Apoptotic Investigation of Brain Tissue Cells in Dogs Naturally Infected by Canine Distemper Virus

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Research

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Abstract

Background

Canine distemper caused by canine distemper virus that belongs to the *Morbillivirus* genus of the Paramyxoviridae family, is still a global epidemic significant infectious disease, especially in pet dogs in China and Seriously harm to the development of the dog industry. It has been known that Apoptosis caused by canine distemper virus can show in culture cells, lymphoid tissues and cerebellum. however, its occurrence in brain tissue cells remains unclear. In order to investigate the relationship among canine distemper infecting brain tissues, apoptosis in brain tissue cells and demyelinating pathogenesis this study was performed.

Methods

16 naturally infected dogs that exhibited clinical signs of CD and tested positive for anti-CDV monoclonal antibody, as well as on six healthy dogs that served as controls were used in the research. Brain specimens were divided into cerebrum, brain stem and cerebellum that was embedded in paraffin and made the sections respectively. Approximately 5 μm-thick sections were stained by haematoxylin–eosin, methyl green pyronin, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling technique and immunohistochemistry. CDV nucleocapsid protein was detected by immune streptavidin-biotinylated peroxidase complex.

Results

alterations in the brain tissues of CDV-infected dogs involved both various cells and nervous fibres. CDV had varying degrees of cytotropism to all brain tissue cells; apoptosis also occurred in all kind of cells in the brain, especially in the endothelia of cerebral vessels, astrocytes, oligodendrocytes and ependymal cells, the heavier infection, the more obvious apoptosis. Serious infections also involved the pyramidal and Purkinje cells. The nervous fibres exhibited demyelinating lesions (showed small multifocal vacuole) as well as some neuron axonal atrophy and disappeared gradually (formed large vacuole).

Conclusions

Apoptosis in brain tissue cells was mainly related to the propagation path and cytotropism of CDV. The apoptosis of astrocytes, oligodendrocytes, and some neurons may play a significant role in the demyelinating pathogenesis in dogs with acute canine distemper. A lot of diverse nervous signs shown in clinic may be in relation to different neuron apoptosis.

Introduction
Canine distemper (CD) is still a global epidemic significant infectious disease, caused by canine distemper virus (CDV) that belongs to the *Morbillivirus* genus of the Paramyxoviridae family [1–3]. The natural host spectrum of CDV comprises in at least six orders and over 20 families of mammals [4–5], in particular all families of the order Carnivora [6–7], including dogs that are susceptible to infection of CDV [8]. According to recent reports [9–12], risk of re-emergence of canine distemper presents in world and some of new strains of canine distemper virus have been isolated in Asia. It seems to tell us must to pay attention to the prevention and control of CD at present.

The pathogenesis of CD mainly involves the central nervous system and immunosuppression. The damage in the central nervous system (CNS) is an important characteristic of CDV-infected dogs. Demyelinating encephalopathy is the most common histopathological lesion of CD in the early or acute stage of the disease [13–15]. Pathologically, CNS alterations in CD frequently involve both white matter that shows multifocal or diffusive demyelination in nerve fiber bundle, and grey matter that mainly displays diverse degeneration of the neurons. Glial cell infection precedes demyelination and is correlated to strong replication of virus in the glial cells of white matter [16]. Nervous signs are the most common clinical manifestations in dogs with CD, including apathy, stupor, behaviour disorder, seizures, ataxia, tetraparesis, tetraplegia, paraplegia, hyperesthesia, myoclonus and incontinence [17–20]. Although the dogs with CD often have diverse neurological manifestations, they are difficult to diagnose based only on neurological signs in clinics.

According to the previous study, CDV has been shown to induce apoptosis in vivo, such as in cerebellum [21] and lymphoid tissue of naturally infected dogs [22–23]. CDV also causes apoptosis in vitro, such as in Vero [24–25] and HeLa cells [21]. However, apoptosis in the brain of dogs naturally infected with CDV remains unclear. This study aimed to determine whether CDV induced apoptosis in the brain of naturally infected dogs and elucidate the apoptotic relationship between CDV infection and demyelinating pathogenesis in the brain.

