ELSEVIER

Veterinary Immunology and Immunopathology
73 (2000) 83–98

Veterinary immunology and immunopathology

www.elsevier.com/locate/vetimm

# Phenotypical characterization of T and B cell areas in lymphoid tissues of dogs with spontaneous distemper

A. Wünschmann[a], E. Kremmer[b], W. Baumgärtner[a,*]

[a]*Institut für Veterinär-Pathologie, Justus-Liebig-Universität, Frankfurter Straße 96, 35392 Gießen, Germany*
[b]*GSF-National Research Center for Environment and Health, Institute of Immunology, 81377 München, Germany*

Accepted 3 November 1999

**Abstract**

CD3, CD4, CD5, and CD8 antigen expression of T cells and IgG expression of B cells and canine distemper virus (CDV) antigen distribution were immunohistochemically examined in lymphoid tissues (lymph node, spleen, thymus, and tonsil) of control dogs and animals with spontaneous canine distemper. In addition, CNS tissue of all animals was studied for neuropathological changes and CDV antigen distribution. Based on the degree of depletion distemper dogs were classified into two groups. Group I represented animals with moderate to marked lymphoid depletion, while group II dogs displayed mild or no depletion. CDV antigen was mainly found in lymphocytes and macrophages of group I dogs, whereas CDV expression was most prominent in dendritic cells of group II animals. In group I dogs, a marked loss of CD3, CD4, CD5, CD8, and IgG expression was noticed, hereby loss of CD4+ cells was more prominent than depletion of CD8+ cells. In the lymphoid tissues of group II animals, a significant increase in the number of T and B cells was observed compared to group I dogs. The number of CD3+, CD4+, and CD8+ cells in group II dogs was similar to the findings in controls, however, CD5 and IgG expression was mildly reduced in T and B cell areas, respectively. Additionally, in groups I and II dogs, CD3+ and CD5− T cells were detected in T cell areas. Whether this cell population represents a cell type with autoimmune reactive potential remains to be determined. Surprisingly in group II animals, viral antigen was found predominantly in dendritic cells indicating a change in the cell tropism of CDV during chronic infection and a possible mechanism of viral persistence. The two patterns of lymphoid depletions correlated to two different types of canine distemper encephalitis (CDE). Group I dogs displayed acute non-inflammatory CDE, whereas group II dogs suffered from chronic inflammatory demyelinating CDE, indicating a pathogenic relationship between lymphocytic depletion and inflammatory brain lesions in distemper. 

* Corresponding author. Tel.: +49-641-99-38202; fax: +49-641-99-38209.
*E-mail address*: wolfgang.baumgaertner@vetmed.uni-giessen.de (W. Baumgärtner).

0165-2427/00/$ – see front matter 
PII: S0165-2427(99)00156-7

*Keywords:* T cell antigens; B cell antigen; Canine distemper virus; Lymphoid tissue; Lymphocytic depletion; Distemper encephalitis

## 1. Introduction

Canine distemper virus (CDV), a pantropic, negative sense, single-stranded RNA morbillivirus, belongs to the family Paramyxoviridae (Pringle, 1999). CDV infection of dogs, generally caused by inhalation of aerosols, may lead to systemic disease with severe immunosuppression following primary replication of the virus in macrophages of the respiratory tract and lymphoid tissues (Krakowka et al., 1985). CDV-induced macroscopic and microscopic changes of lymphoid tissues include thymic atrophy, depletion in the B and T cell areas with loss of secondary follicles, hyperplasia of reticular cells, follicular necrosis, formation of giant cells, and intracytoplasmic inclusion bodies in reticular and lymphatic cells (Lauder et al., 1954; Gibson et al., 1965; Appel, 1969, 1987; Jacoby and Griesemer, 1970; McCullough et al., 1974; Stevens and Osburn, 1976; Krakowka et al., 1977, 1980, 1985; Krakowka and Koestner, 1977; Iwatsuki et al., 1995). Experimental studies indicated that morphological alterations in lymphoid tissues are reversible in animals recovering from CDV infection (Gibson et al., 1965; McCullough et al., 1974; Krakowka et al., 1977, 1980). The mechanism of CDV-associated immunosuppression remains unclear and several hypotheses have been formulated, including virus-mediated T cell cytolysis or disturbed function of CD4+ cells (Mangi et al., 1976; Appel et al., 1982; Cerruti-Sola et al., 1983; Winters et al., 1983). Using double labeling immunohistochemistry, CDV infection mainly of CD4+ cells, but also of CD8+ and B cells has been found in spontaneous distemper, resulting in a marked decrease mainly of CD4+ cells (Iwatsuki et al., 1995). In a recent study it has been shown, that lack of peripheral blood cytokine mRNA transcripts, including IL-1, IL-6, IL-8, IL-12, TNF, and TGF, either due to depletion of cytokine producing cells or virus-induced down-regulation of cytokine genes in virus-infected leukocytes is associated with viremia in canine distemper, indicating that a direct virally-induced effect may be responsible for a diminished immune reaction (Gröne et al., 1998). In addition, continuous impairment of the immune response after virus clearance from lymphoid organs (Krakowka et al., 1975, 1980) may be due to activation of a mononuclear suppressor cell population (Krakowka et al., 1978, 1985).

To further investigate the cellular immune response in dogs suffering from spontaneous distemper, various B and T cell subpopulations in lymphoid tissues were studied immunohistochemically, and results were correlated to the type of encephalitis.

