Archiv für die gesamte Virusforschung 29, 105—108 (1970)

Morphology of Transmissible Gastroenteritis Virus of Pigs

A Possible Member of Coronaviruses

Brief Report

By

M. TAJIMA

Nippon Institute for Biological Science, Akebonocho, Tachikawa, Tokyo, Japan

With 3 Figures

Received June 12, 1969

A definite position of transmissible gastroenteritis (TGE) virus of pigs in the viral classification has not yet been established. The present author studied, with WITTE and EASTERDAY (15), the morphology and development of TGE virus by electron-microscopic techniques of negative staining and thin sectioning. They suggested that TGE virus was similar, in size, shape, and possible mode of replication to the myxoviruses and some oncogenic viruses. However, neither surface projections nor internal helical structure characteristic of the myxovirus could be recognized in their preparations. Recently, OKANIWA *et al.* (11) demonstrated the presence of surface projections on TGE virus, and mentioned that most particles in samples partially purified by the sucrose density gradient centrifugation method have lost their projections. They pointed out the similarity between TGE virus and the myxovirus because of the same size range of both viruses, and the presence of surface projections and of an envelope.

The results described here suggest, together with other properties previously reported, that TGE virus would be placed in the group of coronaviruses. In the present study, considering the fragility of surface projections of TGE virus in the purification procedure, the negative staining method was applied directly to infected culture fluid which contained about $10^{6.5}$ $TCID_{50}$ of virus per ml. Primary cultures of pig kidney cells were infected with TGE virus, TO strain, at a multiplicity of about 10 infective units per cell. After 2 days, the culture fluid was collected and clarified by low speed centrifugation. A small amount of the supernatant was placed on a carbon-Formvar coated grid, and excess fluid was removed with filter paper. The grid was washed with 1% ammonium acetate to remove residual salts. One drop of 2% phosphotungstic acid adjusted to pH 6.2 with normal potassium hydroxide was then applied for negative staining. Most of the samples were examined immediately after harvesting the culture fluid, but some of them, frozen and stored at −20° C for about one month, were subsequently thawed and processed as described above. A JEM-6S electron microscope was used.

Moderate numbers of virus particles were observed in preparations made from the infected culture fluid. They were distributed over the grid surface singly or in small groups (Figs. 1—3). The particles were pleomorphic and variable in size but were predominantly circular in outline with diameters between 100 and 150 mμ, including surface projections. The surface of most particles seen in the specimens prepared immediately after harvesting the culture fluid was covered with distinctive projections approximately 24 mμ long (Figs. 1 and 2). The projections were various in shape but were mostly petal-shaped in outline, and attached to the particle by a very narrow stalk. The widest part measured approximately 10 mμ

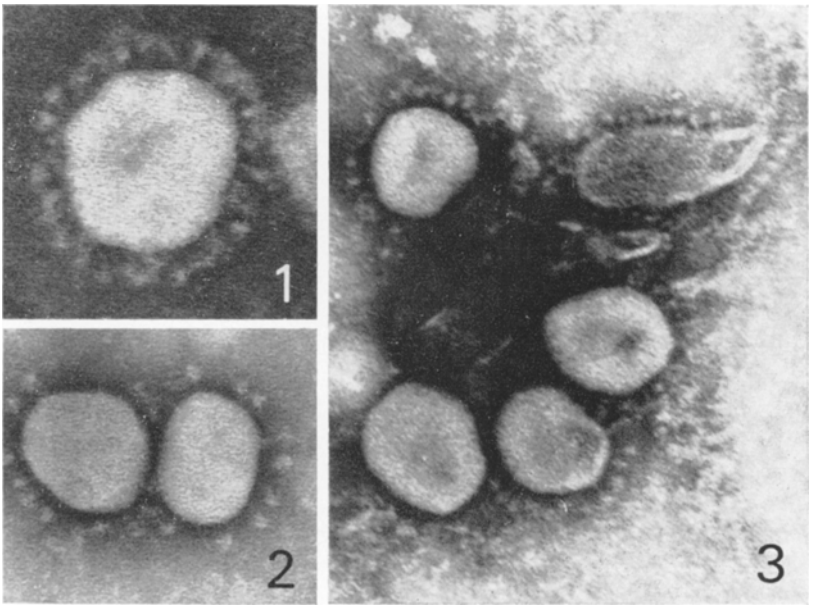

Fig. 1. A single particle of transmissible gastroenteritis virus of pigs. It appears to be intact and is covered with petal-shaped surface projections. × 210,000

Fig. 2. Two particles of transmissible gastroenteritis virus of pigs. They are sparsely covered with the projections, suggesting that some of the projections have been lost. × 150,000.

Fig. 3. A group of virus particles some of which are partly surrounded by projections. One particle in the upper right corner has been partially disrupted and exhibits an envelope. × 150,000

in diameter. The projections seemed to be easily detached from the virus and they were often observed only on a limited area of the particle surface. In the specimens prepared from the culture fluid stored frozen at −20°C for about one month, approximately one half of the particles showed no projections (Fig. 3). As a result of some penetration of phosphotungstate to the interior, an electron-opaque central area and an envelope approximately 10 mμ in thickness were observed in some of the particles (Fig. 3). Spontaneous disruption of the particles was sometimes seen. Even in such disrupted particles, no distinct internal component could be recognized.

The morphology of TGE virus described here agrees well with that of previous authors (11, 15) except that WITTE *et al.* (15) could not demonstrate surface pro-

jections. The projections which give a characteristic appearance to TGE virus seem to be readily lost or damaged and it is probable that the particles observed by WITTE *et al.* (15) lost their projections during the process of purification. It is interesting to note that the particles of TGE virus are morphologically indistinguishable from those of the coronaviruses which have recently been proposed as a new group of viruses (1). TGE virus is ether-labile (5, 7, 8, 12—14), contains ribonucleic acid (no inhibition by deoxyribonucleic acid inhibitors) (7, 15), and replicates in the cytoplasm by a process of budding from membranes of the endoplasmic reticulum and vesicles (15). These properties of TGE virus are in agreement with those of the coronaviruses (1—4, 6, 9, 10), and provide further support for the assumption that it belongs to the coronaviruses.

Acknowledgements

I wish to thank Dr. H. Kuwahara for supplying cell cultures. I also would like to thank Professor B. C. Easterday, Department of Veterinary Science, University of Wisconsin, for his help with this manuscript.

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Author's address: Dr. M. TAJIMA, Nippon Institute for Biological Science, Akebonocho, Tachikawa, Tokyo, Japan.