Immune Responses to *Babesia rodhaini* in Rats

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In *Babesia rodhaini* infection in rats parasitemia remained up to 3 weeks after clinical recovery. The immune responses were demonstrated at least up to one year after recovery.—**Key words: Babesia rodhaini**, Immune response, Rat.

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In the previous paper [3], we reported that mice may be well suited for acute experimental infections because of their extreme susceptibility, while rats were suitable for long-term experiments with *Babesia rodhaini* because of their stable responses to the superinfections regardless of their individualities.

The present study was undertaken to investigate the status of rats clinically recovered from *B. rodhaini* infections. For this purpose, isolation of the organisms, splenectomy and superinfection with the same strain on recovered rats were carried out.

**MATERIALS AND METHODS**

The Australian strain of *B. rodhaini* maintained in mice by serial passages was used in this study, as described previously [3].

Parasitized erythrocytes (PE) were obtained from these mice and were inoculated intraperitoneally into the rats (5-6 weeks old, female, SD strain).

Specimens for assessing parasitemia were prepared daily from tail vein blood of the experimental animals and stained with Giemsa's solution. Hemoglobinuria, being one of the typical clinical signs, was also observed daily. The percentage of PE was determined by the method described previously [3].

All rats clinically recovered from *B. rodhaini* infections after inoculation with $8 \times 10^8$ PE per head (see results) were divided into 5 groups consisting of 8 rats each, and observations were made in the following periods of time after the recovery: Group A. 1–7 days; Group B. 14–21 days; Group C. 30–37 days; Group D. 60–67 days; Group E. 120 days.

**Isolation of organisms:** From two rats in each group, either 0.3 ml of blood or 0.5 ml of 1 : 10 emulsions prepared from their spleen, liver, bone marrow or brain were inoculated intraperitoneally into 5 groups consisting of 6 mice each (5-6 weeks old, female, dd strain).

**Splenectomy:** Splenectomy was performed under ether anesthesia on three rats in each group (groups A, B, C, D and E as mentioned above) by the routine technique.

**Superinfection:** Three rats in each group were superinfected with $8 \times 10^8$ PE per head, respectively.

**RESULTS**

**Recovered rats:** Parasitemia was detected on the 1st day and hemoglobinuria on the 4-6th day after inoculation in all rats. The maximum PE rate, approximately 30% (ranging from 28.3 to 34.5%), was reached on the 5-7th day after inoculation. However, all
These rats recovered on the 10-14th day after inoculation. No parasitemia reappeared, as reported previously [4, 5]. Therefore rats used in the present work were considered to be recovered animals (Table 1).

Isolation of organisms: Four of six mice inoculated with the blood taken from group A rats, showed parasitemia on the 6-7th day after inoculation. These mice also demonstrated hemoglobinuria and died on the 13-14th day after inoculation. However, two mice survived without showing any clinical signs. On the other hand, none of mice inoculated with each of the organ emulsions of group A rats showed parasitemia or hemoglobinuria.

Of six mice inoculated with blood of group B rats, two showed parasitemia on the 8-9th day after inoculation. These mice died on the 12-14th day after inoculation. The other four mice in the same group survived without showing any clinical signs. On the other hand, none of mice inoculated with each of the organ emulsions of group B rats showed parasitemia or hemoglobinuria.

None of the mice inoculated with blood or organ emulsions of groups C, D and E rats died, or showed parasitemia or hemoglobinuria (Table 2).

Splenectomy: As shown in Table 3, parasitemia was detected on the 5th day after splenectomy in two of three rats in group A. The maximum PE rates of these 2 rats were 23.2 and 26.7%, respectively, and those maximum rates were detected on the 12th and 13th day after splenectomy. Though hemoglobinuria occurred in these rats, both of them survived. In the 3rd rat, detection of parasitemia was retarded until the 16th day after splenectomy and the peak parasitemia (8.8%) was reached on the 25th day after splenectomy. This rat showed no hemoglobinuria and survived. The maximum PE rates of all three rats mentioned above were lower than those caused by the primary infection.

Parasitemia was detected on the 11th day after splenectomy in one of the three rats in group B. This rat did not show hemoglobinuria and survived. The maximum PE rate, 4%,

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats examined</th>
<th>Parasitemia</th>
<th>Hemoglobinuria</th>
<th>Mortality</th>
<th>Recovery day after primary infection</th>
<th>Mean maximum PE(^{b}) rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8</td>
<td>8/8</td>
<td>8/8</td>
<td>0/8</td>
<td>12–14</td>
<td>30.4</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>8/8</td>
<td>8/8</td>
<td>0/8</td>
<td>10–13</td>
<td>29.7</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>8/8</td>
<td>8/8</td>
<td>0/8</td>
<td>11–12</td>
<td>28.3</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>8/8</td>
<td>8/8</td>
<td>0/8</td>
<td>12–13</td>
<td>29.5</td>
</tr>
<tr>
<td>E</td>
<td>8</td>
<td>8/8</td>
<td>8/8</td>
<td>0/8</td>
<td>12–14</td>
<td>34.5</td>
</tr>
</tbody>
</table>

a) Parasitized erythrocytes.

