QUANTITATIVE CHANGES IN SELECTED LYMPHOCYTE SUBPOPULATIONS AFTER ADMINISTRATION OF A SOLUBLE PARASITIC ANTIGEN OF BABESIA CANIS TO DOGS

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Abstract

Changes in selected blood lymphocyte subpopulations in dogs administered with a soluble parasitic antigen (SPA) derived from a supernatant of 18S RNA-A and 18S RNA-B Babesia canis cell culture were investigated. The studies included 20 dogs divided into three groups: group I (n=8) - comprised of dogs receiving SPA twice, at 3 week intervals; group II (n=5) - non-vaccinated control dogs, and group III (n=7) - dogs vaccinated twice with a commercial B. canis vaccine. Cytometric analysis revealed that vaccination with SPA derived from B. canis culture had similar effects to the vaccination with a commercial vaccine. The vaccination lowered the percentage of T lymphocytes (CD3+), T helper cells (CD4+), cytotoxic/supressor T cells (CD8+), B lymphocytes (CD21+), and MHC II lymphocytes in the blood in comparison to non-vaccinated dogs. Statistical analysis of the results demonstrated that mean values of the tested parameters at each stage of the study were similar in groups I and III and significantly higher in group II. The lowered level of the lymphocyte subpopulations in groups I and III persisted during the whole period of the study. The results presented that SPA has immunosuppressive effect in the first period after being administrated.

Key words: Babesia canis, soluble parasitic antigen, dogs, lymphocyte, flow cytometry.

Canine babesiosis is a common and clinically significant tick-borne disease caused by haematozoan parasites of the genus Babesia (1). The classification of Babesia sp. places them in the order Piroplasmida within the phylum Apicomplexa. Clinically these pathogens cause remittent fever, progressive anaemia, haemoglobinuria, and marked splenomegaly and hepatomegaly in dogs and, in some cases, death of infected animals (2, 27, 28).

Prevention of the disease is difficult due to the multitude of factors that affect its development (4). Studies on the use of soluble parasite antigens (SPA) from plasma of Babesia-infected animals or derived from in vitro cultures of Babesia organisms in immunoprophylaxis of babesiosis have been in progress for quite some time (5, 15, 16, 22-24). Early observations showed that vaccinations with SPA protected dogs against the development of a critical pathological process characterised by a decrease in packed cell volumes and retention of infected erythrocytes in the microvasculature (18).

Even within one species, Babesia protozoa displays a considerable genetic polymorphism (1, 9), which can be a factor limiting the development of effective immunoprophylaxis of babesiosis in dogs. Because the SPA of a single Babesia canis isolate induces protection against homologous challenge but not heterologous challenge infection (16, 22, 23), it seems logical that a vaccine that is to protect dogs against piroplasmosis in areas where this disease is endemic ought to be developed using parasites coming from these areas. Two commercial vaccines against canine babesiosis have been developed so far: Pirodog® (11) and NobivacPiro® (23). The latter mitigates the course of the disease induced by Babesia canis and Babesia rossi.

The objective of the study was to define changes occurring in selected blood lymphocyte subpopulations (T lymphocytes - CD3+, T helper cells - CD4+, cytotoxic/suppresor T cells - CD8+, B lymphocytes - CD21+, and MHC II lymphocytes) in dogs after administration of the SPA derived from a
supernatant of 18S RNA-A and 18S RNA-B Babesia canis cultures.

Material and Methods

Animals. The study included 20 mixed breed dogs (twelve males and eight females), aged 20-27 months, divided into three groups. Dogs from group 1 (n=8, No. 1-8) were treated subcutaneously, twice at a three-week intervals, with SPA. Dogs from group 2 (n=5, No. 9-13) were used as negative controls and they did not receive the antigen. Group 3 (n=7, No. 14-20) comprised of seven dogs used as positive controls, which received subcutaneously a commercial vaccine at the same time when the dogs from group 1 received SPA.

Preparation of SPA antigen. A process of determination and culture of Babesia canis strains 18S RNA-A and 18S RNA-B was presented in earlier study (4). The supernatant media from in vitro cultures were collected when parasitaemia reached the level of 0.6%, and then mixed. The supernatant media mixture was subjected to centrifugation at 700 g for 30 min to eliminate the red blood cells and possible parasites, as well as the remains thereof.

