Ultrastructural Evaluation of Acute Encephalitis and Hydrocephalus in Dogs Caused by Canine Parainfluenza Virus

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Abstract. Intracranial infection of neonatal dogs with canine parainfluenza virus (strain 78-238) progressed from an acute phase of encephalitis to a chronic phase of internal hydrocephalus. Acute encephalitis was characterized ultrastructurally by laminar cortical necrosis, neuronal degeneration, neocapillary formation, reactive gliosis, and ependymitis. Viral nucleocapsids were seen in the cytoplasm of neurons.

Ultrastructural lesions in hydrocephalic dogs were restricted to ventricular surfaces. The earliest light microscopic change in ependymal cells occurred four weeks post-infection and consisted of segmental loss of ependymal cells. Dogs killed at three and six months post-infection had quantitatively similar ultrastructural alterations in ependymal cells regardless of the extent and severity of hydrocephalus. The shape of these cells changed from cuboidal to squamous. There was progressive loss of cilia and cell organelles along with a concomitant increase of cytoplasmic filaments. Viral nucleocapsids and inflammatory cells were not present in convalescent hydrocephalic dogs. These results suggested that virus-associated hydrocephalus can occur in the absence of obvious viral persistence in ependymal cells and in the absence of inflammation in the ventricular system.

In vivo and in vitro properties of canine parainfluenza virus (CPI 78-238) isolated from cerebrospinal fluid of a dog with posterior paresis have been reported [1, 2, 8]. The data obtained indicate that it could be distinguished from other canine parainfluenza and SV-5 viruses in vitro by its hemagglutination properties [2]. Furthermore, in vivo experiments showed that CPI 78-238 virus is capable of inducing acute encephalitis in neonatal pups, or internal hydrocephalus in convalescent dogs [1]. Other members of the paramyxovirus group are capable of inducing hydrocephalus following intracerebral inoculation into laboratory animals [9, 13, 14, 27, 31]. These studies documented direct viral infection of ependymal cells during the acute phase of infection. Hydrocephalus secondary to viral infection also occurs naturally [12]. The pathogenesis of virus-associated internal hydrocephalus, however, remains unclear.

Aqueductal stenosis as a sequel to a direct effect of virus on ependymal cells would impede outflow of cerebrospinal fluid, thereby causing hydrocephalus [5]. The
noninfectious counterpart of hydrocephalus secondary to blockage of the Sylvian aqueduct has been produced experimentally by intraventricular infusion of particulate materials such as kaolin or silicon oil [10, 29, 30]. Since others have noted that virus-associated internal hydrocephalus can occur in the absence of stenosis [22, 27], factors other than mechanical disruption of cerebrospinal fluid outflow must contribute to intraventricular fluid accumulation.

In dogs, cerebral infection with CPI 78-238 results in internal hydrocephalus with or without accompanying aqueductal stenosis [1]. Thus, detailed morphological examination of the resulting lesions may provide insights into factors contributing to the pathogenesis of virus-associated hydrocephalus. The objectives of this study were three-fold: to confirm the viral specificity of lesions found in the acute stage of infection by demonstrating viral structures in these areas; to observe ultrastructural alterations in ependymal and subependymal cell layers at various times post-infection; and to compare ultrastructural findings in dogs with and without aqueductal stenosis.

Materials and Methods

Virus

The isolation of CPI 78-238 virus from cerebrospinal fluid of a dog with neurological dysfunction and its characterization as a canine parainfluenza virus have been described [2, 8]. The isolate, after eight passages in African green monkey (Vero) cells, was inoculated (0.2 ml of \(10^6.8\) tissue culture infective doses \([TCID]_{50}/0.025\) ml) into six gnotobiotic dogs. For the second experiment, progeny virus from the second in vitro Vero cell passage of CPI 78-238 reisolated from brain suspension material of dogs from experiment 1 was used. This material contained \(10^6.25\) TCID\(_{50}\)/ml.

Maintenance and inoculation of gnotobiotic dogs

Eight colostrum-deprived gnotobiotic dogs from two separate litters were used in this study. Dogs were delivered by cesarean section and maintained in sterile flexible plastic isolation units [16, 17]. Each litter was infected and handled as a separate group. Six neonatal pups (dogs 1–6) were inoculated intracerebrally in the left hemisphere at six days of age. They received 0.2 ml of CPI 78-238 (\(10^6.8\) TCID\(_{50}/0.025\) ml). Dogs 7 and 8 were infected intracisternally with virus recovered from dog 6, following two further in vitro passages of virus in Vero cells (brain suspension pass II, \(10^6.25\) TCID\(_{50}/ml\)).

