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Immunoglobulins in Demyelinating Lesions in Canine Distemper Encephalitis

An Immunohistological Study*

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Summary. The brains of 14 dogs with canine distemper encephalitis were examined with immunohistologic techniques to search for immunogobulin in demyelinating lesions. Four types of lesions presumably representing a temporal sequence of lesion development were distinguished. Immunohistologic findings included immunoglobulin bearing lymphoid cells, amorphous Ig containing material, immunoglobulin bound to the tissue and immunoglobulin containing macrophages and astrocytes. The humoral immune response was absent or very minimal in acute lesions and very intense in chronic lesions. It was concluded that early demyelination in canine distemper encephalitis occurs in the absence of a local humoral immune response but that this response may aggravate and accelerate myelin destruction in the later stages of the disease.

Key words: Canine distemper encephalitis (CDE) – Immunopathology – Demyelination – Immunoglobulin – Local Immune response

Introduction

Canine distemper encephalitis (CDE), a spontaneously occurring and experimentally reproduceable demyelinating disease in dogs is of comparative importance for the study of paramyxovirus associated neurologic disorders in man (Koestner et al. 1975). The pathogenesis of the demyelinating lesions in CDE is not clear. Although distemper virus is clearly present in many demyelinating lesions in distemper (Moulton 1956; Wisniewski et al. 1972; Raine 1976), direct viral destruction of myelin has not been documented (Vandevelde and Kristensen 1977). Indirect destruction of myelin based on viral induced fusion of glial membranes has recently been proposed (Summers et al. 1979). Other workers believe that immune factors play an important role in demyelination in distemper (Koestner et al. 1974), although immunosuppression occurs in the early stages of canine distemper virus infection (Mc Cullough et al. 1974). The occurrence of antimyelin antibodies in serum of dogs with CDE (Krakowka et al. 1973) and demyelination in vitro with CDE serum (Koestner et al. 1974) are perhaps indicative for autoimmune demyelination. Grey matter lesions in experimental CDE became worse after injection of distemper hyperimmune serum (Krakowka et al. 1978). However similar observations concerning white matter lesions are not available. In one study on frozen sections, using fluorescent antibody technique, the occurrence of membrane-bound immunoglobulins in demyelinating distemper was mentioned, but not documented (Koestner et al. 1970, 1974). In order to obtain more direct information on the role of humoral immunity in demyelinating lesions in CDE, we made an immunohistological search for immunoglobulins in distemper lesions at various stages of their development. In order to avoid poor morphologic resolution of frozen sections and unspecific staining of various nervous tissue elements with immunofluorescence methods (Aarli et al. 1975; Traugott et al. 1979) we applied the unlabeled antibody enzyme method (PAP) as developed by Sternberger (1979) to aldehyde fixed paraffin embedded brain tissues of dogs with CDE. It excels most other techniques by high sensitivity, high specificity and its applicability (Sternberger 1979).

Material and Methods

Animals

Fourteen male and female dogs of various breeds, aged from 3 months to 9 years, suffered from spontaneous CDE. The clinical signs

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included spinal and cerebellar ataxia, myoclonus, cranial nerve deficits, visual impairment and generalized convulsions. The total duration of neurologic signs ranged from a few days to 6 months. Several dogs had had signs of systemic distemper infection before onset of neurologic signs. The animals died or were euthanized.

Six 1-year-old male and female Beagle dogs, free of disease and kept in isolation were euthanized and used as negative control animals.

Pathology

All animals were subjected to a complete necropsy. Extraneural organs and CNS tissue were fixed in 10% buffered neutral formalin for at least 1 week and processed for paraffin embedding. Sections were cut and stained with H.-E., Luxol fast blue-Cresyl echt violet and Holmes' silver impregnation.

Immunohistology

Lesions were identified on the histological slides and marked on the corresponding paraffin blocks. From these lesion areas serial 4 µm sections were cut and mounted with 10% Araldite solution in acetone on glass slides (Bancroft and Stevens 1977) and dried for 24 h at 37°C. The sections were deparaffinized and rehydrated in Xylene and graded ethanols and subsequently immersed in 1% Trypsin-Calcium-Chloride solution for 50 min at 37°C (Mepham et al. 1979). After repeated washing in tris-buffered saline, the sections were subjected to the unlabeled antibody peroxidase-antiperoxidase (PAP) method as described by Sternberger (1979). A few selected sections were counter stained with Light green.

Antisera

Immunochemicals were commercially obtained (Miles Laboratories, Elkhart, IN, USA). Rabbit anti-canine IgG (heavy and light chain), anti-canine IgA (heavy chain) and anti-canine IgM (heavy chain) were diluted in tris-buffered saline pH 7.4 containing 1% normal goat serum. The optimal dilution, allowing clear distinction of immunoglobulin with minimal or no background staining, was determined by titration on sections of lymphnodes and was found to be in the 1/4.000–1/16.000 range. Sections of brain lesions incubated with normal rabbit serum in a similar dilution served as negative controls. Goat anti-rabbit immunoglobulin G that served as linkserum and the PAP complex were also diluted in tris-buffered saline to concentrations recommended by Sternberger (1979).

Positive Control Tissues

A hyperplastic canine cervical lymphnode from a case of systemic toxoplasmosis (containing IgG and IgM bearing lymphocytes and plasma cells) and intestine at the ilio-caecal junction (containing IgA bearing plasma cells in the lamina propria) similarly treated as the brain tissues, served as positive control tissues.

