Experimental Hydrocephalus in Suckling Hamster Induced by Myxovirus Infection

II. Pathogenesis of Hydrocephalus Caused by Parainfluenza Virus Type 3

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ABSTRACT Intraperitoneal inoculation of suckling hamsters with parainfluenza virus type 3 (PIV-3) caused hydrocephalus in about 17% of animals thus treated. In the inoculated hamsters, the PIV-3 antigen was found to be located in the choroid plexus and ependymal cells of the ventricular system. This finding suggests that the PIV-3 inoculated intraperitoneally transferred to the choroid plexus from the bloodstream and invades this tissue first, and then gradually affects the ependymal cells of the ventricular system. The pathologic findings obtained in this experiment showed a close similarity with those obtained in our previous experiment on mumps virus induced hydrocephalus. It was conjectured that PIV-3 induced hydrocephalus was caused by the same pathogenesis with the hydrocephalus by mumps virus infection. Our results strongly suggest that the PIV is a probable candidate for human congenital hydrocephalus of unknown origin, since PIV is the most common myxovirus.

Key words: hydrocephalus, aqueductal stenosis, parainfluenza virus type 3, myxovirus, GFAP

In the preceding paper (Takano et al., 1991), we reported that a high frequency of hydrocephalus was caused by the intracerebral inoculation of mumps virus into suckling hamsters, and also discussed in detail the pathogenesis of the hydrocephalus and aqueductal stenosis thus produced. According to previous reports (Johnson and Johnson, 1969), myxoviruses such as influenza, parainfluenza and measles viruses demonstrated experimentally to cause hydrocephalus in rodents subsequent to the selective infection to the ependymal cells surrounding the ventricular system. These results suggest that many of the myxoviruses, which have a selective affinity with the respiratory epithelium, also may carry coincidentally the same affinity with the ependymal cells, subsequently causing damage of these cells and then producing the hydrocephalus.

Parainfluenza virus type 3 (PIV-3), one of the myxoviruses, is known to be a very common pathogenic organism causing respiratory infection in children (Glezen, 1983). Shibuta et al. (1978) demonstrated that
hydrocephalus was produced by the intracerebral inoculation of PIV-3 in suckling hamsters. However, the pathogenesis of it is still a matter of controversy. In this experiment, PIV-3 was inoculated into suckling hamsters intraperitoneally, giving rise to systemic infection, which is the common pattern of the intrauterine fetal infection, and the hamster brains were examined histologically and histochemically to elucidate the pathogenesis of hydrocephalus. In this experiment, newborn Syrian hamsters were used for the reasons which were discussed in the preceding paper.

MATERIALS AND METHODS

The PIV-3 used in this experiment was a standard strain (C-243) isolated from the throat-swab fluid of a patient with respiratory infection. This virus was provided by Dr. M. Matsumoto, Department of Virology and Rickettsiology, National Institute of Health, Tokyo, Japan. The titer of the virus stock was 1.0 × 10⁸ pfu (plaque forming unit)/ml.

Ninety-eight 2-day-old Syrian hamsters were inoculated by injecting them with 50 μl of 1.0 × 10⁷ pfu/ml of PIV-3 intraperitoneally using a 27-gauge needle. For routine histological examination, these animals were anesthetized by pentobarbital, perfused with saline solution and then sacrificed 3, 5, 7, 10, 14, 18 and 20 days after inoculation. Brains were removed and fixed in phosphate-buffered 10% formalin and embedded in paraffin. Serial coronal or saggital sections at 5 μm thickness were stained with hematoxylin and eosin (HE). Two kinds of immunohistochemical studies were carried out on the brain tissue; the identification of PIV-3 antigen using rabbit anti-PIV-3 hyperimmune serum (provided by Dr. M. Matsumoto, National Institute of Health, Tokyo, Japan), and the examination for the reactive changes of glial cells after PIV-3 infection using the rabbit antiserum to the glial fibrillary acidic protein (GFAP; DAKO JAPAN Co., Japan). These immunohistochemical stains were performed by the same methods as those described in the preceding paper (Takano et al., 1991).

RESULTS

Virus concentration, clinical signs and occurrence of hydrocephalus: In the preliminary experiment, about 17% of animals developed hydrocephalus in both experimental groups, which had inoculation at the dosage of 1.0 × 10⁸ and 1.0 × 10⁷ pfu/ml, developed hydrocephalus (Table 1). These animals failed to gain weight, and developed the occipital prominence and squatting-like posture with poor motility (Fig. 1). Most of them died between 20 and 30 days after inoculation. Their brains on 18 days of age showed a bulging cerebral hemisphere with dilated lateral ventricles. The olfactory bulb and the superior and inferior colliculi were
Parainfluenza virus type 3 induced hydrocephalus

atrophic. The cerebellar vermis was displaced downward, this showing an elongated, atrophic and sleeve-like shape (Fig. 2B).

