Decrease of cholesterol and free fatty acids in cortisone-resistant lymphoid cells incubated with allogeneic tumor cells

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Summary. A marked decrease of cholesterol and free fatty acids was found in the cortisone-resistant lymphoid cells from thymus or spleen of mice immunized with Ehrlich carcinoma cells when incubated with the tumor cells.

Many studies have been devoted to the understanding of the mechanism of the lymphocyte-mediated cytolysis, in that the cytotoxic lymphocytes from immune donors kill the relevant target cells[1-3]. However, little is known about the possible role of lipids in the cytotoxic lymphocytes. In a previous paper, we reported that lymphoid cells contain significantly high levels of the cytotoxic free fatty acids as compared with other mammalian cells[4]. In this study, the contents of free fatty acids and other lipids in the total and cortisone-resistant lymphoid cells from thymus or spleen of mice immunized with Ehrlich carcinoma cells were examined after incubation with the tumor cells. It was found that the incubation of lymphoid cells with the tumor cells results in a marked decrease of cholesterol and free fatty acids in the cortisone-resistant lymphoid cells which are cytotoxic to the tumor cells.

Materials and methods. Female mice of ddN strain (non-inbred)[5], 7-8 weeks old, were immunized by the method of Thunold[6], except that the tumor cells in physiological saline were replaced by the cells in emulsion. Ehrlich tumor cells were obtained from mice at 10 days after i.p. inoculation of the cells[7] and suspended in saline (6×10^6 cells/ml). The tumor cell suspension (10 ml) was emulsified with an equal volume of Freund incomplete adjuvant (Difco), and 0.1 ml of the emulsion was injected s.c. into each of 50 mice in a group on the tail. 2 weeks after immunization, the lymphoid cell suspension was prepared from the pooled tissues as described previously[8], using Hanks balanced salt solution (BSS) containing penicillin G (100 IU/ml) and streptomycin (100 µg/ml). The lymphoid cell suspension contained 93-98% of lymphocytes and 2-6% of macrophages, and 94-99% of cells in the suspension were viable with trypan blue test. The cortisone-resistant and total lymphoid cells were obtained from mice with and without treatment of hydrocortisone acetate (12.5 mg/100 g b. wt) 2 days before sacrifice[9,10].

The tumor cells used for incubation were prepared by velocity sedimentation[11-13]. Tumor cells suspended in 40 ml of BSS (1.5×10^7 cells/ml) were gently loaded over the sucrose solution consisting of 2 layers (150 ml of 1.0% sucrose in BSS and 300 ml of 1.5% sucrose in BSS without phenol red) in a 500-ml flask through a needle connected with a 100-ml separatory funnel by tubing. After standing at 4 °C for 1 h, the tumor cells sedimented to the bottom were collected and washed twice with BSS. The lymphoid cells suspended in BSS (pH 7.4) supplemented with 2% bovine albumin fraction V (Armour) in 100-ml flasks were incubated alone or with the tumor cells at 37 °C for 2 h in a shaking water bath (100 rpm). Final concentration of lymphoid cells and tumor cells was approx. 5×10^6 and 5×10^5 cells/ml, respectively.

Table 1. Lipid composition of total and cortisone-resistant lymphoid cells from mice immunized with Ehrlich carcinoma cells after incubation with or without tumor cells[a]

