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# Evidence for multiple sclerosis as an infectious disease

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Evidence for a viral cause of multiple sclerosis (MS) is indirect since no infectious agent has been reproducibly isolated from MS tissues nor has viral genome or antigen been consistently identified. The occurrence of spontaneous human and animal models of demyelination, serologic studies, and epidemiologic data provide pursuasive circumstantial evidence for an infectious trigger in this disease. Potential mechanisms for viral induced demyelination include persistent infection of host tissues or immune mediated organ damage either in the presence or absence of the infectious agent. Any proposed viral candidate should cause demyelination in animals or man and the pattern of infection should be consistent with the unique geographic features of MS epidemiology. In addition, serologic studies should support an infection by the agent and/or viral genome should be detected in MS tissues. At this time no virus can be unequivocally linked to MS but cumulative evidence is more supportive of canine distemper virus than other viruses.

**S. D. Cook, C. Rohowsky-Kochan, S. Bansil, P. C. Dowling**

Department of Neurosciences, UMDNJ-New Jersey Medical School, Newark, VA Medical Center, East Orange, New Jersey, USA

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S. D. Cook, Department of Neurosciences, UMDNJ-New Jersey Medical School, Newark, NJ, USA

Multiple sclerosis (MS) is an acquired inflammatory demyelinating disorder of the central nervous system (CNS) with varied clinical manifestations. The world-wide prevalence of MS is quite distinct with an as yet unexplained crude north-south gradient. The prevalence of MS is increasing in many areas probably due to better case ascertainment, and longer life expectancies. Because of better case ascertainment it is unclear whether or not incidence rates are also changing. Although the etiology and pathogenesis of MS are unknown, accumulating evidence supports the hypothesis that one or more infectious agents triggers the disease in a genetically susceptible host. Subsequently, by mechanisms not yet precisely defined, immune mediated tissue injury is thought to ensue, leading to characteristic recurrent demyelinating lesions in the brain and spinal cord.

At present, evidence for an exogenous cause of MS is indirect. Although numerous infectious agents have been postulated to cause MS no agent has been reproducibly isolated from MS tissues; viral particles have not been convincingly demonstrated by electron microscopy; and neither viral antigen nor genome have been consistently found in MS specimens using sophisticated molecular techniques, including polymerase chain reaction (PCR) (Table 1). Several excellent publications on the virology of MS and other demyelinating diseases have appeared in recent years (1–6). In this paper we review the evidence for a viral cause of MS and propose that canine distemper virus (CDV) is a leading candidate for causing MS in some individuals.

## Evidence for a viral cause of MS

Evidence for environmental factors includes the unique worldwide prevalence of MS, the effect of migration on susceptibility (7) and the relatively low concordance rate of MS in monozygotic twins (31%) even with brain MRI studies and long term

Table 1. Negative or unconfirmed studies for infectious antigen or genome in MS

| | |
|---|---|
| HSV 1, 2 | *Borrelia burgdorferi* |
| HTLV 1, 2 | Measles |
| CMV | Mumps |
| EBV | Rubella |
| Coronavirus | CDV |
| JC | SV5 |

follow-up (8). A similar concordance rate in monozygotic twins has been reported in paralytic poliomyelitis, a disease of known viral cause (9). Suggestive evidence for a viral cause of MS includes the occurrence of spontaneous viral models of CNS demyelination, some of which may be associated with remissions and exacerbations as well as progression (Table 2); increased titers of viral antibodies in the serum and cerebrospinal fluid (CSF) of MS patients, particularly to measles virus (MV) and less commonly to other viruses (1) (Table 3); later onset of childhood infections with MV and other viruses in areas of high MS prevalence (10–15, 16–17) and reports (not always confirmed) of time clustering of MS in several distinct populations (18–23). The inflammatory CNS pathology and the presence of increased IgG and oligoclonal bands in CSF are consistent with an infectious process but can also be seen with immune mediated neurological disorders. In MS the target antigen for oligoclonal IgG, be it a foreign agent or a brain constituent, is unknown.