**Histological examination:** During the first 3 days after inoculation, no pathological findings were detectable in any experimental brains. On 5 days after inoculation, however, mild dilatation of the lateral ventricles became apparent in some of the experimental brains (Fig. 3A). Inflammatory infiltration of monocytes and neutrophils was also noticed in the ventricular system (Fig. 3B) including the cerebral aqueduct (Fig. 4A). When infected hamsters were examined after 7 and 10 days, dilatation of the lateral ventricles was clearly identified. After 14 days, the lateral ventricles showed symmetrical dilatation with remarkable enlargement of the foramen of Monro (Fig. 3C). The denuding of some ependymal layer was observed in the dorsomedial portion and ventral angle of the lateral ventricles (Fig. 3D). Remarkable narrowing of the aqueduct was also observed, although inflammatory infiltration was diminished (Fig. 4B). After 20 days, remarkable edematous changes were present in the subependymal white matter, especially at the dorsomedial portion of the enlarged lateral ventricles (Figs. 3E, 3F). The cerebral aqueduct had lost some of its ependymal cells and become stenotic (Fig. 4C).

In the advanced hydrocephalus 18 days after inoculation, the cerebellum had been flattened and became triangular in shape in the mid-saggital section in comparison to that in the non-hydrocephalic brain (Figs. 5A, 5B). In the cerebellar cortex, the widths of the molecular and granular layers were markedly reduced, although the cytoarchitecture, i.e., the three layers of the molecular, granular and Purkinje cell, was preserved. Each cell of the molecular and granular layer was densely packed, and Purkinje cells were flattened horizontally and burrowed into the surface of the granular layer (Fig. 5b).
Fig. 2  Whole brains and their coronal sections of 18-day-old non-hydrocephalic (A) and hydrocephalic (B) hamsters. Note the atrophy of the superior and inferior colliculi (black arrow), and the elongation of the cerebellar vermis (white arrow).

**Distribution of PIV-3 antigen:** During the first 3 days after inoculation, PIV-3 antigen was not detectable in any experimental hamster brains. The 5th and 7th day after inoculation, PIV-3 antigen became detectable specifically in all ependymal cells of the ventricular system and epithelial cells of the choroid plexus in hydrocephalic brains (Figs. 6B, 6C, 6D). There was no PIV-3 antigen in any non-hydrocephalic brain (Fig. 6A). After 10 days of inoculation, PIV-3 antigen was no longer detectable in the brain.

**GFAP immunohistochemistry:** Five days after inoculation, i.e., 7 days after birth, there were no apparent differences in the distribution of GFAP positive cells between hydrocephalic and non-hydrocephalic brains. After 10 days of inoculation, in non-hydrocephalic hamster brains, some GFAP positive small astrocytes were observed in the subependymal white matter of the lateral ventricles and perivascular area in the cerebral cortex (Figs. 7A, 7B). In the cerebral aqueduct, intense GFAP immunoreactivity was observed at the dorsal portion of the aqueduct, and some GFAP positive small astrocytes were identified in the periaqueductal gray matter (Fig. 8A). On the other hand, in hydrocephalic hamster brains, many GFAP positive astrocytes were noticed in the subependymal white matter and deeper layers, i.e., V and
Fig. 3 Frontal sections of hydrocephalic brain (A, C and E) and a higher magnification of their ventricular surface at the place indicated by a square (B, D and F). HE stain. A and B: Five days after inoculation. Mild dilatation of the lateral ventricles and inflammatory infiltration on the surface of the lateral ventricles (arrows in B) are recognized. C and D: Fourteen days after inoculation. Note the marked dilation of the foramen of Monro and denuding of some ependymal layer (D). E and F: Twenty days after inoculation. Severe symmetrical dilatation of the lateral ventricles and subependymal edema extending to the cortical area (F) is noticed. A, C and E: 7×; B and D: 500×; F: 150×.
VI of the neocortex (Fig. 7C). These GFAP positive cells resembled those of the protoplasmic type of astrocyte (Fig. 7D). Accumulation of GFAP positive astrocytes was also noticed in the periaqueductal gray matter, more abundantly than in the non-hydrocephalic brain (Fig. 8B). Fourteen days after inoculation, when the aqueduct had already become stenotic in the hydrocephalic brain, periaqueductal proliferation of GFAP positive small astrocytes similar to those of the fibrous type was noticed (Fig. 8C).

