ATTEMPTS TO USE THE INTERFERENCE PHENOMENON TO DETECT MOUSE LEUKEMIA VIRUSES IN TISSUE CULTURE

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The virus of Friend leukemia was found in tissue culture by the use of the interference phenomenon with mouse encephalomyocarditis virus.

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The experimental study of mouse leukemia viruses is handicapped by the absence of cytopathic action in tissue cultures. Reproduction of these viruses in tissue culture can be estimated only from the results of biological tests on mice. Indirect methods exist for the detection of viruses in vitro, one of which is based on the use of the interference phenomenon. Evidence that this method can be used for the detection of infectious viruses in tissue cultures is given by several investigations [1, 2, 5]. More recently investigators have directed their attention to problems connected with interference between nontumor and oncogenic viruses, especially leukemogenic. However, these investigations have mainly been concerned with the study of interference between viruses in the animal body [3, 4, 6, 7].

The object of the present investigation was to study the possibility of using interference between certain infectious viruses and the viruses of Friend and Mazurenko leukemias for the detection of the latter in tissue culture.

EXPERIMENTAL METHOD

Monolayer cultures of embryonic fibroblasts of mice of lines BALB/c and CC57BR of the first two subcultures were used in the investigation. The cultures were grown in medium No. 199 with 10% bovine serum. The virus material consisted of a cell-free supernatant prepared from leukemic mouse organs. Viruses of mouse encephalomyocarditis (obtained from T. I. Balezina) and vaccinia virus were used in the interference experiments. The first virus caused cytopathic changes in tissue cultures of mouse embryos (TCD_{50} $10^{8.5}$); vaccinia virus gave a hemadsorption reaction (HAR) with hens' erythrocytes (ID_{50} $10^{6.5}$). From 2 to 3 days after infection with leukemic viruses the tissue culture cells were infected with various doses of encephalomyocarditis or vaccinia virus. Each dilution of virus was used to infect 5 or 6 flasks of tissue culture cells, so that at least 4 flasks per dilution would be available for the final results. The results of interference were based on the determination of the cytopathic action and the HAR. The virus of Friend leukemia was titrated on mice of line BALB/c (ID_{50} $10^{6.5}$). Experiments on neutralization of the interfering action of Friend virus were carried out with the serum of rabbits immunized with concentrated virus preparations.

EXPERIMENTAL RESULTS

The experiments of series I were carried out with tissue culture cells infected with Friend leukemia virus and later infected with mouse encephalomyocarditis virus. After infection of the cultures with the latter virus in dilutions of between 10^{-1} and 10^{-5} ($10^{7.5}$ - $10^{3.5}$ TCD_{50}/ml), a cytopathic action of equal intensity was observed at identical times in the experimental and control cultures. In the subsequent experiments, therefore, smaller doses of encephalomyocarditis virus were used (from $10^{3.5}$ to $10^{0.5}$ TCD/ml). Under these conditions, interference was found in 11 of the 14 experiments between Friend leukemia virus and encephalomyocarditis virus with 10-100 TCD_{50}/ml (Table 1). The presence of Friend virus in the tis-

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TABLE 1. Interference between Friend Leukemia Virus and Encephalomyocarditis Virus in Tissue Culture and Neutralization of this Action by Antiviral (against Friend Virus) Serum (Results of Separate Experiments)

Dilution of encephalomyocarditis virus	Interference phenomenon — Fr. leuk. vir. in subcult.								Neutralization of interference phenomenon with rabbit immune serum													
	Fr_1	C_3	Fr_2	C_3	Fr_3	C_3	Fr_6	C_3	Fr_1	C_1	C_2	C_3	Fr_2	C_1	C_2	C_3	Fr_3	C_1	C_3	Fr_5	C_1	C_3
10^{-4}	4/4	4/4	0	0	0	0	0	0	4/4	4/4	4/4	4/4	0	0	0	0	0	0	0	0	0	0
10^{-5}	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
10^{-6}	3/4	4/4	2/4	4/4	2/4	4/4	2/4	4/4	4/4	0/4	2/4	4/4	4/4	2/4	4/4	4/4	4/4	0/4	4/4	4/4	1/4	4/4
10^{-8}	0/4	2/4	2/4	4/4	2/4	4/4	0/4	4/4	4/4	0/4	0/4	4/4	2/4	0/4	0/4	4/4	2/4	0/4	4/4	3/4	0/4	4/4
10^{-8}	0/4	0/4	0/4	0/4	0/4	2/4	0/4	0/4	1/3	0/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
10^{-9}	0	0	0/4	0/4	0/4	0/4	0	0														

Note: Denominator represents number of cultures infected with that particular dilution of encephalomyocarditis virus, numerator gives number of cultures with cytopathic effect; Fr_1) in experiments with interference, cultures infected with Friend leukemia virus, first subculture; in neutralization experiments, cultures infected with mixture of Friend leukemia virus and antiviral serum; C_1) cultures treated with mixture of Friend leukemia virus and normal serum; C_2) cultures infected with Friend leukemia virus alone; C_3) cultures not infected with Friend leukemia virus.

sue culture was determined by a biological test on mice.

To demonstrate the specificity of the interfering action of Friend leukemia virus, experiments were carried out to study the neutralization of this action by immune serum. The reults are given in Table 1. They show that tissue culture treated with a mixture of Friend virus and immune serum behaved toward encephalomyocarditis virus almost like uninfected cultures. In some experiments very weak inhibition of the cytopathic action of encephalomyocarditis virus was observed, but this could evidently be attributed to the nonspecific action of the serum. In cultures in which Friend virus was treated with normal serum, the interference was the same as when the cultures were infected with Friend virus alone. Consequently, antiserum against Friend virus neutralized that virus, and in such cultures no interference with encephalomyocariditis virus took place.

No interference was observed in tissue culture cells infected with Mazurenko leukemia virus followed by encephalomyocarditis virus.

The viruses of Friend and Mazurenko leukemias gave very weak interference with vaccinia virus in a concentration of 10-100 ID_{50}/ml.

The viruses of Mazurenko and Friend leukemias thus behaved differently in the reaction with the above-mentioned nontumor viruses in tissue culture. Friend leukemia virus gave clear interference with certain doses of mouse encephalomyocarditis virus, the specificity of which was maintained in the neutralization experiments. In tests of Mazurenko virus with encephalomyocarditis virus the interference phenomenon was not observed. The interfering action of these leukemia viruses with vaccinia virus was very weak.

It was not the purpose of the investigation to examine the mechanism of interference between Friend and encephalomyocarditis viruses. Interferon production possibly plays the principal role here. However, interference can take place even without interferon formation. In such cases the interference phenomenon can be explained differently: by changes in cell metabolism, competition for cell components, blocking of receptors, and so on. This problem requires special study.

It may be concluded from the data described above that tests using the interference phenomenon can serve as an indirect method of detection of Friend leukemia virus in tissue culture cells provided that definite doses of encephalomyocarditis virus with a standard infectious titer are used.

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