INTERFERONS PRODUCED BY NEONATAL MOUSE CELLS IN THE PRESENCE OF SERA OF NEWBORN AND ADULT ANIMALS

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Data indicating differences in activity of interferons produced by newborn and adult animals have recently been published [1, 2, 7-9]. It has also been shown that one of the possible factors influencing interferon production in newborn animals is liberation of cathepsin D, which is stimulated by components contained in the serum of such animals [3]. The object of this investigation was to compare the electrophoretic profile of antiviral activity of interferons produced by neonatal mouse cells and to discover the role of age-related serum factors in this process.

EXPERIMENTAL METHOD

Cultures of neonatal mouse fibroblasts were obtained by trypsinization of skin and muscle tissue of newborn mice and subsequent roller culture in bottles (diameter 78 mm, height 130 mm, rotation speed 12 rev/h). The number of cells introduced into each bottle was 30×10^6, and 5-6 days after monolayer formation interferon synthesis was induced with Newcastle disease virus (strain H). The virus was introduced in a dose of 100 CPD_{50} per cell, the virus-containing fluid was poured off 1 h later, the cells were carefully washed, and they were then incubated at 37°C in medium No. 199 containing 2% of serum from sexually mature (version 1 of the experiment) and newborn mice (version 2), previously heated to 56°C for 30 min. The culture fluid was collected 18 h later and the interferon contained in it was determined. To purify the interferon, it was subjected to adsorption chromatography on highly porous glass with a pore size of 40-120 mesh (from Sigma, USA) [6]. After purification the interferon preparations were concentrated by reverse dialysis against polyethylene glycol (mol. wt. 40 kilodaltons) until the protein concentration in the preparation was 1.5 mg/ml. Electrophoretic analysis of the interferon preparations was carried out in 12% sep-

TABLE 1. Purification of Fibroblast Interferon by Adsorption Chromatography on Porous Glass (mean results of 3 experiments)

Parameter	Serum: neonatal	Serum: adult
Initial volume of interferon preparation, ml	200	220
Initial titer, rdu/ml	71	1880
Initial protein concentration, μg/ml	660	1250
Vol. of eluate after purification, ml	40	40
Protein concn. in purified preparations, μg/ml	35	75
Interferon titer in purified preparations, rdu/ml	210	5000
Degree of purification of interferon preparations, expressed as protein	94,2	102,6
Loss of specific activity on interferon preparations after purification, %	40,9	51,7

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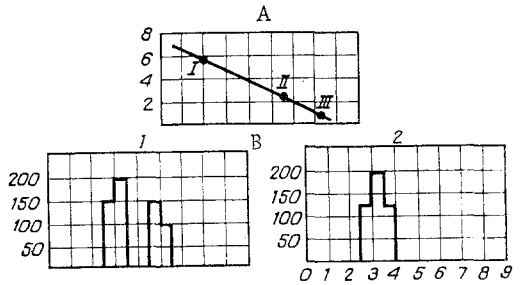

Fig. 1. Distribution profile of antiviral activity of purified interferon preparation in PAG in the presence of sodium dodecylsulfate. Abscissa, length of gel (in cm); ordinate: A) mol. wt. of markers ($\times 10^4$ daltons), B) interferon titer (in rdu). 1) In presence of adult serum; 2) in presence of neonatal sera. A) Markers with known molecular weight: I) bovine serum albumin, II) chymotrypsin, III) cytochrome c. Columns denote antiviral activity of interferon.

arating polyacrylamide gel (PAG) with a ratio of acrylamide to bisacrylamide of 30:0.9 [11]. Excess of ammonium persulfate in the PAG was removed by preliminary electrophoresis with a current of 20 mA for 18 h. Concentrating 5% PAG was formed in the presence of 0.004% riboflavin in light. Glycerol (20%) was added to both gels.

The test preparations of interferon were diluted in Tris-HCl buffer (0.0625 M), pH 6.8, with 1% sodium dodecylsulfate, 10% glycerol, and 0.0012% bromphenol blue, and kept at room temperature for 1 h.

Purified and concentrated preparations of interferon were applied to each division strip of the gel block at the rate of 100-150 μg protein per sample. A current of 15 mA for 18 h was used for electrophoresis. After the end of the procedure the separation strips were cut into pieces 0.5 cm wide and homogenized in a solution containing 1% calf embryonic serum (from the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR), 0.1% Tween-80, 1% glycerol, and antibiotics (penicillin and streptomycin, 100 i.u./ml of each). Material was eluted from each piece of gel for 2 days at 4°C and the interferon titers were determined in the resulting extracts by a micromethod on L cells against 100 CPD_{50} of vesicular stomatitis virus (Indiana strain), using plastic plateaux. The interferon titers were expressed in reciprocal dilution units (rdu).

EXPERIMENTAL RESULTS

The results of the study of interferon preparations produced by fibroblasts of newborn animals in the presence of sera of newborn and adult animals (versions 2 and 1, respectively) are given in Table 1. They provide convincing evidence of significantly higher activity (by 26 times) of interferon production in the first version of the experiments. It is worth noting that purification of the interferon preparations by chromatography on highly porous glass led to virtually equal losses of both protein and initial activity irrespective of the version of interferon formation: The protein concentration in both versions of interferon was 94.2-102.6 times lower in both versions than in the original preparations, and loss of interferon activity in the first version of the experiments was 51.7% compared with 40.9% in the second version.

The results of analysis of the distribution of antiviral activity in PAG as a result of electrophoresis of the interferon preparations representing the two versions of its formation are given in Fig. 1. Interferon from the experiments of version 1 was found to have the whole of its antiviral activity localized in one zone (it was eluted as a single peak) and to be bound with the component whose mol. wt. was 45 kilodaltons. The antiviral activity of interferon obtained in the experiments of version 2 was found in two zones, accounting for 58.4 and 41.6%, respectively, of their total activity; mol. wt. of these components was 41 and 28 kilodaltons respectively, in agreement with data in [6].

The results are convincing evidence that the same cells (neonatal mouse fibroblasts) produced different quantities of interferon depending on the presence of serum (from newborn or adult animals) in the culture medium. It is extremely difficult to provide an unequivocal explanation of this phenomenon. The possibility cannot be ruled out that in the presence of neonatal sera the process of interferon synthesis differs from that which occurs when serum of adult animals is present in the culture medium. Meanwhile it can be tentatively suggested that the serum of newborn animals contains certain components which stimulate inactivation, possibly through hydrolysis of the interferon once it is formed. Such a mechanism is suggested by a fact established previously, namely that liberation of cathepsin D is activated in the presence of neonatal mouse serum [3].

Despite the fact that the mechanism of this phenomenon requires further investigation, it can be postulated that it is the neonatal serum (or the components contained in it) that results in interferon being found for a shorter time in the organs of newborn animals than of adults [4, 7], and it may also be responsible for the absence of an antiviral effect even if interferon is formed in quantities comparable with those in adult animals [11].

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