NOTES
Classification of Mouse Thymic Virus as a Herpesvirus

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Classical herpesvirus particles were observed by thin-section electron microscopy in thymus tissues from mice inoculated with mouse thymic virus. Intranuclear particles measured approximately 100 nm in diameter, and cytoplasmic and extracellular particles measured 135 nm in diameter. The morphology of mouse thymic virus particles together with its properties of heat and ether lability suggest that thymic virus should be classified as a member of the herpesvirus group.

Rowe and Capps (9) isolated and described a new mouse virus which produces an acute massive necrosis of the medulla and cortex of the thymus. The necrosis is greatest at approximately 14 days, after which repair processes and repopulation with thymocytes occur, and by 4 to 6 weeks the normal architecture of the thymus is restored. Whereas thymic necrosis is characteristic of the infection, the salivary glands become chronically infected and excrete virus into the saliva for more than 200 days after infection (unpublished data). We have repeated and confirmed the original observations of Rowe and Capps (9) on the physical properties of heat and ether sensitivity and have observed intranuclear inclusions by cytochemical stains and fluorescent-antibody techniques (unpublished data). In addition, necrosis and pathological changes of the thymus induced by the eighth passage virus used in this study were identical to that originally described by Rowe and Capps (9).

In the original report (9), Banfield observed intranuclear and intracytoplasmic particles, but no further attempts were made to identify or classify the particle. This was primarily the result of a number of technical problems which made it difficult to obtain exact information on the virus. Solutions to many of these problems now have been accomplished and have, to some degree, enabled the pursuit of the present study. This report describes some aspects of the morphology of the virus particle which suggests that mouse thymic virus should be classified as a herpesvirus.

The thymic virus (strain B) used in this study was the eighth passage in suckling mice from the original isolate (9). This virus pool contained 250 mouse infectious doses per ml. Virus was passaged in neonatal mice less than 24 hr of age by intraperitoneal inoculation with 0.05 ml of a 10% organ extract prepared from the livers, spleens, kidneys, and thymuses of infected mice. Virus suspensions were prepared in Eagle basal medium which contained 20% veal infusion broth and antibiotics. It was necessary to use thymic tissue extracts for the source of virus since no cell culture systems have been found which support the growth of thymic virus. The thymic virus pool was tested by one or more of the following procedures for possible contamination by other murine viruses: mouse antibody production (MAP) (3), inoculation and passage in several different cell cultures of murine origin, complement fixation, or animal inoculation. No evidence was found for contamination by any of the following viruses: mouse cytomegalovirus, lactic dehydrogenase, mouse hepatitis, mouse adenovirus, lymphocytic choriomeningitis, reovirus type 3, Sendai, pneumonia virus of mice, mouse pneumonitis (K), Theiler's encephalomyelitis, polyoma, ectromelia, minute virus of mice, or mouse leukemia.

Near term pregnant Swiss-Webster mice were obtained from either Microbiological Associates, Inc., Bethesda, Md., or from the National Laboratory Animal Co., Creve Coeur, Mo. These two colonies have been repeatedly tested and are consistently negative for evi-
dence of natural infection by both mouse salivary gland virus and mouse thymic virus. Mice were housed in filter top cages and were fed apples and sterilized Charles River Prefortified Rat-Mouse Formula (Chesapeake Feed Co., Beltsville, Md.) diet.

For electron microscopy, mice were sacrificed 5 to 8 days after inoculation. The 5th and 8th days were selected since virus reaches its highest titer in the thymus after 7 days, and thymic necrosis does not reach its maximum until day 10 (9; unpublished data). The thymuses were removed, and the majority of cortex was trimmed away leaving principally the medullary portion of the thymus for sectioning. Tissues were fixed overnight in 2% glutaraldehyde in cacodylate buffer (pH 7.4) and then stored in cacodylate buffer (pH 7.4) until processed. Dalton’s chrome-osmium (7) was used as a postfixative followed by dehydration in methyl alcohol and propylene oxide and embedded in Epon-Araldite. Thin sections were mounted on uncoated grids and stained with lead citrate and uranyl acetate before examination with an Hitachi II E-1 electron microscope. A portion of each thymus taken for electron microscopy was sectioned and stained with May-Grünwald-Giemsa (Baker Histology Lab., Great Falls, Va.) and examined in the light microscope for pathognomonic lesions and necrosis induced by thymic virus. In addition, thymuses of three mice from each litter selected for electron microscopy were titrated to confirm the presence of infectious thymic virus. An unsuccessful attempt was made to obtain virus particles from sonically treated clarified thymic tissue extracts for characterization of capsid symmetry by negative-staining techniques.

Virus particles with a morphology and morphogenesis comparable to that described for viruses of the herpesvirus group (5, 8, 12, 13) were seen in thin sections of thymus lymphoid cells taken from mice 5 days after infection with thymic virus (Fig. 1 to 3). The herpesvirus particles were observed only in mice inoculated with thymic virus and were observed in inoculated mice from each of the two mouse colonies used in this study. Intracellular particles approximately 100 nm in diameter were limited by a single membrane and often had a complete nucleoid. The cytoplasmic and extracellular particles had a rough-surfaced outer coat measuring approximately 135 nm in diameter (Fig. 1, 2). The nucleoid of particles in all locations was often a ribbon-shaped, oblong structure measuring approximately 74 by 45 nm (Fig. 1, 2). There was little evidence of margination of nuclear chromatin in any of the infected cells. There were striking accumulations of intranuclear filaments approximately 10 nm in diameter seen in cells with and without evidence of herpes-type particles (Fig. 3). These intranuclear filamentous structures were also observed in the original report on the thymic agent of mice (9). The same type of filamentous structures were occasionally seen in the cytoplasm. Periodic striations which are characteristic of the nucleocapsids of myxoviruses were not observed along the long axis of these tubular structures and there was no evidence of myxovirus type of budding along the cell surfaces even in those cells with a few intracytoplasmic tubules. Therefore, we consider these tubular structures to be associated with the presence of herpes-type virus particles rather than indicating a possible dual infection of herpes and myxovirus. The association of filamentous structures of various morphological configuration, both intranuclear and cytoplasmic, with herpesvirus infection is not unusual (1, 4, 6, 11); however, the role of the filamentous structures in herpesvirus replication is not known.

In addition to mouse thymic virus, there is one other naturally occurring herpesvirus in mice, mouse cytomegalovirus (MCV) (2, 10, 12), that has a morphology which might be indistinguishable from mouse thymic virus. Therefore, it was important to be certain that our animals were infected only with the thymic virus, and that MCV was neither present as a contaminant in the inocula or present as a latent infection in the mice used in the study. No evidence for MCV contamination of the thymic virus seed pool could be detected by the MAP (3) test, mouse embryo cell-culture inoculation, complement fixation, indirect fluorescent-antibody tests, or newborn mouse inoculation. Likewise, no evidence for natural infection of the two mouse breeder colonies used in this study could be found by inoculation and two passages of extracts of salivary glands in newborn mice.

Thus, the morphology of thymic virus particles described in this report together with its properties of heat and ether lability described by Rowe and Capps (9) and confirmed by the present authors suggest that it should be placed in the herpesvirus group (14); however, definitive classification must await studies on capsid symmetry.

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FIG. 1. Intranuclear capsids and filaments. ×57,500.

FIG. 2. Intracytoplasmic capsids. ×32,500. Insert: An extracellular viral particle. ×82,500.

FIG. 3. Striking accumulation of intranuclear filaments in cell with intracytoplasmic capsids at lower left.
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