## 2. Materials and methods

### *2.1. Tissue samples*

Frozen tissue of spleen, thymus, tonsil, and mandibular lymph node of four female and four male healthy Beagles, 7–8 months of age ($n = 8$, Nos. 1–8, control group, CG) and

Table 1
Age, sex, breed, and lymphoid tissue samples of 14 dogs with spontaneous distemper

| Animal No. | Age (months) | Sex | Breed | Lymphoid tissues sampled[a] |
|---|---|---|---|---|
| 9 | 4 | Female | Husky | thy, spl, ln |
| 10 | Puppy | Male | Not known | spl, ln |
| 11 | 5 | Male | Husky | spl, ln |
| 12 | 3 | Male | Bobtail | thy, spl, ln, ton |
| 13 | 2 | Female | Golden Retriever | thy, spl, ln, ton |
| 14 | 4 | Female | Dachshund | thy, spl, ln |
| 15 | 5 | Male | German Shepherd | thy, spl, ln, ton |
| 16 | 5 | Male | Mongrel | thy, spl, ln, ton |
| 17 | 3 | Male | Mongrel | thy, spl, ln, ton |
| 18 | 5 | Female | Mongrel | spl, ln |
| 19 | 9 | Female | Mongrel | spl, ln, ton |
| 20 | Adult | Male | Mongrel | thy, spl, ln, ton |
| 21 | 4 | Male | Jack Russel Terrier | thy, spl, ln, ton |
| 22 | 4 | Female | Mongrel | thy, spl, ln, ton |

[a] thy = thymus; spl = spleen; ln = lymph node; ton = tonsil.

dogs with spontaneous canine distemper ($n = 14$, Nos. 9–22) were used. The control animals served as control group for a toxicological study and were vaccinated and wormed according to standard protocols. Splenic, lymph nodal, thymic and tonsillar tissues were available from 14, 14, 10, and 9 dogs with distemper, respectively (Table 1). In addition the CNS was collected at necropsy and fixed in formalin. The animals were derived from the necropsy material of the Institut für Veterinär-Pathologie of the Justus-Liebig-Universität Giessen, Germany.

### 2.2. Histology and immunohistology

Frozen sections (10 μm) of the lymphoid tissues 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. Immunohistochemistry was performed by employing the avidin–biotin–peroxidase-complex (ABC) method (Alldinger et al., 1996). Monoclonal antibodies (mAb) directed against CDV nucleoprotein (N-4), canine CD4, CD8, CD5, and IgG were used (Table 2). In addition a cross reacting anti-human CD3 antiserum, recognizing the intracytoplasmic portion of CD3, was applied (Table 2). Prior to incubation with mAbs, frozen sections were air-dried, rinsed twice with TRIS-buffered saline (TBS) for 10 min, followed by blocking of the endogenous peroxidase with 0.03% $H_2O_2$ diluted in TBS for 30 min. Before incubation with the anti-CD3 antiserum and the anti-CDV-N-4 mAb sections were preincubated with undiluted pig serum and horse serum, respectively, to block unspecific binding sites. Sections were subsequently incubated with primary antibodies overnight at 4°C, secondary antibodies (biotinylated horse anti-mouse, biotinylated rabbit anti-rat, and biotinylated goat anti-rabbit; Vector Laboratories, Burlingame, California) and the ABC

Table 2
Specificity, clone, and working dilution of the employed poly- and monoclonal antibodies

| Specificity | Clone | Dilution | Reference |
|---|---|---|---|
| huCD3 | Polyclonal | 1 : 500 | Ferrer et al., 1992 |
| caCD5 | Dog 17-4-8 | 1 : 100 | Cobbold and Metcalfe, 1994 |
| caCD4 | YKIX302.9.3.7 | 1 : 100 | Cobbold and Metcalfe, 1994 |
| caCD8 | Dog 10-1-1 | 1 : 1000 | Voß et al., 1993 |
| caIgG | Dog 22-2 | 1 : 500 | Cobbold and Metcalfe, 1994 |
| CDV-N-4 | 3.991 | 1 : 6000 | Örvell et al., 1990 |

(Vector Laboratories, Burlingame, California) for 30 min at room temperature. All monoclonal antibodies were diluted in TBS, while the anti-CD3 polyclonal antibody was diluted in TBS containing 20% pig serum. After visualization of the positive antigen–antibody reaction by incubating the slides in 3,3′-diaminobenzidine-tetrahydrochloride (DAB, Fluka Feinchemikalien GmbH, Neu Ulm, Germany)-$H_2O_2$ 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 the primary antibody, link antibody and ABC or substitution of the specific antibody with ascites from non-immunized Balb/cJ mice. Spleen tissue of a control dog and CDV infected African Green Monkey kidney (Vero) cells served as positive controls.

For routine histology formalin-fixed tissue was embedded in paraffin, cut in 5 μm thick sections, and stained with H & E. Brains of all dogs were additionally stained with Luxol Fast Blue-Cresyl Violet to determine the degree of demyelination. Immunohistochemistry for detection of CDV antigen was performed as described (Baumgärtner et al., 1989).

### 2.3. Scoring and statistical analysis

The degree of depletion in different compartments was evaluated semiquantitatively as follows: 0 = normal tissue architecture, 1 = mild, 2 = moderate, and 3 = severe depletion. Primary and secondary follicles were distinguished in lymph node, tonsil and the white pulp of the spleen. The thymus and T cell areas including the paracortex of the mandibular lymph node, the interfollicular zone of the tonsil, and the PALS (periarterial lymphatic sheaths) of the splenic white pulp were also investigated.

Tonsils and lymph nodes displaying numerous secondary follicles were considered normal. In the germinal center of most follicles the light and dark zone could be distinguished. Mild depletion consisted of loss of secondary follicles, whereas paracortex and medulla were still clearly distinguishable in lymph nodes. A reduction of the cortex in size and lack of recognizable follicles was considered a moderate depletion in lymph nodes. Moderately depleted tonsils were characterized by the presence of only few small follicles. Lack of recognizable cortex and follicles were criteria for severe depletion.

In a normal spleen numerous areas with primary and secondary follicles can be distinguished. Mild depletion was characterized by a reduction of the size of the white

pulp areas and a loss of secondary follicles, whereas a marked reduction of the size of the white pulp and a loss of follicles were indicative of moderate depletion. In severely depleted spleens, the white pulp was no longer recognizable.

For grading thymic alterations, cellularity and distinction of cortical and medullary borders were the most important parameters.

Based on the degree of depletion a 'depletion index' was calculated for each animal (sum of degree of depletion of investigated lymphoid tissues divided by the number of tissues investigated). Accordingly, animals were classified into two groups. Animals with a depletion index > 1 represented group I ($n = 9$, Nos. 9–17), while dogs with a depletion index $\leq 1$ composed group II ($n = 5$, Nos. 18–22).

