Canine distemper virus and multiple sclerosis: A real or an anecdotal association?

A. M. Amude[1,2], A. F. Alfieri[1], A. A. Alfieri[1]

[1] Laboratory of Animal Virology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, P.O. Box 6001, CEP 86051-990, Londrina, Paraná, Brazil
[2] Veterinary Neurology Service, Department of Small Animal Clinical Science, Universidade de Cuiabá, CEP 78015-480, Cuiabá, MT, Brazil.

Canine distemper virus (CDV) has been postulated to play a role in multiple sclerosis (MS), primarily on the basis of epidemiological evidence that has never been confirmed. However, this anecdotal association is still made by physicians and veterinarians. Although morbilliviruses such as CDV and measles virus (MV) potentially use the same cell surface receptor, CDV and others animal morbilliviruses have not yet been definitively implicated in any human disease. In this mini-review, the suggested but unproven involvement of CDV in MS will be addressed, and a possible explanation for the lack of CDV zoonotic infection will also be briefly discussed. The relationship between CDV infection and the development of demyelinating lesions will be revised because distemper is a disease associated with naturally occurring demyelinating encephalomyelitis, and the pathogenesis of distemper-related demyelination has been used for the study of demyelinating conditions in humans. Additionally, distemper-related demyelinating encephalitis shares important neuropathological aspects with MS.

Keywords dog; canine distemper virus; morbillivirus; central nervous system; demyelination; multiple sclerosis; demyelinating encephalomyelitis

1. Introduction

Multiple sclerosis (MS) is a demyelinating condition that affects the central nervous system (CNS) of humans, and it seems to be the most common neurological disease in young and middle-aged adults. Epidemiological observations strongly suggest that MS may be caused by an infectious agent that induces an immune-mediated demyelinating disease [1-3].

Canine distemper virus (CDV) is an important etiological agent of demyelinating encephalomyelitis in dogs [4, 5]. Distemper encephalitis was initially described as “acute MS of the dog” by human neuropathologists [6]. CDV was postulated to play a role in MS primarily on the basis of epidemiological evidence that has never been confirmed. These results may have been biased because no more evidence has been found. However, this unproved association is still occasionally cited in the literature. The association of CDV with MS is anecdotal; however, this anecdotal association is still referred to by both physicians and veterinarian.

Wide geographical differences occur in MS, and the incidence increases further from the equator [7-9]. Thus, it was initially hypothesized that the geographical distribution of MS was due to the stability of CDV, which decreases towards the equator [10]. However, distemper has been efficiently controlled after the widespread use of vaccination [11] and in many developed countries far from the equator, distemper is currently extremely rare [11], although the incidence of MS has apparently not changed [8, 9].

The observation that the incidence of MS increases further from the equator has prompted considerable interest in the factors that might underlie this latitude gradient; potential candidates include population frequencies of disease-associated human leukocyte antigen (HLA) alleles, which are the major genetic component of MS susceptibility [9]. For countries with HLA, UV and smoking data, these three factors were shown to account for 75% of the variance in MS prevalence [9]. Genetic (HLA) and environmental (UV and smoking) risk factors thus interact in a complex manner to determine a large proportion of MS susceptibility within countries from the equator [9].

In addition, the latitude gradient characterized in older studies of MS is decreasing; although higher latitude was associated with higher MS incidence, this latitude gradient was attenuated after 1980, apparently due to increased incidence of MS in lower latitudes [8].

In this mini-review, a possible explanation for the lack of CDV zoonotic infection is discussed, and the suggested but unproven involvement of CDV in MS is addressed. The relationship between CDV infection and the development of demyelinating lesions is also revised, as distemper is a disease associated with naturally occurring demyelinating encephalomyelitis and the pathogenesis of distemper-related demyelination has been used for the study of demyelinating conditions in humans. Additionally, distemper-related encephalitis shares important neuropathological aspects with MS [4].

2. Important considerations about canine distemper virus infection

Morbillivirus such as canine CDV and measles virus (MV) are non-segmented single-stranded negative-sense RNA viruses belonging to the *Paramyxoviridae* family; infection leads to a wide range of clinical signs in their natural hosts (dogs and humans, respectively), varying from a characteristic mild self-limiting infection to death [12]. Both CDV and MV are etiological agents for acute to chronic encephalitis in their respective hosts [13].

