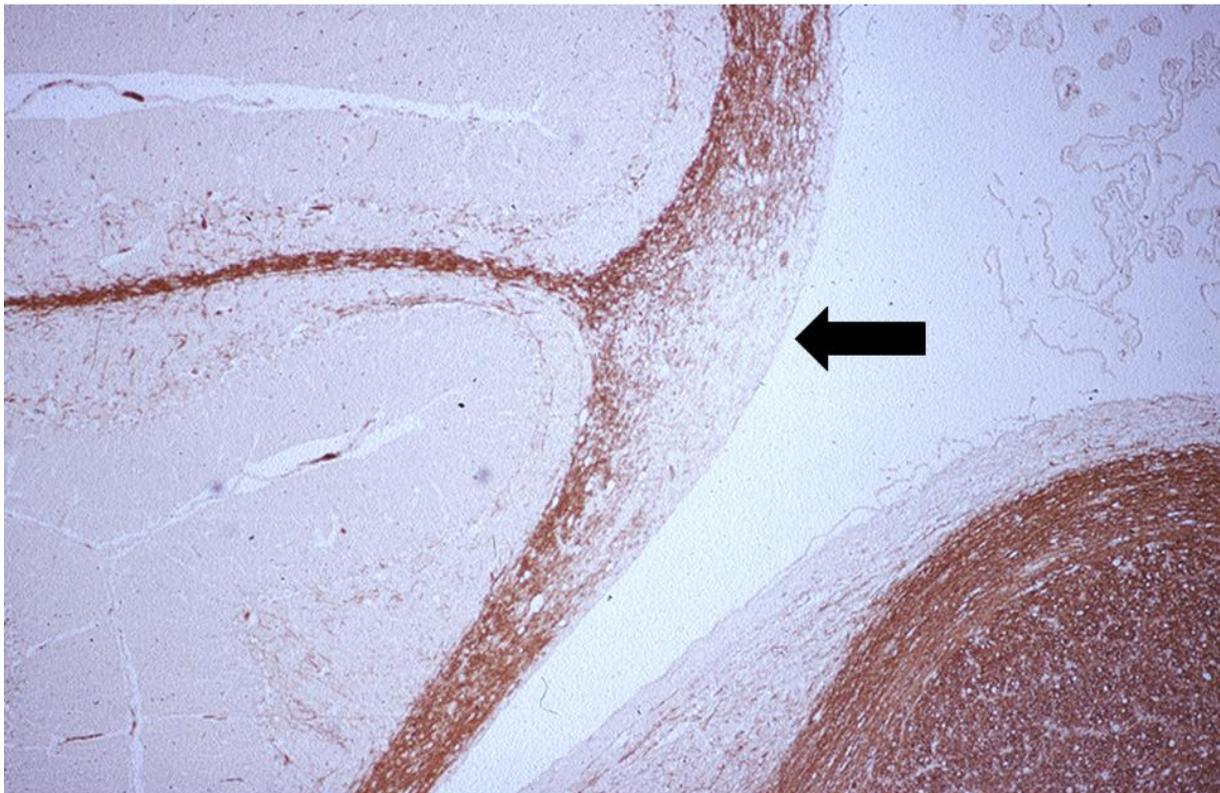


## The pathogenesis of nervous distemper

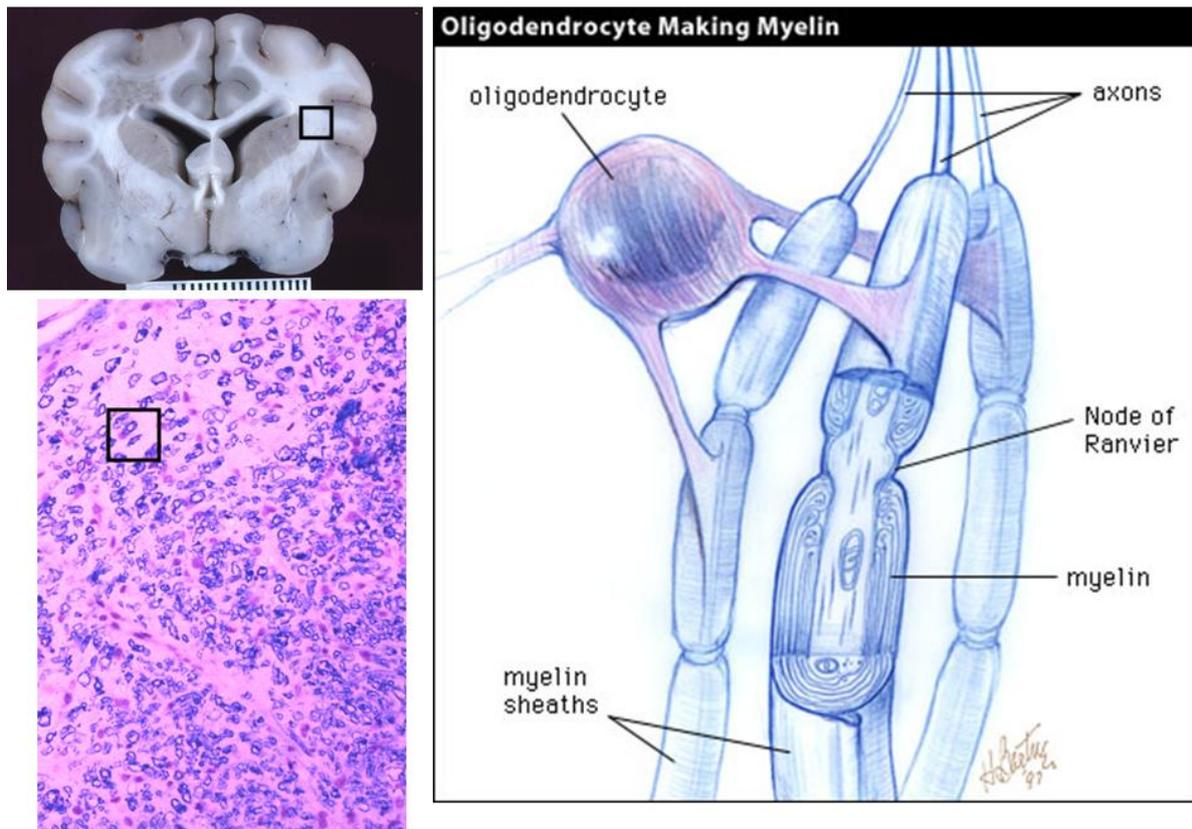
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Canine distemper is a highly contagious viral disease of dogs and of all animals in the Canidae, Mustellidae and Procyonidae families. Canine distemper virus (CDV) is an RNA virus with a nonsegmented, single-stranded genome of negative polarity, and a member of the genus Morbillivirus (Family Paramyxoviridae). CDV is generally transmitted as an aerosol infection to the upper respiratory tract. The primary virus replication takes place in the lymphoid tissues, leading to severe long lasting immunosuppression. T cells are more affected than B cells and CD4+ lymphocytes are rapidly depleted for several weeks whereas CD8+ cells are less severely affected and recover relatively fast. As a result of epithelial infection, a variety of respiratory, intestinal and dermatological signs can occur.



**Figure 1** Typical demyelinating lesion (depicted by arrow) in the cerebellar white matter bordering the fourth ventricle, in acute Distemper. The white matter is immunostained brown with an antibody against a myelin protein. In the lesion, the brown colour, hence the myelin, has completely disappeared.

