STIMULATION OF HUMORAL IMMUNITY TO SURFACE ANTIGENS OF LEUKEMIC CELLS BY INTERFERON-CONTAINING SERUM

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C57BL/6 mice were immunized by a single injection of L-1210 leukemia cells and (CBA × C57BL/6)F_1 mice were immunized by a single injection of leukemia L-1210 and P-388 leukemia cells. For 8 days (including the day of immunization) the animals received daily intraperitoneal injections of 0.4 ml of allogeneic or syngeneic interferon-containing serum, whereas control animals received the same dose of normal serum from intact mice (the interferon-containing serum was obtained from (CBA × C57BL/6)F_1 mice 24 h after intraperitoneal injection of 2 mg tilorone hydorchloride). Some samples of interferon-containing serum were dialyzed for 48 h against physiological saline. The serum interferon titer was 512-1500 units/ml. On the 9th-10th day after immunization the mouse sera were put through the microcytotoxic test against leukemia cells. Definite stimulation of the cytotoxic activity of sera of mice receiving the interferon-containing serum was discovered. The syngeneic interferon-containing serum produced a stronger immunostimulant effect than the allogeneic serum.
KEY WORDS: *interferon; interferon-containing serum; stimulation of antileukemic immunity.*

As was shown previously, injection of interferon, induced in a mouse cell culture, or of interferon-containing serum into mice in conjunction with antigenic stimulation stimulates transplantation and humoral immunity [2-4, 6, 8, 9, 15-17, 19, 21].

The object of this investigation was to attempt to stimulate antileukemic immunity by means of interferon-containing serum.

EXPERIMENTAL METHOD

Mice of strains DBA-2, C57BL/6, and (CBA × C57BL/6)F_1 (females) were used. Lymphatic leukemia L-1210 and P-388 [10, 11] in the ascites form was maintained by a series of passages through DBA/2 mice at weekly intervals. The C57BL/6 and (CBA × C57BL/6)F_1 mice were immunized by intraperitoneal injection of $5 \cdot 10^7$ living leukemia L-1210 cells. The (CBA × C57BL/6)F_1 mice were immunized by intraperitoneal injection of $5 \cdot 10^7$ leukemia P-388 cells. Immune sera were obtained on the 9th-10th day after immunization.

To obtain interferon-containing serum, (CBA × C57BL/6)F_1 mice were given an intraperitoneal injection of 2 mg tilorone hydrochloride,* dissolved in 0.2 ml distilled water.

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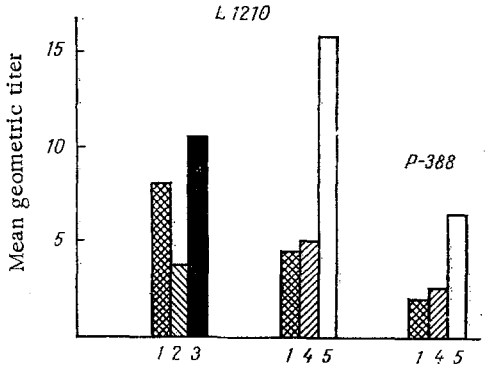

Fig. 1. Effect of interferon-containing serum on antileukemic immunity: 1) immunization with leukemia cells; 2) immunization with leukemia cells + injection of normal allogeneic serum; 3) immunization with leukemia cells + injection of allogeneic interferon-containing serum; 4) immunization with leukemia cells + injection of normal syngeneic serum; 5) immunization with leukemia cells + injection of syngeneic interferon-containing serum.

Blood was taken for obtaining serum after 24 h. Normal serum was obtained from intact (CBA × C57BL/6)F_1 mice.

To rule out any possible contamination of the interferon-containing serum by traces of tilorone and to prove that it in fact contained interferon, several samples of the supposed interferon-containing serum were diluted 1:2 with medium No. 199 and then kept for 2 days at pH 2.0. The sera were then dialyzed against physiological saline for 48 h.

The interferon-containing serum was titrated in a culture of a transplantable strain of L-cells against 100 TCD_{50} of vesicular stomatitis virus [16]. The interferon titer in the mast sera was 512-1500 units/ml. The control serum had no antiviral activity.

The scheme of the experiments was as follows.

A. After immunization with leukemia L-1210 cells, the C57BL/6 mice were divided into 3 groups. The animals of the experimental group, immediately after injection of the cells and daily thereafter, received intraperitoneal injections of 0.4 ml allogeneic interferon-containing serum (8 injections altogether). The animals of the first control group received injections of normal allogeneic serum in the same volumes and at the same times. The mice of the second control group received no injections after immunization. Each group consisted of 5 mice. Five repetitions of the experiments were carried out (altogether 75 mice).

B. After immuniation with leukemia L-1210 cells, the (CBA × C57BL/6)F_1 mice were divided into 3 groups. Syngeneic interferon-containing and normal serum were injected by the scheme described above. The number of experiments was 7 (105 mice altogether).

C. (CBA × C57BL/6)F_1 mice were immunized with leukemia P-388 cells. The experimental conditions were the same as in the previous tests. Four experiments were carried out on 60 mice.

The microcytotoxic test was performed by Terasaki's method in a system of mouse cells [7, 18]. The results were expressed in $\log_2$ units of the dilution of serum that caused 50% lysis of L-1210 and P-388 cells. To compare the levels of cytotoxic antibodies in the different groups, the mean geometric titers of cytotoxic antibodies were calculated.

EXPERIMENTAL RESULTS

The experimental results are given in Fig. 1. Injection of allogeneic or syngeneic interferon-containing serum into mice immunized with L-1210 cells produced a definite immunostimulant effect. For instance, the mean geometric titer of cytotoxic antibodies against surface antigens of L-1210 leukemia cells in the sera of C57BL/6 mice receiving allogeneic interferon-containing serum was 1:10.5, whereas in control mice receiving normal allogeneic serum the titer was 1:3.5.

Following injection of syngeneic interferon-containing serum into the immunized animals the results were even clearer; the mean geometric titer of cytotoxic antibodies after injection of syngeneic interferon-containing serum into (CBA × C57BL/6)F_1 mice was 1:16, compared with 1:4.9 after injection of normal serum. The difference between the values of the mean geometric titers in the experimental and control groups is statistically significant.

Similar results were obtained in experiments in which cells of leukemia P-388 were used for immunization (Fig. 1). For instance, the mean geometric titer of cytotoxic antibodies against surface antigens of leukemia P-388 cells in the sera of mice injected with

syngeneic interferon-containing serum was 1:6.5, compared with 1:2.3 in the sera of mice injected with normal syngeneic serum. The difference between these two titers is statistically significant.

It will be clear from these results that interferon-containing serum is a powerful stimulator of antileukemic immunity. There is no doubt that the main factor possessing an immunostimulant action in these experiments was interferon, for interferon-containing sera treated with acid and dialyzed against physiological saline in order to remove any possible traces of tilorone preserved its antiviral [12] and immunostimulant activity, just as did the untreated sera. These observations are in agreement with results showing the role of interferon in immunity published previously [2-4, 6-8, 15-18, 19, 21].

In the writers' opinion, the results of these experiments will be useful for the development of methods of treatment of acute leukemia in man [1, 5, 13, 14, 19].

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