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PRODUCTION OF ANTIVIRAL FACTOR BY INFECTED CHICK EMBRYONIC FIBROBLASTS

N. V. Gribkova and V. I. Votyakov

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The writers showed previously that infection of chick embryonic fibroblasts by virus VÉL-230 is accompanied by the production of an antiviral factor of protein nature in the culture fluid [2, 9]. The originality of the antiviral factor was confirmed by properties such as the absence of species specificity, preservation of its activity after heating to 100°C for 30 min, and a molecular weight of not more than 10 kilodaltons. The antiviral factor suppressed reproduction of the viruses of influenza, Venezuelan equine encephalomyelitis, vesicular stomatitis, herpes simplex, and the interferon-resistant virus of vaccinia in vitro, without causing any significant stimulation of synthesis of cellular RNA and proteins. Recent investigations have shown that this antiviral effect is unconnected with interferon induction [4].

The aim of this investigation was to continue the study of the production of this cellular antiviral factor.

EXPERIMENTAL METHOD

A primary trypsinized culture of chick embryonic fibroblasts (CEF) and transplantable green monkey kidney cells (Vero), hamster kidney (BHK-21), and human embryonic fibroblasts (HEF) were used in the experiments. The cell cultures were infected with Venezuelan equine encephalomyelitis virus (VÉL-230), maintained by passage in a culture of CEF. The infectious titer of the virus was determined by the plaque-formation method [8]. Antiviral factor (AF) was obtained by successive hydrolysis of the culture fluid at pH 2.0 and 6.0 [1]. The denatured substances were removed by centrifugation and the supernatant was used as the total AF preparation. Antiviral activity of AF was estimated in conventional antiviral units (AU/ml). Activity of AF was taken to be the reciprocal of the maximal dilution of AF causing suppression of reproduction of the test virus by 2 log PFU/ml.

EXPERIMENTAL RESULTS

To study the distribution of the phenomenon of AF production, the ability of other cell cultures to produce similar activity in response to infection was tested. Intact cultures of HEF, Vero, and BHK-21 cells, and also of CEF, produced virtually no AF, since activity of AF was less than 1 AU/ml and did not cause statistically significant suppression of reproduction of the VÉL-230 virus. Activity of the AF preparation obtained from the culture fluid of infected HEF and Vero was comparable and did not exceed 2 AU/ml. Infection of BHK-21 cells did not lead to production of any AF

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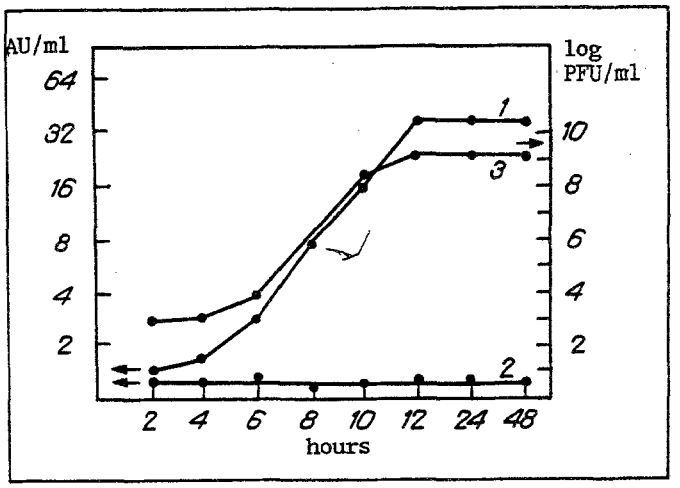

Fig. 1. Dynamics of appearance of activity of AF in culture fluid of CEF. Abscissa, incubation time, in h; ordinate, on left: activity of AF, in AU/ml; on right: infectious titer of virus, PFU/ml. 1) Activity of AF from infected cultures, 2) activity of AF from intact cultures, 3) infectious titer of VÉL-230 virus in culture fluid.

whatever. Only infection of the CEF culture was accompanied by marked production of AF (40 AU/ml), and it was this which determined the choice of this cell culture for the subsequent study of AF production.

In order to study the kinetics of AF production, the antiviral activity of specimens taken at different times after infection of the cell monolayer was determined. As the control of the dynamics of AF production, we used culture fluid of uninfected CEF. The results are given in Table 1. They show that intensive growth of activity of AF was observed in the period between 6-9 h after infection. By 12 h of observation, AF activity reached its maximum. Activity of AF obtained from uninfected CEF was less than 1 AU/ml and was unchanged throughout the period of observation.

In the next experiments we studied dependence of AF production on multiplicity of infection. We found no direct relationship between activity of AF and this particular factor. For instance, with a multiplicity of infection of 30 PFU/cell, activity of the AF preparation obtained reached 20-40 AU/ml. Meanwhile a decrease in the multiplicity of infection to 3 PFU/cell led only to weakening of activity by half.

To study the role of the cell in AF production we used inhibitors of synthesis of cellular macromolecules: actinomycin D (Calbiochem) and cycloheximide (Serva), in final concentrations of 2 μg/ml and 100 μg/ml respectively. A culture of CEF cells was treated with actinomycin D 1 h before infection. After thorough washing the cell monolayer was infected and AF obtained by the method already described. In the case of suppression of cell protein synthesis by cycloheximide, the preparation was added to maintenance medium 1 h before infection and at the time of adsorption of the virus, which was carried out at 4°C. Suppression of biosynthesis of cellular RNA by actinomycin D led to blocking of AF production in the culture medium. To produce AF, cell protein synthesis also is needed, for pretreatment of the cell monolayer during 1 h before infection and addition of cycloheximide during the period of adsorption likewise suppressed AF production.

The investigations thus showed that AF production depends on the cell culture used. For instance, infection of cell cultures was accompanied by marked stimulation of AF production by the CEF culture only. Cells of the BHK-21 line did not produce any AF whatsoever. The low level of activity of AF obtained on CEF and Vero cultures does not allow them to be recommended as cell cultures for production of AF. The marked difference between cells of mammals and birds in the response to infection, manifested as AF production, confirms our previous hypothesis that AF may appear in the early stages of evolution of the animal world [3], and may probably reflect the evolution of immunity.

We found no direct dependence of the level of AF activity on multiplicity of infection. Meanwhile, suppression of biosynthesis of cellular macromolecules by actinomycin D and cycloheximide are evidence that participation of cellular mRNA and proteins in the process of AF production is essential.

We know from data in the literature that fibroblasts produce biologically active substances such as interferons, interleukins, and growth factors [5-7]. However, even though the CEF-VÉL system which we used is, according to data in the literature, a good interferon producer, activity of this cytokine was not found in the total AF preparation [4]. The results of the present investigation, together with data published previously [2-4] indicate, in our view, that chick embryonic fibroblasts, while not belonging to the classical cells of the immune system, can nevertheless produce, in response to infection, a protein factor with antiviral activity, and which, by virtue of its biological properties, can be classed as a noninterferon factor of nonspecific protection.

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