Immunohistological determination of the expression of leukocyte and CDV antigen ('immunoreactivity score') was evaluated semiquantitatively in the different compartments (0 = no; 1 = few; 2 = some; 3 = numerous positive cells; 4 = nearly all/most cells positive). The immunoreactivity of the anti-CDV-N-4 mAb was classified similarly (0 = no; 1 = weak; 2 = moderate; 3 = strong immunoreactivity). In each organ 10 randomly selected areas of each compartment were evaluated at high power by light microscopy.

For the statistical analysis organs without depletion received the 'depletion factor' 4, while organs with mild, moderate and severe cellular loss were assigned to factors 3, 2, and 1, respectively. The 'immunoreactivity index' was obtained by multiplying the immunoreactivity score for each leukocyte differentiation antigen with the organ-specific 'depletion factor'. The immunoreactivity indices were described in form of frequency tables. In order to compare groups, the Kruskal–Wallis H-Test was applied. If the differences were statistically significant, the Wilcoxon–Mann–Whitney U-Test using comparison-related significance levels was performed between all pairs of groups. In all cases a nominal significance level of $p < 0.05$ was used.

## 3. Results

### *3.1. Histology and CDV antigen distribution in lymphoid tissues*

#### *3.1.1. Control animals (Nos. 1–8)*

Lymph node, spleen, thymus, and tonsil of all animals were without significant microscopic lesions and lacked CDV antigen expression. The used CDV-specific mAb showed cross-reactivity with splenic myocytes. The thymuses showed an early stage of involution and few primary follicles were detected in the medullary region.

#### *3.1.2. CDV infected animals (Nos. 9–22)*

A variety of alterations, which can not be described adequately by using the common terminology, were observed in lymphoid tissues. The term 'follicle-like area' was introduced to describe unorganized accumulations of IgG+ lymphocytes.

Group I dogs (Nos. 9–17), characterized by moderate to severe depletion (depletion index > 1), displayed a prominent loss of lymphocytes in both T and B cell areas ($p < 0.01$; Figs. 1 and 2). In thymuses with severe depletion an inverse structure,

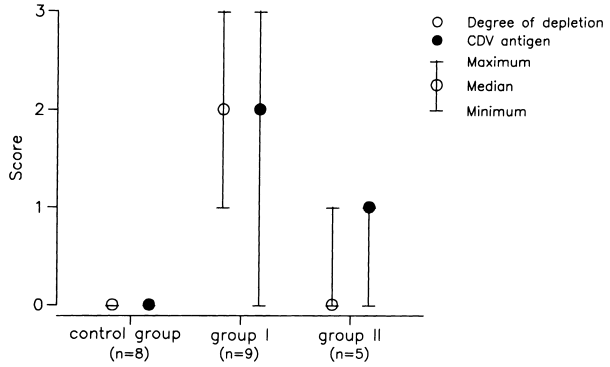


Fig. 1. Degree of depletion and canine distemper virus (CDV) antigen immunoreactivity in the splenic white pulp of dogs of the control group, groups I and II ($n$ = number of animals per group).

characterized by a higher cell density in the medullary region than in the cortical region was noticed.

CDV antigen was found in all thymuses and tonsils of these animals, while two markedly depleted spleens (Nos. 10, 20) and one moderately depleted lymph node (No. 10) were devoid of CDV antigen (Figs. 1 and 2). Viral antigen detection, consisting of a fine granular brown nuclear and cytoplasmic reaction, was more prominent in tonsils and thymuses and more pronounced in follicle-like areas. CDV antigen was observed in lymphocytes, thymocytes, cells of the MPS, thymic epithelial dendritic cells, epithelial cells of the tonsillar mucosa, tonsillar glandular epithelium and fibrocytes (Fig. 3).

Group II dogs (Nos. 18–22) displayed a mild or absent depletion (Figs. 1 and 2) resulting in a low depletion index (<1). Four animals (Nos. 18, 20–22) exhibited only few CDV antigen positive cells including follicular dendritic and interdigitating cells (Fig. 4). Occasionally CDV antigen was noticed in macrophages, lymphocytes/thymocytes and epithelial cells of the tonsils (No. 22). Extraneural CDV antigen was absent in one dog of this group (No. 19).

Lymphocytic depletion was statistically significant in group I dogs (lymph node, spleen, thymus, and tonsil: $p < 0.05$) compared to controls and group II dogs. However,

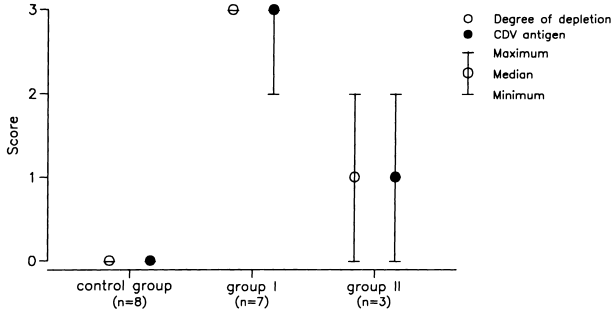


Fig. 2. Degree of depletion and canine distemper virus (CDV) antigen immunoreactivity in the thymus of dogs of the control group, groups I and II ($n$ = number of animals per group).

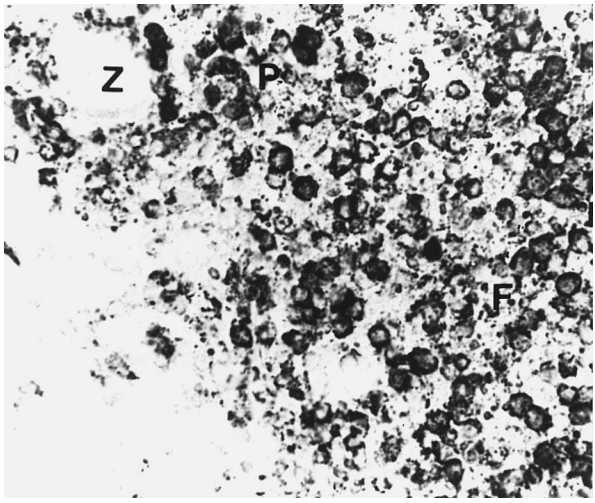


Fig. 3. Spleen, group I dog (No. 13), frozen tissue. CDV antigen is demonstrated in lymphocytes of the PALS and in a follicle like B cell area (F = follicle-like area, P = PALS, Z = central artery). Anti-CDV antibody, ABC method, slightly counter-stained with hematoxylin, ×600.

there was also a significant lymphocytic depletion in group II compared to the controls (lymph node, thymus, and tonsil: $p < 0.05$). The amount of CDV antigen differed significantly between dogs of groups I and II in lymph nodes, thymuses, and tonsils ($p < 0.05$).

