Examination of Virus-Infected Cultured Cells by Scanning Electron Microscopy

CATHERINE ARANYI, JAMES FENTERS, AND VIVIAN TOLKACZ
Life Sciences Division, IIT Research Institute, Chicago, Illinois 60616

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The scanning electron microscope (SEM) was used to detect changes in morphology of BSC-1 cells after infection with vesicular stomatitis virus (VSV) or herpes simplex virus. The morphological changes of the infected cells were related to the length of time of infection and to the virus used. Extensive alteration to the cytoplasm could be seen 24 and 48 hr after infection with 10 and 320 TCID₅₀ of VSV. Within 24 hr after infection with 1 TCID₅₀ of herpes simplex, a few nuclei were swollen. However, 72 hr after infection with 100 TCID₅₀ of herpesvirus, many nuclei were swollen and appeared in large aggregates, probably representing formation of a polykaryocyte. Corresponding samples stained with May-Grunwald-Giemsa were observed in the light microscope and morphological changes were compared to those seen with the SEM.

The scanning electron microscope (SEM) has been increasingly used in different areas of biological research (1–10, 12, 13). The instrument combines the advantages of viewing larger areas of the specimen as in the light microscope, with a magnification range (×50 to ×50,000) and resolving power (200 Å) approaching that of the transmission electron microscope. Because of its depth of focus, images corresponding to three-dimensional visual experience are produced by the instrument. The ease of specimen preparation is an additional significant advantage. Most samples can be examined directly with minimal pretreatment, thus avoiding possible artifacts resulting from the more complex preparation techniques. The image of the sample appears on the screen of a cathode ray tube and can be photographed with a Polaroid camera.

These features of the scanning electron microscope permit a rapid and thorough examination of cell surfaces (3, 13). Visualization of detail and changes in surface structures not attained in the light microscope make it possible to study normal cultured cells and those undergoing pathological changes. This is a report on morphological changes observed in viral-infected tissue culture examined under the SEM and light microscope.

MATERIALS AND METHODS

Tissue culture. The African green monkey kidney cell line, BSC-1, used in these studies was obtained from Industrial Biological Laboratories, Rockville, Md., and from Hope Hopps of the Division of Biologies Standards, Bethesda, Md. The cells were grown on basal medium Eagle (BME) with either Hanks or Earles balanced salt solution (Microbiological Associates, Bethesda, Md.), supplemented with 10% calf serum and 50 units of polymyxin per ml and 100 μg of neomycin sulfate per ml. The maintenance medium was the same as the growth medium, the only exception being that 2% calf serum was used. Cells were grown on coverslips placed in 2 oz. (ca. 60 ml) prescription bottles.

Viruses. The vesicular stomatitis virus (VSV) was obtained from Abbott Laboratories, North Chicago, Ill., and the herpes simplex virus, strain Mayo 1814, was obtained from the Research Reference Reagents Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Md. Both viruses had undergone an undetermined number of passages in BSC-1 cells before use in this study.

Infectivity titrations. The dose of virus employed in the study was determined by simultaneous infectivity titrations. Serial 10-fold dilutions of virus were made in BME, and 0.1 ml of each dilution was inoculated into each of two tissue culture tubes of BSC-1 cells containing 1 ml of maintenance medium. The tubes were incubated at 37 C, and the cells were read for cytopathology at the same time cover slip preparations were being fixed or stained. The infective dose of the virus was calculated by the method of Reed and Muench (11) and was expressed as a 50% tissue culture infectivity dose (TCID₅₀).

Tissue preparation. BSC-1 cells were prepared for examination 24 and 48 hr after infection with VSV and 24 and 72 hr after infection with herpesvirus. Corresponding uninfected BSC-1 cells were prepared simultaneously. After decanting the maintenance medium, the cover slips containing the cells were rapidly rinsed with distilled water and fixed in 3% glutaraldehyde in
sodium phosphate buffer (pH 7.3) for 2 hr at room temperature. The cells were then quickly washed with distilled water and air dried. The cover slips were attached to metal specimen holders of the scanning electron microscope with a mixture of Permount and silver conductive paint. To assure surface conductivity, the samples were coated with a 300-A gold layer by high vacuum evaporation by using a rotating stage. The samples were examined in a model JSM-2 (Japan Electron Optics Laboratory Co., Ltd.)

