IMMUNOPHENOTYPE IN SYMPTOMATIC AND ASYMPTOMATIC DOGS NATURALLY INFECTED BY *Ehrlichia canis*

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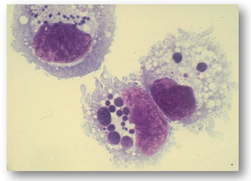
OBJECTIVES



Ehrlichia canis is the main etiologic agent of canine monocytic ehrlichiosis (CME)[1]. Infected animals develop lesions in various organs and tissues and present several clinical signs that may vary depending on the phase of the disease (acute, subclinical or chronic)[2,3]. It has been suggested that the immune response elicited by the host during the infection could influence the clinical signs and laboratory and pathological findings[1,4,5].

The **aim** of the present study was to evaluate the peripheral blood lymphocyte subsets in dogs naturally infected by *E. canis* with (symptomatic) or without (asymptomatic) clinical manifestations of the disease.

METHODS

E. canis-naturally infected dogs were included in the study. Diagnosis was performed using an indirect inmunofluoresence assay (cut-off point 1:80) and/or PCR (Fig. 1 and 2).

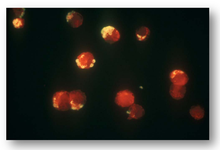


Figure 1. Positive indirect antibody test to *E. canis*

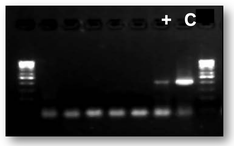


Figure 2. PCR amplification of *E. canis* DNA (C=positive control, +=positive sample)

Figure 3. Symptomatic *E. canis* infected dog with epistaxis

Two groups of animals were evaluated:

- Asymptomatic dogs (n=20), without clinical signs of CME, but with laboratory findings traditionally associated to CME (thrombocytopenia, anemia, and/or hyperproteinemia)
- Symptomatic dogs (n=8), with clinical signs classically associated to CME (pale mucous membranes, fever, lymphadenopathy, weight loss, anorexia, lethargy or signs attributable to bleeding tendencies) (Fig. 3).

Specificity	Isotype	Clone	Lymphocyte phenotype	Conjugate
CD3	Mouse IgG1anti-canine CD3	CA17.2A12	T lymphocytes	FITC
CD4	Rat IgG2a anti-canine CD4	YKIX302.9	Th lymphocytes	RPE
CD8	Rat IgG1 anti-canine CD8	YCATE55.9	Tc lymphocytes	Alexa Fluor® 647
CD21	Mouse IgG1 anti-canine CD21	CA2.1D6	B lymphocytes	RPE
MHC class II	Rat IgG2a anti-canine MCH class II	YKIX334.2	MHCII expression	FITC

Table 1. Monoclonal antibodies used for the flow cytometric study

A multiparametric flow cytometric study using a FACSCalibur flow cytometer was performed to analyze the distribution of the main lymphocyte subsets (T, Th, Tc, B and those that express MHC class II) in each sample. Monoclonal antibodies were supplied by AbD Serotec and are described in Table 1.

Analysis of data was performed with the Statgraphics (CenturionXVI version) software, using the t-student test, considering a level of significance of $p<0.05$.

RESULTS

Despite alterations in hematology, blood biochemistry and protein electrophoresis were higher in this study in dogs with CME in a clinical phase than in animals with subclinical phase of the disease, statistically significant differences between symptomatic and asymptomatic dogs naturally infected by *E. canis* were not detected when evaluating lymphocyte subsets in peripheral blood samples (Table 2).

		Symptomatic CME (n=8)	Asymptomatic CME (n=20)	p value
T lymphocytes (CD3+)	Percentage (%)	73.27	71.98	0.757
	Absolute value (/µl)	2332	2096	0.710
Th lymphocytes (CD3+CD4+)	Percentage (%)	29.08	33.87	0.390
	Absolute value (/µl)	1323	927	0.610
Tc lymphocytes (CD3+CD8+)	Percentage (%)	30.26	27.41	0.703
	Absolute value (/µl)	1985	900	0.344
CD4/CD8 ratio		2.41	1.68	0.613
B lymphocytes (CD21+)	Percentage (%)	10.43	15.13	0.140
	Absolute value (/µl)	251	386	0.165
CMH II+ lymphocytes	Percentage (%)	91.41	92.05	0.763
	Absolute value (/µl)	3993	2656	0.451

Table 2. Relative and absolute average values of lymphocyte subsets in symptomatic and asymptomatic dogs naturally infected by *E. canis*

Although differences were not statistically significant, symptomatic animals showed lower relative and absolute values of B lymphocytes than dogs without clinical signs ($p=0.140$ and $p=0.165$, respectively). These results could support a key role of B cells in host defense during *Ehrlichia* spp. monocytotropic infection, probably related to stimulation of cytokine secretion and proliferation of specific T CD4+ subsets [6,7]. However, it is possible also that these lower relative and absolute values of B cells in peripheral blood in symptomatic dogs could be associated with a higher presence of these lymphocytes and plasma cells in kidney, spleen and bone marrow in clinical phases of CME[1,8].

CONCLUSIONS

The presence or absence of clinical manifestations of CME in dogs naturally infected by *E. canis* does not appear to be related with the peripheral blood distribution of the lymphocyte subsets T, Th, Tc and those that express MHC class II. Further studies are needed to clarify the role of B cells in the pathogenesis and progression of the disease.

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