Protective Immune Response of *Isospora felis*-Infected Mice against *Babesia microti* Infection

Masato TAKAHASHI, Yoshitaka OMATA*, Hiroshi OIKAWA**, Florencia CLAVERIA, Ikuo IGARASHI†, Atushi SAITO, and Naoyoshi SUZUKI†

Department of Veterinary Physiology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080, †Research Center for Protozoan Molecular Immunology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080, and **Aburahi Laboratories, Shionogi Research Laboratories, Shionogi & Co., Ltd., Kohka-cho, Shiga 520–34, Japan

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**ABSTRACT.** Protective response against *Babesia microti* was studied in *Isospora felis*-infected mice. *Isospora felis*-infected mice which were exposed to *B. microti* on the 28th day post-infection showed absolute resistance against *Babesia microti*. Interestingly, these mice showed no anti-*B. microti* antibodies. Mice that received spleen cells from *I. felis*-infected donors that were subsequently exposed to *B. microti* showed lower peak parasitemia (10.3% ± 2.6) compared to those mice that received normal spleen cells (60.9% ± 15.0), and no spleen cells at all (47.3% ± 8.5). Treatment of *I. felis*-infected mice with monoclonal antibodies against L3T4+ cells resulted in a depression of their resistance to *B. microti*, as clearly manifested by high levels of parasitemia. Findings of the present study demonstrate the role of cell mediated immunity, specifically by L3T4+ T-cells induced by the *I. felis* infection, in providing mice protection against *B. microti*.—**KEY WORDS:** Babesia microti, immune response, *Isospora felis*, mouse, natural resistance.

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*Isospora felis*, a feline coccidian parasite, is commonly found in feces of domestic and stray cats. In mammals, this parasite can penetrate and survive in extra-intestinal organs [5]. One of the possible modes of transmission of *I. felis* in cats is the ingestion of infected rodents, as intermediate hosts. Experimental studies of *I. felis* infection in rodents are wanting, probably because of the absence of pathogenicity in the intermediate hosts, and due to the work’s low economic value. Nevertheless, the investigation of immune responses to *I. felis* infection in intermediate hosts is necessary to gain additional knowledge of its transmission mechanism, and to attract epidemiologic interest.

*Coxiella burnetii*, *Nematospiroides dubius* (*N. dubius*) and other species have been shown to induce non-specific immunity to *Babesia microti* infection in mice [2, 6]. Although, the precise defence mechanisms are still unknown, natural killer cells and/or macrophages are believed to interfere with the parasite development within host erythrocytes [6–7].

In the present study, we examined the immune response of *B. microti* infection in mice experimentally inoculated with *I. felis* oocysts. Specifically, we sought to determine whether *I. felis* infection in mice can stimulate the host immune system to induce protective immune reactions against *B. microti*.

**MATERIALS AND METHODS**

Oocysts of *I. felis* were obtained from cats’ feces using ZnSO₄ flotation method, followed by sucrose density gradient centrifugation [1], and stored at 4°C in phosphate buffered saline (PBS). Absence of other coccidian oocysts was microscopically confirmed. Some oocysts were hatched by incubation in PBS supplemented with 0.5% taurhochoic acid and 0.25% trypsin at 37°C for 30 min.

*Babesia microti* parasitized red blood cells (*B. microti* PRBC) obtained from Dr. O. A. Heydon, Berlin Free University, were prepared from infected mice (0.6–0.8 ml blood with a hematocrit of 20–35% and a parasitemia of 50–70%) on the 7th–10th day post infection (p.i.) by heart puncture in heparinized PBS at 4°C, then were washed with PBS three times by centrifugation at 1,200 g for 10 min at 4°C.

Male and female BALB/c and ICR mice, 5–7 weeks of age were used in all experiments. Each group consisted of 4 mice which were infected perorally with 5 × 10⁵ oocysts of *I. felis*. On the 7th and 28th day p.i., they were challenged intraperitoneally with 1 × 10⁵ *B. microti* PRBC. Thereafter, survival of exposed mice was monitored. Percent parasitemia per mouse was examined at one day interval beginning on the 3rd and ending on the 21st day p.i. Level of parasitemia was determined by counting the number of PRBC per 400 cells in tail-blood thin smears stained with Giemsa.

Anti-*I. felis* and anti-*B. microti* IgG titers in sera were measured by indirect immunofluorescence antibody test (IFAT). Test sera were obtained weekly from each mouse and stored at −20°C until use. Serum samples were diluted four-fold in PBS and mounted on glass slides coated with *B. microti* PRBC, and incubated at 37°C for 30 min, washed in PBS for 10 min, then reacted with FITC-conjugated anti-mouse IgG diluted in PBS, at 37°C for 30 min. Anti-*I. felis* IgG antibody titers in the same sera samples were performed following similar procedure.

Spleen cells from either *I. felis* non-inoculated or inoculated BALB/c mice were suspended in RPMI 1640 medium at a concentration of 5 × 10⁶/ml. Four BALB/c
mice were intravenously injected $10^6$ spleen cells of I. felis infected mice, and another four recipient mice received a similar inoculum of spleen cell suspension from I. felis non-infected donors. For the control group, four mice were not injected spleen cells. These three groups of mice were inoculated with $10^6$ B. microti PRBC on the same day of spleen cell transfer. Therefore, the level of parasitemia in each mouse was checked. To establish the absence of I. felis in the spleen cell suspension, two C57Bl/6 mice were also inoculated with the same inoculum dose of spleen cells. Negative anti-I. felis antibody titers were noted in these mice on the 30th day post-spleen cells transfer.

