Theileria sergenti infection in the Bo-RBC-SCID mouse model


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Abstract. We have previously developed the Bo-RBC-SCID mouse model for Theileria sergenti infection. In the present study, this model was further examined to delineate the mode of parasite infection. The Bo-RBC-SCID mice were prepared by periodically transfusing uninfected bovine erythrocytes (Bo-RBCs) into splenectomized SCID mice via the intraperitoneal (i.p.) route. The mice, separated into three groups, were inoculated i.p., intravenously (i.v.), or subcutaneously (s.c.) with T. sergenti-infected Bo-RBCs. Examination of samples of peripheral blood demonstrated that the parasite infected mice inoculated via any one of the three routes. The mice inoculated i.v., however, developed parasite-mia earlier than those inoculated i.p. or s.c. When Bo-RBC-SCID mice prepared without splenectomy were infected with T. sergenti, a high-level parasitemia appeared only once. After that, not only the level of parasitemia but also the number of Bo-RBCs in the peripheral blood rapidly decreased despite the continuation of Bo-RBC transfusions. The results suggest that T. sergenti proliferates primarily in the circulating blood in Bo-RBC-SCID mice and that in response to the parasites growth, the spleen may play an important role in the removal of both parasitized and unparasitized Bo-RBCs from the blood circulation.

Materials and methods

Bovine erythrocytes

An 8-month-old female calf free of Theileria sergenti infection was used as a donor of Bo-RBCs. The calf was born by cesarean section...
and was reared in a separate room in complete isolation from other cattle. The blood samples drawn from the calf were checked for the absence of *T. sergenti* and were used for transfusion into SCID mice within 1 week.

A 12-month-old female calf was splenectomized and then infected with the Chitose stock (Takahashi 1976) of *T. sergenti*. The blood samples obtained from the calf, showing parasitemia of between 4% and 10%, were used to initiate *T. sergenti* infection in Bo-RBC-SCID mice and to prepare erythrocyte-free merozoites. Prior to their inoculation into SCID mice, *T. sergenti*-infected and uninfected Bo-RBCs were washed five times with 0.85% NaCl and the buffy coats were removed.

**Merozoite isolation**

In all, 30 ml blood was collected from the *T. sergenti*-infected calf described above when it showed parasitemia of 15%. The blood cells were washed three times in 0.85% NaCl and the buffy coats were removed. The blood sample was passed through a cellulose column (CF-11, Whatman Biosystems Ltd.) to remove white blood cells and any clotting material. The *T. sergenti* merozoites were released from erythrocytes with *Aeromonas hydrophila* hemolysin and were separated from erythrocyte ghosts by the modified method of Sugimoto et al. (1991). The merozoites were suspended at 3 x 10⁸/ml in phosphate-buffered saline and were inoculated into Bo-RBC-SCID mice.

**SCID mice**

A total of 22 SCID mice (C.B-17 scid/scid, Japan CLEA, Tokyo) 7 weeks of age were used. The mice were housed in a vinyl-film isolator and were provided with γ-ray-sterilized pelleted diet and autoclaved tap water. In all, 11 splenectomized animals and 5 un-splenectomized mice were intraperitoneally (i.p.) transfused with a 1-ml packed cell volume of uninfected Bo-RBCs every 5–7 days. After the third transfusion, the mice were inoculated intravenously (i.v.), i.p., or subcutaneously (s.c.) with *T. sergenti*-infected Bo-RBCs or with erythrocyte-free merozoites as shown in Table 1. As controls, three splenectomized animals and three unsplenectomized mice were periodically transfused with uninfected Bo-RBC but were left uninfected.

Samples of peripheral blood were collected from the tails of the mice every 3–5 days. The percentages of Bo-RBCs and of parasitized erythrocytes were determined by an immunofluorescence antibody technique and by Giemsa staining, respectively, according to the method described elsewhere (Tsuji et al. 1992). All animals were treated according to Laboratory Animal Control Guidelines at Rakuno Gakuen University, which are basically in conformity with the American Association of Laboratory Animal Control Guidelines established by the National Institutes of Health.

**Results**

Successful *Theileria sergenti* infections were induced in the splenectomized Bo-RBC-SCID mice by the inoculation of parasites via any one of the i.p., i.v., and s.c. routes (Figs. 1–3). When the mice were inoculated i.p. with 1 x 10⁸ parasitized Bo-RBCs, the level of parasitemia increased to 1% on days 15–22 and exceeded 10% between days 24 and 30 (Fig. 1). Around day 30, the parasitemia reached a peak level of approximately 40%, whereas the percentage of Bo-RBCs in the SCID mice's peripheral blood cells concomitantly decreased below 30% despite the additional Bo-RBC supply. This Bo-RBC shortage resulted in a temporary decrease in the parasitemia level, but high, albeit greatly fluctuating, levels of parasitemia, again emerged on the continuation of Bo-RBC transfusions.

The Bo-RBC-SCID mice inoculated i.v. developed a high-level parasitemia earlier than those inoculated i.p. Following the i.v. inoculation, the level of parasitemia increased to 1% as early as on day 7, exceeded 10% between days 15 and 17, and reached 20% on days 18–20 (Fig. 2). The early development of parasitemia in the mice inoculated i.v. indicates that *T. sergenti* proliferates primarily in the blood circulation rather than in the peritoneal cavity. To confirm this conclusion, we conducted an identical experiment using three other Bo-RBC-SCID mice. They were inoculated i.v. with *T. sergenti*-infected Bo-RBCs and were transfused with uninfected Bo-RBCs at 7-day intervals. On day 17 postinfection, the mice to which the last Bo-RBC transfusion had been given 3 days before were killed for determination of the numbers of parasitized Bo-RBCs in the peripheral blood and in the peritoneal cavity. The percentage of parasitized Bo-RBCs in the peritoneal cavity was significantly smaller than that in the blood sample (Table 2), supporting the conclusion described above.

The Bo-RBC-SCID mice could also be infected via the s.c. route. However, the lag period before the development of parasitemia varied significantly, as parasitized erythrocytes in the peripheral blood became detectable on days 7, 9, and 27 in the three mice inoculated s.c. (Fig. 3).

Free merozoites isolated from the parasitized erythrocytes were capable of infecting Bo-RBC-SCID mice. Detectable parasitemia, however, appeared at as late as 88 days after the i.v. inoculation (Fig. 4). The parasitemia level increased slowly and reached only 0.16% and 1.87% on day 95. Throughout the experimental period, the percentage of Bo-RBCs in the peripheral blood of those mice could be maintained at over 70% by weekly Bo-RBC transfusions, but the mice seemed to be exhausted and unfortunately died on day 95.

For an exploration of the role of the spleen in *T. sergenti* infection, SCID mice prepared without splene-

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**Table 1. Experimental procedures**

<table>
<thead>
<tr>
<th>Routes of infection</th>
<th><em>Theileria sergenti</em>²</th>
<th>Doses (I.E.)</th>
<th>SCID mice</th>
<th>Treatment</th>
</tr>
</thead>
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<td>Splenectomy</td>
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<tr>
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<td>Splenectomy</td>
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<td>_²</td>
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<td>Splenectomy</td>
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¹ I.E., *T. sergenti*-infected erythrocytes; Merozoites, erythrocyte-free merozoites
² Intraperitoneal transfusion of *T. sergenti*-free bovine erythrocytes