Negative Control Tissues

From normal dogs, areas of the brain containing predilection sites for CDE lesions (fornix, periventricular white matter, cerebellum, optic tracts, cerebral peduncle, medulla oblongata) were identically treated as CDE tissues.

Results

a) *Histology*

A total of 74 demyelinating lesions in CDE dogs as well as 36 brain areas in control dogs were examined. The lesions in all cases of CDE had a multifocal distribution in predilection areas as described before (Innes and Saunders 1962; Fankhauser and Luginbühl 1968). The distribution of the lesions is tabulated (Table 1). As earlier described (Innes and Saunders 1962; Fankhauser and Luginbühl 1968), many lesions bordered directly on the periventricular or subarachnoidal spaces, and nearly all other lesions were also found in the proximity of the CSF pathways. Four major types of lesions were distinguished. For reasons discussed below, these four types were believed to reflect a temporal sequence of lesion development.

Table 1. Anatomic distributions of the lesions

Location	Number of lesions
Corpus callosum	1
Capsula interna	2
Subcortical white matter	5
Optic tract (chiasma-optic radiation)	16
Fornix	4
Cerebral peduncle	13
Cerebellum	20
Medulla oblongata	13
Total	74

Type 1 (acute lesions). Thirty lesions were characterized by varying degrees of vacuolation of the white matter (Fig. 1A). Mostly irregularly shaped vacuoles were widely spaced in some lesions, in others the tissue had a marked spongy appearence. In some lesions there was some reduction in staining intensity of the myelin. Axons were not changed as seen in silver impregnated sections. There were no macrophages in these lesions nor perivascular infiltration with inflammatory cells. There were little or no progressive glial changes. Viral inclusion bodies were found in astrocytic nuclei in approximately 25% of these lesions.

Type 2 (subacute lesions). Twenty lesions in this category had a similar spongy appearence of the white matter as in type 1 lesions. However, reactive changes were well pronounced and consisted of diffuse proliferation of astrocytes and in some areas of microglial cells (Fig. 1B). In 3 lesions there were also a few multinucleated large astrocytes. Macrophages could be found in some lesions. In several lesions there were very moderate numbers of mononuclear inflammatory cells around the bloodvessels. Demyelination as seen on myelin stains was more obvious in these lesions than in type 1. Viral inclusion bodies were found in 50% of these lesions.

Type 3 (chronic lesions). Eleven lesions were characterized by more or less severe motheaten appearence of the white matter which corresponded with marked to

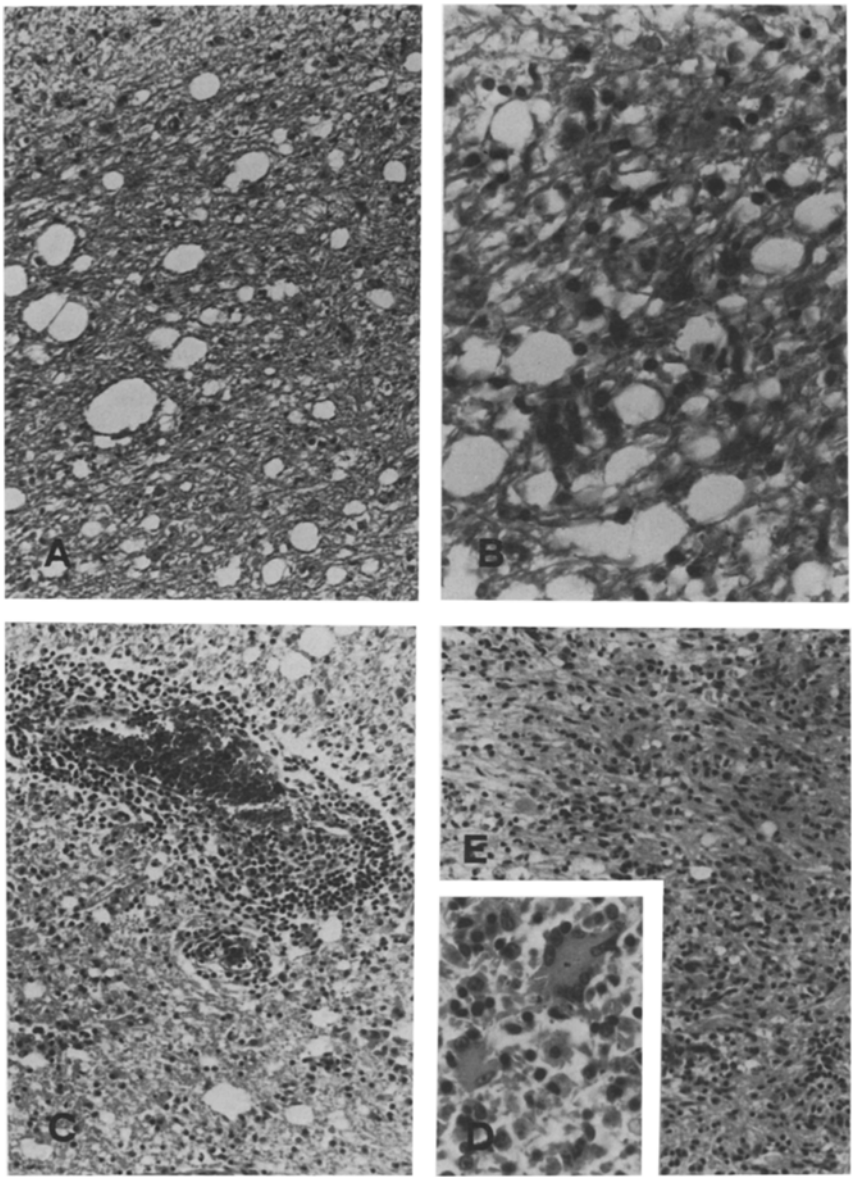

Fig 1. A Type 1 (acute) lesion: vacuolation of white matter without reactive changes. H.-E., ×100. **B** Type 2 (subacute) lesion: Spongy state of the white matter. Desintegration of myelin with beginning cellular infiltration. H.-E., ×250. **C** Type 3 (chronic) lesion: demyelination and large perivascular mononuclear cuffs. H.-E., ×100. **D** Type 3 lesion: multinucleated astrocytes. H.-E., ×250. **E** Type 4 (sclerotic) lesion. Dense astrogliosis of the white matter. H.-E., ×100

severe demyelination as seen on special stains for myelin sheaths.