When moribund, dogs 5 and 6 were euthanatized with sodium pentothal 12 days after inoculation; dogs 3 and 4 were clinically normal when killed four weeks after infection. The remaining four dogs were clinically unaffected throughout the experiment and were killed three months post-infection (dog 7) and six months post-infection (dogs 1, 2, and 8).

Electron microscopy

Samples from various tissues from dogs 1, 2, 5, and 6, including brain and spinal cord, were collected and fixed in 10% buffered formalin. Dogs 3, 4, 7, and 8 were
anesthetized with sodium thiopental and perfused with 4% paraformaldehyde and 5% glutaraldehyde [19]. Organs were removed and stored in chilled fixative. Portions of the brain were minced into 1.0-mm cubes for further fixation in 1.33% osmium tetroxide in s-collidin buffer. After dehydration, the processed tissues were embedded in epon [20]. For light-microscopic studies, sections 1 μm thick were stained with toluidine blue and examined. Areas selected for thin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope.

Results

Gross examination

Dogs 3, 5, and 6 had no gross lesions. Dogs 4, 7, and 8 had a moderate hydrocephalus with dilation of lateral and third ventricles, and dog 8 had dilation of the Sylvian aqueduct. Severe hydrocephalus with enlargement of lateral and third ventricles and accompanying aqueductal stenosis was found in dogs 1 and 2.

Light-microscopic findings

Detailed light-microscopic findings have been published previously [1]. Lesions in two dogs that died of acute encephalitis consisted of multifocal necrosis accompanied by lymphoplasmacytic cellular infiltrates and reactive gliosis in the cerebrum. A flattened, attenuated, and discontinuous ependymal cell lining was the only consistent light-microscopic change seen in convalescent hydrocephalic dogs.

Ultrastructural findings in dogs with acute encephalitis

Ultrastructural evaluation of areas of laminar cortical necrosis in dogs with acute encephalitis after CPI 78-238 infection (dogs 5 and 6) showed neuronal degeneration, necrosis, and status spongiosus, accompanied by proliferating neocapillaries and infiltrates of reactive glial cells. In addition, the Virchow-Robin space around blood vessels contained lymphocytes, macrophages, and a few plasma cells. Viral nucleocapsids were found in the cytoplasm and in transverse sections of cell processes of neuroectodermal cells (fig. 1). Cells containing densely packed areas of viral nucleocapsids (17 nm in diameter) in the cytoplasm had either karyorrhectic nuclei or large nucleoli clearly demarcated from the rest of the karyoplasm. Affected cells had neither condensation of chromatin beneath the nuclear envelope nor bundles of filaments in the cytoplasm. Remnants of synaptic structures contacted degenerate virus-infected neurons.

Ultrastructural evaluation of ependymal lining surfaces in two dogs with acute encephalitis (dogs 5 and 6) showed ependymal cell degeneration accompanied by subependymal edema and infiltrates of lymphocytes and monocytes. Although inadequate fixation (immersion in formalin) precluded detailed analysis of changes in organelles in degenerate ependymal cells, neither viral nucleocapsids nor viral budding was shown convincingly.
Fig. 1: Neuron with viral nucleocapsid in cytoplasm (arrow). Well-defined nucleolus in nucleus. Inset: Cytoplasmic viral nucleocapsids, 17 nm in diameter.

Fig. 2: Normal ependymal cells of dog 4 in lateral ventricle. Ependymal cells lining ventricular surface had cilia (C) and microvilli (M).

Fig. 3: Focal disruption of normal lateral ventricular lining in dog 3, four weeks post-infection. Subependymal edema associated with attenuated ependymal cells. Attenuated ependymal lining cells (E) almost devoid of cilia and microvilli. Subependymal cell structures separated by extra-cellular edema (arrow).

Fig. 4: Ependymal cell lining in lateral ventricle in dog 7, three months post-infection. Pronounced increase of cytoplasmic filaments (F), lack of cilia, and angled course of gap junction (arrow). Ventricular lumen (L).

Ultrastructural findings in hydrocephalic dogs

Ultrastructural examination of brain tissue in hydrocephalic dogs was restricted to the ependymal and subependymal regions. In general, the degree of retrogressive change in all four ventricles observed correlated with the length of the observation period.

