Non-conventional neuropathological manifestations of canine distemper virus infection in dogs

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The conventional neuropathological manifestation of canine distemper virus (CDV) infection is characterized by demyelinating encephalomyelitis. Other recognized non-conventional neuropathological manifestations of distemper include acute encephalopathy, acute encephalitis, polioencephalomalacia, inclusion body polioencephalitis, and old dog encephalitis. With the use of molecular techniques, CDV has been demonstrated in types of non-suppurative meningoencephalitis of unknown etiology that were never previously associated with CDV. Additionally, non-conventional necrotizing encephalopathies affecting the forebrain have been reported in dogs with CDV infection. Because the neuropathology of CDV infection contains many aspects, much of the current literature about distemper has been focused on conventional neuropathological aspects. For these reasons, the authors have concentrated on non-conventional neuropathological features of CDV infection for years; in this mini-review such manifestations of distemper will be addressed.

Keywords dog; canine distemper virus; central nervous system; non-suppurative encephalitis; acute encephalitis; polioencephalomalacia; inclusion body polioencephalitis; old dog encephalitis; neuropathology

1. Canine distemper virus

Canine distemper virus (CDV) belongs to the *Paramyxoviridae* family in the *Morbillivirus* genus together with other viruses such as the extensively studied old morbilliviruses (measles virus, rinderpest virus, and pest de petite ruminants virus) and the new described morbilliviruses (phocine distemper virus and dolphin and porpoise morbillivirus) [1].

CDV is a non-segmented single-stranded negative sense RNA virus. The virion is relatively large (150-240 nm) and surrounded by a lipoprotein envelope derived from virus glycoproteins that incorporate into the cell membrane [1, 2].

The virus has an extensive host range and may infect many mammalian species including members of the *Felidae* (cats and big felids), *Canidae* (dogs, foxes, etc.), *Mustelidae* (weasles), and *Procionidae* (raccoons) families [3, 4]. Although aquatic mammals have their own pathogenic morbilliviruses (phocine distemper virus and dolphin and porpoise morbillivirus), CDV has also been associated with epizootics in these animals [5-7].

Distemper is a multisystemic viral disease associated with viral spread to the central nervous system (CNS) that often results in a progressive and multifocal central nervous system (CNS) disease [8, 9]. Morbilliviruses such as CDV and measles virus (MV) show various clinical signs in their natural hosts (dogs and humans, respectively) varying from the characteristic, mild self-limiting infection to death [10].

Both CDV and MV are etiological agents of acute and chronic encephalitis in their respective hosts. Although CDV and MV produce similar systemic disorders in their hosts, they differ markedly in the frequency of CNS involvement [11]. During viremia, CDV infects the brain in most, if not all, cases, regardless of the clinical signs [12]. Almost half of the dogs with clinical illness due to CDV infection develop nervous distemper [11]. However, MV is only associated with neurological complications in a small minority (about 1/1000) of cases after systemic infection [13].

Recognition that CDV is responsible for several morphologically distinct types of canine encephalitis with similarities to MV-associated disease syndromes in humans has promoted the study of host-CDV relationships as a model for central nervous diseases caused by morbilliviruses [14].

2. Canine distemper virus infection

CDV is generally transmitted as an aerosol infection of the upper respiratory tract. The primary virus replication takes place in the regional lymphoid tissues. Infection of the lymphoid tissues is prevalent and associated with severe, long-lasting immunosuppression. At about 10 days post-infection, CDV starts to spread from the sites of primary replication to various epithelial tissues and to the CNS [2, 15].

During viremia, it is probable that CDV infects the CNS of most, if not all, infected dogs [12], even when typical nervous dysfunction might be not recognized. The authors have detected CDV RNA by RT-PCR and CDV antigens by immunohistochemistry within the CNS of dogs with systemic distemper even in cases where both neurological signs and nervous lesions were absent [16].

3. Non-conventional neuropathological presentations of CDV infection

CDV may be detected within both white and gray matter of the CNS, and different neuropathological presentations have been reported. High incidences of neurological lesions have been documented despite the absence of overt neurological signs [17].

