DEMONSTRATION OF LEUKEMIA-LIKE A AND B VIRUSES IN CELLS OF A STABLE CULTURE OF THE HEp-2 CELL LINE OF HUMAN LARYNGEAL CARCINOMA

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A virus capable of performing a productive cycle in tissue cultures of rabbit kidney cells and human embryonic cells, but with no cytopathological action and not inducing morphological transformation of the cells, was found in a transplantable culture of HEp-2 cells. The virus was found in the cytoplasm of the cells by the indirect fluorescent antibody method and by electron microscopy. The virus population consists of two types of viruses: A and B. Type A nucleoids (500-550 Å in diameter) are formed in the hyaloplasm of interphase and dividing cells. The outer membrane of the type A virion (diameter 850-950 Å) is formed from elements of the cytoplasmic membrane. The membrane of the type B virus nucleoid (diameter 400-450 Å) is formed from the membranous component of the endoplasmic reticulum. The outer membrane of the type B virion (diameter 900-950 Å) is formed from the cytoplasmic membrane. The diameter of the nucleoid of the type B virion is about 300 Å.

Viruses can be isolated from some primary tissue cultures obtained from organs of healthy animals. As a rule, transplantable cells do not contain contaminating viruses [1, 7, 8].

In the investigation described below an attempt was made to demonstrate viruses in a transplantable tissue culture of HEp-2 cells.

EXPERIMENTAL METHOD

A transplantable culture of HEp-2 cells [6], obtained from the D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, was maintained by growth in a nutrient medium consisting of 80% medium No. 199, 10% Hanks' solution with 0.5% lactalbumin hydrolysate, and 10% bovine serum.

Primary tissue cultures of adult rabbit kidneys and cells of 4-month human embryos were obtained from the corresponding tissues by trypsinization. The cell suspension was seeded into flasks in a concentration of 50,000 cells per ml in nutrient medium containing 70% medium No. 199, 10% Hanks' solution with 0.5% lactalbumin hydrolysate, and 20% bovine serum. After incubation for 7-10 days at 37°C, the percentage of serum in the medium used was reduced to ten.

The liquid phase after incubation of the HEp-2 culture was filtered through a G-4 glass filter and applied to a monolayer of rabbit kidney cells or human embryonic tissue. After exposure for 4 h at 37°C the nutrient medium was added and cultivation continued. Uninfected cultures of the same tissues were used as the control.

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Fig. 1

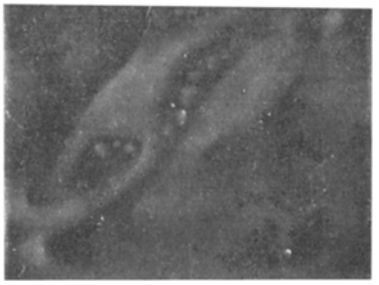

Fig. 2

Fig. 1. Fluorescence of the cytoplasm of rabbit kidney cells on the 30th day after infection in the indirect immunofluorescence reaction with rabbit antivirus serum, 90 ×.

Fig. 2. Control (uninfected) rabbit kidney cells in indirect immunofluorescence reaction with rabbit antivirus serum, 90 ×.

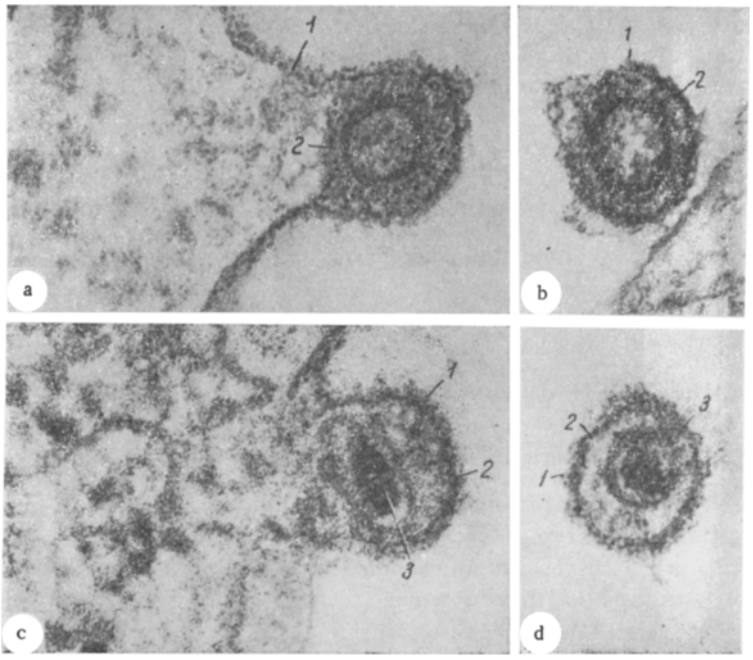

Fig. 3. Formed virus and extracellular virion of types A and B: a) formed type A virus; 1) cytoplasmic membrane; 2) type A nucleoid; b) extracellular type A virion; 1) virion membrane; 2) nucleoid; c) formed type B virus; 1) cytoplasmic membrane; 2) nucleoid membrane; d) extracellular type B virion; 1) virion membrane; 2) nucleoid membrane; 3) nucleoid; 375,000 ×.

Rabbits were immunized with residue of the liquid phase of the rabbit kidney cell culture by injection into a popliteal lymph gland on the 25th day after infection. The residue was obtained by centrifuging 150 ml of the liquid phase, filtered through a G-4 glass filter at 105,000 g for 1 h on a Spinco centrifuge. The material was injected along with Freund's adjuvant (1 : 1). The serum obtained on the 16th day after immunization and also the control (normal) rabbit serum were exhausted with uninfected human embryonic cells (5,000,000 cells to 0.1 ml serum, exposure 1 h at room temperature and 15 min at 37°C). Preparations of 30-day infected human embryonic cultures were fixed for 10 min with acetone and studied by the indirect fluorescent antibody method [3, 5]. Ass anti-rabbit γ-globulin, conjugated with fluorescein isothiocyanate (the commercial preparation supplied by the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR), treated by the method of Éngel'gardt and Abelev [4], was used. Uninfected human embryonic cells were used as the control.

