Changes in the Proportion and Number of Monocytes in the Peripheral Blood of Calves Infected with *Theileria sergenti*

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Monocyte/macrophage play an essential role in host defenses especially in the development of cellular immunity. However, the distribution pattern of this cell population has not been evaluated sufficiently in bovine theileriosis in Japan, caused by *Theileria sergenti*. Although the importance of cellular immunity in *T. sergenti* infection has been discussed [7], the quantitative changes in the monocyte/macrophage population have not been analyzed so far. In this report we describes that the percentage and number of monocytes in peripheral blood increased significantly in the calves after infected with *T. sergenti*.

Five Holstein calves, three to five months old and splenectomised approximately one month before the infection, were used. The Ikeda stock of *T. sergenti* maintained in our laboratory was used to infect these calves [1]. *T. sergenti* was transmitted by subcutaneous injection of a tick-derived sporozoite suspension [2]. Serial blood samples were taken from two calves (Nos. 48 and 50) at intervals of 7 to 10 days to calculate the numbers of erythrocyte and leucocyte, and to estimate parasitemia. Other samples were taken from three calves when the parasitemia progressed remarkably (6.3–27.8%). Blood samples obtained from seven Holstein calves, three to five months old, approximately one month after splenectomy and being negative for the parasite by Giemsa-stained blood smear examination, were used as uninfected control. Blood samples were each taken from the jugular vein into heparinized tubes in the morning. For separating the mononuclear cells (MNC), blood was diluted 1:2 into 10 mM Hepes buffered Hanks balanced salt solution at pH 7.0 (HBSS) and layered on a Ficoll-sodium diatrizoate gradient (Histopaque-1077, GS:1.077, Sigma Chemical Co. St Louis, Mo., U.S.A.). This blood preparation was centrifuged for 60 min at 400 X g, and the cells in the interface were collected and washed 3 times with HBSS and once with RPMI 1640 medium containing bovine fetal serum (15%) and Hepes buffer (10 mM). Smears were prepared by centrifuging the cells for 10 min at 800 rpm in a Shandon Cytocentrifuge (Shandon Southern Instruments Inc., England). At this stage, more than 98% of the cells were verified as MNC by the microscopy of Giemsa-stained cytocentrifuged cell smears. Alpha naphthyl acetate esterase (ANAEE staining was used for the monocyte estimation by the method of Kullenkampf et al. with slight modifications [4, 6]. Cytocentrifuged preparations of cells were fixed in cold buffered formalin-acetone (pH 6.6). Hexactoised para罗斯inile was prepared by mixing 1.2 ml of 4% para罗斯inile in 2.5 M hydrochloric acid with an equal volume of 4% sodium nitrate. Freshly prepared hexactoised para罗斯inile was added to 40 ml of 0.067 M phosphate buffer (pH 5.0) containing 0.4 ml acetone and 10 mg alpha naphthyl acetate, and pH was adjusted to 5.8 by the addition of 2N sodium hydroxide. The slides were then incubated for 3 hrs at 37°C, washed in distilled water and counterstained with 1% toluidine blue. A total of at least 100 cells each was counted three times, and the cells with a diffuse reddish brown cytoplasm color were scored as monocytes. Leucocytes were counted by a Coulter Counter (Model JR; Coulter Electric Co., U.S.A.) and the rates of MNC were estimated by differential counting of at least 300 leucocytes on Giemsa-stained blood smears. The numbers of MNC and monocytes were calculated based on the percentages of MNC in leucocytes, the percentages of monocytes in MNC and the total number of leucocytes. The data obtained in this study was analyzed by Student’s t test.

Table 1 shows the changes in the percentage and number of monocytes together with those in parasitemia, and in the numbers of erythrocyte, leucocyte and MNC with respect to days after infection in the calf No. 48. Table 2 shows the changes in the same parameters as in Table 1 in the calf No. 50. The course of infection and the changes in percentage and number of monocytes were essentially the same in both calves. In the course of infection, the percentage and number of monocytes increased with the progress of parasitemia and anemia. The highest values of the percentage and number of monocytes were recorded on days 27 and 37 when parasitemia and anemia progressed remarkably in calf Nos. 48 and 50 respectively.

