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INVESTIGATIONS ON THE STRUCTURE AND MORPHOGENESIS OF SOME ARBOVIRUSES ISOLATED IN THE U.S.S.R.

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Summary. — The morphology and some stages of morphogenesis in suckling mouse brains of hitherto serologically unclassified viruses Kaspiy (LEIV-A-63), Zavashan (LEIV-Ap-6158), Artashat (LEIV-A-2366), and Paramushir (LEIV-C-2268), isolated in the U.S.S.R., of GM-710 virus isolated in Scotland, and of Sokuluk (LEIV-K-400) virus belonging to the genus *Flavivirus* (family *Togaviridae*) were studied. Virion sizes were determined and changes in infected cells described. Based on their structure and morphogenesis, the viruses Kaspiy, Artashat, Zavashan and Paramushir were referred to the family *Bunyaviridae* and GM-710 virus was referred to the genus *Orbivirus* (family *Reoviridae*).

Key words: Bunyaviridae; *Reoviridae*; *Togaviridae*; *electron microscopy*

Introduction

Electron microscopy may substantially contribute to the identification and classification of viruses especially when serological methods have failed in this respect. In the U.S.S.R., a number of arboviruses were isolated but until the onset of the present investigations they could not be classified serologically. We investigated, therefore, the morphology and some late stages of morphogenesis of the viruses Kaspiy, Artashat, Zavashan, Paramushir and Sokuluk; GM-710 virus isolated in Scotland was also studied.

Materials and Methods

Viruses. Virus Kaspiy was isolated in 1975 from argasid ticks collected in nesting colonies of sea birds on the western and eastern shores of the Kaspian Sea (Lvov *et al.*, 1975). Virus Zavashan was isolated from ixodid ticks in 1976 near the village Zavashan, Artashat region of the Armenian S.S.R. Virus Artashat was isolated in 1972 from argasid ticks and *Larus argentatus* blood in the Artashat region of the Armenian S.S.R. (Lvov *et al.*, 1975). Paramushir virus was isolated from ixodid ticks in 1969—1974 in nesting sites of sea birds on islands in the north-eastern part of the Pacific Ocean basin (Lvov *et al.*, 1976*a*). Sokuluk virus was isolated from bats in the Sokuluk region of the Kirghiz S.S.R. in 1970 (Lvov *et al.*, 1973*b*). GM-710 virus, isolated in Scot-

land, was sent for identification to the Ivanovsky Institute of Virology by Dr. P. Brès, WHO, Geneva.

Electron microscopy. Suckling mice were inoculated intracerebrally with 10% brain suspensions of the individual viruses, whose titres (log LD_{50}/0.01 ml) were 9 (Paramushir), 8 (Zavashan), 6 (Kaspiy), 4.5 (Artashat) and 8 (Sokuluk). GM-710 virus was not titrated. After the appearance of clear-cut clinical signs of brain involvement, pieces of brain cortex, cerebellum and medulla oblongata were dissected for electron microscope examination. These pieces of 1—2 mm^3 were fixed for 2 hr in 2% glutaraldehyde in phosphate buffer pH 7.3, post-fixed for 1 hr in 1% OsO_4 solution in phosphate buffer pH 7.3, and embedded into Araldite. Sections were cut on a Reichert ultramicrotome and stained for 20 min with a 10% uranyl acetate solution in methanol and for 30 min with lead citrate. The preparations were examined in a JEM-100 B electron microscope.

Results

Kaspiy, Paramushir, Artashat and Zavashan viruses

In studying the virion morphology and morphogenesis of these viruses, we found signs of infection in the brain cortex, cerebellum and medulla oblongata of the suckling mice. The infected cells showed vacuolation of the cytoplasm, enlargement of the vesicles and cisternae of the Golgi apparatus, enlargement of the cisternae of the endoplasmatic reticulum, destruction of mitochondria and, occasionally, destruction of nuclear and plasmatic membranes. Local areas of membranes and enlarged cisternae and vesicles of the Golgi apparatus revealed higher electron opacity than other cell membranes. Occasionally we observed virus particles budding into the enlarged cisternae and vesicles.