The centrifuged medium was subjected to sterilising filtration through 0.22 micron membrane and concentrated by ultra-filtration on a membrane of 20,000 Daltons. The concentration was treated with formol in a proportion of 0.12 mg/mL overnight at 4°C, and then lyophilised and divided into doses. Before administration to dogs the lyophilised antigen was dissolved in a solution of saponin at 0.5 mg/mL in apyrogenic sterile water (13).

Vaccination schedule. The antigen (1 ml) was administered subcutaneously in the subscapular region. The vaccination comprised two injections 3 weeks apart on days 0 and 21 of the study.

Determination of surface molecules CD3+, CD4+, CD8+, CD21+ and MHC II on lymphocytes. Blood samples (2 ml) from all dogs were collected into ethylenediaminetetraacetic acid (EDTA) tubes on days 0 (before first vaccination), 1, 3, 7, 14, 21 (before second vaccination), 22, and 28 of the experiment.

Fluorochrome-stained monoclonal antibodies CD8: RPE, CD21: RPE, CD3: FITC, CD4: FITC, and MHC II: FITC (Serotec Immunological Excellence, U.K.) directed against surface molecules on canine lymphocytes were used in the study. The staining was carried out in accordance with the procedure provided by the manufacturer. Tests were performed by means of flow cytometry (Flow cytometer Epics XL Beckman-Coulter, Comesa CH-Werfen Company USA), using one-step cell staining.

Statistical analysis. The results were statistically analysed using Student t-test. The analysed data were the mean values expressing the percentage of lymphocyte subpopulations in particular groups in consecutive days of the study.

Results

Percentage changes in selected lymphocyte subpopulations in blood. After administration of the SPA obtained in own experiments and the commercial vaccine, much lower percentages of CD 3+, CD4+, CD8+, CD21+, and MHC II lymphocytes were observed in the blood of all dogs from groups 1 and 3 in comparison with the control, non-vaccinated animals. Reduced percentage of each of the tested lymphocyte fractions in the whole leukocyte pool was observed at each stage of the study, from the moment of administering the antigen or the commercial preparation for the first time (Figs 1-5). The results of cytometric analysis were very similar in case of animals receiving the own antigen and the commercial antigen (Table 1), which may prove that the SPA has immunosuppressant effects in the first period after their administration. It should be noted that even though in group 2, the mean percentage of CD8+ and CD4+ lymphocytes was higher than in the two remaining groups, 21.5% and 43.6%, respectively (in group 1 it was 18.8% and 37.1%, respectively, and in control group - 19.3% and 39.4%, respectively), the CD4/CD8 ratio in all three groups was similar and was equal to 2. These observations show that the reduced percentage of lymphocytes in the leukocyte pool caused by SPA concerns both analysed T lymphocytes subpopulations. In the control group, comprising of animals that had not received the antigen, the percentage of the lymphocyte subpopulations in white blood cells was similar at each stage of the study and was within the normal limits.

Results of statistical analysis. Statistical analysis showed that administration of SPA to dogs had similar effects as administration of commercial vaccine. It lowered the percentage of CD 3+, CD4+, CD8+, CD21+, and MHC II lymphocytes in the blood of the vaccinated dogs in comparison with non-vaccinated animals. Results of the mean values of these parameters in groups 1 and 3 were very similar but no significant correlation was observed between the results obtained in groups 1 and 2, and 2 and 3 (Table 1).

Figs 1-5 show a graphic presentation of the results obtained. The graphs clearly demonstrate that the mean values of the studied parameters in particular groups at each stage of the study were similar in groups 1 and 3 and significantly higher in group 2.

Fig. 1. Changes in the percentage of CD3+ lymphocytes in groups 1, 2, and 3 on different days of the experiment.
Fig. 2. Changes in the percentage of CD8+ lymphocytes in groups 1, 2, and 3 on different days of the experiment

Fig. 3. Changes in the percentage of CD4+ lymphocytes in groups 1, 2, and 3 on different days of the experiment

Fig. 4. Changes in the percentage of CD21+ lymphocytes in groups 1, 2, and 3 on different days of the experiment

Fig. 5. Changes in the percentage of MHC II lymphocytes in groups 1, 2, and 3 on different days of the experiment

Table 1
Correlations between mean percentage of CD3+, CD4+, CD8+, CD21+, and MHC II lymphocytes in different groups of dogs