Canine distemper has been recognized as a disease entity for over two hundred years. CDV is an important pathogen involved in nervous disturbances of dogs, even when the typical clinical evidence for CDV infection is absent [14, 15]. Although distemper has been successfully controlled by vaccination on developed countries [13], the disease is still endemic in many regions [14, 16, 17] and has also even been recorded in vaccinated dog populations [18, 19]. Moreover, some special forms of chronic distemper encephalomyelitis occur in vaccinated dogs [20-22].

Recognition that CDV infection 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 CNS diseases caused by morbilliviruses [23, 24]. New paramyxoviruses such as the Hendra and Nipah viruses have been discovered to cause lethal meningoencephalitis in human and animals [25-27]. As these viruses show the same pattern of pathogenesis as already-known paramyxoviruses [28], knowledge about the neurobiology and neuropathology of CDV may give insight into disease mechanisms and suggest approaches for prevention and diagnosis of these newly discovered paramyxovirus infections.

3. Distemper-related demyelinating encephalomyelitis

The first description of CDV-associated demyelination originates from 1877 by Gowers and Sankey [4]. The lesions of canine distemper encephalitis fascinated human neuropathologists such as H.J Scherer who quite boldly described it as “acute MS of the dog” [6]. A spontaneous MS-like disease with multifocal demyelinating lesions is rare in domestic animals. Primary demyelination in domestic animals has only been observed for Visna, a retrovirus (lentivirus) infection in sheep, and in CDV infection [29]. While demyelination is a rare complication in the former, it occurs with high frequency in distemper-related encephalomyelitis [4].

The recognized effects of CDV on CNS tissues include acute encephalopathy, acute encephalitis, and acute to chronic demyelinating encephalomyelitis [30]. A rare, distinct chronic non-conventional encephalomyelitis in mature dogs termed *old dog encephalitis* (ODE) [21, 22] and an uncommon form of CDV-induced encephalitis, *inclusion body poliencephalitis* [31, 32], have also been reported.

Although the manifestations of CDV neuropathology appear broad, CDV-related demyelinating encephalitis is the most frequent form of nervous distemper in both naturally occurring and experimentally induced disease [4, 29, 30, 33, 34]. In addition, in most instances the demyelinating lesions occur even in absence of consistent clinical signs. Experimental studies have detected demyelinating lesions even in dogs with subclinical CDV infection (dogs infected with CDV that do not display signs of the disease) [33, 35].

The demyelinating lesions of CDV encephalomyelitis are not only responsible for severe neurological signs but are also thought to be a natural model for human demyelinating conditions such as MS and MV-induced subacute sclerosing pancephalitis [4, 24, 36]. Many of the experimental rodent models that have been proposed for study of the demyelinating conditions of CNS are artificial and not reproducible under natural conditions; however, naturally occurring distemper encephalomyelitis may be used for the study of such demyelinating lesions [4]. For these reasons, the pathogenesis of CDV demyelination has been extensively studied, and the neuropathology of such lesions has been the focus of recent studies [37-50].

3.1 Neuropathological manifestation of CDV demyelination

CDV demyelinating encephalomyelitis is associated with selective loss of myelin sheets accompanied by different degrees of vacuolar change (sponge state, sponge degeneration, or sponge change) within the injured white matter as determined by light microscopy (HE). Different levels of mononuclear infiltration at the perivascular space and nervous parenchyma and various degrees of glial cell reactivity (mainly astrocytes) have also been recognized [4, 51].

Studies have to consider an acute to chronic stage in the development of CDV-induced demyelination according to the degree of demyelination and inflammation within the white matter [4]. According to the degree of demyelination, inflammation, and reactive changes as well as the presence of malacia within the injured white matter, distemper-related demyelinating lesions have been characterized as hyperacute, acute, subacute, and chronic. The spectrum of the CDV-induced demyelinating lesions appears to be wide; however, the neuropathology of spontaneous demyelinating distemper-related encephalomyelitis is remarkably consistent. The variability in neuropathology is largely due to the evolution of the lesion with disease progression [46, 52]. Some variability might be attributed to virus strain differences, although there is little concrete evidence that these play a definite role in natural disease in dogs [52].

