alone, and the degree to which this information is linked with the biological features of this virus, still remain unexplained.

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ACTIVATION OF MACROPHAGES BY BLASTOLYSIN

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In recent years adjuvants, particularly BCG, Corynebacterium parvum, and lipopolysaccharides, have begun to be used for the treatment of certain tumors. It is suggested that macrophages, activated by these adjuvants, either become capable of causing lysis of tumor cells or they begin to secrete mediators which stimulate proliferation of precursors of cytotoxic T-cells [2, 3].

It was shown previously that blastolysin, the main components of which are glycopeptide fragments of the cell wall of Lactobacillus bulgaricus, exhibits antitumor activity against various transplantable and spontaneous tumors of animals [1].

The object of this investigation was to determine whether blastolysin possesses adjuvant activity and whether it can activate cells of the mononuclear phagocytic system and, in particular, macrophages. For this purpose the effect of blastolysin on antibody production, on the ability of macrophages to phagocytose various antigens, and on its ability to exert a cytotoxic action on syngeneic target cells with disturbed growth parameters was studied.

EXPERIMENTAL METHOD

Experiments were carried out on BALB/c and C57BL/6 mice.

Blastolysin was used in different doses: 1 mg per mouse (a dose causing up to 76% inhibition of growth of sarcoma S-180 and completely curing 30-40% of mice), and 4 and 20 mg per mouse. Sheep's red blood cells (SRBC) in a dose of 5×10^6 were used as the antigen.

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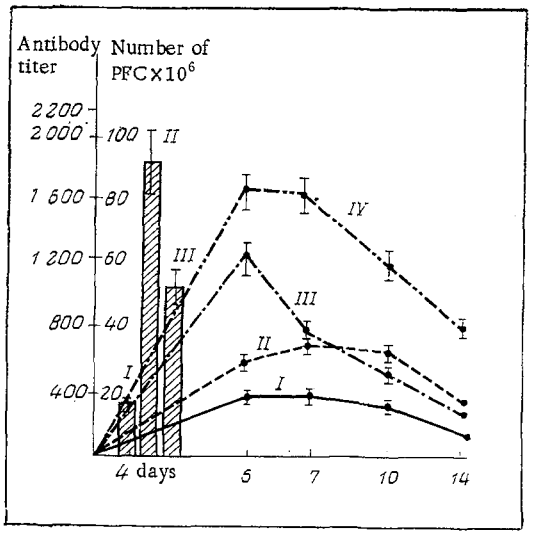

Fig. 1. Effect of blastolysin on production of hemagglutinins and plaque-forming cells (PFC) in the spleen to SRBC. Abscissa, time of observation (in days); ordinate, titer of antibodies (I) and number of PFC (II); I) control; II) blastolysin (1 mg/mouse); III) 4 mg/mouse; IV) 20 mg/mouse.

The objects of phagocytosis were ^{51}Cr-labeled SRBC (0.1 ml of a 20% suspension, 1.5×10^6 cpm) and ^{14}C-labeled typhoid vaccine, which was injected in a dose of 250 million bacterial cells (3×10^5 cpm). Blastolysin and the antigens were injected intraperitoneally. Living BCG vaccine in a dose of 500 μg per mouse was injected both intravenously and intraperitoneally. Peritoneal exudate cells (PEC) were collected on the 4th day after intraperitoneal injection of 2 ml nutrient broth (NB) into mice.

The intensity of antibody formation was judged from the titers of hemolysins and hemagglutinins in the blood serum and the number of antibody-forming cells in the spleen, determined by Jerne's method of local hemolysis in gel.

The rate of phagocytosis and digestion was judged from the level of radioactivity in PEC thoroughly washed to remove free radioactivity, 30 min and 18 h after injection of labeled antigen.

The target cells consisted of a line of fibroblasts, isolated from C57BL mouse embryos and maintained for 1 year by O. S. Gudima, who generously donated the cells. Cell cultures were grown on medium No. 199 with 10% bovine serum and 100 units/ml penicillin. On the 4th day the cells were removed from the flasks with 0.1% trypsin, their viability was determined by means of trypan blue, and they were then labeled by adding 1 μCi of ^{51}Cr (in the form $Na_2{}^{51}CrO_4$) to 2×10^6 cells in 3 ml medium. Incubation continued for 30 min at 37°C.

