

Veterinary Immunology and Immunopathology 67 (1999) 101–116

Identification of CD4+ and CD8+ T cell subsets and B cells in the brain of dogs with spontaneous acute, subacute-, and chronic-demyelinating distemper encephalitis

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Received 22 September 1998; accepted 25 September 1998

Abstract

CD4 and CD8 antigen expression of T cells as well as B cell and canine distemper virus (CDV) antigen distribution were immunohistologically examined in the cerebellum of dogs with spontaneous distemper encephalitis. Cellular and viral antigen expression were evaluated at intralesional and extralesional sites and in the perivascular space. Histologically, acute and subacute non-inflammatory encephalitis and subacute inflammatory and chronic plaques were distinguished. Demyelination was a feature of all subacute and chronic lesions, although the majority of plaques exhibited no or only a low level of active demyelination as demonstrated by single macrophages with luxol fast blue positive material in their cytoplasm. CDV antigen expression, observed in all distemper brains, was reduced in chronic plaques. CD4+, CD8+, and B cells were absent in controls and in some brains with acute encephalitis. A mild infiltration of CD8+ cells was noticed in the neuropil of the remaining brains with acute and all brains with subacute non-inflammatory encephalitis. Single CD4+ cells were found in two brains with acute and in all brains with subacute non-inflammatory encephalitis. Numerous CD8+ and CD4+ cells and few B cells, with a preponderance of CD8+ cells, were detected in subacute inflammatory and chronic lesions. In contrast, in perivascular infiltrates (PVI) of subacute and chronic lesions a dominance of CD4+ cells was detected. The dominating CD8+ cells in acute and subacute non-inflammatory encephalitis might be involved in viral clearance or contribute as antibody-independent cytotoxic T cells to early lesion development. In subacute inflammatory and chronic lesions CD8+ cells may function as cytotoxic effector cells and CD4+ cells by initiating a delayed-type hypersensitivity

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reaction. The simultaneous occurrence of perivascular B and CD4+ cells indicated that an antibody-mediated cytotoxicity could synergistically enhance demyelination. Summarized, temporal and spatial distribution of CD4+, CD8+ and B cells and virus antigen in early and late lesions support the hypothesis of a heterogeneous in part immune-mediated plaque pathogenesis in distemper demyelination. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Canine distemper virus; Demyelinating leukoencephalomyelitis; CD4+ T cells; CD8+ T cells; B cells; Immunopathology

1. Introduction

Canine distemper virus (CDV), a pantropic, negative sense, single-stranded RNA morbillivirus, belongs to the family Paramyxoviridae and is closely related to measles virus (MV; Pringle, 1991). CDV infection of dogs, generally caused by inhalation of infectious aerosols, may lead to systemic disease with severe immunosuppression following primary replication of the virus in macrophages and lymphocytes of the respiratory tract and in lymphoid tissues (Krakowka et al., 1985). During the course of infection, spread of the virus to the central nervous system (CNS) and induction of specific lesions is frequently observed (Appel, 1969; Krakowka et al., 1985). CDVinduced brain lesions can be categorized in acute encephalopathy, subacute to chronic demyelinating encephalitis and inclusion body polioencephalitis (Krakowka et al., 1985; Nesseler et al., 1997). The type of neuropathological alterations is influenced by the age and immune status of the animal at the time of infection (Appel, 1969; Krakowka et al., 1975; Krakowka and Koestner, 1976; Rima et al., 1991) and the virus strain (Summers et al., 1984). Distemper-induced demyelinating encephalitis represents a suitable model for studying the pathogenesis of other demyelinating diseases of unknown etiology that might be triggered by infectious agents such as multiple sclerosis (MS; Dal Canto and Rabinowitz, 1982). Previous studies showed a plaque heterogeneity in demyelinating distemper encephalitis. Early demyelinating lesions are associated with the presence of CDV antigen and -mRNA, lack inflammatory cells and display only weak major histocompatibility complex class II-antigen (MHC-II) expression, whereas late demyelinating lesions are characterized by reduced expression of CDV antigen and mRNA, a strong upregulation of MHC II expression and an immune cell infiltration indicating a virus-independent immunopathological process in chronic alterations (Baumgärtner et al., 1989; Alldinger et al., 1993; Müller et al., 1995; Alldinger et al., 1996). While astrocyte infection can readily be demonstrated, infection of oligodendrocytes has long been doubtful. However, there is rising evidence, that restricted infection of oligodendrocytes, defined as absent or severely diminished viral antigen expression in the presence of viral RNA transcripts, causes myelin loss in early lesions (Zurbriggen et al., 1993, 1998).