In order to reconfirm the results, the percentage and number of monocytes of seven normal and another three experimentally infected calves were evaluated by ANAE staining. As shown in Fig. 1A, the percentage of monocytes in calves (N=5) infected with *T. sergenti* was 21.5±3.0% compared with 6.9±1.0% of the same cells in uninfected control calves (N=7) (P<0.001); that is to say, there was an increase of 14.6% in percentage of monocytes among MNC populations in *T. sergenti* infected calves. A significant difference (P<0.01) in the absolute number of monocytes between the two groups is also shown in Fig. 1B.

Nagahata et al. reported that the optimal density of medium for separating MNC from bovine peripheral
Table 1. Changes in parasitaemia, erythrocyte number, leucocyte number, MNC number and the percentage and number of monocytes of a calf (No. 48) experimentally infected with T. sergenti

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>Parasitaemia(%)</th>
<th>Erythrocyte (x10^6 µl⁻¹)</th>
<th>Leucocyte (µl⁻¹)</th>
<th>MNC (µl⁻¹)</th>
<th>Monocyte (µl⁻¹) (%)³</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>—</td>
<td>845</td>
<td>4599</td>
<td>3564</td>
<td>232</td>
</tr>
<tr>
<td>8</td>
<td>2.8</td>
<td>1014</td>
<td>6001</td>
<td>3001</td>
<td>171</td>
</tr>
<tr>
<td>15</td>
<td>28.8</td>
<td>1002</td>
<td>8597</td>
<td>4823</td>
<td>342</td>
</tr>
<tr>
<td>27</td>
<td>10.7</td>
<td>331</td>
<td>8364</td>
<td>7469</td>
<td>1143</td>
</tr>
<tr>
<td>37</td>
<td>10.7</td>
<td>333</td>
<td>10785</td>
<td>6720</td>
<td>814</td>
</tr>
</tbody>
</table>

a) Percentage of ANAE positive cells among MNC. Results were expressed as mean of three determinations.

Table 2. Changes in parasitaemia, erythrocyte number, leucocyte number, MNC number and the percentage and number of monocytes of a calf (No. 50) experimentally infected with T. sergenti

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>Parasitaemia(%)</th>
<th>Erythrocyte (x10^6 µl⁻¹)</th>
<th>Leucocyte (µl⁻¹)</th>
<th>MNC (µl⁻¹)</th>
<th>Monocyte (µl⁻¹) (%)⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>—</td>
<td>897</td>
<td>5469</td>
<td>3872</td>
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<tr>
<td>8</td>
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<td>7241</td>
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<tr>
<td>15</td>
<td>25.1</td>
<td>1036</td>
<td>7541</td>
<td>4962</td>
<td>496</td>
</tr>
<tr>
<td>27</td>
<td>17.4</td>
<td>612</td>
<td>11447</td>
<td>8059</td>
<td>1088</td>
</tr>
<tr>
<td>37</td>
<td>17.4</td>
<td>353</td>
<td>14430</td>
<td>11688</td>
<td>1344</td>
</tr>
</tbody>
</table>

a) Percentage of ANAE positive cells among MNC. Results were expressed as mean of three determinations.

Fig. 1. The proportion (A) and number (B) of alpha naphthyl acetate esterase (ANAE) positive cells among peripheral blood of infected (N=5) and uninfected (N=7) calves with Theileria sergenti. The proportion of ANAE positive cells (A) is expressed as the percentage among peripheral blood mononuclear cells (MNC). Bars represent the standard error (SE) of the mean.

blood was 1.087 [5]. In our study, the percentages of monocytes obtained in uninfected control calves, however, consisted with that in their sequential report [6]. It was, therefore, considered that the specific density of 1.077 of separating medium used in this study did not affect the results.

Ishimine et al. reported that the enhancement of phagocytic and killing activity of monocytes might be crucial elements in the host defense system against Babesia gibsoni in dogs [3]. Sato et al. also reported that the production of oxidative products such as hydrogen peroxide and superoxide anion, which is the microbicidal system in monocytes, increased remarkably in the course of T. sergenti and B. ovata infection in cattle [7]. It is not clear whether the increase in percentage and number of monocytes observed in this study contributes to the functional activation of this cell population. However, it might be suggested from these results that monocyte/macrophage play an essential role in host defense system against bovine piroplasmosis.

The present study showed that there was a statistically significant increase in the percentage and number of monocytes among MNC populations in T. sergenti infected calves. However, it has not yet clarified how the monocyte contributes to the host defense system in T. sergenti infection. Further studies should be carried out to elucidate the cooperative mechanism of monocytes with the immunity in T. sergenti infection.

REFERENCES