SPECIFIC ACTION OF "IMMUNE" RNA IN TWO DIFFERENT CELL SYSTEMS

V. A. Lyashenko, O. V. Protasova, and M. A. Frolova

UDC 612.017.1 :547.963.32

RNA obtained from the mouse spleen at a time of intensive hemolysin formation induces synthesis of hemolysins in a culture of spleen cells and also modifies the properties of macrophages, stimulating phagocytosis of erythrocytes in a cell culture.

* * *

Recent investigations have shown that RNA isolated from lymphoid organs of immunized animals can induce the formation of antibodies in a culture of lymphoid cells or in intact animals in vivo [2, 3, 5, 8, 9, 15]. RNA can also induce specific changes in phagocyte function [7]. Comparison of the action of one RNA preparation on different types of cells has been undertaken only with RNA isolated from macrophages [13, 14].

In the investigation described below an attempt was made to determine whether RNA from the spleen, in which antibodies are synthesized, can induce immune changes in different types of cells.

EXPERIMENTAL METHOD

F_1 Mouse hybrids (CBA × C57 BL) or noninbred albino mice weighing 16-18 g were used. The RNA-donor mice were immunized by two injections of sheep's erythrocytes in a dose of 0.2 ml of a 10% suspension intravenously at an interval of 5-7 days. RNA was extracted from the spleens of these mice 96 h after the last injection by means of hot phenol and dodecylsulfate by the method of Sherrer and Darnell [16].

Two cell systems were used in the experiments. 1. Spleen cells from intact mice were incubated in RNA solutions in medium No. 199 by the method described by Cohen et al. [5]. RNA was used in a concentration of 30-100 μg/ml. After incubation for 30 min, the number of cells forming hemolysins was determined in parallel samples by the method of local hemolysis [11]. Control samples of cells were incubated in the absence of RNA or in the presence of RNA from the spleen of a nonimmunized mouse.

2. Cells of the peritoneal exudate of intact mice were introduced into tubes with a cover slip, and the cells were allowed to adhere to the glass. The cover slip was then placed in RNA solutions of an empirically selected optimal concentration of 5-10 μg/ml for 30 min at 37°. After incubation the RNA solutions were removed and a suspension of sheep's erythrocytes was poured into the tube in the proportion of ten erythrocytes per cell of peritoneal exudate. The tubes were incubated for 2 h at 37°. The cover slips were then removed and rinsed successively in two vessels containing physiological saline at 4° for 10 sec. After this rinsing, few or no erythrocytes unattached to the cells were found in the specimens. The cover slips were fixed and stained, and the number of erythrocytes adherent to macrophages or ingested by them was counted in the preparations. Erythrocytes discovered in vacuoles in the cytoplasm of the macrophages were regarded as ingested. Cells incubated in medium No. 199 without RNA or with "nonspecific" RNA were used as the control.

I. I. Mechnikov Moscow Research Institute of Vaccines and Sera. (Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 7, pp. 67-70, July, 1970. Original article submitted June 23, 1969.

TABLE 1. Action of Normal and "Immune" RNA on Mouse Spleen Cells (number of zones of hemolysis)

Method of incubation of cells	Index			
	n	M	S*	P
In medium No. 199 without RNA	8	10.1	1.63	
In medium with "normal" RNA, 100 μg/ml	8	12.4	1.26	0.5
In medium with "immune" RNA, 100 μg/ml	7	19.9	2.23	0.01

TABLE 2. Effect of "Immune" RNA on Phagocytosis of Erythrocytes by Macrophages of Nonimmunized Mice

Index studied	Conditions of incubation of samples	Statistical index		
		n	M	$S_{\bar{x}}$
Percentage of active phagocytes	Control (without RNA)	12	6.5	0.82
	RNA "immune" to erythrocytes	12	11.9	1.7
	RNA "immune" to Vi-antigen	6	6.0	0.48
Number of erythrocytes ingested per 100 phagocytes	Control (without RNA)	12	9.0	1.15
	RNA "immune" to erythrocytes	12	17.4	2.25
	RNA "immune" to Vi-antigen	6	6.2	1.41
Number of erythrocytes adherent to 100 phagocytes	Control (without RNA)	12	9.4	1.62
	RNA "immune" to erythrocytes	12	11.6	2.0
	RNA "immune" to Vi-antigen	6	2.9	0.48

EXPERIMENTAL RESULTS

Incubation of spleen cells of intact mice with "immune" RNA approximately doubled the number of cells forming hemolysins (Table 1), while RNA from the spleen of normal mice was inactive. A special Vi-antigen of Salmonella typhi also was inactive.