The intracerebral presence of B cells and their importance for intrathecal antibody production during chronic demyelinating canine distemper leukoencephalomyelitis (DLE) is well documented (Vandevelde et al., 1982, 1986; Alldinger et al., 1996). B cells, mainly producing immunoglobulins of the IgG-subtype, were found, at variance,

within chronic lesions and in perivascular infiltrates (PVI; Vandevelde et al., 1982; Alldinger et al., 1996). Besides a prominent CDV-specific humoral immune response in serum and cerebrospinal fluid (Vandevelde et al., 1982, 1986; Rima et al., 1991), antimyelin specific antibodies were found (Krakowka et al., 1973; Vandevelde et al., 1986). Consequently a mechanism of demyelination based on complement-dependent antibodymediated humoral cytotoxicity was discussed (Vandevelde et al., 1982). However, the pathological significance of anti-myelin antibodies remained doubtful, since high titers of these antibodies were also detected in dogs with resolving lesions (Vandevelde et al., 1986). Furthermore, antibody-dependent T cell-mediated cytotoxicity was suspected, since many intralesional and perivascular lymphocytes were T cells (Vandevelde et al., 1982; Alldinger et al., 1996). A decline of intrathecal antibody production correlated with clinical improvement and it was suggested, that the humoral immune response was rather harmful than beneficial (Vandevelde et al., 1982). So far, only few studies have investigated the cellular immune response in distemper encephalitis. In chronic encephalitis, CD3+ cells, often lacking CD5 antigen expression, dominated the intralesional and perivascular infiltrates in the presence of a pronounced MHC II expression (Alldinger et al., 1996). To further investigate the role of the cellular immune response for the pathogenesis of distemper demyelination the intracerebellar distribution of CD4+, CD8+ and B cells was studied.

2. Materials and methods

2.1. Tissue samples and neuropathological diagnoses

The cerebella of 24 dogs with spontaneous CDV encephalitis and two control animals were investigated by using formalin-fixed, paraffin-embedded and frozen tissues. White matter lesions were classified as acute (focal vacuolation), subacute non-inflammatory (demyelination, astrogliosis with gemistocytes, multinucleated astrocytes and macrophages), subacute inflammatory (demyelination, malacia, gitter cells, macrophages, astrogliosis with gemistocytes, multinucleated astrocytes, and perivascular infiltration of two to three layers thickness), and chronic (demyelination, malacia, gitter cells, macrophages, astrogliosis with gemistocytes, multinucleated astrocytes, and severe perivascular infiltration). In advanced cases of DLE different lesions may occur simultaneously in the same brain. Therefore, final neuropathological diagnosis was based on the most advanced type of white matter lesions.

2.2. Histology and Immunohistology

Paraffin section of $3 \mu m$ thickness were stained with hematoxylin and eosin (H & E) and luxol fast blue (LFB) cresyl echt violet. Frozen sections (10 μ m) were cut on a cryostat (Reichert-Jung, Frigocut 2700), mounted on Superfrost Plus[®] slides (Menzel Gläser, Glasbearbeitungswerk, Braunschweig, Germany), fixed in acetone for 10 min at room temperature and stored at -70° C until used. Following sections were stained with H & E and immunohistochemically employing the avidin–biotin–peroxidase-complex

Specificity	Clone	Dilution	Reference
caCD4 ^a	YKIX302.9.3.7	1:100	Cobbold and Metcalfe, 1994
caCD8 ^a	Dog 10-1-1	1:1000	Voss et al., 1993
caIgG ^a	Dog 17-2	1:500	Cobbold and Metcalfe, 1994
caCD21 ^a (like)	CA2.1D6	1:500	Cobbold and Metcalfe, 1994
CDV-NP ^b	3.991	1:6000	Örvell et al., 1990

Monoclonal antibodies specific for canine lymphocyte surface antigens and canine distemper virus antigen

a ca = canine.

