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Early ependymal changes in experimental hydrocephalus after mumps virus inoculation in hamsters*

Tomoyuki Takano, Yoshiko Mekata, Tsunekazu Yamano, and Morimi Shimada

Department of Pediatrics, Shiga University of Medical Science, Setatsukinowa-cho, Ohtsu 520-21, Japan

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Summary. To elucidate the pathogenesis of early ventricular dilatation in hydrocephalus, we examined early morphological changes in ependymal layers at the lateral ventricles in suckling hamsters without aqueductal stenosis 5 days after the intracerebral inoculation of mumps virus. Mumps virus antigen was detectable in all ependymal cells. The ependymal cilia had almost disappeared and only the microvilli remained. A number of supra-ependymal cells were also observed on the surface of the lateral ventricles. Transmission electron microscopy revealed intracytoplasmic viral-like inclusions in the infected ependymal cells. These results suggest that functional and morphological disturbances in infected ependymal cells may cause early ventricular dilatation before aqueductal stenosis occurs.

Key words: Mumps virus – Hydrocephalus – Aqueductal stenosis – Ependyma – Cilia

Recent clinical studies have implicated mumps or influenza virus as a possible cause of aqueductal stenosis and hydrocephalus [1, 2, 4, 10, 11, 13, 14], but the pathogenesis of hydrocephalus is often obscure. Experimental studies have demonstrated that myxoviruses, such as the mumps and influenza viruses, induce hydrocephalus in suckling mice, rats and hamsters [5–7] and that narrowing of the aqueduct plays an important role in the development of hydrocephalus. Although aqueductal stenosis is considered to be the main cause of severe hydrocephalus, pathogenesis of the mild dilatation of the lateral ventricles that occurs before the aqueductal stenosis appears is controversial [8, 9, 12]. To elucidate the pathogenesis of early ventricular dilatation, we examined the early morphological changes in the ependymal layers in the lateral ventricles of hamsters following experimentally induced mumps virus infection.

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Correspondence to: T. Takano (address see above)

Materials and methods

The strain of mumps virus used in this experiment was isolated from the saliva of a patient with parotitis. Virus infectivity is expressed as the reciprocal of the dilution that causes infection in 50 % of cultures (TCD_{50}).

Twenty-four 2-day-old Syrian hamsters received intracerebral inoculation of 10 µl of 2 TCD_{50} of mumps virus with a 27-gauge needle. The control group consisted of sixteen 2-day-old Syrian hamsters that were injected with the same amount of minimum essential medium in the same manner. Five days after inoculation, animals were anesthetized by ether or pentobarbital and perfused with saline solution. Brains were processed for morphological examination of the ependymal layer of the lateral ventricles.

For routine histological examination, brains were removed and fixed in phosphate-buffered 10 % formalin and embedded in paraffin. Serial 5-µm coronal sections were stained with hematoxylin and eosin (H&E). Tissues were prepared for immunohistochemical study by perfusion with 4 % paraformaldehyde, 0.3 % glutaraldehyde (GA) and 0.2 % picric acid in 0.1 M phosphate buffer (PB) to identify the location of mumps virus antigen. The brains were cut into 20-µm-thick serial coronal sections using cryostat and incubated for 2 days at 4 °C in rabbit anti-mumps virus hyperimmune serum (Denkaseiken Co., Tokyo), which was diluted to 1:1200 in phosphate-buffered saline (PBS). Sections were then incubated with 1:2000 biotinylated anti-rabbit IgG (Vector Lab., Inc., USA) for 2 h and then with avidin-biotin-peroxidase complex (Vector Lab., Inc., USA) for 2 h at room temperature. Finally, sections were incubated in a 0.05 % diaminobenzidine solution containing 0.01 % hydrogen peroxide for 5 min at room temperature.