Usually axons were partially preserved but in some lesions complete necrosis of both myelin and axons had occurred. The characteristic feature of these lesions were very thick perivascular mononuclear cuffs (Fig. 1C). Macrophages were very prominent in these lesions (Fig. 5A) and there were marked progressive astroglial changes. Large multinucleated astrocytes were seen in 3 lesions (Fig. 1D). Viral inclusion bodies were seen in more than 80% of these lesions.

Type 4 (old sclerotic lesions). Well delineated areas of marked myelin loss with partial preservation of axons were found in 12 lesions. The predominant feature of these lesions was very dense astroglial proliferation (Fig. 1E). Astrocytes often occurred in bundles or streams (isomorphic gliosis) (Fig. 1E), and there were also very large giant astrocytes with glassy cytoplasm and plump processes.

There were no macrophages in these lesions. Perivascular mononuclear cuffs were found in about half of the lesions (Fig. 6A), and viral inclusions were found in only one.

Eight dogs had type 1 and type 2 lesions and occasionally a type 3 lesion. Three dogs had many type 3 lesions in addition to type 1 and 2. Three other dogs had only type 4 lesions.

No lesions were found in any of the normal control dogs.

Table 2. Incidence of the different types of immunoglobulin positive material in the different lesion types (numbers reflect only frequency not intensity)

Lesion type	Type 1 (acute) (total 30 lesions)	Type 2 (subacute) (total 20 lesions)	Type 3 (chronic) (total 11 lesions)	Type 4 (sclerotic) (total 13 lesions)
Ig bearing cells not perivascular	3 (10%)	15 (75%)	11 (100%)	12 (92.3%)
Ig bearing cells perivascular	1 (3.3%)	11 (55%)	11 (100%)	8 (61.5%)
Ig bearing cells in meninges	15 (50%)	14 (70%)	11 (100%)	4 (30.7%)
Amorphous Ig containing material	5 (16.6%)	6 (30%)	5 (45.4%)	5 (38.4%)
Ig bound to tissue elements	2 (6.6%)	0 (0%)	4 (36.3%)	5 (38.4%)
Ig in astrocytes	1 (3.3%)	3 (15%)	4 (36.3%)	4 (30.7%)
Ig in macrophages	0 (0%)	1 (0.5%)	8 (72.7%)	1 (0.8%)

Table 3. Immunoglobulin classes involved in different types of Ig positive material in the lesions (numbers reflect only frequency not intensity)

Immunoglobulin class	Number of lesions with IgG	Number of lesions with IgA	Number of lesions with IgM
Ig bearing cells not perivascular	39 (52.7%)	33 (44.5%)	17 (22.9%)
Ig bearing cells perivascular	28 (37.8%)	20 (27%)	7 (9.4%)
Ig bearing cells in meninges	42 (56.7%)	22 (29.7%)	23 (31%)
Amorphous Ig containing material	16 (21.6%)	1 (1.3%)	9 (12.1%)
Ig bound to tissue elements	11 (14.8%)	4 (5.4%)	1 (1.3%)
Ig in astrocytes	12 (16.2%)	0 (0%)	5 (6.7%)
Ig in macrophages	10 (13.5%)	0 (0%)	4 (5.4%)

b) *Immunohistology*

Findings on PAP treated sections of CDE cases included positively labeled cells, positively staining amorphous material, positive staining of tissue elements, macrophages and astrocytes. Only dark brown or black staining material that was clearly standing out against the background was considered to be positive. Further evidence for specific staining was provided by lack of reaction on corresponding slides treated with normal rabbit serum and by lack of staining on sections of normal brain tissue. The results of immunohistology are tabulated and correlated with the 4 main lesion types (Tables 2 and 3).

Lymphoid Cells. Such cells were clearly defined by their dark brown or black staining cytoplasm which appeared as a ring around the unstained nucleus (Fig. 2A). Such cells were considered to be immunoglobulin producing B lymphocytes. Sometimes the cytoplasm of these cells appeared large and excentric on PAP stains which corresponded with the histologic appearence of plasma cells (Fig. 2A). Lymphoid cells were frequently seen in meningeal spaces or sometimes in the choroid plexus (Fig. 3 C), overlying the lesions. Varying amounts of lymphoid cells were located within many lesions. These cells had often no vascular relation but were embedded deeply in the damaged white matter (Fig. 2A,C). Other cells were clearly associated with the perivascular spaces (Fig. 3B). A large proportion of the perivascular cells in type 3 lesions were immunoglobulin bearing cells, but other cells with a distinct lymphoid cell morphology did not stain and were probably in part T lymphocytes. The huge perivascular cuffs in old sclerotic lesions contained very small numbers of immunoglobulin bearing cells (Fig. 6A).

