Suppressed Antibody Response to Sheep Erythrocytes in Experimentally Babesia rodhaini-Infected Mice
Kozo ADACHI, Hiroshi KAWANO, and Susumu MAKIMURA
Department of Veterinary Internal Medicine, Faculty of Agriculture, Miyazaki University, Miyazaki 889-21, Japan
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ABSTRACT. Mice experimentally infected with Babesia rodhaini were immunized with sheep red blood cells (SRBC), and the splenic plaque forming cell (PFC) response to SRBC was investigated. Suppressed splenic antibody response to SRBC accompanied with the elevated parasitemia was recognized. This evidence suggests that an immunosuppression in B. rodhaini infection may be involved in the multiplication of parasites contributing to high mortality of mice infected with B. rodhaini.—KEY WORDS: B. rodhaini infection, mouse, suppressed PFC response.


It is of interest that haemoprotezoan infections induce immune response suppression and enhancement. Focusing on the splenic B lymphocyte response, suppressed antibody response in malaria has been well documented using the plaque forming cell (PFC) assay [5, 7–10], the suppression having been reported to be most pronounced at the peak of parasitemia. In contrast, Yvonne [11] reported the elevated PFC response to sheep erythrocytes (SRBC) in mice during Plasmodium berghei infection. Shimizu et al. [6] have reported that Theileria sergenti lysate antigen has adjuvant effect on antibody production to SRBC in mice. The present study was designed to investigate the relationship between the level of parasitemia and the PFC response to SRBC in Babesia rodhaini-infected mice.

Twelve ICR-SPF female mice, 7 weeks of age, purchased from Kyudo Co., Ltd. (Fukuoka, Japan), were infected intraperitoneally (i.p.) with 10⁶ erythrocytes parasitized with the Australian strain of B. rodhaini (courtesy provided by National Institute of Animal Health, Tsukuba, Japan). The mice, immunized i.p. with 0.2 ml of a 10% suspension of SRBC in 0.01 M phosphate-buffered saline, pH 7.4 at day 0 (n=6) or 2 (n=6) post inoculation, were killed 4 days after the immunization. Six uninfected control mice were also immunized i.p. with a 10% SRBC suspension 4 days before PFC assay. Their splenic cells were suspended in Eagle's MEM (pH 7.4) and assayed for PFC by the method of Cunningham and Szenberg [2]. Lymphocyte count and parasitemia were examined with Wright's-Giemsa-stained blood smears. Gammaglobulin content was measured by the electrophoretic method.

As shown in Fig. 1, the number of anti-SRBC PFC per 10⁶ spleen cells significantly reduced on days 4 and 6 after infection. It may be caused by marked splenomegaly without any change in the number of anti-SRBC PFC per spleen. There was an inverse correlation (p<0.01) between the level of parasitemia and the number of anti-SRBC PFC per 10⁶ spleen cells and spleen. On day 6, the number of anti-SRBC PFC per spleen prominently decreased along with elevated parasitemia and the reduced number of lymphocytes (Table 1). Tanabe et al. [7] reported that depression of the PFC (per spleen) response in the later stage of P. berghei infection is ascribed to decrease in B cells or B and T cells. In the present study, as shown in Table 1, reduction in lymphocyte count was observed on day 4 when the number of anti-SRBC PFC per spleen remained unchanged, indicating that depressed lymphocyte function rather than the

Fig. 1. Time courses of parasitemia (●, %) and splenic plaque cell response (Δ; per 10⁶ spleen cells, ▲; per spleen) to sheep erythrocytes in mice experimentally infected with B. rodhaini. Each point represents mean ± S.D. P values were calculated by the student's t test. Significantly different from controls (pre-infection); *p<0.05, **p<0.01. Number of mice with (at day 4 and 6) and without (pre-infection) B. rodhaini infection is 6, respectively. PI represents pre-infection.

Table 1. Lymphocyte count of mice infected with B. rodhaini

<table>
<thead>
<tr>
<th>Days post infection</th>
<th>Lymphocyte count (×10⁶/mm³)</th>
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</thead>
<tbody>
<tr>
<td>pre-infection</td>
<td>2061±151**</td>
</tr>
<tr>
<td>4</td>
<td>3318±71**</td>
</tr>
</tbody>
</table>

Data represent mean±S.D. **: Significantly different from controls (pre-infection); p<0.01.

Table 2. γ-globulin content of mice infected with B. rodhaini

<table>
<thead>
<tr>
<th>Days post infection</th>
<th>γ-globulin content (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-infection</td>
<td>0.20±0.05</td>
</tr>
<tr>
<td>4</td>
<td>0.22±0.08</td>
</tr>
<tr>
<td>6</td>
<td>0.09±0.03*</td>
</tr>
</tbody>
</table>

Data represent mean±S.D. *: Significantly different from controls (pre-infection); p<0.05.
reduced count may account for the reduction in the number of anti-SRBC PFC per spleen. Tanabe et al. [7] also reported that the suppression of the PFC response (per spleen) to SRBC, thymus-dependent antigens, was observed at the early stage of P. berghei infection (days 0 to 3), and that the PFC response to polyvinylpyrrolidone, a thymus-independent antigen, was not depressed until the later stage of infection. Their results show that the depression of T cell function precedes B cell function impairment in P. berghei infection. In the present experiment, although on day 4 statistically significant reduction in the number of anti-SRBC PFC per $10^6$ spleen cells was observed ($P<0.05$), the number of anti-SRBC PFC per spleen slightly increased rather than reduced (no statistical significance ($\rightarrow$, $P>0.05$). This result indicates that neither T cell nor B cell function is suppressed until the later stage of B. rodhaini infection, this being reflected by the extremely low parasitemia on day 4 after infection. The prominent reduction in the PFC response per spleen to SRBC, thymus-dependent antigens, demonstrated the depression of T or B cell function. Immunosuppression of monocyte activity in malaria [1, 4] and theileriosis [3] was also reported. Immunosuppression in the later stage of B. rodhaini infection may account for an immediate and prominent increase in parasitemia contributing to high mortality of mice infected with B. rodhaini. Unlike the results of Yvonne [11], anti-SRBC PFC response was not elevated in B. rodhaini infection. Furthermore, as shown in Table 2, marked hypergammaglobulinaemia characteristic of protozoan infections was not observed in B. rodhaini infection. On the contrary gammaglobulin content reduced significantly at day 6 ($P<0.05$). This result indicates the suppressed splenic B lymphocyte response in B. rodhaini infection.

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REFERENCES