INCREASE IN SENSITIVITY OF LEUKEMIC CELLS INCUBATED IN INTERFERON TO THE ACTION OF IMMUNE SERUM

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L-1210 and P-388 leukemic cells were incubated in three types of interferon: L-cell and two types of lymphocytic interferon (induced in lymphocytes of intact mice and lymphocytes taken from mice of the same strain on the 10th day after intraperitoneal injection of $5 \cdot 10^7$ L-1210 cells). Pseudointerferon obtained by a method similar to that used to obtain L-cell interferon, but without the virus induction, was used as the control. Cells incubated in interferon were found to be more sensitive to the action of antibodies than cells treated with pseudointerferon. Treatment of cells with lymphocytic interferon induced in lymphocytes of immune mice increased their sensitivity even more than cells treated with interferon obtained in intact lymphocytes.

KEY WORDS: interferon; immune serum; leukemic cells.

It is now firmly established that interferon plays an important role in the development of immunity and in the mechanism of the immune response [1-4, 6-9]. The writers postulated previously [4] that the presence of interferon on a target cell may increase its sensitivity to the action of antibodies.

The object of this investigation was to test this hypothesis.

EXPERIMENTAL METHOD

Male and female DBA/2 and (CBA × C57BL/6)F_1 mice weighing 18-20 g were used.

Three types of interferon were used: L-cell and two types of lymphocytic interferon (induced in lymphocytes of intact and immune mice). Newcastle disease virus [8] was used as the inducer, in a dose of 10-100 virus units per cell.

A continuous culture of L cells was used to prepare the L-cell interferon. Lymphocytic interferon was obtained with the aid of: a) lymphocytes of intact (CBA × C57BL/6)F_1 mice isolated from lymph nodes; b) lymphocytes of (CBA × C57BL/6)F_1 mice immunized with a single dose of $5 \cdot 10^7$ L-1210 cells, isolated from lymph nodes on the eighth day after immunization. The suspension of lymphocytes for obtaining these species of lymphocytic interferon was made up in medium No. 199 in a dose of $1 \cdot 10^6$ cells/ml medium.

The activities of the L-cell and the two types of lymphocytic interferon were determined on L cells by their inhibitory effect on 100 CPD_{50} of vesicular stomatitis virus. The activity of the L-cell interferon was 1000-2000 units/ml, that of the lymphocytic interferon obtained on lymphocytes of intact mice was 128 units/ml, and that of lymphocytic interferon obtained on lymphocytes of mice previously immunized with L-1210 cells was 64 units/ml.

L-cell interferon with an activity of 1:500 was used in the experiments, for which purpose the interferon was diluted with medium No. 199 two to four times times. Both types of lymphocytic interferon were used in a titer of 1:64.

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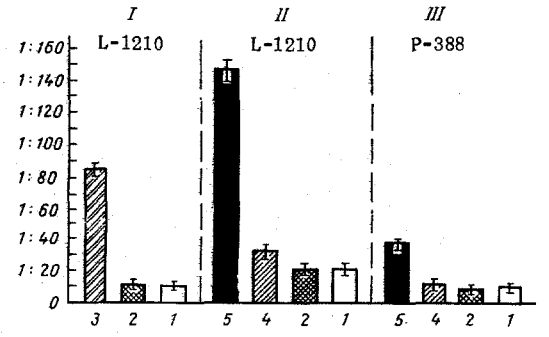

Fig. 1. Cytotoxic activity of mouse sera against L-1210 and P-388 leukemic cells incubated in interferon and pseudointerferon (geometric mean titers). Ordinate, geometric mean titers of sera; abscissa: 1) initial activity of sera; 2) activity of sera against L-1210 and P-388 cells incubated in pseudointerferon; 3) activity of sera against L-1210 cells incubated in L-cell interferon; 4) activity of sera against L-1210 and P-388 cells incubated in lymphocytic interferon induced on lymphocytes of intact mice; 5) activity of sera against L-1210 and P-388 cells incubated in lymphocytic interferon induced on lymphocytes of immune mice.

Pseudointerferon, obtainable by a method similar to that used to obtain L-cell interferon, was used as the control. The only difference was that instead of the inducer virus, allantoic fluid of uninfected chick embryos was used. No interferon activity was discovered in the pseudointerferon by the method of titration based on inhibition of 100 CPD_{50} of vesicular stomatitis virus. When the pseudointerferon was used, it was diluted 2-4 times with medium No. 199 to correspond to L-cell or lymphocytic interferon.