## Proposed mechanisms of viral-induced demyelination

Assuming MS is triggered by an infectious agent there are several major mechanisms whereby demyelination may be induced. The virus may be present in brain or other host tissues but has as yet avoided detection. Failure to identify an infectious agent to date in MS tissue should not be interpreted as proof that a virus is not present. Recent examples of infectious diseases in which the agent was not identified for many years include Heliobacter pylori induced peptic ulcer (24), Lyme disease (25), and cat scratch disease (26). CDV an RNA morbillivirus of dogs; JHM, an RNA mouse coronavirus; and another RNA virus, Theilers murine encephalomyelitis virus are examples of animal demyelinating diseases in which viral genome usually remains detectable in brain (27). In some human demyelinating disorders, not pathologically similar to MS, virus can be readily identified with appropriate probes. This includes PML, HIV and HTLV-1 (28–30).

Alternatively, infectious agents may trigger an autoimmune process and no longer be present in the host target organ when disease is clinically apparent. If such is the case in MS, linking a virus to the disorder may be very difficult and will probably require a combination of serological and epidemiologic evidence of infection. Examples of this mechanism of tissue injury following infectious disease may include postmeasles encephalomyelitis (PMEM), streptococcal induced rheumatic heart disease and the Guillain-Barré syndrome (GBS). In PMEM evidence suggests that MV does not directly invade the CNS. MV has rarely been isolated from the CNS and both immunocytochemical and *in situ* hybridization studies for viral proteins and genome have been consistently negative (2). Instead it has been postulated that the demyelinating lesions in PMEM are immune mediated. Streptococcal induced rheumatic valvular disease may be another model of this mechanism of disease (31). Molecular mimicry may also play a role in GBS. This disorder is frequently associated with an acute infection by cytomegalovirus (CMV), Epstein-Barr virus (EBV), mycoplasma pneumonia (MP) or *Camplobacter jejuni* (32–34). Each of these agents induces the host to produce antibodies to glycolipids or glycoproteins including cold agglutinins reactive to red blood cell membrane components (35) or antibodies to gangliosides including the GMI ganglioside shared by *Camplobacter jejuni* and neural tissue (36). Similarly, peptide homologies have been recognized between a number of viruses including hepatitis B, MV, CDV, EBV and several CNS antigens (11).

Lastly, the virus may be immunomodulating or immunosuppressive through its effect on host lymphocytes thereby altering immune surveillance allowing the emergence of autoreactive T cells or antibodies. MV and CDV are examples of pathogens having this property (2, 37).

Table 2. Human and animal models of inflammatory CNS demyelinating diseases

| | | |
|---|---|---|
| Human – | ADEM-PIEM (PML, HIV, HTLV1) | |
| Animal – | CDV | Visna |
| | JHM | Other |
| | Theilers | |

Table 3.

| | |
|---|---|
| MS serum antibody significantly higher than controls | |
| Measles | Herpes simplex |
| CDV | Adenovirus |
| Vaccinia | Varicella |
| EBV | Influenza |
| Rubella | Parainfluenza |
| MS CSF antibody increased vs. controls | |
| Measles | Human coronavirus |
| Rubella | Influenza A, B |
| Herpes-zoster | Respiratory syncytial virus |
| Vaccinia | Adenoviruses |
| Herpes simplex | Parainfluenza 1, 2, 3 |
| Mumps | EBV |
| Mycoplasma pneumonia | |
| Cytomegalovirus | |

### Candidate agents

In GBS known to be triggered by multiple infectious agents, a remarkably uniform baseline worldwide incidence is seen, as well as a wide age range of host susceptibility (38). In contrast the unusual global distribution of MS and peak susceptibility in young adults is consistent with a single agent or relatively few agents as a cause of MS worldwide.

