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IMMUNOPHENOTYPICAL CHARACTERIZATION OF MONOCYTES IN CANINE DISTEMPER VIRUS INFECTION

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ABSTRACT

Canine distemper virus (CDV) infection induces multifocal demyelination in the central nervous system (CNS). It is thought that the resident macrophages of the CNS, the microglia, as well as invading monocytes associated with the inflammatory reaction may play a central role in the demyelinating process. To evaluate changes in peripheral monocytes in CDV infection their immunophenotype was characterized by flow cytometry during the course of an experimental CDV infection in dogs. The highest number of CDV infected monocytes was found in dogs developing demyelinating lesions. In CD18, CD45, CD44, and CD14 neither up- nor down-regulation was observed. Marked up-regulation occurred in a number of surface molecules including CD1c, B7-1 and B7-2, MHC I, and CD11b. Peak expression was found at 4 to 5 weeks post infection (PI), regardless of clinical outcome. All these molecules play an important role in the host's immune response, notably antigen presentation and cell adhesion. These results demonstrate that CDV infection in vivo may enhance several macrophage functions. This could lead to more effective clearance of the virus but may also increase demyelination through a bystander effect in animals that accumulated significant amounts of CDV in the CNS.

Keywords: canine monocytes, canine distemper virus infection (CDV), pathogenesis of CDV, immunophenotype

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INTRODUCTION

Canine distemper virus (CDV) is a single-stranded RNA morbillivirus belonging to the family of Paramyxoviridae (Pringle, 1999). In the acute phase of the disease CDV infection induces multifocal demyelination in the central nervous system (CNS) against a background of a systemic disease with severe immunosuppression and lymphoid depletion (Vandevelde et al., 1982; Krakowka et al., 1985; Greene and Appel, 2006). The pathogenesis of CDV-associated immunosuppression remains unclear (Greene and Appel, 2006). Replication of virus in the immunocompetent cells leads to disturbed function or lymphocytic apoptosis (Wunschmann et al., 2000; Kumagai et al., 2004; Schobesberger et al., 2005) whereby $CD4^+$ cells are more affected than $CD8^+$ cells (Tipold et al., 2001). In the subacute and chronic stage of the infection, an antiviral immune response is initiated and is associated with additional inflammatory damage to the white matter (Vandevelde and Zurbriggen, 2005). The contributing factors in the pathogenesis of demyelination in distemper include viral replication in astrocytes (Vandevelde et al., 1982), restricted infection of oligodendrocytes (Zurbriggen et al., 1998), down-regulation of myelin gene transcription (Graber et al., 1995), dysregulation of cytokines, metalloproteinases and their inhibitors (Markus et al., 2002; Gröters et al., 2005) and the intrathecal antiviral immune response (Vandevelde and Zurbriggen, 2005). There is little doubt that the microglial/macrophage compartment plays a pivotal role on the effector side of the demyelinating process (Vandevelde and Zurbriggen 2005). As monocytes and microglia are both members of the macrophage-lineage they show cross-reactivity of many surface markers (Dijkstra et al., 1985; Perry and Gordon, 1991; Flaris et al., 1993; Fedoroff, 1995; Stoll and Jander, 1999), and upon activation similar effector functions can be exerted (Graeber and Streit, 1990). Microglial cells are activated in distemper (Tipold et al., 2001, Stein et al., 2004), and show an up-regulation of MHC

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II (Alldinger et al., 1996) and various other surface molecules (Stein et al., 2004), as well as enhanced secretion of reactive oxygen species (Stein et al., 2004). In the course of the inflammatory response in demyelinating lesions in distemper, peripheral monocytes/macrophages invade the CNS, complementing the local microglia. The central role of macrophages in myelin destruction was proposed by earlier investigators in distemper (Wisniewsky, 1972), and supported by in vitro studies, showing ADCC in CDV-infected canine brain cell cultures (Griot-Wenk et al., 1991). Not much is known about the effect of CDV on peripheral blood monocytes. In contrast to the overt CDV-induced lymphopenia, monocyte numbers are not significantly decreased and monocytosis is very rarely observed (McCullough et al., 1973; Tipold, 1996; Greene and Appel, 2006). This might be due to minimal replication of CDV in these cells (Von Messling et al., 2004). Cultured macrophage functions seemed not to be impaired and may even be enhanced as a result of CDV infection (Brugger et al., 1992).

The objective of the present study was to examine peripheral monocytes isolated from experimentally CDV-infected dogs at various stages of the infection and to characterize changes which may be relevant to the pathogenesis of demyelination.

MATERIAL AND METHODS

Animals

Twenty 5-6-months-old experimentally CDV-infected SPF Swiss-Beagle dogs, 10 males and 10 females from two litters were derived from a DNA vaccine study with challenge infection with the virulent CDV-strain A75/17 (111/99/Berne/CH; Stein et al. 2004; experiment see Cherpillod et al., 2000). All animals were regularly physically and neurologically examined and rectal temperature was measured daily. Blood

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samples for monocyte isolation were taken every 3-4 days. All dogs were euthanized 20-31 days post infection (PI).

All but one dog (no. 18) showed a bi- to triphasic febrile body temperature from three days PI on (see Fig. 1) which was accompanied by lymphopenia in 13 dogs, and clinical signs consistent with systemic CDV infection such as diarrhea, rhinitis, conjunctivitis, coughing and weight loss. In six dogs the clinical signs resolved and they showed no abnormalities at the time of euthanasia. The remaining 14 dogs developed additional neurological signs indicating forebrain and/or brainstem lesions. According to the clinical signs after remission of initial disease the dogs were assigned to three examination groups.