**Methods**

**Animals**

The 22 dogs used in this research were divided into two groups. Group 1 comprised 16 dogs with neurological signs (Nos. 1–16, experimental group) and suffering from spontaneous acute CD diagnosed by anti-CDV monoclonal antibody in clinic. Group 2 consisted of six healthy Beagle dogs (three males and three females, age of 6 months, Nos. 17–22, control group) that served as control animals from a study on traditional Chinese herb medicine. These dogs were vaccinated and dewormed according to standard protocols.

**Sample collections and histology**
Brains were immediately collected from animals at necropsy and fixed by immersing in 10% neutral-buffered formalin for 72 h. To observe the brain lesions in detail, the fixed brain was split into left and right hemispheres along the longitudinal fissure. Each part was then divided into cerebrum, brain stem and cerebellum and sliced into 0.3 cm thickness. All tissues were dehydrated through graded alcohols, embedded in paraffin and sectioned at 5 µm thicknesses by Leica microtome. The sections were stained by haematoxylin–eosin (HE). Control animals (Nos. 17–22) were mercy killing and their brains were subjected to the same histopathological procedures.

**In situ detection of genome fragmentation**

The terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) technique was performed for in situ detection of DNA fragmentation using a commercial ApopTag Plus in situ apoptosis detection kit (DAKO EPOS). The reaction was carried out according to the manufacturer’s descriptions. In brief, the sections were incubated with proteinase K at room temperature (RT) for 15 min, and endogenous peroxidase was quenched with 3% H₂O₂ in methanol at RT for 5 min. Terminal deoxynucleotidyl transferase and deoxynucleotides were applied, and the sections were placed in humid atmosphere at 37°C for 30 min. The reaction was stopped by a blocking buffer. The sections were treated with peroxidise, streptavidin, conjugate plus diaminobenzidine (DAB) and lightly counterstained with hematine.

**Methyl green–pyronin (MGP) stain**

MGP stain was used to elucidate the DNA and RNA change in the nucleolus and cytoplasm when apoptosis occurred. The sections from the brain tissues were deparaffinized, hydrated with distilled water, covered with MGP solution (Bi Tian-yun, China) for 4 min, then blocked and air dried at RT for 5 min, differentiated with distilled water, dehydrated and mounted.

**Detection of CDV by immunohistochemistry**

To detect the CDV antigens and confirm the CDV infection, the streptavidin-biotinylated peroxidase complex (sABC) method was used by a monoclonal antibody against CDV nucleocapsid protein (NP). Sections were autoclaved in citrate buffer at 121°C for 5 min for anti-CDV immunostaining. The endogenous peroxidase activity was quenched with 3% (w/v) H₂O₂ diluted in methanol at RT for 10 min. The sections were then incubated with primary antibody (mouse anti-CDV monoclonal antibody 1:400; Chemicon International, Inc.) at 4°C for 24 h and secondary antibodies (biotinylated goat anti-mouse and rabbit IgG; Chemicon International, Inc.) at RT for 10 min. Finally, the sections were visualised with DAB and lightly counterstained with haematoxylin.

**Anti-glial fibrillary acidic protein (GFAP) assay**

Anti-GFAP assay was performed to investigate the metabolism of astrocytes. In brief, the rehydrated sections were dipped in 3% hydrogen peroxide in methanol solution at RT for 15 min, blocked with normal goat serum diluted (1:20) in PBS at RT for 20 min, incubated with primary antibody (mouse monoclonal
anti-GFAP; DAKO EPOS) at RT for 80 min, incubated with secondary antibody (goat anti-mouse IgG peroxidase conjugate) at RT for 40 min, visualised by DAB and lightly counterstained with haematoxylin.

