Acta Neuropathol (Berl) (1983) 59:269–276

Acta Neuropathologica

Glial Proteins in Canine Distemper Virus-induced Demyelination

A Sequential Immunocytochemical Study*

M. Vandevelde[1], P. Bichsel[1], S. Cerruti-Sola[1], A. Steck[2], F. Kristensen[3], and R. J. Higgins[4]

[1] Institute of Comparative Neurology, University of Berne, Bremgartenstr. 109a, P.O. Box 2735, CH-3000 Berne, Switzerland
[2] Dept. of Neurology, University of Lausanne, CH-1000 Lausanne, Switzerland
[3] Institute of Clinical Immunology, Inselspital, University of Berne, CH-3000 Berne, Switzerland
[4] School of Veterinary Medicine, Davis, California, USA

Summary. A temporal series of demyelinating lesions in experimental canine distemper virus (CDV) infection was examined with immunohistological techniques demonstrating myelin basic protein (MBP), myelin-associated glycoprotein (MAG), and glial fibrillary acidic protein (GFAP) on serial sections. The earliest lesions were characterized by decreased MBP and MAG and increased GFAP. During the further progression of the disease, MBP and MAG losses continued to match each other. There was no indication of MAG loss preceding the disappearance of MBP. In the more advanced lesions there was a marked decrease of GFAP positive cells. Since these findings differed considerably from similar immunohistochemical studies in progressive multifocal leukoencephalopathy (PML) where demyelination results from oligodendroglial infection, it was concluded that the oligodendroglial cell body is not the primary target of CDV. The marked astroglial changes were also considered to contribute to demyelination in CDV infection but the mechanism by which this happens remains unknown.

Key words: Canine distemper – Demyelination – Immunohistology – MBP – MAG – GFAP

Introduction

Although demyelination in canine distemper virus (CDV) infection in dogs has been known for more than a century (Saunders 1973; Fankhauser 1982) its pathogenesis remains enigmatic. Immunopathologic reactions may play a role in the chronic stage of the disease (Krakowka et al. 1973; Vandevelde et al. 1982a, b) but recent evidence (Summers et al. 1979; Higgins et al. 1982; Vandevelde et al. 1982a, b) leaves little doubt that the initial demyelinating lesions in distemper are directly virus-induced. It is not apparent how CDV causes myelin destruction. Ultrastructural studies (Wisniewski et al. 1972; Raine 1976; Summers 1979; Higgins et al. 1982) have consistently demonstrated CDV particles in the lesions, but have rarely (Raine 1976), if at all (Higgins et al. 1982), provided evidence for oligodendroglial infection or cytolysis. One fluorescent antibody study (Vandevelde and Kristensen 1977) failed to show a pattern of oligodendroglial infection in CDV-induced lesions as has been demonstrated with similar techniques in corona virus infection in mice (Nagashima et al. 1978). A very important recent finding, however, was the demonstration of segmental demyelination in early distemper lesions suggesting some form of oligodendroglial alteration (Higgins et al. 1982). In another ultrastructural study (Summers et al. 1979) it was suggested that CDV-induced fusion of glial cell membranes may lead to myelin destruction.

Recent advances in immunocytochemistry using antibodies against different myelin proteins (Sternberger et al. 1979) have been applied to pathologic tissues (Itoyama et al. 1980, 1982). These studies have shown that primary lesions of the oligodendroglial cell body result in a specific immunohistological pattern of myelin protein alteration. In the present study we applied similar techniques on a series of demyelinating lesions after experimental CDV infection in the hope to shed some light on their pathogenesis.

Materials and Methods

Dogs

The material used in this study originated from two different experiments involving a total of 20 inbred beagle dogs. The dogs had been infected with Cornell A75-15 CDV (kindly provided to us by Dr. M. Appel, Cornell University, Ithaca, NY, USA). The details of the experimental procedure have been described elsewhere (Vandevelde et al. 1982a, b; Cerruti-Sola et al. 1983).

* Supported by the Swiss National Science Foundation (Grant No. 3.809.81) and the Swiss M. S. Society
Offprint requests to: M. Vandevelde, DVM (address see above)

