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Role of CD4$^+$ T Cells in the Control of Primary Infection with *Babesia microti* in Mice

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ABSTRACT

The role of T cells for the resolution of acute primary infection with *Babesia microti* was investigated in the present study. BALB/c mice exhibited a peak parasitemia of approximately 40% of parasitemia and subsequently recovered naturally from their primary infection. Nude mice, however, could not resolve primary infection and developed persistent high parasitemia. Mice depleted of CD4$^+$ T cells with monoclonal antibody (mAb) had high parasitemia and failed to control the infection. However, depletion of CD4$^+$ T cells one week after infection did not affect the course of infection. Depletion of CD8$^+$ T cells showed no apparent effect on the course of infection. High concentration of IFN-γ was demonstrated in the culture supernatant of spleen cells from untreated and anti-CD8 mAb treated mice, but not from anti-CD4 mAb treated mice. Mice treated with anti-IFN-γ mAb showed higher peak parasitemia and remained above 10% of parasitemia until days 26 after infection. These results suggest that CD4$^+$ T cells play an essential role in the resolution of *B. microti* acute primary infection and that IFN-γ produced by CD4$^+$ T cells is partially responsible for control of early stage of acute infection with *B. microti*.

INTRODUCTION

Babesia, a tick-transmitted hemoprotozoan parasite, causes enormous economic losses in domestic animals throughout the world (McCosker 1981). *Babesia microti*, a species that parasitizes rodents, produces transient high parasitemia in mice and they naturally recover from the acute infection (Ruebush and Hanson 1979). The importance of cell-mediated immunity in *B. microti* infection has been reported (Irvin et al. 1981; Eugui and Allison 1980). Congenitally athymic nude mice (Clark and Allison 1974), lethally irradiated, thymectomized mice recon-stituted with anti-theta serum-treated bone marrow cells (Ruebush and Hanson 1980) or the administration of anti-lymphocyte serum in hamsters (Wolf 1974) fail to suppress infection with *B. microti*.

Likewise, B cell-dificient mice acutely infected with *B. microti* undergo remission with similar kinetics of parasitemia as immunologically intact mice (Cavacini et al. 1990). These results suggest that T cell-mediated immunity play a more significant role than the antibody-dependent immunity in the resolution of acute *B. microti* infection. However, the mecha-nism of mediating control of the primary infection with *B. microti* has not been fully understood.

In the present study, we focused on role of T cells in the control of primary infection with *B. microti* using immunocompetent BALB/c mice, and immuno-deficient athymic nude mice and SCID mice. Specifically, we sought to identify T cell subpopulation(s), and effect of in vivo depletion of T cell subpopulation(s) in the course of infection using anti-CD4 and anti-CD8 monoclonal antibodies (mAb). Also, the T cell subsets controlling the course of infection were identified through cytokine production in spleen cells and by monitoring the effect of administration of mAb against cytokines produced by T cells.

MATERIALS AND METHODS

Mice and parasite: Female BALB/c mice used in the study were purchased from CLEA (Tokyo, Japan). Histocompatible female BALB/c nu/nu, and nu/+ mice were obtained from CLEA. Female mutant mice having severe combined immune deficiency (SCID), and background CB-17 mice were purchased from CLEA, or were kindly provided by Central Institute for Experimental Animal (Kawasaki, Japan). All mice used ranged between 5-7 wks old at the start of the experiment. *B. microti* (Munich strain) was kindly provided by Prof. A. O. Heydorn of the Institute of Parasitology and Tropical Veterinary Medicine, Free University of Berlin, Germany. Mice were inoculated intraperitoneally (IP) with $1x10^7$ parasitized erythrocytes (PRBC). Parasitemia was estimated by counting PRBC of Giemsa-stained mouse blood smears, and was monitored at 2 days interval.