Each type of CDV-related demyelinating lesion (hyperacute, acute, subacute, and chronic) is described below. For lesion characterization, the authors adapted a neuropathological score [34] following parameters previously reported [30, 46, 49-54].

3.1.1 Hyperacute demyelination

In both naturally occurring and experimentally induced hyperacute demyelinating lesions, no white matter changes are present with the exception of scattered few small vacuoles [46]. In such a neuropathological presentation, morphological features of inflammation are absent. The myelin content as assessed by special myelin stain (luxol fast blue) is normal or only slightly reduced compared to non-affected control regions. There is no pallor of the white matter on HE staining, and CDV inclusion bodies may be often recognized [34].

3.1.2 Acute demyelination

Acute demyelinating encephalomyelitis is characterized by focal or multifocal to diffuse demyelinating plaques accompanied by obvious white matter vacuolization (different degree of spongy change). Mostly irregular shaped vacuoles are widely spaced in some lesions, and in others, the tissue has a marked spongy appearance [51]. Occasionally, an increased number of reactive astrocytes, a few gemistocytes, and mild microgliosis may be observed [30, 53].

In most cases, perivascular cuffs are lacking; however, scattered lymphocytes occasionally might be found around blood vessels, forming not more than one layer of cells [52]. In some unusual situations, scattered cuffs with three to five layers of lymphocytes may be recognized episodically within the neuraxis in the absence of other inflammatory, necrotizing, and reactive changes [34]. In such demyelinating lesions, foci of malacia with Gitter cell infiltration are not present [54]. Myelin staining shows a mild to moderate decrease in myelin content within the affected areas. On HE staining, mild to moderate pallor of the demyelinated white matter may be recognized and CDV inclusion bodies may or may not be observed [50].

3.1.3 Subacute demyelination

In subacute demyelinating encephalomyelitis, the demyelinating plaques are characterized by a moth-eaten appearance (severe spongy degeneration), foci of malacia with Gitter cell infiltration, varying degrees of astrocytic hypertrophy and hyperplasia (astrogliosis and astrocytosis) with gemistocytes and multinucleated astrocytes [51, 54]. In addition, perivascular mononuclear cuffs may be absent or observed in some plaques [30, 50, 53]. Histiocytic cells with different signs of reactivity may be seen around the same blood vessels, and occasionally scattered lymphocytes may be present [49]. However, not all changes are present in each individual lesion. The myelin content is markedly reduced compared to hyperacute and acute lesions, and the HE stain is marked by severe pallor of the vacuolated white matter. Caution should be taken with pallor on HE staining as this measure is very subjective and does not necessary imply tissue destruction. Pale staining or improper fixation can have the same effect [34].

3.1.4 Chronic demyelination

In chronic demyelinating encephalomyelitis, the invasion of inflammatory cells is prominent. Influx of lymphocytes may be observed around blood vessels (Virchow-Robin space), forming partly multi-layered perivascular cuffs, and immune cells (lymphoplasmacytic cells) diffusely invade the parenchyma [4, 49, 51]. Astrocytic gliosis (fibrous astrocytes) is prominent, and gemistocytes and Gitter cells also may be observed [51, 54].

Chronic demyelinating plaques may be observed to be markedly spongy with relatively small vacuoles or more diffuse with large vacuoles, probably due to tissue edema. Severe destruction of the white matter may also be observed with large areas of malacia and severe Gitter cell infiltration [46]. Frequently, malacia is accompanied by a severe inflammatory reaction with intense infiltration of lymphoplasmocytic and/or lymphohistiocytic cells. In such foci of chronic demyelinating lesions with malatic and inflammatory changes, the edema and severe destruction of the white matter may obscure the vacuolar change, which makes the spongy state less obvious [46, 50].

The myelin content as assessed by special myelin staining is moderately reduced in areas of diffuse demyelination and may almost completely vanish in areas of compact demyelination. Pallor of the white matter should be judged cautiously on HE staining, as artifacts or edema due to inflammatory reactions may mimic pallor [34].