For electron microscopic studies, animals were perfused with 4 % GA in 0.1 M PB, and brains were sectioned coronally to expose the ependymal layer of the lateral ventricles. The specimens for scanning electron microscopy were dehydrated with graded solutions of ethanol, dried by the critical point CO_2 method, and sputter-coated with gold-palladium. For transmission electron microscopy, specimens were dehydrated in 100 % ethanol and embedded in Epon. Ultrathin sections from selected areas were cut and stained with uranyl acetate and lead citrate.

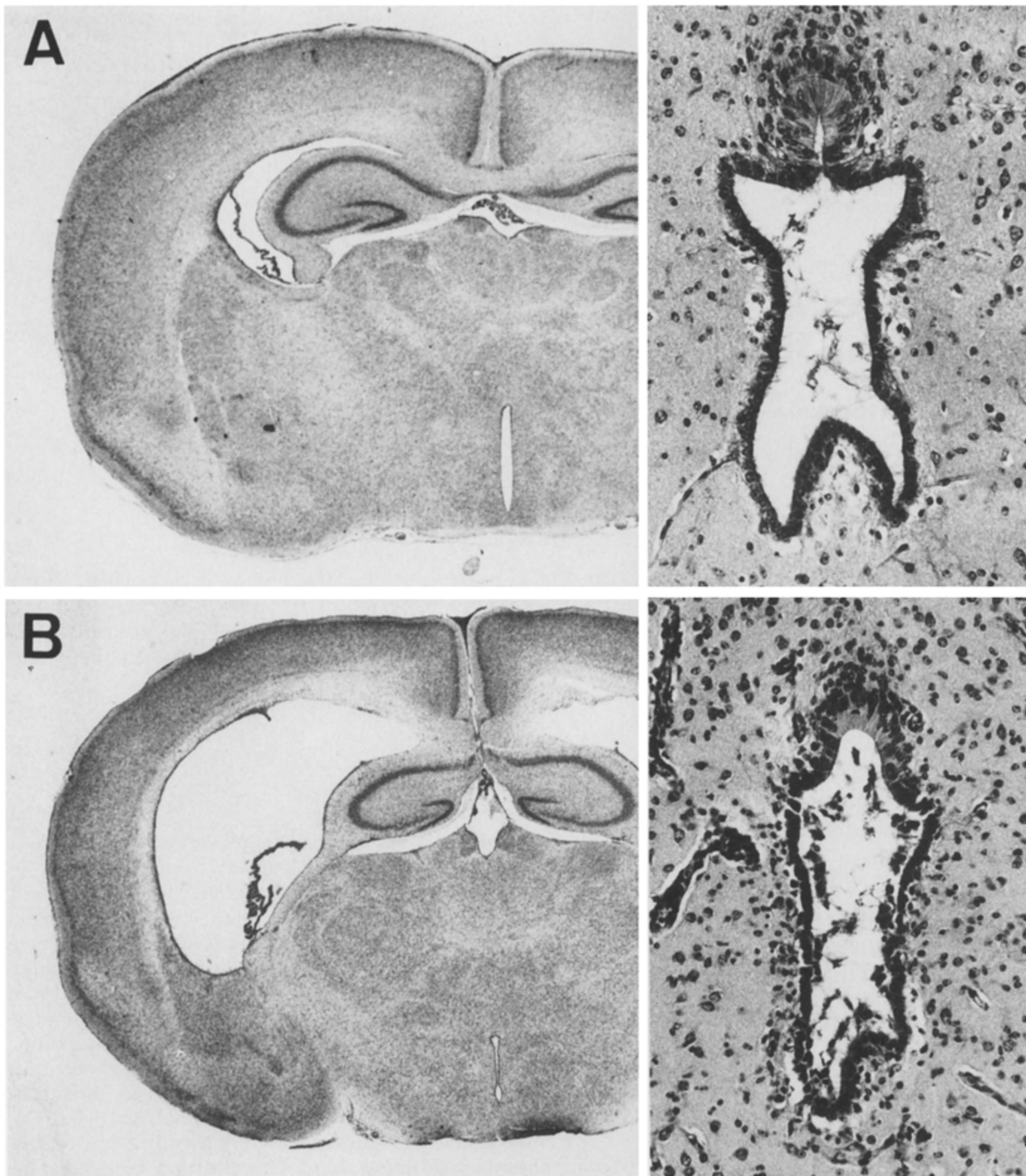
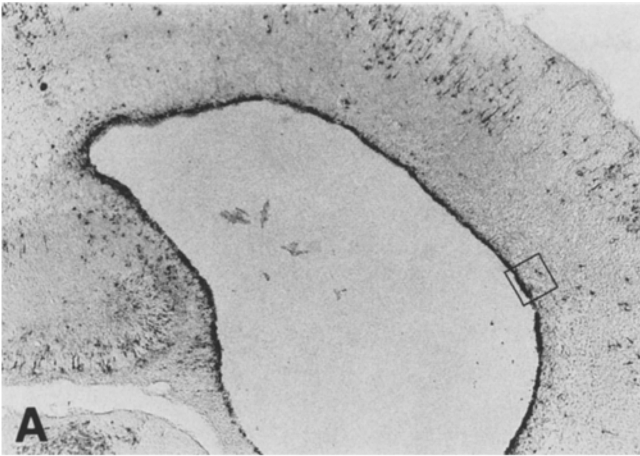