<table>
<thead>
<tr>
<th></th>
<th>Group 1 – Group 2</th>
<th>Group 2 – Group 3</th>
<th>Group 1 – Group 3</th>
</tr>
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<tbody>
<tr>
<td>CD3</td>
<td>-0.458 (0.302)</td>
<td>-0.536 (0.215)</td>
<td>0.981 (&lt;0.0005)</td>
</tr>
<tr>
<td>CD8</td>
<td>-0.185 (0.692)</td>
<td>-0.458 (0.301)</td>
<td>0.911 (0.004)</td>
</tr>
<tr>
<td>CD4</td>
<td>-0.809 (0.027)</td>
<td>-0.871 (0.011)</td>
<td>0.988 (&lt;0.0005)</td>
</tr>
<tr>
<td>CD21</td>
<td>-0.392 (0.384)</td>
<td>-0.517 (0.234)</td>
<td>0.936 (0.002)</td>
</tr>
<tr>
<td>MHC II</td>
<td>-0.612 (0.144)</td>
<td>-0.676 (0.096)</td>
<td>0.968 (&lt;0.0005)</td>
</tr>
</tbody>
</table>

Discussion

It is assumed that SPA are toxic substances released in the host's body by Babesia protozoa, responsible for clinical signs of the disease. SPA dilates blood vessels and leads to hypotension and blood clots in vessels. Such disorders predispose parasite-infected erythrocytes to deposition on vessel endothelium. This antigen can indirectly increase the adhesiveness and agglutination tendency of red blood cells. The clinical picture of babesiosis largely depends on the systems and organs where SPA-induced pathological reactions take place (19, 22, 23). In dogs vaccinated with SPA, the presence of antibodies specific to this antigen was detected (15). Experimental studies indicated that their highest titres appeared approximately on the 5th or 6th d after the experimental infection of the immunised animals and their level depended on the dose of antigen used for vaccination (17, 21). Initially, a drop in haematocrit and swellings of the lymph nodes and spleen were observed in the dogs that had been vaccinated and then infected with virulent protozoa. As antibodies specific to SPA appeared in the serum of the studied animals, the number of erythrocytes increased, the swellings of lymph nodes and spleen receded, and the blood flow through blood vessels improved (18). These results clearly demonstrate that the SPA antibodies, formed as a consequence of antigen...
stimulation, prevent the development or exacerbaton of the pathological processes presented above. Vaccination with SPA also stops the chain of disorders leading to a circulatory shock (21). Numerous clinical observations confirm the efficacy of SPA in the prevention of piroplasmosis in animals. This antigen was first used to prevent piroplasmosis by Sibenovic et al. (25). Studies on its application in the prevention of canine babesiosis have been carried out for years (18, 22, 23). So far, the way of inducing immunity against babesiosis by this antigen has not been studied in dogs in details. The literature provides no data on the impact of administering SPA to dogs on changes in the percentage composition of blood cells. The majority of works on animal vaccination with this antigen focused on assessing the efficacy of this method in disease prevention, ignoring other aspects connected with its application (18, 20, 22, 23). Assessment of the efficacy of the SPA obtained in the experiments undertaken in the prevention of canine babesiosis will be the subject of a separate article. At the moment, the attention should be paid to the changes, which SPA application may induce in the lymphocyte level in blood. This may be another step towards a better understanding of the mechanism of developing immunity against piroplasmosis.

It seems to be interesting that administration of Babesia canis SPA obtained in own experiments, which was derived from cell culture, as well as the commercial vaccine did not lead to any increase in the percentage of CD3+, CD4+, CD8+, CD21+, and MHC II lymphocytes in the blood of the dogs used in the experiment. Similar observations were noticed by Garcia et al. (12), who showed that in cattle infected with Babesia bigemina, in contrast to in vitro conditions (7, 8), no differences were observed in the levels of CD4+ and CD8+ lymphocytes in comparison with the healthy animals.

Effective control of babesiosis depends on innate and acquired immunity with a well-balanced cytokine production (6). Lack of an increase in the number of lymphocytes in the total pool of leukocytes is not a definitive criterion for assessing the efficacy of the antigen in babesiosis prevention. The results obtained in the group of animals vaccinated with own antigen proved to be almost identical to the results obtained in the group of animals receiving the commercial vaccine, effective in babesiosis prevention. A similar drop in the percentage of particular lymphocyte subpopulations was observed after the vaccination of dogs with the commercial preparation against leishmaniasis (10) and this phenomenon indicates only that some vaccines can have immunosuppressant effects in the first period following vaccination (14, 26).

References