Group I comprised seven dogs with no clinical signs (dog nos. 3, 4, 6, 9, 11, 16, 18; see Tab. 1). These dogs showed the lowest mean body temperature in the course of the disease and after initial fever no second phase of elevated body temperature occurred (see Fig. 1). No lesions were found on histopathological examination of the CNS. The six dogs of **group II** displayed mild clinical signs such as conjunctivitis and diarrhea (dog nos. 5, 8, 10, 12, 13, 14; see Tab. 1), their mean body temperature was slightly higher than that of group I (see Fig. 1). There were no lesions in their brains demonstrated by histopathology. Seven dogs confined to **group III** showed severe clinical signs (dog nos. 1, 2, 7, 15, 17, 19, 20; see Tab. 1). The dogs of group III showed highest rectal temperatures in the course of the CDV infection (see Fig. 1) with a triphasic elevation of the temperature and severe neurological signs. Histopathological examination of the latter dogs showed active lesions: the brain of the dogs nos. 2, 15, and 17 displayed small multifocal demyelinating lesions with vacuolation of the white matter, astrocytic swelling, mild gliosis and occasional macrophages (see Tab. 1, nos. of dogs in non-bold) whereas the dogs nos. 1, 7, 19,

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and 20 exhibited relatively large multifocal plaques with complete myelin loss, marked gliosis and invasion with macrophages (see Tab. 1, nos. of dogs in bold).

Differential haemogram

For evaluation of the differential blood count blood smears were prepared over the course of the vaccine study for each individual dog and assessed consistently by one person. The slide was stained with Diff-Quik (Dade Behring, Eschborn) and cells were counted.

Antibodies

MAbs used for immunophenotypic analyses were either specific for dog cell surface markers or cross-reacting with them. Monoclonal mouse antibodies directed against CD11b, CD18, CD1c, B7-1 (CD80), B7-2 (CD86), CD3, CD4, CD8α, and CD21 were provided by Prof. Peter F. Moore, University of Davis, USA. MAbs against CD11b, CD18, CD4, CD8α, CD21 and MHC class I were characterized at the first international canine leukocyte antigen workshop (Cobbold and Metcalfe, 1994). The following primary antibodies were purchased: mAb directed against CD45 (conjugated with biotin), CD44 (both rat mAb, Serotec, Eching, Germany), MHC class I (mouse mAb, VMRD, Inc. Pullman, USA) and CD14 conjugated with R-PE (mouse mAb, Dako, Glostrup, Denmark). The clones of the monoclonal antibodies are summarized in Table 2. The murine mAb D110 was prepared from cell culture supernatant (Bollo et al., 1986) and used for detection of an epitope of the CDV nucleocapsid protein which is resistant to tissue fixation and embedding procedures. All antibodies were used for flow cytometry (FACS) analysis. Human normal immunoglobulin G (Globuman Berna, Berne, Switzerland) served to block non-specific binding. Secondary antibodies were purchased: R-Phycoerythrine

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conjugated F(ab´)2 fragment goat anti-mouse IgG (gam-PE; Dianova, Hamburg, Germany), R-PE conjugated F(ab´)2 fragment rabbit anti-rat (rar-PE) and streptavidine conjugated fluorescein-isothiocyanate (FITC, both from Serotec, Eching, Germany) for flow cytometry.

Isolation of peripheral blood mononuclear cells (PBMCs) and flow cytometry

Peripheral blood was examined once or twice weekly until euthanasia. Approximately 5 ml of blood was collected by venipuncture into heparinized tubes and subjected to discontinuous density gradient centrifugation according to Wunderli and Felsburg (1989) modified by Somberg et al. (1992). Briefly, Pancoll (density 1.077; Cytogen, Ober-Moerlen, Germany) and Histopaque (density 1.119; Sigma, Deisenhofen, Germany) were used and centrifuged at 400 x g for 20 min at room temperature. After centrifugation PBMCs accumulated as a white cell-layer at the interphase between plasma and Pancoll. Following two washing steps with PBS the supernatant was discarded and cells were available for further examination. Monocyte and lymphocyte subsets were differentiated as two cell populations by their differing size (forward scatter, FSC) and complexity (side scatter, SSC), and monocytes were selectively examined after gating this population in flow cytometry (see Fig. 5A). Low percentages of the gated cells for the expression of CD3, CD8α, CD21 and CD4 served to exclude contamination with lymphocytes.

Monocytes were examined every third to forth day in the course of the CDV infection. They were stained as previously described for lymphocyte subsets (Tipold et al., 1999). After Fc-receptor blockade with 10 mg/ml of human IgG, primary antibodies were incubated for 30 min at 4°C and washed twice with Hanks´ buffered saline (Sigma, Deisenhofen, Germany). Secondary antibodies conjugated either with FITC

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(CD45), rar-PE (CD44), or gam-PE (all other mAbs) were diluted 1:100, added and incubation was continued for 30 min. Following two washing steps, cells were resuspended in FACS flow solution and analysed immediately after staining without fixation with a FACSCalibur™ using the Cell-Quest®-Software (both: BD Biosciences, Heidelberg, Germany). Negative control samples were stained with the secondary antibodies only. Cell viability was assessed by propidium iodide exclusion. Analysis gates were adjusted to not exceed 2% positive staining with negative controls (see Fig. 5B). For each sample 10^4 events were analysed.