Fig. 1. Frontal sections of control brain (**A**) and mildly hydrocephalic brain (**B**) 5 days after inoculation. *Insets:* Aqueducts of control and hydrocephalic brains. Inflammatory cells were observed on the surface of the aqueduct and the microglial activation was seen in the underlying ependyma in the hydrocephalic brain. Aqueducts were not stenotic. H&E staining. Frontal sections: × 10; aqueducts: × 400

Results

Mild dilatation of the lateral ventricles was apparent as early as 5 days after inoculation with mumps virus. Inflammatory infiltration of the monocytes and neutrophils was observed in and around the ventricular system, including the aqueduct. However, no apparent histological changes were detected in the H&E-stained sections of the ependymal layer. No stenosis of the cerebral aqueduct was observed (Fig. 1). Mumps virus antigen was found in almost all ependymal cells of the ventricular system (Fig. 2) and in some pyramidal cells in the cerebral cortex and hippocampus.

When the surface of the lateral ventricles was examined by scanning electron microscopy, an orderly arrangement of ciliary clusters was detected in control animals (Fig. 3). In experimentel hamsters, the ependymal cilia had almost disappeared and only the microvilli remained. A number of supraependymal cells with long processes were also observed on the surface of the lateral ventricles (Fig. 4). Transmission electron microscopy revealed a number of intracytoplasmic viral-like inclusions in the infected ependymal cells, although these abnormal structures were not detected in the junctional complex at the gap junction or the zonula adhaerens. No abnormalities were observed in cytoplasmic organelles, such as the mitochondria and the Golgi apparatus (Fig. 5).