**Anti-galactocerebroside (GalC) assay**

Anti-GalC assay (GalC detection kit; DAKO EPOS) was used to examine the type and metabolic state of oligodendrocytes. In brief, the deparaffinsed-rehydrated sections were dipped in 3% hydrogen peroxide in methanol solution at RT for 10 min, autoclaved in citrate buffer at 121°C for 5 min, incubated with primary antibodies at RT for 60 min, added with secondary labelled antibody at RT for 30 min and visualised with DAB.

**Results**

During examination in the clinic all 16 dogs with natural CD displayed several neurological manifestations, which varied in range and severity. Some dogs showed mild signs, whereas others developed serious features similar to those\textsuperscript{[15–16, 20]} reported for CD. The most common symptoms were apathy(16/16), anorexia(16/16), myoclonus(16/16), seizures(12/16), ataxia(10/16), tetraparesis(8/16), nystagmus(7/16), pedal movements(5/16) and opisthotonos(5/16) (Table 1). The control animals showed a negative reaction to antibody against CDV. None of the control animals displayed any neurological signs.
| Cases No. | Age (months) | Sex* | Breed        | Anti-CDV antibody | Main neurological signs in clinic                                                                 |
|-----------|--------------|------|--------------|-------------------|--------------------------------------------------------------------------------------------------|
| 1         | 5            | M    | Mongrel      | +                 | Spastic tetraparesis, cervical rigidity, myoclonus, apathy                                     |
| 2         | 7            | M    | Labrador     | +                 | Myoclonus in bilateral thoracic limb, tetraparesis, anorexia                                    |
| 3         | 4            | F    | Beagle       | +                 | Spastic tetraplegia, myoclonus in pelvic limb, apathy                                           |
| 4         | 6            | M    | Poodle       | +                 | Myoclonus in truncal and limb, nystagmus, apathy, anorexia                                      |
| 5         | 4            | F    | Mongrel      | +                 | Tremor, ataxia, myoclonus in truncal and limb, anorexia                                         |
| 6         | 6            | F    | Mongrel      | +                 | Disorientation, myoclonus in pelvic limb, nystagmus                                             |
| 7         | 5            | M    | Shiba        | +                 | Spastic tetraparesis, ataxia, myoclonus in pelvic limb                                         |
| 8         | 5            | F    | Shin Tzu     | +                 | Spastic tetraplegia, myoclonus in thoracic limbs, apathy                                        |
| 9         | 3            | F    | Labrador     | +                 | Apathy, pedal movement, disorientation, dementia                                                |
| 10        | 4            | M    | Shin Tzu     | +                 | Apathy, pedal movement, disorientation, dementia                                                |
| 11        | 7            | M    | Mongrel      | +                 | Spastic tetraplegia, myoclonus in pelvic limbs, seizures                                       |
| 12        | 4            | M    | Pekinges     | +                 | Tremor, spastic paraparesis, ataxia, opisthotonus                                               |
| 13        | 5            | F    | Pekinges     | +                 | Tremor, ataxia, myoclonus in pelvic limbs, pedal movement                                        |

*M = male; F = female.
### Table of Neurological Signs and Cases

| Cases No. | Age (months) | Sex* | Breed       | Anti-CDV antibody | Main neurological signs in clinic                          |
|-----------|--------------|------|-------------|-------------------|------------------------------------------------------------|
| 14        | 7            | F    | Poodle      | +                 | Myoclonus in cervical and thoracic limbs                  |
| 15        | 6            | M    | Shin Tzu    | +                 | Myoclonus in truncal and limb ataxia, apathy              |
| 16        | 5            | M    | Mongrel     | +                 | Apathy, anorexia, seizures, nystagmus, opisthotonus         |

*M = male; F = female.

