NOTES

Equine Infectious Anemia Virus from Infected Horse Serum

HIDEO NAKAJIMA, TOMOO YOSHINO, AND CHUZO USHIMI

Equine Infectious Anemia Research Division, National Institute of Animal Health, Kodaira, Tokyo 187, Japan

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Equine infectious anemia virus was purified from infected horse serum samples. Electron microscope observation on negatively stained preparations of purified virus showed roughly spherical particles sized between 100 and 200 nm in diameter. In disrupted particles, an envelope was visible but no internal structure could be resolved. Since the purified virus fraction had a strong antigenic activity to antisera in immunodiffusion reaction, these particles are thought to be the causative virus of equine infectious anemia.

Equine infectious anemia (EIA) is a viral disease of horses characterized by a lifelong persistent infection, intermittent fever, anemia, and progressive weakness. Histopathologically, proliferative lymphoid cells are generally observed in the reticuloendothelial system. The virus does not infect any experimental animals other than Equidae nor propagate in cultivated cell lines in vitro, with a few exceptions.

The etiological agent originated from EIA-infected horse materials has been demonstrated by electron microscopy (1, 2, 8, 9), but no reliable results have been obtained, mainly because of difficulties in preparing purified virus (7).

In this investigation, we tried to purify EIA virus from infected horse serum, and we observed the purified fraction under electron microscope. Immunodiffusion reaction was mainly carried out for quantitative determination of EIA virus because titration of virus infectivity in infected materials is quite difficult.

Serum samples were obtained from six horses experimentally infected with EIA virus at the first febrile stage of disease. Before the virus was used for purification study, virus antigenicity was examined on all the samples by immunodiffusion reaction. For the determination of virus antigenicity, EIA virus was precipitated from the serum at 100,000 × g for 180 min. Most of the virus was recovered in sediments by this procedure (7). The sediment was suspended to 1/100 the original volume in phosphate-buffered saline containing 0.1% Tween 80 and shaken for 10 min with 2 volumes of diethylether as previously described (6). The resultant liquid phase was diluted twofold with phosphate-buffered saline, and its antigenicity was titrated. Procedures for immunodiffusion have been described (5). Results indicated that only one sample from horse 583 showed strong antigenicity (1:4) and another showed weak antigenicity (1:1); the other four samples did not show antigenic activity. In the current investigation, therefore, serum obtained from horse 583 was mainly used for purification study.

After several examinations (4, 7, 10), EIA virus was purified in almost the same manner as described previously (3). Briefly, the virus was sedimented by centrifugation at 100,000 × g for 180 min from 500 ml of the serum and suspended in a small amount of 0.01 M phosphate buffer (pH 7.4). The suspension was loaded onto a diethylaminoethyl-cellulose column, and EIA virus was eluted with a 1.0 M NaCl solution after removal of contaminating proteins with a 0.15 M NaCl solution. The eluate was concentrated and centrifuged in a cesium chloride solution at 40,000 rpm for 22 h in an SW50.1 rotor of the Beckman model L2 ultracentrifuge. As the virus banded at around a density of 1.15 g/ml, three fractions (0.75 ml) near the density were collected as the purified sample. The antigenicity of the sample was 1:16 in immunodiffusion reaction. The experiment was repeated and the same result was obtained.

After the purified material was dialyzed against 1% ammonium acetate and stained with 2% phosphotungstic acid, the preparation was observed under electron microscope. There were many particles which were roughly spherical with diameters between 100 and 200 nm (Fig. 1), although some of them showed considerable pleomorphism. They seemed to be readily dam-
aged and spontaneous disruption was frequently seen. Particles consisted of internal structures and an envelope partly covered with fine surface projections. The internal structural details could not be made out morphologically. Some particles had a "tail" (Fig. 1b), which is often observed in ribonucleic acid tumor viruses.

The particles described above were considered to be the etiological virus of EIA since they were found in the fraction with strong antigenic activity that is specific for EIA virus (5), and they were morphologically similar to those prepared from infected horse leukocyte cultures (3).

LITERATURE CITED