Amorphous Material. In a limited number of lesions small amounts of ill defined positively staining material was present. This material had often a globular shape but occasionally it appeared as irregularly shaped accumulations of dark brown amorphous material (Fig. 4C). The material was sometimes found immediately beneath the pia or on the ependymal surface extending into the border area of lesions. However, there was never a systematic or massive occurrence of this material throughout a demyelinating lesion.

Staining of Structural Elements. In a number of lesions of type 3 and type 4, strong staining of parts of the tissue itself was found. In several severe lesions there was a diffuse strong brown staining of the whole area (Fig. 4A). Staining was to a large extent due to labeled fibrous structures (Fig. 4D), which seemed to be nerve

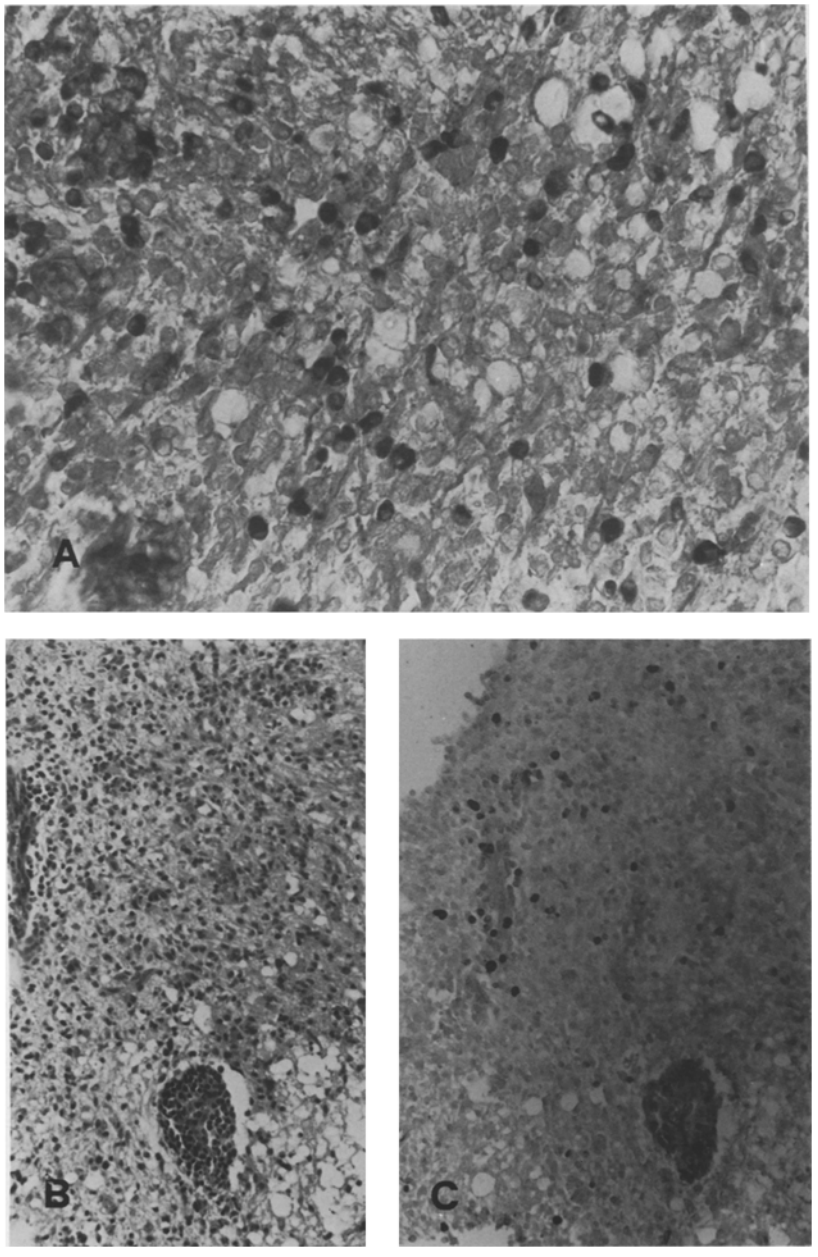

Fig 2. A Type 3 lesion: infiltration of degenerating white matter with Ig bearing cells. Anti-canine IgG. PAP, ×250. **B** Type 3 lesion: dense infiltration with inflammatory cells. H.-E., ×100. **C** Same area as B: scattered IgA bearing lymphoid cells. Anti-canine IgA. PAP, ×100

fibers, but it was not possible to identify these structures with certainty. Since extensive demyelination had occured in such lesions already, it was questionable whether myelin sheaths or parts of these were stained or whether perhaps axons had reacted. The reaction product was only in very few instances associated with vacuoles in demyelinating lesions (Fig. 4B). Immunoglobulins bound to degenerating white matter was practically nonexistent in the lesions of type 1 and 2.

Positively Reacting Macrophages. Because of their size and foamy cytoplasm, macrophages could be well recognized on the immunohistologic stains of many type 3 lesions. Macrophages were in part positively stained on the cell surface. In other lesions macrophages contained irregularly shaped clumps of positively stained material in their cytoplasm (Fig. 5B).

Astrocytes. Strongly positive staining of large reactive astrocytes was found especially in the type 3 and the type 4 lesions. The staining consisted of homogeneous dark brown to black staining of the astrocytic cytoplasm and part of their processes (Fig. 6A, B).

