# Effects of Depletion of T Cell Subpopulations on the Course of Infection and Anti-Parasite Delayed Type Hypersensitivity Response in Mice Infected with *Babesia microti* and *Babesia rodhaini*

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ABSTRACT. To elucidate the role of T cell subpopulations in the protective cell-mediated immune response at the initial phase of infection with *Babesia microti* (BM) and *B. rodhaini* (BR), the changes in the course of infection and anti-parasite delayed type hypersensitivity (DTH) response after BM or BR inoculation were investigated in Lyt-2$^+$ T cell- or L3T4$^+$ T cell-depleted mice. Depletion of Lyt-2$^+$ T cells strongly enhanced the resistance to BM infection, whereas it increased the susceptibility to BR infection. In contrast, depletion of L3T4$^+$ T cells increased susceptibility to BM infection, while it enhanced resistance to BR infection. The anti-parasite DTH response in BM-infected mice was significantly enhanced by depletion of Lyt-2$^+$ T cells, while significantly reduced by depletion of L3T4$^+$ T cells. No effects of depletion of either Lyt-2$^+$ or L3T4$^+$ T cells on DTH response was observed in BR-infected mice. From these results, it was suggested that the roles of Lyt-2$^+$ and L3T4$^+$ T cells in the protective cell-mediated immune response at the initial phase of infection were different between BM- and BR-infected mice, resulting in the difference in their course of infection. — KEY WORDS: *Babesia microti*, *Babesia rodhaini*, mouse, T cell subpopulation.

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Intraerythrocytic protozoa, *Babesia microti* (BM) and *Babesia rodhaini* (BR) are the causative agents of murine babesiosis. It is well documented that these two species cause different course of infection in mice [1, 2, 5, 28]. Briefly, BM infection is chronic and non-lethal, whereas BR infection is acute and lethal. Many investigators reported that splenic cells played definitive roles in the protective immunity to BM and BR infection, since splenectomized mice were found to be more susceptible to both BM and BR infection compared with intact mice [3, 4, 13, 15, 26].

In addition, the enhancement of anti-parasite DTH response that characterized cell-mediated immune response was suggested to be closely associated with resistance to both BM and BR infection [17, 18, 22]. Ruebush and Hanson [16] reported that splenic T cells largely contributed to the protective mechanism in BM infection from the observations that anti-T cell serum abrogated the protective activity of the immunized splenic cells, as had been reported in BR infection [29].

Our previous study [23] demonstrated that the splenic L3T4 positive (L3T4$^+$) T cells/Lyt-2 positive (Lyt-2$^+$) T cells ratio in BM-infected mice increased from day 3 to 6 after inoculation (ai), whereas the ratio in BR-infected mice decreased during this period. From these results, the difference between the course of infection with BM and BR was suggested to be correlated with the increase of suppressive activity of splenic Lyt-2$^+$ T cell at the initial phase of infection. Ruebush *et al.* [18] reported that splenic L3T4$^+$ T cells enhanced cell-mediated immunity at day 6 ai in BM infection, but not with BR infection. On the other hand, splenic L3T4$^+$ T cells inhibited cell-mediated immune response at the initial phase of *Plasmodium chabaudi* AS [25] and *Leishmania major* infections [10, 11, 21]. Therefore, to elucidate the mechanism of difference between the course of infection with BM and BR, it was necessary to examine the role of T cell subpopulations, Lyt-2$^+$ and L3T4$^+$ T cells, in the protective cell-mediated immune response at the initial phase of infection (0 to 6 days ai) with these two parasites.

In this study, the course of infection and DTH response were comparatively examined in Lyt-2$^+$ or L3T4$^+$ T cell-depleted mice infected with BM or BR.

## MATERIALS AND METHODS

*Mice*: Male BALB/c mice aged 7 weeks were supplied from Nippon SLC Inc. (Shizuoka, Japan).

*Parasites*: Munich strain of *Babesia microti* and Australian strain of *B. rodhaini* were maintained in our laboratory by serial passages of parasitized blood to BALB/c mice.