Alterations in the brain tissues of CDV-infected dogs involved both various cells and nerve fibers. The changes in all kinds of brain tissue cells were mainly CDV infection, degeneration and apoptosis. The nerve fibers mainly exhibited demyelinating lesions (showed small multifocal vacuole) as well as some neuron axonal atrophied and disappeared gradually (formed large vacuole). CDV had varying degrees of cytotropism in all brain tissue cells, and apoptosis also occurred in all kinds of cells in the brain. The more serious CDV infected, the more apoptotic cells occurred.

The severe CDV-infected cells in brain tissue were cerebral blood vessel endothelia, astrocytes and ependymal cells. The intense positive reactive endothelia, showing brown cells or dark brown cells (Fig. 1A), were primarily examined in small vessels of brain tissues, when the sections were stained by anti-CDV immunostaining. Following infectious exacerbation, the endothelium and astrocytic foot processes that appeared like line symbols and encircled in blood vessel showed intense positive reaction stained by anti-CDV immunostaining, representing these cells had been infected by CDV. Astrocytes were also stained as intense positive cells and became darker brown star-like pattern (Fig. 1B) by anti-CDV immunostaining, indicating that CDV had infected astrocytes and started to replication in cytoplasm of astrocytes. Anti-GFAP staining exhibited that the astrocytes in the demyelinating areas were in metabolic disorder and had several anti-GFAP strong positive substances (weak positive reaction in control dogs) in their cytoplasm (Fig. 1C). Some of endothelia of small vessels and astrocytes around the blood vessels had strong positive reaction (Fig. 1D) by TUNEL staining, showing dark brown cells and demonstrating their apoptosis.

MGP staining revealed that some nuclei of endothelia and astrocytes around the blood vessels showed pyknosis and appeared dark red, indicating they were in an apoptotic status because RNA of plasmosome in these cells become to condensation.

The ependymal cells of the control animals appeared like columnar cells with microvilli on its free surface (Fig. 2A) and had a negative reaction with anti-CDV and TUNEL staining. However, in CDV-infected dogs, the ependymal cells became cubic or flat cells, in which microvilli in cellular membrane surface
disappeared, and several eosinophilic inclusion bodies were observed in their cytoplasm and nucleos (Fig. 2B). Anti-CDV positive reaction matter was observed in the eosinophilic inclusion bodies of ependymal cells (Fig. 2C), proving that ependymal cells had been infected by CDV and the eosinophilic inclusion bodie was a kind of substance produced by CDV replication and metabolism. When the cells were seriously infected numerous ependymal cells died via apoptosis, and the nucleus was stained dark brown (Fig. 2D) by TUNEL staining.

The secondary CDV-infected cells in brain tissue were oligodendrocytes, microglias and neurons that mainly located in the nervenucleus of white matter in brain stem. The oligodendrocytes of brain stem in the control dogs were arranged as a string of beads along the nerve fibers and exhibited a negative reaction for anti-CDV and TUNEL staining as well as a weak positive reaction for GalC staining. By contrast, in CDV-infected dogs, some oligodendrocytes in demyelinating areas were in dispersive state and had a strong positive reaction for anti-GalC staining, which might indicate that bipolar immature oligodendrocytes increased (Fig. 3A). However, multipolar mature oligodendrocytes decreased in some initial demyelinating areas via apoptosis, showing a intense positive reaction by MGP (Fig. 3B) and TUNEL staining(Fig. 3C). Following the apoptosis of oligodendrocytes, local and diffuse demyelination were observed in white matter of brain stem and cerebrum, showing lots of small vacuoles and some of bigger vacuoles in demyelinating areas stained by HE. A few microglia were present in demyelinating areas and appeared among oligodendrocytes, some of which showed a strong positive reaction for anti-CDV and TUNEL staining. Some of the neurons in the nervenucleus of white matter were often infected by CDV, in which shrunken neurons with condensed nuclei exhibited a strong positive reaction for anti-CDV (Fig. 3D) and TUNEL staining, indicating that neurons severely infected by CDV were in apoptosis.