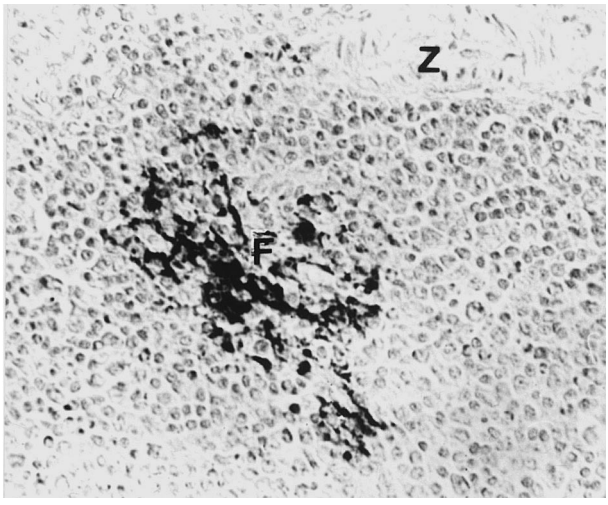


Fig. 4. Spleen, group II dog (No. 21), frozen tissue. CDV antigen is demonstrated in follicular dendritic cells (F = follicle, Z = central artery). Anti-CDV antibody, ABC method, slightly counter-stained with hematoxylin, ×380.

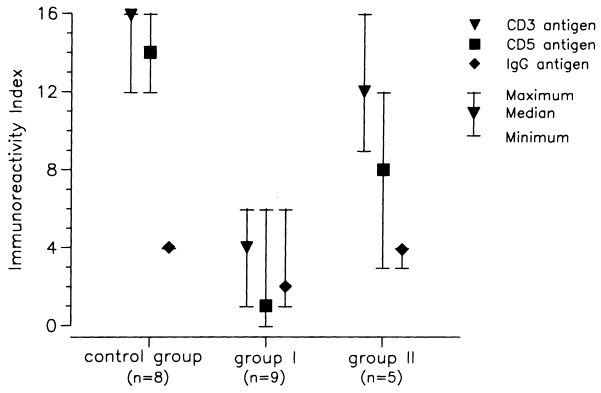


Fig. 5. CD3, CD5, and IgG expression ('immunoreactivity index') in the splenic PALS of dogs of the control group, groups I and II ($n$ = number of animals per group).

### 3.2. Distribution of CD3+, CD5+, CD4+, CD8+, and IgG+ cells in lymphoid tissues

CD3, CD4, CD5, CD8, and IgG expression was demonstrated on lymphocyte surface membranes. In addition, IgG antigen was found in the cytoplasm of plasma cells. The anti-CD4 mAb reacted with lymphocytes and the cytoplasm of large round cells, resembling macrophages. Such cells were found at the cortico-medullary junction and in the medulla of the thymus and in follicles of control and CDV-infected dogs. Additionally, in germinal centers and the marginal zone dendritic cells and macrophages were stained by this mAb. CD4 expression of neutrophils was equivocal.

#### 3.2.1. Control animals

In T cell areas (paracortex, PALS, interfollicular zone) and the thymic medulla, the vast majority of cells expressed CD3, CD4, and CD5 antigen, while only few cells were CD8 and IgG antigen positive (Figs. 5–8). The CD4+/CD8+ cell ratio was about 3 : 1. In the thymic medulla solitary primary follicles, consisting of IgG+ cells, were observed. In the thymic cortex most cells were CD3, CD4, and CD8 antigen positive and numerous cells expressed the CD5 antigen (Figs. 9 and 10), whereas IgG+ cells were absent.

In splenic primary follicles and the mantle zone of secondary follicles the vast majority of cells expressed IgG (Fig. 11). Single CD3+, CD4+ and CD5+ cells were found in these compartments, while CD8+ cells were absent (Figs. 11 and 12). In the dark zone of the germinal centers CD3+, CD4+, CD5+ and CD8+ cells were not detectable. In the light zone numerous CD4+ cells and some CD3+ and CD5+ cells were found. Weak IgG antigen expression was observed on the majority of cells in the dark and light zone of the germinal center.

#### 3.2.2. CDV infected animals

In T cell areas of group I (Nos. 9–17) a substantial loss of CD3+, CD4+, CD5+ and CD8+ cells was found compared to controls ($p < 0.01$; Figs. 5–10). Hereby, the loss of

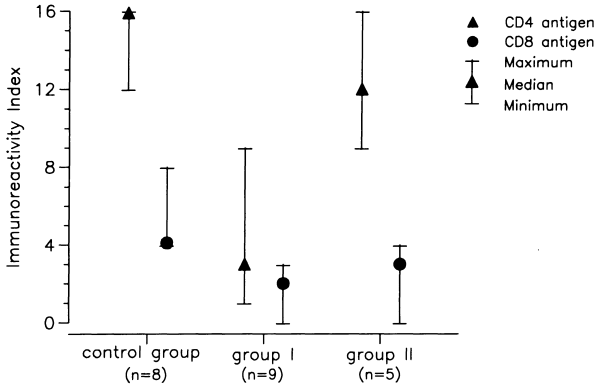


Fig. 6. CD4 and CD8 expression ('immunoreactivity index') in the splenic PALS of dogs of the control group, groups I and II ($n$ = number of animals per group).

CD4+ cells was more prominent than the loss of CD8+ cells, leading to a preponderance of CD8+ cells in the interfollicular zone of two animals (Nos. 15, 17). The number of IgG+ cells was mildly increased (Fig. 5). However, the thymic medulla did not contain any IgG+ cells.