*Inoculation*: Mice were inoculated by peritoneal injection with $1 \times 10^4$ parasitized erythrocytes (PE) in 0.2 m*l* of physiological saline per head.

*Depletion of Lyt-2$^+$ and L3T4$^+$ T cells in vivo*: Monoclonal antibodies (mAb) of rat anti-mouse Lyt-2 and L3T4, and rat IgG as a control antibody were obtained from BIOSYS S.A. (France) and Cappel Inc. (Westchester Penna., U.S.A.), respectively. The Lyt-2$^+$ T cell- and L3T4$^+$ T cell-depleted mice (referred to as D-Lyt-2 and D-L3T4, respectively) were prepared by intraperitoneal administration of anti-mouse Lyt-2 and anti-mouse L3T4 mAb (500 $\mu$g/head at 5 days, 250 $\mu$g/head at 4 days and 1 day before PE

Table 1. Cell numbers of splenic T cell subpopulations in T cell subpopulation-depleted mice at day 6 after *B. microti* and *B. rodhaini* inoculation

| Treated mice | Cell number ( $\times 10^7$, mean ± SD, n=3) | |
|---|---|---|
| | L3T4$^+$ T cell | Lyt-2$^+$ T cell |
| *B. microti*-inoculated mice | | |
| Intact | 4.11 ± 0.31 | 0.91 ± 0.12 |
| Rat IgG | 3.98 ± 0.65 | 0.87 ± 0.12 |
| D-Lyt-2 | 2.75 ± 0.59 | 0.15 ± 0.07* |
| D-L3T4 | 0.16 ± 0.01* | 0.30 ± 0.01 |
| *B. rodhaini*-inoculated mice | | |
| Intact | 4.96 ± 0.55 | 2.62 ± 0.33 |
| Rat IgG | 5.03 ± 0.59 | 2.87 ± 0.27 |
| D-Lyt-2 | 3.85 ± 0.42 | 0.37 ± 0.09* |
| D-L3T4 | 0.28 ± 0.17* | 1.37 ± 0.34 |

Intact: Intact mice, Rat IgG: rat IgG-injected mice, D-Lyt-2:anti-Lyt-2 mAb-injected mice, D-L3T4: anti-L3T4 mAb-injected mice.
*: Significant difference compared with intact mice ($p<0.001$).

inoculation for each antibody). Intact mice and the mice administrated with rat IgG in the same way (Rat IgG) served for controls. Each of control and mAb administrated groups consisted of 6 mice. The preliminary study by flow cytometric analysis demonstrated that the number of splenic Lyt-2$^+$ T cells in D-Lyt-2 and that of splenic L3T4$^+$ T cells in D-L3T4 were significantly reduced at day 6 after BM or BR inoculation as compared to those in controls (Table 1).

*Parasitemia and packed cell volume (PCV)*: Parasitemia was determined by Giemza-stained blood smears at an interval of 3 days ai and PCV at an interval of 6 days ai. In BR inoculated mice, if any mice died, parasitemia and PCV were also determined on the day of their death.

*Babesia lysate antigen (BLA)*: The blood with parasitemia exceeding 80% was collected by cardiac puncture in heparinized syringe and washed 3 times with physiological saline by centrifugation (400 g, 8 min, 4°C). The PE pellet was freezed and thawed with liquid nitrogen for 3 times. The lysate was finally centrifuged at 144,000 g for 30 min at 4°C. The supernatant served for BLA in anti-parasite DTH response analysis.

*Anti-parasite DTH response*: Since the degree of anti-parasite DTH response at day 6 ai were shown to be closely related to the resistance of mice against BM and BR infection [18, 22], all mice were injected intracutaneously with 50 $\mu l$ of BLA (containing about 2 $\mu g$ of protein) in the right footpad and equal volume of physiological saline in the left footpad at day 6 ai. The thickness of their footpads were measured before and 24 hr after injection. The swelling rate was determined by the following formula: Footpad swelling rate (%) = (thickness of right footpad 24 hr after injection — thickness of right footpad before injection) × 100 / thickness of right footpad before injection.