The agent(s) causing MS may infect man frequently with MS being an uncommon complication similar to paralytic poliomyelitis after poliovirus infection. Alternatively, the agent(s) may infrequently infect man, but when it does may commonly cause neurological disease. An example of this type of disorder is human rabies. In the former instance it may be difficult to link a virus to MS by serological methods, whereas in the latter situation it would be much easier.

### Human viruses

While no virus can be clearly linked to MS at present, several ubiquitous human viruses remain as possible candidates in causation since they trigger acute demyelinating disease of the CNS or PNS, serologic studies show elevated antibody titers to these viruses in MS patients or because infections with these viruses may explain the global pattern of MS. MV, the human coronavirus 229E, and EBV fulfill some of these criteria.

Measles is the virus most commonly associated with post infectious encephalomyelitis, an acute demyelinating disorder in which lymphocytes reactive with myelin basic protein are present in peripheral blood (2). In several epidemiologic studies measles infections have been shown to occur at a later age in MS patients than controls, particularly in geographic areas where MS is more common (10–17, 38). MV antibodies are frequently elevated in MS serum and CSF whereas lymphocytes from MS patients have a reduced cytotoxic effect on MV infected cells (39). Measles antibodies are elevated to many measles antigens, demonstrated with various serological methods (1, 40–42). MV peptides also share amino acid sequences with human MBP (11). Although MV genome has been detected in some MS brain specimens (43,44), genome has not been identified in other studies even using sensitive PCR techniques (45–47). Evidence against measles as the sole cause of MS includes documented cases of MV infection post MS, the lack of correlation of MV infections with the worldwide pattern of the disease (i.e., north-south gradients and clustering) and most importantly the failure of measles vaccine to prevent MS (48).

The coronavirus 229E is a ubiquitous agent causing upper respiratory infections in humans. Although no increase in serum antibody to 229E has been demonstrated, in one unconfirmed study CSF antibody to this virus was detected in 26% of MS patients but not in controls (49). A receptor for 229E has been demonstrated in brain synaptic membranes and cultured neural cells can be infected by this virus. A mouse coronavirus, JHM can cause demyelinating disease in mice, rats and primates (27). In one study human coronavirus 229E genome was found in 4 of 11 MS brain samples as compared to none of 11 controls (50), but others have been unable to confirm this observation using PCR (47). Although these findings are provocative, in our opinion more seroepidemiologic evidence or confirmation of viral genome in MS, but not control brain, is needed before considering 229E as a serious candidate agent in MS.

EBV can produce acute demyelinating disease in man, elevated EBV antibody has been demonstrated in MS serum and CSF (51–53), EBV infection has been reported to occur at a later age in MS patients than controls (17), and in one study 5 patients developed an MS-like illness following a neurological illness associated with an EBV infection (54). EBV also shares common amino acid sequence with MBP (11). Unfortunately, the serologic findings are difficult to interpret because of the ubiquitousness of EBV infections, the high rate of positive serology in controls, and the possibility that latent EBV may be reactivated due to immune alterations naturally occurring in MS or associated with MS immunosuppressive therapies. Nevertheless, EBV cannot be excluded as a viable candidate agent in MS.

### Animal viruses

The ideal characteristics for an animal virus proposed to cause MS are shown in Table 4. Several animal viruses can produce CNS demyelinating disease with remissions and exacerbations as well as progression. These include the coronavirus, mouse hepatitis, CDV, Theilers virus and visna (Table 2). Visna, an RNA lentivirus of sheep is unlikely to cause MS since human-sheep exposure would not explain the worldwide pattern of MS, and neither serologic evidence of visna antibodies nor viral genome has been reported in MS. Antibodies to mouse coronavirus and Theilers virus have not been shown to be increased in MS sera or CSF, and there is no obvious epidemiologic relationship between these viral infections of mice and MS (50). Although in one study mouse hepatitus genome was identified in 12 or 22 MS brain samples by *in situ* hybridization using cloned coronavirus cDNA probes, and antigen was identified

in two patients using a monoclonal antibody to JHM (55), coronavirus genome was not detected using PCR (47). To date, Theilers virus genome has not been found in MS tissues (55). Thus, in our opinion, these animal viruses are unlikely to cause MS.

