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24 **ABSTRACT**

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

41

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

44 INTRODUCTION

45 Canine distemper virus (CDV) is a single-stranded RNA morbillivirus belonging to the
46 family of Paramyxoviridae (Pringle, 1999). In the acute phase of the disease CDV
47 infection induces multifocal demyelination in the central nervous system (CNS)
48 against a background of a systemic disease with severe immunosuppression and
49 lymphoid depletion (Vandeveldel et al., 1982; Krakowka et al., 1985; Greene and
50 Appel, 2006). The pathogenesis of CDV-associated immunosuppression remains
51 unclear (Greene and Appel, 2006). Replication of virus in the immunocompetent cells
52 leads to disturbed function or lymphocytic apoptosis (Wunschmann et al., 2000;
53 Kumagai et al., 2004; Schobesberger et al., 2005) whereby CD4⁺ cells are more
54 affected than CD8⁺ cells (Tipold et al., 2001). In the subacute and chronic stage of
55 the infection, an antiviral immune response is initiated and is associated with
56 additional inflammatory damage to the white matter (Vandeveldel and Zurbriggen,
57 2005). The contributing factors in the pathogenesis of demyelination in distemper
58 include viral replication in astrocytes (Vandeveldel et al., 1982), restricted infection of
59 oligodendrocytes (Zurbriggen et al., 1998), down-regulation of myelin gene
60 transcription (Graber et al., 1995), dysregulation of cytokines, metalloproteinases and
61 their inhibitors (Markus et al., 2002; Gröters et al., 2005) and the intrathecal antiviral
62 immune response (Vandeveldel and Zurbriggen, 2005). There is little doubt that the
63 microglial/macrophage compartment plays a pivotal role on the effector side of the
64 demyelinating process (Vandeveldel and Zurbriggen 2005). As monocytes and
65 microglia are both members of the macrophage-lineage they show cross-reactivity of
66 many surface markers (Dijkstra et al., 1985; Perry and Gordon, 1991; Flaris et al.,
67 1993; Fedoroff, 1995; Stoll and Jander, 1999), and upon activation similar effector
68 functions can be exerted (Graeber and Streit, 1990). Microglial cells are activated in
69 distemper (Tipold et al., 2001, Stein et al., 2004), and show an up-regulation of MHC

70 II (Aldinger et al., 1996) and various other surface molecules (Stein et al., 2004), as
71 well as enhanced secretion of reactive oxygen species (Stein et al., 2004). In the
72 course of the inflammatory response in demyelinating lesions in distemper,
73 peripheral monocytes/macrophages invade the CNS, complementing the local
74 microglia. The central role of macrophages in myelin destruction was proposed by
75 earlier investigators in distemper (Wisniewsky, 1972), and supported by in vitro
76 studies, showing ADCC in CDV-infected canine brain cell cultures (Griot-Wenk et al.,
77 1991). Not much is known about the effect of CDV on peripheral blood monocytes. In
78 contrast to the overt CDV-induced lymphopenia, monocyte numbers are not
79 significantly decreased and monocytosis is very rarely observed (McCullough et al.,
80 1973; Tipold, 1996; Greene and Appel, 2006). This might be due to minimal
81 replication of CDV in these cells (Von Messling et al., 2004). Cultured macrophage
82 functions seemed not to be impaired and may even be enhanced as a result of CDV
83 infection (Brugger et al., 1992).

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

87

88 **MATERIAL AND METHODS**

89 **Animals**

90 Twenty 5-6-months-old experimentally CDV-infected SPF Swiss-Beagle dogs, 10
91 males and 10 females from two litters were derived from a DNA vaccine study with
92 challenge infection with the virulent CDV-strain A75/17 (111/99/Berne/CH; Stein et al.
93 2004; experiment see Cherpillod et al., 2000). All animals were regularly physically
94 and neurologically examined and rectal temperature was measured daily. Blood

95 samples for monocyte isolation were taken every 3-4 days. All dogs were euthanized
96 20-31 days post infection (PI).