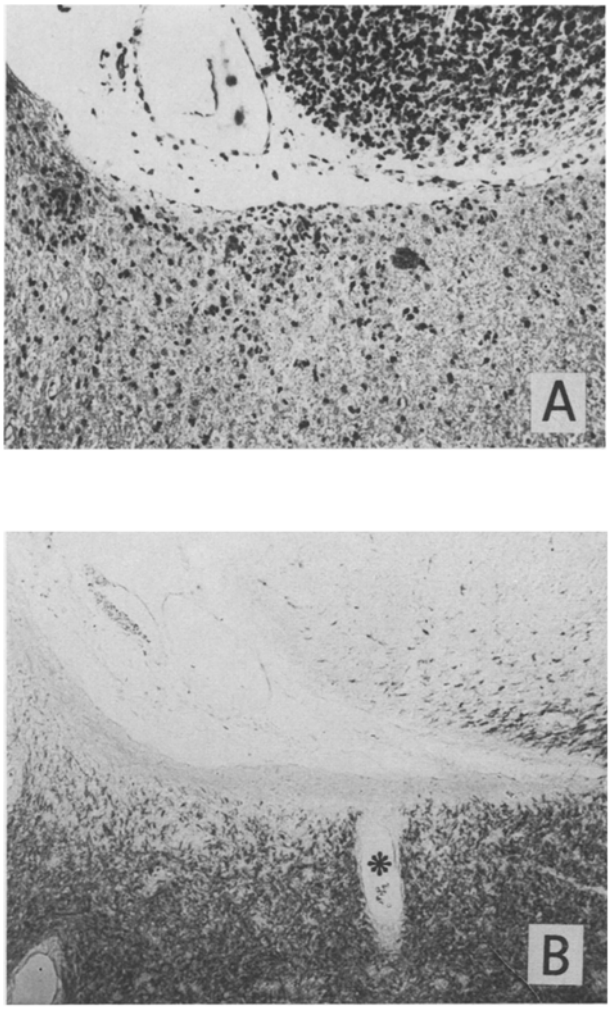

Fig. 1. A Lesion in the subpial cerebellar white matter; 21 days p.i. Diffuse and focal hypercellularity. HE, ×100. **B** Same lesion as in **A**. Marked subpial demyelination. Anti MBP–PAP, ×100. **C** Adjacent section to **B**. Blood vessel (*asterisk*) serves as landmark. Loss of MAG matches disappearance of MBP. Anti MAG–PAP, ×100

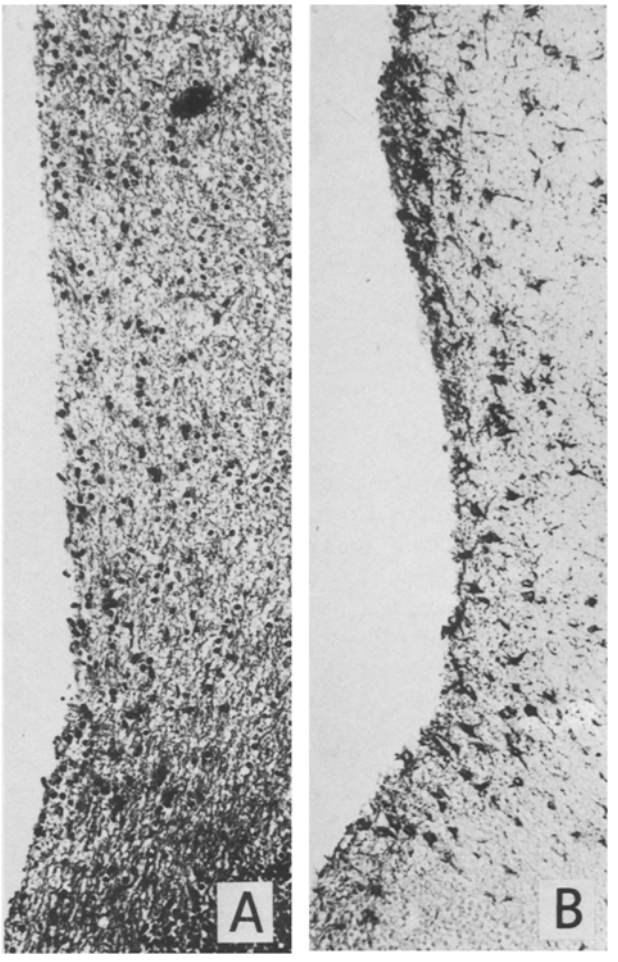

Fig. 2. A Subpial white matter in the cerebellum; 16 days p.i. Diffuse hypercellularity. HE, ×100. **B** Same lesion as in **A**. Prominence of subpial astrocytes. Anti GFAP–PAP, ×100

Brain

In all dogs the brains had been removed immediately after death and fixed in 10% $CaCo_3$ neutralized formalin or 4% PBS-buffered paraformaldehyde solution. Representative areas were processed for paraffin embedding. Sections stained with HE were examined, and a total of 22 representative lesion areas were selected from 14 dogs for immunohistological staining. These dogs had been killed at 16, 21, 24, 29, 31, 33, 34, 42, 56, and 63 days after infection. All lesions were located in the classical subpial or periventricular predilection areas including the cerebellar central and foliated white matter, the cerebellar peduncles, and the white matter in the vicinity of the IVth ventricle. Sections were also cut from the same predilection sites in two dogs that survived the infection without brain lesions and in five normal noninfected beagle dogs.

Antisera

Rabbit antiserum against glial fibrillary acidic protein (GFAP) (Dakopatts, Danmark) and human IgM (Dakopatts, Danmark), goat antirabbit IgG (Miles Laboratories) as well as PAP complex (Miles Laboratories) were commercially purchased.