Single virions or their aggregates (3—5 in the section thickness) occurred both within vacuoles, cisternae and vesicles of the cytoplasm and intercellularly (Figs 1—5). In sections, the virions appeared rounded or oval. The diameter of the former was 95 $\pm$ 5 nm. The virions possessed an envelope consisting of an osmiophobic and two osmiophilic layers. The envelope was about 15 nm thick. The inner core of the virions displayed low or moderate electron opacity. Electron-dense structures of 8—10 nm diameter occurred in the core of some virions. Most frequently these structures were adjacent to the virion envelope (see Figs 1—5).

Sokuluk virus

In suckling mice infected with Sokuluk virus, the most marked morphological changes were observed in cells of the brain cortex and medulla oblongata. In the cytoplasm of infected cells there occurred accumulations of small vacuoles, virus precursors of 25—30 nm diameter and Sokuluk virions. The cell organelles were at various stages of degeneration and occasionally the plasma membrane was destroyed.

The vacuoles usually occurred in the perinuclear area of the cytoplasm and accumulated in foci; as compared with other cell membranes, their membranes were more electron opaque (Fig. 6). In some cells we found budding particles of Sokuluk virus; virus precursors participated in their formation. Budding of virus particles was only observed on membranes of vacuoles.

In cell sections, Sokuluk virions 42—45 nm in diameter usually had a rounded shape. The virions possessed a core 30—32 nm in diameter, separated by a narrow light rim from the outer envelope. The latter was about 5 nm thick and its surface was not smooth, appearing as effaced (Fig. 7).

GM-710 virus

Examination of ultrathin sections of suckling mouse brains infected with GM-710 virus showed the infection being accompanied by the formation in the cytoplasm of characteristic virus "factories", vacuolation of the cytoplasm, enlarged width of the channels and cisternae of the smooth endoplasmic reticulum and destruction of cytomembranes.

The viral "factories" were usually located in the perinuclear area of the cytoplasm; their shape was irregular and they were not limited by a membrane. The "factories" most frequently consisted of a finely granular matrix, viral particles and filamentous threads (Fig. 8). The amounts of finely granular matrix and virus particles in the "factories" varied, but the less finely granular matrix, the more virus particles in the "factories" and vice versa.

The filamentous threads 16—18 nm thick were situated parallelly to each other and in our sections were up to 1.6 μm long (Fig. 9). The morphology of virus particles localized in the "factories" varied. Some had the shape of virions, i. e. of virus particles with a core, the others were empty particles, i.e. they contained no core. Virions and "empty" particles frequently occurred in crystalline arrays (from 20 to 30 in the section layer). The GM-710 virions had 60—65 nm in diameter; they contained a highly electron-opaque core 30—35 nm in diameter surrounded by a light zone; the outer envelope of the virions was about 14 nm thick. The "empty" particles had the same outer diameter as the virions. The diameter of the inner space was 40—42 nm. As distinct from the virions, the "empty" particles occurred only among the finely granular matrix. No morphological changes were visible in the nuclei of affected cells.

Discussion

Reproduction of GM-710 virus in suckling mouse brain cells was associated with the formation in the cytoplasm of characteristic "factories" which contained finely granular matrix, filamentous threads, virions and "empty" virus particles. Formation of such "factories" is characteristic of infections caused by viruses belonging to the genus *Orbivirus* of the family *Reoviridae* (Gushchina *et al.*, 1977).

The role of the matrix and of the filamentous threads in the morphogenesis of orbiviruses has not yet been elucidated. Cromack *et al.* (1971) assume that they represent a substrate, including proteins used in the formation of virus particles.

According to Fields *et al.* (1971), the "empty" virus particles represent structures free of nucleic acid. We observed that the "empty" virus particles

were localized only among the finely granular matrix. We assume, therefore, that they are formed from proteins occurring in the finely granular matrix and represent virus particles at the first stage of virion formation.

The virions of GM-710 virus had 60—65 nm in diameter, contained an electron-dense core 30—35 nm in diameter, separated from the 14 nm thick outer envelope by a narrow light zone. A comparison of these results with reported data (Verwoerd *et al.*, 1979) allows the conclusion that GM-710 virus belongs to the genus *Orbivirus* (family *Reoviridae*).

According to our recent serological investigations, GM-710 virus represents a new strain of Ochotskiy virus. The latter was isolated in 1971 and referred to the antigenic group Kemerovo of the genus *Orbivirus* (Lvov *et al.*, 1973*a*).