3.2 Pathogenesis of acute demyelination

The acute initial myelin lesions develop during a period of severe immunosuppression and are not inflammatory [55]. This mechanism of acute demyelination has been examined and it was shown that the initial lesions are directly virus-induced, as there is a clear correlation between the occurrence of demyelination and CDV replication in the cells of the white matter [33].

The most likely explanation for demyelination would be oligodendroglial infection. Segmental demyelination [56] and degenerative oligodendroglial changes in acute foci [57] are strongly suggestive of a primary oligodendroglial lesion. However, despite considerable efforts using immunocytochemical and ultrastructural techniques, CDV proteins and viral nucleocapsids were rarely observed within oligodendrocytes when compared to astrocytes and microglial cells, which easily support CDV infection [33, 58, 59].

Advanced studies *in vitro* and *in vivo* on oligodendroglial pathology in canine distemper revealed a restricted CDV infection (CDV mRNA expression without viral protein production) of oligodendrocytes that led to a massive down-regulation of myelin gene expression [38, 60]. However, the number of CDV mRNA-expressing oligodendrocytes found in infected but not-yet demyelinated areas seems rather small (8% of all oligodendrocytes in the infected area) to explain ensuing demyelination [38].

Most infected cells in the CNS are astrocytes [46, 57, 61], which are important for maintaining tissue homeostasis. Alteration of the astrocyte population might contribute to the development of demyelination, and an indirect mechanism may participate in oligondendrocyte dysfunction [4, 5, 52, 54].

Findings in acute non-inflammatory demyelination included viral replication in astrocytes [35], restricted infection of low numbers of oligogendrocytes [38], mild diffuse invasion with CD8+ cells [44], up-regulation of the CD44 hyaluronate receptor [42], and up-regulation of reactive oxygen species (ROS) [62]. However, none of these findings by themselves has provided a satisfactory explanation for myelin destruction in distemper.

Diffuse up-regulation of major histocompatibility complex class II (MHC II) in the white matter in the early state of distemper [37], up-regulation of metalloproteinases [48, 63], and immunocytochemical evidence of microglial hyperplasia in initial demyelinating lesions [39] suggest that microglia could be involved in the pathogenesis of early demyelination. Stein et al. [47, 62] showed a clear correlation between up-regulation of various microglial functions and the presence of demyelination.

Yarin et al. [43] demonstrated that progesterone concentration decreased in SNCs from dogs with CDV demyelination. The decrease in progesterone concentration may play a role in distemper demyelination, as progesterone has neuroprotective effects including augmentation of myelination in the central and peripheral nervous system. Despite extensive studies and speculation, the mechanism of acute demyelination in distemper is not completely understood.

3.3 Pathogenesis of chronic demyelination

The chronic lesions of distemper-related demyelinating encephalomyelitis are characterized by the influx of inflammatory cells, mostly mononuclear, and coincide with the recovery of the immune system [55]. A detailed immunophenotyping study of the associated cells revealed that, dependent on the age of the demyelinating plaque, CD4+, CD8+, and B cells show a different spatial distribution [41]. Accordingly, CD8+ lymphocytes invade the brain earlier and are the dominant cell population in the neuropil, while in the perivascular space the lymphocytic infiltrate is mainly composed of CD4+ and B cells.

The inflammatory reaction in the chronic demyelinating lesions leads to progression of the tissue damage and plays an important role in demyelination at this stage of the lesion due to immunopathological complications that occur in the attempt to remove CDV from the CNS. Tissue damage and demyelination may result from bystander effects of infiltrating virus-specific T and B cells and their cytokine products [52].