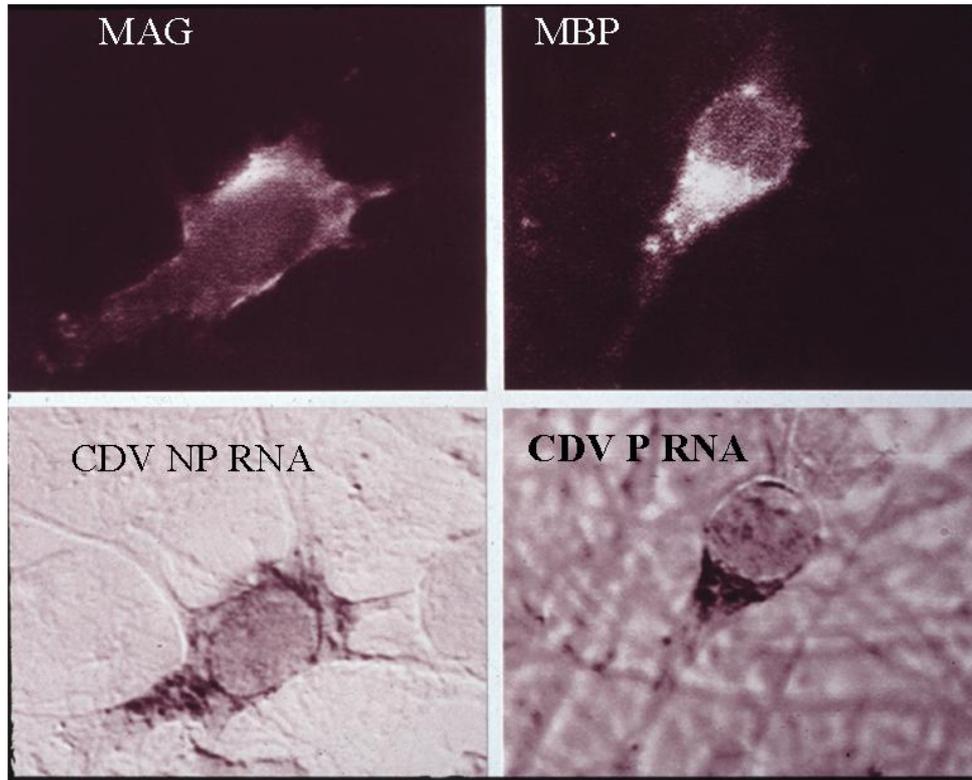
At about 10 days p.i., CDV starts to spread from the sites of primary replication to various epithelial tissues and the central nervous system (CNS). CDV causes multifocal lesions in the gray as well as in the white matter of the CNS. The demyelinating lesions are not only responsible for severe neurological signs but are also thought to be a model for human demyelinating conditions such as Multiple Sclerosis. Therefore, the pathogenesis of demyelination in distemper has been closely investigated.



**Figure 2** The structure of the normal white matter. Upper left: transverse section through a fixed dog brain showing grey and white matter. Lower left: microscopic preparation showing white matter in cross section consisting of myelin sheets (blue ringlets) surrounding nerve fibres (clear round structures). Right: schematic drawing showing myelin sheets which are derived from oligodendroglial processes wrapping themselves around the nerve fibres. A lesion of the oligodendrocytes leads to demyelination

