IMPAIRMENT OF NEUTROPHIL AND LYMPHOCYTE FUNCTIONS IN DOGS WITH UNCOMPLICATED AND PYODERMA COMPLICATED DEMODICOSIS

POŠKODENIE FUNKCIE NEUTROFILOV A LYMFOCYTOV U PSOV S NEKOMPLIKOVANOU DEMODIKÓZOU A DEMODIKÓZOU KOMPLIKOVANOU S PYODERMOU

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ABSTRACT: The objective of investigation was to determine the degree of phagocytic ability of neutrophils and lymphocyte blastogenesis in dogs with different duration of uncomplicated localized (LD) and generalized (GD) demodicosis, in comparison with the values in dogs with demodicosis complicated with pyoderma and the clinically normal dogs. At the time of analysis, the dogs received no medical therapy. Pyoderma was detected in the group of dogs which exhibited GD over five weeks from the appearance of clinical signs. Depression of phagocytic activity (of phagocytosis) and ingestion capacity (index of phagocytic activity) have been demonstrated in the dogs suffering from GD, but not in the LD dogs. Neutrophil dysfunction depended on the duration of clinical signs manifestation. Neutrophils from the dogs with pyoderma complicated GD exhibited significantly lower ingestion capacity than those from the dogs with uncomplicated GD. It was proposed that the presence of bacterial infection may be related to the neutrophil alteration initiated by demodicosis. Lymphocyte blastogenesis suppression to Con A accompanied not only uncomplicated GD, but also LD. This immunosuppression develops with the duration of clinical disease. Lymphocytes from the dogs with uncomplicated GD and pyoderma complicated GD responded to Con A equally, however their response was significantly lower in comparison with the control. We do not assume that the immunosuppression in the investigated dogs with complicated GD was due to the secondary bacterial infection. In conclusion, the dysfunction of both lymphocytes and neutrophils could predispose to the development of secondary pyoderma, a common sequela to generalized demodicosis.

dogs; demodicosis; neutrophils; lymphocytes; phagocytic activity; blastogenesis

ABSTRAKT: Predmetom štúdie bolo určiť úroveň fagocytárnej schopnosti neutrofilov a blastogenézy lymfocytov u psov s rozdielnym trváním nekomplikovanej lokalizovanej (LD) a generalizovanej demodikózy (GD) v porovnaní s hodnotami u psov s demodikózou komplikovanou pyodermou a klinicky zdravých psov. Počas doby sledovania psy neboli podrobené žiadnej terapii. Pyoderma bola zistená u skupiny psov s generalizovanou demodikózou trvajúcou viac ako päť týždňov od objavenia sa klinických príznakov. Zniženie fagocytárnej aktivity (percento fagocytózy) a ingesnej kapacity (index fagocytárnej aktivity) bolo preukázané u psov s GD, ale nie u psov s LD. Dysfunkcia neutrofilov bola zvýšená na trvání manifestácie klinických príznakov. Neutrofile psov s GD komplikovanou s pyodermy mali signifikantne nižšiu ingesnú kapacitu ako neutrofile psov s nekomplikovanou GD. Predpokladáme, že prítomnosť bakteriálnej infekcie je vo vzťahu k alterácii neutrofilev inicíiované demodikózou. Supresia fagocytárnej blastogenézy ku Con A doprevádzala nielen nekomplikovaný GD, ale aj LD. Táto inmunosupresia sa vyvíja so trváním klinického ochorenia. Lymfocity psov s nekomplikovanou GD a GD komplikovanou pyodermou reagovali na Con A podobne, avšak ich reakcia bola signifikantne nižšia v porovnaní s kontrolou. Nepredpokladáme, že inmunosupresia u vyšetrovaných psov s komplikovanou GD bola dôsledkom sekundárnej bakteriálnej infekcie. Záverom je možné konštatovať, že dysfunkcia lymfocytov a neutrofilov mohla byť predispozičným faktorom pre rozvoj sekundárnej pyodermie, obvyklého následku generalizovanej demodikózy.