The follicle-like areas were almost exclusively composed of IgG+ cells with single scattered CD3+, CD4+, CD5+ and CD8+ cells. The expression of all surface antigens except of the CD8 antigen was reduced in B cell areas compared to the control animals ($p < 0.01$; Figs. 11 and 12). In two animals, follicles or follicle-like areas were absent.

Expression of all investigated T cell surface antigens of group II dogs (Nos. 18–22) was significantly increased in the paracortex and interfollicular zone compared to group I animals ($p < 0.05$). CD3, CD4, and CD5 expression in the PALS ($p < 0.01$; Figs. 5 and 6), CD4 and CD8 expression in the thymic medulla ($p < 0.05$; Fig. 8) and CD3 and CD8

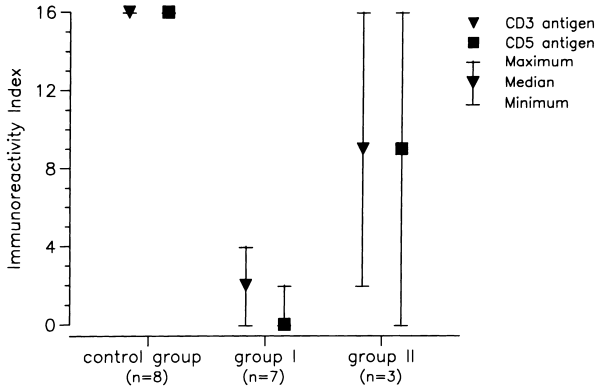


Fig. 7. CD3 and CD5 expression ('immunoreactivity index') in the thymic medulla of dogs of the control group, groups I and II ($n$ = number of animals per group).

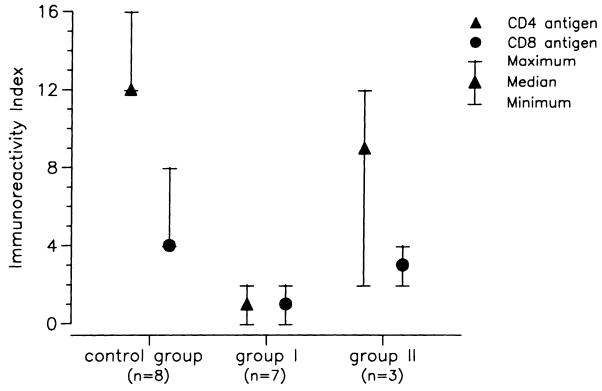


Fig. 8. CD4 and CD8 expression ('immunoreactivity index') in the thymic medulla of dogs of the control group, groups I and II ($n$ = number of animals per group).

expression in the thymic cortex ($p = 0.01$; Figs. 9 and 10) was lower in group I dogs compared to group II dogs. However, compared to controls, CD3, CD4, CD5, and CD8 expression in the paracortex ($p < 0.01$) and thymic medulla ($p < 0.05$; Figs. 7 and 8), CD5 ($p < 0.01$) and CD8 expression ($p < 0.05$) in the PALS (Figs. 5 and 6), and CD4 and CD5 expression in the interfollicular zone ($p < 0.01$) showed also a significant decline in group II animals.

In the primary follicles of lymph node, spleen, and tonsil of group II dogs the IgG expression was reduced ($p < 0.05$; Fig. 11), while CD8 expression increased ($p < 0.05$; Fig. 12) compared to the controls. In addition, a decrease of CD5 expression in the primary follicles of the lymph node ($p < 0.05$) and of the CD3 and CD4 expression in the primary follicles of the tonsil ($p < 0.05$) was noticed.

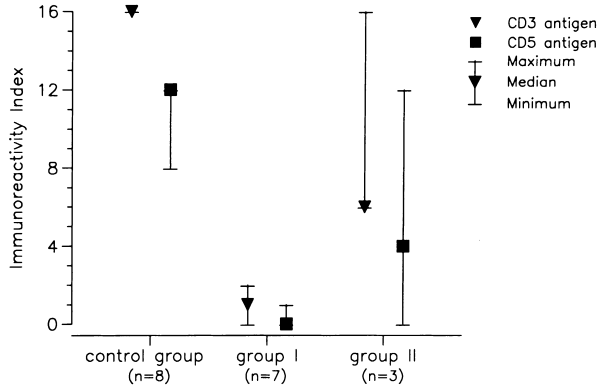


Fig. 9. CD3 and CD5 expression ('immunoreactivity index') in the thymic cortex of dogs of the control group, groups I and II ($n$ = number of animals per group).

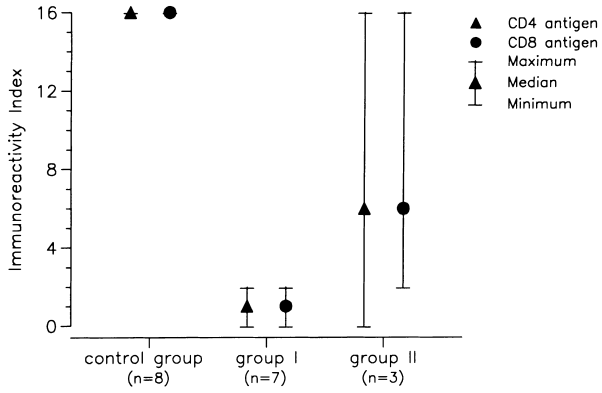


Fig. 10. CD4 and CD8 expression ('immunoreactivity index') in the thymic cortex of dogs of the control group, groups I and II ($n$ = number of animals per group).

### 3.3. Histology and CDV antigen distribution in the CNS of distemper dogs

CDV antigen was detected in the CNS of all animals of groups I and II. Two types of CNS lesions were found. Group I dogs exhibited focal vacuolation of the cerebellar white matter with abundant CDV expression. Viral antigen was present in astrocytes and less frequently in neurons, ependymal cells, chorioid plexus epithelial cells, and microglia. In group II dogs, lesions were plaque-like and characterized by demyelination, malacia, macrophage and gitter cell infiltration, astrogliosis with gemistocytes, multinucleated astrocytes and moderate to severe perivascular lymphoplasmacytic and histiocytic infiltration. Few CDV antigen positive astrocytes were found at the edges of the plaques.