Table 1

^b CDV-NP = canine distemper virus nucleoprotein.

(ABC) method (Alldinger et al., 1996). Monoclonal antibodies (mAbs) directed against CDV nucleoprotein (NP), canine CD4 and CD8 antigen were employed (Table 1). B cells were characterized by the use of anti-canine CD21 (like)- and anti-canine IgG mAbs (Table 1).

Briefly, frozen sections were air-dried, rinsed twice in Tris buffered saline (TBS) for 10 min, followed by blocking of endogenous peroxidase with 0.03% H₂O₂ diluted in TBS for 30 min at room temperature prior to incubation with the primary antibody. Before incubation with the anti-CDV-NP antibody sections were preincubated with undiluted horse serum to block unspecific binding sites for 10 min at room temperature. Sections were subsequently incubated with the primary antibody overnight at 4°C, secondary antibody (biotinylated horse anti-mouse and biotinylated rabbit anti-rat, Vector Laboratories, Burlingame, CA), and the ABC (Vector Laboratories, Burlingame, CA) for 30 min at room temperature. All antibodies were diluted in TBS. After visualization of the positive antigen–antibody reaction by incubating the slides with 3,3' diaminobenzidine-tetrahydrochloride (DAB, Fluka Feinchemikalien GmbH, Neu Ulm, Germany), H₂O₂ in 0.1 M imidazole (Fluka Feinchemikalien GmbH, Neu Ulm, Germany), pH 7.1, for 10 min, sections were slightly counterstained with hematoxylin.

Controls included omission of primary antibody, link antibody and ABC or substitution of specific antibodies with ascites from non-immunized Balb/cJ mice. Canine spleen tissue and CDV infected African Green Monkey kidney (Vero) cells served as positive controls. Immunohistological expression of leukocyte differentiation antigens was evaluated quantitatively in three compartments (intralesional and extralesional sites and perivascular space). The number of cells stained per square unit of tissue within each compartment were counted using an ocular morphometric grid. Values represented the number of cells per mm². In the perivascular infiltrates (PVI) the number of stained and unstained cells was recorded and the percentage of immunohistologically detectable cells was calculated for each PVI. CDV antigen distribution was scored semiquantitatively (- = negative, + = single positive cells, ++ = moderate number of positive cells, +++ = numerous positive cells).

2.3. Statistical analysis

Statistical analysis of the data obtained by cell counting was performed by using the program BMDP (Dixon, 1993). Since the distribution of the data was skewed to the right

a logarithmic transformation was performed. For data description the geometric mean and dispersion factor were calculated and plotted. To evaluate differences between various plaque types and cell populations a two-way factor analysis of variance was computed. When significant differences were seen, a one-way factorial comparison of groups was added, employing the Wilcoxon–Mann–Whitney-test. A level of p < 0.05 was considered significant.

3. Results

3.1. Neuropathology

CDV antigen positive brains were categorized in acute (n = 10), subacute noninflammatory (n = 3), subacute inflammatory (n = 3), and chronic (n = 8) encephalitis. Lesions were located in the white matter, but occasionally extended into the granular layer of the gray matter. In contrast to formalin-fixed, paraffin-embedded tissue differentiation of acute and subacute non-inflammatory lesions was not always possible in frozen sections. However, focal CDV accumulation in these sections was suggestive of a corresponding lesion. Due to this difficulty and the similarity of immune cell infiltration in brains with acute and subacute non-inflammatory encephalitis the findings in these brains will be presented together by referring to the type of encephalitis and not to the type of plaque. In contrast, subacute inflammatory (n = 11) and chronic lesions (n = 22)were readily recognized in frozen tissue. Demyelination was observed in subacute and chronic plaques. The majority of plaques was inactive or displayed, at a low level, active demyelination as demonstrated by LFB-positive material within the cytoplasm of macrophages. In most active lesions only single phagocytes containing LFB-positive myelin degradation products were observed. Their number seemed to be increased in subacute inflammatory and some chronic lesions. In these plaques, LFB-positive macrophages showed a random intralesional distribution, while focal accumulations were rarely detected.