On the 20th day of cultivation of the monolayer the tissue cultures were fixed for 5 min with 1% glutaraldehyde in phosphate buffer (pH 7.2) and then for 45 min with 1% OsO_4 in acetate–veronal buffer (pH 7.2). The subsequent treatment of the tissue culture for electron-microscopic examination was as described previously [2].

A disk of polymethacrylate, with the tissue culture embedded in it, was examined under the phase-contrast microscope and selected groups of cells or single cells were studied in the electron microscope. Ultrathin sections were cut on an LKB-4800 ultratome, shadowed for 2-3 h with a 2% aqueous solution of uranyl acetate and then with lead citrate by Venable's method [9], and investigated in the GEM-7 electron microscope giving an instrumental magnification of 5000, 30,000, and 50,000 times.

EXPERIMENTAL RESULTS

Observations on the infected rabbit kidney and human embryonic tissue cultures continued for 3 months. During this period the infected cells were indistinguishable morphologically from the controls.

Fluorescence of the cytoplasm of the infected human embryonic cells was found by the indirect fluorescent antibody method in the reaction with antiserum. The fluorescence was localized principally in the perinuclear zone and appeared as fine granules. Sometimes the antigen was found as large fluorescent deposits. In some cells the fluorescence was distributed throughout the cytoplasm (Fig. 1). No fluorescence of the nuclei was observed. In all 11 specimens studied, persistent fluorescence of antigen, located in the cytoplasm of the infected cells, was found. The antisera did not cause fluorescence of the control cells (Fig. 2). Normal rabbit serum did not induce fluorescence in the cytoplasm of infected human cells.

On electron-microscopic examination, viruses of types A and B were found in the culture of HEp-2 cells and also in the infected rabbit kidney and human embryonic cultures. No such viruses could be detected in the control cultures. Virus nucleoids of type A, 500-550 Å in diameter, were present in the cytoplasm of interphase and dividing cells. The nucleoids were formed in the hyaloplasm and then migrated toward the surface of the cell, formed buds, and were liberated into the extracellular medium (Fig. 3a). The outer membrane of the virion was formed from elements of the cytoplasmic membrane. The diameter of the extracellular type A virus was 850-950 Å (Fig. 3b).

The type B virions also were finally formed on the cell surface (Fig. 3c). The outer membrane of the virion was formed from elements of the cytoplasmic membrane, while the membrane of the nucleoid was evidently formed from the membranous component of the endoplasmic reticulum. The diameter of the extracellular virion was 900-950 Å, the diameter of the nucleoid membrane 400-450 Å, and the diameter of the nucleoid itself 300 Å (Fig. 3d). In its submicroscopic organization, the virus was similar to Bittner's virus. The virus was considerably larger in the cells of the primary cultures than in the cells of the HEp-2 culture. Type A virions were predominant in the human embryonic cells.

Virus was thus found in the cells of stable cell line HEp-2 of human laryngeal carcinoma. It can be postulated that the antiserum contained antivirus antibodies, although the presence of antibodies against various elements of the rabbit kidney cell in it cannot be ruled out. Fluorescence of the cytoplasm of the infected human embryonic cells evidently acts as a test for the virus. This is confirmed by the absence of fluorescence in uninfected human embryonic cells. Viruses of types A and B were revealed by electron microscopy in the original culture of HEp-2 cells and after subculture into rabbit kidney cells and human embryonic cells, and enabled all stages of formation of the virions to be examined.

The conditions for replication and maturation of virus are created in primary cultures of rabbit kidney cells and human embryonic cells. The productive cycle, culminating in liberation of the fully formed virion into the external medium, evidently does not lead to death of the cell. The virus probably does not induce transformation of the investigated cells. By persisting in the cells of primary cultures the virus accumulates in much larger quantities than in the original HEp-2 tissue. However, the conditions for performance of the productive cycle of the virus were different in the primary tissues, as shown by predominance of type A virions over type B virions in the human embryonic cells, which was not observed in the rabbit kidney cells.

It is difficult at present to draw any definite conclusions regarding the role of the virus in pathology. This problem may be solved after identification of the virus and the study of its properties.

LITERATURE CITED

1. O. G. Andzhaparidze, V. I. Gavrilov, B. F. Semenov, et al., Tissue Culture in Virological Research [in Russian], Moscow (1962).
2. A. F. Bykovskii, Vopr. Virusol., No. 4, 500 (1961).
3. É. M. Vardosanidze and I. S. Irlin, Vopr. Virusol., No. 5, 556 (1963).
4. N. V. Éngel'gardt and G. I. Abelev, Byull. Éksperim. Biol. i Med., No. 5, 94 (1962).
5. A. H. Coons, in: General Cytochemical Methods, Vol. 1, New York (1958), p. 399.
6. A. Moore, L. Sabachewsky, and E. Toolan, Cancer Res., 15, 598 (1955).
7. W. Rowe, R. Huebner, L. Gilmore, et al., Proc. Soc. Exp. Biol. (New York), 84, 570 (1953).
8. W. Rowe, I. Hartley, S. Waterman, et al., Proc. Soc. Exp. Biol. (New York), 92, 418 (1956).
9. J. Venable and R. Coggeshall, J. Cell. Biol., 25, 407 (1965).