Detection of CDV in monocytes

CDV detection in monocytes was performed as previously described (Cherpillod et al., 2000). Briefly, monocytes were fixed with 2% paraformaldehyde for 20 min at 4°C. After two washing steps with PBS, cells were resuspended in 50µl PBS containing 0.03% Saponin (Merck, Hannover, Germany) for cell permeabilisation. Following Fc-receptor blockade with 10 mg/ml human IgG, 10µl of the mAb D110 (in a dilution of 1:16) was added, and the cells incubated for 30 min at 4°C. After two washing steps with PBS-Saponin-solution secondary goat-anti-mouse IgG antibody conjugated with PE was added in a dilution of 1:50 and again incubated. Negative control samples were stained with secondary antibody only. After a final washing step monocytes were immediately measured with flow cytometry.

RESULTS

Detection of CDV in canine monocytes

In all but three dogs of group I and II with no or mild clinical signs CDV nucleocapsid protein was detected in only 0 - 5.5% of the monocytes on day 6 PI. A few days later

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CDV-positive monocytes could no longer be detected with the exception of dog nos. 4 and 6 of group II, showing positive staining of monocytes on day 26 to day 35 PI. In contrast, all dogs of group III showed up to 33.3% CDV-positive monocytes at all time points during the course of the infection (see Fig. 1B). In two dogs peak levels of CDV-positive monocytes were preceded by or concurrent with significant elevation of body temperature (dogs no. 17 and 20).

Immunophenotypic characterization of monocytes during CDV infection

A differential haemogram revealed that the absolute monocyte cell count was not grossly altered in any of the dogs irrespective of showing signs of systemic disease or fever in the course of CDV infection (see Fig. 1C).

Immunophenotypical characterization was performed on the basis of two different parameters: percentage of positive cells for the expression of a certain surface molecule and the mean fluorescence intensity (measured by means of fluorescent channel numbers) in flow cytometry as a relative mean for the intensity of expression of a certain molecule. Monocyte surface molecule expression was evaluated in each individual dog on nine time points over a period of five weeks. The mean of the values for each sampling day was assessed for each group and compared. Changes in surface molecule expression were correlated with changes in body temperature. The results are depicted in Figures 2 and 3.

The ***percentage* of monocytes expressing CD18**, **CD11b**, **CD45**, **CD44**, **B7-2** (CD86), and **MHC I** did not show a significant change in the course of the infection in any of the examination groups (data not shown). CD14 showed an initial increase and remained constant during the experiment; group III had generally lower percentages of expression than the other two groups (Fig. 2). **CD1c** and **B7-1** (CD80) were expressed in a continuously increasing percentage of positive cells for

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all three examination groups during the course of the CDV infection peaking towards the end of the fourth week PI followed by a decline during the last week of the experiment (see Fig. 2A + B). **CD1c** and **CD14** were found in a generally higher percentage of monocytes in group I (mean values of the whole examination period are given; CD14 $\bar{x}$ = 74.7%; CD1c $\bar{x}$ = 63.4%) and group II (CD14 $\bar{x}$ = 72.9%; CD1c $\bar{x}$ = 57.4%) compared to group III (CD14 $\bar{x}$ = 53.9%; CD1c $\bar{x}$ = 46.7%; see Fig. 2A + C). ***Expression intensity*** of **CD1c** also steadily increased in all groups reaching its maximum at the end of the fourth week PI (see Fig. 3A). Likewise, the surface molecules, **CD11b**, **B7-2** (CD86), and **MHC I** showed an up-regulated expression intensity peaking at the end of the third week PI, followed by a gradual down-regulation in all three examination groups except for MHC I, which remained high until the end of the experiment (see Fig. 3B-D). For **CD18**, **CD45**, **CD44**, **CD14**, and **B7-1** (CD80) no up- or down-regulated expression intensity was measured in any examination group.

Correlation between up-regulation of surface antigens and body temperature

Peak levels of MHC I, CD11b, B7-2 (CD86), and CD1c expression intensity were preceded by or concurrent with elevations of body temperature in several animals. This correlation was particularly striking for B7-2 which was detected in almost all of the dogs in each examination group (see Fig. 4).

DISCUSSION

Previous studies strongly suggest that microglia and peripheral macrophages invading the CNS play an important role in the pathogenesis of demyelination in canine distemper virus infection (Stein et al., 2004; Vandevelde and Zurbriggen, 2005). Not much is known on the effect of CDV on peripheral macrophages.

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The differential haemogram in distemper characteristically reveals a lymphopenia eventually in combination with a leukopenia (Greene and Appel, 2006). In contrast, monocyte numbers are hardly affected and may even be increased in distemper (McCullough et al., 1973; Appel, 1987; Tipold 1996; Greene and Appel, 2006). One study showed a mild transient decrease of CD14-positive monocytes early in the course of experimental CDV infection (Schobesberger et al., 2005). The present study confirms that the absolute monocyte numbers may oscillate during the course of CDV infection but never reach subcritical values neither in dogs with severe clinical signs and widespread lesions in the CNS (group III) nor in dogs with no or mild clinical signs without alteration of the CNS (group I and group II).

