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**SERUM INTERLEUKIN-12 LEVELS AND SPLENIC HELPER T CELL
SUBPOPULATION IN *BABESIA RODHAINI* INOCULATED MICE PREIMMUNIZED
WITH *BABESIA MICROTI***

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ABSTRACT

Changes of serum interleukin-12 (IL-12) level and splenic helper T cell (Th cell) subpopulation were examined in the early phase of inoculation with *Babesia rodhaini* in mice preimmunized with *B. microti*. Only slight increase of the percentage in parasitized red blood cell was observed in *B. rodhaini* inoculated preimmunized mice. Serum IL-12 level significantly increased from 6 hr with a peak at 12 hr after the inoculation. In addition, the mice showed a differentiation of helper T cell type 1 (Th 1 cell) and the suppression of helper T cell type 2 in splenic Th cells on day 3 after the inoculation. These results suggested that IL-12 produced in the early phase of infection induced the differentiation of Th 1 cell, by which cellular protective immunity enhanced against *Babesia rodhaini* infection in preimmunized mice.

Key words: serum, IL-12, *Babesia rodhaini*, preimmunized mice

INTRODUCTION

Interleukin-12 (IL-12), a cytokine secreted from antigen presenting cells including macrophage, is well known to induce the differentiation of helper T cell (Th cell) into helper T cell type 1 (Th 1 cell) (Seder et al. 1993; Heinzel et al. 1995; Scharton-kersten et al. 1995; Maruo et al. 1996), resulted in the activation of cell mediated protective immunity. In the early phase of infection with protozoa, especially *Babesia* spp infection [Hsieh et al. 1992; Shimada et al. 1996], there were quite differences in species. Briefly, *Babesia microti* and *B. rodhaini* infected mice developed predominantly Th 1 cell and helper T cell type 2 (Th 2 cell) in splenic Th cells, respectively.

On the other hand, it has been generally accepted that the resistance against *B. rodhaini* lethal infection was observed in preimmunized mice with *B. rodhaini* and also with *B. microti*. The former mice showed Th 1 cell differentiation (Shimada et al. 1991), however the latter protective mechanism was not clearly understood (Cox et al. 1969). Since no cross antigen-antibody reactions was observed between *B. microti* and *B. rodhaini* infected mice, cell mediated immune response, especially Th 1 cell activation, was considered to be an important factor for the resistance (Shimada et al. 1991, 1996; Hashiguchi-Kato et al. 1999). This note deals with serum IL-12 levels and splenic Th cell subpopulation in the early phase of infection in *B. rodhaini* inoculated mice preimmunized with *B. microti*.

MATERIALS AND METHODS

Mice, protozoa and preimmunization: Male BALB/c mice, 8 weeks old, were supplied by SLC Inc. (Shizuoka, Japan). *Babesia microti* (Munich strain) and *B. rodhaini* (Australian strain) have been maintained in our laboratory by serial passages of parasitized blood to mice. Preimmunization for mice was performed by peritoneal inoculation with 1×10^4 of *B. microti* parasitized red blood cell (PRBC) to intact BALB/c mice. The mice were clinically healthy without detection of protozoa in peripheral blood until approximate 3 months after the inoculation.

Experimental inoculation: The preimmunized mice were inoculated by peritoneal injection with 1×10^4 of *B. rodhaini* PRBC/head in 0.2 ml of sterile saline.

Parasitemia: The percentage of PRBC was monitored by Giemsa's-stained smears of peripheral blood from *B. rodhaini* inoculated preimmunized mice and intact mice.

Serum IL-12 levels: Serum samples were obtained from *B. rodhaini* inoculated preimmunized mice by cardiac puncture at 0, 3, 6, 12, 24, 48, 72, 144, 266 and 298 hr after the inoculation. The serum samples were selected randomly from 4 or 5 mice at each point. Interleukin-12 levels were measured by the solid phase sandwich enzyme linked immunosorbent assay (ELISA) KIT (BioSource Cytoscreen Mouse IL-12 KIT; CA, USA).

Cytokine assay for Th cell population: For cytokine assay of the Th cell population, spleen cells were collected from *B. rodhaini* inoculated preimmunized mice on day 3 after the inoculation. The spleen cells were suspended in phosphate buffered saline (PBS; 0.01 M, pH 7.2) with 5% fetal bovine serum (FBS). Then, the cell suspension was eluted by a cell enrichment immunocolumn (CELLECT-PLUS MOUSE CD4 KIT) for obtaining Th cell as a CD4 positive ($CD4^+$) T cell (Cytovax, Biotechnologies Inc., Alberta, Canada). The number of $CD4^+$ T cell was adjusted to 1×10^6 cell/ml in RPMI 1640 (pH 7.2) supplemented with 10% FBS, 5 mM HEPES, 40 mg/l of gentamicine, 0.3 g/l of glutamine and 30 ml/l of 7% sodium bicarbonate solution. One milliliter of the cell suspension per well was incubated with 5 mg/ml concanavaline A (Con A) in 24-well culture plate at 37 °C in 5% CO₂ air for 24 hr. The culture supernatant was collected after the incubation and measured IL-4 and interferon γ (IFN- γ) levels by solid phase sandwich enzyme linked immunosorbent assay (ELISA) KIT (BioSource Cytoscreen Mouse IL-4 and IFN- γ KIT; CA, USA) as a marker of Th 1 and Th 2 cell, respectively.