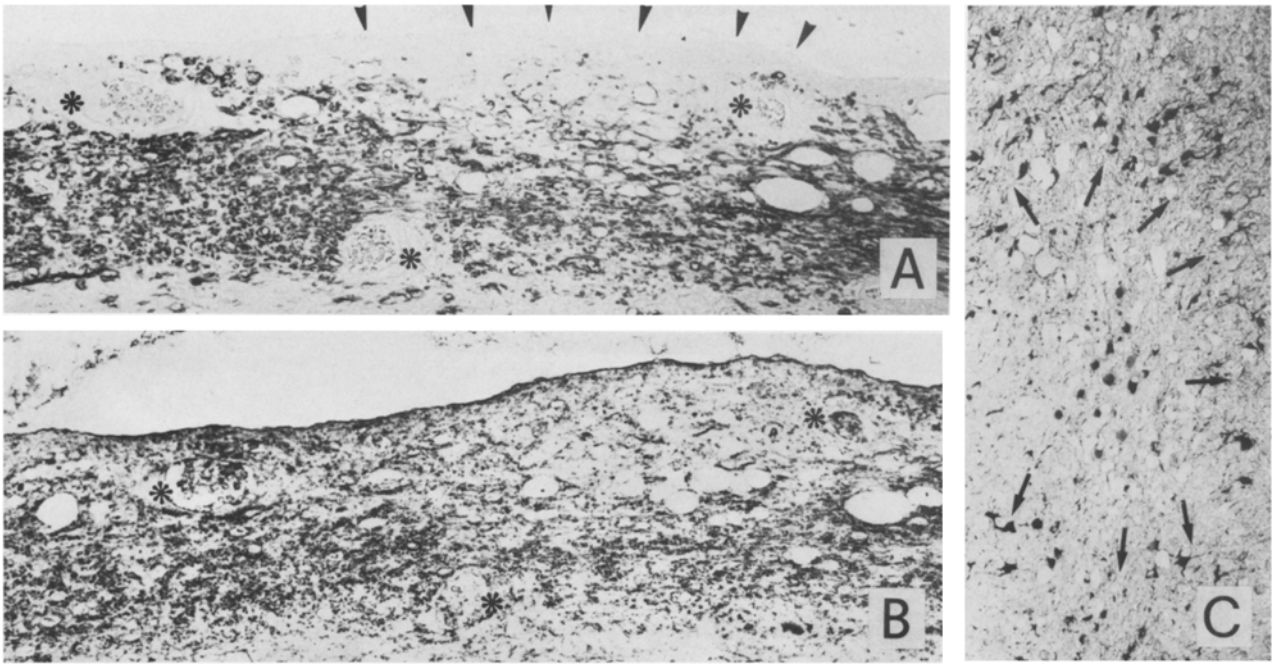

Fig. 3. A Subpial white matter in cerebellar peduncle; 31 days p.i. (pial surface indicated by *arrow heads*). Severe spongy state and demyelination. Anti MBP – PAP, × 100. **B** Adjacent section to **A**. Loss of MAG does not noticeably exceed that of MBP (three asterisks in the vicinity of blood vessels serve as landmarks). Anti MAG – PAP × 100. **C** Spongy lesion in the cerebellar foliated white matter; 42 days p.i. Edge of lesion is roughly indicated by *arrows*. Loss of GFAP-positive cells as compared to the surrounding tissue. In the middle of the lesion a group of rounded cells

As antiserum against the myelin associated glycoprotein (MAG) served a human monoclonal antibody (IgM K) from a patient with peripheral neuropathy. This serum binds specifically to MAG as shown by immunoblotting studies (Steck et al., submitted). In addition, immunocytochemical studies with this serum showed strong binding to myelin in several animal species including the dog with selective labeling of the periaxonal space (Steck et al., submitted). Antimyelin basic protein (MBP) was prepared by repeated injections of human MBP in complete Freund's adjuvans into rabbits and was a kind gift of Dr. C. Beranek, University of Berne.

Immunohistological Technique

Four-micrometer serial sections were cut of the selected lesion areas and mounted with chrom-alum-gelatine (Sofroniew and Schrell 1982). After deparaffinization the sections were covered with diluted antisera for 48 h at 4°C. The PAP method was then performed essentially as described by Sternberger (1979). For the anti MAG staining, a 4-layer technique was applied where the primary antiserum was followed by rabbit antihuman IgM (Steck et al. submitted).

Results

Clinical Observations

Of the 14 dogs from which lesions were selected, eight showed neurologic signs including localized and generalized myoclonus, ataxia, visual deficits, tremor, and generalized convulsions. The details of the clinical course have been reported elsewhere (Vandevelde et al. 1982a, b; Cerruti-Sola et al. 1983). The remaining dogs had no neurologic signs, Two dogs that were killed at 56 and 63 days post infection (p.i.) showed gradual clinical improvement after an initial period of neurologic progression.