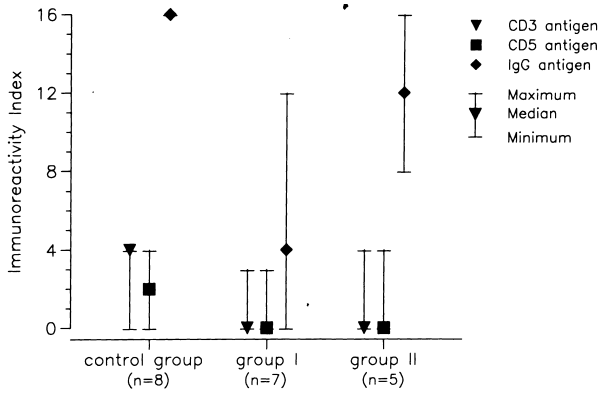


Fig. 11. CD3, CD5, and IgG expression ('immunoreactivity index') in the B cell area of the spleen of dogs of the control group, groups I and II ($n$ = number of animals per group).

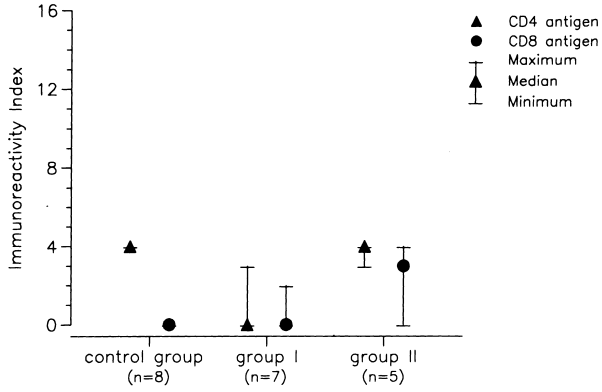


Fig. 12. CD4 and CD8 expression ('immunoreactivity index') in the B cell area of the spleen of dogs of the control group, groups I and II ($n$ = number of animals per group).

## 4. Discussion

The present study showed that lymphoid organs of dogs with distemper displayed two different depletion patterns. Group I animals exhibited moderate to severe loss of lymphocytes in the presence of moderate to abundant amounts of CDV antigen, whereas lymphoid organs of group II dogs displayed no or only mild depletion with few CDV antigen positive cells. In group II dogs, CDV infection seemed to be restricted to dendritic cells, while in group I animals, the vast majority of CDV-infected cells were lymphocytes and macrophages. In group I dogs, a marked depletion of all lymphocyte populations was noticed, although loss of CD4+ cells compared to CD8+ cells seemed to be more pronounced. The lymphoid organs of group II dogs showed a significant increase in CD3+, CD4+, CD5+, CD8+ and IgG+ cells, compared to group I dogs. In some compartments, the numbers of CD3+, CD4+, and CD8+ cells were similar to the findings in control animals. However, the number of CD5+ cells was consistently reduced in T cell areas compared to the controls. The two patterns of lymphoid alterations were strongly correlated to two different types of distemper encephalitis. Group I dogs suffered from acute non-inflammatory CDE, whereas group II animals exhibited chronic inflammatory demyelinating CNS lesions.

The distribution pattern of T cell subsets and B cells in control dogs was in accordance to previous observations (Rabanal et al., 1995). CD4 expression of neutrophils though reported by others was equivocal in the present study (Cobbold and Metcalfe, 1994). However, neutrophilic invasion is not a major feature of canine distemper and has not been observed histologically in the present cases. Therefore, possible false positive results due to potential CD4 expression of neutrophils can be neglected in the present study.

In CDV-infected animals T and B lymphocytes and macrophages are thought to represent the primary target cells for CDV infection (Baumgärtner, 1993; Iwatsuki et al., 1995; Moritz et al., 1998). In contrast, dendritic cells seemed to serve as host cells for the virus in group II dogs. Whether this change in cell tropism reflects a consequence of the

immune response and represents a mechanism of viral persistence as described for neurons and oligodendrocytes remains to be investigated in future studies (Zurbriggen et al., 1993, 1998; Nesseler et al., 1997, 1999). Absence of CDV mRNA in follicular dendritic cells would indicate antigen trapping by uninfected follicular dendritic cells as has been described for MV (Griffin, 1995; Heinen et al., 1995).

Similar to a previous study, a more pronounced loss of CD4+ compared to CD8+ cells was noticed in the present investigation (Iwatsuki et al., 1995). This decrease of CD4+ cells may be due to direct virus-mediated cell damage or indirect effects mediated by virus-infected dendritic cells. Thymic epithelial dendritic cell infection by CDV may result in compromised maturation and selection of T cells, leading to the release of immature T cells, including potentially auto-reactive cells. CD5 antigen negative T cells in lymphoid organs of CDV-infected animals may represent such a cell type. Studies with virus-infected human and murine thymic epithelial cells support this interpretation (Numazaki et al., 1989; Kyuwa et al., 1991; Auwaerter et al., 1996; Fugier-Vivier et al., 1997; Grosjean et al., 1997). CD5 is expressed later in the T cell development within the thymus than the CD3 antigen (Weiss et al., 1987; Arber and Weiss, 1995) and a strong CD5 antigen expression may be correlated to the terminal selection processes (Kersh and Hedrick, 1995).

It has been assumed, that after initial lymphoid depletion in the acute stage of CDV infection a regeneration of lymphoid organs may develop in the chronic phase of the disease (Gibson et al., 1965; McCullough et al., 1974; Krakowka et al., 1977, 1980). Nevertheless lymphoid repopulation and virus clearance from lymphoid tissue does not result in complete functional regeneration of the immune response (Krakowka et al., 1975, 1980). This might be reflected in the slightly modified phenotypical composition of T cell areas of group II dogs compared to controls. In addition, mononuclear suppressor cells and decrease of IL-1-production by macrophages were thought to be responsible for a transient immunosuppression in these dogs (Krakowka, 1982; Krakowka et al., 1987). In measles, long term impaired immune response is associated with increased secretion of IL-4 and IL-6 by mononuclear cells, indicating a shift of the T helper cells towards the Th2 type (Borrow and Oldstone, 1995).

