INVESTIGATION OF HUMORAL IMMUNITY IN MICE WITH TRANSPLANTABLE LEUKEMIA DURING IMMUNOCHEMOTHERAPY

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The effect of cyclophosphamide (CP), Freund's complete adjuvant (FCA), and a combination of both on development of transplantable leukemia—hemocytoblastosis of mice and on some humoral immune responses was studied. If CP (on the third day after transplantation of leukemia) and FCA (on the tenth day after transplantation of leukemia) were adminstered at certain times the mean life span of the mice was increased to 93.9 days (from 8.5 days in the control) and 42.9% of the animals survived over 180 days. During immunochemotherapy the intensity of humoral immunity is increased. Against the background of severe immunodepression following administration of CP + FCA an increase was found in the number of antibody-forming cells and the cytotoxic activity of the serum IgM in the reaction with leukemic target cells was significantly increased after administration of CP and FCA.

KEY WORDS: leukemia; immunochemotherapy; cyclophosphamide; Freund's complete adjuvant; antibody formation.

A factor which considerably limits the treatment of malignant neoplasms with drugs is that antitumor preparations depress immunity, which plays an important role in the resistance of the organism to tumors.

One or two studies of the mechanism of action of carcinolytic compounds, notably cyclophosphamide (CP), on certain immunological responses of tumors with animals have been published [2, 3].

The object of this investigation was to study the effect of CP, Freund's complete adjuvant (FCA), and a combination of both on the development of transplantable leukemia—hemocytoblastosis and on certain humoral immunological reactions of the tumor-bearing animal.

EXPERIMENTAL METHOD

Experiments were carried out on female C57BL/6 mice weighing 20-24 g. All the animals were divided into several groups. The animals of group 1 were inoculated with leukemic spleen cells from mice infected with leukemia—hemocytoblastosis of the La Puyman strain. The animals of group 2 were inoculated with leukemia and, three days later, received an intraperitoneal injection of CP in a single dose of 250 mg/kg. The animals of group 3 received an intraperitoneal injection of FCA (Difco, USA) seven days after injection of CP (10 days after inoculation with leukemia).

At various times after transplantation of leukemia and during the period of immunochemotherapy, blood was taken from the retro-orbital sinus of the mice, or sheep's red blood cells (RBCs) were injected intravenously, and on the fourth day after injection of the RBCs the local hemolysis in gel test was carried out as described by Jerne and Nordin [7].

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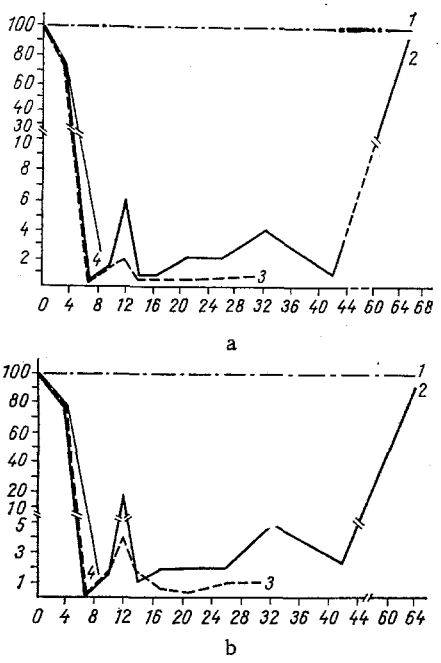

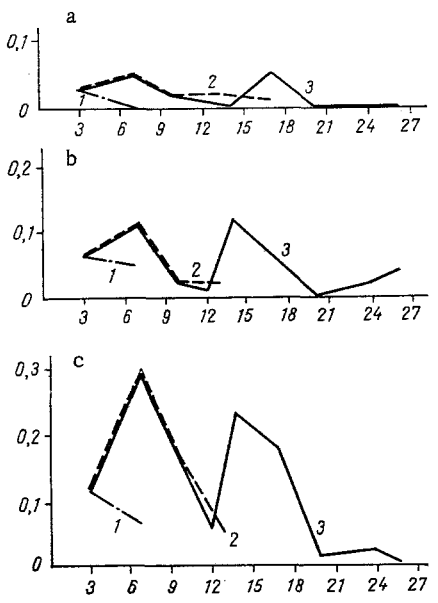

Fig. 1 Fig. 2

Fig. 1. Number of AFCs in intact mice (1), in leukemic mice (4); and leukemic mice treated with CP alone (3) and with CP +FCA (2): a) count per million spleen cells; b) count per whole spleen. Abscissa, days of experiment; ordinate, number of AFCs (in % of control).