The type of immunoglobulin class involved in the lesions is tabulated (Tables 3 and 4). Although IgA and

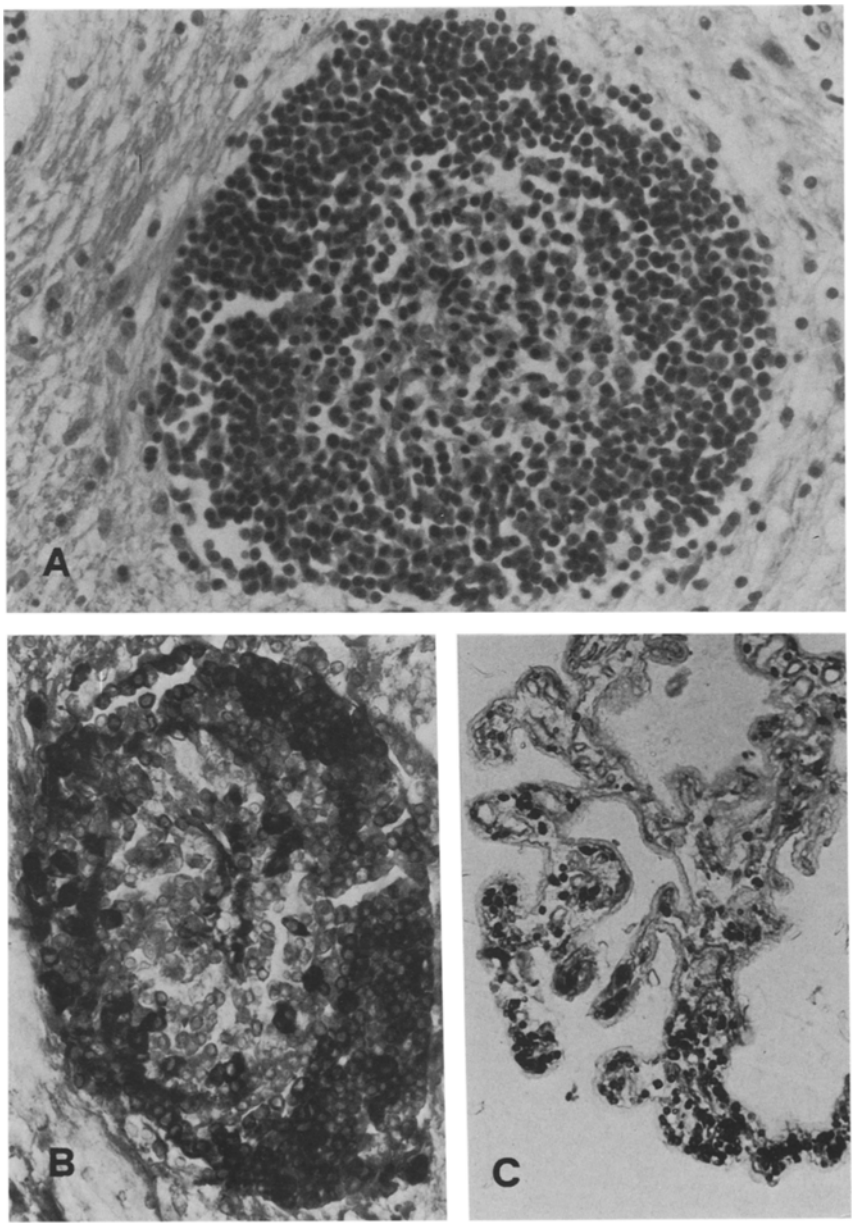

Fig 3. A Type 3 lesion: large perivascular cuff. H.-E., ×250. **B** Same cuff as in **A**: many cells contain immunoglobulin anti-canine IgG. PAP, ×250. **C** Chloroid plexus in IV ventricle in vicinity of type 2 lesion. Many Ig bearing cells. Anti-canine IgG. PAP, ×100

IgM bearing cells were seen in many lesions, they were by far less numerous than IgG bearing cells. Only in four dogs there was a notable IgM response, in one of these, with only acute lesions, IgM bearing cells prevailed. In two other dogs IgA bearing cells were moderately numerous.

Amorphous immune complexes contained predominantly IgG and IgM. Staining of structural tissue elements was mostly IgG. Positively stained astrocytes contained mostly IgG, and much less frequently IgM as well.

In acute lesions, positive immune staining was mainly limited to small numbers of immunoglobulin bearing cells in the overlying meninges. Moderate numbers of perivascular lymphoid cells as well as immunoglobulin bearing cells without perivascular relationship occured in many type 2 lesions. There were also several subacute lesions that contained amorphous Ig containing material. Massive invasion with immunoglobulin bearing cells was typical for the type 3 lesions. In many of these chronic lesions there were immune complex containing macrophages, amorphous Ig containing material and diffuse staining of tissue elements and astrocytes. The latter was also found in several sclerotic lesions where immunoglobulin bearing lymphoid cells were only present in moderate numbers.