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

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

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

122

123 **Differential haemogram**

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

128

129 **Antibodies**

130 MAbs used for immunophenotypic analyses were either specific for dog cell surface
131 markers or cross-reacting with them. Monoclonal mouse antibodies directed against
132 CD11b, CD18, CD1c, B7-1 (CD80), B7-2 (CD86), CD3, CD4, CD8 α , and CD21 were
133 provided by Prof. Peter F. Moore, University of Davis, USA. MAbs against CD11b,
134 CD18, CD4, CD8 α , CD21 and MHC class I were characterized at the first
135 international canine leukocyte antigen workshop (Cobbold and Metcalfe, 1994). The
136 following primary antibodies were purchased: mAb directed against CD45
137 (conjugated with biotin), CD44 (both rat mAb, Serotec, Eching, Germany), MHC class
138 I (mouse mAb, VMRD, Inc. Pullman, USA) and CD14 conjugated with R-PE (mouse
139 mAb, Dako, Glostrup, Denmark). The clones of the monoclonal antibodies are
140 summarized in Table 2. The murine mAb D110 was prepared from cell culture
141 supernatant (Bollo et al., 1986) and used for detection of an epitope of the CDV
142 nucleocapsid protein which is resistant to tissue fixation and embedding procedures.
143 All antibodies were used for flow cytometry (FACS) analysis. Human normal
144 immunoglobulin G (Globuman Berna, Berne, Switzerland) served to block non-
145 specific binding. Secondary antibodies were purchased: R-Phycoerythrine

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

150

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

152 Peripheral blood was examined once or twice weekly until euthanasia. Approximately
153 5 ml of blood was collected by venipuncture into heparinized tubes and subjected to
154 discontinuous density gradient centrifugation according to Wunderli and Felsburg
155 (1989) modified by Somberg et al. (1992). Briefly, Pancoll (density 1.077; Cytogen,
156 Ober-Moerlen, Germany) and Histopaque (density 1.119; Sigma, Deisenhofen,
157 Germany) were used and centrifuged at 400 x g for 20 min at room temperature.
158 After centrifugation PBMCs accumulated as a white cell-layer at the interphase
159 between plasma and Pancoll. Following two washing steps with PBS the supernatant
160 was discarded and cells were available for further examination. Monocyte and
161 lymphocyte subsets were differentiated as two cell populations by their differing size
162 (forward scatter, FSC) and complexity (side scatter, SSC), and monocytes were
163 selectively examined after gating this population in flow cytometry (see Fig. 5A). Low
164 percentages of the gated cells for the expression of CD3, CD8 α , CD21 and CD4
165 served to exclude contamination with lymphocytes.

166 Monocytes were examined every third to fourth day in the course of the CDV infection.
167 They were stained as previously described for lymphocyte subsets (Tipold et al.,
168 1999). After Fc-receptor blockade with 10 mg/ml of human IgG, primary antibodies
169 were incubated for 30 min at 4°C and washed twice with Hanks' buffered saline
170 (Sigma, Deisenhofen, Germany). Secondary antibodies conjugated either with FITC

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

179

180 **Detection of CDV in monocytes**

181 CDV detection in monocytes was performed as previously described (Cherpillod et
182 al., 2000). Briefly, monocytes were fixed with 2% paraformaldehyde for 20 min at
183 4°C. After two washing steps with PBS, cells were resuspended in 50µl PBS
184 containing 0.03% Saponin (Merck, Hannover, Germany) for cell permeabilisation.
185 Following Fc-receptor blockade with 10 mg/ml human IgG, 10µl of the mAb D110 (in
186 a dilution of 1:16) was added, and the cells incubated for 30 min at 4°C. After two
187 washing steps with PBS-Saponin-solution secondary goat-anti-mouse IgG antibody
188 conjugated with PE was added in a dilution of 1:50 and again incubated. Negative
189 control samples were stained with secondary antibody only. After a final washing
190 step monocytes were immediately measured with flow cytometry.

191

192 **RESULTS**

193 **Detection of CDV in canine monocytes**

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

196 CDV-positive monocytes could no longer be detected with the exception of dog nos.
197 4 and 6 of group II, showing positive staining of monocytes on day 26 to day 35 PI. In
198 contrast, all dogs of group III showed up to 33.3% CDV-positive monocytes at all time
199 points during the course of the infection (see Fig. 1B). In two dogs peak levels of
200 CDV-positive monocytes were preceded by or concurrent with significant elevation of
201 body temperature (dogs no. 17 and 20).