Monoclonal Antibodies (mAb): GK1.5 and 53-6.72 mAb were used against mouse CD4 and CD8, respectively, while XMG1.2 and 11B1 were used against interferon-gammma(IFN-γ) and interleukin(IL)-4, respectively (Waki et al. 1992). MAb were obtained from mouse ascites (Mueller et al. 1986) and were purified by 50% ammonium sulfate precipitation and subsequently dialized against phosphate-buffered saline (PBS). Protein con-centration was determined with Bradford method (Bradford 1976) using bovine serum albumin, as standard.

Treatment with mAb: Mice were depleted of T cell subsets by IP injection with 0.5 mg of anti-CD4 or anti-CD8 mAb for three successive days prior to the infection, and with additional same dose every three days post-inoculation. Control mice were inoculated with 0.5 mg normal rat IgG (Caltag Laboratories, South San Francisco, CA). Mice were injected with 1.0 mg of anti-IFN-γ and anti-IL-4 mAb for four consecutive days PI, and then every three days till the end of experiment.

Preparation of spleen cells: Infected and control mice were killed under anesthesia, and their spleens were removed aseptically, minced with scissors, and squeezed between two frosted glass slides. The cell suspension was filtered through a sterile stainless mess to remove tissue fragments. Contaminated erythrocytes were lyzed with 0.83% ammonium chloride, washed with Hank's balanced salt solution (HBSS) and resuspended to a concentration of $5x10^6$ cells/ml in RPMI 1640 medium (Gibco, Grand Island, NY).

Flow cytometric analysis of T cell subsets: Spleen cells suspended in RPMI 1640 containing 2.0% fetal bovine serum (FBS) were incubated with FITC-conjugated anti-CD4 or anti-CD8 mAb for 30 min on ice. After washing with HBSS, cell samples were analyzed by flow cytometry (FACScan, Becton Dickinson, Mountain View, CA).

Preparation of *Babesia*-lysate antigen (BLA): Blood from mice with about 80% parasitemia was obtained through cardiac puncture using heparinized syringes and washed three times. Pelleted PRBC were frozen with liquid nitrogen and thawed, this step repeated three times. The thawed sample was centrifuged at 144,000g for 30 min at 4°C, and the supernatant obtained was designated as BLA.

Detection of IFN-γ and IL-4 activity in spleen cell culture: $5x10^6$/ml spleen cells from infected and control mice were prepared and cultured in RPMI 1640 containing 5.0 % FBS, 100 units penicillin/ml and 100 mg streptomycin/ml, in the presence of BLA. Culture supernatant was harvested 72 hr post-incubation and stored at -80°C until use. Concentrations of IFN-γ and IL-4 were measured using ELISA kits (Holland Biotechnology, Netherlands; Endogen Inc., Boston, MA, respectively).

RESULTS AND DISCUSSION

BALB/c nu/+ mice infected with *B. microti* developed acute infection registering 41.75% peak parasitemia on day 10, subsequently followed with remission, while nude mice failed to resolve infection (Fig. 1). Although parasitemia in nude mice decreased to 38.2% on day 12, a high level of infection was sustained ranging between 45-60% until the 40th day PI. SCID mice developed rapid acute infection with peak parasitemia of 83.2% and failed to control primary infection as the nude mice did, with immunocompetent mice showing spontaneous remission (Fig. 2). These results confirm earlier observation of the failure of nude mice to resolve *B. microti* and *Plasmodium berghei yoelli* infection (Clark and Allison 1974). SCID mice having no functional T and B cells failed to resolve primary infection registering similar levels of parasitemia as nude mice. Cavacini et al.

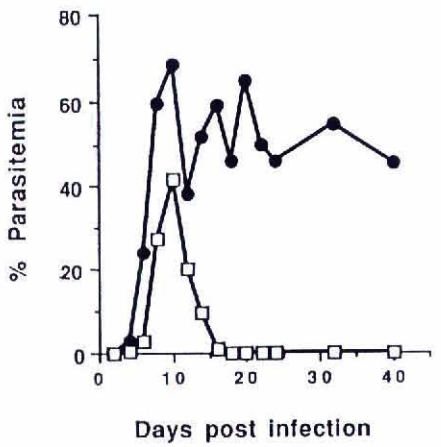

Figure 1. Course of *B. microti* infection in BALB/c nu/+ (□) and nu/nu (●) mice. Parasitemia was determined in groups of five mice every two days.

