SERUM INTERLEUKIN-12 LEVELS IN MICE INOCULATED WITH BABESIA MICROTI AND BABESIA RODHAINI

J. Protozool. Res.14, 50-54 (2004)

SERUM INTERLEUKIN-12 LEVELS IN MICE INOCULATED WITH *BABESIA MICROTI* AND *BABESIA RODHAINI*

RIE HASHIGUCHI-KATO, PHAM NGOC THI, TAKASHI OHMORI, SATOSHI TAMAHARA, MIZUKI TOMIHARI, TERUMASA SHIMADA, NAOAKI MATSUKI and KENICHIRO ONO

Department of Veterinary Clinical Pathobiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

Corresponding Author: Kenichiro ONO, Department of Veterinary Clinical Pathobiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan。 Fax: 03-5841-8187, E-mail: aken@mail.ecc.u-tokyo.ac.jp

ABSTRACT

Serum Interleukin-12 (IL-12) levels were measured in *B. microti* and *B. rodhaini* inoculated mice, since it is well known as an early responsible cytokine to induce the differentiation of helper T cell (Th cell) into helper T cell type 1 (Th 1 cell). Serum IL-12 levels in *B. microti* inoculated mice revealed two significant peaks at 3 and 24 hr after inoculation, while no significant change was observed in *B. rodhaini* inoculated mice. From our previous report, Th 1 cell and helper T cell type 2 (Th 2 cell) was predominant in splenic Th cells of mice on 5 day after inoculation with *B. microti* and with *B. rodhaini*, respectively. Interleukin-12 might initiate the differentiation of splenic Th 1 cell in the early phase of infection in mice infected with *B. microti* and closely related to the host protective cellular immunity.

INTRODUCTION

Intraerythrocytic protozoa, *Babesia microti* and *B. rodhaini*, are causative agents of murine babesiosis. It is well known that the course of infection is quite different between them (Cox and Young 1969; Allison 1984; Inchley et al. 1987). *Babesia microti* infection is non-lethal with a transient parasitemia, while *B. rodhaini* infection is lethal with severe parasitemia and anemia. Many researchers suggested that the difference of this was closely related to the cell mediated protective immunity in the early phase of infection, based on the results of a delayed type hypersensivity and splenic helper T cell (Th cell) subpopulation (Shimada et al. 1991; Hashiguchi-Kato et al. 1999). Briefly, helper T cell type 1 (Th 1 cell) and helper T cell type 2 (Th 2 cell) was predominant in splenic Th cells from *B. microti* and *B. rodhaini* infected mice, respectively (Hsieh et al.1993; Shimada et al. 1996).

Interleukin-12 (IL-12), a heterodimeric cytokine secreted from antigen presenting cells included macrophage, is well known as an early responsible cytokine to induce the differentiation of Th cell into Th 1 cell (Hsieh et al. 1992; Seder et al. 1993; Henzel et al. 1995; Scharton-kersten et al. 1995). This note deals with serum IL-12 levels in early phase of infection in *B. microti* and *B. rodhaini* inoculated mice and discusses with our previous results on splenic Th cell subpopulation.

MATERIALS AND METHODS

***Mice and protozoa*:** 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.

Experimental inoculation: Each intact mouce was inoculated by peritoneal injection with approximate $1x10^4$ parasitized red blood cell (PRBC)/head of *B. microti* or *B. rodhaini* in 0.2 ml of sterile saline. The percent PRBC was monitored by Giemsa's-stained smears of peripheral blood in *B. microti* and *B. rodhaini*.inoculated mice. The percent IRBC in B. rodhaini inoculated mice increased from day 6 to 12 after the inoculation, causing the death, whereas that in B.microti transiently increased from day 9 with a peak on day 17.

Serum IL-12 levels: Serum samples were obtained from *B. micronti* or *B. rodohaini* inoculated mice at 0, 3, 6, 9, 12, 18, 24, 48, 72 and 96 hr after inoculation by cardiac puncture. The samples were selected randomly from 4 or 5 mice at each time. IL-12 levels were measured by the solid phase sandwich enzyme linked immunosorbent assay (ELISA) KIT (BioSource Cytscreen Mouse IL-12 KIT; CA, USA). Student's t-test (unpaired) was used to evaluate the statistical significance.

RESULT AND DISCUSSION

Changes of serum IL-12 level: Changes of serum IL-12 level in *B. rodhaini* and *B. microti* inoculated mice are shown in Fig. 1. Serum IL-12 level in *B. microti* revealed two significant peaks at 3 and 24 hr after inoculation, while no significant change was observed in *B. rodhaini* inoculated mice.