Statistics: Student's t-test (unpaired) was used to evaluate the statistical significance.

RESULTS AND DISCUSSION

Parasitemia: The percentage of PRBC in *B. rodhaini* inoculated preimmunized mice slightly increased from day 9 to day 20 after the inoculation, whereas it remarkably increased in intact mice from day 6 to day 12 after the inoculation, causing the death (Fig. 1).

Changes of serum IL-12 level: Changes of serum IL-12 level in *B. rodhaini* inoculated preimmunized mice are shown in Fig. 2. Serum IL-12 level significantly increased from 6 hr after the inoculation with a peak level at 12 hr compared to that in the preinoculation level, and then decreased gradually by 72 hr.

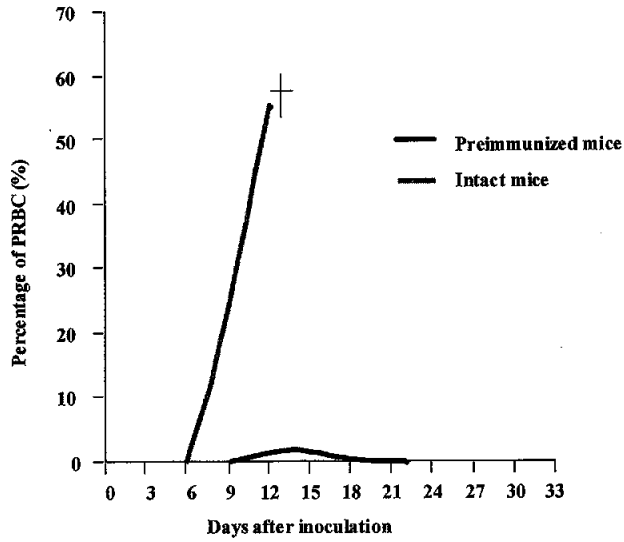


Fig. 1. Changes of PRBC in *B. rodhaini* inoculated mice preimmunized with *B. microti* and in *B. rodhaini* inoculated intact mice.

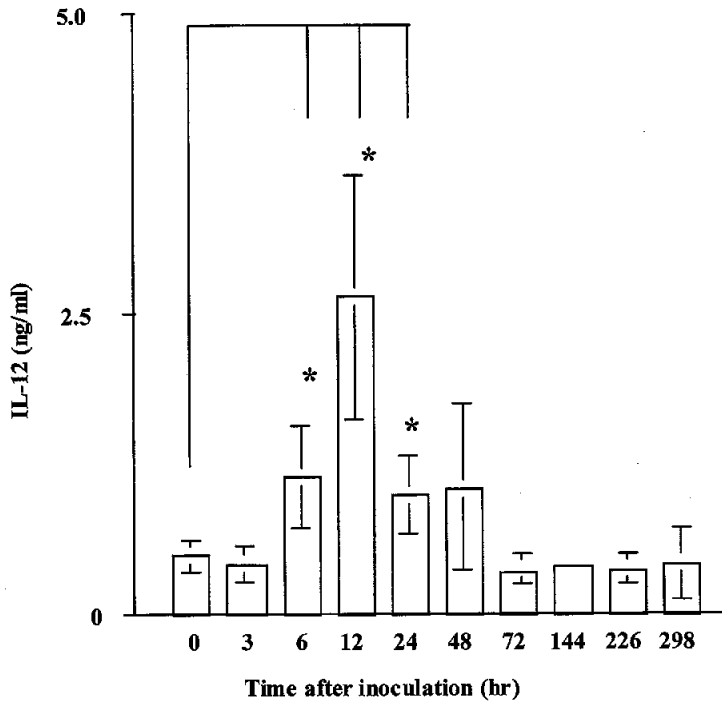


Fig. 2. Changes of serum IL-12 level in *B. rodhaini* inoculated mice preimmunized with *B. microti*.
*: Significant difference compared with pre-inoculated level