Lymphoid cells Cell groups[b]	Tissues	Incubation of lymphoid cells with (+) or without (−)[c] Tumor cells	EDTA	Lipids/lymphoid cells (mg/10^{10} cells)[d] Cholesterol	Fatty acids	Triglycerides	Cholesterol esters	Phospholipids
Total cells	Thymus	−	−	9.5±0.3	11.7±0.4	7.0±0.3	9.5±0.7	47.8±2.7
		+	−	9.2±0.1	11.7±0.6	7.3±0.9	9.6±0.7	49.8±2.1
	Spleen	−	−	10.3±0.4	9.0±0.4	5.4±0.5	10.5±1.0	42.2±0.8
		+	−	10.2±0.3	9.2±0.4	6.6±0.5	10.5±0.9	44.2±1.0
Cortisone-resistant cells	Thymus	−	−	24.6±0.4	38.0±0.8	27.2±0.9	27.2±0.9	112.0±2.7
		+	−	12.5±0.8	16.4±0.7	28.7±1.1	27.6±1.6	114.0±3.9
		−	+	25.0±0.3	39.1±1.6	27.6±0.7	27.5±2.0	113.0±3.5
		+	+	24.8±0.4	38.8±1.3	27.8±0.5	26.5±1.7	115.5±3.2
	Spleen	−	−	11.3±0.3	10.0±0.3	8.6±0.6	8.6±0.7	46.6±2.2
		+	−	6.2±0.2	4.9±0.3	8.8±0.8	8.7±0.5	48.2±1.2
		−	+	10.6±0.5	10.0±0.4	8.6±0.4	9.2±0.8	44.6±1.0
		+	+	10.3±0.6	9.5±0.3	8.8±0.4	9.6±0.6	45.8±1.1

[a] Mice were immunized with Ehrlich carcinoma cells and killed 14 days later. Lymphoid cells from thymus or spleen of immunized mice were suspended in Hanks balanced salt solution (pH 7.4) containing 2% bovine albumin fraction V (5×10^6 lymphoid cells/ml), and incubated alone or with the tumor cells at 37 °C for 2 h in the absence or presence of 2 mM EDTA (tumor cells/lymphoid cells: 1/10). [b] Cortisone-resistant and total lymphoid cells were prepared from mice with and without treatment of hydrocortisone acetate (12.5 mg/100 g b.wt) 2 days before sacrifice. [c] After incubation, the mixture of lymphoid cells and tumor cells were separated into each cell group by velocity sedimentation. [d] Each value represents mean ± SE of 6 experiments.

Table 2. Effect of total and cortisone-resistant lymphoid cells on the growth of Ehrlich tumor cells in mice[a]

Lymphoid cells mixed with tumor cells Cell groups	Tissues	Presence (+) or absence (−) of EDTA in the incubation medium	Diameter (mm) of tumors in mice after inoculation of cell mixture[b] (at weeks) 1	2	3	4	5
Total cells	Thymus	−	6.8±0.4	10.6±0.8	13.6±0.6	17.7±1.1	21.4±0.9
	Spleen	−	7.3±0.5	10.1±0.5	13.3±0.8	17.3±1.4	22.0±1.8
Cortisone-resistant cells	Thymus	−	0	0	0	0	0
		+	7.1±0.3	10.5±0.7	12.2±1.0	15.6±1.5	19.3±1.5
	Spleen	−	0	0	0	0	0
		+	7.2±0.5	10.3±0.6	12.9±0.8	15.9±0.8	19.4±0.6
(tumor cells alone)			7.2±0.6	10.6±0.9	14.2±0.9	17.9±2.2	22.4±2.0

[a] Lymphoid cells from mice immunized with Ehrlich carcinoma cells were suspended in Hanks balanced salt solution (BSS, pH 7.4) containing 2% bovine albumin fraction V (5×10^6 lymphoid cells/ml), and incubated with the tumor cells at 37 °C for 2 h in the presence or absence of 2 mM EDTA (tumor cells/lymphoid cells: 1/10). After incubation, the cell mixture was washed with BSS, and injected s.c. into non-immunized mice on the right flank (10^6 tumor cells/mouse). Control animals received an equal number of the tumor cells incubated for 2 h (tumor cells alone). [b] Each value represents mean±SE of 8 mice. Tumor diameter = (short diameter + long diameter)/2.

The lymphoid cells were also incubated alone or with tumor cells in the presence of 2 mM EDTA-2 Na (ethylenediamine tetraacetic acid disodium). After incubation, the cells collected by centrifugation were washed twice and suspended in BSS ($1\text{-}2\times10^7$ lymphoid cells/ml). The mixture of lymphoid cells and tumor cells was vigorously pipetted to obtain a single cell suspension, and separated into each cell group by velocity sedimentation. The cell mixture (5 ml) was loaded over 2 layers of sucrose solution (20 ml of 0.6% sucrose in BSS without phenol red and 20 ml of 1.0% sucrose in BSS) in 50-ml test tubes, and allowed to stand at 4 °C for 90 min. The cells present in the overlayer were then collected gently, washed twice with BSS and used for lipid determination[4]. Almost all the recovered cells (approximately 99%) were lymphoid cells, of which the recovery was about 94% (88–99%).

