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The Number of MHC class II Positive Splenic Antigen Presenting Cell in *Babesia microti* and *Babesia rodhaini* infected mice

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ABSTRACT

To elucidate helper T (Th) cell activation by the class II major histocompatibility complex (MHC), Ia molecules, positive antigen presenting cells (APC), including macrophages and activating B cells, changes of the number of Ia positive APC in spleen cell were examined by flowcytometric analyses in *Babesia microti* and *Bebesia rodhaini* infected mice. The number of neither Ia positive macrophages nor Ia positive B cells showed significant difference between *B. microti* and *B. rodhaini* infected mice until day 9 after inoculation. However, the splenic Th cell developed IFN-γ Producing Th cell type 1 (Th1) in *B. microti* and IL-4 producing Th cell type 2 (Th2) in *B. rodhaini* infected mice. These results demonstrated that the proliferation of APC is not major factor for the differentiation of Th cell into Th1 or Th2 cell and suggested that some humoral factors regulate the differentiation in the early phase of infection with *B. microti* and *B. rodhaini*.

INTRODUCTION

Babesia microti and *B. rodhaini* are well known as the causative agents of murine babesiosis. Although these two protozoa are classified into the same genus, they induce different course of infection. Our previous studies (Shimada et al. 1996) have demonstrated that these differences mainly caused by the early phase activation of splenic Th cell subsets. Briefly, *B. microti* infected mice showed principally Th1 cell which expresses IFN-γ mRNA, while *B. rodhaini* infected mice developed Th2 cell which expresses IL-4 mRNA.

It has been widely accepted that the activation and proliferation of antigen-specific Th cell are regulated by several antigen presenting cells (APC), such as macrophage/dendritic cells, B cells, and Kupffer cells expressing Ia molecules on the surface membrane (Ashwell et al. 1984; Kaye et al. 1988; Fiorentino et al. 1991; Croft et al. 1992; Macatonia et al. 1993). Several reports suggested that macrophages and B cells as APC mainly promote Th cell into Th1 cell and Th2 cell, respectively (Gajewski et al. 1991; Croft et al. 1992; Hsieh et al. 1992, 1993 ; Schmitz et al. 1993). However, there are few reports on the proliferation and population of Ia positive APC in spleen cells after infection with *Babesia* spp. In this study, changes of the number of total spleen cells, and the expression of Ia positive splenic macrophages and B cells were examined in *B. microti* or *B. rodhaini* infected mice in the early phase of infection.

MATERIALS AND METHODS

Animals and protozoa

Male BALB/c mice, 8 weeks old, were supplied by SLC Inc. (Shizuoka, Japan). *B. microti* (Munich strain) and *B. rodhaini* (Australian strain) have been maintained in our laboratory by serial passages of parasitized blood to mice.

Experimental infection

Mice were inoculated by peritoneal injection with 10^4 parasitized erythrocytes of *B . microti* or *B. rodhaini* in 0.2 ml of sterile saline diluted per head. The percent parasitemia was monitored by Giemsa's-stained smears of peripheral blood.

Preparation of flowcytometric analysis for Ia positive APC

The total spleen cells were obtained and pooled from 3 randomly chosen mice every 3 day after inoculation (ai) as described previously (Shimada et al. 1992). The number of the collected spleen cells were counted by hemocytometer. A portion of the spleen cell suspension was used for the staining of Ia-positive B cells. The splenic adherent cells for the staining of Ia-positive macrophages were obtained by the method of Kono et al. (1964). Briefly, 10^8 of the spleen cells were plated in 9 cm culture dish with 15 ml of RPMI 1640 medium. After the incubation at 37 °C in 5% CO_2 air for 90 min, non-adherent cells were removed by vigorously washing with warm RPMI 1640 for 3 times, and then remaining adherent cells were collected by scraping with rubber policeman and washed with phosphate buffered saline (PBS), pH 7.2. The pelleted 4×10^6 of both adherent cells and total spleen cells were stained with 50 ml of 50-fold diluted phycoerythrin (PE)-labeled mouse anti-mouse I-A^d monoclonal antibody (PharMingen, San Diego, CA) on ice for 30 min. The cells were washed with PBS containing 0.1% fetal bovine serum (FBS) (GIBCO, NY) for 3 times. Then, the adherent cells were further stained with 50 μl of 50-fold diluted fluorescein isothiocyanate (FITC)-labeled rat anti-mouse macrophage (MAC-1) monoclonal antibody (Cedarlane, Ontario, Canada), and the total spleen cells were stained with 100-fold diluted FITC-labeled rat anti-mouse IgG monoclonal antibody (Cedarlane, Ontario, Canada) on ice for 30 min, respectively. These cells were washed and suspended with PBS to the final volume of 1 ml for flowcytometric analyses.

Flowcytometric analyses

The percentage of double positive cells for PE and FITC was determined with CYTO ACE (Japan Spectroscopic Co, Tokyo, Japan) by analyzing 20,000 cells at each time. The number of splenic Ia-positive macrophages or B cells were calculated from its percentage and the total count of collected adherent cells or spleen cells, respectively.