Inflammation is also associated with intrathecal immunoglobulin synthesis. It has been known for a long time that antiviral antibodies play a dominant role in immunity against CDV. Bollo et al. [64] found that the occurrence of anti-CDV antibodies in the cerebrospinal fluid coincided with the clearance of CDV and CDV-containing cells from the inflammatory lesions. Because most oligodendrocytes do not express CDV proteins [38], the progression of demyelination cannot be effectively explained by an antiviral cytotoxic reaction with destruction of infected oligodendrocytes. Other types of antiviral immune response could be responsible for the inflammatory tissue damage seen in distemper. Macrophages, which are very numerous in distemper lesions, could play an important role. It was shown that antiviral antibodies bound to the surface of CDV-infected cells interacted with the Fc receptors of neighboring macrophages via their Fc domains [65-67]. This interaction resulted in a respiratory burst of the macrophages with release of reactive oxygen radicals, which can be harmful to oligodendrocytes. Thus, the humoral antiviral immune response could also lead to destruction of oligodendrocytes through a bystander effect [52].

Viral persistence is the key to the pathogenesis of chronic lesions and seems to contribute to lesion maintenance and progression [68]. The mechanism of CDV persistence is not yet completely understood. *In vitro* studies have suggested that persistence of CDV in the neural cells is favored by non-cytolitic spread of virus, with reduced viral spread to extracellular space. Thus, very little or no virus is freely released outside of neural cells [69-71]. *In vitro* ultrastructural examination confirmed that release of virus particles by budding is a rare event in primary dog brain cell cultures infected with virulent CDV strains [69, 70]. These mechanisms studied *in vitro* could enable the spread of CDV within nervous tissue *in vivo* without requiring virus release to the extracellular space. In chronic cases, virulent CDV probably spreads from cell to cell via cellular processes, with very little release of infectious particles into the extracellular space and without cytolysis [72]. This mechanism of viral persistence in chronic encephalomyelitis allows CDV to remain in the CNS even in the presence of an effective antiviral inflammatory response. Thus, CDV escapes from virus-hostile sites to other sites where inflammation has not yet occurred [33, 53, 64, 72].

Molecular studies comparing the virulent A75/17 CDV strain with the attenuated Onderstepoort strain have been performed to identify molecular mechanisms of virus persistence. Initially, the nucleocapsid (N) protein was suggested to be the molecular determinant of persistence [73]. However, other wild-type CDV proteins are also involved in the mechanisms of viral persistence. Persistent noncytolytic infection is associated with very limited cell-cell fusion, and it was found that fusion efficiency was markedly attenuated by the structure of the fusion (F) protein of the neurovirulent A75/17 CDV strain [74]. Moreover, it was also observed that the interaction of the surface glycoprotein [hemagglutinin (H) and F] with the matrix (M) protein of the persistent strain greatly influenced fusion activity. Site-directed mutagenesis showed that the C-terminus of the M protein is of particular importance in this respect. Interestingly, although the N protein alone did not affect H/F-induced cell-cell fusion, maximal inhibition occurred when the latter was added to a combination of glycoproteins with M protein [75]. Thus, very limited fusogenicity in virulent CDV infection, which favors persistence by limited cell destruction, may involve complex interactions between all viral structure proteins.

Evidence of autoimmunity is not unusual in viral infections in different organ systems, including the brain [3]. The role played by autoimmunity was already questioned with respect to the pathogenesis of CDV demyelination. Although such a discussion may still be occasionally found in studies that discuss the pathogenesis of CDV demyelination, it has never been proven that autoimmunity plays a real role on CDV demyelination [4, 52]. Autoantibodies have been found in dogs with distemper. Anti-myelin antibodies in the serum have long been known to occur in distemper [76]. Vandevelde et al. [77] also found such antibodies in the cerebrospinal fluid of dogs with distemper; these antibodies were found to be locally produced in the inflammatory brain lesions. One mechanism by which anti-myelin antibodies could induce demyelination would be via antibody-dependent cytotoxicity; however, such antibodies fail to elicit antibody-dependent cytotoxicity [66]. The autoimmune reactions in distemper are probably epiphenomena that are not primary involved in the chronic demyelinating process [4, 52].

4. CDV and human infection

The host range of CDV includes domestic and non-domestic carnivores [78]. There is a single report of natural CDV encephalitis in a monkey (*Macaca fuscata*) [79], and at the time of that description, the possibility of human infection was raised. However, evidence for the ability of CDV to cause systemic infection in humans is very weak [12].

