ANTIGEN-SPECIFIC LYSIS in vitro OF LYMPH GLAND CELLS FROM INTACT MICE INDUCED BY RNA FROM INTACT LYMPH GLAND CELLS OF IMMUNIZED ANIMALS

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The supernatant obtained after centrifugation of a suspension of viable lymph gland cells from immunized animals induced sensitivity to lysis in vitro by specific antigen in lymph gland cells from intact mice in vivo. After chromatography of the supernatant on Sephadex G-200, this property was localized in fraction 3 (molecular weight about 30,000). Deoxyribonuclease, trypsin, and deproteinization did not affect this fraction, but ribonuclease deprived it of its inducing properties.

KEY WORDS: antigen-specific lysis; RNA of lymph gland cells.

After immunization of mice with various antigens cells undergoing lysis when treated with large doses of specific antigen appear in their lymph glands [2-5]. This antigen-specific lysis of these cells is due to a cytophilic factor of nonimmunoglobulin nature with a molecular weight of about 30,000, located on the surface of its carrier cells in a relatively weakly bound state [1]. Treatment of intact lymph gland cells in vitro with this factor renders them capable of lysis by specific antigen [1].

The immunological properties of this factor were studied by injection of suspensions of lymph gland cells from immunized animals and also of various fractions of the "washings" of those cells into syngeneic mice.

EXPERIMENTAL METHOD

Male and female CC57W and CBA mice weighing 16-18 g were immunized subcutaneously with bovine serum albumin (BSA) or egg albumin (EA) in a dose of 2-3 mg per mouse. On the eighth day after immunization, when the number of cells undergoing lysis by specific antigen in vitro in the lymph glands is greatest [3, 4], the regional lymph glands were removed and cell suspensions made from them ($6-9 \cdot 10^8$ cells/ml) in Hanks's solution, pH 7.0 (90-95% of the cells were viable). In different experiments, suspensions of these cells or supernatants prepared from them, and their fractions, were injected intravenously into intact mice. The mice received: 1) 20-30 million cells of an unwashed suspension of lymph glands from immunized mice; 2) the same number of similar cells, but previously washed three to four times with Hanks's solution; 3) 0.5-0.7 ml of supernatant (SW) obtained after centrifugation of a suspension of lymph gland cells ($5-6 \cdot 10^8$ million cells/ml) from immunized mice at 6000 g for 15 min; 4) the cell residue was washed three times with Hanks's solution, resuspended to its initial volume, and frozen and thawed five times, then centrifuged at 6000 g for 15 min, and the resulting supernatant of these disintegrated cells (SD) was injected into mice in a dose of 0.5-0.7 ml; 5) in subsequent experiments the SW was chromatographed on Sephadex G-200; the three fractions obtained after chromatography were dialyzed against distilled water, concentrated to the initial volume, equilibrated with NaCl to an isotonic solution, and injected in a dose of 0.5-0.7

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TABLE 1. Induction of Specific Sensitivity of Lymph Gland Cells of Intact Animals to Lysis by Antigen in vitro by Means of Suspensions of Lymph Gland Cells from Immunized Mice or Cell-Free Preparations Made from Them*

Days after injection of material	Material injected					
	lymph gland cells of immunized mice		SW	SD	serum of immunized mice	
	unwashed	washed			1 ml	2 ml
1-	0,16±0,03	0,07±0,04	0,16±0,03	0,04±0,02	0,02±0,02	0,13±0,01
3-	0,17±0,05	0,17±0,02	0,20±0,04	0,03±0,02	0,05±0,04	0,17±0,04
5-	0,17±0,02	0,15±0,05	0,15±0,04	0,14±0,06	0,03±0,04	0,14±0,05
10-	0,10±0,03	0,12±0,03	0,13±0,02	0,15±0,04	0,05±0,02	0,15±0,04
15-	0,05±0,02	0,08±0,06	0,04±0,01	0,09±0,05	0,05±0,02	0,02±0,04

*Mean cytotoxic indices of series of experiments with confidence limits at $P < 0.05$ (8-10 mice in group) are shown; cytotoxic indices were calculated relative to control (the same cells were incubated with "intact" antigen or without antigen).

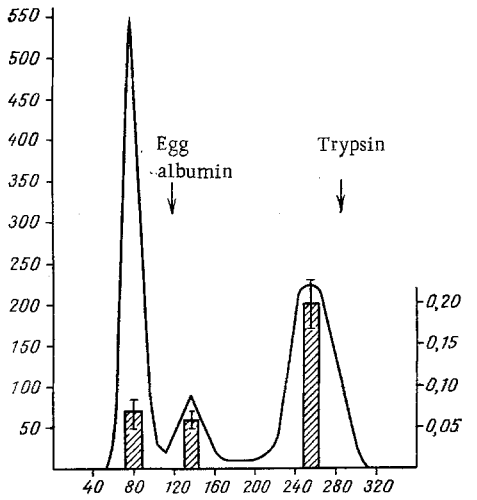

Fig. 1. Transfer of specific sensitivity to antigen-dependent lysis to lymph gland cells of intact mice by fraction 3 obtained after chromatography of SW on Sephadex G-200. Ordinate: absorption at 280 nm (left), cytotoxic index (right); abscissa, elution volume (ml).

ml (250-300 μg protein, after Lowry) into intact mice; 6) fraction No. 3, previously treated for 30 min with deoxyribonuclease (DNase, 0.1 mg/ml), ribonuclease (RNase, 0.1 mg/ml), or trypsin (1 mg/ml) at 37°C, was injected; 7) fraction No. 3 was deproteinized with perchloric acid [6], the supernatant was dialyzed against distilled water, concentrated to its initial volume, restored to an isotonic solution with NaCl, and injected into intact mice; 8) serum of immunized mice was injected in a dose of 1-2 ml. In control experiments intact mice were injected with the cell suspension SW or SD obtained from lymph glands of intact animals or mice immunized with another antigen.