psy; demodikóza; neutrofily; lymfocyty; fagocytárna aktivita; blastogenéza

INTRODUCTION

Demodicosis is a frequent skin disease of dogs (Sischo et al., 1989; Toman et al., 1996) associated with modulation of host’s immunity (Lemarie, 1996; Paulík et al., 1996a). The most frequently studied immune aspect of this disease is a suppression of cellular immunity, especially in view of lymphocytic reactivity to T-cell mitogens (Scott et al., 1974, 1976; Corbett et al., 1975, 1976; Healey and Gaafar, 1977; Barta et al., 1981; Barriga et al., 1992; Paulík et al., 1996b, c and others) and recently in view of suppression of interleukin-2 production (Lemarie and Horohov, cit. Lemarie, 1996).
There is only a limited number of reports dealing with the activity of non-specific immunity in demodicosis, even those available are in contradiction. While Scott (cit. Muller et al., 1989) and Toman et al. (1995) did not report any deficiency in the functional activity of neutrophils, others demonstrated suppression of spontaneous and chemotactic motility (Lotimer et al., 1983; Schmitt et al., 1994) and decrease in phagocytic activity (Schmitt et al., 1994) of these blood cells.

Pathogenesis of demodicosis has not yet been completely understood. Many questions have not yet been explained. They concern for example the always proclaimed (Muller et al., 1989; Georgi and Georgi, 1992; Giné, 1996), but not yet proved hereditary defect of T-cells; the principle (an effect of serum factor only) of immunosuppression (Hirsch et al., 1975; Kražič, 1987; Paulík et al., 1996b) and the immunopathogenesis of juvenile-onset or adult-onset of demodicosis (Scott et al., 1976; Wilkie et al., 1979; Krawiec and Gafnar, 1980; Duclos et al., 1994); and even the very cause (uncomplicated demodicosis or pyodermia associated demodicosis) of immunosuppression associated with clinical disease (Scott et al., 1976; Barta et al., 1983; Barriga et al., 1992; Paulík et al., 1996c; Toman et al., 1996).

This study was undertaken: 1) To assess the degree of phagocytic ability of blood neutrophils in demodicetic dogs exhibiting clinical signs of varying duration, taking into consideration blood lymphocyte reactivity to mitogen; 2) To assess an association between the suppression of lymphocyte blastogenesis and demodicosis without or with pyodermia taking into consideration the phagocytic ability of neutrophils.

MATERIAL AND METHODS

Animals and protocol

The observations were carried out on 12 healthy dogs (7 breeds) and 28 dogs with demodicosis. Three of the healthy dogs were males and nine were females. Average age of healthy dogs was 1.3 years (4 mo. to 4 yr. old). These dogs came from private owners and were examined together with sick dogs.

Fifteen dogs (5 breeds; 6 males and 9 females; of average age 1.1 years, 6 mo. to 2 yr. old) from the sick dogs were diagnosed clinically as having active localized demodicosis (LD; persisted for 1–10 weeks) and 13 (6 breeds; 8 males and 5 females; of average age 1.4 years, 5 mo. to 5.0 yr. old) as having active generalized demodicosis (GD; persisted for 4–16 weeks). The diagnosis was confirmed parasitologically. None of the dogs with LD and GD with the disease duration of less than 5 weeks showed clinical signs of pyogenic dermatitis at the time of investigation. From the GD dogs that had skin lesions for more than 5 weeks, superficial pyodermia was clinically and bacteriologically (Staphylococcus aureus) diagnosed in 4 dogs. The phagocytic ability of blood neutrophils and the blastogenesis of blood lymphocytes in demodicosis affected dogs were investigated (according to acceptance of sick dogs at our clinic for small animals) by the same procedures as in clinically healthy dogs. At the time of analysis, the dogs did not receive any pharmacotherapy. Animals were divided to groups and evaluated according to Tab. 1.

Examination

Blood samples were obtained from the v. cephalica and placed into a tube containing heparin (5 units/ml of blood).

Phagocytic ability of blood neutrophils. Evaluation was carried out as described by Větvička (1982), using the phagocytosis of 2-hydroxyethylmetacrylate copolymer particles (MSHP, diameter: 1.2 μm; ARTIM Prague). Fresh heparinized blood in volume of 0.1 ml was mixed with 0.05 ml of MSHP suspension. The mixture was incubated for 1 hour at 37 °C with occasional shaking. Phagocytic activity (PA) was expressed from the number of neutrophils (by differentiation of 200 leukocytes) as the percentage of cells able to phagocytize more than 3 MSHP. The index of phagocytic activity (IPA) was calculated as the ratio of the