Our results concerning the structure and some stages of morphogenesis of Sokuluk virus allow the conclusion that it belongs to the genus *Flavivirus* because it can be differentiated from the genus *Alphavirus* based on several characteristic symptoms. This concerns especially the fact that the vacuoles in Sokuluk virus-infected cells were smaller and less numerous than in alphavirus infections. In addition, the vacuoles observed in Sokuluk virus infections were distributed in foci in the cytoplasm of infected cells. Another characteristic feature was the diameter of Sokuluk virions of 42—45 nm, which is somewhat less than in members of the genus *Alphavirus* (Porterfield *et al.*, 1978). The focal accumulation of small vacuoles probably represents a "factory" on the membranes of which precursors of Sokuluk virus are built. In our experiments we occasionally observed budding virus particles, virus precursors having participated in their assembly. Budding of the virus particles occurred only on the membranes of vacuoles.

In the final stage of infection the vacuoles were filled with virions which were arranged into paracrystalline aggregates. According to Matsumura *et al.* (1971), the latter can migrate to the cell surface. Our observations thus confirmed the data by Lvov *et al.* (1973*b*) that Sokuluk virus is a member of the genus *Flavivirus* (fam. *Togaviridae*).

The unclassified viruses Kaspiy, Artashat, Paramushir and Zavashan had virions 95 ± 5 nm in diameter with a lipoprotein envelope about 15 nm thick. In cell sections, this lipoprotein envelope consisted of an osmiophobic and two osmiophilic layers. According to Korolev *et al.* (1976) and Reingold *et al.* (1976) the electron-dense structures 8—10 nm in diameter, occurring in the inner space of the virions, represented cross-sections of helical ribonucleoprotein. As to the size and shape of virions, Kaspiy, Artashat, Paramushir and Zavashan viruses do not differ from other members of the family *Bunyaviridae* (Murphy *et al.*, 1973; Obijeski and Murphy, 1977).

Virions of the viruses examined, like in other bunyaviruses, were formed by budding from membranes of cisternae and vesicles in the region of the Golgi apparatus (Porterfield *et al.*, 1976; Lvov *et al.*, 1976*a*, *b*; Chastel *et al.*, 1979).

A comparison of our data concerning Artashat, Kaspiy, Paramushir and Zavashan viruses with reports in the literature allows the conclusion that they can be referred to the family *Bunyaviridae*.

It should be mentioned that the use of electron microscopy in the identification of unknown viruses is especially important with new bunyaviruses. This family namely contains a great number of viruses which are antigenically unrelated to each other. Serological relationships exist only between members of a given virus group. Consequently an identification by serological methods of a newly isolated virus which shows no antigenic relationship to known groups appears impossible. This was the case with Kaspiy, Artashat and Zavashan viruses which are serologically unrelated to each other and cannot be included into any known antigenic group of bunyaviruses.

References

Cromack, A. S., Blue, J. L., and Gratzek, J. B. (1971): A quantitative ultrastructural study of the development of bluetongue virus in Madin-Darby bovine kidney cells. *J. gen. Virol.* **13**, 229—244.

Chastel, C., Rogues, X., and Beaucournu, J. C. (1979): Morphology and morphogenesis of Soldado virus (Hughes group); similarities with viruses of the *Bunyaviridae* family. *Arch. Virol.* **60**, 153—159.

Fields, B. N., Raine, C. S., and Baum, S. G. (1971): Temperature-sensitive mutants of reovirus type 3: defects in viral maturation as studied by immunofluorescence and electron microscopy. *Virology* **43**, 569—578.

Gushchina, E. A., Tsilinsky, Ya. Ya., Usmanov, R. K., Gushchin, B. V., Lvov, D. K., and Klimenko, S. M. (1977): Study on the morphological aspects of Ochotskiy virus replication in chick fibroblast culture (in Russian). *Vop. Virus.* **22**, 87—91.

Korolev, M. B., Donets, M. A., Rubin, S. G., and Chumacov M. P. (1976): Morphology and morphogenesis of Crimean hemorrhagic fever virus. *Arch. Virol.* **50**, 169—172.

Lvov, D. K., Timopheeva, H. L., Gromashevsky, V. L., Tsyrkin, Yu. M., and Veselovskaya, O. V. (1973*a*): Ochotskiy' virus, a new arbovirus of the Kemerovo group isolated from *Ixodes* (*Ceratixodes*) *putus* Pick-Camb. 1978 in the Far East. Arch. *Virusforsch.* **3**, 160—164.

