EFFECT OF VIRUS SV 40 ON CULTURES OF GREEN GUENON LUNG CELLS

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After inoculation of a culture of green guenon lung cells with virus SV 40 chronic infection and transformation of the cells developed, and virus antigen and specific intranuclear tumor antigen were detected in them. Injection of the transformed cells into green guenons did not lead to tumor development.

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Virus SV 40 of monkeys, isolated in 1960 from a culture of kidney cells of Macaca rhesus monkeys [13], is a latent agent for these animals. Investigations [4, 5, 7, 9] have shown that large doses of virus (of the order of 10,000 TCD_{50}) cause the development of sarcomas in newborn hamsters and the African rodents of the genus Mastomys 4-9 months later at the site of injection. In cultures of embryonic cells of man, hamsters, and certain other animals, SV 40 leads to transformation [8, 10-12]. Hemoimplantation of even a small number of transformed hamster embryonic cells into adult animals was accompanied by growth of tumors. In a culture of green guenon kidney cells, highly sensitive to this virus, distinct cytopathic changes (formation of intranuclear DNA-containing inclusions, vacuolation of the cytoplasm) developed after infection, leading to death of the culture [2, 6, 12].

The object of the present investigation was to study morphological changes in a strain of green guenon lung (Russian abbreviation LZM) cells when infected with SV 40, because the use of tissue cultures can facilitate detection of the specific features of interaction between this virus and cells.

EXPERIMENTAL METHOD

Strain A-426 of virus SV 40 was used for the experiments. Cells of strain LZM were obtained from organs of young animals by G. I. Avgustinovich by trypsinization in the usual way, and the strain was cultivated on Eagle's medium, with 10% bovine serum. Cells were infected with virus at the 4th passage in suspension.

The method of the cytological and cytochemical investigations was published previously [2]. At different passages of the strain, the number of nucleoli, the number of normal and abnormal mitoses, the content of intranuclear inclusions, and the number of multinuclear cells were counted. The results of the counts were subjected to statistical analysis.

The direct method of fluorescent antibodies was also used to detect virus (with rabbit anti-SV 40 antiserum) and tumor antigens. In the latter case the sera of hamsters with tumors induced by injection of hamster embryonic cells transformed by SV 40 were used. These sera contain antibodies against tumor T-antigen but do not contain antivirus antibodies.

EXPERIMENTAL RESULTS

Strain LZM consisted of polygonal and fusiform cells with round, or less frequently, oval nuclei. Most cells ($94.1 \pm 1.6\%$) had 1-3 nucleoli. The nuclei were enlarged in 2-5% of cells. Giant cells with multiple or highly fragmented nuclei were absent. The mitotic activity in the formed monolayer was 4.5 ± 3 throughout the passages. Solitary abnormal mitoses were found but not in every preparation. No intranuclear inclusions were seen. Glycogen granules and lipid droplets were present in the cytoplasm. Neither virus nor tumor antigens were present.

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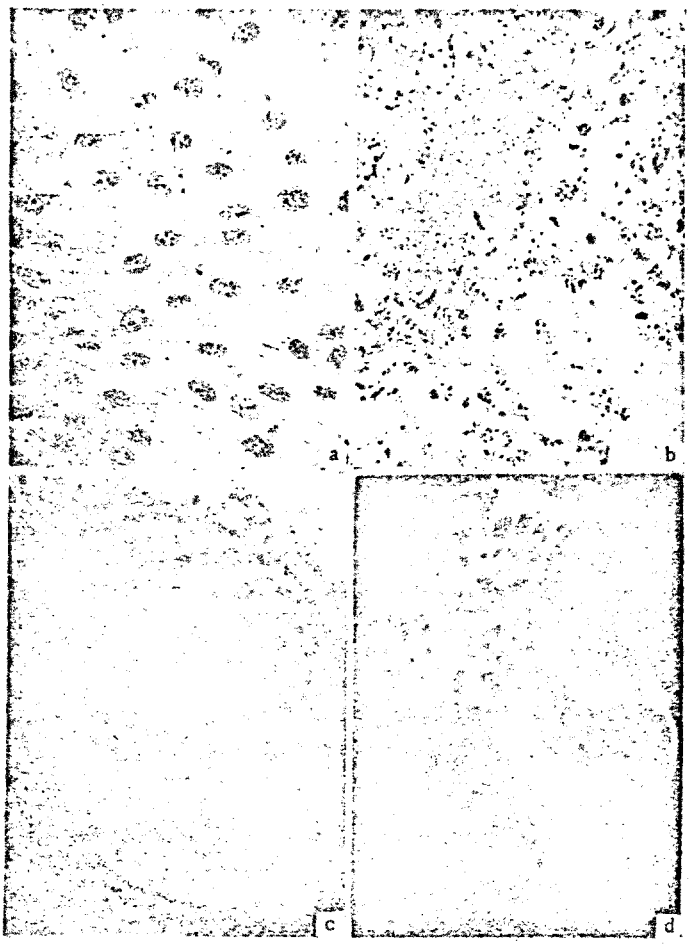

Fig. 1. Culture of green guenon lung cells. a) Control; b) transformed by SV 40; c) intranuclear inclusions in cells of transformed culture; d) fluorescence of cells of transformed culture after treatment with labeled globulin from anti-SV 40 rabbit serum. Hematoxylin-eosin. Direct method of fluorescent antibodies. 10× (a and b) and 90× (c and d).