A monolayer of macrophages was obtained by adding $(2-3) \times 10^6$ PEC in 0.5 ml to flasks containing 1.5 ml medium No. 199. After incubation for 1 h at 37°C the medium was poured off and the adherent cells were washed twice with fresh medium.

Labeled target cells, washed to remove free radioactivity, were added to the monolayer of macrophages. The cell mixture was incubated for 18 h at 37°C in 2 ml of the above-mentioned medium.

The cytotoxicity of the macrophages was determined from the percentage of chromium liberated from the labeled target cells into the culture medium, by the usual formula [3].

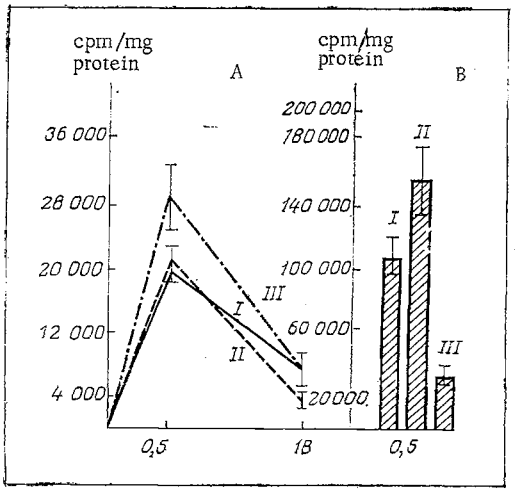

Fig. 2. Action of blastolysin on ingestion and and digestion of ^{51}Cr-SRBC (A) and ^{14}C-labeled typhoid vaccine (B) by PEC. Abscissa, time of observation; ordinate, ingestion of antigen (in cpm/mg protein). A: I) control, II) 1 mg/mouse, III) 4 mg/mouse; B: I) control, II) 4 mg/mouse, III) 20 mg/mouse.

TABLE 1. Cytotoxicity of PEC Macrophages of C57BL Mice after Injection of Blastolysin

Target cells	Macrophages	Number of samples	Cytotoxicity (M ± m, %)
L- cells	After freezing and thawing 5 times	10	78±1,0
	Intact	10	27±0,8
	2 h after injection of blastolysin	10	46±0,9
	2 weeks after injection of BCG	10	72±0,6
C57BL embryonic fibroblasts	After freezing and thawing 5 times	5	86±0,7
	Intact	5	14±0,9
	2 h after injection of blastolysin	5	55±1,0
	2 weeks after injection of BCG	5	70±0,6

EXPERIMENTAL RESULTS

To study the adjuvant properties of blastolysin it was injected intraperitoneally in the above-mentioned doses simultaneously with a small dose of SRBC into BALB/c mice.

As the results given in Fig. 1 show, practically all doses of blastolysin tested stimulated antibody formation. Intraperitoneal injection of 1 mg blastolysin into mice simultaneously with 5×10^6 SRBC increased the formation of plaques of hemolysis (calculated per 1×10^6 spleen cells) fivefold. In these experiments blastolysin in a dose of 1 mg per mouse stimulated antibody formation by a greater degree than a dose of 4 mg per mouse, injection of which caused a threefold increase in the number of hemolysis plaques. These experiments showed that blastolysin possesses adjuvant properties.

In the next series of experiments the action of blastolysin was studied on the phagocytic and digestive power of the macrophages. In groups of animals in which the effect of blastolysin on the ingestion and digestion of ^{51}Cr-labeled SRBC was investigated, PEC were collected 30 min and 18 h after intraperitoneal injection

