EFFECT OF "IMMUNE" RNA ON CELLS OF DIFFERENT TYPES INTERACTING IN THE IMMUNE RESPONSE

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Simultaneous injection of bone marrow antigenic cells incubated with "immune" RNA, thymus cells, and sheep's red cells into irradiated CBA or DBA/2 mice leads to greater accumulation of antibody-forming cells in the recipients' spleen than in mice injected with intact bone marrow cells or the same cells incubated with normal RNA. Pre-incubation of thymus cells with "immune" RNA does not cause any change in the accumulation of antibody-producing cells. Incubation of peritoneal exudate cells with "immune" or normal RNA likewise had no effect on the character of accumulation of antibody-forming cells.

KEY WORDS: antibody formation; immunocompetent cells; "immune" RNA.

The possibility of induction of an immune response by RNA isolated from the tissues of an immunized donor has several times been demonstrated [1, 5, 8, 13]. The mechanism of action of this RNA is open to discussion [4, 9-12]. One aspect of its activity could be connected with processes determining the high efficiency of interaction between immunocompetent cells at the beginning of development of the process. When the study of RNA from this standpoint is contemplated, differences in its effect on cells of various types participating in immunogenesis must be considered.

The object of this investigation was to assess the effect of "immune" RNA on each of the three types of interacting cells (A, B, and T) on a model of cell cooperation in vivo.

TABLE 1. Number of AFCs in Spleen of Irradiated CBA and DBA/2 Mice after Transplantation of BM Cells Incubated with Normal and "Immune" RNA (M ± m)

Expt. No.	Mice	Cells injected		
		BM + T + SR	(BM + nRNA) + T + SR	(BM + iRNA) + T + SR
1	CBA	1678±445	2149±244	3718±461
2	CBA	1142±100	1768±56,1	3205±104,2
3	DBA/2	640±145	1420±116,6	2297±237

Legend. Here and in Tables 2 and 3 the brackets denote that the cells were incubated with the RNA Preparation. T) thymus cells; SR) sheep's red cells; nRNA) normal RNA; iRNA) immune RNA; PE) cells of peritoneal exudate.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA and DBA/2 mice obtained from the "Stolbovaya" nursery of pure-line animals, Academy of Medical Sciences of the USSR. As recipients in a syngeneic cell-transfer model, mice lethally irradiated on the "Stebel'-3a" apparatus were used. The CBA mice were irradiated in a dose of 900 R and the DBA/2 mice in a dose of 1050 R.

Bone marrow cells were obtained from the femora by flushing them out with medium No. 199. Thymocytes were obtained from the thymus. The macrophages were cells of a peritoneal exudate obtained from mice after preliminary intraperitoneal injection of a peptone–glycogen mixture as described by Argyris [7].

Preparations of "immune" RNA were extracted by Scherrer's method [6] from the spleen of mice killed on

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TABLE 2. Number of AFCs in Spleen of Irradiated CBA Mice after Injection of Thymus Cells Incubated with "Immune" RNA (M ± m)

Expt. No.	Cells injected		
	BM+T+SR	(T+iRNA)+ BM+SR	(BM+iRNA)+ T+SR
1	1536±252	1563±331	3015±741
2	1080±258	1058±136	1933±427
3	1256±371	1556±412	—
4	1529±266	1564±294	—

TABLE 3. Number of AFCs in Spleen of Unirradiated CBA and DBA/2 Mice after Injection of Peritoneal Exudate Cells, Processing Sheep's Red Cells, after Incubation with Normal and "Immune" RNA (M ± m)

Expt. No.	Mice	Cells injected		
		PE	PE+ nRNA	PR+ iRNA
1	CBA	144±13,6	167±8,5	118±11,7
2	CBA	136±13,1	150±19,3	174±21,6
3	CBA	230±10,2	—	177±18,8
4	DBA/2	74±14,3	80±11,3	58±6,6

the 4th day after immunization with sheep's red cells. Normal RNA was obtained from intact animals by the same method. Spectrophotometry of the preparations was carried out on the SF-16 instrument at wavelengths of 230, 260, and 280 nm to determine the concentrations of RNA and protein. In all cases the protein concentration was below 0.5-1%. Preparations with a higher protein content were not used. The cells were incubated with RNA at 37°C for 30 min. The quantity of RNA added to the cell suspension was 500 μg to 3×10^8 cells.

In all cases, irrespective of the form of the experiment, syngeneic cells were used. The number of antibody-forming cells (AFCs) in the spleen of the irradiated mice was determined on the 8th day after injection of the cells; in the work with unirradiated animals the number of AFCs was determined on the 5th day after injection.

EXPERIMENTAL RESULTS

Series I. Irradiated CBA or DBA/2 mice were injected intravenously with 5×10^6 BM cells incubated with normal or "immune" RNA. At the same time, the same mice were injected with 20×10^6 thymus cells and 2×10^8 sheep's red cells. The results in Table 1 show that preincubation of the BM cells with "immune" RNA led to a marked increase in intensity of the immune response. Incubation of BM cells with normal RNA also led to some increase in the number of AFCs, although the increase was not so great as with the "immune" RNA.

Series II. Irradiated CBA mice were injected intravenously with 5×10^6 intact BM cells, 20×10^6 thymus cells incubated with "immune" RNA, and 2×10^8 sheep's red cells. All four experiments (Table 2) yielded similar results. Preincubation of the thymus cells with "immune" RNA caused no change in the accumulation of AFCs. The validity of these relationships was confirmed by data for groups in which the "immune" RNA was incubated with BM cells.

Series III. Intact CBA or DBA/2 mice were injected intravenously with 5×10^6 peritoneal exudate cells, processing the antigen, after incubation with normal or "immune" RNA. The results in Table 3 show that "immune" RNA, once it has interacted with phagocytes, just as with thymus cells, has no effect on the process of AFC accumulation. No change in the strength of the immune response was found after injection of peritoneal exudate cells incubated with normal RNA.

The usual method of studying activity of "immune" RNA in vivo is by injecting it directly into an intact animal. This approach does not permit a direct analysis of the type of immunocompetent cells in which the RNA manifests its activity. The method chosen in this investigation was preincubation of one type of immunocompetent cell (B, T, or A) with the RNA preparation in vitro. In this way the cell type that responds most actively to exogenous RNA could be identified.

Modern views on the initial stage of immunogenesis are based on the idea that interaction between three types of cells is necessary [2, 3]. The predominant interacting factor in this case is the cell surface receptors. In lymphocytes of bone-marrow origin the receptor function is played by the immunoglobulins of the cell surface.

It can be concluded from the results of these experiments that "immune" RNA, when incubated with BM cells, increases the pool of AFC precursors carrying specific receptors for a particular antigen (sheep's red cells). It is probably this factor that determines the great strength of the immune response. The absence of change in the immune response after incubation of RNA with thymus cells and cells of the peritoneal exudate could be explained by the following hypotheses: 1) messenger RNA cannot determine additional specific synthesis in these types of cells because of the character of their differentiation; 2) the functional state of these cell types is such that, even in the presence of additional immunoglobulin synthesis, the form of participation of these cells in the immune response is strictly programmed and cannot be switched to the track of the antibody producers.

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