3.2. CDV antigen distribution

Both control brains were negative for CDV antigen. In brains with acute, subacute noninflammatory and subacute inflammatory alterations CDV antigen showed a diffuse or multifocal distribution (Fig. 1(A)). In acute and subacute non-inflammatory encephalitis, CDV antigen positive cells were detected in the white matter and granular layer of the gray matter. Virus antigen expression was mainly observed in astrocytes and less frequently in neurons, ependymal cells, choroid plexus epithelial cells, and microglia. The number of CDV antigen positive cells varied from few to many (Fig. 2). In brains with subacute inflammatory plaques CDV antigen was only found in lesions (Fig. 3(A)). A reduction of CDV antigen expression with almost complete clearance in the center was observed in chronic lesions. Single CDV antigen positive astrocytes were seen at the edge of these lesions (Fig. 4(A)). Since the latter were included in the virus scoring, the obtained values may appear disproportionally high, despite a dramatic loss of viral



Fig. 1. Cerebellum, acute canine distemper encephalitis, following sections, frozen tissue. CDV antigen (A) displayed a diffuse distribution and was located mainly in astrocytes, endothelial cells or pericytes of the white and gray matter. Few CD8+ (B) and single CD4+ cells (C, arrows), randomly distributed, were present in the white matter. G = gray matter; A–C: ABC-method, bar = $110 \,\mu$ m.

antigen in the majority of lesions (Fig. 2). Perivascular infiltrating lymphocytes were CDV antigen negative in all brains investigated.

3.3. Distribution of inflammatory cells

No lymphocytes were detected in the CNS of control dogs. CD8, CD21 (like) and IgG antigen-specific cell membrane-bound expression was demonstrated on lymphocytes only. CD4-specific reaction was found on lymphocytes and as a faint brown cytoplasmic precipitate in gitter cells. Cells with granulocyte morphology were not present in distemper brains and there was no immunoreactivity with a canine granulocyte-specific monoclonal antibody (Cobbold and Metcalfe, 1994; dog 15, data not shown). IgG antigen expression was occasionally found in the cytoplasm of plasma cells. However,



Fig. 2. Number of CD4+, CD8+, and B cells and semiquantitatively determined amount of CDV antigen positive cells in acute and subacute non-inflammatory encephalitis and in subacute inflammatory and chronic demyelinating lesions in distemper encephalitis. In acute and subacute non-inflammatory encephalitis no or only few CD8+ and single CD4+ cells were found in the presence of abundant CDV antigen expression. In subacute inflammatory lesions a strong infiltration of CD8+ and CD4+ cells was noticed (CD8+/CD4+ cell ratio: 3 to 1). Few B cells migrated into the neuropil. In chronic lesions with a declining CDV antigen expression, CD4+ and CD8+ cell infiltration into the neuropil was reduced. Geometric mean and dispersion factor are demonstrated.

interpretation of IgG antigen immunohistochemistry was severely hampered in most cases by a diffuse background staining due to serum leakage from the vasculature into the neuropil. Therefore, the anti-CD21 (like) mAb was chosen for quantitative evaluation of B cell distribution.

Immunohistologically detectable immune cells were absent in five brains with acute encephalitis. Few CD8+ cells were found diffusely distributed in the white matter and granular layer of the gray matter in the remaining brains with acute and subacute non-inflammatory encephalitis (Fig. 1(B)). Scattered single CD4+ cells were seen in two brains with acute and all brains with subacute non-inflammatory encephalitis (Fig. 1(C)). In general, the number of CD8+ cells exceeded the number of CD4+ lymphocytes (p = 0.04; Fig. 2). B cells were not detected in these brains. Although infiltrating cells seemed to accumulate in single plaques, the majority of cells exhibited a random distribution and there was no clear morphological correlation between plaque distribution, virus antigen expression and cellular infiltrates.