## RESULTS

*Parasitemia and PCV*: In BM inoculated-D-Lyt-2 mice (BM D-Lyt-2), parasitemia at day 15 ai was significantly lower than that in BM inoculated-intact and -Rat IgG mice (BM controls) ($p<0.01$). The PCV at day 15 ai in BM D-Lyt-2 was significantly higher than that in BM controls ($p<0.01$). In contrast, BR inoculated-D-Lyt-2 mice (BR D-Lyt-2) showed higher parasitemia than those of BR inoculated controls (BR controls) at day 9 ai. At day 10 ai, PCV in these mice revealed significantly lower than those of BR controls ($p<0.05$). All of the BR D-Lyt-2 died at day 10 ai, 2 days earlier than BR controls (Fig. 1).

On the other hand, BM inoculated-D-L3T4 mice (BM D-L3T4) showed significantly higher parasitemia than that of BM controls at day 9 ai ($p<0.05$). This tendency persisted until day 21 ai, when already no parasitemia was detected in BM controls. The PCV was significantly lower than that in controls ($p<0.05$) at day 21 ai. Contrarily, BR inoculated D-L3T4 mice (BR D-L3T4) showed significantly lower parasitemia and higher PCV at day 10 ai compared to those in BR controls (Fig. 2).

*DTH response*: In our previous study [22], the footpad swelling rates at day 6 after BR inoculation in resistant mice, which were produced by transferring the BR-immunized splenic cells, was about 30%, whereas about 10% in the susceptible mice. In this study, the footpad swelling rates of BM intact and Rat IgG mice were 30.2 ± 5.2% and 29.4 ± 4.4%, respectively. BM D-Lyt-2 showed significantly higher (46.4 ± 9.6%), while BM D-L3T4 showed significantly lower footpad swelling rate (15.4 ± 6.4%) than BM controls ($p<0.05$, $p<0.01$, respectively). On the other hand, the rates of BR intact and Rat IgG mice were 14.6 ± 5.2% and 13.7 ± 7.2%, respectively. No significant difference was observed either between BR control and BR D-Lyt-2 (14.8 ± 6.4%), or between BR control and BR D-L3T4 (14.3 ± 8.0%) (Fig. 3).

## DISCUSSION

The decrease of the number of each T cell subpopulation at day 6 ai was considered to be caused by the administration of the mAbs. However, administration of each mAb also reduced the number of uncorresponding T cell subpopulation. This reduction may be due to the imbalance of cytokine network caused by the depletion of counterpart subpopulation, since cytokines produced by Lyt-2$^+$ and L3T4$^+$ T cells have been reported to regulate the activation and proliferation of each other population [3, 6, 12, 14].

Our previous study on BM and BR suggested that the increase of splenic Lyt-2$^+$ T cell number at the initial phase of infection enhanced the susceptibility against BR infection, and thus resulting in the difference in the course of infection with BM and BR. The present study demonstrated that the depletion of Lyt-2$^+$ T cells strongly enhanced the resistance of mice against BM infection, whereas it increased the susceptibility to BR infection. Moreover, depletion of Lyt-