**FIG. 1.** Scanning electron micrographs of BSC-1 cells 24 hr after virus infection. (A) Control, (B) VSV-infected, (C) herpes-infected.
scanning electron microscope using a beam accelerating voltage of 25 kv and a tilt angle of 40°. Identical cell culture samples on cover slips were fixed for 5 min in absolute methanol, stained with May-Grunwald-Giemsa, and examined in a light microscope.

RESULTS AND DISCUSSION

After 24 hr of infection with VSV, the cells showed a 5% or less cytopathogenic effect (CPE) as indicated by rounding of cells. The infectivity
FIG. 4. Light micrographs of stained (May-Grunwald-Giemsa) BSC-1 cells. Controls: (A) 24 hr, (D) 48 hr, and (F) 72 hr. Infected with VSV: (B) 24 hr and (E) 48 hr. Infected with herpesvirus: (C) 24 hr and (G) 72 hr.
titrations indicated a virus dose of 10 TCID₅₀. The unusual field depth resulting in a three-dimensional-like appearance in scanning electron micrographs is evident in Fig. 1. Uninfected controls for the 24-hr period (Fig. 1A) showed monolayered BSC-1 cells with the nucleus and nucleoli standing out clearly above the plane of the cytoplasm. In comparison, the VSV-infected cells showed alterations in the cytoplasm and the nuclei had a tendency to round up and appear more discrete (Fig. 1B).

The effect of herpesvirus on BSC-1 cells 24 hr after infection is shown in Fig. 1C. No CPE was noted in the cells on cover slips 24 hr after infection although simultaneous infectivity titrations indicated a TCID₅₀ of 1. However, at the same time-period, some morphological changes could be seen under the SEM. A few of the nuclei were swollen and damaged, although most cells in the surrounding area appeared normal.

In Fig. 2, the effect of VSV is demonstrated 48 hr after infection. At this time, the VSV had a TCID₅₀ of 320. The cells, prior to fixation, were viewed with the light microscope and showed 75 to 80% CPE with considerable sloughing. Prominent extension-like processes appeared in the 48-hr uninfected cell control (Fig. 2A). In infected cells (Fig. 2B), the damage to the cytoplasm was somewhat more extensive than in the 24-hr infected cells.

Cell monolayers on the cover slips infected with 100 TCID₅₀ of the herpesvirus showed 50% CPE, and the cytological damage caused by the virus was much more extensive after 72 hr, as shown in Fig. 3B. The aggregated nuclei probably represented formation of a polykaryocyte. The corresponding cell control is shown in Fig. 3A.

Photographs of the stained cells viewed in the light microscope are shown in Fig. 4. Twenty-four hours after infection with VSV (Fig. 4B), no apparent effects on the cells were seen when compared with the uninfected control (Fig. 4A). However, after 48 hr of infection (Fig. 4E), cytoplasmic changes were apparent in comparison with the control cells (Fig. 4D).

Light micrographs of the stained cells 24 hr after infection with herpesvirus (Fig. 4C) show slight nuclear changes when compared to the cell control (Fig. 4A), whereas nuclear as well as cytoplasmic changes were noted 72 hr after infection (Fig. 4G). The 72-hr cell control is shown in Fig. 4F.

This study showed that morphological changes in tissue culture caused by viral infection can be determined with the SEM 24 hr after infection. However, when corresponding samples were examined under the light microscope, CPE was not apparent with herpes infection and minimal CPE was noted with the VSV infection. Additional studies should be performed to determine the minimum time after viral infection that morphological changes may be observed.

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