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REPLACEMENT OF THE HELPER FUNCTION OF T-CELLS BY RNA-CONTAINING ANTIGEN-SPECIFIC LYSIS FACTOR

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The supernatant obtained after centrifugation of a suspension of viable lymph node cells from immunized mice was chromatographed on Sephadex G-200 and the fractions were deproteinized. The third fraction (molecular weight 30,000 daltons) specifically stimulated antibody formation in intact mice immunized with sheep's red cells and restored ability to form antibodies in lethally irradiated intact mice protected with syngeneic bone marrow. The activity of this fraction disappeared after treatment with RNase but not with DNase or trypsin. The first and second deproteinized fractions of supernatant of the suspension of viable lymph node cells from immunized animals nonspecifically inhibited antibody formation in intact mice immunized with sheep's red cells.

KEY WORDS: helper T-lymphocytes; antigen-specific RNA-containing factor.

During immunogenesis substances containing antigen-specific information and capable of influencing the development of the immune response in intact animals appear in the lymphoid tissue. RNA preparations capable of inducing antibody synthesis both in vitro and in vivo have been isolated from extracts of lymphoid tissue of immunized animals [8-10,14]. On the other hand, antigen-specific [13,19] and antigen-nonspecific [15,20] factors replacing the helper function of T-cells in antibody formation have been isolated from the supernatant of lymphocyte cultures.

The writers showed previously [1,2,6] that the supernatant obtained after centrifugation of a suspension of viable lymph node cells from immunized animals contains an RNA-containing factor capable of inducing sensitivity to lysis by specific antigen in the lymph node and thymus cells of intact animals. The object of the present investigation was to study the effect of this factor and of other deproteinized preparations from such a supernatant on the primary immune response of mice to sheep's red cells.

EXPERIMENTAL METHOD

CBA mice weighing 16-18 g were immunized subcutaneously in the inguinal region with sheep's red cells in a dose of 200-300 million cells or with bovine serum albumin (BSA) in a dose of 0.5-1.0 mg per mouse. On the 8th day after immunization, when a considerable quantity of RNA-containing factor had accumulated in the lymph nodes [1,2], the regional lymph nodes were removed and a cell suspension ($6-9 \cdot 10^8$ cells/ml) prepared from them in Hanks' solution (90-95% of the cells were viable). The suspension was centrifuged at 6000g for 15 min and the resulting supernatant dialyzed at 4°C against 0.0175 M Na-phosphate buffer, pH 6.5. The

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TABLE 1. Effect of Deproteinized Fractions of Supernatant on Number of AFC in Intact Mice after Immunization with Sheep's Red Cells (M ± m)

Substance injected	Fraction No.	Source of supernatant: lymph node cells from mice immunized with sheep's red cells	Source of supernatant: lymph node cells from mice immunized with BSA	Control
Deproteinized fractions of supernatant and sheep's red cells	1	51±17	48±20	
	2	63±21	57±19	
	3	536±67	80±23	
The same, fractions treated with enzymes:				
trypsin	1	64±23	—	
	2	55±20	—	
	3	517±49	—	
DNase	1	57±19	—	
	2	45±17	—	
	3	497±57	—	
RNase	1	198±27	—	
	2	207±32	—	
	3	217±31	—	
Sheep's red cells and enzymes:				
trypsin	—	—	—	205±49
DNase	—	—	—	237±54
RNase	—	—	—	246±61
Sheep's red cells	—	—	—	231±38

Note. 1) Supernatant obtained after centrifugation of suspension of viable lymph node cells from immunized mice for 15 min at 6000g. 2) Mean number of AFC per 10^8 nucleated spleen cells in 3-5 experiments (with 8-10 mice in each experiment) and confidence intervals at P = 0.05 given in the table.

supernatant was then chromatographed on Sephadex G-200 (column 65 × 2.5 cm), equilibrated with the same buffer. Three fractions obtained after gel-chromatography (molecular weight over 100,000, 60,000, and 30,000 respectively) were concentrated to the original volume and deproteinized for 20 min on a boiling waterbath with 0.1 M perchloric acid [7]. The deproteinized fractions were dialyzed against distilled water, concentrated to the original volume, and restored to isotonicity with dry Eagle's medium.

In the experiments of series I 0.3-0.5 ml of one of the resulting deproteinized fractions was injected intravenously into intact mice. The animals were killed 2 days later, the spleen was removed, and the number of antibody-forming cells (AFC) against sheep's red cells was determined by Jerne's test [16].

In the experiments of series II, 2 days after injection of the deproteinized fractions of supernatant of lymph node cells, intact mice were immunized intraperitoneally with a dose of $(2-4)\cdot10^6$ sheep's red cells, and the number of AFC was determined in their spleen 4 days later. In some experiments before injection the deproteinized fractions were treated for 30 min at 37°C with DNase (0.1 mg/ml), RNase (0.1 mg/ml), or trypsin (1 mg/ml). The enzymes were obtained from the firms Difco and Reanal.

In the experiments of series III and IV lethally irradiated (900 R; ^{60}Co source) CBA mice were used. After 4-5 h each animal was given an intravenous injection of $(2-3)\cdot10^6$ syngeneic bone marrow cells together with 0.3-0.5 ml of the corresponding deproteinized preparations or with $2\cdot10^6$ intact syngeneic thymus cells. Each mouse was given an intraperitoneal injection of $1\cdot10^7$ sheep's red cells 24 h after irradiation. The animals were killed on the 8th day, the spleen was removed, and the number of AFC against sheep's red cells was counted.