Helper T cell population characterized by cytokine assay

For in vitro cytokine measurements, spleen cells were collected on day 5 ai from *B . microti* or *B. rodhaini* infected mice and suspended in PBS with 5% FBS and then eluted by cell enrichment immunocolumns, CELLECT-PLUS MOUSE CD4 KIT, for obtaining helper T cells as $CD4^+$ T cells (Cytovax, Biotechnologies Inc., Alberta, Canada). Splenic $CD4^+$ T cells from intact mice were also used as a control. The number of $CD4^+$ T cells were adjusted to 10^6/ml in RPMI 1640 (pH 7.2) supplemented with 10% FBS, 40 mg/L of gentamicin and 0.3 g/L of glutamine. One ml of the cell suspension per well was incubated with 5 μg/ml concanavalin A (Con A) in 24-well culture plate at 37 °C in 5% $C0_2$ air for 24 hr. The culture supernatant was collected after the incubation and measured IL-4 and IFN-γ levels by the solid phase sandwich enzyme linked-immuno-sorbent assay (ELISA) KIT (BioSource Cytoscreen Mouse IL-4 and IFN-γ kit; CA).

RESULTS

Changes of the number of APC cells:

The number of total splenic cells increased after *Babesia* infection, however, it showed no statistically significant difference between *B. microti* and *B. rodhaini* infected mice throughout the experimental periods (Fig. 1).

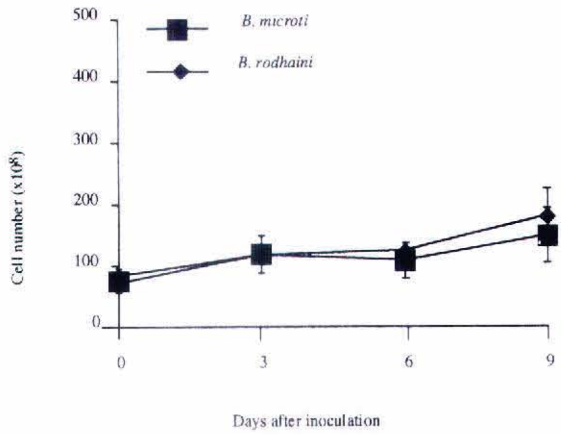

Fig. 1. Changes of the number of total spleen cells in *B. microti* and *B. rodhaini* infected mice. Closed square and diamond indicate the spleen cell number in *B. microti* and *B. rodhaini* infected mice, respectively. Spleen cells were obtained and pooled from 3 randomly chosen mice. Each value represents the mean ± SD.

In addition, the number of neither Ia-positive macrophages nor Ia-positive B cells measured by flowcytometry showed significant difference between *B. microti* and *B . rodhaini* infected mice until day 9 ai (Fig. 2).

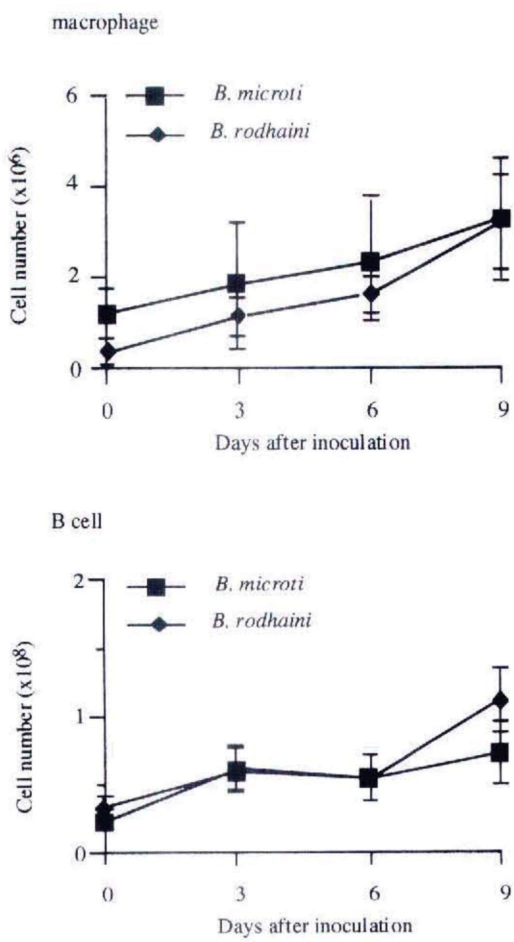

Fig. 2. Changes of the number of splenic Ia positive macrophage and B cell in *B. microti* and *B. rodhaini* infected mice. Closed square and diamond indicate the cell number in *B. microti* and *B. rodhaini* infected mice, respectively. The cell numbers were determined on the spleen cells pooled from 3 randomly selected mice at each period. Each value represents the mean ± SD.