<table>
<thead>
<tr>
<th>Groups of dogs</th>
<th>Time affected</th>
<th>Number of dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. both groups (2. + 3.)</td>
<td>1–10</td>
<td>5.6 ± 2.7</td>
</tr>
<tr>
<td>2. to 5 weeks</td>
<td>1–5</td>
<td>3.3 ± 1.5</td>
</tr>
<tr>
<td>3. over 5 weeks</td>
<td>6–10</td>
<td>7.6 ± 1.5</td>
</tr>
<tr>
<td>GD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. both groups (2. + 3.)</td>
<td>4–16</td>
<td>8.2 ± 3.9</td>
</tr>
<tr>
<td>2. to 5 weeks</td>
<td>4–5</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>3. over 5 weeks</td>
<td>6–16</td>
<td>10.5 ± 3.1</td>
</tr>
<tr>
<td>3a. (GD)</td>
<td>6–16</td>
<td>10.5 ± 4.4</td>
</tr>
<tr>
<td>3a. (GD + SP)</td>
<td>9–12</td>
<td>10.5 ± 1.7</td>
</tr>
</tbody>
</table>

LD = localized demodicosis
GD = generalized demodicosis
GD + SP = GD complicated with superficial pyodermia
number of phagocytized MSHP and the number of phagocytizing cells.

**Blastogenic response of blood lymphocytes to mitogen.** The lymphocytes were isolated using the Ficoll (Pharmacia Biotech AB, Sweden), according to the method described by Procházková (1979). The majority (> 95%) of isolated cells were defined as mononuclear cells. Viability of these cells exceeded 95%, as determined by trypan blue exclusion. The cultivation, mitogen stimulation and the measurement of response of lymphocytes to Con A by ethidium bromide (EB) fluorescence assay were investigated, as described in our previous paper (Paulik et al., 1996b). Briefly, separated cells were resuspended in culture medium (RPMI 1640 containing 15% autologous dog serum) to give 1 x 10⁶ cells per ml. Five hundred microliters of the lymphocyte suspension were cultured in the presence of 500 μl of the mitogen concanavalin A (25 μg/ml; Sigma Chemical Co., USA). As a control, 500 μl of the culture medium was added instead of the mitogen. The cultures were set up in triplicate and incubated at 37 °C and 5% CO₂ in humidified air for 96 hours. After incubation cell pellets of samples were solubilized with sodium decyl sulphate (Aldrich Chemie, Germany) solution and kept at room temperature for 30 minutes. Afterward, EB (Aldrich Chemie, Germany) solution was added. Mixtures without lymphocytes were used as a background of fluorescence intensity (FI). FI was measured by a spectrofluorometer (Jasco FP-550, Japan). Stimulation index was calculated as follows: (A-C)/(B-C); where A is mean FI with mitogen, B is mean FI without mitogen, C is background FI.

**Statistical analysis**

Data obtained were expressed as mean ± standard deviation and analysed by Student’s t-test. Only the P values equal to 0.05 or smaller than 0.05 were considered statistically significant.

**RESULTS**

The comparison of parameters of phagocytic ability of blood neutrophils and blood lymphocyte blastogenesis in investigated groups of dogs with demodicosis and the control group (C group) is shown in Tab. II. The percentage of phagocytic neutrophils was significantly lower only in the GD dogs (groups 2) when compared with C group. The comparison of groups (i.e. 1 and 2) between LD and GD dogs revealed a significant reduction in the percentage of phagocytic neutrophils in GD dogs in all the cases. A significantly higher number of neutrophils phagocytized in the LD and GD dogs that showed signs of clinical disease for less than 5 weeks from the appearance of the first skin lesions (i.e. group 1 in LD and GD) in comparison with the dogs manifesting clinical signs over 5 weeks (i.e. group 2 in LD and GD). The index of phagocytic activity was significantly decreased only in GD dogs (group 2) when compared with the C group. Significantly lower IPA values were found in the GD dogs of group 2 when compared with the same groups (i.e. group 2 in LD and GD). The index of phagocytic activity was significantly decreased only in the GD dogs of group 2.

A significant decrease in SI values was observed in all groups of LD and GD dogs when compared with those in the group C. Significantly lower blastogenesis of lymphocytes was observed in group 1 of GD dogs when comparing groups 1 and 2 between LD and GD dogs. No significant difference was observed between groups 2 of LD and GD dogs. In both LD and GD dogs, SI values were significantly higher in dogs with shorter duration of clinical disease (i.e. group 1).