EXPERIMENTAL RESULTS

The experiments of series I showed that deproteinized fractions of supernatant of a suspension of viable lymph node cells from mice immunized with sheep's red cells did not transmit ability to form antibodies to intact mice. The number of AFC in the spleen of mice receiving these preparations was the same as in the spleen of intact animals (20-25 AFC/10^8 nucleated spleen cells).

Preliminary treatment of the intact mice with deproteinized preparations of fractions 1 and 2 of supernatant of the suspension of viable lymph node cells from animals immunized with sheep's red cells or BSA nonspecifically inhibited the development of an immune response during immunization of the animals with sheep's red cells (Table 1). The analogous preparation of fraction 3 (molecular weight about 30,000) of the supernatant, however, considerably strengthened the immune response of the animals when immunized with

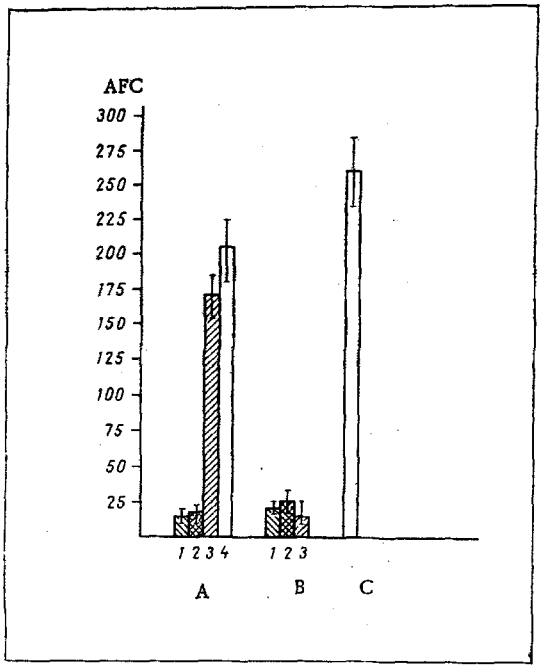

Fig. 1. Restoration of immune response in lethally irradiated CBA mice protected with syngeneic bone marrow cells. A) Deproteinized preparations of fractions of supernatant of lymph node cells from mice immunized with sheep's red cells (1-3, fraction numbers; 4, thymus cells from syngeneic mice); B) deproteinized preparations isolated from fractions of supernatant of lymph node cells from mice immunized with BSA (1-3, fraction numbers); C) antibody formation in unirradiated mice immunized with sheep's red cells. Ordinate, number of AFC per 10^8 nucleated spleen cells with confidence intervals at P = 0.05.

sheep's red cells. Treatment of the deproteinized preparations with RNase, but not with DNase or trypsin, abolished their effect on the development of the immune response in mice immunized with sheep's red cells (Table 1).

Lethally irradiated mice were used in the experiments of series III and IV. Their antibody-forming ability was restored if the deproteinized preparation of the third fraction, but not of the first or second fractions, of the supernatant was injected simultaneously with the bone marrow cells of intact syngeneic mice (Fig. 1). Antibody formation was not restored if deproteinized fraction 3 of the supernatant of a suspension of viable lymph node cells from animals immunized with BSA was used (Fig. 1). The increase in the number of AFC in irradiated mice protected with syngeneic bone marrow under the influence of the tested doses of RNA-containing preparation (50-100 μg RNA) of fraction 3 of the supernatant was approximately the same as after injection of syngeneic thymocytes into the animals (Fig. 1).

The investigation thus showed that none of the deproteinized fractions obtained after gel-chromatography of the supernatant of a suspension of viable lymph node cells from immunized mice could transmit ability to form antibodies against sheep's red cells to intact animals. However, the first and second deproteinized fractions of this supernatant had a nonspecific immunodepressive effect on development of the immune response against sheep's red cells in intact mice. This effect disappeared after treatment of these preparations with RNase but not with DNase or trypsin. It can thus be tentatively suggested that it was due to RNA. A similar nonspecific effect on antibody formation is shown by certain high-molecular-weight RNA preparations of heterologous origin [12]. Other RNA preparations and polynucleotides can nonspecifically potentiate antibody formation [9,11] and resistance to infection [5]. On the other hand, specific immunologic reactivity can be transferred in vitro and in vivo by RNA-enriched extracts of lymphoid organs of immunized animals [8,9,17,18].

By contrast with this, RNA-containing factor inducing specific stimulation of antibody formation in intact mice against sheep's red cells was found in the deproteinized third fraction (initial molecular weight 30,000) of

supernatant of a suspension of viable lymph node cells from mice immunized with sheep's red cells. Such a preparation was able to restore antibody formation in lethally irradiated mice protected with bone marrow cells of intact syngeneic animals. Consequently, it can replace the function of helper T-cells, and this property distinguishes it from preparations of normal and "immune" RNA, which can evidently stimulate AFC precursors in the presence of thymus cells [3,4].

This RNA-containing factor is bound with T-lymphocytes of the lymph nodes and thymus of intact mice and can transfer ability to perform antigen-specific lysis to these cells both in vitro and in vivo [1,2,6].

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