**DISCUSSION**

In the previous studies on experimental hydrocephalus caused by parainfluenza virus (PIV), types 1 and 2 were used (Friedman et al., 1975; Kristensson et al., 1984; Margolis and Kilham, 1977). Recently PIV type 3 was also demonstrated to cause hydrocephalus in high frequency in mice when this virus was inoculated intracranially (Shibuta et al., 1978, 1985). These experiments suggest that the fetal infection with parainfluenza virus, which is one of the most common pathogenic viruses in respiratory infections, may cause congenital hydrocephalus. In this experiment, the possible occurrence of hydrocephalus was examined in sucking hamsters by intraperitoneally inoculated with PIV-3. Intraperitoneal inoculation causes a systemic infection such as that seen in the fetus after intrauterine infection. Of these hamsters, seventeen per cent had hydrocephalus. The PIV-3 antigen in these brains was confirmed to be localized specifically in the choroid plexus and ependymal cells of the ventricular system.

Experimental studies using the method of intraperitoneal virus inoculation offer us some important suggestions with respect to the processes of virus invasion into the brain from the blood stream. Lipton and Johnson (1972), who studied the pathogenesis of rat virus infection by the intraperitoneal route, reported that the virus antigen was found first in the choroid plexus, and then in the ependymal cells and meninges. Wolinsky et al. (1976) demonstrated that in the brains of newborn hamsters, which had intraperitoneal

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**Fig. 4** Coronal sections of the cerebral aqueduct in the hydrocephalic hamster brain. Five (A), 14 (B) and 20 (C) days after inoculation. HE stain. 200×.
Parainfluenza virus type 3 induced hydrocephalus

Fig. 5 Mid-saggital sections of cerebellar cortices of non-hydrocephalic (A) and hydrocephalic (B) hamsters and their higher magnification (a and b) 18 days after inoculation. HE stain. Purkinje cells are flattened and buried in the surface of the granular layer (arrows in b). m: molecular layer; P: Purkinje cell layer; g: granular layer; A and B: 50 ×; a and b: 500 ×.
Fig. 6 Immunohistochemical demonstration of the PIV-3 antigen 7 days after inoculation. A: The cerebral cortex in the non-hydrocephalic brain. The PIV-3 antigen is not detected. B, C and D: The cerebral cortex (B), higher magnification of the ependymal cells and choroid plexus in the lateral ventricles (C) and the cerebral aqueduct (D) in the hydrocephalic brain. Note the PIV-3 antigen in all ependymal cells of the ventricular system and in epithelial cells of the choroid plexus. A and B: 30×; C: 500×; D: 150×.
Parainfluenza virus type 3 induced hydrocephalus

Fig. 7 Immunohistochemical demonstration of GFAP in the cerebral cortex of non-hydrocephalic (A and B) and hydrocephalic (C and D) hamster brains 10 days after inoculation. Note many GFAP positive astrocytes resembling those of protoplasmic type (D). A and C: 20×; B and D: 200×.

inoculation of neuroadapted mumps virus, the virus antigen was detected first in the choroid plexus and ependymal cells, and then throughout the brain parenchymal cells. In our experiment, the antigen of PIV-3 was found in the choroid plexus and ependymal cells after 5 and 7 days of inoculation. PIV-3 antigen was not detectable in the brain after 10 days. These results confirm that the choroid plexus seems to be a specific site where virus can easily pass through and invade the central nervous system from the blood.

The cerebral capillaries lack the fenestrations seen in other vessels; they have tight junctions and dense membranes and are tightly packed against astrocytic footplates (Johnson, 1982). These morphological structures correspond to the conceptual “blood-brain-barrier (BBB)”. On the other hand, the endothelial cells of the choroid plexus resemble more closely those of the extraneural tissues which contain fenestrae, lack basement membranes, and are surrounded by loosely arranged stromal cells (Wolinsky and Johnson, 1980). In our experiment, in which the PIV-3 was inoculated intraperitoneally, PIV-3 antigen was found almost exclusively in the choroid plexus and then to spread to the ependymal cells of the ventricular system. These results also indicate that the endothelial cell of the choroid plexus, which lacks the BBB, enables PIV-3 to pass through.

The pathologic findings obtained in this experiment had a close similarity to those in our preceding experiment on mumps virus induced hydrocephalus, in terms of the histological changes and affinity of the
virus antigen with ependymal cells and distribution of GFAP. However, the mumps virus antigen was also detected on some neurons in our previous experiment, in which the virus was inoculated intracerebrally. These results suggest that PIV-3 induced hydrocephalus is caused by the same pathogenesis with the hydrocephalus by mumps virus infection. Considering results, it is conjectured that other myxoviruses may also cause hydrocephalus with a similar pathology if they were inoculated into suckling animals.

Recently a human case of congenital hydrocephalus confirmed to be caused by a maternal influenza virus infection (Conover and Roessmann, 1990). This case showed ependymal damage, aqueduct forking, hydrocephalus and neuronal heterotopias. Moreover, the immunohistochemical examination disclosed the presence of influenza virus antigens in the brain. It goes without say that most of common viral infections usually terminate without clinical manifestations even during pregnancy. We should make more efforts to find these microbial pathogens in those cases of congenital hydrocephalus of unknown origin.

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Parainfluenza virus type 3 induced hydrocephalus

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