In the present study the percentage of expressing monocytes as well as the expression intensity of nine different surface molecules were examined in the course of a CDV challenge infection. While a somewhat lower proportion of monocytes expressed CD1c and CD14 in dogs of group III as compared to group I and group II (see Fig. 2A+C, Table 3), the frequency of expression of other surface markers was not reduced. On the contrary, CD11b, B7-1 (CD80), B7-2 (CD86), and MHC I were enhanced in all dogs (see Table 3), including those with severe clinical signs and demyelinating lesions in the CNS (group III). This finding is consistent with the reported preserved or even enhanced functions such as phagocytosis, release of reactive oxygen species and procoagulant activity of CDV-infected macrophages cultured in vitro (Brugger et al., 1992). Microglia in demyelinating CDV infection in our previous studies showed distinct signs of activation by an upregulated expression of CD18, CD11b, CD11c, CD1c, MHC class I and MHC class II and a tendency for increased expression intensity of ICAM-1 (CD54), B7-1 (CD80), B7-2 (CD86) (Stein et al. 2004). Immunocytochemical evidence for activation of microglia in demyelinating CDV infection was also found by Alldinger et al. (1996) and Tipold et

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al. (1999). Thus, these reported findings in microglia, the resident phagocytic cells in the CNS, are consistent with our observations in monocytes derived from the peripheral blood in the present study. However, there are some important differences in up-regulated expression of surface molecules between the two cell populations, for example CD18 expression was not at all altered in monocytes but up-regulated in microglial cells in distemper (Stein et al., 2004). Such distinctions in the expression profile reflect fundamental functional differences between microglia and peripheral monocytes.

The surface markers which were found to be up-regulated in either percentage of positive cells or expression intensity on monocytes in the course of CDV infection in the present study included CD1c, B7-1 and B7-2, MHC I, and CD11b. All play an important role in the host's immune response. Monocytes act as antigen presenting cells and therefore, are capable of expressing different molecules such as MHC I, and CD1. MHC I is responsible for processing and presenting cytosolic antigen, predominantly viral antigen, and it mediates the development of T cells (Janeway and Travers 1997). CD1c is structurally related to MHC I and serves to present lipidic and glycolipidic antigens to T cells and natural killer (NK) cells. Both surface markers are also known to be upregulated on microglial cells in CDV infection (Stein et al., 2004). B7-1 (CD80) and B7-2 (CD86) are expressed by antigen presenting cells (APCs) and function as important co-stimulatory molecules for T cell activation (Abbas and Lichtman, 2003). They are absent or expressed at low levels on resting APCs and are induced by various stimuli such as cytokines in the course of microbial or viral infections. Up-regulation of B7-1 and B7-2 was demonstrated on monocytes in the course of CDV infection in the present study and was also found on microglia in dogs with demyelinating CDV infection (Stein et al., 2004). These costimulatory molecules are thought to play an important role in the pathophysiology of Multiple Sclerosis

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(Windhagen et al., 1995), for which CDV infection is an established animal model (Appel et al., 1981). CD11b is an integrin which plays an important role for the adhesion of leukocytes (Abbas and Lichtman, 2003). Enhanced expression of CD11b might support the invasion of infected monocytes into the CNS and spreading of the virus. Expression of the upregulated surface molecules generally increased over time during the experiment reaching highest levels around four weeks PI. Peak expression of B7-2 and CD11b seems to coincide with phases of viremia reflected by increased rectal temperatures and peak levels of CDV-positive monocytes. Up-regulation of various surface markers occurred to a similar extent and with similar kinetics in the monocytes of all three groups. The mechanism leading to this effect is not clear. A direct correlation with CDV infection in these cells is not likely since only dogs with severe clinical signs and demyelinating CNS lesions (group III) had a high percentage of infected monocytes (up to 33%) and over a much longer time span as compared to the other groups, in which only transient infection in small numbers of monocytes was seen. This is remarkably consistent with our previous studies in isolated microglia, where dogs with demyelinating CNS lesions displayed a significantly higher virus load in microglial cells (up to 18%) compared to dogs with no histological abnormalities in the CNS (Stein et al., 2004). The high number of CDV-infected cells in the microglia/monocyte compartment probably reflects the continuous increase in viral load in many organs in immunosuppressed animals unable to mount an effective antiviral immune response.

The observed upregluation of antigens typically expressed on active macrophages probably supports clearance of the infection. However, in animals that accumulate significant amounts of CDV in the CNS however, such antiviral macrophage activity can lead to detrimental bystander damage (Wisniewsky 1972, Griot-Wenk et al 1991). It is of interest to note in this respect, that peak expression of the above

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named surface markers occured at a point in time of the infection where invasion of inflammatory cells, including monocytes in the CNS becomes established and inflammatory demyelination is initiated (Vandevelde and Zurbriggen, 2005).

In conclusion, our study has shown that CDV infection markedly alters the expression of surface molecules of peripheral blood monocytes generally in the sense of an up-regulation. Up-regulation of certain markers may favour entry of these cells in the CNS and/or enhance their effector functions. Thus this study supports the notion that macrophages, either derived from microglia or peripheral monocytes, play a central role in the demyelinating process in CDV infection.

