ENHANCEMENT OF THE IMMUNE RESPONSE BY IMMUNIZATION WITH L1210 CELLS PREINCUBATED IN INTERFERON

S. V. Skurkovich, I. A. Kalinina,
E. I. Eremkina, and T. I. Bulycheva

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Mice were given a single intraperitoneal injection of L1210 cells preincubated either in interferon or in "pseudointerferon." On the tenth day the mice were killed and the cytotoxicity of the sera against cells of leukemia L1210 was determined by the microcytotoxic test (a modification of Terasaki's method). Leukemic cells treated with interferon before immunization induced a more marked immune response than cells treated under the same conditions with "pseudointerferon." The immune response, judging from the results of the cytotoxic test, was enhanced by two to four times.

KEY WORDS: interferon; leukemic cells; immune response.

Stimulation of the immune response to an antigenic stimulus is a topical line of research at the present time. This is particularly so with respect to systems including tumor or leukemic antigens. The writers have shown [3-5, 10, 11] that injection of small doses of interferon into animals against the background of antigenic stimulation induces the stimulation of humoral and transplantation immunity.

The object of this investigation was to study the immune response of mice immunized with L1210 leukemic cells preincubated with interferon.

EXPERIMENTAL METHOD

Experiments were carried out in September-November, 1974, on DBA/2 and (CBA × C57BL/6)F_1 mice, both male and female, weighing 18-20 g.

Interferon induced in a culture of L cells by Newcastle disease virus [6] was used. The titers of interferon obtained were 1000-2000 units/ml. For the experiment the interferon was diluted two to four times with medium No. 199, i.e., the preparation used had a titer of 500 units/ml. "Pseudointerferon" was used as the control. It was prepared by a similar method to the interferon itself. The difference was that allantoic fluid of uninfected chick embryos was used instead of the inducer virus. Investigation of the "pseudointerferon" from an initial dilution of 1:4 failed to show any interferon titer. The "pseudointerferon," like the true interferon, was diluted three to four times with medium No. 199 for the experiments.

Cells of mouse ascites leukemia L1210, transplanted weekly into DBA/2 mice by the usual method [7], were used for immunization.

Treatment of the leukemic L1210 cells with interferon and pseudointerferon and immunization of the animals were carried out as follows. Ascites cells were suspended in Hanks's solution and sedimented by centrifugation (800g for 10 min). The red blood cells were hemolyzed by the addition of 0.83% NH_4Cl solution, after which the cell suspension was washed twice with Hanks's solution. Interferon or pseudointerferon respectively was then added to the cell residue in the proportion of 1 ml to $5 \cdot 10^7$ cells and, after resuspension, the cells were incubated at 4°C for 18 h. The supernatant was removed after incubation and the residue was treated with medium No. 199 at the rate of 0.2 ml per $5 \cdot 10^7$ cells. The viability of the

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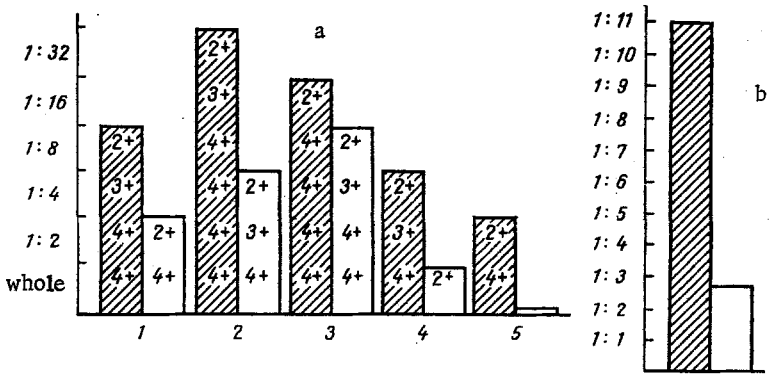

Fig. 1. Cytotoxic activity against L1210 cells of sera from mice immunized with leukemic cells treated with interferon and pseudointerferon: a) results of five separate experiments; b) mean results of these experiments expressed as geometric mean titer. Abscissa: a) number of experiment; ordinate: a) dilutions of sera; b) geometric mean titer of sera; unshaded columns represent cytotoxic activity of sera of mice immunized with L1210 cells preincubated with pseduointerferon; shaded columns — the same with cells preincubated in interferon. Results of cytotoxic tests shown inside columns.

cells was 80-85% on staining with supravital dyes (a mixture of equal parts of 0.1% solutions of Trypan Blue and eosin, added to the cell suspension in the proportion of 10:1).