202

203 **Immunophenotypic characterization of monocytes during CDV infection**

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

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

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

222 all three examination groups during the course of the CDV infection peaking towards
223 the end of the fourth week PI followed by a decline during the last week of the
224 experiment (see Fig. 2A + B). **CD1c** and **CD14** were found in a generally higher
225 percentage of monocytes in group I (mean values of the whole examination period
226 are given; CD14 \bar{x} = 74.7%; CD1c \bar{x} = 63.4%) and group II (CD14 \bar{x} = 72.9%; CD1c \bar{x}
227 = 57.4%) compared to group III (CD14 \bar{x} = 53.9%; CD1c \bar{x} = 46.7%; see Fig. 2A + C).
228 **Expression intensity** of **CD1c** also steadily increased in all groups reaching its
229 maximum at the end of the fourth week PI (see Fig. 3A). Likewise, the surface
230 molecules, **CD11b**, **B7-2** (CD86), and **MHC I** showed an up-regulated expression
231 intensity peaking at the end of the third week PI, followed by a gradual down-
232 regulation in all three examination groups except for MHC I, which remained high
233 until the end of the experiment (see Fig. 3B-D). For **CD18**, **CD45**, **CD44**, **CD14**, and
234 **B7-1** (CD80) no up- or down-regulated expression intensity was measured in any
235 examination group.

236

237 **Correlation between up-regulation of surface antigens and body temperature**