The light CDV-infected neurons were pyramidal cells and Purkinje cells. When the infection was serious, the whole cortical matter was infected by CDV, in which lots of CDV-infected positive cells (Fig. 4A), including cells situated in meninges, molecular, external granular, and pyramidal layers, were detected by anti-CDV staining. The pyramidal cells located in grey matter, especially the large ones, were often infected by CDV, in whche CDV-infected pyramidal cells became to degeneration showing satellite phenomenon and neuronophagia (Fig. 4B), or were shrunken and small with condensed nuclei stained dark red (Fig. 4C) by MGP staining. Some of the pyramidal cells were positive reaction for anti-CDV immunostaining and exhibited apoptosis (Fig. 4D)stained by TUNEL staining, which indicated that the pyramidal cells severely infected by CDV were easily to become apoptotic cells, following apoptosis of some pyramidal cells, big vacuole-like demyelination was observed in brain white matter, particularly under brain grey matter. Furthermore, a few of Purkinje cells were also infected by CDV, showing that Shrunken cells with condensed nuclei, in which the chromatin was compacted in the periphery of the nuclei, had a positive reaction for anti-CDV staining in the Purkinje cell layer.

Discussion
Relationship between apoptosis and demyelinating pathogenesis in brain tissues

Brain tissue demyelination in CDV-infected dog is a common encephalopathy and can be considered as a characteristic encephalopathic lesion caused by CDV. The mechanism of demyelination in dogs with CD has been investigated by several researchers. They found that demyelination of nerve fiber is mainly related to astrocytes and oligodendrocytes. The present study proved that apoptosis of neurons and glial cells is related to demyelination in brain tissues. Several studies [13,27–28] demonstrate that astrocyte is the main target of CDV in the CNS. In the white matter, astrocytes surrounded by myelin sheath have a positive effect in neuronal and oligodendroglial survival. Infected astrocytes fail to produce neurotrophic factors that was required for life of neurons and oligodendrocytes. Thus, CDV-infected astrocytes may have an important function in the mechanism of demyelination. The present study [29–30] proved that astrocytes were the principal CDV-infected target of in brain tissues and had a strong positive reaction for anti-CDV immunostaining. In this research the metabolic disorder of astrocytes in CDV-infected dogs, showing a strong positive reaction but in control dogs weak positive reaction stained by anti-GFAP immune technique, were observed in the white matter, especially in demyelinating areas. A considerable number of astrocytes showed a strong positive reaction for TUNEL staining, indicating that the positive-reactive astrocytes had been in apoptosis. Similar to the measles virus [31], CDV can disrupt the glial fibrillary acidic protein cytoskeleton and cause astrocyte apoptosis. Thus, when several astrocytes died via apoptosis, neurotrophic factors were not transmitted to oligodendrocyte for myelin maintenance, and the demyelination process were prompted gradually.

Oligodendrocytes are directly related to demyelination of the brain in dogs with CD. In the demyelinating areas, negative regulation of myelin gene transcription, inhibition of specific enzymatic activity in oligodendrocytes [32], oligodendrocyte degeneration and reduction of the population of oligodendrocytes suggest the involvement of oligodendrocytes in acute distemper demyelination process [33]. This study found that the oligodendrocytes in the demyelinating area were disordered and lost normal structure situation. Multipolar mature oligodendrocytes decreased because of apoptosis. By contrast, bipolar oligodendrocyte precursor cells increased in the demyelinating area, showing a strong positive reaction for GalC with anti-GalC staining. Pearce-Kelling et al [34] reported that bipolar oligodendrocyte exhibits high susceptibility for certain CDV strains. Similarly, another bipolar cell population obtained from adult canine brains revealed an increased susceptibility for CDV in vitro studies [35]. A considerable number of CDV infections in white matter can result in oligodendroglial apoptosis that expressed strong positive cells by TUNEL staining in demyelinating parts. Oligodentrocytes can produce many substances that are necessary for the formation of myelin sheath. Thus, they have a significant function in maintaining the integrity of myelin sheath. Furthermore, this study found apoptosis in many pyramidal cells in gray matter and neurons in nervous nuclei, indicating a strong positive reaction by TUNEL staining. Their axons were degenerated, dissolved and died, which led to the disintegration of myelin sheaths around the axons and formation of large vacuoles in the demyelinating areas [30].
Relationship between apoptosis and CDV infection in brain tissues