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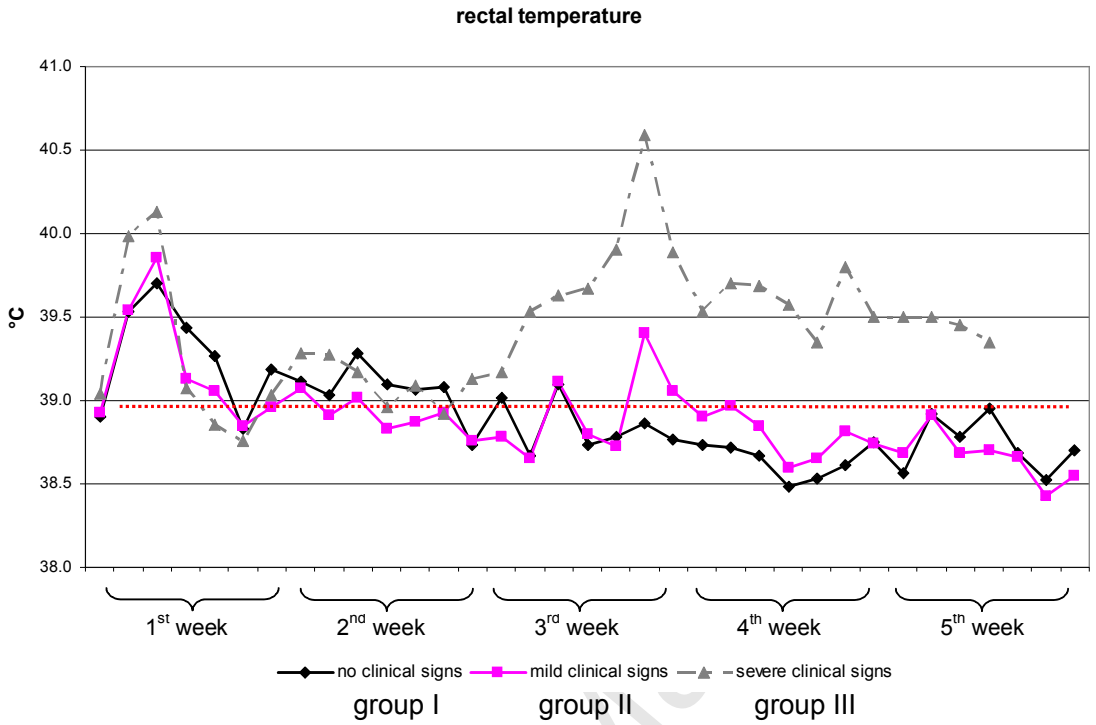
A



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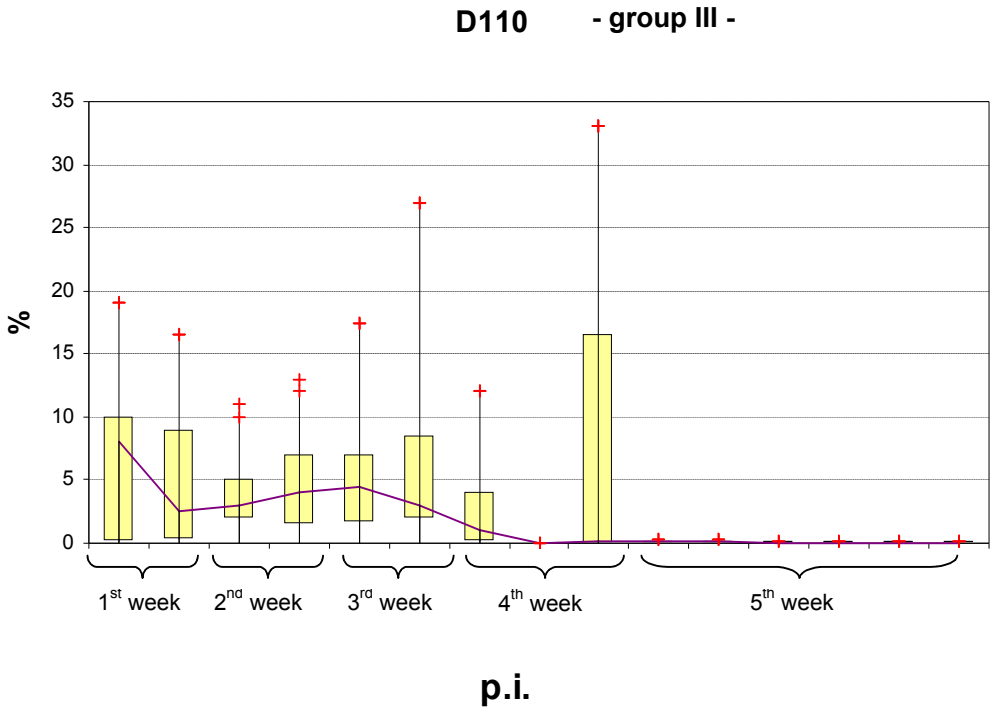

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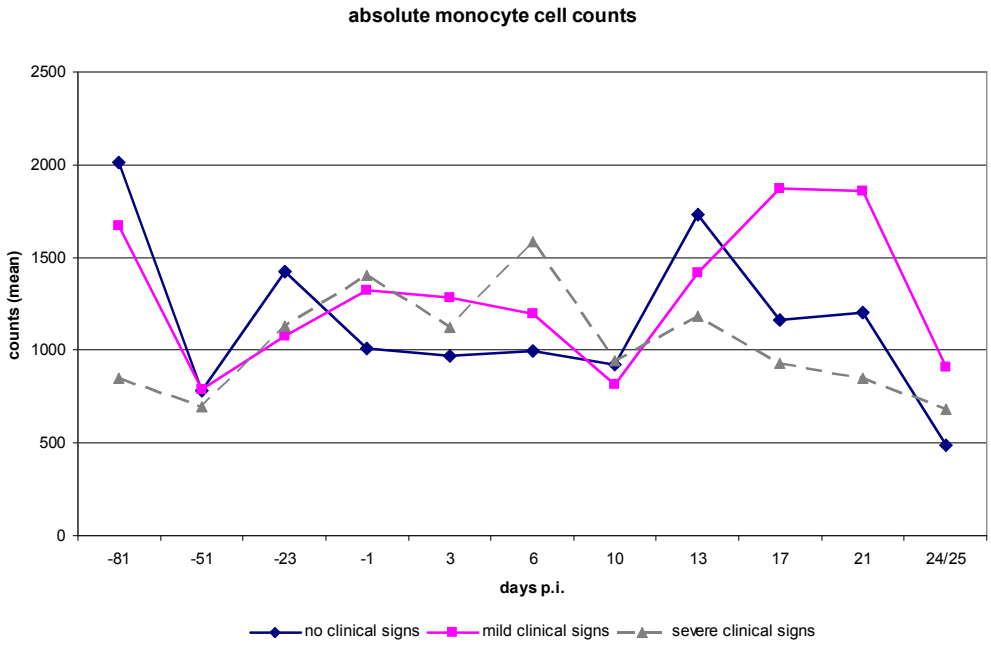

group I group II group III

Figure 1.