The conventional neuropathological manifestation of distemper is characterized by acute to chronic demyelinating encephalomyelitis [12, 15, 18-22]. Other recognized non-conventional neuropathological manifestations of distemper include acute encephalopathy, acute encephalitis [21, 23], polioencephalomalacia [24], inclusion body polioencephalitis [25], and old dog encephalitis [26, 27]. With the use of molecular techniques, CDV was demonstrated in types of non-suppurative meningoencephalitis of unknown etiology that were never previously associated with CDV [28]. In addition, the authors occasionally observed non-conventional necrotizing encephalopathies affecting the forebrain of dogs with CDV infection, in which the CDV-related necrotizing changes mimicked the microscopic and even the gross findings of necrotizing encephalitis in Pug, Yorkshire and Maltese dogs [16]. In such cases, CDV was characterized within the CNS lesions by a combination of immunohistochemistry and RT-PCR [16].

3.1 Acute Encephalopathy and Acute Encephalitis

Both acute encephalopathy and acute encephalitis have been observed in spontaneous CDV infection of mustelids [29]. CDV-induced changes affect the rostral brain, sparing the cerebellum and caudal brainstem. While encephalopathy is a non-inflammatory CDV-induced change, encephalitis is accompanied by a mild to moderate inflammatory reaction [19, 21].

In dogs, these neuropathological manifestations have not been frequently observed under natural conditions of CDV infection [19, 21] but have been described experimentally [23]. Acute encephalopathy and acute encephalitis are non-demyelinating nervous conditions that are relatively unusual when compared to the high incidence of demyelinating CDV-related disease. In the last eight years of studying nervous distemper, acute encephalopathy and acute encephalitis were only observed in our lab under natural conditions of the disease (spontaneous wild-type CDV strain infection) in two immature dogs (3 to 6 months) [16].

3.1.1 Neuropathology of acute encephalopathy and acute encephalitis

Acute encephalopathy is a non-inflammatory and non-demyelinating form of CDV infection characterized by a few foci of single-cell neuronal necrosis [19]. Other changes that may also be occasionally recognized in acute CDV encephalopathy include mild gliosis (astrogliosis and astrocytosis), satellitosis, neuronophagia, and reactivity of the endothelial cells [swelling of endothelial cells (hypertrophy) in the presence or absence of endothelial hyperplasia] [16]. Gray matter is mainly affected, and the nervous system changes are more prone to occur in the forebrain with sparing of the hindbrain [16].

Acute encephalitis is a non-demyelinating CDV encephalitis associated with mild lymphomonocytic perisvascular cuffing [21]. In acute encephalitis, most of the lesions are restricted to the gray matter of the cerebral cortex (forebrain). A significant inflammatory reaction is not evident in neuroanatomical sites of the hindbrain (caudal brainstem and cerebellum). Discrete to moderate degrees of neuronal necrosis, satellitosis, and neuronophagia; varying degrees of gliosis; and hypertrophy and hyperplasia of endothelial cells might also be observed within the forebrain [16]. Although the perivascular inflammations tend not to pass through the Virchow-Robin space, foci of mononuclear inflammatory cellular aggregates may be recognized within the nervous parenchyma. Vacuolar changes are not observed within the white matter of the brain (H&E stain), although mild glial reaction and endothelial reactivity may be occasionally recognized [16].

3.1.2 Pathogenesis of acute encephalopathy and acute encephalitis

As acute encephalopathy and acute encephalitis have not been frequently described, the pathogenesis of these lesions has not been studied; thus, their occurrence has no current explanation. In the two instances registered by the authors, both RNA and antigens of CDV were detected in all the neuroanatomical sites of the brain (cerebrum, cerebellum, mesencephalon, pons, medulla) despite the presence of neuropathological changes only in forebrain. However, these results were not specific for acute encephalopathy and acute encephalitis; similar molecular findings were observed in different neuropathological presentations [16].

3.2 Old dog encephalitis

Non-usual CDV chronic disseminated encephalitis observed in mature dogs has been termed old dog encephalitis (ODE) because the disease was initially described in old dogs [26]. ODE is neuropathologically similar to measles virus-related subacute sclerosing panencephalitis (MV-related SSPE) with respect to the type, nature, and neuroanatomical distribution of the lesions within the brain [11, 30]. Although ODE was initially described in old dogs, the disease was recorded in five 2 to 3-year-old dogs [30, 31]. Cordy [26] also reported the disease in dogs aged from 2

years on. In addition, Axthel and Krakowka [14] experimentally reproduced the disease by inoculating the R252-CDV strain in an 11-month-old gnotobiotic Beagle dog. Due to the occurrence of the disease in non-old dogs and the neuropathological similarities of ODE with MV-related SSPE, ODE might be designated as CDV-related sclerosing panencephalitis [16].

