ELECTRONMICROSCOPICAL CHARACTERISATION OF SIMIAN PAPOVAVIRUS SA12

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PLATES XXXVI AND XXXVII

THE isolation of SA12, a papova-like virus, from uninoculated kidney-cell cultures derived from the vervet monkey (*Cercopithecus aethiops pygerythrus*) was reported by Malherbe, Harwin and Ulrich (1963). Nuclear changes in the infected cells resembled those produced by SV40, but the basophilic inclusion material was more clearly defined and cytoplasmic vacuolation was not prominent. SA12 grew more slowly than SV40 in vervet monkey-kidney cultures, and was not neutralised by SV40 antiserum. It has since been shown to be serologically distinct from SV40 and the human papovaviruses (Shah, personal communication).

More recently, Rangan *et al.* (1974) and Reissig *et al.* (1976) isolated a papovavirus from uninoculated, stump-tailed-macaque kidney cultures. This virus, too, is serologically distinct from the human papovaviruses and SV40 and, in addition, possesses an outer envelope. This communication describes the ultrastructural features of SA12 in primary cultures of vervet monkey-kidney cells and compares them with those of other papovaviruses.

MATERIALS AND METHODS

SA12 virus was grown on monolayer cultures of primary vervet monkey-kidney cells in Hanks' medium supplemented with 1% foetal calf serum. Cells were harvested when cytopathic changes were prominent, usually after 8 days. A portion of the cell suspension was pelleted by low-speed centrifugation, fixed in 3% glutaraldehyde, washed in phosphate-buffered saline, and post-fixed in 1% osmium tetroxide; after dehydration in ethanol, the cells were embedded in Epon 812 and thin sections were cut and double-stained with uranyl acetate and lead citrate for examination in a Philips EM300 electronmicroscope (EM). The remainder of the infected cells were pelleted as before and disrupted by rinsing and then suspending in 0.5-ml volumes of distilled water. A drop of the material was then mixed with one drop of 3% phosphotungstic acid, pH 6, and layered on carbon-coated formvar grids; excess fluid was removed and, after being allowed to dry, the grids were examined in the EM. Particle diameters were measured on photographic negatives with a Nikon 6C comparator.

RESULTS AND DISCUSSION

From observations on the ultrastructural development of various animal and human papovaviruses we believe that they produce significantly different cytopathology in the nucleus of the infected cell, but that the cytoplasmic changes are similar for most if not all of them. Malherbe *et al.* (1963) noted differences in the nuclei of cells infected with SV40 and SA12 by light microscopy. In the present EM studies, infection with SA12 did not give rise to intranuclear virus crystals, although these have been observed in cells infected with SV40 (Grandboulan *et al.*, 1963), with mouse polyoma virus (Mattern, Takemoto and Daniel, 1966), and with the human papovaviruses, JC (ZuRhein, 1969), BK (Lecatsas, Prozesky and

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Scheepers, 1974) and MG (Lecatsas, Schoub and Prozesky, 1976). Tubular forms of SA12 virus were also absent although these have been reported for most other papovaviruses. On the other hand, certain cytopathological features were produced by SA12, SV40 and the human papovaviruses, namely, electron-dense clumps of chromatin in the nucleus (figs. 1 and 2), the loss of the nuclear envelope after the appearance of virus particles in the cytoplasm (fig. 2) and the intimate association of the virus particles with the endoplasmic reticulum (fig. 3). Liberation of the virus from the cell occurred after alignment of closely packed particles in one or more layers on the cytoplasmic side of the cell membrane, which then ruptured to release clusters of particles (fig. 4). Structures composed of whorled membranes that could have lysosomal functions were commonly found in the cytoplasm (fig. 2). Negative-contrast studies showed SA12 particles to be typical papovaviruses, with a diameter of approximately 43 nm (fig. 5).