<table>
<thead>
<tr>
<th>Group (Days after recovery)</th>
<th>A (0–7)</th>
<th>B (14–21)</th>
<th>C (30–37)</th>
<th>D (60–67)</th>
<th>E (120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood(^{b})</td>
<td>4/6</td>
<td>2/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Spleen(^{b})</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Liver(^{b})</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Bone marrow(^{b})</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Brain(^{b})</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
</tbody>
</table>

a) Inoculation with 0.3 mL per head.
b) Inoculation with 0.5 mL per head.
was reached on the 21th day after splenectomy. The other two rats survived without showing any clinical signs.

None of rats of groups C, D and E died, or showed parasitemia or hemoglobinuria.

Superinfection: As shown in Table 4, none of each group died, or showed parasitemia. However, when 3 recovered rats were superinfected at one year after recovery, they all showed slight parasitemia. The numbers of their PE were only 1-2 in 10 microscopical fields examined on the 3rd and 4th day after inoculation, and parasitemia could be demonstrated only for 2 days thereafter.

DISCUSSION

The present paper deals with the status and the immune responses of latent infections after recovery from B. rodhaini infection in rats. For this purpose, isolation of organisms, splenectomy and superinfection with the same strain of organisms on recovered rats were carried out.

In order to isolate parasites from recovered rats, mice were used as receiver animals in this work, because of their extremely high susceptibilities to B. rodhaini infection as described previously [3, 5].

As shown in Table 2, when mice were inoculated with blood of recovered rats, parasitemia and hemoglobinuria were detected in group A (4/6) and group B (2/6), followed by death, but no such effects were observed in groups C, D and E. On the other hand, when mice were inoculated with emulsions of each organ of recovered rats, none of them showed parasitemia, hemoglobinuria or death.

As shown in Table 3, when recovered rats were splenectomized, parasitemia and hemoglobinuria reappeared only in group A (3/3) and group B (1/3). However, these clinical signs were not detected at all in other groups after splenectomy.

The results of these experiments indicate that the parasites in recovered rats are latently persist in the blood up to 3 weeks after clinical recovery.

All the recovered rats of groups A, B, C, D and E could survive superinfection without
showing clinical signs. However, 3 rats superinfected at one year after recovery showed slight parasitemia with a few PE for only 2 days. But in these rats no hemoglobinuria, being one of the most remarkable clinical signs, was observed during the course of the experiment.

These results suggest that the rats infected with *B. rodhaini* may eliminate the parasite completely as early as 3 weeks after recovery but retained the immunity for one year or more. Hussein [6] reported a similar phenomenon in mice infected with *B. microti*.

Ishihara [7] and Soulsby [11] reported that latent infection of *Babesia* species in cattle can persist for a considerable period and parasites usually may be demonstrable by the injection of blood into splenectomized animals. Some workers [1, 2, 10] also reported that resistance to superinfection with *Babesia* species is dependent on a state of premunition in which a few numbers of parasites persist in the blood or tissues after recovery. However, premunition can easily be broken down by splenectomy [8, 9].

In this experiment, we could not demonstrate the state of premunition in rats. This could possibly be due to the strain of parasite used. The Australian strain has been maintained by subsequent passages in mice, and not transmitted by ticks, for a long time. Therefore, it may be considered that some biological changes have occurred in the nature of parasites.

Besides, it may be speculated that resistance to superinfection with *B. rodhaini* in rats is dependent on a state of sterile immunity in which parasites are eliminated from the organs after clinical recovery.

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REFERENCES

要約

_Babesia rodhaini_ 感染耐過ラットの免疫獲得について：細 相彥（日本獣医畜産大学寄生虫学教室）
——_B. rodhaini_ 感染耐過ラットについて，マウスへの虫体回収実験，摘脾の影響，攻撃感染に対する反応を検討した結果，次の成績が得られた。（1）血液・脾・肝・骨髄・脳の乳剤をマウスに接種したところ，耐過後3週間は虫血症が潜在的に持続していた。 （2）耐過後3週間までは摘脾により再発病することがなかった。（3）耐過後少なくとも1年間はラットは寄生マウス赤血球 8×10⁸ の攻撃接種に耐えることが示された。