3.2.1 Neuropathology of ODE

ODE is a non-conventional chronic CDV-related encephalitis that has been described as disseminated non-suppurative encephalitis characterized by severe diffuse gliosis and moderate to extensive perivascular cuffing of mononuclear inflammatory cells, mainly lymphocytes [11, 27, 32]. Such inflammatory changes occur within both the white and gray matter of the forebrain with relative sparing of the hindbrain (cerebellum and caudal brainstem) and marked involvement of the cerebral hemispheres and cranial brainstem. The thalamus, mesencephalon, and pons are relatively more affected than the medulla and corpus striatum. In some cases, the cerebral lesions are prevalent and the cranial brainstem lesions are mild to absent. In the cerebral cortex, the sensoriomotor and paleocortex are more affected than the visual area [26, 27]. Usually, there is no particular relationship to the meningeal surface or to the ventricle system [26]. ODE also resembles multiple sclerosis in humans [27, 30].

Within the cortical lesions of ODE, the inflammatory cellular infiltrate is extensive around the Virchow-Robin space and also invades the nervous parenchyma (neuropil) [26, 31, 33]. In the subcortical areas, perivascular mononuclear cell infiltrates extend into the white matter. This white matter change is accompanied by astroglial cell hypertrophy and hyperplasia, microglial proliferation, and chronic demyelination [27, 34]. In the cortical gray matter, infiltration of mononuclear cells is accompanied by marked astrogliosis, neuronal loss, neuronal degeneration, and necrosis with an increased number of perineuronal astrocytes and macrophages [26, 27]. CDV inclusion bodies might be found in neurons and/or glial cells, but the absence of typical CDV inclusion bodies has been reported in some cases [14, 26, 31].

Occasionally, multinucleated giant cells might be observed within ODE lesions [14, 30], similarly to some human nervous conditions such as multiple sclerosis and MV-related SSPE [30]. Axthelm and Krakowka [14] suggested that the multinucleated giant cells might be syncytial cells of glial origin; however, immunohistochemical characterization of the multinucleated giant cells was never performed to clarify their origin. Recently, in an ODE case characterized by our group [27], immunohistochemistry demonstrated that these multinucleated giant cells were glial fibrillary acidic protein (GFAP)-negative but were strongly immunoreactive to macrophage-activating factor and vimentin, indicating their hematopoietic origin.

3.2.2 Pathogenesis of ODE

The naturally occurring incidence of ODE is low, the pathogenesis is controversial, and the precise host-CDV relationship is unknown. Most neuropathological manifestations and CDV-induced lesions have been reproduced experimentally; however, ODE was only reproduced once [14] despite several attempts. The isolation of the virus by conventional virological methods from nervous tissue has never been accomplished [12, 31, 34], and co-cultivation techniques should be performed to increase the chances of recovering the virus [14]. Additionally, ODE and conventional distemper were not reproduced by inoculation of susceptive animals (dog and ferret) with brain homogenates or recovered virus from ODE cases [14, 33].

Different hypotheses have been proposed to explain the occurrence and pathogenesis of ODE; however, its origins remain uncertain. This disease might represent classical chronic and subclinical CDV encephalitis or a persistent infection by a replication-defective wild-type virus strain [14, 34, 35]. Vandevelde et al. [34] could not isolate the virus from naturally occurring ODE even with the use of a co-cultivation technique with explanted brain cells. Axthelm and Krakowka [14] recovered CDV from the SNC of an experimental ODE after additional effort with multiple passages using prolonged co-cultivation and passage of brain explant monolayers within Vero cells. Because of the difficulty in recovering CDV from the brains of dogs with ODE, it has been suggested that a replication-defective form of CDV (non-productive infection) might be involved on the pathogenesis of ODE [11], similarly to MV in SSPE [13].

Viral persistence plays an important role in both ODE and MV-related SSPE. In contrast to MV-related SSPE, in which the mechanisms of viral persistence have been clarified and a genetically distinct mutant MV strain (replication-defective form) has been reported [13], no conclusive evidence has been published for ODE.