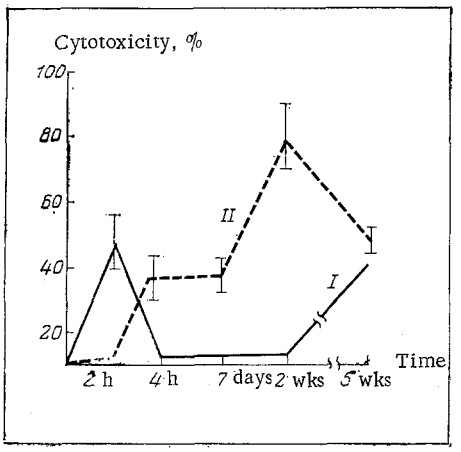

Fig. 3. Trend of appearance of cytotoxicity of macrophages after injection of blastolysin (I) and BCG (II) into C57BL mice. Abscissa, time of observation; ordinate, cytoxicity (in %).

of different doses of blastolysin simultaneously with the labeled SRBC. Phagocytosis of ^{14}C-typhoid vaccine by macrophages was investigated once only, 30 min after injection. Animals into which antigens were injected without blastolysin served as the control (Fig. 2).

The results showed that blastolysin, in a dose of 1 mg, increased phagocytosis of both typhoid vaccine and SRBC by PEC. Meanwhile, an increase in the dose of blastolysin (4 mg per mouse in experiments with SRBC and 20 mg per mouse in the experiments with typhoid vaccine) did not lead to increased ingestion of antigens by PEC, and indeed, in a dose of 20 mg, led to marked inhibition of phagocytosis.

Injection of blastolysin in a dose of 1 mg per mouse not only increased ingestion of antigens, but also slightly accelerated digestion of SRBC, for the quantity of radioactive material in the PEC of animals receiving blastolysin fell more rapidly than in the control.

These results indicate that blastolysin, in certain doses, activates cells of the mononuclear phagocytic system, but in order to establish whether they can be effector cells for the lytic action of blastolysin of tumors in vivo, in the next series of experiments the aim was to discover whether macrophages of animals receiving blastolysin acquired the property of destroying cells with disturbed growth parameters in allogeneic and syngeneic systems in vitro.

PEC were obtained: 1) 2 h after injection of blastolysin, 2) 2 weeks after injection of BCG into the animals, and 3) from groups of intact animals. The results of these experiments are given in Table 1.

Macrophages obtained from the animals 2 h after injection of blastolysin caused considerable lysis of the labeled target cells not only in allogeneic, but also in syngeneic systems. The cytotoxicity, in per cent, was a little lower than that of macrophages from animals receiving BCG 2 weeks before the experiment, although it significantly exceeded the control.

In the next experiments the dynamics of acquisition and length of preservation of cytotoxicity by macrophages of animals receiving a single injection of BCG or blastolysin was compared. The results of these experiments (Fig. 3) indicate that the trend of appearance of cytotoxicity differed after injection of blastolysin and BCG. Only 2 h after injection of blastolysin the PEC of the mice had a cytotoxic action on syngeneic target cells, after 4 h, their cytotoxicity was sharply reduced, but it reappeared after 2 weeks, to reach a maximum by the 5th week of the investigation. Cytotoxicity of the macrophages began to appear not earlier than 4 h after injection of BCG. It remained at this level for the first week, reached a maximum after 2 weeks, and still remained high after 5 weeks.

There is insufficient evidence at present to judge whether cells of the same type are activated by blastolysin and by BCG at different times after their administration. At the same time, there is no doubt that the trend of appearance of cytotoxicity of peritoneal exudate macrophages after injection of blastolysin is biphasic in character: The first phase is short but sufficiently clearly defined (from 21 to 55% cytotoxicity in different experiments). After a rapid decline in cytotoxicity, it begins to reappear after 2 weeks and remains at about

the same level for a long time. These results suggest activation of different types of cells. However, it should be pointed out that several workers have shown that besides macrophages, the so-called natural killer cells, which were described previously in the spleens and lymph nodes of unstimulated mice [5], may also have a cytotoxic action in peritoneal cells in the early stages after injection of BCG vaccine. However, this suggestion requires special verification.

The present investigations suggest that blastolysin, which possesses considerable antitumor activity, is a highly promising preparation, for its mechanism of action is based not on its toxic effect on cells, but on the ability of blastolysin to cause nonspecific stimulation of immunogenesis and to activate cells of the mononuclear phagocytic system, which plays an important role in the maintenance of cellular homeostasis of the body.

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