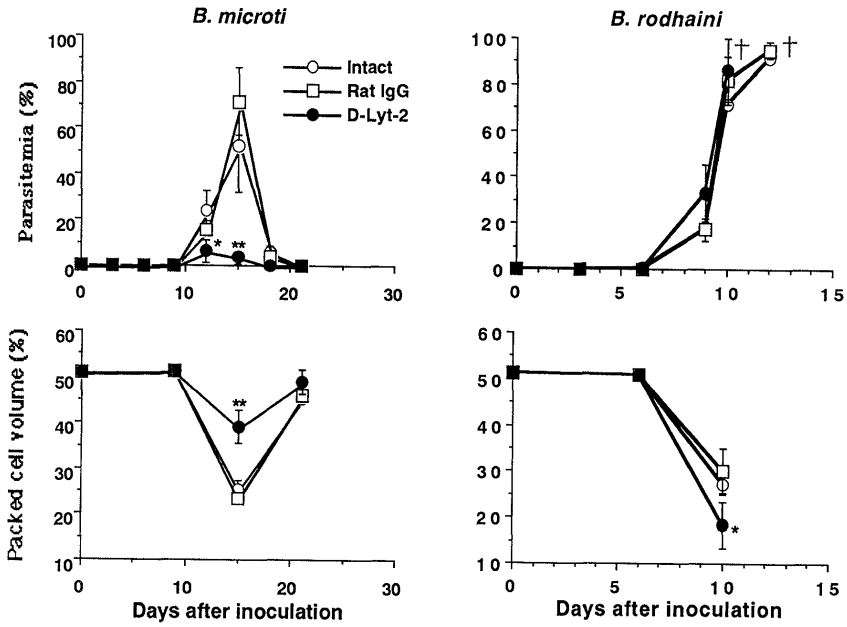


Fig. 1. Changes of parasitemia and packed cell volume after *B. microti* and *B. rodhaini* inoculation in Lyt-2 positive cell-depleted mice. ○—○: intact mice (Intact), □—□: Rat IgG-infected mice (Rat IgG), ●—●: Lyt-2 positive cell-depleted mice (D-Lyt-2). * and **: significant difference between intact and Lyt-2 positive cell-depleted mice. (*: $p<0.05$, **: $p<0.01$)

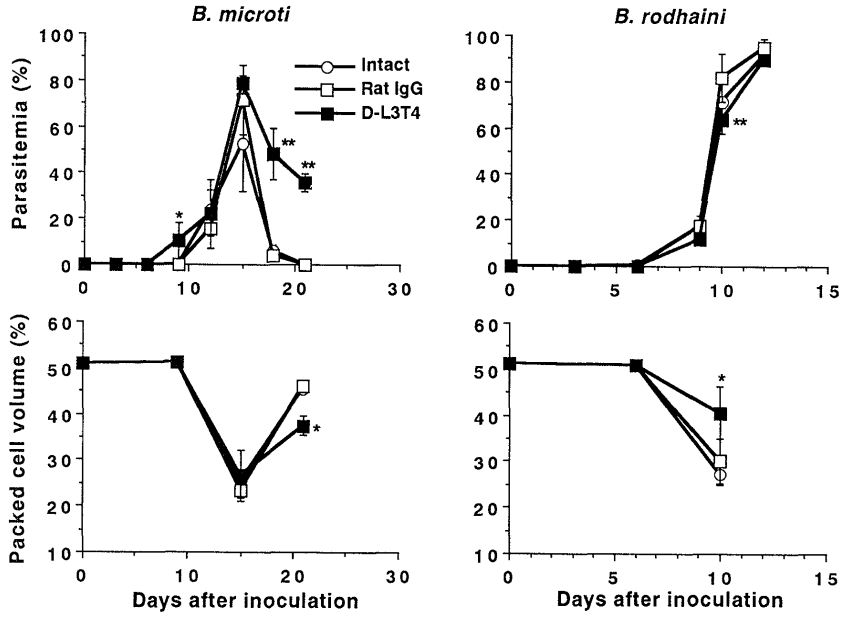


Fig. 2. Changes of parasitemia and packed cell volume after *B. microti* and *B. rodhaini* inoculation in L3T4 positive cell-deleted mice. ○—○: intact mice (Intact), □—□: Rat IgG-injected mice (Rat IgG), ■—■: L3T4 positive cell-depleted mice (D-L3T4). * and **: Significant difference between intact and L3T4 positive cell-depleted mice. (*: $p<0.05$, **: $p<0.01$)