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

242

243 **DISCUSSION**

244 Previous studies strongly suggest that microglia and peripheral macrophages
245 invading the CNS play an important role in the pathogenesis of demyelination in
246 canine distemper virus infection (Stein et al., 2004; Vandeveld and Zurbriggen,
247 2005). Not much is known on the effect of CDV on peripheral macrophages.

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

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

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

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

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

321 The observed upregulation of antigens typically expressed on active macrophages
322 probably supports clearance of the infection. However, in animals that accumulate
323 significant amounts of CDV in the CNS however, such antiviral macrophage activity
324 can lead to detrimental bystander damage (Wisniewsky 1972, Griot-Wenk et al
325 1991). It is of interest to note in this respect, that peak expression of the above

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

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

335

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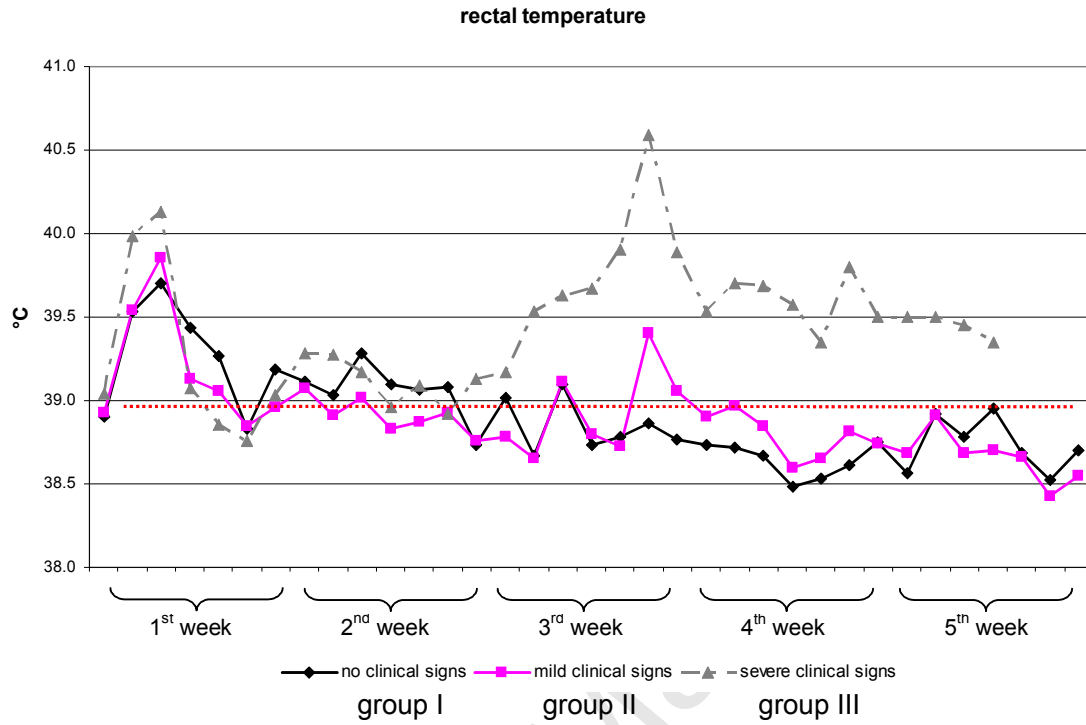
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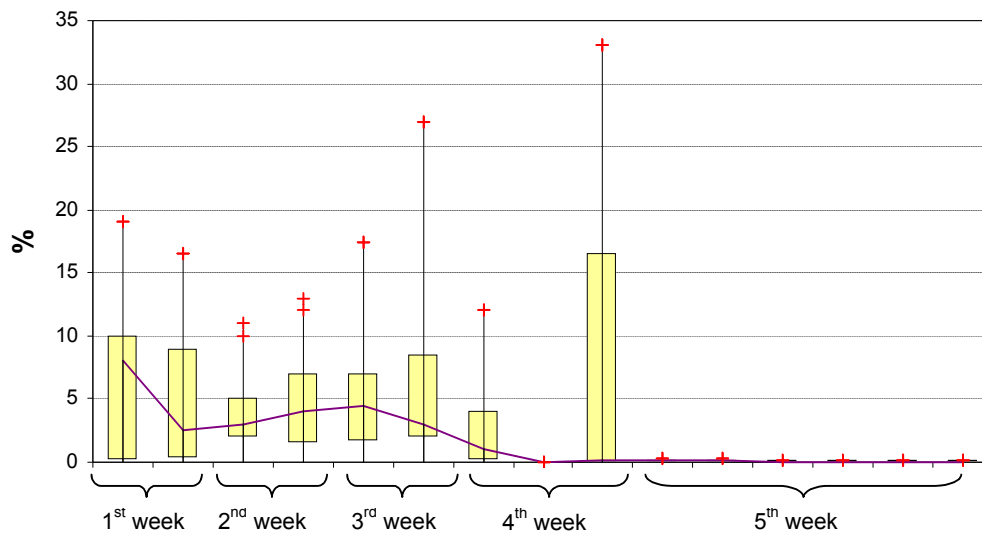
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D110 - group III -