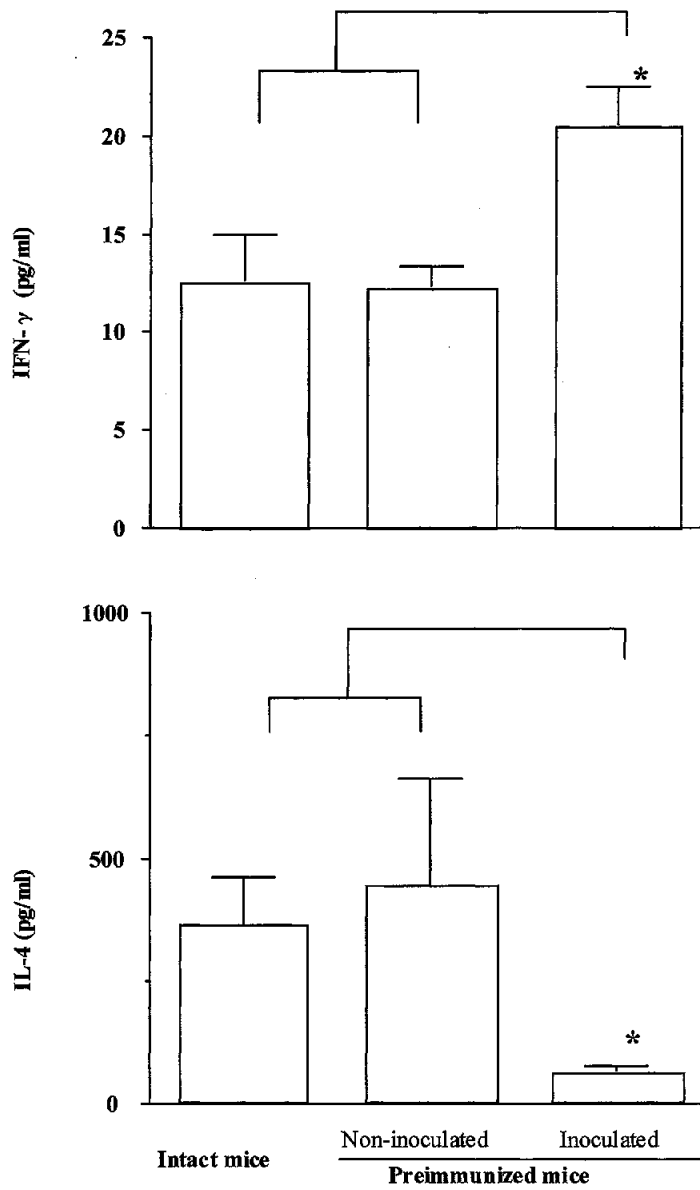


Fig. 3. IFN- γ and IL-4 production from splenic Th cells collected as CD4 positive T cells from *B. rodhaini* inoculated mice preimmunized with *B. microti*, and from non-inoculated preimmunized mice and intact mice as controls.

*: Significant difference compared to non-inoculated preimmunized and intact mice.

IFN- γ and IL-4 levels in CD4⁺ T cells cultured medium: Splenic CD4⁺ T cells were obtained from *B. rodhaini* inoculated preimmunized mice, and also from non-inoculated preimmunized and intact mice as controls. Both IL-4 and IFN- γ levels in culture medium of CD4⁺ T cells from non-inoculated preimmunized mice were similar to those from intact mice. In contrast, significant higher IFN- γ level and remarkable lower IL-4 level were observed in *B. rodhaini* inoculated preimmunized mice compared to those in non-inoculated preimmunized mice and intact mice (Fig. 3).

Our previous report demonstrated that no significant increase of serum IL-12 level with Th 2 cell differentiation in splenic Th cells was observed in *B. rodhaini* infected intact mice (Hashiguchi-Kato, et al. in press). Many investigators reported that the resolution of protozoa infection was mediated by T cell dependent cellular immunity (Cavacini et al. 1990; Brown et al. 1991; Shimada et al. 1991, 1996; Taylor-Robinson et al. 1992, 1994; Hashiguchi-Kato et al. 1999). On the other hand, the protozoa infections were resolved by protective antibodies as for the cross protective immunity in *Babesia* and *Plasmodium* spp infection (Zivkovic, et al. 1983; Vercammen et al. 1997). However, no significant cross antigen reactive antibody was detected between *B. microti* and *B. rodhaini* infected mice by western blotting analysis (data not shown). Cox et al. (1969) and Clerk et al. (1998) also reported that the cross-reacting antibody titer was usually low or could not detect in *Babesia* and/or *Plasmodium* spp infection. In this study, splenic CD4⁺ T cells from *B. rodhaini* inoculated mice preimmunized with *B. microti* on day 3 after the inoculation significantly produced INF- γ and reduced IL-4 production compared to those from both non-inoculated preimmunized and intact mice. Therefore, the activation of Th 1 cell was strongly suggested to induce the protective activity against *B. rodhaini* challenge inoculation. The decrease of Th 2 cell, which suppressed Th 1 cell proliferation, was also observed in *B. rodhaini* inoculated preimmunized mice. In addition, significant increase of IL-12 level observed at 6 hr after the inoculation indicated that *B. rodhaini* inoculated preimmunized mice showed the similar on Th 1 cell activation to that in mice infected with *B. microti* in the early phase of infection, in which early IL-12 production followed by Th 1 cell activation was observed. The Th 1 cell differentiation in splenic Th cell was reported to induce the resistance against *Toxoplasma gondii* infection in mice (Gazzinilli et al. 1994). Sypek et al. (1993) also reported that the administration of IL-12 during the first week before Th 2 cell development led susceptible BALB/c mice to be resistance against *Leishmania major* infection. Therefore, the results from this study suggested that IL-12 produced in early phase of infection promotes the differentiation of Th cell into Th 1 cell, by which cellular protective immunity is enhanced against *Babesia rodhaini* infection in mice preimmunized with *Babesia microti*.

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