After inoculation of the culture with SV 40 chronic infection developed. Infective virus was isolated at all passages. Oxyphilic intranuclear inclusions characteristic of SV 40 sections were formed in the cells (Fig. 1c). The inclusions contained DNA and protein and were surrounded by a translucent halo. During passages of the culture the number of inclusions increased: at the second passage after inoculation inclusions were present in $1 \pm 0.3\%$ of cells, and at the 4th-5th passage in $2.3 \pm 0.4\%$.

The number of nucleoli did not differ significantly from their number in the control, and it increased only slightly during subsequent passages: at the 2nd passage $96.9 \pm 1.4\%$, at the 4th-5th $97 \pm 0.9\%$, and at the 12th passage $88.4 \pm 3.2\%$ of cells contained 1-3 nucleoli. Only by the 12th passage, when transformation had developed, was there an increase in the number of cells with 5 or more nucleoli to $5.8 \pm 2.2\%$, compared with $0.9 \pm 0.6\%$ in the control. Massive vacuolation of the cytoplasm, characteristic of the cytopathogenic action of this virus, was not observed. The content of glycogen granules and lipids in the infected cultures was the same as in the controls.

In some cells (2.5%) virus antigen was discovered, localized mainly in the nuclei (Fig. 1d). In their shape, the fluorescent areas sometimes resembled intranuclear inclusions. Tumor antigen could be detected in the nuclei of some cells. Fluorescence was removed by preliminary treatment with specific native immune serum before introduction of the globulin–fluorochrome conjugate, and was absent in the control preparations. Fluorescence likewise was not observed in transplantable hog embryonic kidney cultures (HEK), infected with tick-borne encephalitis virus (Table 1).

TABLE 1. Immunofluorescence of Cultures Using Different Sera

Serum	Cultures		
	Control LZM	LZM infected with SV 40	HEK infected with tick-bone encephalitis virus
Labeled anti-SV 40	–	+	–
Native anti-SV 40	–	–	–
Labeled anti-SV 40			
Labeled serum of hamster with tumors*	–	+	–
Native serum of hamster with tumors	–	–	–
Labeled serum of hamster with tumors			
Native serum of hamster with tumors	–	+	–
Labeled anti-SV 40			
Native anti-SV 40	–	+	–
Labeled serum of hamster with tumors			
Labeled serum of control hamsters	–	–	–

Legend: +) specific fluorescence, –) absence of fluorescence.
*Tumors induced by injection of cultures of hamster embryonic cells transformed by SV 40.

During cultivation islands of transformation appeared. If the culture was kept without passage the islands grew in size and became monolayer in form. The transformed culture consisted of polymorphic cells of different shapes and sizes. About 11-15% were cells with greatly enlarged nuclei in which fragmentation was often observed. However, giant cells characteristic of cultures of hamster embryonic cells transformed by SV 40 were rare. Most cells contained from 1-3 nucleoli (88.4%).

Intranuclear inclusions were found in $0.2 \pm 0.07\%$ of cells in which signs of degeneration were often observed. The mitotic activity of the cultures at the stage of the formed monolayer was faily high, amounting to 48 ± 19 in different preparations. Anomalies of mitosis were found in $25 \pm 4\%$ of all dividing cells. Multipolar mitoses, anaphase and telophase bridges, and loss of chromosomes were observed. No significant differences in the glycogen and lipid content compared with the control were found.

Subcutaneous transplantation of transformed cells into guenons, performed by G. I. Avgustinovich, led to the formation of small nodules which rapidly underwent regression. In no case was transplantation followed by progressive growth and tumor formation.

Comparison of the results of this investigation with the changes induced by SV 40 in other tissue cultures from various animals and man shows that the results of interaction between cells and virus is dependent on other factors than species specificity and dose of agent. Organ specificity, influencing sensitivity of the cells to the virus, is also important.

Changes similar to those described above were observed after infection of a diploid culture of human embryonic cells and tissue cultures of cells of various animals with large doses of SV 40 [1, 3]. Hence, the process of transformation of cells in various cultures after infection with SV 40 follows different courses and does not always lead to malignant change.

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