To obtain the immune serum, cells of mouse ascites leukemia L-1210 and P-388, transplanted weekly into DBA/2 mice in a dose of $1.5 \cdot 10^6$ cells per mouse, were used. The ascites cells were added to Hanks' solution and sedimented by centrifugation (200g, 10 min). The supernatant was removed and the erythrocytes lysed by the addition of 0.83% NH_4Cl solution. The cell suspension was then washed twice with Hanks' solution and the number of cells in 1 ml was counted. (CBA × C57BL/6)F_1 mice were immunized by a single intraperitoneal injection of $5 \cdot 10^7$ L-1210 or $7.5 \cdot 10^7$ P-388 leukemic cells. The mice were killed on the 10th day in order to obtain the immune serum.

Treatment of the leukemic cells with interferon was carried out as follows: Interferon (experiment) or pseudointerferon (control) was added to the residue of the freshly obtained and washed mouse ascites leukemic cells in a dose of 1 ml to $5 \cdot 10^7$ cells and they were incubated for 2 h at 18-20°C. After incubation the cells were sedimented by centrifugation and then suspended in Hanks' solution for subsequent testing. The sensitivity of the cells to the cytotoxic action of the immune serum was determined in a microcytotoxic test (a modification of Terasaki's method [9, 10]. As initial activity the activity of the serum was determined against leukemic cells suspended in Hanks' solution. Reactions with more than 50% of dead cells were taken to be positive. The results were compared relative to the geometric mean titer of the sera in the reaction with cells incubated in interferon, in psuedointerferon, and in Hanks' solution.

EXPERIMENTAL RESULTS

The results of 30 experiments to study the cytotoxic action of sera obtained against L-1210 and P-388 leukemic cells against the corresponding leukemic cells incubated in interferon and in the control media are shown in Fig. 1.

As Fig. 1 shows, the geometric mean titer of immune serum against L-1210 leukemic cells suspended in Hanks' solution in the experiments of series I was 1:10, whereas the titers of the immune serum against L-1210 cells incubated in L-cell interferon and in pseudointerferon were 1:84 and 1:10.6, respectively; i.e., incubation of the leukemic cells in interferon considerably increased their sensitivity to the immune serum (difference statistically significant, $P < 0.05$).

Incubation of L-1210 and P-388 cells in lymphocytic interferon in series II and III also was accompanied by an increase in their sensitivity to the cytotoxic activity of the immune sera compared with the initial sensitivity of cells suspended in Hanks' solution. A statistically significant ($P < 0.05$) increase was found in the

sensitivity of the L-1210 leukemic cells to the cytotoxic action of the immune serum if they were incubated in interferon obtained on lymphocytes of immune mice (geometric mean titer 1:147) compared with that in interferon obtained on lymphocytes of intact mice of the same strain (geometric mean titer 1:34). If, on the other hand, P-388 cells were incubated in lymphocytic interferon obtained on immune lymphocytes, although an increase in their sensitivity to the cytotoxic action of the immune serum was observed (geometric mean titer 1:39) compared with the sensitivity of P-388 cells incubated in lymphocytic interferon obtained on lymphocytes of intact mice (geometric mean titer 1:13), this difference was not statistically significant.

It is difficult at present to explain the mechanism of the increased sensitivity of the antigenic determinants to antibodies after treatment with interferon. However, these observations fit in to some degree with the writers' hypothesis [5] that for an immune reaction to take place interferon must be present on the target cell and the immune lymphocyte.

In conclusion, these experiments confirm yet again the importance of interferon in the formation of immunity and of immune responses. In this context it is a very demonstrative fact that interferon obtained on the lymphocytes of mice previously immunized with L-1210 cells increased sensitivity to antibodies more than interferon obtained on lymphocytes of intact mice. In the writers' view, the presence of interferon on the target cell in vivo may be of great importance in ordinary immune reactions and also in allergic and autoimmune processes in man [5]. The possibility cannot be ruled out that in the latter case the quantity of interferon present on target cells plays an important role in increasing the sensitivity of cell systems to autoantibodies, even with low activity.

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