### The canine-distemper-multiple sclerosis hypothesis

Canine distemper virus, a measles-like morbillivirus in dogs, or a closely related virus has been proposed as a likely candidate in the causation of MS. The evidence for this controversial hypothesis is based on the following:

#### Demyelination with morbillivirus infections

Morbilliviruses produce spontaneous CNS inflammatory or demyelinating disease in a variety of species including man, seal, porpoise, dog, ferret, tiger, lion, and raccoon (2, 56–62). CDV is one of the most neurotropic forms of morbillivirus, with some strains causing demyelination in up to 90% of infected dogs (63) as compared to the 0.1% rate of post measles encephalomyelitis in man. CDV can cause CNS disease in a wide range of animals including primates (64), and can cause CNS demyelination with an acute, or relapsing-progressive course in dogs (65). CDV induced demyelination can occur weeks or months following an acute or subclinical systemic infection. Animals may have seizures, myoclonus, ataxia, paralysis, tremors or optic neuritis (66). As in MS, dogs with CDV infections of the CNS may have an increase in CSF IgG levels (67). In dogs with a relapsing-progressive course, plaques can form in the CNS and the pathologic lesions can be difficult to distinguish from those seen in MS (65). CNS demyelination is usually multifocal and periventricular in location. Although virus is usually found in the acute demyelinating brain, it may be difficult to detect or undetectable by conventional techniques in the relapsing-progressive form of this CNS demyelinating disease (65). It is generally believed that the acute demyelinating lesions are due to the direct lytic effect of CDV, whereas the chronic demyelinating lesions are probably immune mediated.

#### Dog exposure and MS

The CDV-MS hypothesis would imply that MS incidence and prevalence should be highest in cultures where dogs with CDV have the greatest contact with genetically susceptible individuals. Thus, MS should be more common in areas with high dog density, where distemper epidemics occur frequently, particularly in geographic regions with cold, damp weather when dog-human contact is apt to be maximized by dogs being kept indoors. Conversely, MS should be less common in areas where dogs are absent or present in low density, where dog human contact is less common because of cultural attitudes towards dogs or weather conditions, where dogs are kept outdoors, and where CDV occurs uncommonly.

Although limited data is available on dog demographics, MS and indoor dog density are presumed to be greater in Europe and North America than in India, China and Japan. Studies in the United States have shown a latitudinal relationship of dog demographics with a significantly higher proportion of dogs kept indoors in the colder northern than in the warmer southern United States, with intermediate rates being found in the mid tier states (68, 69). Although CDV, like measles, occurs in all climates, in a given area CDV peaks in colder, damper months, conditions conducive to greater human-ill dog contact (66, 70). A latitudinal relationship has been documented for other dog zoonosis. For example, hydatidosis in man is more common in the colder northern areas of Kenya where dogs are often kept indoors than in the warmer southern areas where dogs are primarily kept outdoors (71).

Although by no means as common as 20–30 years ago, distemper is still one of the more important infectious disease of dogs in Western Europe and North America despite the widespread use of vaccines (72). Epidemics of CDV have been followed by a significant increase in MS incidence rates in a number of islands including Newfoundland (20), Key West (73), Sitka (21), the Faroe Islands (74), and Iceland (75), although Poser denies an epidemic of MS and Kurtzke denies an epidemic of CDV in the Faroes (76, 77), and Benediktz disputes the existence of MS epidemics in Iceland (78).

