DETECTION OF MYELOBLASTOSIS VIRUS ANTIGEN IN CELL CULTURES

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It has been shown by the method of fluorescent antibodies that myeloblastosis virus begins to be synthesized in the cytoplasm of chick fibroblasts 24 h after infection, and that synthesis takes place in all the cells after 48-72 h. Myeloblastosis virus cannot multiply in myodermal fibroblasts of mouse embryos or heart cells of Cynomolgus monkeys, although it is adsorbed onto the surface of these cells.

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Viruses of the fowl sarcoma-leukemia groups are widely distributed in nature and can contaminate material used in virologic investigations and vaccines prepared on chick embryos. The question of the spectrum of species pathogenecity of these viruses has become particularly important at the present time.

The object of this investigation was to use the method of fluorescent antibodies as an indicator of one of the viruses of this group, myeloblastosis virus, in cell cultures. The choice of the fluorescent antibody method was determined by the fact that it can not only demonstrate antigen of myeloblastosis virus, but can also demonstrate its accumulation, localization, and synthesis in cell cultures in vitro.

EXPERIMENTAL METHOD AND RESULTS

Cell Cultures. The following cells were used in the experiments: chick fibroblasts from 11-day embryos, myodermal cells from a mouse embryo (MEC), and heart cells of a Cynomolgus monkey (CMH). The cultures were grown at 37° on cover slips in test tubes with nutrient medium of the following composition: 60 ml lactalbumin hydrolysate, 30 ml medium No. 199, 10 ml bovine serum with penicillin and streptomycin at the rate of 200 units each/ml. After formation of a monolayer, the cultures were washed with Hanks's solution and infected with myeloblastosis virus. The virus material, consisting of plasma from a leukemic chicken diluted 1 : 100 with nutrient medium without serum, was introduced into the tubes for 90 min at room temperature, and then poured off and replaced by nutrient medium of the previous composition.

Virus. Myeloblastosis virus (strain BAI) subcultured through day-old chicks of the Russian White breed in the Laboratory of Oncogenic Viruses was used [4]. Filtered plasma taken from a chick at the height of the disease and kept until required at −70° was used for infecting the cultures. As a virus control plasma of normal 30-day chicks was used.

Immune Sera. Immune chicken serum against myeloblastosis virus was obtained from chickens surviving infection with a large dose of virus [8], and from chickens aged 3-4 months which had received four intravenous injections of leukemic plasma, each of 1 ml, at intervals of one week. γ-Globulin was isolated from the serum by the method of L. B. Mekler and V. K. Naumova [2]. A rabbit serum against myeloblastosis virus was obtained by means of a cycle of intravenous injections of plasma from leukemic chickens into 6-month rabbits. The plasma was injected in a dose of 2 ml on four occasions at weekly intervals. A fluorescent ass serum against rabbit globulin was obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology.

The preparations were fixed in acetone at room temperature, but in some cases unfixed preparations were examined. They were stained by the direct or indirect Coons' method. The usual staining technique was used [5].

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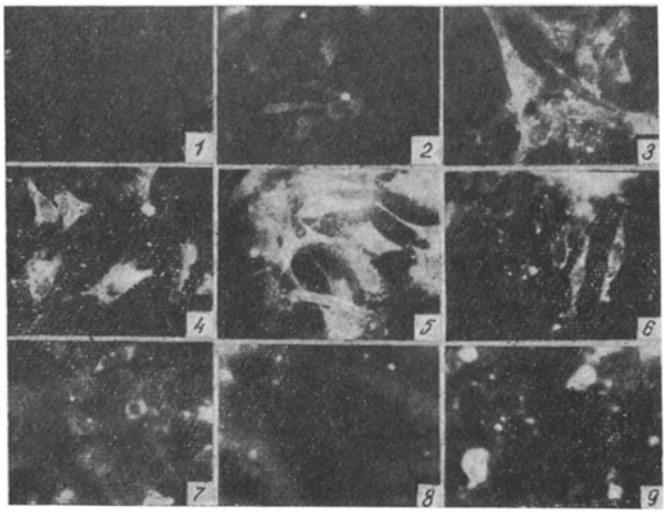