Dogs 3 and 4 were killed four weeks post-infection and, although only one of these two dogs had moderate hydrocephalus by gross examination, ultrastructural lesions were similar in both dogs. The surface of the ventricle was lined, for the most part, by cuboidal to columnar ciliated ependymal cells (fig. 2). Adjacent ependymal cells
Fig. 5: Ultrastructural changes in ependymal cells from dog 7, three months after infection. Supra-ependymal cell (arrow) of uncertain origin overlies attenuated ependymal lining cells (E). Large cytoplasmic membrane-bound vacuoles (V) within subependymal cells.

formed interdigitations with zonulae adherents and gap junctions, and irregular microvilli projected from the luminal surface between the cilia. Both dogs had focal interruptions in the normal ependymal lining surface. In these areas the ventricular lining consisted of flattened cytoplasmic projections lacking the typical features of ependymal cells (fig. 3). These cytoplasmic projections were almost devoid of organelles and possessed only occasional microvilli and no cilia. Extracellular edema distorted and separated the underlying normal architecture of the subependymal region. In spite of these alterations, there was no direct contact between cerebrospinal fluid and white matter because the underlying basement membrane remained intact.

One dog with moderate hydrocephalus (dog 7) was examined for lesions three months post-infection. Although a few normal cuboidal ependymal cells were found, most of the ventricular surface consisted of a poorly organized, but continuous, ependymal cell lining (fig. 4). Altered ependymal cells were characterized by marginal nuclear chromatin condensation and an increase of filaments in the cytoplasm, especially in the basal part of the cells. Cell elements lining the ventricular surfaces lacked cilia, basal bodies, and ciliary rootlets, and had reduced numbers of mito-
Fig. 6: Area containing relatively normal cuboidal ependymal cells from dog 1, six months post-infection. Increased number of cytoplasmic filaments (F) in these cells; cytoplasmic vacuoles (V) in cytoplasm of subependymal cells. Ventricular lumen (L).

Chondria and microvilli. In addition, gap junctions between adjacent cells were oblique or parallel to the ventricular surface rather than perpendicular as in controls. Occasionally, attenuated ependymal cells were covered by an additional cell layer of supraependymal cells of uncertain origin characterized by large nuclei and cytoplasmic extensions along the ventricular surface (fig. 5). Subependymal cells contained large membrane-bound vacuoles. In addition, electron-microscopic examination of neutrophils found within subependymal brain capillaries showed intracytoplasmic aggregates of viral nucleocapsid-like material.

Similar but more severe alterations in ependymal cell architecture occurred in dogs 1, 2 and 8, killed six months after infection. Degenerative changes in ependymal cells were similar to those described earlier (dogs 3, 4, and 7) except that subependymal vacuoles were more distinct. In all dogs, these vacuoles were separated from the ventricular lumen by cytoplasmic processes.

Occasionally, focal areas of almost normal ependymal cells were seen (fig. 6). Even in these areas, however, subependymal edema was present and ependymal cells contained increased numbers of cytoplasmic filaments.

In no instance of hydrocephalus were recognizable viral particles or nucleocapsids seen within the ependyma. The subependymal fibrillar area also was devoid of viral structures and inflammatory cell infiltrates.

Discussion

A number of investigators have studied the neuropathological features of parainfluenza virus infection in laboratory rodents [9, 14]. Both acute encephalitis and
Ependymitis have been recognized [7, 28, 32]. Further, hamsters with virus-associated ependymitis subsequently develop internal hydrocephalus [14, 21].

This report describes the ultrastructural findings in dogs with acute encephalitis and hydrocephalus following infection with a canine parainfluenza virus. In dogs with acute encephalitis, intracytoplasmic viral nucleocapsids without viral budding were found in areas of laminar cortical necrosis in the cerebrum. These findings confirm the virus-induced nature of these lesions previously found by light microscopy [1]. Identification of the cell type and cell processes containing nucleocapsids was difficult in some areas. On the basis of the subcellular features of glial cells and neurons [18, 24], the virus-infected cells were identified as neurons in various stages of degeneration. Severely affected cells, however, lacked synaptic connections that would confirm their origin as neurons. Inadequate fixation precluded accurate delineation of ependymal cell alterations in acutely affected dogs. It can be assumed, however, that productive viral infection with accompanying cell degeneration did in fact occur in ependymal cells because CI 78-238 viral antigen was found in them by immunofluorescence methods, three to seven days after infection [1]. Thus, ultrastructural features of CI 78-238-induced acute encephalitis in our dogs were similar to those in other host-parainfluenza virus systems.

Normal ultrastructural features of the subependymal plate and associated ependyma in the dog have been described by others [3, 24]. In hydrocephalic dogs examined four weeks after infection, normal cuboidal ependymal cells were found regularly. Progressive alterations in the ependymal lining, in general, correlated with the duration of the hydrocephalus (as reflected in months post-infection). Change from a cuboidal to a squamous pattern occurred with time. Accompanying this change in cell morphology was a progressive loss of cilia, a reduction of subcellular organelles, and an increase in cytoplasmic fibrils. Adjacent cell connections (i.e., gap junctions) were maintained; however, they assumed an oblique instead of a vertical orientation to the ventricular surface. At no time did cerebrospinal fluid come into direct contact with subependymal tissue. Changes in ependymal structure and in the subependymal fibrillar layer were not accompanied by subependymal axonal degeneration, reactive astrocytosis, or inflammatory cell infiltration as has been described in other models of parainfluenza-associated ependymitis and hydrocephalus in laboratory animals [10, 31].