To the authors' knowledge, in contrast to MV-SSPE, the host-CDV-relationship, and not the CDV strain, is the most important factor in determining ODE. The replication-defective form of CDV is probably generated within the SNC after nervous system infection with a non-replication-defective CDV (conventional wild-type CDV strain). The main evidence for such a mechanism is the observation of ODE could be reproduced experimentally by intraperitoneal inoculation of conventional demyelinating CDV strain (R252-CDV strain) in an 11-month-old dog [14]. The R252-CDV strain was originally recovered from a naturally occurring conventional case of chronic demyelinating encephalitis in 1972 by direct inoculation of brain homogenate into CDV-susceptible gnotobiotic dogs [36]. This virus strain has been extensively characterized with respect to virulence and *in vivo* and *in vitro* growth properties [36-38]. It is a potent immunosuppressive agent [39] and produces conventional acute [20] and chronic [36] neurologic disease in CDV-susceptible dogs. In addition, all virion proteins are produced and tissue culture-adapted R252-CDV infection is

productive, suggesting the original lack of a replication-defective CDV form. It is possible that the reproduction of ODE was not the objective of the authors. Speculatively, they were probably studying the pathogenesis of CDV infection; however, one of the six beagle dogs infected at that time developed chronic nervous disease and at euthanasia 988 days after inoculation, the brain showed lesions compatible with what is known as ODE [14].

Other strong evidence that the CDV strain plays a minor role in the pathogenesis of ODE is provided by phylogenetic analysis of the CDV nucleoprotein (NP) gene from both ODE and conventional demyelinating distemper cases, which showed no differences in cluster formation [32]. Thus, ODE and the conventional wild-type CDV strain may cluster in the same branch of the phylogenetic tree [27]. However, when phylogenetic trees were reconstructed with both conventional and SSPE-related MV strains, using the same region of the NP gene from both morbilliviruses (CDV and MV), an obvious segregation pattern in clustering was observed in MV [16]. Phylogenetic analysis of the MV NP gene showed two distinct clusters: one with the conventional wild-type MV strains and the other with the SSPE-related MV strains [16]. On the other hand, a similar study with the same NP region of the CDV gene showed no differences in cluster formation, and both ODE and conventional wild-type CDV strains were found to cluster in the same branch of the phylogenetic tree [16, 27].

3.3 Polioencephalomalacia associated with CDV Infection

Polioencephalomalacia in dogs is an infrequently reported neuropathological condition [40]. The lesions have been described mainly in association with lead and cyanide poisoning, thiamine deficiency, cardiac arrest, hypoglycemia, and ischemia due to meningitis and thromboembolic disease [41-43]. However, dogs with polioencephalomalacia have been described with CDV infection [44].

In the most CDV-free polioencephalomalacia conditions, there is predominant involvement of the neocortex, especially of the dorsal gyri. In such CDV-free conditions, neocortical involvement may also take place with necrosis of both the neo- and paleocortex. In contrast to other conditions, CDV-related polioencephalomalacia lesions have been found in the cerebral grey matter, predominantly involving the ventral parts of the cerebral cortex (paleocortex). Such lesions occur in a bilateral and sometimes symmetrical pattern [24]. The pyriform cortex and Ammon´s horn are the most affected areas. Lesions may also be recognized in the amygdala, olfactory lobes, claustrum, and septal area. In some dogs, there is also involvement of the ventral part of the sylvian gyrus [24, 44].

3.3.1 Neuropathology of CDV-related polioencephalomalacia

The lesions in the paleocortex consist of selective degeneration of scattered nerve cells, massive selective destruction of most neurons in some areas, and occasionally intense invasion with macrophages (Gitter cells), mainly in pronounced encephalomalacia. In the hippocampus, the large pyramidal cells of Sommers´ sector may be affected. In some dogs, there is also considerable necrosis of the dentate gyrus. The degenerating neurons have a strongly eosinophilic granular cytoplasm and are often shrunken; their nuclei appear karyorrhectic and are sometimes triangular with small dark-staining nuclei [44]. The surrounding neuropil generally is edematous with a spongy appearance due to vacuolation, especially around the blood vessels and necrotic neurons. Most of the dogs also have swelling and proliferation of the endothelial and adventitial cells of the microvasculature in affected areas. Mild perivascular cuffing by mononuclear cells around some vessels may be found [44].