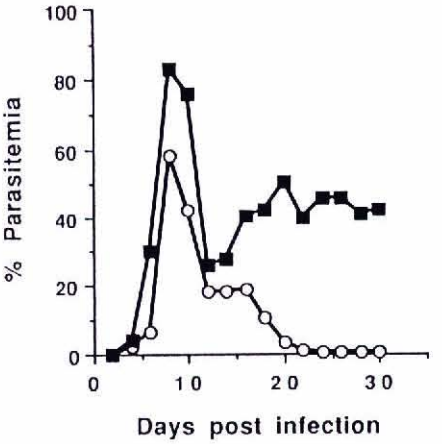

Figure 2. Course of *B. microti* infection in SCID (■) and CB-17 (○) mice. Parasitemia was determined in groups of three mice every two days.

(1990) demonstrated that B-cell deficient mice could control *B. microti* as well as *P. chabaudi* infection. These data put together apparently indicate that T cell-mediated mechanism(s) may indeed play a vital role in the resolution of *B. microti* in mice.

To identify T cell subpopulation in the control of *B. microti* infection, BALB/c mice were treated with either anti-CD4 or anti-CD8 mAb. This treatment with mAbs resulted in depletion of more than 92% of CD4+ or CD8+ T cells in the spleens and had no detectable affects on the opposite subpopulation when compared with those of untreated mice. Treatment with normal rat IgG gave essentially similar results with those of untreated mice (data not shown). Depletion of CD4+ T cells in BALB/c mice resulted in a course of infection similar to that of nude mice (Fig. 3). CD4-depleted mice had the highest peak parasitemia of 69.5% on day 10 PI, and they failed to clear the infection exhibiting between 34-47% parasitemia. In contrast, mice depleted of CD8+ T cells showed a similar pattern of parasitemia until day 8 PI, but cleared primary infection, similar to that observed in normal rat IgG-treated or untreated control groups. A requirement for CD4+ T cells in protective immunity has been demonstrated in *P. berghei* (Waki et al 1992), *P. chabaudi* (Cavacini et al 1986; Süss et al 1988) and *P. yoelli* (Jayawadena et al 1982; Vinetz et al 1990). Our results also demonstrate the importance of CD4+ T cells in the clearance of infection with *B. microti*. While involvement of CD8+ T cells have been suggested to contribute to the development of protective immunity against *P. chabaudi* (Podoba and Stevenson 1991), our present findings indicate that CD8+ T cells seem to have no effect on the course of *B. microti*.

In order to examine whether CD4+ T cells are required throughout a primary infection to control the rise of infection, treatment of mice with anti-CD4 mAb was started three days prior to, and seven and 14 days after *B. microti* inoculation. As shown in Figure 4, the administration of anti-CD4 mAb prior to the infection resulted to the development of high parasitemia just like in nude mice, however,

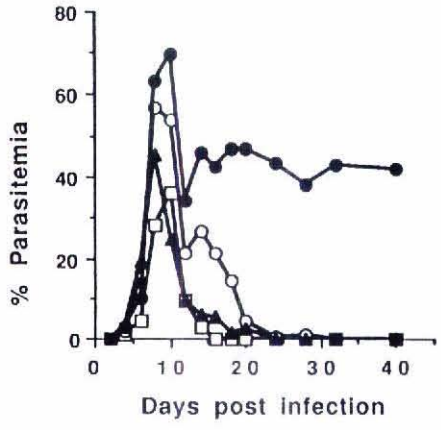

Figure 3. Course of *B. microti* infection in mAb-untreated BALB/c mice (□) or mice treated with normal rat IgG (○), anti-CD4 (●) and anti-CD8 (▲) mAb. Parasitemia was determined in groups of three mice every two days.