Various cytokines were considered to regulate the Th cell differentiation into Th 1 cell in the early phase of infection, however IL-12 primarily played a central immunoregulatory role in initiating and maintaining Th 1 cell responses (Hsieh et al. 1992; Macatonia et al. 1995; Hino et al. 1996; Maruo et al. 1996). Gazzinelli et al. (1994) indicated that macrophage-derived IL-12 induced the differentiation of Th cell into Th 1 cell, showing the resistance against *Toxoplasma gondii* infection in mice. The administration of IL-12 during the first week before Th 2 cell development also led susceptible BALB/c mice to be resistance against *Leishmania major* infection (Sypek et al. 1993). In this study, serum IL-12 level in *B. microti* inoculated mice significantly increased as early as 3 hr after inoculation, whereas no remarkable increase of its level was observed in *B. rodhaini* inoculated mice throughout the experimental period. The early expression of IL-12 mRNA in the spleen cells was also observed in *B. microti* inoculated mice compared with that in *B. rodhaini* inoculated mice (data not shown). On the other hand, our previous report (Hashiguchi-Kato 1999) indicated that Th 1 cell activation was developed in splenic Th cells on day 5 after inoculation in *B. microti* infected mice, and Th 2 cell activation was observed in *B. rodhaini* infected mice. Therefore, deficiencies of IL-12 production in *B. rodhani* infected mice might induce Th 2 cell differentiation. Many investigators also reported that the resolution of protozoa infection was mediated by Th 1 cell dependent cellular immune mechanism (Cavacini et al. 1990; Brown et al. 1991; Shimada et al. 1991; Taylor-Robinson et al. 1994). Considering these reports and our results, in which *B. rodhaini* infected mice failed to induce IL-12 and selected Th 2 cell development, 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 microti* infection in mice.

REFERENCES

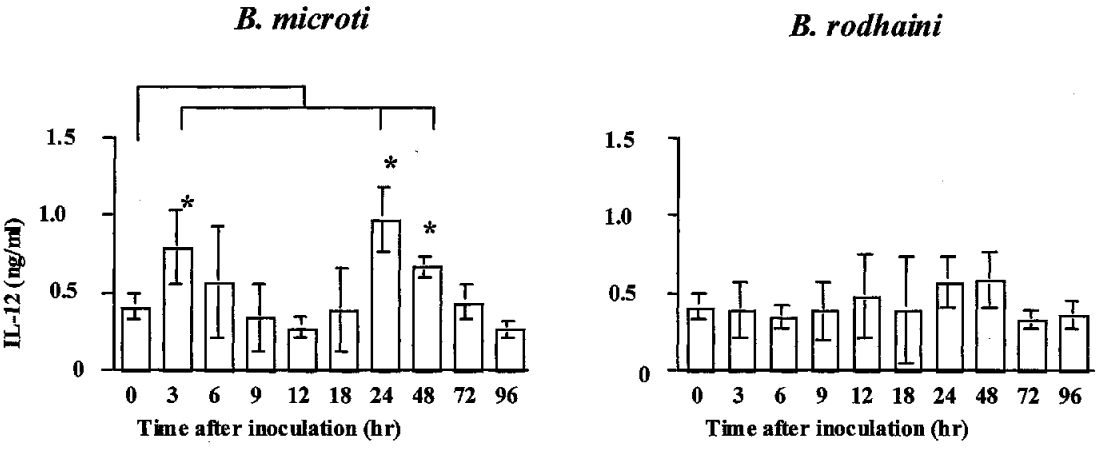

Fig. 1. Changes serum IL-12 levels in mice inoculated with *B. microti* and *B. rodhaini.*
*: Significant difference ($p<0.05$) to initial value

Allison, A. C. 1984. Cellular immunity to malaria and babesia parasites: a personal viewpoint. *Contemp. Top Immunobiol.* 12: 463-490.

Brown, W. C., Logan, K. s., Wagner, G. G. and Tetzlaff, C. L. 1991. Cell mediated immune response to *Babesia bovis* merozoite antigens in cattle following infection with Tick-derived or cultured parasites. *Infect. Immun.*, 59: 2418-2426.

Cavacini, L. A., Parke, L. A. and Wedanz, W. P. 1990. Resolution of acute malarial infections by T cell-dependent non-antibody-mediated mechanisms of immunity. *Infect. Immun.*, 58: 2946-2950.