Previous studies [29,36−38] have proven that brain tissues are infected by CDV mainly through bloodstream and cerebrospinal fluid. The present study demonstrated that the apoptotic cells in brain tissues were related to the CDV-infected route. The earlier the cell contact with CDV, the more serious the CDV infection was, the more obvious apoptosis was. According to this research the blood-born infection, in which many cells were infected, is earlier and severer than cerebrospinal fluid infection. Krakowka et al [39] verified that neuron invasion of CDV occurs predominantly through the hematogenous route. Summers et al [40] and Axthelm et al [41] demonstrated that viral antigen is first detected in the brain capillary tissues and venular endothelia at 5 and 6 days pi and/or in perivascular lymphocytes, astrocytic foot processes and pericytes at 8 days pi. The present research found that CDV followed by blood circulation could intrude the endothelium of small blood vessels in the brain tissues and replicated in there, resulting in lots of CDV in the endothelium and a strong positive reaction was visualised for anti-CDV immunostaining. This pathological cellular lesion could finally lead to severe CDV-infected endopithelium to death via apoptosis. The CDV in the endothelium of blood vessels could also infect the astrocytes via the astrocytic foot processes, resulting in serious infection of astrocytes and apoptosis, as demonstrated by anti-CDV and TUNEL immunostaining. With this pattern, CDV could destroy the blood–brain barrier, enter into the brain tissues, and infect other neurons and glial cells.

Vandevelde et al [42] investigated the route of CDV in the brain tissues and found that ependymal and subependymal white matter infections could be infected by CDV. They verified that the brain tissues can be infected by the cerebrospinal fluid pathway. Moreover, a replicated-CDV infection of the choroid plexus endothelium with the release of progeny virus into the cerebrospinal fluid followed by ependymal infection and spread of virus to the subependymal white matter can be observed at 10 days pi [42−43]. The present study found that the pathological morphology of ependymal cells infected by CDV were changing from columnar to cubiform, then becoming flat cells, in which several eosinophilic inclusion bodies showed a strong positive reaction in their cytoplasm by anti-CDV immunostaining. When the CDV infection was serious, many ependymal cells on the ventricular surface died via apoptosis and expressed intense positive by TUNEL staining. This phenomenon indicates that the CDV from the cerebrospinal fluid invaded the ependymal cells and replicated, resulting in ependymal cells to infection and apoptosis. In this approach, the CDV entered the subependymal white matter by destroying the ependymal membrane barrier. Thus, the demyelination around the ventricle was more serious than in the other parts of the white matter in brain tissues.

In addition, this research revealed that the neuronic apoptosis in nervenucleus or grey matter and demyelination in white matter occurred randomly in brain tissues and no particular district and position of brain tissue were as some constant region of lesion through surveying the sections of cerebrum, brain stem and cerebellum of dogs with CDV in different parts. So in clinical practice no particular neurological manifestation could been used as diagnostic symptom for dogs with CD, though a lot of diverse nervous
signs were examined clinically in CDV-infected dogs. On the side it had been reported that neurological signs of dogs with CD were able to caused by other factors\textsuperscript{[13,44]}.