Gross examination showed internal hydrocephalus as a clinically unrecognized sequel to CI 78-238 infection in five of six dogs. Two dogs infected at six days of age developed severe hydrocephalus with accompanying aqueductal stenosis. Two littermates, examined four weeks post-infection, had no stenosis of the Sylvian aqueduct, although one had a moderate enlargement of the lateral and third ventricles. Since these two pups had similar ultrastructural lesions, it was concluded that the expression of virus-associated hydrocephalus at necropsy may vary and that hydrocephalus is not correlated with the extent or degree of ependymal involvement. Dogs infected at six months of age developed a moderate hydrocephalus without
aqueductal stenosis. This finding suggests that the type of hydrocephalus induced by CPI 78-238 may vary with the age of susceptible dogs at the time of inoculation. At the ependymal cell level, however, no significant ultrastructural differences between moderate (without stenosis) and severe (with stenosis) hydrocephalus were found. Thus, the extent of damage to ependymal structures was related more to the duration of the disease process than to the severity of the grossly visible hydrocephalic change. Other investigators have reached similar conclusions [29].

In the later stages of hydrocephalus, cells lining the ventricular system did not possess the structural features of normal ependyma. The new lining cells were either altered ependymal cells or a newly developed, less differentiated, ependymal-cell type. In either case, these cells apparently are severely deficient in normal ependymal-cell function and, as a consequence, the absorption of cerebrospinal fluid may be compromised. Their functional defect may result in an increase in cerebrospinal fluid pressure and result in progressive development of hydrocephalus [23].

In our studies, aqueductal stenosis occurred only in dogs inoculated neonatally. Another study reported a similar age-related event in Mycoplasma sp.-associated hydrocephalus in rats [15]. In that study, inoculated newborns developed severe hydrocephalus, whereas weanling rats were relatively resistant. The authors suggested that the loss of cranial elasticity with age influences the magnitude of hydrocephalus. This concept is also supported by work on mechanisms of development of kaolin-induced hydrocephalus in cats with and without an intact cranial vault [11]. An intact skull limited the extent and severity of kaolin-induced hydrocephalus, presumably through mechanical means.

Stenosis of the aqueduct may occur by two different mechanisms. Virus-mediated ependymal cell destruction along the aqueduct may facilitate contact among subependymal cell elements. Cell adhesions with resultant disruption of normal aqueductal architecture may produce a stenotic lesion. Another factor that may contribute to aqueductal stenosis is virus-induced cerebral and subependymal edema secondary to acute encephalitis or cerebrospinal fluid leakage from the ventricular system into the surrounding neuropil. The net increase in extraventricular pressure would tend to force the walls of the aqueduct into close juxtaposition [25], resulting in stenosis.

The observation that age at the time of onset determines whether aqueductal stenosis will occur is supported by the documented occurrence of a high incidence of stenosis and hydrocephalus in young dogs regardless of cause [26]. In contrast, acquired hydrocephalus in older dogs is less pronounced and may be missed at gross examination [4].

It is important to stress that the apparent viral cause of hydrocephalus in our dogs was not readily apparent upon ultrastructural examination. Neither viral nucleocapsids nor accompanying hematogenous inflammatory cell infiltrates were found in ependymal structures. Intracytoplasmic inclusions compatible with parainfluenza nucleocapsids were found only in the cytoplasm of intravascular neutrophils in one dog (dog 7). These structures may represent an artifact, however, since viremia was not shown in these experiments [2].
We have noted previously that high levels of antibody to canine parainfluenza virus can be shown in the cerebrospinal fluid of hydrocephalic CPI 78-238-infected dogs. This finding may be interpreted either as a reflection of local immunoglobulin synthesis in the brain or as a consequence of leakage of plasma proteins into the cerebrospinal fluid. The data, to date, do not permit differentiation between these two possibilities.

The results of this investigation and related studies [1, 2, 8] confirm the neuropathogenic potential of canine parainfluenza virus in dogs. The high incidence of hydrocephalus in convalescent, clinically normal dogs indicates that sequelae to inapparent viral infection may manifest themselves weeks to months after the infectious event. The fact that the virus could not be recovered from hydrocephalic dogs [1] nor could any evidence of viral infection or accompanying inflammation be detected at the light [1] or ultrastructural levels clearly does not obviate the underlying viral cause of this syndrome. Thus, future studies must be directed toward improved methods of virus detection in this disease of dogs and in comparable disorders in man.

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References


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