Enveloped virus particles, as reported recently for the stumptailed macaque virus, were not found in our preparations of SA12; consequently SA12, in its cytopathology, bears a stronger resemblance to SV40 and the human papovaviruses than to the macaque virus. Cytopathology can serve as a valuable means of gauging the relationship of viruses to each other, and fewer and finer differences in the cell pathology suggest closer evolutionary affinities. Mattern *et al.* (1966) stressed the remarkable affinity of mouse polyoma virus for various cell membranes. The strong attachment of viruses such as SA12 and SV40 to cellular membranes, particularly the cell membrane, suggests that papovaviruses, including the human agents, are membranotropic and that this may play some role in the pathogenic effects noted in demyelinating diseases such as progressive multifocal leucoencephalopathy (PML) in which JC and SV40-like viruses are thought to play an aetiological role. A similar situation exists for the macaque monkey, in which spontaneous PML-like lesions have been reported, containing polyoma-like virus particles (Gribble *et al.*, 1975).

SUMMARY

Ultrastructural studies of simian virus SA12, growing in primary cultures of vervet monkey-kidney cells, have demonstrated a typical papovavirus capsid morphology and a diameter of 43 nm. The cytopathic nuclear characteristics, with the absence of virus crystals and elongated capsid forms, distinguish it from SV40 and the human papovaviruses. Like other papovaviruses, SA12 shows a strong affinity for cellular membranes.

REFERENCES

- GRANDBOULAN, N., TOURNIER, P., WICKER, R. AND BERNHARD, W. 1963. An electron microscopic study of the development of SV40 virus. *J. Cell. Biol.*, **17**, 423.
- GRIBBLE, D. H., HADEN, C. C., SCHWARTZ, L. W. AND HENRICKSON, R. V. 1975. Spontaneous progressive multifocal leucoencephalopathy (PML) in macaques. *Nature, Lond.*, **254**, 602.
- LECATSAS, G., PROZESKY, O. W. AND SCHEEPERS, F. 1974. The cytopathology and development of a human polyoma virus (B.K.). *Arch. ges. Virusforsch.*, **45**, 319.
- LECATSAS, G., SCHOUB, B. D. AND PROZESKY, O. W. 1976. Development of a new human polyoma virus strain (MG). *Archs Virol.*, **51**, 327.
- MALHERBE, H., HARWIN, R. AND ULRICH, M. 1963. The cytopathic effects of vervet monkey viruses. *S. Afr. med. J.*, **37**, 407.
- MATTERN, C. F. T., TAKEMOTO, K. K. AND DANIEL, W. A. 1966. Replication of polyoma virus in mouse embryo cells: electron microscopic observations. *Virology*, **30**, 242.
- RANGAN, S. R. S., LOWRIE, R. C., ROBERTS, J. A., JOHNSTON, P. B. AND WARRICK, R. P. 1974. Virus from stumptailed monkey (*Macaca arctoides*) kidney cultures. *Lab. Anim. Sci.*, **24**, 211.
- REISSIG, M., KELLY, T. J., DANIEL, R. W., RANGAN, S. R. S. AND SHAH, K. V. 1976. Identification of the stumptailed macaque virus as a new papovavirus. *Infect. Immun.*, **14**, 225.
- ZU RHEIN, G. M. 1969. Association of papova-virions with a human demyelinating disease (progressive multifocal leucoencephalopathy). *Prog. med. Virol.*, **11**, 185.

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FIG. 1.—Primary culture of vervet monkey-kidney cells infected with SA12, showing dispersed virus particles (V) and characteristic electron-dense chromatin patches in the nuclei (N). Electron-micrograph (EM). $\times 25\,000$.

FIG. 2.—SA12 virus particles (V) in the nucleus and cytoplasmic matrix of an infected cell. Note the absence of the nuclear envelope and the presence of a central vacuole, containing virus particles, and dense chromatin patches. Typical whorled-membrane structures (L) occur in the cytoplasm. EM. $\times 30\,000$.

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FIG. 3.—Infected cell showing intimate association of SA12 virus particles with cytoplasmic membranes. The cell membrane (C) is visible. EM. $\times 75\ 000$.

FIG. 4.—Virus particles being released from the cell. Note the invariable arrangement of the particles along the cell surface in one or more layers. The arrow indicates the cell membrane immediately below the virus particles on the cell surface. EM. $\times 75\ 000$.

FIG. 5.—SA12 virus particles showing typical papovavirus morphology and a diameter of 43 nm. Negatively stained. EM. $\times 200\ 000$.