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PRÉSENCE D'ANTICORPS ANTI-ACIDE RIBONUCLÉIQUE DANS  
LES IMMUNOSÉRUMS ANTIRIBOSOMES

PRESENCE OF ANTI-RIBONUCLEIC ACID ANTIBODIES IN ANTIRIBOSOMAL IMMUNE SERA

by

Emanoil Barbu and Jacques Panijel

Summary

The immune sera prepared against ribosomes contain one or several antibodies precipitating with ribonucleic acid (RNA). The specific role of the ribonucleic acids as antigens also brings to light their role as inhibitors in precipitation reactions of heterologous ribosomes with immune sera.

The problem of the existence of specific antibodies for nucleic acids is still very much debated. Recently, Seligson<sup>1</sup> and Ueicher et al.<sup>2</sup> showed that lupus erythematosus sera contain substances precipitating with deoxyribonucleic acid (DNA), which have been considered by these authors to be genuine antibodies. Nevertheless, these sera cannot be obtained by immunization with DNA preparations. This makes it difficult to exclude completely the hypothesis that it is a question of secondary reactions which are immunologically nonspecific.

A similar type of objection has been raised with regards to the existence of anti-ribonucleic acid antibodies (anti-RNA). In the present note, it has been demonstrated in fact that there exists in antisera against ribosomes such antibodies. The ribosomes used for immunization are essentially ribonucleoprotein particles containing 50 - 60% RNA.

### Methods

(A) Ribosomes were prepared, starting with bacterial cultures (E. coli K12, E. coli B, Streptococcus A<sub>23</sub>) and from animal tissues (rabbit and rat livers) by the methods already described<sup>3,4</sup>.

(B) Antisera were obtained by intravenous immunization of rabbits with ribosomes.

(C) RNA was obtained from ribosomes by removing the protein with phenol by the method of Schramm et al.<sup>5</sup>

(D) The immunochemical studies were carried out using the qualitative ring test, and with the quantitative method for RNA content of specific precipitates as described earlier<sup>3,4</sup>.

### Results

(A) Qualitative Method-- The ring test was effective with various antisera and with different dilutions of RNA in 0.145 M NaCl at neutrality. In less than 10 min at ambient temperature, a distinct positive reaction was observed with solutions containing 5 µg per ml RNA or more. Normal sera did not give a reaction under these conditions. The reaction occurred even though the RNA had been heated for 2 min at 100°C, but disappeared completely after treatment with RNase even when the RNA concentration was as high as 0.5 mg per ml.

(B) Quantitative Method-- The amount of RNA precipitated by the immune sera depended on both the serum and the RNA preparations

1 ml of antiserum no. 62 for E. coli K12 ribosomes precipitated from 20 to 22 µg rat liver RNA, 18 to 20 µg E. coli B RNA, and 12 to 14 µg Streptococcus A<sub>23</sub> RNA.

1 ml of antiserum no. 57 (for E. coli K12 (3300) ribosomes) precipitated 30 to 35  $\mu$ g rat liver RNA and 22 to 24  $\mu$ g E. coli B RNA.

1 ml of Streptococcus A23 antiribosomal serum no. 31 precipitated only 6 to 8  $\mu$ g of the various RNA preparations.

The percentage of RNA recovered in the specific precipitated decreases when the concentration of RNA is increased. In the case of serum no. 62, it is 44 % with 40  $\mu$ g per ml antigen, 24 % with 80  $\mu$ g per ml and 6 % with 200  $\mu$ g per ml.

After treatment of the preparations with RNase, no precipitate was obtained with antiribosomal sera.

(C) Inhibition Reaction-- The specific intervention of RNA as an antigen can likewise be presented as evidence for inhibition reactions. The experiment consisted of adding various excess quantities of RNA to the antisera and then adding homologous and heterologous ribosomes.

Table I shows the percent inhibition of ribosome precipitation as a function of the amount of RNA added (the RNA was extracted from E. coli B ribosomes.)

TABLE I

	ribosomes	RNA ( $\mu$ g/ml)	% inhibition
Antiserum for <u>E. coli</u> K12 ribosomes	<u>E. coli</u> B	1500	0
		750	0
	<u>Streptococcus</u> A23	3000	55
		1500	70
		375	43
		94	17
Antiser for <u>Streptococcus</u> A23 ribosomes	<u>E. coli</u> B	1500	38
		750	39
	<u>Streptococcus</u> A23	1500	0
		750	0

It can be seen that inhibition reaches very important levels in the case of precipitin reactions with heterologous ribosomes but has no effect in the case of homologous reactions. This is conveniently explained if one accepts that the antisera contain several groups of antibodies, some of which are only specific for RNA while others are specific for the protein constituents of the ribosomes. Thus, the common antigen for ribosomes of diverse origin will be just RNA while the protein antigens will vary considerably depending on the type of ribosome. Inhibition by RNA is affected the most by the proportion of anti-RNA antibodies in the immune serum. Nevertheless, it happens that ribosomes of different origins react differently from a quantitative point of view<sup>3,4</sup> indicating that they may contain, in addition to the common ribonucleic acid antigen, protein antigens of varying amounts.

### Conclusions

All the experiments demonstrate that RNA, which by itself seems incapable of inducing the formation of precipitating antibodies, is nevertheless antigenic when it is present in the form of ribosomes. The fact that it is possible to induce the appearance of anti-RNA antibodies poses the problem as to what degree such an antibody can influence the synthetic processes particularly at the cellular level.

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