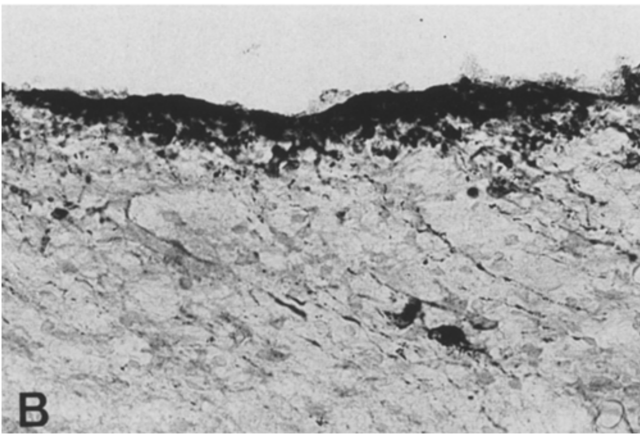
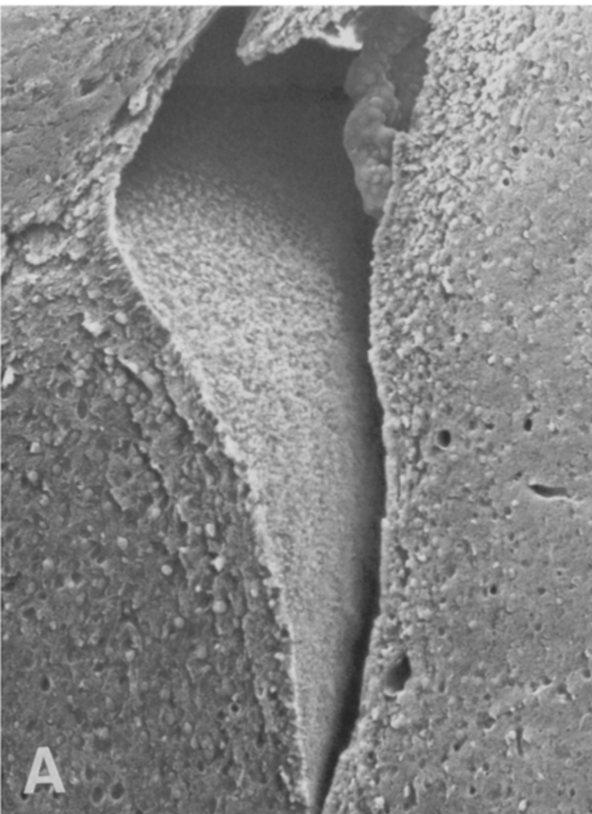


Fig. 2A,B. Immunohistochemical demonstration of the mumps virus antigen 5 days after inoculation. **A** Frontal section; **B** higher magnification of the part of the ependymal layer indicated by the square in **A**. Note the strong accumulation of mumps virus antigen in the ependymal layer. **A** × 40; **B** × 500



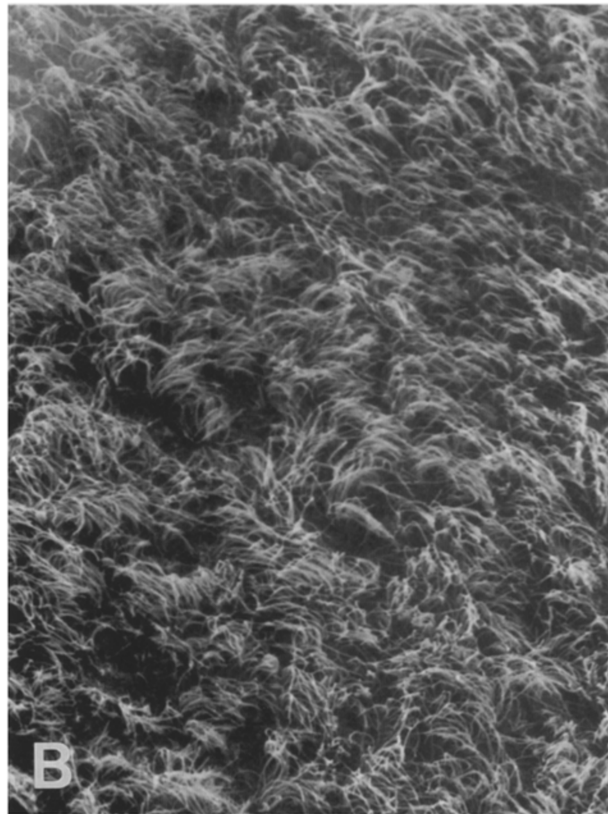


Fig. 3. Scanning electron micrographs of coronally sectioned control brain (**A**) and ependymal layer (**B**) 5 days after inoculation. An orderly arrangement of ciliary clusters was seen (**B**). **A** × 250; **B** × 2000

