DETECTION OF SPECIFIC VIRUS ANTIGEN IN CELLS OF WISTAR RATS INFECTED WITH FOWL ERYTHROBLASTOSIS VIRUS

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A specific virus antigen has been found by the immunofluorescence method in tumors developing in Wistar rats infected at birth with fowl erythroblastosis virus. The antigen was found in tumor cells after repeated transplantation in rats and also after passage of the cells in vitro.

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Penetration of a virus into cells is not necessarily associated with the development of pathological signs. Its reproduction need not be accompanied by the development of disease. Notwithstanding this, specific virus antigens can be detected in the cells by means of immunological methods.

The object of this investigation was to detect antigen of fowl erythroblastosis virus in cells of Wistar rats infected with it. The presence of infection was judged by the results of an immunofluorescence test.

EXPERIMENTAL METHOD

Fowl erythroblastosis virus (strain R), adapted to hens of the Russian White breed [6], was used in the experiments. Newborn Wistar rats* were infected during the first days of life with plasma from birds with erythroblastosis in a dose of 0.2 ml subcutaneously or intraperitoneally. In some experiments repeated injections of virus in a dose of 0.3 ml were given to the animals on the next 1 or 2 days. Rats with signs of tumor growth were sacrificed. Tumor tissue, ascites cells, and tissue of the parenchymatous organs of the animals with tumors were used for immunofluorescence analysis with fluorescein isothiocyanate-labeled γ-globulin from the sera of hens immunized with erythroblastosis virus [3-5]. In addition, impressions from transplanted rat tumors and material from monolayer cultures obtained from tumor cells and ascites cells of rats infected with erythroblastosis virus were investigated. The tissue impressions and slides with monolayer cultures were fixed in a mixture of absolute ethanol (95 parts) with glacial acetic acid (5 parts).

EXPERIMENTAL RESULTS

Of the 74 rats infected in the neonatal period with erythroblastosis virus and surviving the period of early development, 37 developed tumors which were studied for the presence of antigen of avian erythroblastosis virus by the immunofluorescence method.

Fluorescence was detected in cells of primary tumors of Wistar rats and ascites cells of rats with tumors when stained with immune γ-globulin from the sera of hens immunized with avian erythroblastosis virus conjugated with fluorescein isothiocyanate, predominantly in the perinuclear zone (Fig. 1). No such fluorescence was present in control preparations from normal tissues of rats not infected with erythroblastosis virus. Fluorescence of the tumor cells and ascites cells of Wistar rats infected with erythroblastosis virus in the neonatal state was blocked by unlabeled sera obtained from hens immunized with leukemia

* The Wistar rats were obtained from the Nursery of the Academy of Medical Sciences of the USSR, where they were inbred.

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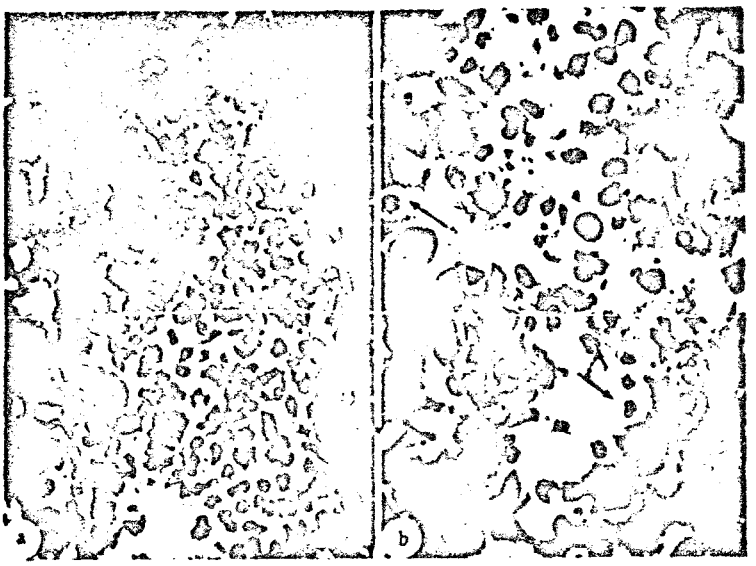

Fig. 1. Immunofluorescence of primary tumor of Wistar rat inoculated in the neonatal state with erythroblastosis virus. a) Normal rat spleen; b) tumor.

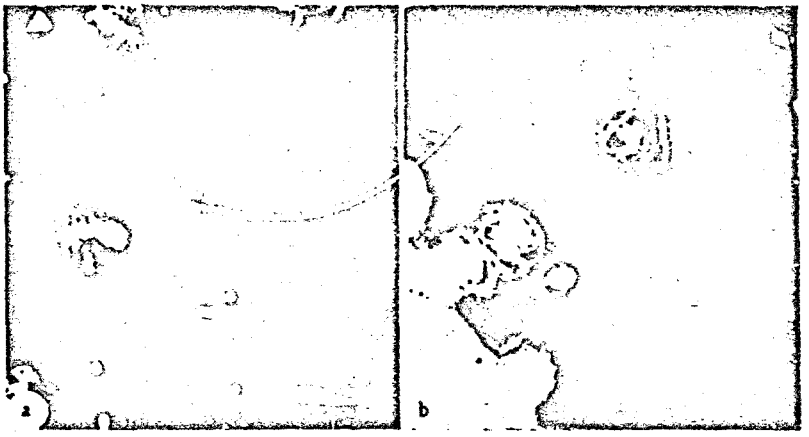

Fig. 2. Immunofluorescence of transplantable tumor of Wistar rat. a) Cells of normal mesenteric lymph gland of rat; b) cells of transplantable tumor from 12th successive passage.

virus. Unlabeled γ-globulin from normal hen sera did not block fluorescence; preliminary treatment with this γ-globulin did not suppress fluorescence of the preparations when stained with fluorescent γ-globulin from the serum of hens immunized with leukemia virus.