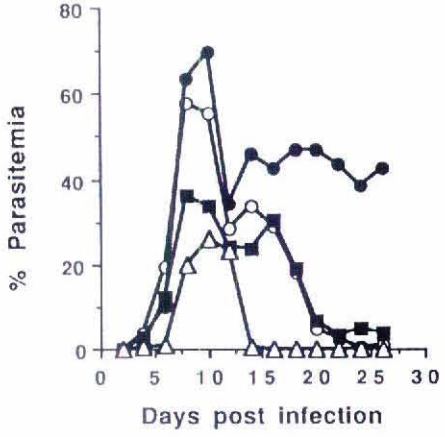

Figure 4. Course of *B. microti* infection in mAb-untreated BALB/c mice (○), or mice trated with anti-CD4 mAb three days prior to parasite inoculation (●), and on day 7 (■), and day 14 (△) post inoculation.

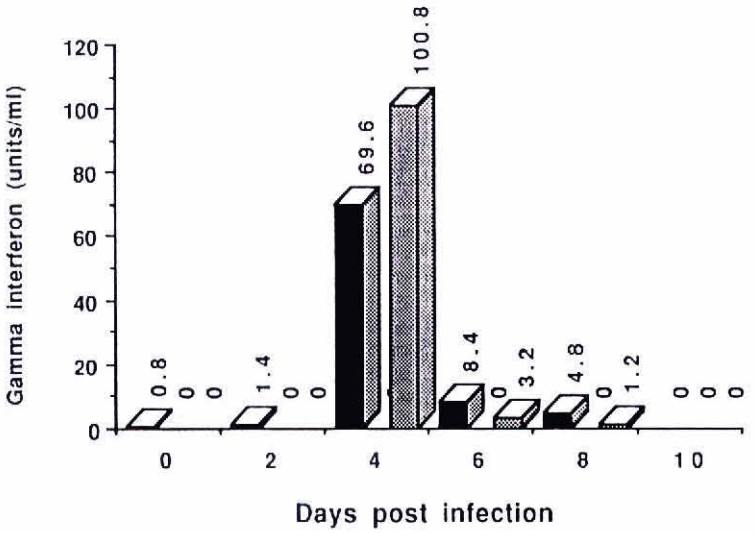

Figure 5. IFN-γ production in the supernatant of spleen cell cultures obtained from untreated BALB/c mice (■), or from mice treated with anti-CD4 (▧), and with anti-CD8 mAb (▩).

when treatment was started at 7 and 14 days PI, infection was under control, similar to that observed in mAb untreated mice. While the presence of CD4+ T cells at early phase of acute infection is necessary in the resolution of *P. chabaudi* (Langhorne et al 1990), in the present study, depletion of CD4+ T cells at seven and 14 days PI did not affect their ability to control of parasitemia. These results also suggest that CD4+ T cells play a major role during the prepatent and early patent periods of *B. microti* primary infection.

The role of CD4+ T cells that contribute to the resolution of acute phase of *B. microti* is not known. In *P. chabaudi* infection, two subsets of CD4+ T cells have been suggested to be responsible for the control of primary infection at different stage, showing Th1 cells predominating until after peak parasitemia, and Th2 cells predominating after the decrease of parasitemia (Langhorne et al 1989). Th1 cells mediate delayed-type of hypersensitivity (DTH) or activate macrophages and produce IFN-γ and IL-2, while Th2 cells act as helper cells for antibody production and produce IL-4 and IL-5 (Mosmann and Coffman 1987). To determine the role of CD4+ T cell subsets in *B. microti* infection, lymphokine production in spleen cells was assayed during the first 10 days PI. Production of IFN-γ and IL-4 in the supernatant of spleen cell culture in the presence of specific *Babesia* antigen obtained from anti-CD4, anti-CD8 mAb treated, or untreated mice was determined. High concentration of IFN-γ was detected in cultures on day 4 from untreated and anti-CD8-treated mice, but production of IFN-γ of spleen cells from CD4-depleted mice was completely inhibited (Fig. 5). IL-4 was not detectable in all groups during the first 10 days, except that a very low concentration of IL-4 was noted in untreated and anti-CD8 treated mice only on day 8 PI, (data not shown). These data demonstrated that IFN-γ is apparently produced by CD4+ T cells during early phase of *B. microti* infection and suggest that Th1 cells are apparently

