PATTERN OF MITOSIS IN DKLCh-4 CULTURES INFECTED WITH STRAIN K OF HERPES SIMPLEX VIRUS

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Strain K of herpes simplex virus (second antigenic group) reproduces in DKLCh-4 diploid cells without cytopathogenic effect. Starting from 24 h, mitotic activity in the infected cultures is depressed, but the increase in number of pathological mitoses observed following infection by strains of the first antigenic group is not observed.

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Changes in mitotic activity of cell cultures infected with herpes simplex virus [3, 5-7] and the appearance of pathological mitoses in infected cultures, mostly colchicine-like metaphases [2, 5, 6] have been reported in the literature. A colchicine-like effect has also been observed in cultures of DKLCh-4 cells (a strain of diploid cells from human embryonic lung) infected with strains Tolstova, Berezina, and L_2 of herpes simplex virus, belonging to the first antigenic group [1, 4]. These strains were propagated in a selected system giving the characteristic cytopathogenic effect (appearance of intranuclear inclusions, cytoagglomeration, syncytium-formation, total cell destruction).

It was decided to study the mitotic pattern in DKLCh-4 cultures infected with a strain of herpes virus (K) belonging to the second antigenic group; viruses of this group multiply in cell cultures without exerting any cytopathogenic action on them [1, 4], but accumulation of virus in the culture fluid can be demonstrated by intracerebral injection into mice.

EXPERIMENTAL METHOD

Herpes simplex virus, strain K, belonging to the second antigenic group, was used in the experiments. For primary infection, cultures of human embryonic lung diploid cells (strain DKLCh-4), grown in penicillin flasks with cover slips and in Povitskaya's flasks on Eagle's medium for diploid cells were inoculated with a brain suspension from infected mice (1:10) in physiological saline. The multiplicity of infection was 0.2 LD_{50} per cell. Later the DKLCh-4 culture was infected with culture fluid of the preceding passage. The virus was titrated in mice weighing 7-8 g and the culture fluid injected intracerebrally. Statistical analysis of the results was carried out by the method of Reed and Muench. The cover slips with control and infected cultures growing on them were fixed in Shabadash's neutral mixture or Carnoy's fluid and stained with hematoxylin-eosin. Cytological changes and mitotic activity of the control and infected cultures were studied (in each case 5000-10,000 cells were counted), and the number of pathological mitoses and their predominant forms determined. Statistical analysis of the data was carried out by the Fisher – Student method.

EXPERIMENTAL RESULTS

No signs of cytopathogenic action could be found in DKLCh-4 cultures infected (primary inoculation and subcultures) with herpes simplex virus, strain K. Stained control and infected cultures were indistinguishable: they consisted of regularly arranged fibroblast-like cells with pale, oval nuclei, containing finely dispersed chromatin and 1-3 nucleoli.

Neither intranuclear inclusions, margination of the chromatin, rounding of the cells, cytoagglomeration, nor syncytium-formation was found in the infected cultures. Nevertheless, the results of titration in

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mice (intracerebral inoculation) showed that strain K of herpes virus accumulated in the culture fluid in considerable amounts ($10^{3.8}$-$10^{4.5}$ LD_{50}/ml). The mitotic index of the infected cultures 12 h after infection (third subculture) was $13.6 \pm 1.1^0/_{00}$, and the mitotic activity of the control cultures $12.8 \pm 0.9^0/_{00}$; 18 h after infection the corresponding values were 15.1 ± 0.6 and $14.6 \pm 0.8^0/_{00}$. In neither case was the difference statistically significant ($P > 0.1$). The mitotic activity of the infected cultures 20 h after inoculation was slightly below the control level (12.3 ± 1.2 and $14.2 \pm 0.8^0/_{00}$, respectively; $P > 0.1$). A highly significant decrease in mitotic activity compared with the control was observed 24 h after inoculation with the virus (6.2 ± 0.7 and $14.01 \pm 1.2^0/_{00}$; $P < 0.001$). At all subsequent times of observation (48, 72, 96, and 120 h) the mitotic activity of the infected DKLCh-4 cultures was much below that of the controls. Starting from 72 h, no mitoses whatsoever were found in several specimens of cultures infected with strain K.

At no time of observation was a significant increase in the number of pathological mitoses found in the infected cultures (4% of pathological mitoses in the infected cultures and 3% in the controls after 18 h; 2% of pathological mitoses among dividing cells in the infected cultures and in the control after 24 h). Hardly any colchicine-like metaphases were found. No signs of blocking of cell division at the metaphase stage likewise were discovered: the ratio between the phases of mitosis $\left(\frac{\text{prophase}+\text{metaphase}}{\text{anaphase}+\text{telophase}}\right)$ between 12 and 14 h after infection varied from 3 : 1 to 4 : 1.

The results must be compared with those of observations of changes in the mitotic pattern in DKLCh-4 cultures infected with herpes virus strains belonging to the first antigenic group (Tolstova, Verezina, and L_2). These strains 12 and 18 h after inoculation caused a statistically significant increase in mitotic activity, and only after this was the mitotic index of the infected cultures lowered. In DKLCh-4 cultures infected with strain K, no significant changes in mitotic activity were observed in the early periods, and from 24 h onward it was depressed. Also when strains of the first antigenic group were used, a sharp rise in the number of pathological mitoses was observed (mainly colchicine-like metaphases), whereas in cultures infected with strain K the number of pathological mitoses was just as small as in the control cultures. The differences observed evidently reflect differences between strains of herpes simplex virus described previously [1, 4]. The appearance of pathological mitoses in cultures infected with strains of the first antigenic group is probably one of the earliest signs of the cytopathogenic action of these strains on the chosen cell system. Both the chromosomes themselves (marked shortening and deformation are observed) and the apparatus of division (scattering of chromosomes in the cytoplasm of cells held back in metaphase) are affected in these cases. These changes precede the apperance of the typical signs of herpetic infection in vitro such as the formation of intranuclear inclusions, margination of the chromatin, rounding of the cells, and so on. To judge from the patterns observed, reproduction of strain K in DKLCh-4 cultures was not accompanied by damage to the chromosomes or to the achromatin apparatus of division. The problem of a link between the cytopathogenic effect and the disturbance of the mitotic cycle in cell cultures infected by different strains of herpes simplex virus belonging to different antigenic groups requires further study.

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