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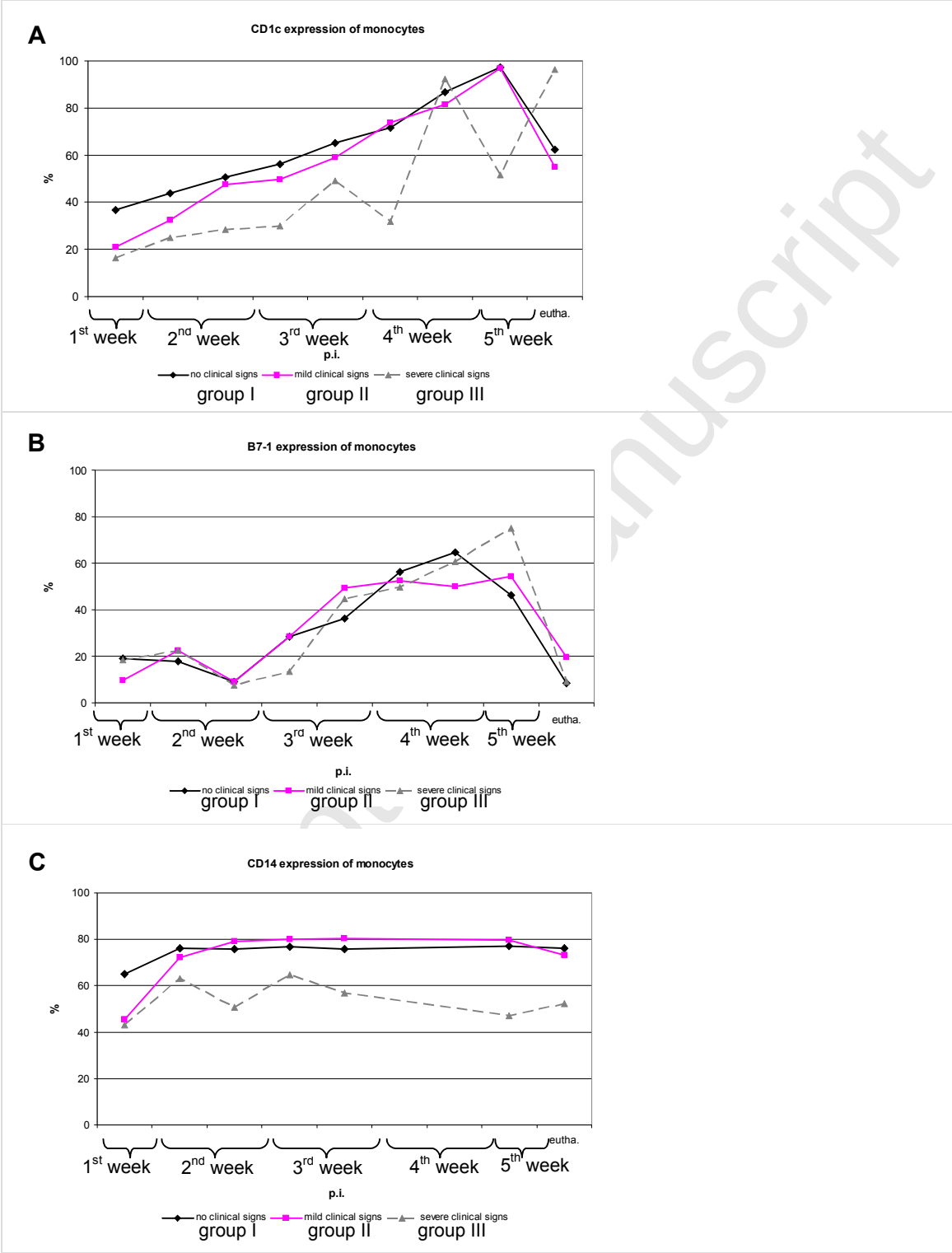