Th cell subsets in spleen cells:

In *B. microti* infected mice, the CD4+ T cells from day 5 ai produced significantly IFN-γ compared with that from control mice and *B. rodhaini* infected mice. The cell cultured supernatant also showed significantly high IL-4 levels, which indicate Th2 cell activation in both *B. microti* and *B. rodhaini* infected mice (Fig. 3).

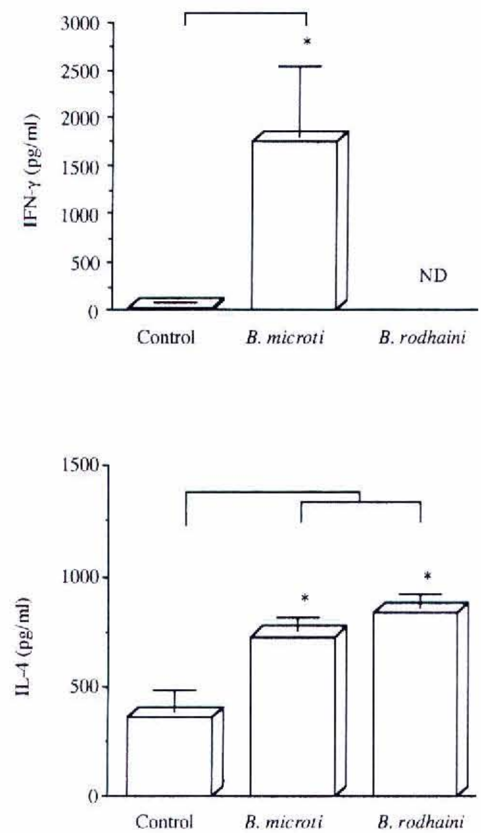

Fig. 3. IFN-γ and IL-4 productions by splenic CD4+ T cells in *B. microti* and *B. rodhaini* infected mice. Splenic CD4+ T cells were collected on day 5 after inoculation with *B. microti* or *B. rodhaini* infected mice and cultured (106/ml) with 5 μg/ml Con A in RPMI 1640 with 10% FBS for 24 hr. The cultured supernatant was collected after the incubation and measured IFN-γ and IL-4 level by the ELISA. The column height and bar indicate the mean ± SD of cytokines production. (*) indicates statistically significant ($p<0.05$) of IFN-γ and IL-4 production from that in control mice. (ND) indicates not detected.

DISCUSSION

The differentiation of Th cell into Th1 or Th2 cell has been reported by several investigators to be regulated by distinct APC population. They also suggested that macrophages stimulate proliferation of antigen-specific Th1 cell, while B cells stimulate Th2 cell (Gajewski et al. 1991; Croft et al. 1992; Hsieh et al. 1992; 1993; Schmitz et al. 1993). Consistent with this notion is the fact that prior depletion of B cells from susceptible BALB/c mice in vivo renders them to be resistant, since antigen-specific Th1 cell is able to resolve *Leishmania* infection (Sacks et al. 1984).

In this study, flowcytometric analyses have demonstrated that the number of neither Ia-positive macrophages nor Ia-positive B cells showed significant difference between *B. microti* and *B. rodhaini* infected mice until day 9 ai. In addition, the intensity of the Ia expression on each of these cells also showed no difference between these mice during the experimental period (data not shown). In several *Leishmania* species, it is generally accepted that the number of Ia-positive spleen cells do not affect the differential proliferation of Th cell subsets, since no significant difference is observed in the number of Ia-positive splenic cells between susceptible BALB/c mice and resistant C57BL/6 mice in the early phase of infection (Lang et al. 1991 ; Sunderkotter et al. 1993). Corresponding to the reports in leishmaniasis, the proliferation of Ia-positive APC and/or the density of Ia molecule on each cell are not considered to regulate directly the activation of distinct Th cell subsets in the early phase of infection with *Babesia* spp.

The outcome of the infection with certain intracellular protozoa is reported to depend on the differential activation of Th cell subsets in the early phase of host immune response. The resistant C57BL/6 and C3H/HeN mice showed a Th1 response, whereas susceptible BALB/c mice mounted Th2 response in the early phase of infection with *Leishmania major* (Scott et al. 1988). In *Plasmodium chabaudi* AS infection, resistant C57BL/6 mice developed the activation of Th1 cell, while susceptible J/A mice induced a strong Th2 response in the early phase of infection, resulted in a severe and lethal course of malaria (Stevenson et al. 1993).

In this study, the cytokine production by splenic helper T cells revealed that significant IFN-γ production was observed on day 5 in *B. microti* infected mice, while marked IL-4 production was detected and no significant IFN-γ production was observed in *B. rodhaini* infected mice. The cytokine production by splenic Th cell showed that *B. microti* infected mice activated Thl cells, while *B. rodhaini* infected mice led to activate Th2 cell in the early phase of infection, however, no changes of the proliferative response in Ia positive APC was observed.

Therefore, these results suggested that some humoral factors such as cytokines might be the strong interactive factors for the differentiation of Th cell subsets in the early phase of infection with *Babesia* spp.

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