Neuropathology and Immunocytochemical Studies

The earliest lesions from 16 to 21 days p.i. (three dogs) were characterized by diffuse hypercellularity of the white matter (Figs. 1a, 2) as described earlier (Vandevelde et al. 1982a, b). MBP was clearly diminished (Fig. 1b) or focally absent in those lesions, which was closely matched by a clear absence of MAG in the same areas (Fig. 1c). On GFAP staining, there was a clear increase of astrocytic processes and cell bodies (Fig. 2b). From 24 to 31 days p.i. (three dogs) the lesions had a pronounced spongy appearance, and one lesion contained a few swollen axons. There were also several astrocytes with a large glassy looking cytoplasm and sometimes containing two or more nuclei. MBP was completely abolished in the spongy regions (Fig. 3a) and so was MAG in exactly the same area (Fig. 3b). Both myelin proteins were still present in the immediate vicinity of the spongy areas. Astrocytic

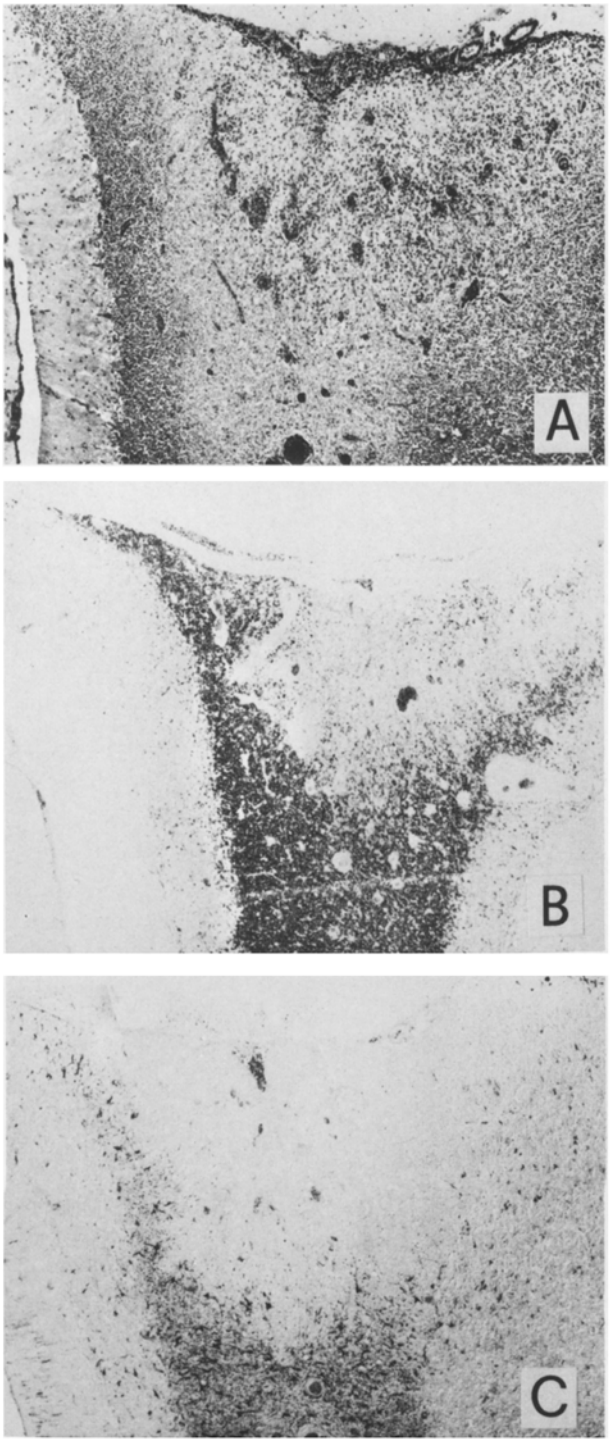

Fig. 4. A Severe inflammatory lesion in the cerebellar white matter; 29 days p.i. HE, × 40. **B** Same lesion as in **A**. Massive demyelination. Anti MBP–PAP, × 40. **C** Adjacent section to **B**. Marked paucity of astrocytes. Anti GFAP–PAP, × 40