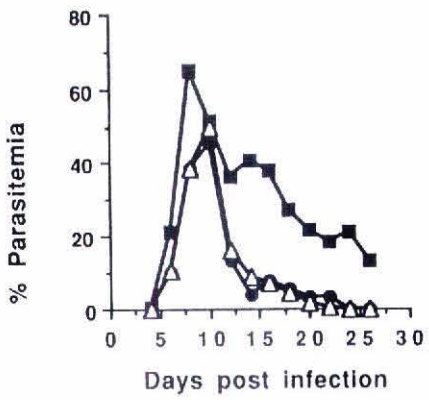

Figure 6. Course of *B. microti* infection in untreated BALB/c mice (●), in mice treated with anti-IFN-γ (■) and anti-IL-4 (△) mAb.

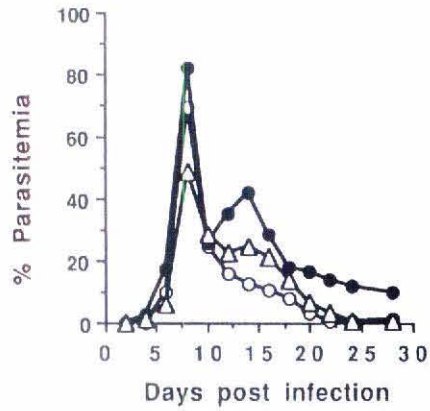

Figure 7. Course of *B. microti* infection in untreated BALB/c mice (○), in mice injected with anti-IFN-γ mAb beginning on the day of inoculation (■), and on day 8 (△) post inoculation.

involved in the recovery of acute infection with *B. microti* and collaborate previous reports that DTH response occurs in parallel with resistance against *B. microti* (Ruebush et al 1986).

To confirm the role of IFN-γ produced by CD4+ T cells in the resolution of *B. microti*, mice were treated with anti-IFN-γ or anti-IL-4 mAb. As shown in Fig. 6, treatment of mice with anti-IL-4 mAb resulted in the resolution of infection, similar to that of normal rat-IgG treated or untreated groups. Mice treated with anti IFN-γ mAb, however, showed higher peak parasitemia (65.0%) compared to untreated mice (46.0%) and to anti-IL-4 mAb treated group (50%), and was sustained at greater than 10% parasitemia until the 26th day PI. However, the effect of anti-IFN-γ mAb was less marked among mice that received lower dose of anti-CD4 mAb. If treatment of mice with anti-IFN-γ mAb was started on the 7th day PI, mice showed lower parasitemia than those mice that received the treatment on day 0 (Fig. 7). The role of IFN-γ on the blood stage of malaria infection has been reported (Waki et al 1992; Clark et al 1987; Shear et al 1989; Meding et al. 1990) and has been suggested to activate macrophages (Beutler et al 1986). In the present study, treatment of mice with anti-IFN-γ alone showed higher parasitemia relative to the control group, however, obstructive effect of anti-IFN-γ on the recovery of infection was not significant compared to the effect of anti-CD4. It may be supposed that one type of antibody is insufficient to deplete all IFN-γ activity in vivo, thus the need to prepare other types of mAb against IFN-γ. Also, it seems that IFN-γ alone cannot replace the capacity of CD4+ T cells in clearing acute primary infection, and that additional factors such as IL-2, tumor necrosis factor as suggested in malarial infection (Taverne et al 1990; Taverne et al 1987) may be necessary in the resolution of *B. microti*.

Based on our results, we conclude that CD4+ T cells play an essential role in the resolution of *B. microti* acute primary infection and that IFN-γ produced by CD4+ T cells, at least in part, is responsible for the control of early stage of acute *B. microti* infection in mice.

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