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Short Communication

Immunophenotypic evaluation of working Labrador Retrievers and German Shepherd dogs living in the same environment

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ABSTRACT

Multiparametric flow cytometry was used to compare peripheral blood lymphocyte subset distribution between healthy working police Labrador Retrievers (LRs; $n = 12$) and German Shepherd dogs (GS; $n = 11$) living in the same environment. The CD4/CD8 ratio was significantly higher in LR than in GS because of the lower percentage of CD8+ T lymphocytes in LR. GS showed the highest relative percentage of CD3−/CD21− lymphocytes, whereas LR had the highest percentages of MHC II+ lymphocytes. Because age, sex, environmental and housing conditions, dietary patterns, and training or working routines were similar in both breeds in the study, differences in peripheral blood lymphocyte subset distribution could be attributed to the influence of breed on the immune system.

Age, sex, race/ethnicity, genetics, circadian rhythm, dietary patterns, altitude, and exercise affect peripheral blood lymphocyte subset distribution in humans (Maini et al., 1996; Tsegaye et al., 1999; Bain et al., 2000). Although the effects of age on the canine immune system have been assessed (Greeley et al., 1996, 2001; Byrne et al., 2000; Faldyna et al., 2001; HogenEsch et al., 2004; Blount et al., 2005), little information is available with regard to potential breed variability in dogs (Byrne et al., 2000; Faldyna et al., 2001). The aim of the present study was to investigate differences in peripheral blood leukocyte subsets between German Shepherd dogs (GS) and Labrador Retrievers (LRs) living in the same environment.

The study included 12 LR (3 females, 9 males) and 11 GS (4 females, 7 males). Dogs came from different families, were in police service (National Police, Madrid), were maintained in similar housing and environmental conditions (including dietary patterns and exercise routines) and had received routine anti-parasite drugs and vaccination at least 2 months before blood sampling. All dogs were clinically healthy according to physical examination, haematology and blood chemistry evaluation, and were negative to *Ehrlichia/Anaplasma/Neorickettsia* spp. and *Leishmania* spp. by serology and polymerase chain reaction (PCR). All procedures were approved by the Animal Research Committee of the Complutense University of Madrid.

Blood samples were drawn by cephalic venepuncture at 14.00–16.00 h, collected in tubes with EDTA and heparin, and processed for flow cytometry (FC) within 2 h of drawing the blood. Multiparametric flow cytometry (FC) was performed to analyse different lymphocyte subsets (T, Th, Tc, B, those that express MHC class II, and non-T, non-B lymphocytes) in each sample. Absolute values were calculated using white blood cell (WBC) analysis in combination with FC data.

Monoclonal antibodies to canine lymphocyte cell surface antigens were obtained from AbD Serotec (Table 1). The FC protocol employed was standardized before the beginning of the study, and the specificity of primary antibody binding was also evaluated using the isotype controls suggested by the manufacturer (data not shown). Aliquots of blood in EDTA (100 μL) were exposed to three different combinations of appropriate amounts of antibodies (Table 1). A fourth blood aliquot was not exposed to any antibody. After 30 min of incubation in the dark, red blood cells were lysed with Erythrolyse red blood cell lysing buffer (AbD Serotec) following the manufacturer's instructions. Finally, cells were resuspended in 100 μL of cell fixing solution (BD CellFIX, Becton Dickinson) and analysed using FACSCalibur FC (Becton Dickinson) and Cell-Quest software. A total of 10,000 events were acquired for each preparation. Fluorescence in FL1, FL2 and FL4 was measured on lymphocytes gated on the basis of forward and side scatter light characteristics.

Normal distribution of data was previously confirmed evaluating skewness and kurtosis. Data analysis was performed using Student's *t* test or Wilcoxon test, considering a level of significance of $P < 0.05$ and using the Statgraphics software (Centurion XVI version).

The mean age in the LR and GS dogs were 4.8 ± 1.6 years and 5.7 ± 1.1 years, respectively. The sex and age distribution in both

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Table 1
Monoclonal antibody combinations used in the flow cytometry study.

Tube	Fluorescence 1	Fluorescence 2	Fluorescence 4
1	Anti-CD3 (clone CA17.2A12):FITC (3 μL)	Anti-CD4 (clone YKIX302.9):PE (5 μL)	Anti-CD8 (clone YCATE55.9):AlexaFluor 647 (5 μL)
2	Anti-CD3 (clone CA17.2A12):FITC (3 μL)	CD21 (clone CA2.1D6):PE (2 μL)	–
3	MHC II (clone YKIX334.2):FITC (5 μL)	CD21 (clone CA2.1D6):PE (2 μL)	–

The amount of each antibody is given in parentheses. FITC, fluorescein isothiocyanate; PE, phycoerythrin.

populations was similar ($P = 0.475$ and $P = 0.387$, respectively). No sex or age-related differences were identified in absolute counts or percentage of lymphocyte subsets.