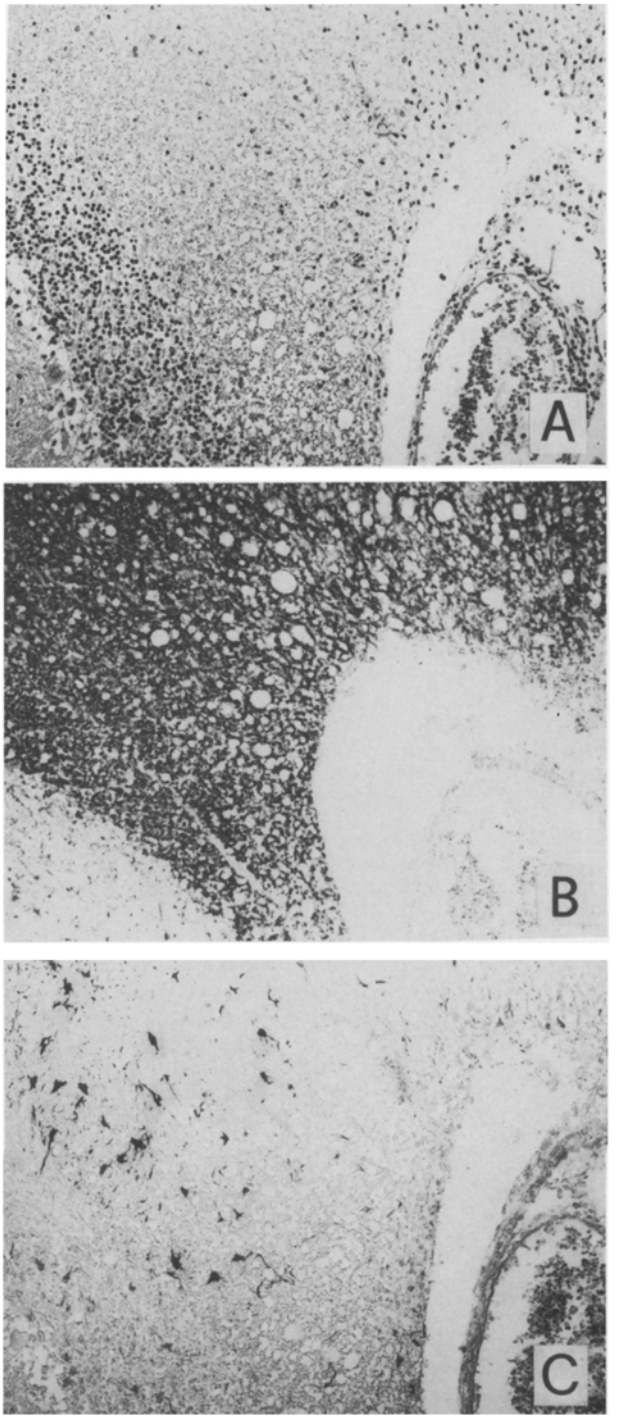

Fig. 5. A Chronic lesion in the subpial cerebellar white matter; 50 days p.i. Vacuolated appearance of the tissue and hypercellularity. HE, × 100. **B** Same lesion as in **A**. Diffuse loss of myelin. Anti MBP–PAP, × 100. **C** Adjacent section to **B**. Marked loss of GFAP–-positive cells in the lesion. Anti GFAP–PAP, × 100

processes were very prominent in the general area as seen on GFAP-stained sections, but in the spongy foci there was a clear decrease in GFAP staining (Fig. 3c). A few plump cells stained very faintly GFAP positive. In one dog killed on day 29 p.i. marked perivascular and meningeal accumulation of lymphocytes and plasma

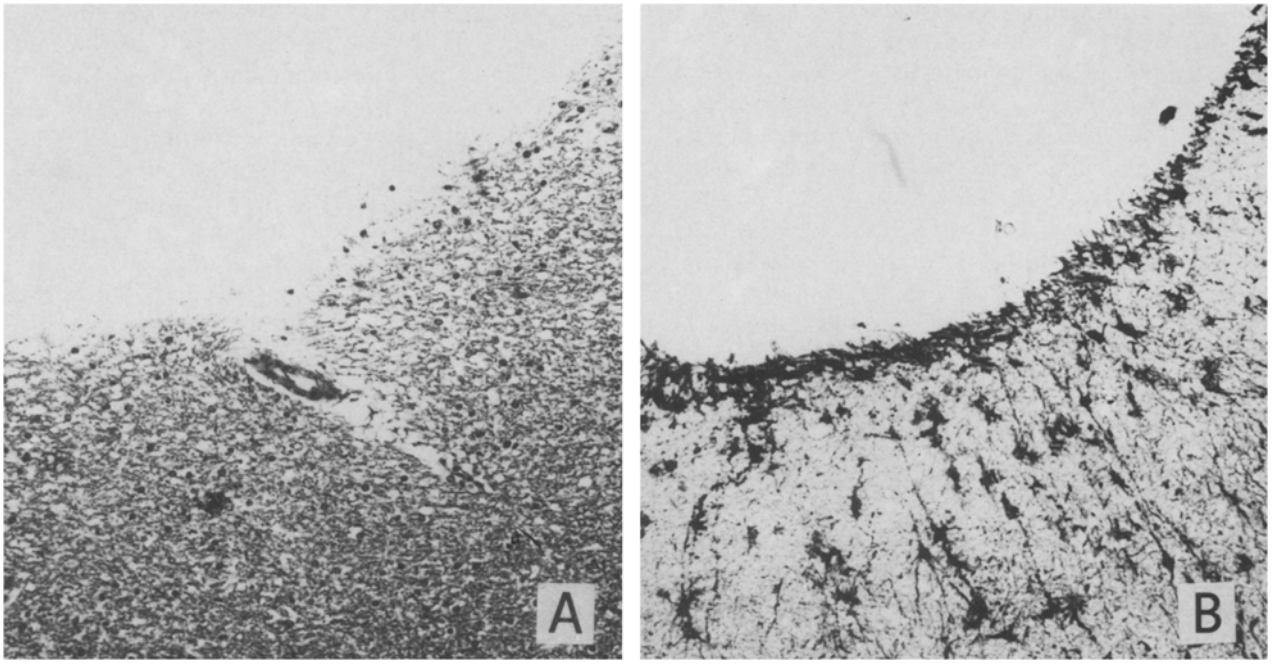

Fig. 6. A Chronic lesion in the subpial cerebellar white matter; 63 days p.i. Loose texture of the subpial tissue. HE, ×100. **B** Same area as in **A**. Prominent astrocytes. Anti GFAP–PAP, ×100

cells had occurred in the demyelinating lesions (Fig. 4a). There were also large numbers of macrophages. In these inflammatory lesions there was marked loss of MBP and MAG (Fig. 4b), as well as a remarkable paucity of GFAP-positive cells (Fig. 4c). One dog killed at 33 days p.i. had moderate inflammatory changes, and MBP and MAG were clearly diminished. There were also fewer GFAP-positive cells in these lesions.