Typical distemper-related demyelinating lesions in the cerebellopontine angle may occur in some affected dogs in addition to the polioencephalomalacia lesions [44]. However, CDV-related polioencephalomalacia lesions may also occur in the absence of other typical neuropathological findings of nervous distemper such as demyelinating lesions [24].

3.3.2 Pathogenesis of CDV-related polioencephalomalacia

The neuronal changes in the affected areas look like those of ischemia-hypoxia and may occur due to impaired cerebral circulation in affected regions. The cerebral cortex and hippocampus are recognized as areas especially vulnerable to cerebral hypoxia. The importance of local phenomena, such as swelling of astrocytic end-feet, in response to focal seizure activity in the induction of ischemic neuronal damage should be considered. The large clear spaces around blood vessels (and neurons) observed in previous reports [24, 43, 44] may represent severe astrocytic end-feet swelling, which may lead to collapse of the capillaries. In addition, endothelial and adventitial proliferation in the affected areas may contribute to the ischemia [24].

Experimental and natural studies in animal and human models have shown that polioencephalomalacia may occur secondary to seizure activity [40]. It is therefore speculated that CDV-related polioencephalomalacia might be due to seizure-induced changes [45, 46], as all the dogs reported with such conditions presented with seizure disorders ranging from “chewing” fits to status epilepticus. However, it is unknown whether this seizure activity is the cause of the observed histopathological changes or whether the seizure is just one clinical sign of preexisting CDV-induced cortical necrosis.

The pattern and localization of seizure-induced necrosis is different from the lesion distribution in CDV-related polioencephalomalacia. Neuronal necrosis associated with status epilepticus has been described in a laminar pattern. Such laminar necrosis mainly involves cell layers III and IV of the cerebral cortex in experimental rat studies [47-49]. Experimental studies in baboons have shown layers III, V, and VI to be the most affected [50, 51], while dogs with genetic epilepsy dying of status epilepticus primarily show involvement of layers II and III [52]. In the classical CDV-related polioencephalomalacia, there is no predilection for a specific neuronal layer [24]. In contrast, in most CDV-free polioencephalomalacia conditions, there is predominant involvement of the neocortex, especially of the dorsal gyri [40]. CDV-related polioencephalomalacia lesions have been found in the cerebral grey matter, predominantly in the ventral parts of the cerebral cortex (paleocortex/ rhinencephalon) [24, 44]. These differences suggest that seizures alone may not be used to explain the CDV-related polioencephalomalacia and that the virus probably plays a real role in the pathogenesis of CDV-related polioencephalomalacia.

Based on human evidence of viral neurobiology, Lisiak and Vandevelde [24] suggested that the rhinencephalic locations of CDV-related polioencephalomalacia lesions could be explained on the basis of viral entry via the olfactory pathways as described in human viral infections [53]. At that time (1979), much of the knowledge about CDV neurobiology, as well as the exact mechanisms of CDV entrance within nervous tissue, was not yet available. However, their interpretation using human models of viral neurobiology was probably correct. In 2006, Rudd et al. [54] added new concepts of CDV neurobiology that described anterograde CNS invasion via the olfactory nerve.

Different mechanisms have been proposed for several CDV-free polioencephalomalacia conditions [49, 55]; however, the exact role played by CDV in polioencephalomalacia lesions is not known. Brunner et al. [56], studying molecular events in CDV infection by *in vitro* studies, found that neurons and astrocytes were clearly infected and that the infection spread only slowly to neighboring cells. Interestingly, they observed that CDV infection caused massive neuronal death, including non-infected neurons. Brunner et al. [56] also found that antagonists of N-methyl-D-aspartate (NMDA)-type or alpha-amino-3-hydroxy-5-methylisoxazole-4-propinate (AMPA)-type glutamate receptors could inhibit neuronal loss, indicating an involvement of the glutaminergic system in the induction of cell death in infected and non-infected cells. Finally, they showed that following CDV infection, there was a steady increase in extracellular glutamate in infected cultures. The results of Brunner et al. [56] indicate that CDV infection induces excitotoxic insults on neurons via glutaminergic signaling. It can be speculated that such deleterious molecular events may take place *in vivo* in naturally occurring nervous distemper. Thus, the neuropathological aspects and neuroanatomical distribution of CDV-related polioencephalomalacia lesions might be due to the association of: i) neuronal death via CDV-induced glutaminergic imbalance [56]; and ii) anterograde CNS invasion via the olfactory pathway [54].