Anti-mouse L3T4 monoclonal antibody (mAb) (rat hybridoma GK-1, 5) and anti-mouse Lyt 2, 2 mAb (rat hybridoma 53-6,72) were purified using 33% ammonium sulfate precipitation method. Each of the I. felis-infected mice on the 28th day p.i. were depleted of T-cell subset through inoculation with 0.5 mg/0.25 ml of anti-L3T4 mAb or anti-Lyt2,2 mAb intraperitoneally for three successive days prior to challenge with $10^6$ B. microti PRBC. Treatment with mAb was continued for the next 27 days post-challenge with B. microti at two days interval. Depletion of L3T4 or Lyt2,2 positive (L3T4$^+$, Lyt2,2$^+$) cells in mouse spleen was monitored by fluorocytometric analysis using FITC-conjugated mAbs.

RESULTS

As shown in Fig. 1, B. microti-infected mice which were inoculated with I. felis on the 7th day p.i. showed a peak parasitemia of 29.8% $\pm$ 6.48 on the 11th day B. microti p.i. The control group, likewise, registered a peak parasitemia of 42.5% $\pm$ 0.7 on the same day. BALB/c mice inoculated with I. felis and then exposed to B. microti on the 7th day p.i. also showed lower parasitemia compared to that of the control (Fig. 1b). Interestingly, I. felis-inoculated mice which were exposed to B. microti PRBC on the 28th day I. felis p.i. registered absolute resistance to the establishment of B. microti infection (Fig. 1c). With ICR mice, we noted less than 1.0% and 60.0% parasitemia in the experimental and control groups, respectively (Fig. 1d). Also, ICR mice showed anti-I. felis antibody IgG titer of 4$^3$. Anti-B. microti antibody IgG was detected on the 7th day post-inoculation with B. microti.

Figure 2 shows mice that received spleen cells from I. felis-infected mice had lower peak parasitemia (10.3% $\pm$

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**Fig. 1.** Mean parasitemia ± SD of B. microti infected mice. ■: B. microti exposed only. Bar: standard deviation per group samples. 1a. □: BALB/c mice infected with B. microti and inoculated with I. felis 7 days post-infection. 1b. □: BALB/c mice inoculated with I. felis and then exposed to B. microti 7 days post-inoculation. 1c. □: BALB/c mice inoculated with I. felis and exposed with B. microti 28 days post-inoculation. 1d. □: ICR mice inoculated with I. felis and exposed with B. microti at 28 days post inoculation. IFA titer of anti-B. microti IgG (○) and anti-I. felis IgG (△) in I. felis-infected mice; anti-B. microti IgG (●) and anti-I. felis IgG (▲) in control.
2.6) on the 11th day *B. microti* p.i., compared to those that received normal spleen cells (60.9% ± 15.0) and to those mice that received no spleen cells at all (47.3% ± 8.5).

The effects of *in vivo* treatment with anti-L3T4 mAb or anti-Lyt 2,2 mAb on the resistance pattern of *I. felis*-infected mice against *B. microti* are shown in Figure 3. The treatment of *I. felis*-infected mice with anti-L3T4 mAb (Fig. 3a) resulted in a depressed resistance against *B. microti*, as evidenced by severe parasitemia. Two of the four mice in this group had about 85% parasitemia on the 13th day post-challenged and was sustained at 50% parasitemia until the end of the experiment. One mouse also showed high parasitemia at about 70%, on the 23rd day post-challenged. In comparison, *I. felis*-infected mice inoculated with anti-Lyt 2,2 mAb (Fig. 3b), with normal rat IgG (Fig. 3c), or without-inoculation (Fig. 3d) either had significantly low parasitemia or no parasitemia until the end of the observation period. Using fluorocytometric analysis, the treatment of mice with either anti-L3T4 mAb or anti-Lyt 2,2 mAb resulted in more than 90% of the restricted depletion of the corresponding population (data not shown).

**DISCUSSION**

Despite the numerous findings of the existence of natural resistance of hosts to babesiosis *microti*, the precise defence mechanism underlying such phenomenon remains controversial, to date. Mzembe et al. [6] reported the role of macrophages in suppressing the growth of *B. microti* in *N. dubius*-infected mice by releasing soluble factors which show parasiticidal effect when tested *in vitro*. On the contrary, macrophages obtained from *Schistosoma mansoni*-infected mice failed to suppress the *in vitro* growth of *B. microti* [4]. Studies by Ruebush and Burgess [7] on the determination of interferon-γ (INF-γ) levels and parasitemia in experimental *B. microti* infection in beige mice provided valuable information that led them to suggest the probable contribution of NK cells in the expression of host natural resistance.

In the present study, infection of mice with *I. felis* induced significant host protection against *B. microti* infection. Interestingly, we found no cross reactive antibodies with *B. microti*, thus indicating no apparent participation of humoral antibodies. Likewise, results of spleen cells transfer and the control of T-cell subsets with mAb’s revealed the importance of spleen cells, specifically the L3T4+ cells in providing protection against *B. microti*. Our findings strongly demonstrate that non-pathogenic parasites such as *I. felis* has the capability to stimulate host’s immune response.

While L3T4+ cells are generally associated with production of INF-γ, tumor necrotic factor (TNF), IL-2 and of other lymphokines in the presence of antigens and induction of activation of macrophages, NK cells or L3T4+ own cells through lymphokines, we have no data to explain the role of L3T4+ cells in the present study. Thus, studies related to the interaction between L3T4+ cells and effector cells are necessary to understand the mechanism(s) underlying natural resistance of mice against *B. microti*.

**REFERENCES**