Neuropathology revealed two different types of canine distemper encephalitis characterized by acute non-inflammatory and chronic inflammatory demyelinating encephalitis in groups I and II dogs, respectively. T lymphocytes represent the major inflammatory cell type in the brain of dogs with CDE (Alldinger et al., 1996; Gaedke et al., 1999). While CD8+ lymphocytes have been identified predominantly in the parenchyma of the white matter of dogs with non-inflammatory and inflammatory CDE, CD4+ cells and B lymphocytes were present abundantly in the perivascular infiltrates of chronic inflammatory white matter CDE-lesions (Wünschmann et al., 1999). The CD8+/CD4+ cell ratio in the parenchyma of the white matter of dogs with chronic inflammatory distemper encephalitis was about 3–1 (Wünschmann et al., 1999). This is almost the opposite to the CD8+/CD4+ cell ratio observed in T cell areas of group II animals and indicates a selective rather than a random migration of CD8+ cells into the parenchyma.

The present results confirmed previous observations that the absence of intracerebellar inflammatory infiltrates coincides with severe lymphocytic depletion in lymphoid tissues,

whereas repopulation of lymphoid tissue is associated marked lymphoplasmacytic encephalitis (McCullough et al., 1974; Krakowka et al., 1980; Fankhauser, 1982; Summers and Appel, 1994; Gaedke et al., 1999). Future studies will have to address the importance of T cell subtypes for initiation, progression and termination of CDV infection in the early and late phases of distemper infection by analysis of their respective cytokine profile and the role of lymphoid repopulation for the development of chronic demyelinating lesions.

## 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 statistical analyses. We thank Dr. S. Cobbold for kindly providing the anti-CD4 mAb (clone YKIX302.9.3.7). 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).

## References

Alldinger, S., Wünschmann, A., Baumgärtner, W., Voss, C., Kremmer, E., 1996. Up-regulation of major histocompatibility complex class II antigen expression in the central nervous system of dogs with spontaneous canine distemper virus encephalitis. Acta Neuropathol. 92, 273–280.

Appel, M.J.G., 1969. Pathogenesis of canine distemper. Am. J. Vet. Res. 30, 1167–1182.

Appel, M.J.G., 1987. Canine distemper virus. In: Horzinek, M.C.M. (Ed.), Virus Infections of Vertebrates, Vol. 1. Elsevier, Amsterdam, Oxford, New York, Tokyo.

Appel, M.J.G., Shek, W.R., Summers, B.A., 1982. Lymphocyte-mediated immune cytotoxicity in dogs infected with virulent canine distemper virus. Infect. Immun. 37, 592–600.

Arber, D.A., Weiss, L.M., 1995. CD5: a review. Appl. Immunohistochem. 3, 1–22.

Auwaerter, P.G., Kaneshima, H., McCune, J.M., Wiegand, G., Griffin, D.E., 1996. Measles virus infection of thymic epithelium in the SCID-hu mouse leads to thymocyte apoptosis. J. Virol. 70, 3734–3740.

Baumgärtner, W., Örvell, C., Reinacher, M., 1989. Naturally occurring canine distemper virus encephalitis: distribution and expression of viral polypeptides in nervous tissue. Acta Neuropathol. 78, 504–512.

Baumgärtner, W., 1993. Virale Infektionskrankheiten bei Welpen und Junghunden unter besonderer Berücksichtigung der Staupevirusinfektion. Prakt. Tierarzt 74, 26–32.

Borrow, P., Oldstone, M.B.A., 1995. Measles virus-mononuclear cell interaction. Curr. Top. Microbiol. Immunol. 191, 85–100.

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. 4, 77–90.

Cobbold, S., Metcalfe, S., 1994. Monoclonal antibodies that define canine homologues of human CD antigens: summary of the first international canine leukocyte antigen workshop (CLAW). Tissue Antigens 43, 137–154.

Fankhauser, R., 1982. Hundestaupe: Geschichte einer Krankheit. Schw. Arch. Tierheilk. 124, 245–256.

Ferrer, L., Fondevila, D., Rabanal, R., Ramis, A., 1992. Detection of T lymphocytes in canine tissue embedded in paraffin wax by means of antibody to CD3 antigen. J. Comp. Pathol. 106, 311–314.

Fugier-Vivier, I., Servet-Delprat, C., Rivailler, P., Rissoan, M.C., Liu, Y.J., Rabourdin-Combe, C., 1997. Measles virus suppresses cell-mediated immunity by interfering with the survival and functions of dendritic cells. J. Exp. Med. 186, 813–823.

Gaedke, K., Zurbriggen, A., Baumgaertner, W., 1999. Lack of correlation between virus nucleoprotein and mRNA expression and the inflammatory response in demyelinating distemper encephalitis indicates a biphasic process. Eur. J. Vet. Pathol. 5, 9–20.

Gibson, J.P., Griesemer, R.A., Koestner, A., 1965. Experimental distemper in the gnotobiotic dog. Pathol. Vet. 2, 1–19.

Griffin, D.E., 1995. Immune response during measles virus infection. Curr. Top. Microbiol. Immunol. 191, 117–133.

Gröne, A., Frisk, A.L., Baumgärtner, W., 1998. Cytokine mRNA expression in whole blood samples from dogs with natural canine distemper virus infection. Vet. Immunol. Immunopathol. 65, 11–27.

Grosjean, I., Caux, C., Bella, C., Berger, I., Wild, F., Banchereau, J., Kaiserlian, D., 1997. Measles virus infects human dendritic cells and blocks their allostimulatory properties for CD4+ T cells. J. Exp. Med. 186, 801–812.

Heinen, E., Bosseloir, A., Bauzahzah, F., 1995. Follicular dendritic cells: origin and function. Curr. Top. Microbiol. Immunol. 201, 15–47.

Iwatsuki, K., Okita, M., Ochikubo, F., Gemma, T., Shin, Y.S., Miyashita, N., Mikami, T., Kai, C., 1995. Immunohistochemical analysis of the lymphoid organs of dogs naturally infected with canine distemper virus. J. Comp. Pathol. 113, 185–190.