Lymph glands from all mice receiving the suspensions of cells or their fractions were taken at different times, suspensions were prepared from them, and the lympholysis test was carried out with them [3-5]. For this purpose equal volumes (0.05 ml of each) of the suspensions of lymph gland cells and 1% solution of the antigens (BSA in the experimental series, EA as the "intact" antigen in the control, or vice versa) were mixed. The mixtures were incubated for 30 min at 37°C and, after addition of trypan blue, the cytotoxic index was calculated.

EXPERIMENTAL RESULTS

Lymph gland cells taken from intact mice 24 h after intravenous injection of an unwashed suspension of lymph gland cells from immunized animals because sensitive to lysis in vitro by specific antigen (Table 1). However, the same suspension, if washed three or four times with Hanks's solution before injection into

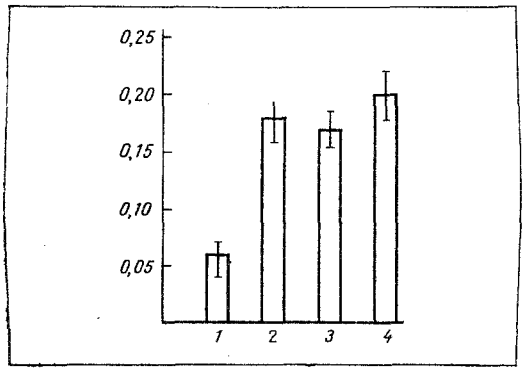

Fig. 2. Effect of enzyme treatment and deproteinization of fraction 3 on transfer of ability to antigen-specific lysis to lymph gland cells of intact mice. Ordinate, cytotoxic index; abscissa, treatment of fraction 3 with: 1) RNase, 2) DNase, 3) trypsin, 4) perchloric acid (deproteinization).

the intact mice, conferred the same sensitivity on the lymph gland cells only after 3 days. Consequently, washing temporarily rendered the cells unable to induce antigen-dependent lysis in the cells of intact animals.

Supernatant of the "washings" (SW) of lymph gland cells of immunized mice, if injected intravenously into intact animals, also induced in their lymph gland cells after 24 h the ability to undergo lysis in vitro in the presence of specific antigen. Lymph gland cells of intact mice kept this ability for 10 days (Table 1). The same property of transmitting sensitivity to antigen-specific lysis was possessed by fraction 3 (molecular weight about 30,000), but not by fractions 1 or 2 obtained after chromatography of this same SW on Sephadex G-200 (Fig. 1).

Enzyme treatment of fraction 3, active in the transfer of antigen-specific lysis by lymph gland cells of intact mice, showed that neither DNase nor trypsin affect this property, whereas treatment with RNase abolishes it (Fig. 2). Deproteinization of fraction 3 did not reduce its ability to induce sensitivity of the cells to lysis in vitro by specific antigen in the lymph glands of intact mice (Fig. 2). These results indicate that the factor conferring sensitivity to specific lysis under the influence of antigen on lymph gland cells of intact animals is evidently an RNA-containing substance.

The supernatant of the washed and disintegrated cells (SD) of lymph glands obtained on the eighth day after immunization of mice, if injected intravenously into intact animals, induced in the lymph gland cells of these mice the ability to undergo lysis in vitro by specific antigen, but not before the fifth to sixth day after injection. This late effect of SD evidently had a different mechanism from the transfer of ability to undergo lysis by means of SW. It coincided in the time of appearance of the antigen-lysable cells in the lymph glands with active immunization [4, 5]. It can therefore be explained by active immunization of the recipient with the antigen or its fragments associated with certain cell fractions contained in SD.

Lymph gland cells of intact mice could become capable of antigen-specific lysis after injection of large doses (1.5-2 ml, not 1 ml) of serum obtained on the eighth day after immunization of mice (Table 1).

Suspensions of lymph gland cells, SW, SD, or the serum of intact animals did not transfer specific sensitivity to lysis by antigen in vitro to lymph gland cells of syngeneic recipients.

The results obtained thus show that activity of the factor inducing ability to undergo antigen-specific lysis in lymph gland cells of intact mice in vivo is associated with the RNA fraction of the "washings" of lymph gland cells of the immunized animals. Preparations of RNA obtained from lymphocytes of immunized mice are known to be capable of inducing ability to exhibit various immunological phenomena, including antibody formation, in the cells of intact animals both in vivo and in vitro [7, 11]. However, these RNA preparations were obtained from cells by disintegration. An RNA preparation actively transferring antigen-specific lysis was obtained by the present writers under conditions favoring maximal viability of the cells, by washing them with Hanks's solution, pH 7.0, in the usual way. It is evident, therefore, that this RNA fraction lies on the surface of the lymphocytes and also, possibly, in the intercellular fluid. The ability of this RNA fraction, with a molecular weight of about 30,000, to bind itself in vitro to the surface

of intact lymphocytes of thymus origin and to induce in them the ability to undergo antigen-specific lysis as early as after 20 min is very interesting [1]. It is suggested that this agent does not induce the formation of new antigen-binding receptors in the intact cells, but itself possesses specific antigen-binding ability. Considering the binding of this factor with lymphocytes of thymus origin [1, 4], it can be postulated that it is one of the receptors of the T-lymphocytes.

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