In two dogs killed on day 34 p.i. and two others killed on day 42 p.i. there were no inflammatory changes but the demyelinating lesions had spread considerably deeper into the brain parenchyma. Such lesions still had a spongy appearance and contained many macrophages. Both MAG and MBP staining were almost completely lost in these large demyelinating lesions. Lack of MAG staining was partially more pronounced but did not noticeably exceed the border of the MBP-deficient area. On GFAP-stained sections there was also a marked decrease of astroglial processes and cell bodies.

One dog killed on day 42 p.i. had several small glial nodules in the cerebellar white matter without apparent demyelination. Also, the myelin appeared unremarkable on the corresponding immunohistological sections. At 56 days p.i. (two dogs) the lesions still had a spongy appearance, but the individual vacuoles were smaller giving the tissue a "loose textured" appearance (Fig. 5a). There were a few macrophages in these lesions and a slight hypercellularity. In one of these dogs there were also mild inflammatory changes. MBP was diffusely reduced in these 56 day old lesions (Fig. 5b). MAG was also deficient but appeared to be less affected in one lesion. In two of the three lesions examined in these two dogs. GFAP-positive cells were markedly reduced, although within one lesion there was a focal area of astrocytic proliferation (Fig. 5c).

The remaining two dogs were killed on day 63 p.i. The lesions in these animals were characterized by a mildly loose texture of the tissue (Fig. 6a) with very mild hypercellularity. In one of these dogs there were very mild inflammatory changes. MBP deficiency was not obvious in these lesions but possibly reduced (fainter staining), whereas on MAG-stained sections there was nothing remarkable. The GFAP-positive cells were very prominent in the lesions at 63 days p.i. (Fig. 6b).

Discussion

In progressive multifocal leukoencephalopathy (PML) in man the virus infects predominantly oligodendrocytes (Lampert 1978), although some degree of astrocytic infection has also been documented (Mászló and Tariska 1982).

In a recent elegant study, Itoyama et al. (1982) clearly demonstrated that MAG is the first myelin

protein to be affected in PML lesions. Thus, a pattern of demyelination in which the destruction of MAG precedes the one of the major myelin protein MBP, is indicative of a lesion at the level of the oligodendroglial cell body. There is some discrepancy in this hypothesis: although a similar sequence of events as in PML seems to take place in MS lesions (Itoyama et al. 1980), a recent ultrastructural study showed surviving and even multiplying oligodendrocytes in an active MS lesion (Raine et al. 1981) suggesting that the myelin sheath itself is the primary target in MS. However, in contrast to PML, the cause and pathogenesis of MS are largely unknown. Therefore, we believe that it is justified to compare the findings in PML (Itoyama et al. 1982) with those obtained by means of similar techniques in distemper lesions, which – for reasons discussed above – are also virus-induced.

Our results show simultaneous involvement of MBP and MAG in CDV infection. Even in the earliest lesions there was no clear indication for primary affection of MAG. Although in some lesions the loss of MAG seemed more obvious than the one of MBP, there was never any major discrepancy in MAG and MBP stainings as in the PML lesions studied by Itoyama et al. (1982). The staining differences in a few of our lesions could be explained by the fact that MAG constitutes only a very small portion of the total myelin protein fraction, whereas MBP accounts for 30% (Braun and Bostroff 1977). In conclusion, our present study does not provide support for a primary lesion of the oligodendroglial cell in CDV infection similar to the one in PML. Our observations are consistent with previous ultrastructural (Wisniewski et al. 1972; Raine 1976; Summers et al. 1979; Higgins et al. 1982) or fluorescent antibody studies (Vandevelde and Kristensen 1977), which failed to provide conclusive evidence for oligodendroglial infection in canine distemper. However, Higgins et al. (1982) clearly demonstrated segmental demyelination in early distemper lesions similar to the one in experimental corona virus infection in mice, a disease in which oligodendrocytes are known to be the primary target (Lampert 1978; Dal Canto and Rabinowitz 1982). It is conceivable that CDV induces primary lesions in the distal portions of the oligodendrocyte. Virus-induced fusion of glial membranes at this level, as proposed by Summers et al. (1979), could play an important role.

