble cytoplasmic ribonucleoprotein antibodies remains to be clarified.

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13-*cis*-Retinoic Acid: Inhibition of Bladder Carcinogenesis Induced in Rats by *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine

Abstract. *Transitional cell carcinoma was induced in the bladders of male Fischer rats by 12 oral doses of the carcinogen* N-*butyl*-N-*(4-hydroxybutyl)nitrosamine. Feeding of 13-*cis*-*retinoic acid after completion of carcinogen treatment diminished the number and severity of cancers and other proliferative lesions of the bladder.*

In a previous study we demonstrated that 13-*cis*-retinoic acid can inhibit the development of bladder cancer induced by *N*-methyl-*N*-nitrosourea in female Wistar/Lewis rats (*1*). A high percentage of these malignancies were squamous cell carcinomas (30 to 40 percent), while in humans squamous bladder cancers account for much less of the total (*2*). We therefore wished to verify our results in another experimental system in which carcinogenesis produces almost entirely transitional cell tumors (*3*). In the study reported here, bladder cancer was induced in Fischer male rats with 12 intragastric doses of 200 mg of *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (OH-BBN) (*4*) over a period of 6 weeks (Table 1). One week following the final dose, 21 of the 43 treated rats were placed on diets containing 240 mg of 13-*cis*-retinoic acid per kilogram of diet. The animals were killed 6 months after the initial carcinogen treatment. The bladders were inflated with 10 percent formalin, and after fixation were transected with two cuts in a dorsoventral plane to yield an anterior and a posterior dome-shaped area and a median cylindrical area. Each of the three areas was serially sectioned (5 μm) in the plane of the original cuts, and stained with hematoxylin and eosin. The slides were randomized, coded, and evaluated by three of us (G.M.F., D.G.G., and S.F.S.) using two similar semiquantitative scoring systems. One system has been described previously (*5*), and the other will be described at length in a later report. There was excellent agreement between all three pathologists, and the results were therefore averaged.

The transitional cell carcinomas and other proliferative epithelial lesions were ranked according to their relative grade or atypia score (Table 1). These were determined, in part, by the degree of epithelial differentiation and of cellular anaplasia and by the number of mitotic figures. The results indicate that 13-*cis*-retinoic acid inhibited the development of transitional cell carcinomas in the bladder. Significantly fewer animals fed this retinoid had severe (high-grade) cancers. The same was true for the other proliferative epithelial lesions; fewer animals fed

Table 1. Inhibition of bladder carcinogenesis by 13-*cis*-retinoic acid. Beginning at 6 weeks of age, male Fischer rats were dosed twice weekly, intragastrically, each time with 200 mg of OH-BBN (*4*), dissolved in 0.5 ml of 20 percent ethanol. A total of 2400 mg was given over a period of 6 weeks. One week after the final dose, 21 of the rats were placed on a diet containing 240 mg of 13-*cis*-retinoic acid (Hoffmann-La Roche) per kilogram of diet. The retinoid was obtained as a gelatinized beadlet preparation and blended into a powdered diet (Wayne Lab Meal). The control group was fed the same diet containing gelatinized beadlet material without retinoid. Animals were killed 6 months after the initial carcinogen treatment. Histological sections from the anterior, median, and posterior areas of each bladder were randomized, coded, and evaluated by three pathologists using similar semiquantitative scoring systems, and the results were averaged. Values for *P* were derived from chi-square and Student *t*-tests. The diagnosis of transitional cell carcinoma was based on the presence of invasion of underlying connective tissue or invasion of smooth muscle, and of moderate to marked histologic or cytologic atypia. Atypia was scored on a scale from 0 to 5 depending on the presence of increased cellularity, hyperchromasia, prominent nucleoli, pleomorphism of cellular and nuclear size and shape, loss of cell polarity and orientation, and presence of mitoses. Low-grade cancers had atypia scores below 3; high-grade, 3 and above. Other proliferative epithelial lesions included noninvasive papillomas and discrete hyperplastic lesions that did not meet the diagnostic criteria necessary to be classified as carcinomas. Lesions were considered to have atypical epithelia if atypia scores were 2 or above. An atypia score of 1 was considered minimal; a score of 2, moderate; and a score above 2, severe. Criteria for scoring atypia have been described in detail (*5*). In addition to the rats described here, 25 rats, which received no carcinogen, were fed 13-*cis*-retinoic acid. Bladders from these animals showed no histopathological abnormalities. Bladders from 23 rats, which received neither carcinogen nor retinoid for the duration of the experiment, were also histologically normal.

Treatment	Rats (No.)	Rats with transitional cell carcinoma (No.)		Rats with other proliferative epithelial lesions (No.)		Total proliferative epithelial lesions, including cancers (No.)	Rats with epithelial atypia (No.)			Mean atypia score
		Low-grade	High-grade	Typical epithelium	Atypical epithelium		Minimal	Moderate	Severe	
OH-BBN alone	22	1	9	4	9	47	5	6	11	2.0
OH-BBN and 13-*cis*-retinoic acid	21	3	4	7	3	24	10	6	5	1.1
P			<.05		<.01	<.01	<.025		<.025	<.01