INTERFERON IN THE MOUSE BONE MARROW

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Nucleated bone marrow cells of mice produce interferon in vitro in response to injection of Newcastle disease, influenza, and Sendai viruses but not vesicular stomatitis virus. Highest interferon concentrations are found 8-26 h after injection of Newcastle disease virus (NDV). The highest interferon titers are obtained after injection of NDV with a multiplicity of 1:10 PFU/cell, with a cell concentration of 10^7/ml, and in medium no. 199 containing $\geq 5\%$ serum. Ability of the bone marrow cells to produce interferon depended on the time when they were taken after the animal's death and it diminished as the cells were kept. Partial inactivation of NDV by ultraviolet irradiation or by the action of acid (pH 2.0) did not alter the ability of the virus to induce interferon formation in the bone marrow cells.

Investigations have shown that the tissues of animals and man produce interferon in vivo and in vitro in response to injection of corresponding virus or synthetic interferonogens. Leukocytes possess the same property.

In view of these observations, and also of the results of an investigation of interferon formation in radiation chimeras [9], it was decided to study the ability of bone marrow cells to form interferon.

EXPERIMENTAL METHOD

The following viruses were used as interferonogens: Newcastle disease virus (NDV; strains H, B_1, Beaudette VAR), influenza (strain A/WSN), vesicular stomatitis (strain Indiana), and Sendai.

All the experiments were carried out with bone marrow cells obtained from the tibias of CBA mice weighing 12-16 g. The cells were counted in a Goryaev's chamber. The number of viable cells was determined by staining with neutral red. Up to 2.5×10^7 nucleated cells were obtained from each mouse. Cells were diluted with medium no. 199 with 10% bovine serum to a concentration of 1.0×10^7/ml.

TABLE 1. Relationship between Production of Interferon in Bone Marrow and Cell Concentration and Multiplicity of Infection by Strain H of Newcastle Disease Virus

Cell concentration (no. of cells/ml)	Interferon titer with multiplicity of infection given below			
	0.1	1.0	10.0	100.0
10^5	<1:5	<1:5	1:80	1:80
$5 \cdot 10^5$	< 5	1:20	1:160	1:160
10^6	1:10	1:160	1:320	1:640
$5 \cdot 10^6$	1:160	1:320	1:640	—
10^7	1:640	1:1280	1:1280	—

To obtain interferon, 0.5 ml of a suspension of bone marrow cells of the required concentration was treated with 0.5 ml virus, the dose of which per cell had been determined previously. After incubation for 24 h at 37°C the suspension was treated with 4 ml medium no. 199 with 2% serum and then centrifuged for 5-10 min at 1000 rpm. The culture fluid was drawn off and the pH adjusted to 2.0 with 1% HCl solution. After three to four days the pH of the culture fluid was adjusted to neutral by 1% NaOH solution. Activity of the bone marrow interferon was determined in a three day culture of L cells against 100-1000 TCD_{50} vesicular stomatitis virus.

The virus was irradiated with a BUF-60 lamp with a power of 9.2 erg/mm^2/sec. Irradiation continued for between

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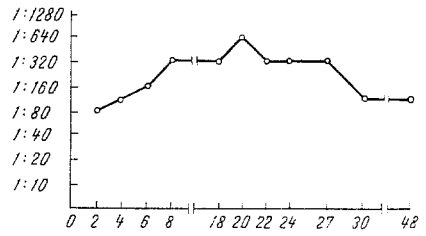

Fig. 1

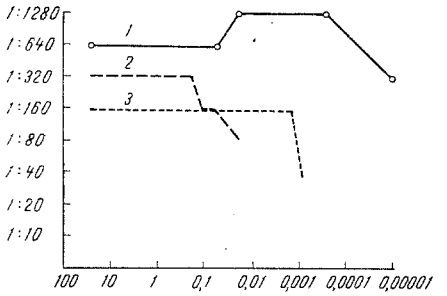

Fig. 2

Fig. 1. Dynamics of interferon formation by mouse bone marrow. Ordinate, interferon titer; abscissa, time (in h).

Fig. 2. Interferonogenicity of partially inactivated strain H of NDV in bone marrow cells of mice and rats: 1) titers of interferon induced in mouse bone marrow cells by NDV partially inactivated by ultraviolet irradiation; 2) the same, with the use of NDV partially inactivated by treatment with acid (pH 2.0); 3) titers of interferon induced in bone marrow cells of rats by NDV partially inactivated by ultraviolet irradiation. Ordinate, interferon titer; abscissa, multiplicity of infection.