Our study supports previous observations according to which astroglial hypertrophy and hyperplasia (Summers et al. 1979; Vandevelde et al. 1982b; Higgins et al. 1982) are components of the earliest myelin lesions in distemper. Surprisingly, during the further progression of demyelination regressive astroglial changes became conspicuous. In lesions with a pronounced spongy state there was a very clear decrease of GFAP-positive cells and processes. In addition we observed very faintly staining plump rounded cells in such lesions. Since all ultrastructural studies so far have shown abundant evidence for widespread astroglial infection in distemper (Wisniewski et al. 1972; Raine 1976; Summers et al. 1979; Higgins et al. 1982) it seems reasonable to explain the astrocytic changes in this study as a result of a profound cytopathic effect of CDV. It remains unclear if or how astroglial changes could be associated with demyelination. It has been known for a long time that fibrous astrocytes are an integral part of the white matter (Spielmeyer 1922), where they are considered to provide structural support (Peters et al. 1976). Although desmosome-like junctions between oligodendrocytes and astrocytes have been observed (Raine 1977) it is not clear how the latter cells would contribute to the stability and/or maintenance of the myelin sheath. The striking spongy appearance of advanced acute demyelinating lesions in distemper is ultrastructurally characterized by increased extracellular space and ballooning of myelin sheaths (Summers et al. 1979). The vacuolated appearance of such lesions is reminiscent of brain edema associated with other noninfectious diseases in various animal species (Fankhauser and Luginbühl 1968). Considering the role of astrocytes in maintaining fluid and electrolyte balance in the brain (Bradbury 1975), the present findings support the hypothesis that astroglial infection and alteration in CD may result in edema and subsequent degeneration of myelin (Frauchiger and Fankhauser 1970). There are some similarities in this respect between CDV infection and demyelination in mice after experimental herpes simplex virus infection (Townsend 1981). In this model, astroglial infection and cytolysis with vacuolation of myelin sheaths precede demyelination. It is thought that astroglial cytolysis activates macrophages which then cause myelin destruction by release of proteolytic enzymes (Townsend 1981). However, all these considerations cannot explain the earliest lesion in distemper which consists of segmental demyelination in the absence of edema and in which hypertrophic astrocytes themselves participate in the separation of the myelin sheath from the axon (Higgins et al. 1982).

The interpretation of the findings in our four dogs with chronic lesions (56–63 days p.i.) is difficult in respect to recovery and remyelination. Two of these dogs had no clinical signs, the other two had clinically improved after an initial period of severe neurologic impairment. However, as shown on previous occasions (Summers et al. 1979; Vandevelde et al. 1982b; Higgins et al. 1982) clinico-pathologic correlations are often lacking in distemper. We cannot exclude that these four dogs had only mild lesions in the acute stage of infection. Thus, our immunohistological findings

showing only minor myelin changes in these dogs cannot be taken as evidence of remyelination which is known to occur in distemper (McCullough et al. 1974; Higgins et al. 1982), without corresponding ultrastructural studies of the same lesions.

Our study also included a few dogs with inflammatory lesions. In these lesions no clear discrepancy between MBP and MAG destruction was to be observed either. It has been well established that inflammatory changes occur later on in the disease (Campbell 1957; McCullough et al. 1974; Summers et al. 1979; Vandevelde et al. 1982a, b) and that they coincide with immunologic recovery (Vandevelde et al. 1982b; Cerruti-Sola et al. 1983) following profound immunosuppression which has been well documented in CDV infection (Krakowka et al. 1980). Some dogs with inflammatory lesions may recover clinically as literature data (McCullough et al. 1974; Appel et al. 1982) and the results of the present study indicate, while a few dogs develop severe progressive inflammatory demyelination as seen in some cases of spontaneous distemper. Such local immunologic reaction may enhance myelin destruction perhaps on an autoimmune basis as some studies indicate (Krakowka et al. 1973; Vandevelde et al. 1982a, b). In the present study only one dog was examined at the stage of intense inflammation. Therefore, our immunocytochemical data are not sufficient to shed some light on the possibility of an immunologic attack on the oligodendrocyte in inflammatory canine distemper lesions.

Our study has shown that the immunohistochemical pattern of demyelination in canine distemper differs from the one described by Itoyama et al. (1982) in PML, a disease resulting from oligodendroglial infection. We have also provided additional evidence for astroglial pathology in distemper. Further ultrastructural studies will be necessary to clarify the pathogenesis of virus-induced demyelination in CDV infection.

Acknowledgements. The authors wish to thank Mrs. E. Vandevelde and Miss U. Affolter for technical, and Mr. Hebeisen for photographic assistance. We also thank Mrs. L. Etter for typing the manuscript.

References

Appel MJG, Shek WR, Summers BA (1982) Lymphocyte-mediated immune cytotoxicity in dogs infected with virulent canine distemper virus. Infect Immun 37:592–600

Bradbury MWB (1975) Ontogeny of mammalian brain-barrier systems. In: Cserr HF, Fenstermacher JD, Fencl V (eds) Fluid environment of the brain. Academic Press, New York San Francisco London, pp 81–103

Braun PE, Brostoff SW (1977) Proteins of myelin. In: Morell P (ed) Myelin. Plenum Press, New York London, pp 201–231

Campbell RSF (1957) Encephalitis in canine distemper. Br Vet J 113:143–162

Cerruti-Sola S, Kristensen F, Vandevelde M, Bichsel P, Kihm U (1983) Lymphocyte responsiveness to lectin and myelin antigens in canine distemper infection in relation to the development of demyelinating lesions. J Neuroimmunol (in press)

Dal Canto MC, Rabinowitz SG (1982) Experimental models of virus-induced demyelination of the central nervous system. Ann Neurol 11:109–127