Since Steiner originally noted high dog exposure in MS patients (78), and Chan postulated MS was spread from dog to man via infected urine (79), there have been at least 21 studies in the literature which have studied dog exposure in MS patients as compared to controls. Seven of 21 studies showed significantly more exposure to dogs, indoor dogs, small dogs or dog exposure 5 to 10 years before onset or probable onset of MS as compared in controls (80–89). No study showed significantly more dog exposure in controls. This result is remarkable in that dog exposure is generally high in the western world with 60–80% of controls having prior dog ownership in some European and US studies. Because the risk of developing MS is probably quite low even when an individual is exposed to the factor(s) causing MS, the high level of ex-

posure to dogs in the population makes retrospective small case control studies relatively insensitive for testing the CDV-MS hypothesis.

While dog exposure *per se* would be expected to be important if the CDV-MS hypothesis is correct, the more important factor would be exposure to dogs with CDV or a distemper-like illness. In this regard, 3 studies have noted significantly more CDV or a CDV-like illness prior to onset of MS in patients then in controls (69, 82, 90) and three other studies have noted more CDV exposure in MS patients, although not achieving statistical significance (91–93).

### Search for CDV antigen/genome in MS brain tissues

Several groups have unsuccessfully attempted to identify CDV antigen and genome in MS brain specimens using radioimmunoassay, Southern blots, in situ hybridization and highly sensitive PCR techniques (44, 94–96). However, the vaccine strain of CDV which was used for genomic studies may not recognize wild strains of CDV (96). Thus, it is not yet certain that wild strains of CDV are not present in MS tissue, and even if absent does not exclude the possibility that CDV acts as a triggering agent in MS inducing autoimmune demyelination by several possible mechanisms, i.e., viral coating with brain antigens, molecular mimicry, or alteration of immune regulation. In this regard, direct infection of brain periventricular white matter is known to occur in dogs with CDV infections (63,65), peptide homologies exist between CDV nucleocapsid and bovine proteolipid protein (11) and as with measles virus infection in man, host immunosuppression usually accompanies acute infection (2, 37).

### Older CDV serologic studies

Although a large number of studies have confirmed the finding of elevated measles antibody levels in MS serum, relatively few studies measured antibody to CDV, despite the fact that these viruses share common nucleocapsid antigens which serologically cross-react. Using a tissue culture neutralization assay, we carried out the first large scale study of CDV and measles virus neutralizing antibodies in patients with MS (97). The results of our study revealed that sera from 142 patients with MS had significantly higher CDV neutralization titers than age- and sex-matched normal or neurologic controls. Significantly higher measles antibody titers were also found in the MS group confirming earlier reports.

Several other studies have also shown a trend towards or a significant increase in CDV neutralizing antibodies in MS patients as compared to controls (12, 93, 98–100). In one study, the highest antibody titers were noted to virulent rather than vaccine strains of CDV (99), with no significant increases in antibody titer to 6 other dog viruses. Smaller studies or those utilizing different techniques found no difference in serum CDV titers between patients and controls. Unfortunately, these serological studies were unable to clearly distinguish between CDV antibody and cross reacting MV antibodies.

### New studies for CDV specific antibodies in MS

The nucleotide sequence and the deduced amino acid sequence of the hemagglutinin (H) gene of CDV were reported several years ago (101). We have analyzed the amino acid sequence of the H protein using the Hopp & Woods algorithm which predicts antigenic determinants based on the hydrophilic nature of the amino acids (102). Linear peptides composed of 15 to 16 amino acids corresponding to the three most hydrophilic regions of the CDV H protein were synthesized. Two of the three CDV-H peptides have one amino acid sequence in common with the corresponding MV peptides whereas the third has none. We have used these CDV-H peptides as antigens in ELISA assays to determine whether specific CDV-H peptide anti-