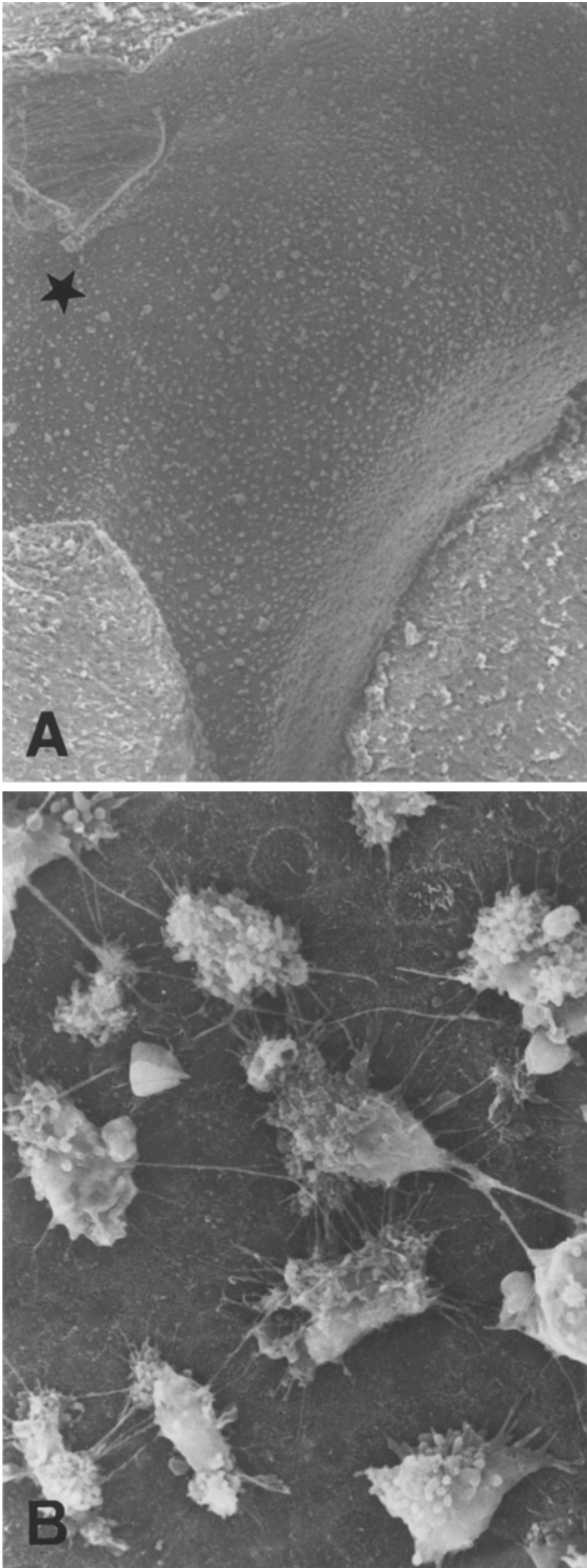


Fig. 4A,B. Scanning electron micrographs in coronally sectioned hydrocephalic brain (**A**) and its ependymal layer (**B**) 5 days after inoculation. The foramen of Monro was markedly dilated (*asterisk* in **A**). Note the destruction of ciliary clusters and the accumulation of supraependymal cells (**B**). **A** × 250; **B** × 2000