Fig. 1. Dynamics of accumulation of myeloblastosis virus antigen in culture of chick fibroblasts demonstrated by fluorescent antibody method. 1) Untreated control culture 24 h after beginning of experiment (absence of fluorescence); 2) control culture 24 h after treatment with healthy chicken plasma (outlines of cells can be seen, fluorescence absent); 3) experimental culture 24 h after infection with myeloblastosis virus (fluorescence of cytoplasm of individual cells; 4) experimental culture 48 h after infection (fluorescence of cytoplasm of most cells); 5) experimental culture 72 h after infection (fluorescence of cytoplasm of all cells of monolayer, nucleus remains dark); 6) experimental culture 96 h after infection (diminution of fluorescence of cell cytoplasm and fluorescence of extracellular material); 7) experimental culture 120 h after infection (appreciable decrease of fluoroescence). All preparations mentioned above were fixed in acetone at room temperature for 10 min; 8) unfixed control culture 72 h after treatment with plasma of healthy chickens (absence of specific fluorescence, cell outlines faintly visible); 9) unfixed experimental culture 72 h after infection with myeloblastosis virus (fluorescence of surface of infected cells).

Experiments on Chick Fibroblasts. Experiments were performed with two controls: an untreated culture and a culture treated with normal chicken plasma.

During the first 24 h after infection no fluorescence was seen in the experimental or control cultures. After 24 h clear fluorescence of the cell cytoplasm appeared in the experimental culture, without fluoroescence in the nuclei (Fig. 1, 1-3). Fluorescence was absent from the control preparations, and only faint outlines of the cells and slight autofluorescence could be seen (Fig. 1, 1 and 2). Fluorescence of the cytoplasm of individual cells was observed in the experimental preparation, and the dark nuclei could be clearly distinguished (Fig. 1, 3). On the following day the fluorescence in the experimental preparations intensified and by 48 h nearly all cells of the monolayer were brightly fluorescent (Fig. 1, 4). After 72 h all the cells were brightly fluorescent (Fig. 1, 5). Later the fluorescence of the infected cells weakened (Fig. 1, 6 and 7). In the control preparations no fluorescence was present throughout the period of investigation.

To determine the more precise localization of antigen in the cells, unfixed cultures were stained. In this way antigens located only on the surface of the cells were demonstrated (Fig. 1, 8 and 9).

The specificity of fluorescence in our experiments was confirmed by its blocking as a result of preliminary treatment of the preparations with nonfluorescent immune globulin to myeloblastosis virus.

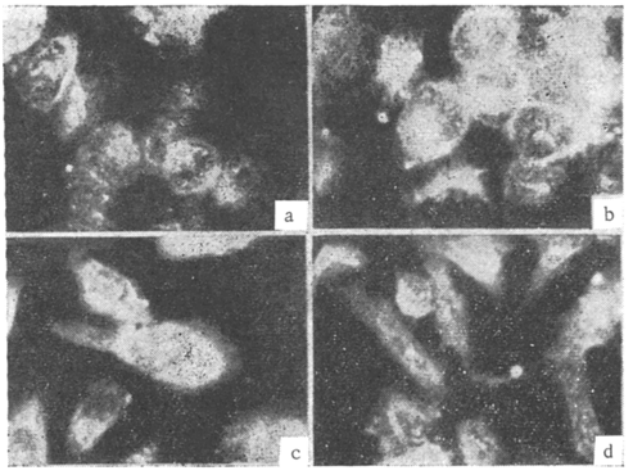

Fig. 2. Cultures of mammalian cells infected with myeloblastosis virus and stained with fluorescent chicken serum against this virus. a) Control CMH culture 4 h after treatment with plasma of healthy chickens (autofluorescence of cells, specific fluorescence absent); b) experimental CMH culture 4 h after infection (negligeable fluoroescence of cytoplasm of infected cells); c) control MEC culture 4 h after treatment with plasma of healthy chickens (autofluoroescence of cells); d) experimental MEC culture 4 h after infection (faint fluorescence of cell cytoplasm).

Experiments on Heterologous Cultures. Preparations were made 24, 48, and 96 h after infection. At no time was fluorescence observed. Cultures were then investigated earlier, and this showed that fluororescence appeared (Fig. 2, b and d) in both experimental cell cultures soon (2-4 h) after infection. By 18-24 h after infection it completely disappeared. Only autofluorescence was present in the control preparations (Fig. 2, a and c). The basic assumption made before investigation of the heterologous cell cultures was that mammalian cells can be infected with viruses of the fowl neoplasm complex [1, 3, 7].

According to available information [5], myeloblastosis virus, when infecting cultures of mammalian cells, cannot multiply in these cells and induce their conversion. By using a different test, the fluorescent antibody method, we found that myeloblastosis virus is adsorbed on the surface of myodermal fibroblasts of the mouse embryo and on Cynomolgus monkey heart cells, but does not multiply in them.

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