The fluorescence of tumor cells of rats infected with erythroblastosis virus was thus specific in character. The specificity of fluorescence was also confirmed in a control experiment with rat tumors of different origin. The tumors used were of rat strain P-KO 20^{100}, derived from a tumor in a CC57BR mouse [2], and bred in the laboratory. Tumor cells of this strain did not give fluorescence with fluorescein isothiocyanate-labeled γ-globulin from serum of hens immunized with erythroblastosis virus. An additional control was set up with another fowl virus – the virus of Newcastle disease. Treatment of preparations from tumors of Wistar rats with antiserum against Newcastle disease virus did not reduce the fluorescence on subsequent testing with labeled γ-globulin obtained from hen leukemia antiserum. Fluorescence could thus be blocked only by means of unlabeled leukemia antiserum.

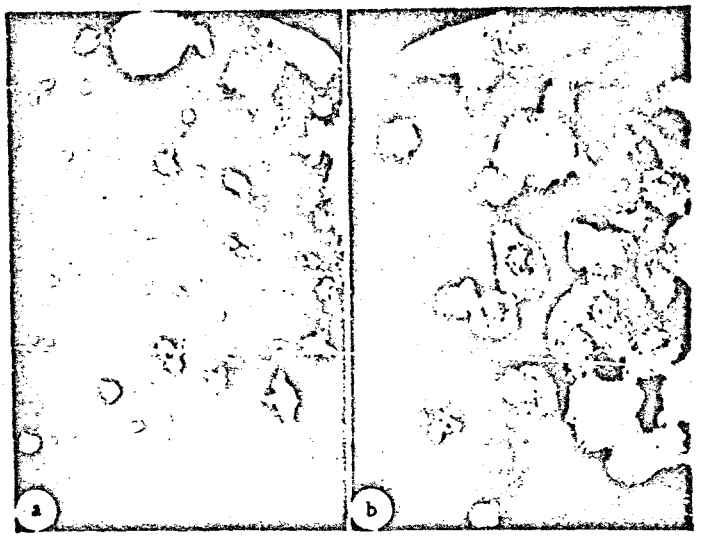

Fig. 3. Immunofluorescence of tumor cells of Wistar rat in tissue culture. a) Culture of hamster CASV 40 tumor cells; b) tumor cells from tissue culture.

Primary tumors developing in Wistar rats infected with avian erythroblastosis virus were subjected to repeated passage through newborn and young rats. Specific fluorescence was found in the transplanted tumors of both the first and suosequent passages when treated with labeled γ-globulin from leukemia antiserum. This fluorescence persisted even after 12 successive passages (Fig. 2). It also was blocked only by unlabeled γ-globulin from the sera of hens immunized with avian leukemia virus, and was not blocked by globulin from normal hen serum.

Ascites cells and tumor cells from Wistar rats were used to obtain monolayer cultures. These cultures, when tested by the direct Coons' method with labeled γ-globulin from leukemia antiserum gave bright fluorescence. This fluorescence was blocked by unlabeled leukemia antiserum (Fig. 3). No such fluorescence was present in cells of control monolayer cultures from the tissue of animals not infected with erythroblastosis virus and grown under the same conditions as the experimental cells. Fluorescence was absent in the cells of hamster tumor CASV 40 made available by V. I. Gavrilov. The hamster tumor was primarily induced by SV 40 virus [1].

The results of these experiments show that a specific virus antigen can be demonstrated by the use of labeled γ-globulin of immune hen sera in the cells of rats (infected in a neonatal state with avian erythroblastosis virus), in which tumors subsequently developed. This antigen persists for a long time in the tumor cells and is found after repeated passages of the tumors and during cultivation of ascites cells from the rats in tissue culture.

The detection of a specific virus antigen in the cells of rat tumors developing after infection of the animals in the earliest period of their postembryonic development with fowl erythroblastosis virus raises the question of the ability of erythroblastosis virus to infect these cells under certain conditions. It may be concluded from the results obtained that the cells of mammals and, in particular, the cells of Wistar rats possess receptors necessary for their infection with avian erythroblastosis virus.

Further investigations are being conducted to discover the etiological connection between erythroblastosis and tumors of Wistar rats infected with it.

LITERATURE CITED

1. V. I. Gavrilov, N. N. Vasil'eva, N. N. Dodonova, et al., Vopr. Virusol., No. 5, 583 (1963).
2. V. M. Zhdanov and V. A. Parnes, Byull. Éksp. Biol., No. 4, 82 (1963).
3. D. M. Levina and V. A. Parnes, In: Relationships between Virus and Cell [in Russian], Riga (1965), p. 60.

4. D. M. Levina and V. A. Parnes, In: Current Problems in Veterinary Virology [in Russian], Moscow (1965), p. 133.
5. V. A. Parnes and D. M. Levina, In: Diseases of Birds [in Russian], Leningrad (1960), p. 60.
6. V. A. Parnes and D. M. Levina, In: Current Problems in Veterinary Virology [in Russian], Moscow (1965), p. 132.