Other groups of (CBA × C57BL/6)F_1 mice received a single intraperitoneal injection of L1210 cells ($5 \cdot 10^7$ cells per mouse), treated with interferon and pseudointerferon. These mice were killed on the tenth day and the cytotoxicity of their sera against cells of leukemia L1210 was determined in the microcytotoxic test (a modification of Terasaki's method [10,13]). Reactions estimated as 2+ or more were considered to be positive. The results of the tests were subjected to statistical analysis: the negative logarithm of the geometric mean titer to base 2 was calculated for the experimental and control groups.

EXPERIMENTAL RESULTS

The results of the cytotoxic tests of the sera of mice immunized with L1210 cells preincubated in interferon and in pseudointerferon are given in Fig. 1. They show that leukemic cells, treated with interferon before immunization, induced a stronger immune response than cells treated under the same conditions with pseudointerferon. The immune response in the cytotoxic test was enhanced by two to four times. Differences in the activity of the sera in the two groups of mice compared in these experiments were statistically significant.

Although the results are original in character, they are definitely associated with the authors' previous results and hypotheses concerning the role of interferon in immunity [3-5, 10-12]. The possibility cannot be ruled out that leukemic cells, with interferon on their surface, carry it to the lymphoid system and that this process is accompanied by stimulation of the function of the lymphoid cells or involvement of additional numbers of lymphocytes in the immune process. There is another possible interpretation of the findings. Additional antigenic determinants may be formed or newly exposed on the surface of the leukemic cell under the influence of interferon, and this may lead to enhancement of the immune response.

Recent preliminary experiments showed that washing the cells once after incubation in interferon does not prevent them from inducing an immune response on the same scale as if unwashed. The final interpretation of these experimental results requires further investigations.

However, the data obtained with the mouse system suggest that it would be useful to use interferon as a stimulator of the antileukemic immune response in man.

Having regard to the extensive studies of active immunization of patients with acute leukemia with allogeneic leukemic cells currently in progress [1, 2, 8, 9, 11], the preliminary treatment of the cells with interferon may possibly be indicated.

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DETERMINATION OF HYPERSENSITIVITY TO ANTIGENS BY COUNTING CELLS MIGRATING FROM CAPILLARY TUBES

D. K. Novikov, G. P. Adamenko, and V. I. Novikova

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Migration of leukocytes and macrophages from capillary tubes placed in a well containing medium was measured by counting the number of cells coming out into the medium. The method is highly sensitive and enables a small number of cells to be used with a large number (up to 600 or more) of capillary tubes. Sodium azide, phytohemagglutinin, and incubation at 4°C inhibited migration of the cells from the capillary tubes. Dead cells did not migrate from the tubes into the medium. Tuberculin and Bacillus Calmette-Guerin (BCG) vaccine inhibited migration of macrophages of guinea pigs immunized with BCG but not with staphylococcal allergen, and vice versa.

KEY WORDS: hypersensitivity of delayed type; migration of cells from capillary tubes.

Determination of migration of leukocytes and macrophages has been extensively applied in the study of reactions of hypersensitivity of delayed type (HDT). In this state, sensitized lymphocytes cultivated *in vitro* with the specific antigen liberate substances which inhibit or, less frequently, stimulate the migration (mobility) of leukocytes and macrophages [8, 12, 13]. Existing methods of determination of this state and evaluation of the inhibition of cell migration are based on measurement with a planimeter or by projecting on paper the zones of cells migrating onto the surface of a slide from horizontally placed capillary tubes in special chambers [2, 7, 14, 18, 19], from fragments of the spleen and clots of plasma [3, 10,

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