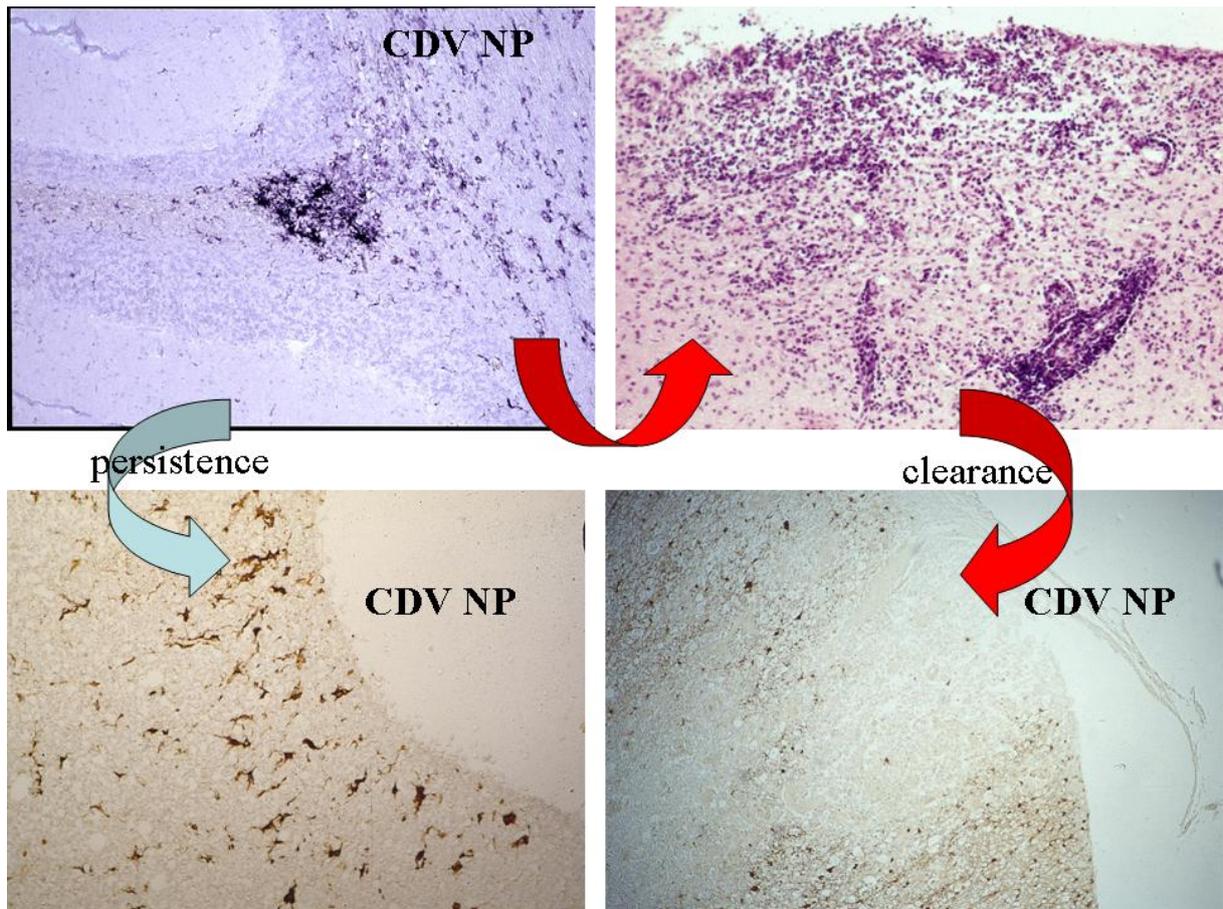
The initial myelin lesions develop during a period of severe immunosuppression and are not inflammatory. Several immunocytochemical studies and recent in situ hybridization work in spontaneous and experimental distemper have clearly shown that demyelination coincides with replication of CDV in the glial cells of the white matter. Spatio-temporal studies leave no doubt that the initial white matter lesions are associated with viral activity and that their development is highly predictable. The obvious explanation for the phenomenon of demyelination would be infection of oligodendrocytes, the myelin producing cells. Therefore, research has focused on finding evidence of CDV in oligodendrocytes.

## Restricted CDV infection of oligodendrocytes



**Figure 3** Oligodendrocytes in brain tissue cultures infected with CDV. Double labelling was performed combining immunofluorescence with antibodies against myelin proteins (MBP, MAG) to identify the oligodendrocytes with in situ hybridisation (complementary probes against Nucleoprotein and Phosphoprotein) showing mRNA of the virus (black dots). Viral protein is not present in these cells. Therefore this is a restricted infection.

It has been shown by light microscopy that the majority of infected cells are astrocytes. Most electron microscopical studies agree that oligodendroglial infection is rare in distemper. Only very few oligodendrocytes containing CDV protein were found by light microscopy. However, ca 8% of the oligodendrocytes at the perimeter of lesions contained CDV mRNA. We had found this restricted oligodendrocyte infection earlier in dog brain cell cultures, in which virulent CDV causes a slowly spreading non-cytolytic infection. CDV proteins or viral nucleocapsids were only very rarely found in oligodendrocytes in culture, in contrast to astrocytes and microglial cells which readily support CDV infection. We concluded from these in vivo and in vitro studies that CDV causes a restricted infection of the oligodendrocyte, which is possibly responsible for the phenomenon of demyelination. Why the production of viral protein does not take place in these cells remains to be clarified.