Jacoby, R.O., Griesemer, R.A., 1970. Effects of adrenalectomy on the lymphoid lesions in dogs with experimentally induced canine distemper. Am. J. Vet. Res. 31, 1825–1833.

Kersh, G.J., Hedrick, S.M., 1995. Role of TCR specificity in CD4 versus CD8 lineage commitment. J. Immunol. 154, 1057–1068.

Krakowka, S., 1982. Mechanisms of in vitro immunosuppression in canine distemper virus infection. J. Clin. Lab. Immunol. 8, 187–196.

Krakowka, S., Axthelm, M.K., Johnsen, G.C., 1985. Canine distemper virus. In: Olsen, R.G., Krakowka, S., Blakeslee, J.R. (Eds.), Comparative Pathology of Viral Diseases, Vol. 2. CRC press, Boca Raton.

Krakowka, S., Cockerell, G., Koestner, A., 1975. Effects of canine distemper virus infection on lymphoid functions in vitro and in vivo. Infect. Immun. 11, 1069–1078.

Krakowka, S., Cockerell, G., Koestner, A., 1977. Intradermal mitogen response in dogs: correlation with outcome of infection by canine distemper virus. Am. J. Vet. Res. 38, 1539–1542.

Krakowka, S., Higgins, R.J., Koestner, A., 1980. Canine distemper virus: review of structural and functional modulation in lymphoid tissue. Am. J. Vet. Res. 41, 284–292.

Krakowka, S., Koestner, A., 1977. Comparison of canine distemper virus strains in gnotobiotic dogs: effects on lymphoid tissues. Am. J. Vet. Res. 38, 1919–1922.

Krakowka, S., Ringler, S.S., Lewis, M., Olson, R.G., Axthelm, M.K., 1987. Immunosuppression by canine distemper virus: modulation of in vitro immunoglobulin synthesis, interleukin release and prostaglandin $E_2$ production. Vet. Immunol. Immunopathol. 15, 181–201.

Krakowka, S., Wallace, A.L., Koestner, A., 1978. Syncytia inhibition by immune lymphocytes: an in vitro test for immunity to canine distemper. J. Clin. Microbiol. 7, 292–297.

Kyuwa, S., Yamaguchi, K., Yoyoda, Y., Fujiwara, K., 1991. Induction of self reactive T cells after murine coronavirus infection. J. Virol. 65, 1789–1795.

Lauder, I.M., Martin, W.B., Gordon, E.D., Lawson, D.D., Campbell, R.S.F., Watrach, A.M., 1954. A survey of canine distemper, Vet. Rec. 66, 607–611 and 623–631.

Mangi, R.J., Munyer, T.P., Krakowka, S., Jacoby, R.O., Kantor, F.S., 1976. A canine distemper model of virus induced anergy. J. Infect. Dis. 133, 556–563.

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

Moritz, A., Frisk, A.L., Baumgärtner, W., 1998. Beurteilung diagnostischer Möglichkeiten bei der Staupevirusinfektion des Hundes. Kleintierpraxis 43, 153–172.

Nesseler, A., Baumgärtner, W., Gaedke, K., Zurbriggen, A., 1997. Abundant expression of viral nucleoprotein mRNA and restricted translation of the corresponding viral protein in inclusion body polioencephalitis of canine distemper. J. Comp. Pathol. 116, 291–301.

Nesseler, A., Baumgärtner, W., Zurbriggen, A., Örvell, C., 1999. Restricted virus protein translation in canine distemper virus inclusion body polioencephalitis. Vet. Microbiol. 69, 23–28.

Numazaki, K., Goldman, H., Bai, X.Q., Wong, I., Wainberg, M.A., 1989. Effects of infection by HIV-1, cytomegalovirus, and human measles virus on cultured human thymic epithelial cells. Microbiol. Immunol. 33, 733–745.

Örvell, C., Blixenkrone-Möller, M., Svansson, V., Have, P., 1990. Immunological relationships between phocid and canine distemper virus studied with monoclonal antibodies. J. Gen. Virol. 71, 2085–2092.

Pringle, C.R., 1999. Virus taxonomy — 1999. Arch. Virol. 144/2, 421–429.

Rabanal, R.M., Ferrer, L., Else, R.W., 1995. Immunohistochemical detection of canine leukocyte antigens by specific monoclonal antibodies in canine normal tissue. Vet. Immunol. Immunopathol. 47, 13–23.

Stevens, D.R., Osburn, B.I., 1976. Immune deficiency in a dog with distemper. J. Am. Vet. Med. Assoc. 168, 493–498.

Summers, B.A., Appel, M.J.G., 1994. Aspects of canine distemper virus and measles virus encephalomyelitis. Neuropathol. Appl. Neurobiol. 20, 525–534.

Voß, C., Kremmer, E., Hoffmann-Fezer, G., Schumm, M., Günther, W., Kolb, H.J., Thierfelder, S., 1993. Identification and characterization of a mouse monoclonal antibody (M10) directed against canine (Dog) CD8+ lymphocytes. Vet. Immunol. Immunopathol. 38, 311–325.

Weiss, A., Dazin, P.F., Shields, R., Fu, S.M., Lanier, L., 1987. Functional competency of T cell antigen receptors in human thymus. J. Immunol. 139, 3245–3250.

Winters, K.A., Mathes, L.E., Krakowka, S., Olsen, R.G., 1983. Immunoglobulin class response to canine distemper virus in gnotobiotic dogs. Vet. Immunol. Immunopathol. 5, 209–215.

Wünschmann, A., Alldinger, S., Kremmer, E., Baumgärtner, W., 1999. 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. Vet. Immunol. Immunopathol. 67, 101–116.

Zurbriggen, A., Schmid, I., Graber, H.U., Vandevelde, M., 1998. Oligodendroglial pathology in canine distemper. Acta Neuropathol. 95, 71–77.

Zurbriggen, A., Yamawaki, M., Vandevelde, M., 1993. Restricted canine distemper virus infection of oligodendrocytes. Lab. Invest. 68, 277–284.