Lvov, D. K., Tsyrkin, Yu. M., Karas, F. R., Timopheev, E. M., Gromashevski, V. L., Veselovskaya O. V., Osipova, N. Z., Fomina, K. B., and Grebenyuk, Yu. I. (1973*b*): "Sokuluk"-virus, a new group B arbovirus isolated from *Vespertilio pipistrellus* Schreber, 1775, bat in the Kirghiz S.S.R. *Arch. Virusforsch.* **41**, 170—174.

Lvov, D. K., Timofeeva, A. A., Gromashevsky, V. L., Karas, F. R., Skvortsova, T. M. *et al.* (1975): New arboviruses isolated in the U.S.S.R. in 1969—74 (in Russian), pp. 322—324. In *Voprosy med. Virusologii, Tezisy Konf. Inst. Poliomielita i virusnykh Entsefalitov*, Moscow.

Lvov, D. K., Sasonov, V. L., Gromashevsky, V. L., and Sovortsova, T. M. (1976*a*): "Paramushir"-virus, a new group arbovirus, isolated from ixodid ticks in nesting sites of birds on Pacific Ocean basin. *Arch. Virol.* **51**, 157—161.

Lvov, D. K., Sidorova, G. A., Gromashevsky, V. L., and Kurbanov, M. B. (1976*b*): Virus-"Tamdy" — a new arbovirus, isolated in the Uzbek S.S.R. and Turkmen S.S.R. from ticks *Hyalomma asiaticum* Shulze et Schottke, 1929, and Hyalomma plumbeum plumbeum Panzer, 1796. *Arch. Virol.* **51**, 15—21.

Matsumura, T., Stolar, V., and Schlesinger, R. M. (1971): Studies on the nature of dengue viruses. V. Structure and development of dengue virus in Vero cells. *Virology* **46**, 344—355.

Murphy, F. A., Harrison, A. K., and Whitfeld, S. G. (1973): *Bunyaviridae*: Morphologic and morphogenetic similarities of Bunyamwera serologic supergroup viruses and several other arthropod-borne viruses. *Intervirology* **1**, 297—316.

Obijeski, J. F., and Murphy, F. A. (1977): *Bunyaviridae*: Recent biochemical developments. *J. gen. Virol.* **37**, 1—14.

Porterfield, J. S., Casals, J., Chumakov, M. P., Gaidamovich, S. Ya., Hannoun, C., Holmes, I. H., Horzinek, M. C., Mussgay, M., Oker-Blom, N., and Russel, P. K. (1976): Bunyaviruses and *Bunyaviridae*. *Intervirology* **6**, 13—24.

Porterfield, J. S., Casals, J., Chumakov, M. P., and Gaidamovich, S. Y. (1978): *Togaviridae*. *Intervirology* **9**, 129—148.

Reingold, V. N., Shestopalova, N. M., Smirnova, S. E., Averina, S. M., and Chumakov, M. P. (1976): Intranuclear localization, morphology and morphogenesis of Khazara virus (in Russian), pp. 71—73. In *Tezisy Dokladov* **10** *vsesoyuznoi Konf. po elektronnoi Mikroskopii*, Tashkent.
Verwoerd, D. W., Huismans, H., and Erasmus, B. J. (1979): Orbiviruses, pp. 285—345. In H. Fraenkel-Conrat and R. R. Wagner (Eds): *Comprehensive Virology* vol. 14, Plenum Press, New York and London.

Explanations of Electron Micrographs (Plates XXIII—XXV):

Figs 1—5. Ultrathin sections of the suckling mouse brain cortex.
1 — Zavashan virus virions accumulated intercellularly. × 60 000.
2 — Kaspiy virus virions accumulated intercellularly. × 90 000.
3 — Part of the cytoplasm of a Paramushir-virus infected cell. × 45 000.
4 — Paramushir virus virions in enlarged vesicles of the Golgi apparatus. × 90 000.
5 — Artashat virus virions localized apparently in enlarged vesicles. × 90 000.
Figs 6 and *7*. Ultrathin sections of the medulla oblongata from suckling mice infected with Sokuluk virus.
6 — Accumulation in the perinuclear area of small vacuoles. × 45 000.
7 — Sokuluk virus virions within vacuoles. × 90 000.
Figs 8 and 9. Ultrathin sections of GM-710 virus-infected suckling mouse brains.
8 — Accumulation of parallelly situated filamentous threads. × 45 000.
9 — Cytoplasmic "factory" containing finely granular matrix, aggregated virus particles and filamentous threads. × 90.000.