In animals with subacute inflammatory and chronic plaques immune cells were detected in the perivascular space, within plaques and at extralesional sites. In the latter

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Fig. 3. Cerebellum, subacute inflammatory demyelinating lesion in distemper encephalitis, following sections, frozen tissue. Moderate number of CDV antigen positive cells (A) restricted to the lesion, extending from the white matter to the granular layer of the gray matter. Many CD8+ (B), few CD4+ (C), and single B cells (D). G = gray matter; A–D: ABC-method, Bar = 70 µm.

compartment, CD8+ and CD4+ cells were observed diffusely distributed in the brain parenchyma. Their numbers, distribution and the ratio between both T cell subsets were similar to the findings in acute and subacute non-inflammatory encephalitis.

In subacute inflammatory lesions the majority of lymphocytes displayed the CD8+ T cell phenotype (Fig. 3(B)). CD4+ cells were regularly found in these lesions, but in smaller numbers (p = 0.03; Fig. 3(C)). The CD8+/CD4+ cell ratio was about 3 to 1. B cells were only seen in 3 out of 11 subacute inflammatory lesions (Fig. 3(D)). The PVI contained significantly more CD4+ (27%, dispersion factor; 1.7) than CD8+ cells (7%, dispersion factor: 1.9, p = 0.0008), and no B cells (Fig. 5).

In chronic lesions a reduction of CD8+ and CD4+ cells per mm² was noticed compared to subacute inflammatory lesions (Figs. 2 and 4(B) and (C)). Although a

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Fig. 4. Cerebellum, chronic demyelinating lesion in distemper encephalitis, following sections, frozen tissue. Clearance of CDV antigen (A) from the center of the lesion, note single CDV antigen positive cells at the edge of the plaque. Moderate number of CD8+ (B), and CD4+ lymphocytes (C), and single B cells (D) in the neuropil. Perivascular infiltrates (PVI) were composed of slightly more CD4+ (C) than CD8+ cells (B). B cells (D) were found in single PVI in a notable number. Note variation of cellular composition of PVI. G = gray matter; A–D: ABC-method, Bar = 170 μ m.

variation concerning the intralesional composition was found within different plaques, in general, the number of CD8+ cells was higher compared to CD4+ cells (p = 0.0001; Fig. 2) and the CD8+/CD4+ cell ratio remained about 3 to 1 (Fig. 2). B cells were seen regularly in chronic lesions, but less frequently than CD8+ and CD4+ cells (p < 0.0001; Figs. 2 and 4(D)). The prominent PVI of chronic lesions were composed of about 27% CD4+ cells (dispersion factor: 2.0), 18% CD8+ cells (dispersion factor: 2.4) and 19% B cells (dispersion factor: 2.6; Figs. 5 and 6(A)–(D)). The difference between the percentage of CD4+ cells compared to CD8+ (p = 0.0016) and B cells (p = 0.02; Fig. 5), was despite the high dispersion factor significant. Moreover, the percentage of



Fig. 5. Percentage of CD4+, CD8+, and B cells in cerebellar perivascular infiltrates (PVI) of subacute inflammatory and chronic demyelinating lesions in distemper encephalitis. In PVI of subacute and chronic demyelinating lesions a dominance of CD4+ cells was detected. In PVI of chronic lesions the percentage of CD8+ and B cells increased. Geometric mean and dispersion factor are demonstrated.

CD8+ (p = 0.0042) and B cells (p = 0.0067) increased significantly in PVI of chronic compared to subacute inflammatory lesions (Fig. 5).

4. Discussion

The present study confirms and extends previous findings that the intracerebellar immune response in spontaneous DLE is T cell-dominated (Alldinger et al., 1996). Cellular infiltrates displayed marked phenotypical differences in various types of plaques and in the investigated CNS compartments such as intra- and extralesional sites and perivascular space. In brains with acute and subacute non-inflammatory encephalitis only few CD8+ and CD4+ cells were observed; hereby, CD8+ cells seemed to enter the CNS earlier than CD4+ cells. In subacute inflammatory and chronic lesions a strong infiltration of CD8+ and CD4+ cells (CD8+/CD4+ cell ratio: 3 to 1) was noticed, whereas a preponderance of CD4+ cells was detected in PVI. As shown in previous studies the paucity of intracerebellar inflammatory infiltrates in acute and subacute non-inflammatory distemper encephalitis coincides with severe lymphocytic depletion in lymphoid tissues, whereas inflammatory brain lesions are associated with recovery and repopulation (McCullough et al., 1974; Krakowka et al., 1980; Fankhauser, 1982; Summers and Appel, 1994). Cellular infiltrates except for few macrophages and a mild