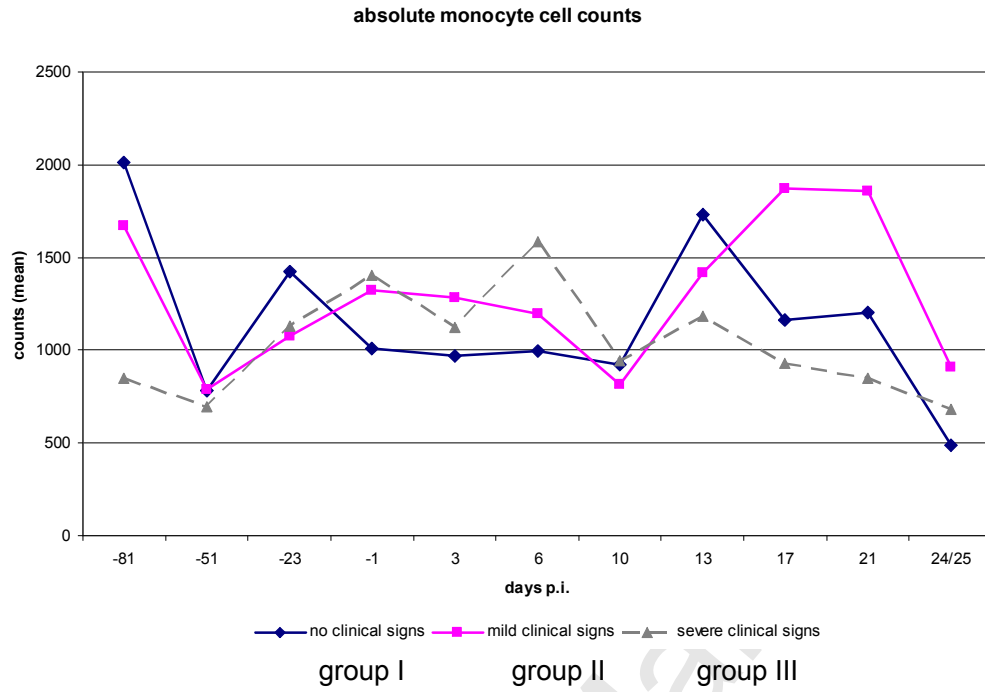


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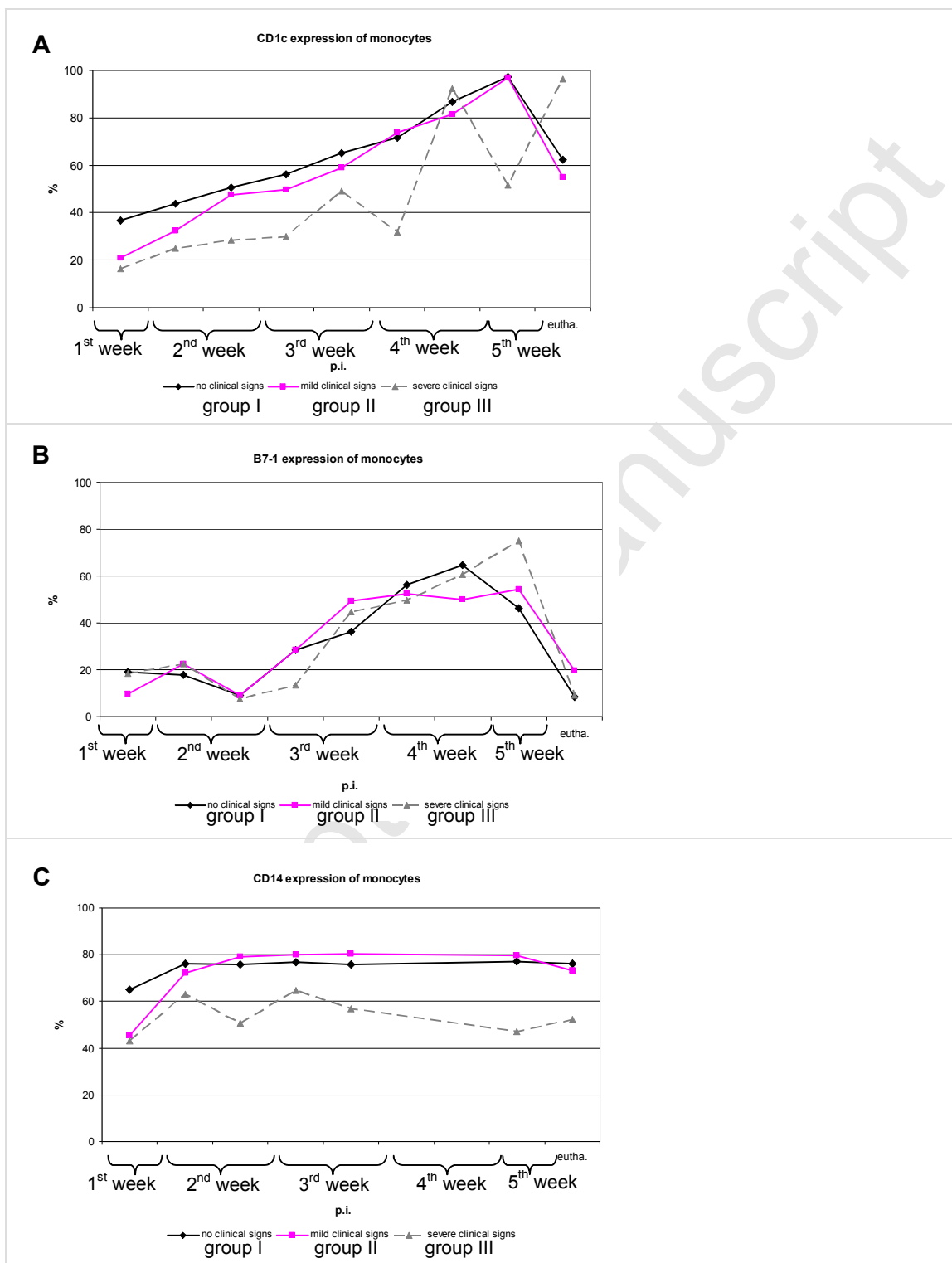
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490 **Figure 1.**