Cox, F. E. G. and Young, A. S. 1969. Acquired immunity to *Babesia microti* and *Babesia rodhaini* in mice. *Parasitology*, 59: 257-268.

Gazzinilli, R. T., Wysocka, M., Hayashi, S., Denkers, E. Y., Hieny, S., Casper, P., Trinchieri, G. and Sher, A. 1994. Parasute-induced IL-12 stimulates early IFN-γ synthesis and resistance during acute infection with *Toxoplasma gondii. J. Immunol.*, 153: 2533-2543.

Hashiguchi-Kato, R., Shikano, S., Shimada, T., and Ono, K. 1999. The number of MHC class□ positive splenic antigen presenting cell in *Babesia microti* and *Babesia rodhaini* infected mice. *J. Protozool Res.*, 9: 41-48.

Heinzel, F. P., Rerko, R. M., Ahmed, F. and Pearlman E. 1995. Endogenous IL-12 is required for control of Th2 cytokine responses capable of exacerbating leishmaniasis in normally resistant mice. *J. Immunol.*, 155: 730-739.

Hino, A. and Nariuchi, H. 1996. Negative feedback mechanism suppresses interleukin-12 production by antigen-presenting cells interacting with T helper 2 cells. *Eur. J. Immunol.*, 26: 623-628.

Hsieh, C. –S., Heinberger, A. B., Gold, J. S., O'Garra, A. and Murphy, K. M. 1992. Differential regulation of T helper phenotype development by interleukins 4 and 10 in an αβ T-cell-receptor transgenic system. *Pro. Natl. Acad. Sci. USA,* 89: 6065-6069.

Inchley, C. J., Grieve, E. M. and Preston, P. M. 1987. The proliferative response of mouse lymphoid tissues during infection with *Babesia microti* or *Babesia rodhaini. Int. J. parasitol.* 17: 945-950.

Macatonia, S. E., Hoskin, N. A., Litton, M., Vieira, P., Hsieh, C. -S., Culpepper, J. A., Wysocka, M., Trinchieri, G., Murphy, K. M. and O'Garra, A. 1995. Dendritic cells produce IL-12 and direct the development of Th1 cells from native $CD4^+$ T cells. *J. Immunol.*, 154: 5071-5079.

Maruo, S., Toyo-oka, K., Oh-hara, M., Tai, X. –G., Iwata, H., Takenaka, H., Trinchieri, G. and Fujiwara, H. 1996. IL-12 produced by antigen-presenting cells induces IL-2-independent proliferation of T helper cell clones. *J. Immunol.*, 156: 1748-1755.

Scharton-Kersten, T., Afonso, L. C. C., Wysocka, M., Trinchieri, G. and Scott, P. 1995. IL-12 is required for natural killer cell activation and subsequent T helper 1 cell development in experimental leishmaniasis. *J. Immunol.*, 154: 5320-5330.

Seder, R. A., Gazzinelli, R., Sher, A. and Paul. W. E. 1993. Interleukin 12 acts directly on $CD4^+$ T cells to enhance priming for interferon γ production and diminish interleukin 4 inhibition of such priming. *Pro. Natl. Acad.* Sci. USA, 90: 10188-10192.

Shimada, T., Igarashi, I., Maki, Y., Claveria, F. G., Saito, A. and Suzuki, N. 1991. Cellular subsets involved in protective imunity to *Babesia rodaini* infection in BALB/c mice. *J. Protozool Res.,* 1: 35-44.

Shimada, T., Shikano, S., Hashiguchi, R., Ohashi, F., Matsuki, N. amd Ono, K. 1996. Expression of

gammma-interferon and interleukin-4 messenger RNA in splenic L3T4^{+} T cells from *Babesia microti-* and *Babesia rodhaini-* infected mice. *J. Protozool Res.,* 6: 25-30.

Sypel, J. P. Chung. C. L., Mayor, S. E. H., Subramanyam, J. M., Goldman, S. J., Sieburth, D. S., Wolf, S. F. and Schaub, R. G. 1993. Resolution of cutaneous leishmaniasis: Interleulin 12 initiates a protective T helper type 1 immune response. *J. EXP. Med.,* 177: 1797-1802.

Taylor-Robinson, A. W. and Phillips, R. S. 1992. Fuctional characterization of protective CD^{4} T-cell clones reactive to the murine malaria parasite *Plasmodium chabaudi. Immunology,* 77: 99-105.