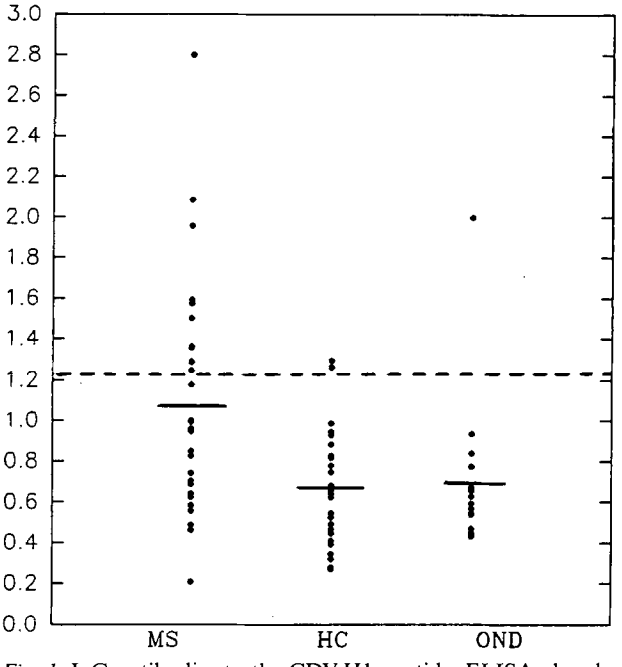


*Fig. 1.* IgG antibodies to the CDV-H1 peptide. ELISA absorbance values of CDV-H1 IgG antibodies in sera of MS patients, healthy controls (HC) and OND patients. Short solid line in each column denotes mean value of respective group. Long dashed line across figure represents absorbance values 2 standard deviations above mean of healthy controls.

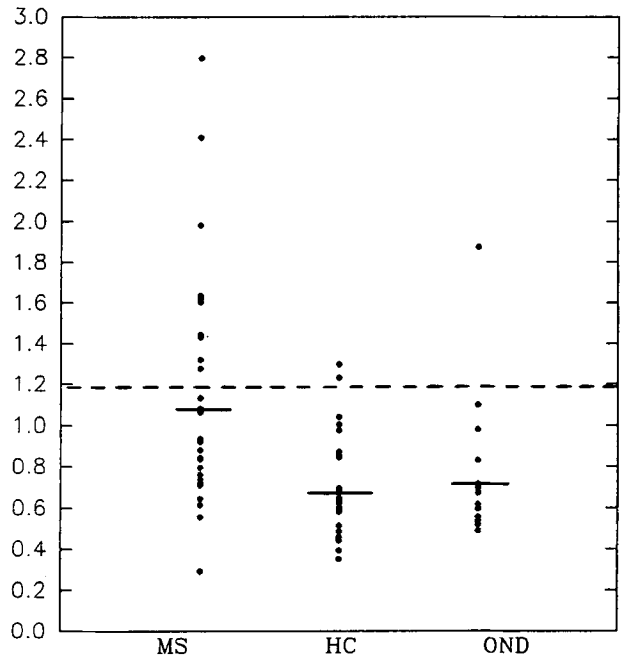


*Fig. 2.* IgG antibodies to the CDV-H2 peptide. ELISA absorbance values of anti-CDV-H2 in sera of MS patients, HC and OND patients. Short solid line in each column denotes mean value of respective group. Long dashed line across figure represents absorbance values 2 standard deviations above the mean of healthy controls.