Fig. 6. Cerebellum, perivascular infiltrate in a chronic demyelinating lesion in distemper encephalitis, following sections, frozen tissue. Severe infiltration of mononuclear cells in the space of Virchow Robin (A). Few CD8+ (B), numerous CD4+ (C) and moderate number of B cells (D). A: Hematoxylin-Eosin. B–D: ABC-method. A–D: Bar = $170 \,\mu$ m.

transient perivascular infiltration of lymphocytes are rarely observed in early lesions using conventional techniques (Summers et al., 1979). Similarly, a mild infiltration of randomly distributed single T cells, predominantly of the CD8 phenotype, were immunohistologically detected in brains with non-inflammatory lesions in the present study. Although there seemed to be an increase of inflammatory cells in some plaques, the overall random distribution of these cells suggested that they played no major contributing role in early lesion development. The immunohistologically detected T cells might be part of the normal T cell surveillance of the CNS as it occurs following T cell activation in the periphery (Hickey and Kimura, 1987; Hickey et al., 1991) or represent an attempt of the host to terminate viral CNS infection as has been described for mouse hepatitis (MHV), Theiler's murine encephalomyelitis (TMEV), and murine lymphocytic

choriomeningitis virus infection (Oldstone et al., 1986; Lindsley and Rodriguez, 1989; Sedgwick and Dörries, 1991; Borrow et al., 1992; Dethlefs et al., 1997). However it still remains a possibility that CD8+ cells may be cytotoxic effector cells when they encounter cells presenting viral antigens or epitopes similar to viral proteins (molecular mimicry; e.g. myelin basic protein, heat shock protein) (Sheshberadaran and Norrby, 1984; Jahnke et al., 1985).

Associated with recovery from lymphoid atrophy a notable cellular infiltration and increased MHC II expression leading to virus clearance in chronic plaques can be observed in DLE (Fankhauser, 1982; Cerruti-Sola et al., 1983; Bollo et al., 1986; Alldinger et al., 1996). The CD8+/CD4+ cell ratio in subacute inflammatory and chronic plaques was about 3 to 1. This is almost the opposite to the CD8+/CD4+ cell ratio observed in T cell areas of lymphoid tissues in control dogs (Rabanal et al., 1995) indicating a selective migration of CD4+ and CD8+ cells into the neuropil. Several immune-mediated mechanisms including anti-myelin antibodies in serum and cerebrospinal fluid (Krakowka et al., 1973; Vandevelde et al., 1986), increased MHC II expression, and both non-specific and CDV antibody-mediated generation of reactive oxygen species in macrophages (by-stander demyelination) have been described as possible mechanisms for the pathogenesis of chronic demyelinating distemper lesions (Cerruti-Sola et al., 1983; Griot et al., 1989; Alldinger et al., 1996). The present findings showed that possibly cytotoxic CD8+ effector cells contribute to the progression of the lesion. In addition, CD4+ cells may activate macrophages as the final effector cells in the course of a delayed-type hypersensitivity response. A similar pathogenesis has been proposed for MHV- and TMEV-induced demyelination (Oleszak et al., 1995; Houtman and Fleming, 1996; Tsunoda and Fujinami, 1996). Infiltration of CD8+ and CD4+ lymphocytes in inflammatory lesions despite reduced viral antigen expression suggested that the cellular immune response may not only be directed against and triggered by virus- but also self-antigens. The occurrence of T cells, responding to self-antigens, could be the consequence of disturbed thymic negative selection, following virus infection of thymic dendritic cells, as shown for MV and MHV infection (Kyuwa et al., 1991; Auwaerter et al., 1996).