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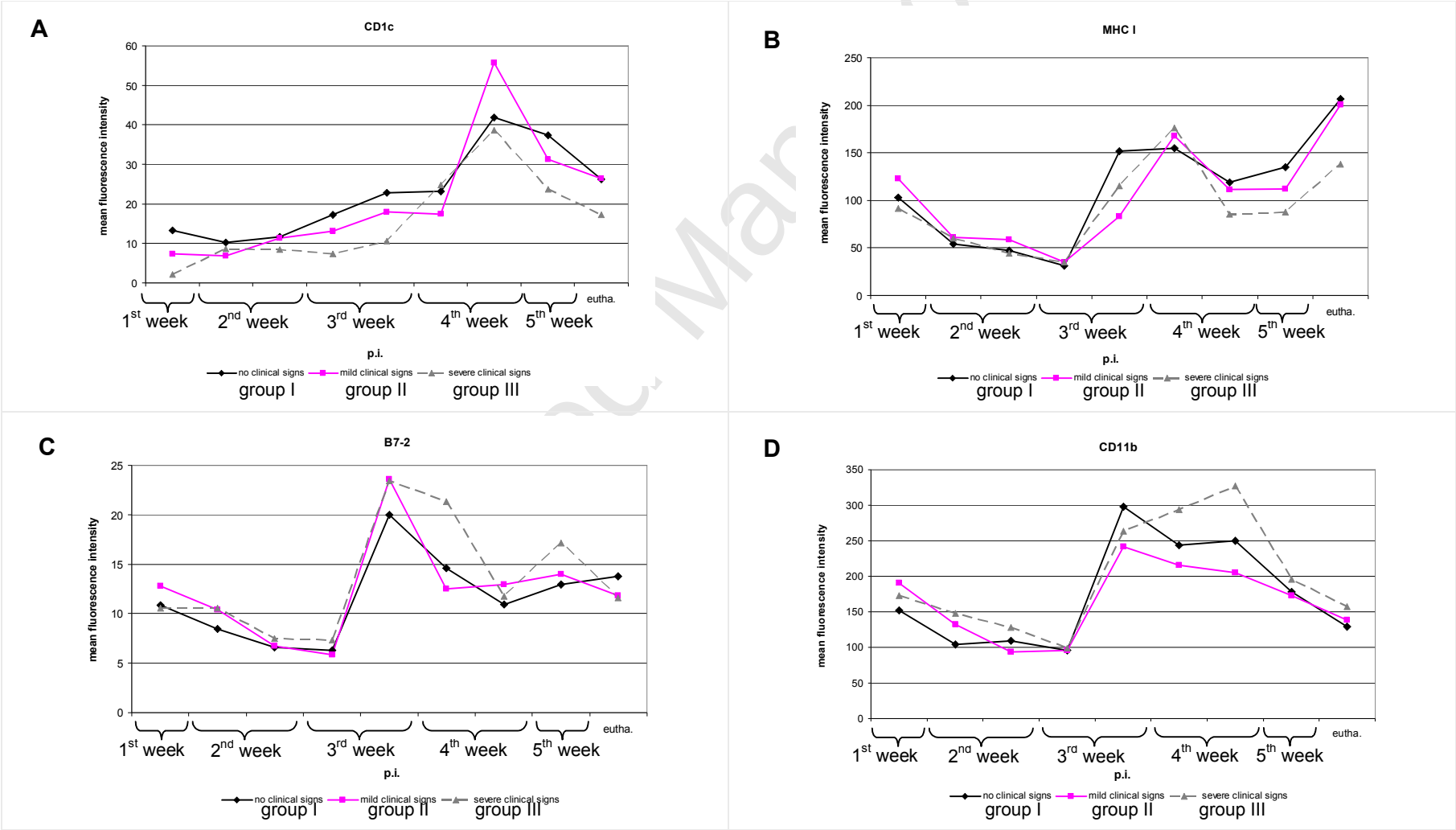

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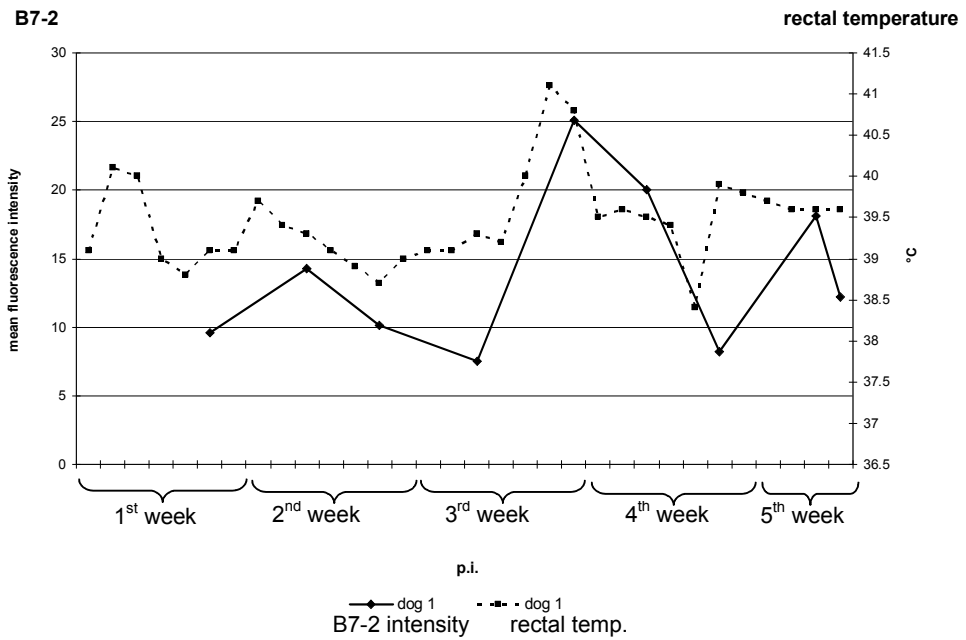

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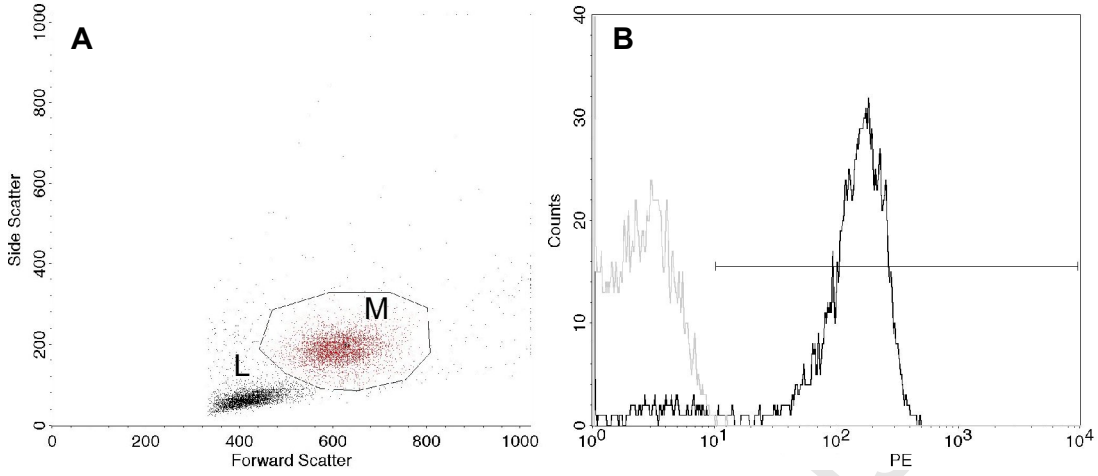

Figure 5.