**Figure 4** Further evolution of the lesion. Upper left: acute stage with virus replication in the white matter (blue labelled cells). Upper right: massive inflammation in the chronic stage with additional destruction of the tissue. Lower right: Inflammation leads to clearance of the virus in the centre of an infected area (brown labelled cells). Lower left: a persistently infected area (brown labelled cells) in the chronic stage. Obviously, the immune response has not (yet) found this lesion. Persistence of the virus continues to drive the immune response and tissue damage, provoking a chronic progressive disease.

Between 20 and 30 days p.i., cultured oligodendrocytes, which grow superimposed on a layer of astrocytes in mixed dog brain cell cultures, start to degenerate and disappear although the supporting culture remains a continuous cell sheet. Ultrastructural studies revealed microvacuolation and loss of organelles in these oligodendrocytes. The morphological changes are preceded by metabolic dysfunction of these cells, because the activity of cerebroside sulfotransferase - an oligodendrocyte specific enzyme - decreased markedly soon after infection, and myelin transcription was strongly reduced in infected brain cell cultures. In vivo we showed that CDV infection led to a massive downregulation of myelin gene transcription. The fate of these oligodendrocytes remains unclear; there is no concrete evidence that these cells undergo necrosis or apoptosis. There is little doubt, however, that a change of these cells lies at the base of the demyelinating process, although its mechanism is not yet understood.

The contribution of the immune response to early lesion development is not clear. While an effective antiviral neutralizing antibody response is lacking in the acute phase of distemper, anti CDV IgM antibodies occur within the first 2 weeks of infection. Despite severe immunosuppression and lack of perivascular cuffing, numerous CD8+ cells are found in acute demyelinating lesions and also diffusely distributed in the brain parenchyma, roughly correlating with areas of viral infection. In the CSF of such animals, high IL8 concentrations are found. It was suggested that initial microglial cell activation which occurs in distemper may trigger invasion of T cells in the CNS.

The initial intrathecal immune response during the immunosuppressive stage of the disease consists of a diffuse invasion of CD8+ T cells. During immune recovery a mature immune reaction develops by perivascular infiltration of CD4+ cells and subsequent recruitment of large numbers of plasma cells and strong antibody synthesis. The titers of CDV neutralizing antibodies in the CSF often exceed those in the serum. Binding studies have shown that antibodies are made against all CDV proteins. The occurrence of anti-CDV antibodies in the CSF coincides with the clearance of CDV - and of CDV containing cells - from the inflammatory lesions.

The antiviral immune response should be beneficial to the host: we could clearly demonstrate that

CDV is removed from the tissue in the inflammatory lesions. However, our studies also showed that CDV can persist in white matter areas outside of the inflammatory demyelinating lesions. It appears therefore that a chronic progressive disease develops if the intrathecal immune response keeps lagging behind viral replication. Thus viral persistence is the key to the pathogenesis of the chronic lesion. We found that persistent CDV spreads in a non-cytolytic manner by way of cell processes with very limited budding and release of infectious virus, like attenuated viruses. In contrast to attenuated CDV, virulent virus induces very little cell-to-cell fusion, eventually leading to cell destruction. In addition, CDV is capable to produce a restricted infection in neurons without expressing viral protein. Others have found a restricted expression of surface proteins in the CNS. Thus CDV appears to do everything to avoid being recognized by the immune system. It appears that both the limited shedding of infectious virus and the lack of cell-to-cell fusion is related to a restricted expression of fusogenic complexes in the cytoplasmic membrane. The responsible molecular determinants of the viral fusion protein are presently being characterized in our lab.

#### **Treatment and prevention of canine distemper**

Much has been learned about the development of the CNS lesions in distemper. Some of these findings may provide a base for the development of therapeutic strategies, in particular at the immunomodulatory level. However, while modification of the detrimental inflammatory response may become feasible, the basic problem remains the presence of the virus in the CNS. Effective antiviral therapies against Morbilliviruses are not available yet. Therefore, the most important veterinary intervention remains prevention. Whereas vaccines against CDV have been available since a long time and have greatly reduced the incidence of the disease, avianized strains may offer inadequate protection against the nervous form of CDV and other vaccine strains may cause postvaccinal encephalitis. We have recently developed a DNA vaccine based on sequences from virulent CDV, and preliminary studies have shown that it is capable to protect dogs against the clinical manifestations of the infection.

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