CD8+ cells dominated the infiltrates in the neuropil during all stages of DLE. A functional diversity of these cells including cytotoxicity and immunoregulatory activities (Kemeny et al., 1994) can be assumed because of their spatial and temporal distribution. Besides cytotoxicity, a protective role of CD8+ cells by downregulating the severity of chronic demyelinating lesions in experimental allergic encephalomyelitis (EAE) and TMEV has been described (Sun et al., 1988; Borrow et al., 1992). Whether these activities can be correlated with a cell type-specific set of cytokines in early and late distemper lesions needs to be investigated (Kemeny et al., 1994; Dutton, 1996).

The perivascular space in subacute and chronic demyelinating lesions contained predominantly CD4+ and B cells. These tightly packed cuffs provide a microenvironment for the effective utilization of CD4+ cell-derived cytokines by B cells, resulting in a strong antibody production (Esiri and Gay, 1990). The synergistic action of the cellular and humoral immune response represents an essential component for the development of demyelinating lesions in EAE and a similar mechanism might operate in chronic distemper lesions (Linington et al., 1988). The spatial distribution of CD4+ cells in the

perivascular space and in the neuropil may be influenced or requested by a subset-specific cytokine repertoire (Romagnani, 1995).

A certain percentage of perivascular cells did not express the CD4, CD8 and CD21 (like) antigen. These cells consisted of endothelial cells, macrophages and other not yet identifiable mononuclear cells. Whether these are CD4+ and CD8+ cells with a subthreshold expression of the investigated cell surface differentiation antigens or belong to different cell lineages, e.g. natural killer cells or $\gamma\delta$ T cells, which may lack both differentiation antigens remains undetermined (Fowlkes and Pardoll, 1989). The latter plays an important role in various autoimmune disorders (Olive, 1995).

Whether the immune response triggers the damage or is a consequence of the disease process represents a key question for the pathogenesis of primary demyelination. The lack or the presence of only single LFB-positive macrophages suggested that most lesions were inactive or displayed, only at a low level, active demyelination. The latter seemed to be more prominent in subacute inflammatory and some chronic lesions, indicating that the observed inflammatory response was associated with active demyelination in these plaques. Whether the inflammatory cells represent a primary or secondary phenomenon, and to what extent active demyelination is directly correlated to lymphocyte and macrophage activation in DLE, needs to be determined in future studies.

In distemper encephalitis distribution of T cell subsets varied depending upon the plaque type and the CNS compartment and displayed a plaque-specific spatial and temporal distribution. In brains with acute and subacute non-inflammatory distemper encephalitis only randomly distributed T cells, although at a low level, were detected. Phenotypically these cells belonged predominantly to the CD8 subpopulation. The occurrence of these cells can be interpreted as an attempt of the host to terminate viral CNS infection. However, they could also represent an immunopathological cytotoxic antibody-independent T cell response, which acts synergistically with the direct virusinduced cytolytic process in the pathogenesis of early lesions. In subacute inflammatory and chronic demyelinating DLE the intralesional infiltrates were dominated by CD8+ cells, while CD4+ and B cells were more prominent in perivascular cuffs. CD4+ cells may support antibody production by B cells and stimulate macrophages to evoke a delayed-type hypersensitivity reaction depending upon their occurrence in the perivascular space or within lesions, respectively. The distribution of phenotypically identical and different T cell subsets in the various CNS compartments as well as in early and late lesions indicated a highly complex most likely cytokine controlled cellular immune response in various plaque-types in demyelinating distemper encephalitis.

Acknowledgements

The authors wish to thank Annette Artelt and Sandra Heinz for excellent technical assistance, Ute Zeller for photographic support and Dr. K. Failing and H. Heiter for their help and advice for the statistical analyses. We thank Dr. P.F. Moore for kindly providing the anti-CD21 (like) mAb (clone CA2.1D6). A. Wünschmann received a research award from the Justus-Liebig-Universität, Giessen, Germany. This study was supported by grants of the Deutsche Forschungsgemeinschaft (Ba 815/4-1 and 815/4-2) and the Gemeinnützige Hertie-Stiftung.

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