Incubation of macrophages with "immune" RNA in a concentration of 5 μg/ml led to an increase in the number of macrophages engaged in phagocytosis of erythrocytes, and an increase in the number of erythrocytes ingested per 100 macrophages (Table 2). The difference between the mean percentage of phagocytic macrophages in the control samples and in samples incubated with "immune" RNA is statistically significant ($P = 0.01$), from which it can be concluded that a definite stimulant effect was present [1]. "Nonspecific" RNA had no such action.

The RNA preparations which were used contained about 3% protein. Regarding this protein conventionally as serum protein, control experiments were carried out in which the serum of the RNA donors was added to the incubation medium in concentrations of 1 : 3000 and 1 : 30,000. After the addition of serum, an increase mainly in the number of erythrocytes adherent to macrophages was observed. Typical "rosettes," not observed after treatment with RNA, appeared (Fig. 1). Consequently, the serum differed from RNA in the character of its action.

Hence, RNA preparations from the spleen, in which intensive antibody synthesis was taking place, induced specific immunological changes in cells of two types.

The difference between the optimal concentrations of RNA in these two systems can be explained by the fact that samples containing macrophages had far fewer cells than samples containing spleen cells, and the number of RNA molecules per cell was greater.

RNA causes structural changes in only a few cells in the population; Their number is comparable with the number of cells possessing immune properties before treatment with RNA. These are perhaps cells "cloned" to the particular antigen. For this reason, in accordance with views expressed by some investigators [4, 10, 17], an antigen-RNA complex is most likely to be the active principle of the preparations studied.

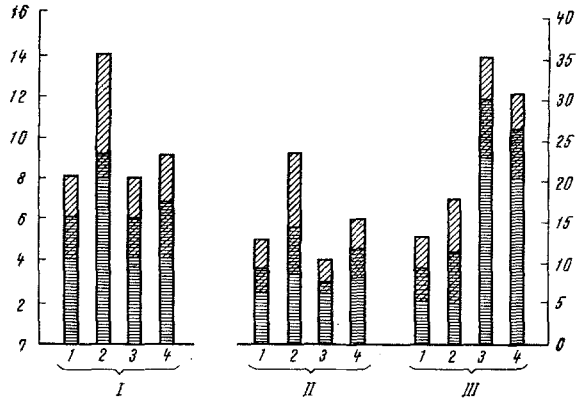

Fig. 1. Effect of "immune" RNA and antiserum on indices of phagocytosis of erythrocytes by macrophages. Ordinate, on left: percentage of macrophages engaged in phagocytosis, on right: number of erythrocytes undergoing phagocytosis per 100 macrophages. Horizontally shaded columns in diagram represent mean values (media). Oblique shading represents confidence limits the median [1]. I) Mean percentage of cells engaged in phagocytosis in cultures; II) mean number of erythrocytes ingested per 100 macrophages; III) mean number of erythrocytes adherent to 100 macrophages. 1) In samples without RNA and serum (control); 2) in samples with "immune" RNA; 3) in samples with antiserum in dilution 1 : 30,000; 4) in samples with antiserum in dilution of 1 : 3000.

The question whether macrophages can possess immunologic specificity not due to cytophilic antibodies has been discussed in the literature [6]. The results of the present investigation, in agreement with other data [12] concerning the formation of an antibody-like substance in macrophages after contact with antigen in vitro, are in favor of such a possibility.

LITERATURE CITED

1. I. P. Ashmarin and A. A. Vorob'ev, Statistical Methods in Microbiological Investigations [in Russian], Leningrad (1962).
2. B. D. Stanchev, Molekul. Biol., 1, No. 1, 3 (1967).
3. F. L. Adler, M. Fishman, and S. Dray, J. Immunol., 97, 554 (1966).
4. B. A. Askonas and J. M. Rhodes, Nature, 205, 470 (1965).
5. E. P. Cohen, R. W. Newcomb, and L. K. Crosby, J. Immunol., 95, 583 (1965).
6. D. C. Dumonde, Brit. Med. Bull., 23, 9 (1967).
7. M. Fishman, R. A. Hamerstron, and N. P. Bond, Nature, 196, 549 (1963).
8. J. Fong, D. Chin, and S. S. Elberg, J. Exp. Med., 118, 371 (1963).
9. H. Friedman, Biochem. Biophys. Res. Commun., 17, 272 (1964).
10. J. S. Garvey and D. H. Campbell, J. Exp. Med., 105, 361 (1957).
11. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
12. D. Jacherts, Z. Med. Mikrobiol., 152, 1 (1966).
13. D. Jacherts and H. Noltenius, Z. Med. Mikrobiol., 152, 112 (1966).
14. D. Jacherts, Z. Med. Mikrobiol., 152, 262 (1966).
15. J. A. Mannick and R. H. Egdahl, Science, 137, 976 (1963).
16. K. Sherrer and J. Darnell, Biochem. Biophys. Res. Commun., 3, 486 (1962).
17. D. E. Thor and S. Dray, J. Immunol., 101, 461 (1968).