Morbilliviruses such as CDV and MV potentially use the same cell surface receptor [80, 81]; however, CDV and others animal morbilliviruses have not been yet been definitively implicated in any human disease [12]. However, CDV has been suggested to be associated with human bone diseases such as Paget's disease [11, 12].

Morbilliviruses are readily cross-neutralized; this property was exploited in the past in the use of MV vaccine to protect cattle from rinderpest when no appropriate vaccine was available. A heterotypic MV vaccine was already used to immunize puppies against distemper [82], and the administration of egg-adapted CDV (Lederle-CDV strain) to children conferred a degree of protection against natural infection with measles [83]. Hence, early exposure of most of the human population to MV in the presence of a maternal antibody against MV in association with widespread vaccination against MV vaccination suggests that anti-MV antibodies could neutralize CDV would hinder or prohibit infection by CDV.

5. Why was CDV suspected to cause MS?

Although, CDV probably does not naturally infect humans, it has been proposed to be an etiological agent of MS. The CDV-MS association was raised because of an apparent connection between prolonged contact with pet animals and some cases of MS [84]. In addition, exposure to animal illness, specifically canine distemper, was a factor found to be associated with the development of MS [85]. In subsequent reports, dogs and CDV specifically became the focus of attention.

CDV was postulated to play a role in MS primarily on basis of epidemiological evidence that has not yet been confirmed. The results used to derive such evidence were probably biased because no further results have been found. A flurry of epidemiological and serological studies largely failed to support a dog-MS association [86, 87]. MS continues to be seen in some countries in which dog numbers have diminished (such as Iceland) and more widely in the western world despite the dramatically reduced incidence of CDV infection in dogs following the introduction of effective vaccines four decades ago [12].

The detection of anti-CDV-like antibodies in the cerebrospinal fluid (CSF) of MS patients suggests a possible link to CDV [88], but this study probably was confounded by the cross-reactivity of CDV with MV-specific antibodies [12]. In addition, increased levels of antibodies to a number of viruses including MV have been observed in serum and CSF samples from MS patients [89, 90]. However, these appear to be non-specific reactions, as antibodies to a number of others viruses are also increased [3,12].

CDV and MV have also been suspected as etiological agents of MS because both viruses (distemper and measles) can demonstrate persistent infection within the CNS, resulting in a progressive and multifocal demyelinating disease [71, 72, 91]. However, the role of CDV and MV in MS has never been proven [3, 11].

Brain samples from patients with MS were examined by immunohistochemistry using a panel of MV and CDV monoclonal antibodies [92]. All antibodies were negative except for one anti-MV antibody that bound to 8/9 MS plaques and 2/5 herpes simplex virus encephalitis brain samples, but not to six controls or samples from four patients with ischemic stroke. However, no evidence for the presence of MV in MS plaques was obtained by RT-PCR, calling into question the specificity of the antibody. Geeraedts et al. [92] suggested that this monoclonal anti-MV antibody might recognize an epitope of an as-yet unrecognized morbillivirus present in the human CNS that might be implicated in MS pathogenesis. Alternatively, a protein up-regulated during inflammation might be responsible for the observed effect due to non-specific immunolabeling [92]. The latter is the most likely scenario, as Sheshberadaran and Norrby [93] identified a cross-reactive epitope on a 79 kD stress protein.

6. Final considerations

A direct link was unequivocally established between CDV and MS, and CDV infection of humans is probably not widespread under natural conditions. The neuropathological features and pathogenesis of CDV demyelination were reviewed because distemper-related demyelinating encephalomyelitis shares some neuropathological aspects with MS, such as acute non-inflammatory demyelinating lesions followed by the chronic progression of demyelination marked by non-suppurative inflammation with perivascular cuffing and mononuclear invasion of the parenchyma [4, 94]. Additionally, up-regulation of MHC II, the CD44 hyaluronate receptor, and metalloproteinases, as well as microglial cell activation, has been shown to play a role in pathogenesis of demyelination in both distemper and MS [37, 42, 47, 48, 62]. Moreover, naturally occurring distemper-related demyelinating encephalomyelitis may be used for the study of demyelination conditions of the CNS [4].

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