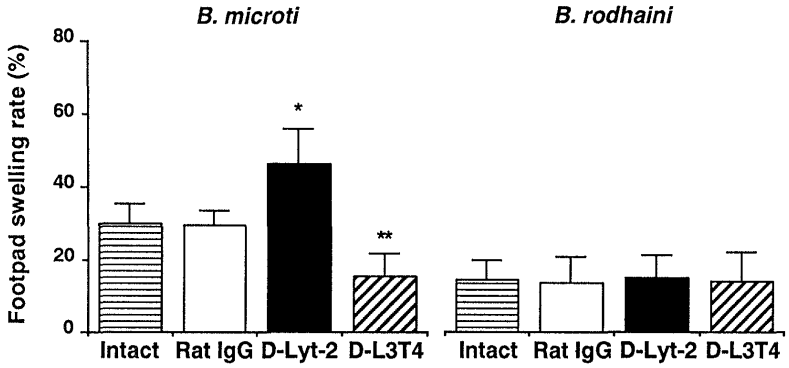


Fig. 3. Delayed-type hypersensitivity response to homologous parasite antigens in T cell subpopulation-depleted mice inoculated with *B. microti* or *B. rodhaini*. Intact: intact mice, Rat IgG: rat IgG-injected mice, D-Lyt-2: Lyt-2 positive-T cell depleted mice, D-L3T4: L3T4 positive T cell-depleted mice. * and **: significant difference between intact and T cell subpopulation-depleted mice. (*: $p<0.05$, **: $p<0.01$)

$2^+$ T cell significantly enhanced anti-parasite DTH response in BM-infected mice, while it gave no effect on that response in BR-infected mice. Recently, the inhibitive function of Lyt-$2^+$ T cells have been discussed in the cell-mediated immune response to various infectious pathogens [3, 5, 27]. Stevenson and Tam [24] demonstrated that CD8 (Lyt-2)$^+$ T cells suppressed cell-mediated immunity at the initial phase of *Plasmodium chabaudi* AS infection. The Lyt-$2^+$ T cells obtained by skin biopsy from patients with leprosy also inhibited cell-mediated immune response against *Mycobacterium Leprae* and exacerbated the disease [19]. Therefore, Lyt-$2^+$ T cells in BM-infected mice were considered to inhibit the anti-parasite cell-mediated immune response at the initial phase of infection, and to reduce the resistance against BM infection.

On the other hand, in BR-infected mice, the Lyt-$2^+$ T cells may be associated with the protective immune response at the initial phase of infection and give resistance to BR infection, for example by producing gamma-interferon ($\gamma$-IFN) [3, 6], the cytokine required for the control of peak parasitemia in *Plasmodium chabaudi* AS-infected mice [24]. However, further investigations are necessary to clarify the role of Lyt-$2^+$ T cells in the protective immune response to BR infection.

It has been reported that depletion of L3T4$^+$ T cells attenuated the cell-mediated immune response and reduced the resistance against several parasite infections including BM [7, 9, 18, 21]. The depletion of the these cells, however, was also reported to enhance the cell-mediated immune response and increase the resistance against *Leishmania major* infection [10, 11]. In our study, depletion of L3T4$^+$ T cells increased the susceptibility to BM infection, while it reduced the susceptibility to BR infection. Furthermore, depletion of L3T4$^+$ T cells significantly reduced anti-parasite DTH response in BM-infected mice, while it gave no effect in BR-infected mice. Therefore, it was suggested that L3T4$^+$ T cells enhanced the anti-parasite cell-mediated immune response at the initial phase of BM infection and, on the other hand, the lack of this enhancement in BR infection was associated with its fatal outcome.

It is well documented that mouse L3T4$^+$ T cells can be divided into two subsets, helper T cell type-1 (Th1 cell) and type-2 (Th2 cell), on the basis of their cytokine production and regulation of immune responses [8, 12, 14, 20, 25]. Briefly, Th1 cells produce $\gamma$-IFN and interleukin (IL-2), and enhance cell-mediated immune response, whereas Th2 cells produce IL-4, IL-5 and IL-6, and regulate the humoral one. Many investigators reported the development of Th1 cell at the initial phase of infection with *Leishmania major* in naturally resistant mice, while Th2 cell developed in susceptible mice [10, 11, 21]. The similar results were reported in *Plasmodium chabaudi* AS-infected mice [24]. These reports, together with our data, supports the idea that L3T4$^+$ T cells developing at the initial phase of BM and BR infection are Th1 like and Th2 like cells, respectively. Further studies are required to determine the type of developing helper T cells in BM- and BR-infected mice.

In conclusion, it was suggested that the roles of Lyt-$2^+$ and L3T4$^+$ T cells on the cell-mediated immune response at the initial phase of infection were different between BM- and BR-infected mice, resulting in the difference in their course of infection.

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