3.4 Inclusion body polioencephalitis

The lesions of inclusion body polioencephalitis are predominantly found in the grey matter with minimal to absent white matter involvement. Such lesions are localized mainly in the nuclear regions of the brainstem (diencephalon, mesencephalon, and medulla oblongata) and additionally in the cerebral cortex of some dogs [25, 57]. Although less evident, occasional lesions may be present in the cerebellar nuclei. Not all brainstem nuclei are equally affected. There is some individual variation in the severity and extent of the pathological changes. Brainstem lesions are always present, but cerebral cortex involvement may be absent in some cases [25, 57].

Initially, inclusion body polioencephalitis lesions were recorded in post-vaccinal encephalitis cases. Cornwell et al. [58] reported cases confirmed by virological assay as real cases of post-vaccinal encephalitis in which the main lesions consisted of irregular encephalomyelitis in the brain and spinal cord; the white matter was spared and the areas most severely affected were the deep neuronal layers of the cerebral cortex, the thalamus, the nuclei of the mesencephalon and medulla oblongata, and the dorsal horns of the spinal cord. Hartley [59] also reported cases of post-vaccinal inclusion body encephalitis in which the lesions were prominent in brainstem, mainly in the ventral pontine gray matter. However, cases have also been described [25, 57] where inclusion body polioencephalitis lesions were not considered as post-vaccinal cases because of the difference in the age of the animals and the time of vaccination.

3.4.1 Neuropathology of inclusion body polioencephalitis

The lesions of inclusion body polioencephalitis are characterized by mild to moderate perivascular cuffing of lymphohistiocytic infiltrates, glial nodules or diffuse reactive gliosis, edema, neuronophagia, loss of neurons, and foci of acute neuronal degeneration (normal or swollen neurons that are intensely acidophilic) and neuronal death (neuronal necrosis with swollen or shrunken and angular cells). Otherwise comparatively normal, several neurons present with large eosinophilic intranuclear or, less commonly, intracytoplasmic inclusion bodies [25, 57-59]. In addition, malatic lesions and blood-born mononuclear inflammatory and activated microglial cells may be observed diffusely scattered within the lesions. Lymphohistiocytic meningitis may also be observed [25, 57-59].

3.4.2 Pathogenesis of inclusion body polioencephalitis

The pathogenesis of such grey-matter lesions is not totally clear. The immune/inflammatory response within the neuraxis is dominated by T cells and is associated with strong major histocompatibility complex II (MHC II) up-regulation [57]. It was speculated that distemper-related inclusion-body polioencephalitis might be due to a non-productive CDV infection of neurons that is characterized by abundant expression of CDV nucleoprotein (NP) mRNA and reduced translation of the corresponding viral protein [25].

To date, the abundant expression of all viral protein mRNA and reduced protein translation, especially that of matrix proteins, is the most important finding in inclusion body polioencephalitis [57]. The restricted viral infection in the gray matter might represent a mechanism for viral persistence in distemper polioencephalitis. CDV persistence within the CNS may play role in the inflammatory response and other changes in affected areas. The reason why viral persistence and respective lesions in inclusion-body encephalitis are restricted to grey matter sites in brainstem nuclei and occasional cerebral cortical areas remains unclear.

4. Final considerations

The neuropathological manifestations of nervous distemper are diverse, and the causes of CDV neuropathology are varied and not fully elucidated. Many efforts have focused on conventional neuropathological aspects because CDV demyelinating encephalomyelitis may be used to model demyelinating conditions in man; however, there are also many non-conventional neuropathological manifestations of CDV infection. In Brazil, distemper is an endemic disease in urban canine populations [22, 60-62]; consequently, the possibility of observing non-conventional manifestations of CDV-induced neuropathological lesions can be elevated in urban dog populations in South America. Retrospective and prospective investigations have been carried out by the authors to understand the role of CDV infection in the development of non-conventional neuropathology in dogs from Brazil.

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