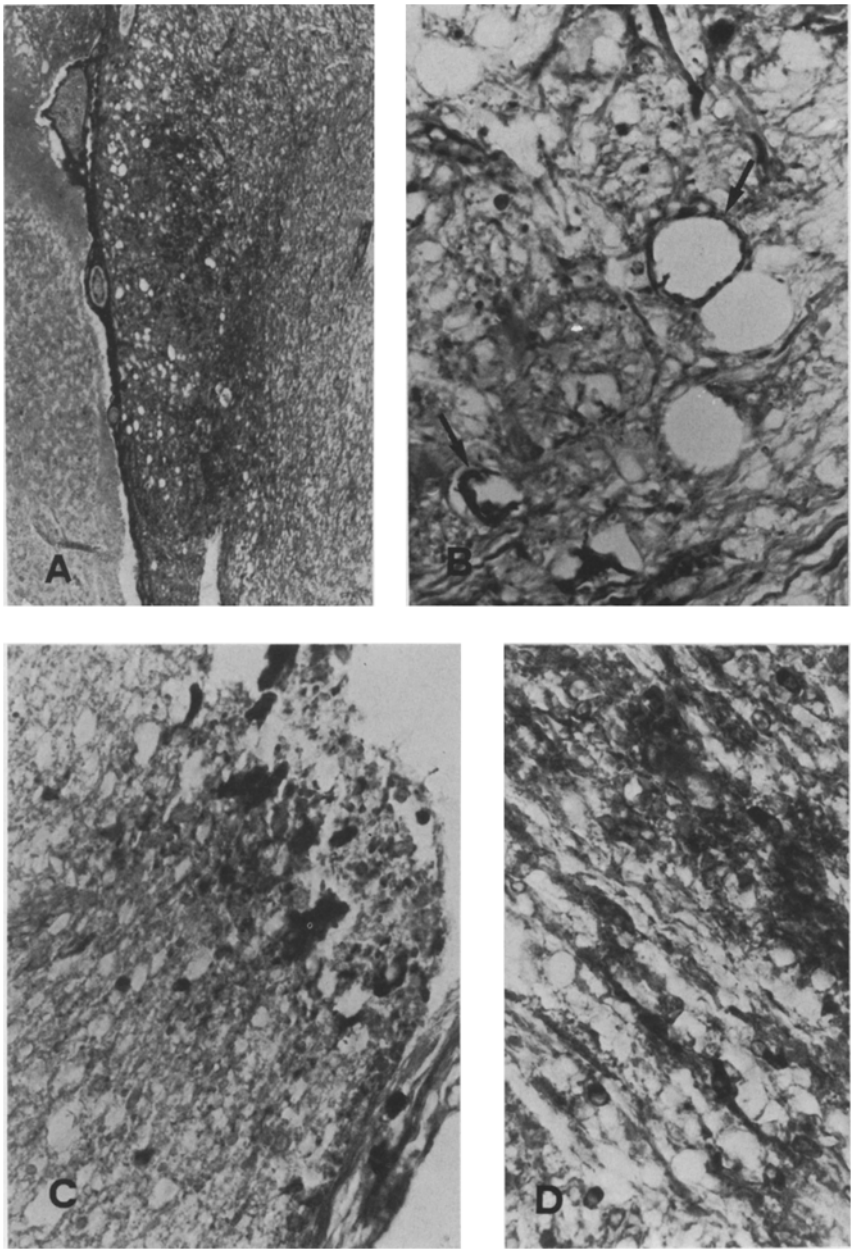

Fig 4. A Strong diffuse staining of a type 3 lesion. Anti-canine IgG. PAP, ×250. **B** Type 3 lesion: reaction product associated with a few vacuolated myelin sheaths (*arrows*). Anti-canine IgG. PAP, ×250. **C** Type 2 lesions: amorphous immunecomplexes at the edge of the lesions. Anti-canine IgM. PAP, ×250. **D** Type 3 lesions: diffuse staining partially due to positively staining fibrous structures. Anti-canine IgG. PAP, ×250

In the control dogs an occasional immunoglobulin bearing lymphoid cell was found in the meninges. A few – mostly IgG bearing – cells were seen in the choroid plexus in several sections.

Discussion

Because of the great variation in onset of neurologic signs after CDV infection (Appel and Gillespie 1972; Mc Cullough et al. 1974; Martin and Nathanson 1979) and of the frequent lack of clinico-pathologic correlation in CDE in our own experience, our temporal classification of the lesions was mainly based on morphologic criteria derived from observations on spontaneous and experimental CDE. According to experimental studies on CDE (Gillespie and Rickard 1956; Summers et al. 1979) vesiculation of white matter as in type 1 and 2 lesions of our study was considered to be an early change.

Observations on spontaneous (Campbell 1957) as well as on experimental CDE (Mc Cullough et al. 1974) strongly suggest that pronounced perivascular cuffing with mononuclear cells, as in type 3 lesions, occurs later on in the disease. Sclerotic lesions of the 4th type have been described in CDE with prolonged survival time (Vandevelde et al. 1979). The immunopathologic find-