30 sec and 20 min. The virus was first centrifuged to remove coarse debris and diluted 1:2 with physiological saline.

For acid treatment of the virus 1% HCl solution was used. After 3-30 min the pH was adjusted to neutral with 1% NaOH solution.

EXPERIMENTAL RESULTS

Of the viruses tested, the four strains of NDV gave rise to the highest interferon production in the mouse bone marrow cells (interferon titers from 160 to 320 units/ml). Smaller quantities of interferon (40-80 units/ml) were induced by influenza (strain A/WSN) and parainfluenza (Sendai) viruses. Vesicular stomatitis virus was unable to induce interferon production in the mouse bone marrow cells.

Interferon was found in response to injection of strain H of NDV (multiplicity 10, cell concentration 10^7/ml) 2 h after the injection, and by 8 h its concentration reached its maximum, at which level it remained until 27 h (Fig. 1).

Interferon production was largely dependent on the dose of the virus interferonogen and on the cell concentration (Table 1). The highest interferon titers were obtained by the use of a high cell concentration (10^7/ml). In this case even the injection of comparatively low doses of virus (0.1-1 PFU/cell) led to intensive interferon production. If cell concentrations of $\leq 5 \times 10^6$/ml were used, the dose of virus required to be injected in order to obtain interferon production was 10-100 PFU/cell.

Production of interferon by the bone marrow, like that of interferon obtained from other sources, was dependent also on the serum content of the medium. A concentration of 5-10% serum was optimal in these investigations, and the use of lower concentrations led to a decrease in titer. The serum could be added to the medium 1 h after addition of the virus or at once, during actual preparation of the bone marrow cell suspension. Similar results have been obtained in experiments on leukocytes [11].

The highest titers of interferon were obtained by the use of fresh bone marrow, and also after the suspension of extracted bone marrow cells (concentration 10^7/ml) had been kept for 3 h at 4-6°C. The ability to form interferon was reduced by half if the cells were kept under the same conditions for 6-24 h, and by three-quarters if they were kept for 48 h. If serum was present in the medium (10-40%) the cells preserved their ability to produce interferon better. The results of tests of the viability of the cells by staining with neutral red also confirmed the necessity of keeping them in medium with serum (by 48 h in medium without serum only 28% of cells remained viable, compared with 35-39% in medium with serum).

The ability of the bone marrow cells to produce interferon fell sharply with an increase in the time between obtaining the bone marrow and death of the mice. For instance, after 3 h the cells produced only one-quarter as much interferon, and after 6 h only one-eighth as much. Bone marrow taken 24 h after the animal's death (the mice were kept at 4-6°C) was unable to produce interferon.

Partial inactivation of NDV by ultraviolet irradiation or by the action of acid (pH 2.0) considerably increases interferonogenicity of the virus in chick embryonic cells [3, 8, 13]. Different results were obtained in bone marrow cells of mice and rats, in which the partially inactivated virus induced the formation of the same amount of interferon as the original virus (Fig. 2). More intensive inactivation led to a decrease in the interferonogenicity of the NDV for the bone marrow cells of the animals studied. Inactivation of the virus by ultraviolet irradiation gave better preservation of its interferonogenicity for these cells than inactivation with acid.

Bone marrow cells of animals (mice and rats) are thus good (if not better) producers of interferon in vitro. The highest interferonogenicity in these cells was exhibited by NDV, which is also the best inducer of serum interferon in mice [7].

By contrast with the experiments on chick embryonic cells [3, 8, 13], partial inactivation of the NDV did not increase its interferonogenicity in the bone marrow cells, just as in the leukocytes of the peripheral blood or peritoneal exudate [1, 12]. Bone marrow interferon, like interferon produced by leukocytes and microphages [4, 10], was found in the cell suspension very soon (starting from 2 h) after injection of the virus and high concentrations were reached after 8 h.

The intensity of formation of bone marrow interferon, like that of leukocyte interferon [6, 12], was dependent on the concentration of cells and multiplicity of infection. However, the titers of mouse leukocyte interferon [2, 5] were lower, as a rule, than the titers obtained in bone marrow cells. This fact, combined with the ease of obtaining nucleated bone marrow cells (approximately 2×10^7 cells from a mouse weighing 16 g) are not only of theoretical, but also of practical importance.

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