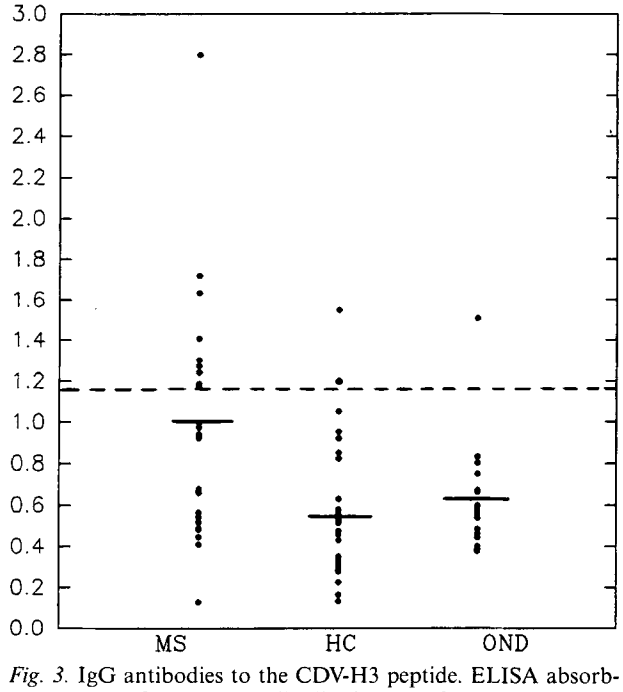


*Fig. 3.* IgG antibodies to the CDV-H3 peptide. ELISA absorbance values of CDV-H3 antibodies in sera of MS patients, HC and OND patients. Short solid line in each column denotes mean value of respective group. Long dashed line across figure represents absorbance values 2 standard deviations above the mean of healthy controls.

bodies can be detected in the sera of animals immunized with CDV and in human sera. Appropriate blocking studies and assays of human (SSPE, MV infection) and hyperimmune animal sera with high titers of CDV or MV antibodies showed the specificity of the assays for CDV.

Sera from 31 patients with clinically definite MS as defined by the Poser committee criteria (103), from 17 patients with other neurological disorders (OND) and 27 healthy controls were screened for CDV specific antibodies. Sera from MS patients exhibited significantly ($P<0.001$) higher levels of CDV-H1 peptide antibody than sera from OND patients or healthy individuals (Fig. 1). Ten of 30 (33%) MS patients had absorbance values for anti-CDV-H1 IgG greater than 2 SD from the mean of healthy controls as compared to 2 of 27 (7%) healthy individuals and 1 of 17 (6%) of OND patients. Similar elevated levels of CDV-H2 and CDV-H3 peptide antibodies were also observed in MS patients compared to the control groups (Fig. 2, 3). Thirty-three percent of MS patients had anti-CDV-H2 and anti-CDV-H3 antibodies compared to 7% healthy individuals and 6% OND patients. The majority of patient and control sera reactive to one CDV H peptide reacted to the other two CDV H peptides as well. Analysis of several serum samples from CDV positive and negative MS patients over several years demonstrated no significant change in CDV antibody titer. No increase in measles antibody was found in MS patients with highest CDV titers compared with controls. While it is possible that human sera could react to one synthetic peptide of CDV shared with a ubiquitous bacterial or viral protein, it is highly unlikely that this mechanism would explain why positive sera react to all three structurally unrelated CDV H peptides. A search through peptide data banks reveals no common protein sharing peptide sequences with our three CDV H peptides. Thus, these preliminary results suggest that CDV infection can occur in man. It also appears that CDV-H protein specific antibodies are more frequent in MS pa-

Table 4. Ideal characteristics for a candidate animal virus

| | |
|---|---|
| Epidemiology: | – Naturally occurring<br>– Common, widespread<br>– Explains MS prevalences, epidemics<br>– Close human contact |
| Clinical features in animal: | – Optic neurotis, myelitis<br>– Acute, R&E, CP<br>– CSF IgG increase |
| Animal pathology: | – Periventricular demyelination<br>– Plaques |
| Evidence for human infection: | – Serology<br>– Viral identification or transmission |

tients compared to OND patients and healthy individuals. This raises the possibility that CDV may trigger MS in some patients since this virus fulfills most of the characteristics of the ideal animal virus candidate (Table 4) in MS causation. Clearly, more studies are indicated to determine the spectrum of specific antibodies to H and other CDV peptides in MS patients from several geographic areas, as well as to assess the relationship between CDV specific antibodies, disease activity, dog exposure and HLA haplotypes.

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