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Diagnosis Techniques of the Neutropenia Syndrome – Immobility of the Leucocytes with Dogs

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Abstract. Primary immunodeficits related to the phagocitary function, signaled in dogs, constitute a cause in the activation of certain variable, uncharacteristic clinical signs, with an uncertain therapeutic response. An immunodeficit condition framable in this group is the neutropenia syndrome – immobility of leucocytes which can be diagnosed through functional evaluation techniques of the neutrophiles. In this sense there was used the evaluation technique of migration through filtering layer and under agarose, the evaluation technique of the capacity of embedment of carbon particles and the evaluation technique of the total bactericide capacity.

Keywords: phagocitosis, neutrophiles, migration, immunodeficit

INTRODUCTION

Primary immunodeficits signaled in dogs are affections determined mostly genetically, with unclear causality. In the group of functional deficits signaled in dogs'neutrofiles there is also the neutropenia syndrome – immobility of leucocytes, also called the „lazy" leucocyte syndrome. With no high incidence, the syndrome was signaled and diagnosed especially in people. With dogs, the anomaly can be categorized within the group of functional immunodeficits of the phagocitary cells, with a hereditary predisposition in the Irish setter and Doberman.

Starting from the observations noted in the reference literature and the casuistry within the clinic of the Faculty of Veterinary Medicine – Bucharest, five Irish setters were tested so as to identify the functional tests which might lead to this diagnostic.

MATERIAL AND METHODS

The biologic material used was represented by five dogs of the Irish setter breed, 17-23 months old, unrelated, out of which three dogs displayed clinical signs of lymphadenitis, gingivitis and supurate dermatitis. The other two exemplars were clinically healthy in well-maintained condition.

The samples collected for the diagnostic of the immunodeficit were venous blood collected on heparin in three phases at intervals of two weeks.

The techniques used for diagnosing the immunodeficit mainly sought to establish the number of neutrofiles and their functional evaluation. With this objective, leucogramas were

carried out through the May-Grunwald-Giemsa technique and the neutrofiles in the collected samples were tested functionally.

The separation of the neutrofiles in the total blood was performed by the separation technique by Percoll environment. This is a biphase environment which allows a separation in discontinuous density gradient. The separation density used was 1,087, the fraction of the collected netrofiles having a purity over 95%. This technique is also advantageous as it does not functionally affect the separated cells.

In order to analyze the phagocitary functions the phagocytosis mechanism was tested sequencially, evaluating the migration capacity, the endocytosis and intracellular digestion. The evaluation of the migration capacity was performed by two techniques, respectively through filtering layer and the migration technique under agarose. The first one is based on the capacity of the phagocytes to cross a transparent glass filter (Boyden filter) after a chemotactic standardized solution of formyle-metionil-leucil-phenylalanine was previously filtered (FMLP). The suspension of tested neutrofiles was introduced in the filtering system and, after a 37^{o}C incubation, the proportion of migrated cells was measured based on having measured the optical density of the filtering system. The evaluation technique of migration under agarose additionally alows the evaluation of the correctness of the migrating direction. The migration support was constituted of a double gelatine and agarose layer, laid on a glass blade. The tested cells and the chemotactic solution (FMLP) are deposited in the wells cut in the migration layer. The evaluation of the migration both as number of cells and as migration speed was performed using a microscope with micrometric eyepiece.

The evaluation of the endocytosis capacity was performed using the embedment test of the carbon particles. The source of these particles was the supernatant of a China-ink centrifugate. The reduction of the quantity of carbon particles in a mixture of neutrofile suspension and China-ink, evaluated spectrophotometrically, allows the evaluation of the embedment capacity of the sample neutrofiles.

In order to evaluate the capacity of intracellular digestion there was used the technique of determining the global bactericide activity, using as reference bacteria a strain of *Staphylococcus aureus*. Practically, there were placed in contact, in the initially liquid then solid culture environment, the phagocyte suspension and the reference bacteriological strain. In order to interpret the level of intracellular digestion there are counted the units forming colonies grown on the solid culture environment.

RESULTS AND DISCUSSIONS

The values obtained upon counting the neutrofiles indicate an insignificant reduction of approximately 10-12% for the three animals in the lot suspected of immunodeficit, in comparison with the clinically healthy ones. Even in this situation, the values recorded for all the tested animals are framed within the normal physiological limits known for dogs. Practically, the quantitative determination of the neutrofiles does not lead to the diagnostic of obvious neutropenia.

For an easier interpretation of the results a table system was used, which comprises the data resulted after the functional evaluation of the analyzed neutrofiles.

Subjects 1, 2 and 3 represent the animals suspected of immunodeficitary syndrome, while subjects 4 and 5 represent the clinically healthy animals.

Within the evaluation of the migration capacity through filtering layer (Table 1) there was observed, in the case of the three animals suspected of immunodeficit, that the optical density of the filtering system increased by only 33-35%, in comparison with the healthy

animals for which the increase was over 240%. The evaluated parameters in all the other samples varied insignificantly.

The evaluation test of the migration capacity under agarose layer (Table 2) indicates major differences in what concerns the migration distances crossed by the neutrophils of the healthy animals in comparison with those suspected of immunodeficit. In this sense there are significant the increases by over 230% of the migration distance. The unspecific migration was observed in all the tested subjects and set to approximately equal values for the two categories of animals.