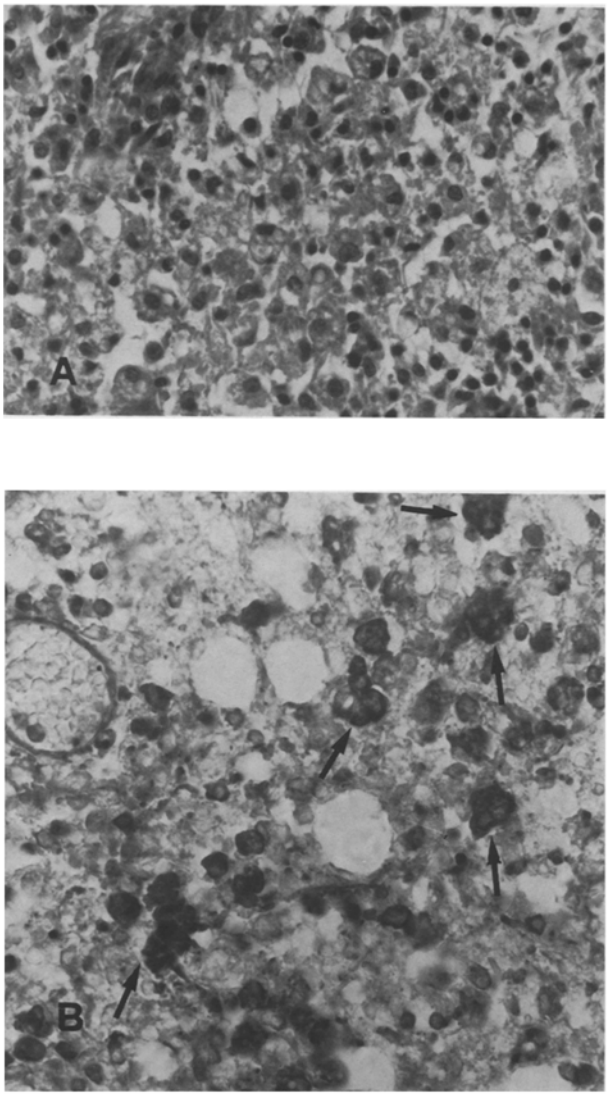

Fig 5. A Type 3 lesions: macrophages. H.-E., × 250. **B** Same lesion as **A**: many macrophages contain immunecomplexes (*arrows*). Anti-canine IgG, × 250

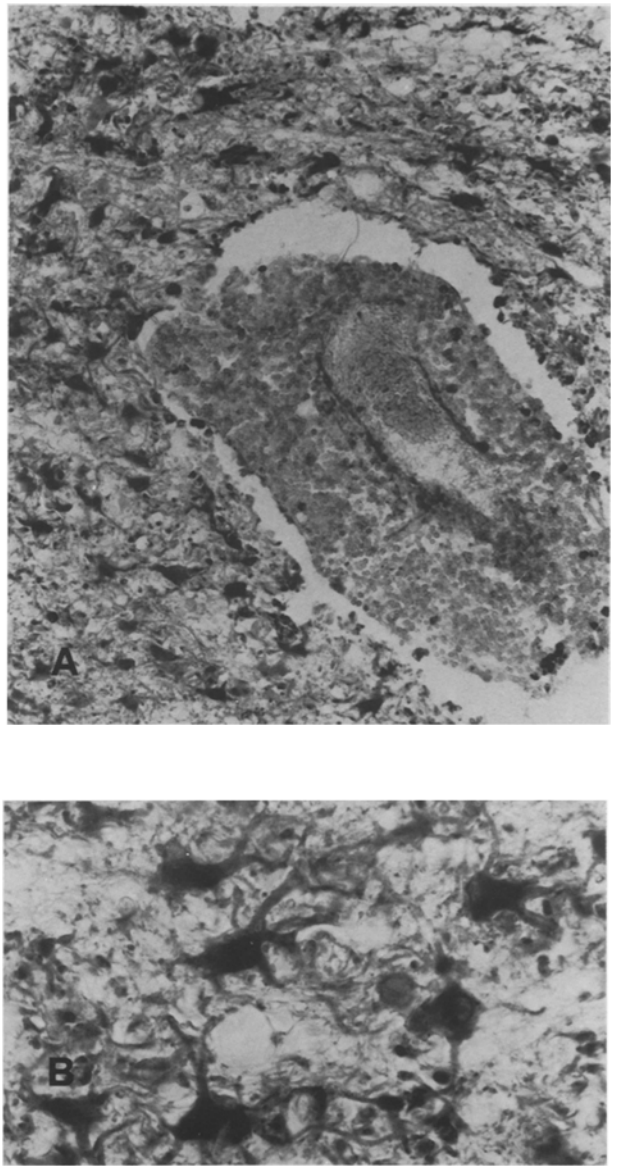

Fig 6. A Type 4 lesions: large perivascular cuff with only very few Ig bearing lymphoid cells. Strong positive staining of astrocytes. Anti-canine IgG. PAP, × 100. **B** Same lesion as A: positively staining astrocytes. Anti-canine IgG. PAP, × 250

Table 4. Distribution of Ig classes among different Ig containing lesion types (numbers reflect only frequency, not intensity)

	Total number of lesions with Ig	Number of lesions with IgG	Number of lesions with IgA	Number of lesions with IgM
Type 1 lesion (acute)	19	17 (89%)	5 (26.3%)	13 (68.4%)
Type 2 lesion (subacute)	18	18 (100%)	11 (61%)	11 (61%)
Type 3 lesion (chronic)	11	11 (100%)	10 (90.9%)	7 (63.6%)
Type 4 lesion (sclerotic)	13	13 (100%)	13 (100%)	2 (15.3%)

ings of a more pronounced IgM response in earlier lesions than in older lesions further support our temporal classification since a typical humoral immune response is initiated by IgM antibody formation, which then is followed by IgG antibody formation (Weiser et al. 1971).

The presence of immunoglobulins in many lesions in this study, undoubtedly indicates that the humoral immune system plays a significant role in CDE. It is probable that this local response is in part directed against canine distemper virus. Indeed, inclusion bodies indicating the presence of virus (Appel and Gillespie 1972) were found in many lesions. It is also known that antibodies against CDV are present in serum and CSF in CDE (Appel and Gillespie 1972; Krakowka et al. 1975). On the other hand, the fact that only small or undetectable amounts of virus may be present in active demyelinating lesions (Vandevelde and Kristensen 1977) and the positive correlation between severity of demyelination and severity of local inflammatory response found in this study, suggests that a local immune response plays an active role in CDE lesions, as indicated by others (Wisniewski et al. 1972; Koestner et al. 1974; Krakowka et al. 1978). It must be stressed, however, that although abundant evidence was found for the presence of immune reactions in many active lesions, none or only very weak responses were found in the earliest lesions, suggesting that antibody-dependent myelin destruction is not involved in the initial stages of demyelination in CDE.