**Conclusion**

To our knowledge, the present study is the first to show that natural CDV infection resulted in apoptosis in all kinds of brain tissue cells. The data obtained by HE, methyl green–pyronin, TUNEL technique, immune sABC and immunohistochemistry supported that apoptosis had an important function on demyelinating pathogenesis. CDV could infect the endothelium of the brain vessel and astrocytic food process that surrounded the blood vessels. Severe infection could lead to apoptosis, resulting in blood–brain barrier damage and viral entry into the grey and white matter. CDV could also invade the ependymal cells, induce apoptosis, damage the cerebroventricular barrier and intrude the white matter. CDV that entered the brain tissues could continue to infect the glial cells, neuron in nervenucleus, pyramidal cells and Purkinje cells, and cause some of serious infecting cells to apoptosis. Thus, apoptosis in brain cells was mainly related to the route of transmission and cytotropism of CDV. The apoptosis of astrocytes, oligodendrocytes, and some neurons play a significant role in the demyelinating pathogenesis in dogs with acute CD.

**Abbreviations**

CDV
canine distemper virus

CD
canine distemper

CNS
central nervous system

HE
haematoxylin–eosin

DAB
diaminobenzidine

TUNEL
TdT mediated dUTP Nick End Labeling

MGP
Methyl green–pyronin

NP
nucleocapsid protein

PBS
phosphate buffer saline

RT
room temperature
Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Contributions

Conception of the study and supervising the progress of study: YP and XL. Conduct experiments: YP, SW, PL, FY and YZ. Sample collection: PL, FY, YZ and BP. Draft of manuscript: YP and XL. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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Ethics declarations

The animal experimental study was conducted in accordance with a study protocol approved by the Animal Ethics Committee of Xinxiang University (approval number 20170305).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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**Figures**

Severe CDV-infected endothelia and astrocytes A. CDV antigen intense positive reaction in the endothelium (↑) of blood vessel. sABC staining, Bar = 20 μm. B. CDV antigen intense positive reaction of endothelia (↑) and astrocytic food processes (□) surrounding the blood vessels and astrocytes. sABC staining, Bar = 20 μm. C. Astrocytes located in mild and moderate demyelinating areas are intense positive reaction (↑) against GFAP. Anti-GFAP staining, Bar = 50 μm. D. Apoptosis of endothelia (□) and star-patterned astrocytes (↑) are detected in primary demyelinating areas. TUNEL staining, Bar = 50 μm.
Figure 2

Severe CDV-infected ependymal cells A. Normal ependymal cells appear like columnar cells (↑) with microvilli on its free surface. HE staining, Bar = 20 μm. B. There are some of eosinophilic inclusion bodies (↑) in cytoplasm and nucleus of ependymal cells. HE staining, Bar = 20 μm. C. CDV antigen intense positive reaction (↑) in ependymal cells with inclusion bodies. sABC staining, Bar = 20 μm. D. A lot of apoptotic cells (↑) are found among ependymal cells. TUNEL staining, Bar = 20 μm.
Figure 3

Moderate CDV-infected oligodendrocytes and nervenucleus cells A. Oligodendrocytes show an anti-GaIC strong positive reaction in demyelinating areas. Anti-GaIC staining, Bar = 50 μm. B. Some of intense positive oligodendrocytes (↑) were observed in initial demyelinating areas. MGP staining, Bar = 50 μm. C. A lot of apoptotic multipolar mature oligodendrocytes (↑) present in demyelinating areas. TUNEL staining, Bar = 50 μm. D. Strong positive neurons for anti-CDV antigens in the nervenucleus cells situated in white matter. sABC staining, Bar = 50 μm.
Figure 4

mild CDV-infected pyramidal cells A. Lots of CDV-antigen positive cells were observed in cerebral cortical layer. sABC staining, Bar = 100 μm. B. The lesions characteristics of satellite phenomenon (↑) and neuronophagia (↓) are detected in CDV-infected pyramidal cells. HE staining, Bar = 20 μm. C. Small shrunken pyramidal cells (↑) show dark red, situating in the apoptotic states. MGP staining, Bar = 20 μm. D. Some of apoptotic pyramidal cells (↑) with pyknosis appear strong positive reaction staining dark brown. TUNEL staining, Bar = 20 μm.