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492 **Figure 2.**

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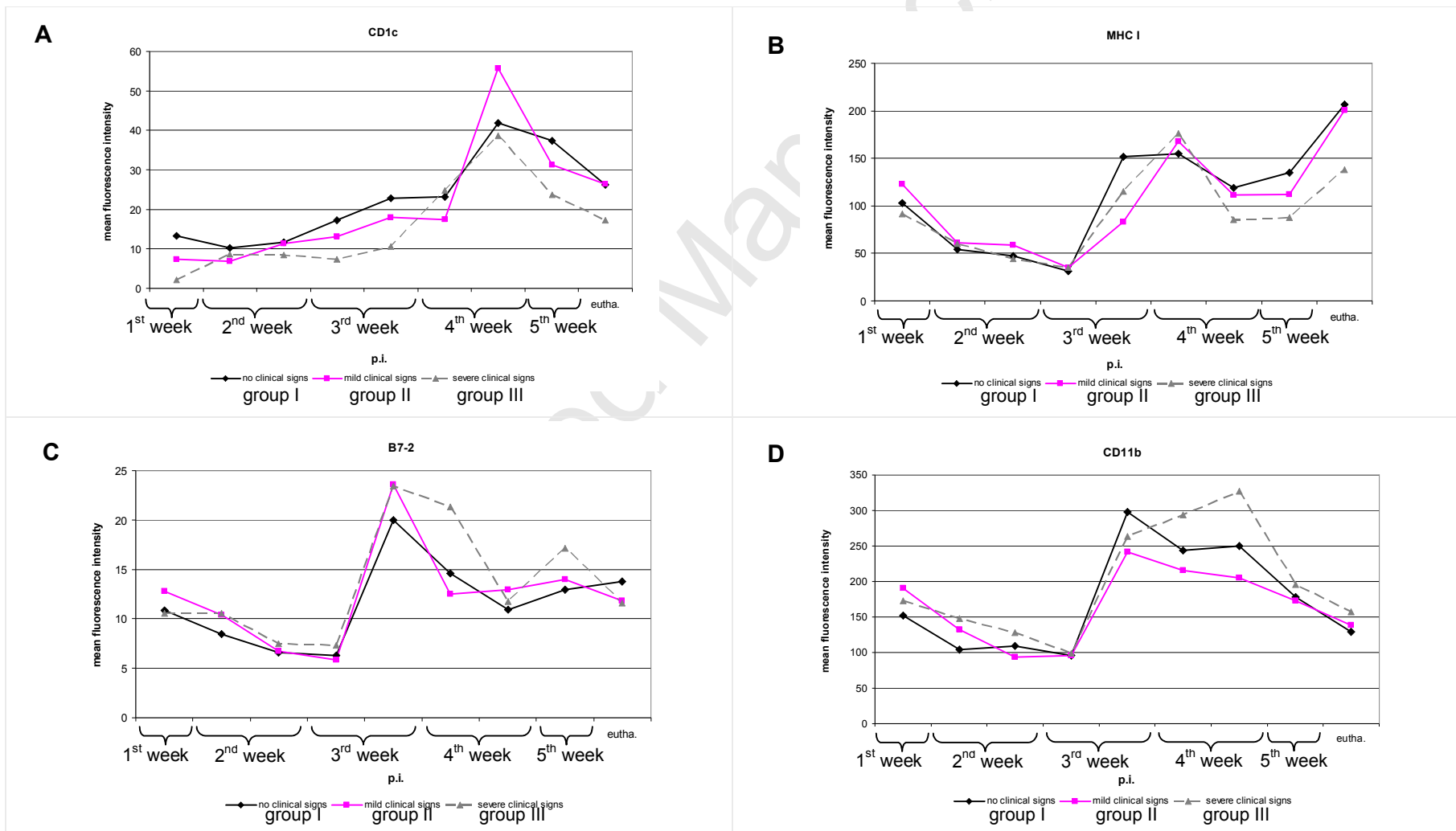


Figure 3.