Since the bulk of the immunoglobulins found in these lesions appeared to be associated with lymphoid cells, it is likely that migration of these cells and local production of immunoglobulin in the tissue take place in CDE. Immunoglobulin bearing cells in CDE lesions as well as in the adjacent CSF spaces could explain the presence of high titers of specific antibodies in CSF as in several neurologic diseases in man (Link 1978; Schliep and Felgenhauer 1979) and has been indicated by serologic studies on CSF in CDE (Cutler and Averill 1968).

Further interpretation of the immunohistologic data to explain how immunoglobulin could have caused tissue changes was difficult. Although immunoglobulin appeared to be bound to tissue components in several severe lesions, distinct coating of myelin with reaction product as in isolated brain prisms and tissue cultures (Johnson et al. 1979) in experiments on serum induced demyelination was not unequivocally demonstrated in our study. This may perhaps be partially due to technical distortion of myelin sheaths during tissue processing for paraffin embedding. It is possible that immunoglobulin was non-specifically bound in these lesions. Simultaneous demonstration of complement would allow a more direct interpretation of these findings. Although complement fixation may be the most attractive way to explain antibody mediated cell destruction, other mechanisms by which antibodies cause tissue damage are possible. Experimental studies using the rabbit eye model have shown that antibodies against myelin components in conjunction with nonspecific lymphocytic infiltrates can cause demyelination (Brosnan et al. 1977; Wisniewski et al. 1980).

Since anti-myelin antibody is present in serum of dogs with CDE (Krakowka et al. 1973) and since a vigorous inflammatory response with Ig-bearing cells as well as non Ig-bearing lymphoid cells – presumably in part T cells – occurred in chronic lesions in our study, such antibody-dependent cell-mediated myelin destructive mechanism could play a role in advanced CDE. The same rabbit eye model has been used to show that demyelination may occur as a result of nonspecific stimulation of macrophages by the presence of inflammatory cells in the vicinity of myelinated tissues (Kristensson et al. 1979). The release of proteolytic enzymes by stimulated macrophages resulting in so called bystander demyelination has also been demonstrated in vitro (Cammer et al. 1978). Since active macrophages are obviously present in advanced distemper lesions such a bystander effect could also be considered in the pathogenesis of chronic CDE.

Other evidence for immune mediated demyelination was provided by the finding of positive staining material in macrophages in some lesions. The question can be raised whether strong positive staining of astrocytes in several lesions is also based on phagocytosis of Ig containing material, since it is known that astrocytes are involved in active phagocytosis of myelin in CDE (Raine 1976). Similar staining of astrocytes has been observed in MS lesions (Dubois-Dalq et al. 1975; Prineas and Raine 1976). Although it appears that reactive astrocytes readily bind serum proteins in a nonspecific way (Aarli et al. 1975; Traugott et al. 1979), we cannot believe that the strong astrocyte staining in CDE lesions is altogether nonspecific. The binding was only seen with antiserum against IgG and to a lesser degree with anti IgM. It was not seen with anti-IgA antiserum nor with control serum. In addition, unspecific staining should be effectively blocked in the PAP method by treating the sections with concentrated link serum before specific antisera are applied (Sternberger 1979). The staining of astrocytes could of course be due to nonspecific absorption of immunoglobulin molecules by these cells in vivo, but this would also indicate that antibodies had been present in the lesions. Although autolytic changes were minimal in our material, post mortem uptake of immunoglobulin by astrocytes cannot be excluded. Immunohistologic studies with material obtained after perfusion – fi-

xation of living animals could resolve this question. Of interest was the frequent finding of IgA bearing cells in the lesions in our dogs. IgA bearing plasma cells have also been found in the perivascular cuffs in Multiple Sclerosis (Mussini et al. 1977). Although IgA cells were by far outnumbered by IgG containing lymphocytes it would still be tempting to speculate on the existence of a secretory like immune system in CNS infections similar to the secretory system in other organs (Weiser et al. 1971).

We conlude from our study that antibodies are probably not involved in the initial stages of demyelination in canine distemper. Early demyelination could be based on astroglial changes as proposed by Summers et al. (1979). The crucial role of the astrocytes in CDE has also been stressed by others (Frauchiger and Fankhauser 1971; Raine 1976; Vandevelde and Kristensen 1977). Since astrocytes exhibit phagocytic activity in CDE (Raine 1976) it is conceivable that these cells may also produce chemotactic factors attracting immunocompetent cells to the lesions to initiate a response against canine distemper virus and perhaps against altered and/or normal brain components. The individual immunogenetic background is probably responsible for the intensity of this immune response and predisposition for auto-immune reactions. The humoral immune response could result in additional tissue damage in the later stages of CDE as suggested by this study.

Further investigations correlating immunopathologic findings with immunologic parameters in vivo in dogs with spontaneously occurring CDE are in progress. Further support of the role of antibodies in myelin lesions could be provided by techniques such as elution and characterization of immunoglobulins from affected brain tissues (Gilden and Tachovsky 1979; Mehta et al. 1978; Link 1979). Immunohistologic techniques avoiding excessive post mortem changes of the white matter must be developed and a search should be made for complement proteins in the lesions.

We are aware of the limitations of investigations based on spontaneously occurring disease. We believe, however, that some useful information was obtained giving indications for research on experimentally induced CDE.

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