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examination group	histopathological findings in the CNS	dog no.
group I **no clinical signs** (n = 7)	no active lesions	3, 4, 6, 9, 11, 16, 18
group II **mild clinical signs** (n = 6)	no active lesions	5, 8, 10, 12, 13, 14
group III **severe clinical signs** (n = 7)	demyelinating lesions	**1**, 2, **7**, 15, 17, **19**, **20**

Table 1.

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antigen	clone
CD1c	CA13.9H11
B7-1 (CD80)	CA24.5D4
B7-2 (CD86)	CA24.3E4
CD11b	CA16.3E10
CD18	CA1.4E9
CD45	YKIX 716.13
CD44	YKIX 337.8.7
CD14	TÜK4
MHC I	H58A
CD3	CA17.2A12
CD4	CA13.1E4
CD8α	CA9.JD3
CD21	CA2.1D6

Table 2.

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antigen	monocytes
CD18	no effect
CD11b	upregulated intensity
CD45	no effect
CD44	no effect
CD14	higher % in groups I+II compared with group III
CD1c	upregulated intensity and percentage; higher % in groups I+II compared with group III
B7-1	upregulated percentage
B7-2	upregulated intensity
MHC I	upregulated intensity

Table 3.

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Figure – Legend

Figure 1. Rectal temperatures (A), percentage of D110-positive monocytes in dogs of group III (severe clinical signs with demyelinating lesions due to CDV infection in the CNS) (B), and absolute monocyte cell counts (C) in the course of the CDV infection of dogs of group I (no clinical signs and no CDV lesions in the CNS, mean value of n = 7, black line) compared with dogs of group II (mild clinical signs and no CDV lesions in the CNS, mean value of n = 6, solid grey line), and dogs of group III (severe clinical signs and demyelinating lesions due to CDV infection in the CNS, mean value of n = 7, dashed grey line). The abscissa shows the time points of rectal temperature measurement (A), and of the examination of blood samples (B and C). Negative numbers in C represent days prior to infection with CDV. Values above the dotted line in A indicate the presence of fever. In B the boxplots display minimums and maximums, lower and upper quartiles, and medians which are connected. The box contains the middle 50% of the sample values. The results for group I and II are not shown as there was only a very low percentage (0-6%) of D110-positive cells. p.i. = post infection

Figure 2. Percentage of CD1c (A), B7-1 (CD80, B), and CD14 (C) expressing monocytes in the course of the canine distemper virus (CDV) infection for dogs of group I (mean value of n = 7, black line) compared with dogs of group II (mean value of n = 6, solid grey line), and dogs of group III (mean value of n = 7, dashed grey line). The abscissa shows the time points with examination of blood samples. p.i. = post infection, euth. = day of euthanasia

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Figure 3. Mean fluorescence intensity (log values; measured by mean fluorescent channel numbers) as a relative means for expression intensity of CD1c (A), MHC class I (B), B7-2 (CD86; C), and CD11b (D) in the course of the CDV infection for dogs of group I (mean value of n = 7, black line) compared with dogs of group II (mean value of n = 6, solid grey line), and dogs of group III (mean value of n = 7, dashed grey line). The abscissa shows the time points with examination of blood samples. p.i. = post infection, euth. = day of euthanasia

Figure 4. Mean fluorescence intensity of B7-2 for dog no. 1 of group III which demonstrates an elevation of the expression intensity at day 21 p.i. which was preceded by an elevation of the rectal temperature above 41°C in this dog at day 20 p.i. The solid line represents the value of B7-2 expression intensity, the dashed line symbolizes the rectal temperatures of this dog. The abscissa shows the time points with examination of blood samples. p.i. = post infection

Figure 5. **A** Dot plot with forward scatter (FSC) displayed at the abscissa and side scatter (SSC) at the y-axis. Cells with comparable size (FSC) and granularity (SSC) form populations which can be differentiated: lymphocytes (L) which are slightly smaller and less granulated and monocytes (M) which are gated for further evaluation. **B** Histogram displaying the staining result of negative control (grey line) and CD11b (black line). The analysis gate was adjusted to not exceed 2% positive staining with negative controls. PE = Phycoerythrine

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Table - Legend

Table 1. Dogs of the vaccine challenge experiment were divided into three examination groups according to clinical findings. Histopathological findings in the central nervous system (CNS) corresponded to the clinical signs as dogs with severe clinical signs developed the most severe neurological signs and demyelinating lesions in the CNS. A negative control group consisting of two uninfected dogs served as a reference for the comparison of monocytes and microglial cells at the day of euthanasia. Numbers in bold of group III indicate dogs with large multifocal plaques and severe demyelination.

Table 2. Designation of the monoclonal antibodies used in the study

Table 3. Overview over the effects of the CDV infection on regulation of surface antigens on canine monocytes.

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