The values resulted after testing the endocytosis capacity (Table 3) indicate approximately equal values for the two categories of animals, including in the final phase of evaluating the optical density. These results suggest a weak chemotactism of the carbon particles.

The values of the phagocytosis index obtained after the intracellular digestion of a bacteriological standardized strain are different for the two categories of animals (Table 4). The clinically healthy animals have an increased phagocitary index with values between 22 and 32%, in comparison with the animals suspected of immunodeficit syndrome.

CONCLUSIONS

- In the case of the three animals suspected of a deficit of the phagocitary function, the evaluation tests of the migration capacity through filtering layer indicated very low values in comparison with the clinically healthy animals; the optical density of the phagocytes of the healthy animals, engaged in the filtering system, was 2.4 higher than in the case of those animals suspected of immunodeficit.
- In the case of the evaluation through filtering layer, the migration distance recorded in the healthy animals was approximately 2.3 higher than in those suspected of immnodeficit; the unspecific migration maintained at values similar to both categories of animals, which proves that it was not the chemotactic factors which influenced the results, within the experiment.
- The endocytosis capacity tested by carbon particles as phagocyte element did not indicate significant differences between the two categories of animals; it is possible that this test be not probative with dogs.
- The phagocitary index determined by the evaluation of the intracellular digestion capacity of a reference bacteriological strain indicates values 20-30% higher in clinically healthy animals, in comparison with those suspected of immunodeficit syndrome.
- In case of suspecting the lazy leucocyte syndrome, the tests which evaluate the migration capacity of neutrofiles can represent correct and efficient methods to diagnose this immunodeficit; we suggest using in this sense the technical variants based on migration in filtering layer and under agarose layer.

Table 1.

Evaluation of the migration capacity through filtering layer

sampling	subject	Optical density		
		initial	after 2 of migrations	final
1	1	0.370	0.481	0.412
	2	0.374	0.522	0.443
	3	0.381	0.499	0.427
	mean	*0.375*	*0.500*	*0.427*
	4	0.369	0.902	0.407
	5	0.380	0.921	0.435
	mean	*0.374*	*0.912*	*0.421*
2	1	0.365	0.492	0.431
	2	0.388	0.502	0.440
	3	0.380	0.487	0.465
	mean	*0.378*	*0.494*	*0.445*
	4	0.367	0.922	0.421
	5	0.381	0.931	0.420
	mean	*0.374*	*0.927*	*0.421*
3	1	0.372	0.495	0.402
	2	0.384	0.564	0.415
	3	0.392	0.502	0.407
	mean	*0.383*	*0.520*	*0.408*
	4	0.388	0.956	0.434
	5	0.382	0.945	0.431
	mean	*0.385*	*0.951*	*0.432*

Table 2.

Evaluation of the migration capacity under agarose

sampling	subject	Distance migration (μm)		
		specific	unspecific	final
1	1	710	720	690
	2	670	690	700
	3	690	710	670
	mean	*690*	*707*	*687*
	4	1720	880	1790
	5	1810	820	1840
	mean	*1765*	*850*	*1815*
2	1	720	800	840
	2	690	740	740
	3	680	720	810
	mean	*697*	*753*	*797*
	4	1800	770	1920
	5	1790	910	1860
	mean	*1795*	*840*	*1890*
3	1	690	770	610
	2	740	710	800
	3	610	630	670
	mean	*680*	*703*	*693*
	4	1750	850	1910
	5	1790	870	1800
	mean	*1770*	*860*	*1855*

Table 3:

Evaluation of the ingestion capacity of carbon particles

sampling	subject	Optical density	
		initial	after endocytosis
1	1	0.632	0.512
	2	0.622	0.518
	3	0.650	0.532
	mean	*0.635*	*0.521*
	4	0.620	0.404
	5	0.618	0.396
	mean	*0.619*	*0.400*
2	1	0.598	0.488
	2	0.587	0.494
	3	0.602	0.517
	mean	*0.596*	*0.500*
	4	0.599	0.403
	5	0.605	0.387
	mean	*0.602*	*0.395*
3	1	0.622	0.508
	2	0.617	0.522
	3	0.605	0.515
	mean	*0.615*	*0.515*
	4	0.632	0.401
	5	0.615	0.394
	mean	*0.624*	*0.398*

Table 4.

Evaluation of the intracellular digestion capacity

sampling	subject	Phagocytosis index determined after	
		60 minutes	120 minutes
1	1	0.620	0.650
	2	0.680	0.680
	3	0.590	0.610
	mean	*0.630*	*0.637*
	4	0.710	0.840
	5	0.690	0.880
	mean	*0.700*	*0.860*
2	1	0.580	0.610
	2	0.600	0.630
	3	0.580	0.580
	mean	*0.587*	*0.607*
	4	0.730	0.910
	5	0.740	0.890
	mean	*0.735*	*0.900*
3	1	0.550	0.640
	2	0.590	0.620
	3	0.570	0.590
	mean	*0.570*	*0.617*
	4	0.750	0.880
	5	0.720	0.900
	mean	*0.735*	*0.890*

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