According to the tumor neutralization test (Winn test)[14], the cytotoxicity of lymphoid cells was examined. After incubation at 37 °C for 2 h in the presence or absence of 2 mM EDTA, the mixture of lymphoid cells and tumor cells was washed twice and suspended in BSS (2×10^6 tumor cells/ml). The cell mixture (0.5 ml) was injected s.c. into each of 8 normal mice on the right flank, and the tumor growth in mice was observed for 6 weeks. Control animals received an equal number of the tumor cells incubated for 2 h.

Results and discussion. In the total lymphoid cells from thymus or spleen, the contents of each lipid fraction in the lymphoid cells incubated with tumor cells were nearly equal to those in the corresponding cells incubated alone (table 1). The amounts of triglycerides, cholesterol esters or phospholipids in the cortisone-resistant lymphoid cells incubated with tumor cells were also similar to those in the corresponding cells incubated alone, within each of the lymphoid cells examined.

On the other hand a marked difference was found in the levels of cholesterol and free fatty acids between 2 groups of the cortisone-resistant lymphoid cells from thymus or spleen; the contents of these lipids in the cortisone-resistant cells incubated with tumor cells were less than 50% of those in the corresponding cells incubated alone ($p<0.01$). In the presence of 2 mM EDTA, however, no significant difference was found in the levels of cholesterol and free fatty acids between 2 groups of the cortisone-resistant lymphoid cells from thymus or spleen. Further, the lipid composition of the cortisone-resistant lymphoid cells incubated alone was quite similar to that of the corresponding cells without incubation, irrespective of the original tissues (lipids in the former/lipids in the latter: 0.96–1.05). Therefore, it may be concluded that the cortisone-resistant lymphoid cells from thymus or spleen of mice immunized with Ehrlich carcinoma cells lose a large amount of cholesterol and free fatty acids when incubated with the tumor cells, and EDTA inhibits the decrease of these lipids in the cortisone-resistant lymphoid cells incubated with the tumor cells. The group containing cortisone-resistant lymphoid cells from thymus or spleen of mice amounted to about 5 or 20% of total lymphoid cells[15,16].

The effect of lymphoid cells on the growth of Ehrlich tumor cells in mice was then examined (table 2). A progressive increase of tumor size was observed in mice injected with the tumor cells alone, or with the mixture of the tumor cells and total lymphoid cells from thymus or spleen. Animals given the tumor cells, which had been incubated with the cortisone-resistant lymphoid cells from thymus or spleen in the presence of EDTA, showed the tumor growth, too.

However, no tumor growth was observed in mice receiving the tumor cells that had been incubated with the cortisone-resistant lymphoid cells from thymus or spleen in the absence of EDTA. These results indicate that the cortisone-resistant lymphoid cells from thymus or spleen of mice immunized with Ehrlich carcinoma cells are cytotoxic to the relevant tumor cells[17], and the effect of the cortisone-resistant lymphoid cells on the tumor cells is inhibited by EDTA[1–3]. In the cytotoxic lymphocytes from mice immunized with allogeneic tumor cells, therefore, cholesterol and free fatty acids may be involved in the cytotoxicity against the relevant tumor cells[18]. Interestingly, free fatty acids from lymphocytes have been reported to be highly cytotoxic to the tumor cells[4,19,20].

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Direct evidence for pH-dependent Fc receptors on proximal enterocytes of suckling rat gut

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Summary. By means of an erythrocyte-antibody rosette technique, Fc receptors, functional at pH 6.0 but not at 7.2, were shown to be present on enterocytes isolated from duodenum and jejunum (but absent from ileum) of 12–20-day-old suckling rats.