Results of WBC distribution and lymphocyte subpopulations for both breeds are shown in Tables 2 and 3. GS showed higher percentage of CD8+ T (Fig. 1) and non-T, non-B (CD3−/CD21−) lymphocytes compared to LR. The percentage of CD8+ T cells obtained in GS were comparatively higher than those previously reported in this breed (Chabanne et al., 1995; Faldyna et al., 2001), while in LR, our results were in agreement with those previously reported by Blount et al. (2005), but were lower than those found by Greeley et al. (1996) in LR of similar age. CD3−/CD21− cells can be defined as non-T, non-B lymphocytes, which include NK cells. However, no clear markers for canine NK cells are available (Reis et al., 2005). The fact that GS showed the highest percentages of lymphocytes characteristic of cellular immune responses (CD8+ T and NK lymphocytes) contrasts with the previous description of a diminished cellular response in GS (Nyindo et al., 1980). Related to that finding, it has been suggested that a weaker activity of circulating lymphocytes in GS and a low concentration of IgA could be related to the predisposition of this breed to deep pyoderma (Chabanne et al., 1995; Faldyna et al., 2001).

The CD4/CD8 ratio obtained in GS in the present study was lower than in LR and also lower than what has been previously reported in this breed (Chabanne et al., 1995; Faldyna et al., 2001). However, the ratio in LR from our study was similar to that previously reported (Blount et al., 2005). Differences between studies could be explained by differences in monoclonal antibody clones and sources, methods of sample treatment and result analysis, characteristics of the evaluated animals and differences in geographic areas (Byrne et al., 2000; Reis et al., 2005).

The percentage of MHC II+ lymphocytes was higher in LR than in GS. It has been suggested that canine MHC genes have a role in breed susceptibility to diabetes mellitus and various autoimmune diseases (e.g., chronic superficial keratitis in GS) (Catchpole et al., 2008; Jokinen et al., 2011). The significance of the higher percentages of MHC II+ lymphocytes in LR needs further evaluation. However, it is noteworthy that this percentage was high in both groups and is not related to differences in the absolute values of these cells.

Our results should be interpreted with caution considering the absence of differences in the absolute values of the assessed lymphocyte subsets and the low number of dogs included in the study. In addition, all animals in the current study were working dogs and

Table 2
Differences in peripheral blood cell counts of leukocyte subset populations between Labrador Retrievers and German Shepherd dogs (mean ± SD).

		Labrador Retriever dogs ($n = 12$)	German Shepherd dogs ($n = 11$)	P
Leukocytes $\times 10^3/\mu L$		11.7 ± 1.4	11.1 ± 2.1	0.639
Neutrophils	%	65 ± 3.1	64 ± 2.9	0.742
	$\times 10^3/\mu L$	7.5 ± 0.8	7 ± 1.2	0.441
Lymphocytes	%	24 ± 2.2	24 ± 2.8	0.929
	$\times 10^3/\mu L$	3.1 ± 0.7	2.9 ± 0.4	0.614
Monocytes	%	4.3 ± 0.6	4.9 ± 0.5	0.175
	$\times 10^3/\mu L$	0.5 ± 0.1	0.6 ± 0.1	0.340
Eosinophils	%	6 ± 2	6 ± 2	0.989
	$\times 10^3/\mu L$	0.7 ± 0.1	0.6 ± 0.1	0.570

Table 3
Differences in immunophenotype of peripheral blood lymphocytes between Labrador Retrievers and German Shepherd dogs (mean ± SD).

		Labrador Retriever dogs ($n = 12$)	German Shepherd dogs ($n = 11$)	P
T lymphocytes (CD3+)	%	69 ± 4.8	69 ± 2.8	0.779
	$\times 10^3/\mu L$	2.1 ± 0.4	1.9 ± 0.2	0.527
Th lymphocytes (CD3+/CD4+)	%	39 ± 4	35 ± 2.9	0.166
	$\times 10^3/\mu L$	1.2 ± 0.3	1 ± 0.1	0.193
Tc lymphocytes (CD3+/CD8+)	%	19 ± 2.6	26 ± 2.7	0.004
	$\times 10^3/\mu L$	0.6 ± 0.1	0.6 ± 0.1	0.630
CD4/CD8 ratio		2.1 ± 0.3	1.4 ± 0.3	0.005
B lymphocytes (CD21+)	%	15 ± 2.8	14 ± 2.6	0.617
	$\times 10^3/\mu L$	0.5 ± 0.1	0.4 ± 0.1	0.476
MHC II+ lymphocytes	%	91 ± 2.4	86 ± 3.3	0.025
	$\times 10^3/\mu L$	2.9 ± 0.7	2.5 ± 0.5	0.402
Non-T, non-B lymphocytes (CD3−/CD21−)	%	12 ± 2.5	17 ± 2.5	0.014
	$\times 10^3/\mu L$	0.4 ± 0.2	0.5 ± 0.1	0.511