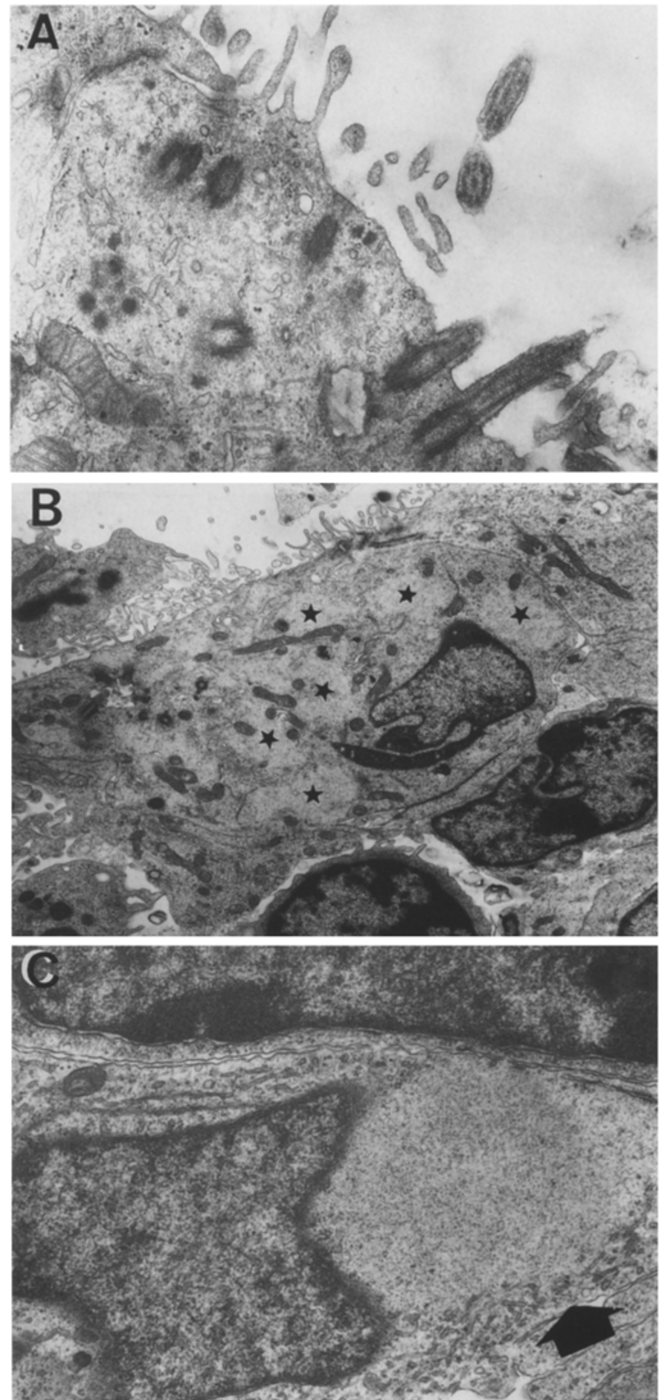


Fig. 5A–C. Transmission electron micrographs of ependymal cells 5 days after inoculation in control (**A**) and hydrocephalic (**B,C**) animals. Note the intracytoplasmic viral-like inclusions in the ependymal cell (*asterisks* in **B**) and one of their higher magnification (*arrow* in **C**). **A** × 9500; **B** × 7000; **C** × 14 000

Discussion

Although hydrocephalus has been experimentally induced by viral infection, the pathogenesis of hydrocephalus, and especially of the mild ventricular dilatation that occurs before aqueductal stenosis appears, remains controversial [8, 9, 12]. We observed ciliary damage on ependymal cells and the infiltration of supraependymal cells in the ependymal layers surrounding the lateral ventricles in hydrocephalic brains without stenotic aqueducts. We also observed intracytoplasmic viral-like inclusions in the ependymal cells of the lateral ventricles. These results suggest that the ependymal layer is affected during the acute phase of infection before aqueductal stenosis occurs.

The ependymal cilia are important in maintaining the flow of the intraventricular cerebral fluid [3]. It is possible that effective circulation and reabsorption of cerebrospinal fluid (CSF) require the presence of intact ependymal cells without intracytoplasmic viral inclusions and resulting ciliary damage. This study did not reveal how infected ependymal cells actually disturb the circulation of CSF. However, our results suggest that morphological changes in the infected ependymal cells may induce functional disturbances of ependymal cells, subsequently leading to the early ventricular dilatation that occurs before aqueductal stenosis.

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