When LD dogs of group 1 were divided into 2 subgroups (2A = disease lasting for 1-3 weeks, n = 4; 2B = disease lasting for 4-5 weeks, n = 3) regarding skin lesion duration, only SI value in dogs of subgroup 2B

| III. Phagocytic capacity of blood neutrophils and blastogenesis of blood lymphocytes in dogs with different duration of demodicosis |
|---|---|---|---|
| Groups of dogs | Parameters |   |   |
|               | PA (%) | IPA | SI |
| LD            |        |     |    |
| 1. to 5 weeks | 66.0 ± 8.2a,b | 7.0 ± 1.7 | 4.7 ± 1.6a,b |
| 2. over 5 weeks | 50.1 ± 12.0c | 6.2 ± 1.9a,b | 2.1 ± 0.8a,b |
| GD            |        |     |    |
| 1. to 5 weeks | 53.4 ± 7.3a,b | 6.0 ± 1.4a,b | 2.9 ± 0.5a,b,c |
| 2. over 5 weeks | 37.4 ± 11.6a,b,c | 3.1 ± 1.3a,b,c | 1.4 ± 0.4a,c |
| Control       | 59.2 ± 8.4 | 7.2 ± 2.8 | 7.8 ± 1.8 |

LD and GD = see Tab. I; PA (%) = phagocytic activity; IPA = index PA; SI = stimulation index

x = P < 0.001 (vs. control)

PA: a, b, c = P < 0.05, a, d = P < 0.025

IPA: b, c = P < 0.01, a = P < 0.005

SI: c = P < 0.05, b, c = P < 0.001 (the values designated by the same letter are significantly different)
was significantly lower in comparison with the group C (Fig. 1). In four dogs from group 3 (n = 8, Tab. I) of dogs with GD, dermodicosis was complicated with superficial pyoderma. When these dogs (group GD + SP, disease lasting on average 10.5 ± 1.7 weeks) were compared to four dogs with uncomplicated GD (group GD, disease lasting on average 10.5 ± 4.4 weeks) no significant difference was found in lymphocyte blastogenesis. However, lower IPA (P < 0.05) and PA (P > 0.05) values were observed. All parameters investigated in GD + SP and GD groups were significantly lower in comparison with the control dogs (Tab. III).

DISCUSSION

The results reported in this paper demonstrate the degree of phagocytic ability of blood neutrophils in the dogs with different duration of uncomplicated LD and GD with regard to lymphocyte blastogenesis, in comparison with those in the clinically healthy dogs and the dogs with demodicosis complicated with pyoderma.
blastogenesis, because blastogenesis was significantly suppressed also in other groups of dogs with uncomplicated GD and LD. Prolonged exposure of neutrophils to high concentrations of inflammatory mediators could result in generalized deactivation of all cellular functions due to receptor down-regulation (Donabedian and Gallis, 1981). In our study depression of phagocytic ability of neutrophils has been demonstrated in dogs suffering from GD, but not in LD dogs. Neutrophil dysfunction was a secondary phenomenon with respect to the disease process and it depended on the duration of clinical signs. Thus, the presence of staphylococcal infection may be related to the neutrophil alteration, which may be induced by mediators of the severe inflammatory process that accompanied longer durative GD.

Initial studies showed that GD is associated with serum substance (s) - induced T-cell suppression (Scott et al., 1974, 1976; Hirsh et al., 1975; Corbett et al., 1975 and others). It was demonstrated that the dogs with LD and early GD did not exhibit any T-cell suppression (Scott et al., 1976) and the relationship between the lymphocyte response to mitogens and the clinical state of GD was indicated (Scott et al., 1976; Krawiec and Gañár, 1980). Our results demonstrate that the lymphocyte blastogenesis suppression to Con A was associated not only with GD, but also with LD and that this immunosuppression developed with the duration of clinical disease in both forms of demodicosis. These findings confirm the results of recent reports in this respect (Barriga et al., 1992; Paulik et al., 1996b). Generalized demodicosis is often associated with secondary pyoderma. There is an indication that the lymphocyte blastogenesis suppression in demodicosis is due to the secondary bacterial infection and is absent in dogs with uncomplicated demodicosis (Barta et al., 1983; Toman et al., 1995). In contrast, in our study lymphocytes from dogs with uncomplicated and complicated GD responded to Con A equally but showed significantly depressed responses to mitogen as compared with the control dogs (Tab. III). In addition, lymphocytes from dogs with bacterial pyoderma had a normal response to Con A (Barriga et al., 1992; Toman et al., 1995), whereas the dogs with uncomplicated GD responded poorly (Barriga et al., 1992). Therefore, we cannot assume that the immunosuppression observed in the superficial pyoderma complicated GD dogs was due to the secondary bacterial infection. In conclusion, both lymphocyte blastogenesis suppression and depression of phagocytic activity of neutrophils could predispose to the development of secondary pyoderma, a common sequela to generalized demodicosis.

REFERENCES


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