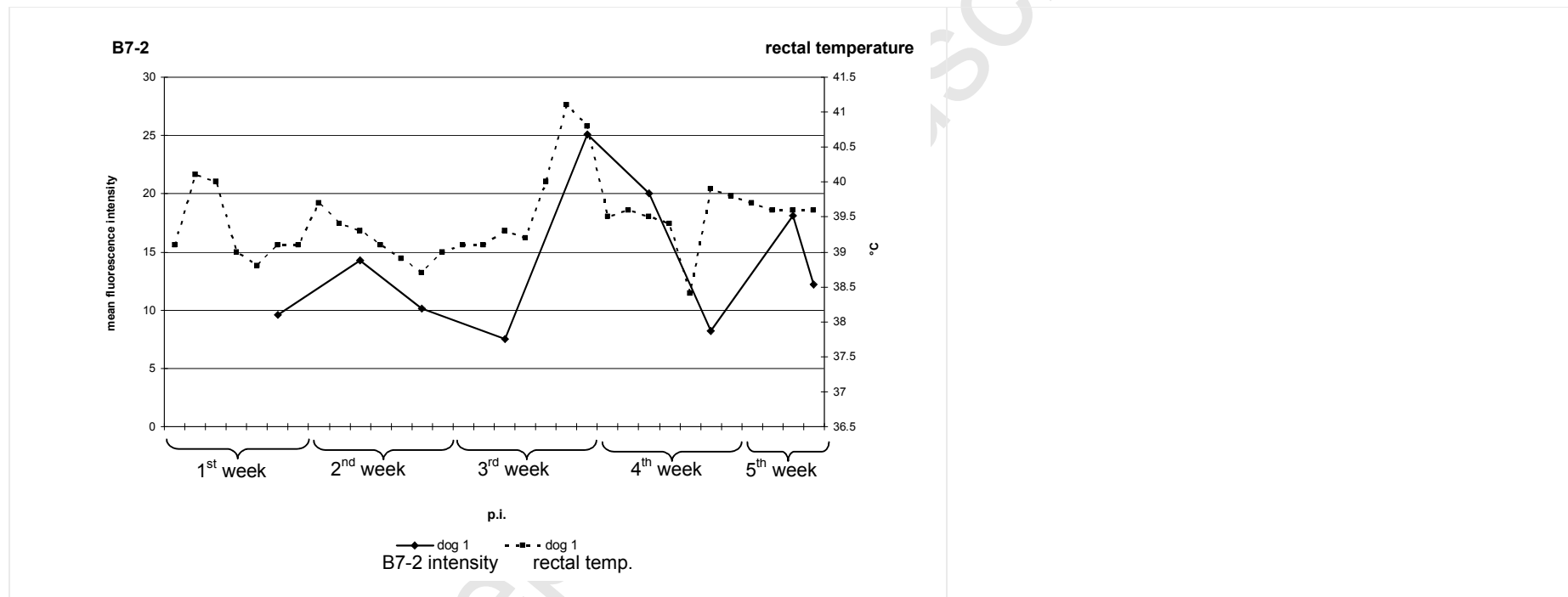


Figure 4.

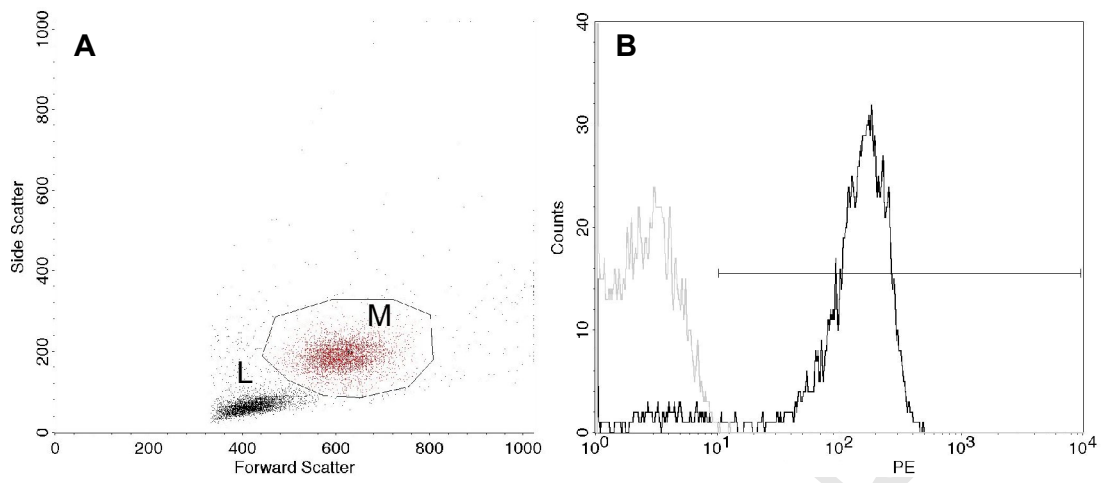


Figure 5.

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.

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.

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.

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

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

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.