Fig. 2. CTA of serum (a) and its IgM (b) and IgG fractions (c) in C57BL/6 mice with La Puyman leukemia: 1) mice with leukemia (control); 2) mice treated with CP; 3) treated with CP + FCA. Abscissa, days of experiment; ordinate, cytotoxic index.

TABLE 1. Effect of CP and Its Combination with FCA on Life Span of C57BL/6 Mice with La Puyman Leukemia

Group of mice	Mean life span of mice (in days)	% of mice surviving to 180th day	P (relative to control)
Control	8,5±0,34	0	—
Receiving CP	30,5±4,73	0	0,02
Receiving FCA	10,9±0,18	0	0,8
Receiving CP + FCA	93,9±16,73	42,9±9,1	0,001

Blood serum obtained from the animals of each group at different times was fractionated by column gel chromatography on Sephadex G-200 into IgM and IgG fractions, which were tested for their cytotoxic activity (CTA) by the method of Gorer and Gorman in Lezhneva's modification [4].

Some animals from each group were left in order to determine their life span. The results were subjected to statistical analysis [5].

EXPERIMENTAL RESULTS

As Table 1 shows injection of CP into mice in a single dose of 250 mg/kg on the third day after transplantation of leukemia increased the mean life span of the animals to 30.5 days but did not prevent development of leukemia. Injection of FCA alone had no effect on the course of the leukemia. The mean life span of the mice in this group was almost the same as that of the control group. Meanwhile, in the group of mice receiving FCA seven days after the injection of CP, a marked increase in the mean life span of the mice was observed (to 93.9 days); moreover, 42.9% of the animals of this group survived more than 180 days after inoculation with leukemia.

The study of the antibody-forming activity of the spleen cells from mice of the various groups showed certain differences (Fig. 1). In mice receiving leukemic spleen cells

only, the number of antibody-forming cells (AFCs) fell steadily during development of the leukemia, to reach a minimum by the time of the animals' death.

In the animals receiving CP and CP + FCA the number of AFCs changed in a wave-like manner. Against the background of the severe immunodepression produced by the development of leukemia and by treatment with CP, on the 12th day of the experiment (the ninth day after injection of CP and the second day after injection of FCA) a transient increase in the number of AFCs was observed during the recovery period; after injection of FCA the number was two to three times greater ($P = 0.02$) than the number of AFCs in animals receiving CP only. On the following days, however, the number of AFCs fell in both groups and by the 14th day of the experiment there was no significant difference between them. Starting from the 15th-18th day of the experiment the number of AFCs rose again in the animals treated with CP + FCA, and by the 20th-32nd day it was greater than the number of AFCs in the third group by a statistically significant degree ($0.1 > P > 0.05$). In the mice receiving CP alone the number of AFCs in the spleen was almost unchanged in this period.

The whole period of recovery of the initial level of AFCs in the spleens of mice receiving CP + FCA lasted 65-70 days.

When the titer of hemagglutinins and hemolysins in the blood serum of these animals was determined no significant differences could be found in mice receiving CP or CP + FCA.

The study of the serum IgM and IgG levels in the experimental animals in the cytotoxicity test with leukemic target cells showed that injection of CP caused a temporary increase in CTA of the serum IgM ($P = 0.01$), which fell until the 12th day after transplantation of leukemia, whereas IgG and the whole serum possessed no significant CTA ($P > 0.5$; Fig. 2).

Injection of FCA (on the seventh day after CP injection) into the animals led to a further significant ($P < 0.05$) increase in CTA of IgM only.

The results show that humoral immunity is strengthened during immunochemotherapy.

Investigations in the writers' laboratory have shown that injection of FCA leads to stimulation of the specific humoral immunological response, aimed against group-specific antigen (GSA) of mouse leukemias. Progression of leukemia under these circumstances is evidently due to increased production of IgG antibodies against GSA, blocking the cytotoxic effect of the IgM antibodies of the same specificity. Regression of leukemia on the other hand, is accompanied by a greater production of IgM antibodies against GSA, which are replaced later than antitype-specific antibodies [1, 6].

The considerable therapeutic effect observed in these experiments can conjecturally be linked mainly with the ability of FCA to stimulate humoral immunological responses directed against the antigens of leukemic cells.

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