Fankhauser R (1982) Hundestaupe – Geschichte einer Krankheit. Schweiz Arch Tierheilkd 124:245–256

Fankhauser R, Luginbühl H (1968) Pathologische Anatomie des zentralen und peripheren Nervensystems der Haustiere. Parey, Berlin Hamburg, pp 191–462

Frauchiger E, Fankhauser R (1970) Demyelinating diseases in animals. In: Vinken P, Bruyn GW (eds) Handbook of clinical neurology, vol 9. North Holland, Amsterdam

Higgins RJ, Krakowa SG, Metzler AE, Koestner A (1982) Primary demyelination in experimental canine distemper virus induced encephalomyelitis in gnotobiotic dogs. Acta Neuropathol (Berl) 58:1–8

Itoyama Y, Sternberger NH, Webster HdeF, Quarles RH, Cohen SR, Richardson EP (1980) Immunocytochemical observations on the distribution of myelin-associated glycoprotein and myelin basic protein in multiple sclerosis lesions. Ann Neurol 7:167–177

Itoyama Y, Webster HdeF, Sternberger NH, Richardson EP, Walker DL, Quarles RH, Padgett BL (1982) Distribution of papovavirus, myelin-associated glycoprotein, and myelin basic protein in progressive multifocal leukoencephalopathy lesions. Ann Neurol 11:396–407

Krakowka S, McCullough B, Koestner A, Olsen R (1973) Myelin-specific autoantibodies associated with central nervous system demyelination in canine distemper virus infection. Infect Immun 8:819–827

Krakowka S, Higgins RJ, Koestner A (1980) Canine distemper virus: Review of structural and functional modulations in lymphoid tissues. Am J Vet Res 41:284–292

Lampert PW (1978) Autoimmune and virus-induced demyelinating diseases. Am J Pathol 91:175–208

Mászló M, Tariska I (1982) Are astrocytes infected in progressive multifocal leukoencephalopathy (PML)? Acta Neuropathol (Berl) 56:45–51

McCullough B, Krakowka S, Koestner A (1974) Experimental canine distemper virus-induced demyelination. Lab Invest 31:216–222

Nagashima K, Wege H, Meyermann R, ter Meulen V (1978) Corona virus induced subacute demyelinating encephalomyelitis in rats: A morphological analysis. Acta Neuropathol (Berl) 44:63–70

Peters A, Palay SL, Webster HdeF (1976) The fine structure of the nervous system: The neurons and supporting cells. Saunders, Philadelphia London Toronto

Raine CS (1976) On the development of CNS lesions in natural canine distemper. Neurol Sci 30:13–28

Raine CS (1977) Morphological aspects of myelin and myelination. In: Morell P (ed) Myelin. Plenum Press, New York London, pp 1–89

Raine CS, Scheinberg L, Waltz JM (1981) Multiple sclerosis – Oligodendrocyte survival and proliferation in an active established lesion. Lab Invest 45:534–546

Saunders LZ (1973) Some historical aspects of the neuropathology of canine distemper. Schweiz Arch Neurol Psychiatr 112:341–352

Sofroniew MV, Schrell U (1982) Long-term storage and regular repeated use of diluted antisera in glass staining jars of increased sensitivity, reproducibility, and convenience of single- and two-color light-microscopic immunocytochemistry. J Histochem Cytochem 30:504–511

Spielmeyer W (1922) Histopathologie des Nervensystems. Springer, Berlin

Sternberger LA (1979) Immunocytochemistry. 2nd edn. Wiley & Sons, New York Chichester Brisbane Toronto, pp 122–129

Sternberger NH, Quarles RH, Itoyama Y, Webster HdeF (1979) Myelin-associated glycoprotein demonstrated immunocytochemically in myelin and myelin-forming cells of developing rat. Neurobiology 76:1510–1514

Summers BA, Greisen HA, Appel MGJ (1979) Early events in canine distemper demyelinating encephalomyelitis. Acta Neuropathol (Berl) 46:1–10

Townsend JJ (1981) The relationship of astrocytes and macrophages to CNS demyelination after experimental Herpes simplex virus infection. J Neuropathol Exp Neurol 40:369–379

Vandevelde M, Kristensen B (1977) Observations on the distribution of canine distemper virus in the central nervous system of dogs with demyelinating encephalitis. Acta Neuropathol (Berl) 40:233–236

Vandevelde M, Kristensen F, Kristensen B, Steck AJ, Kihm U (1982a) Immunological and pathological findings in demyelinating encephalitis associated with canine distemper virus infection. Acta Neuropathol (Berl) 56:1–8

Vandevelde M, Higgins RJ, Kristensen B, Kristensen F, Steck AJ, Kihm U (1982b) Demyelination in experimental canine distemper virus infection: Immunological, pathologic, and immunohistological studies. Acta Neuropathol (Berl) 56:285–293

Wisniewski HM, Raine CS, Kay WJ (1972) Observations on viral demyelinating encephalomyelitis. Canine distemper. Lab Invest 26:589–599

Received December 21, 1982/Accepted December 28, 1982