In the rat, passive immunity is transferred from mother to young largely after birth and for a time period that extends up to 18–21 days post-partum[1]. When the young rat suckles during this time, immunoglobulin (IgG) present in the colostrum and milk has somehow to be selectively transported across the cellular barriers constituting the gut wall. The sites of transmission within the gut and the mechanism whereby selection is effected are still matters of controversy. A growing body of evidence based on ultrastructural[2,3], autoradiographic[4] and quantitative[5-7] assessment of IgG transport points to the proximal region (jejunum) of the small intestine and involvement of receptors specific for the Fc region of IgG that have dependency upon an acid pH for IgG-receptor binding[8,9]. However, a recent report[10] still places emphasis on the distal region (ileum) as a site of transport and suggests that specific receptors are not involved in the selective entry of immunoglobulin into enterocytes. In a previous study[11] it was shown that Fc receptors could be detected on endodermal cells of rabbit yolk sac by means of an erythrocyte-antibody (EA) rosette technique, and that such receptors were associated with selective immunoglobulin transport. In the present preliminary investigation we have applied the EA rosette technique to enterocytes removed from different regions of suckling rat gut to see whether or not they too have detectable Fc receptors.

Initial studies were carried out on 12-day-old suckling Wistar strain rats. These were isolated from the mother for 2 h and then killed by cervical dislocation. The intestine was dissected out, freed of mesentery, and the gut lumen perfused with cold isotonic phosphate buffered saline, pH 7.2, in order to remove debris. It was then divided into segments comprising duodenum, jejunum, lower jejunum and upper ileum, and ileum, and each segment everted over the sealed drawn-out end of a Pasteur pipette. The everted sacs so formed were tied with ligatures at both ends and incubated in serology tubes containing 0.25% trypsin (Difco) and 10% foetal calf serum (FCS) in Eagles minimal essential medium (MEM) for 45 min at 37 °C. The medium was then gently agitated for a few min to dislodge enterocytes and centrifuged at 250 × g for 3 min to pellet the cells. These were then washed 3 times in MEM/10 FCS, where necessary passed through a hypodermic needle to break up clumps, and finally re-suspended at $4–5 \times 10^5$ cells/ml. Although this isolation procedure resulted in some cell fragmentation, many cells were left intact with a readily visible brush border; ileal cells could be distinguished from jejunal cells by the presence of a large supranuclear vesicle

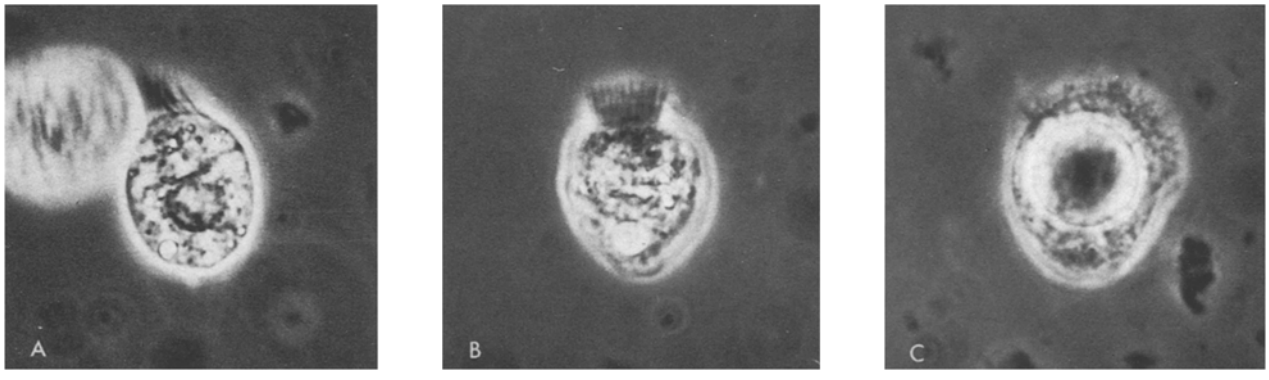

Fig. 1. Appearance of enterocytes obtained from *A* duodenum, *B* jejunum and *C* ileum, as seen under phase microscopy.