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The Role of Scavenger Receptor Type A (SR-A) during Infection with *Babesia microti*

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ABSTRACT

The class A macrophage scavenger receptor (SR) binds an extraordinary wide range of ligands including bacterial pathogens and mediates macrophage adhesion. In the present study, SR knock-out mice were infected with *Babesia microti* to clarify its role in host defense mechanisms against protozoan infection. Several aspects of the immunological responses of mice during infection were studied. In *B. microti* infection, the packed cell volumes of SR-/- mice at 16-30 days after infection were significantly higher than those of SR+/+ mice, although the course of parasitemia was not different between these mice. Increase in spleen weights after infection were less obvious in SR-/- mice than in SR+/+ mice at 20 days after infection. Flow cytometric studies revealed that the decreases in the percentages of Thy1.2$^+$ as well as CD4$^+$ T cells in spleen after *B. microti* infection were higher in SR+/+ mice than that in SR-/- mice, whereas there were no difference in the rates of B220$^+$ cells between SR-/-and SR+/+ mice. The peritoneal macrophages from SR+/+ mice exhibited higher phagocytic activity than those from SR-/- mice. These results indicate that scavenger receptor enhances immune responses and phagocytosis of parasitized erythrocytes, although it does not directly play an important role of protection against *B. microti* infection.

INTRODUCTION

Babesia, a tick-transmitted heamoprotozoa 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 and IFN-γ in *B. microti* infection has been reported (Irvin et al. 1981; Eugui and Allison 1980). However, the mechanism of mediating control of the primary infection with *B. microti* has not been fully understood. We previously reported that $CD4^+$ T cell plays a very important role in immune response of host to protozoan infection (Igarashi et al. 1994). At the mean time, IFN-γ appears to take part in the response (Orinda et al 1994). However, not only IFN-γ, but other cytokines may involve in the immune response. Therefore, it is important to examine the other factors including TNF-α. In the present study, we focused on the role of macrophages in the control of primary infection with *B. microti* using knock-out mice. The role of macrophage for the resolution of acute primary infection with *B. microti* was investigated in this study.

The class A macrophage scavenger receptor (SR-A) is a trimeric integral membrane glycoprotein which exists in two forms, type I and II generated by alternative splicing of a single gene product (Naito et al. 1992). This family of receptors has recently been extended through the discovery of several additional scavenger receptors and now includes at least three independent SR classes (Kodama et al. 1988; Kodama et al. 1990). Scavenger receptor is defined according to their ability to bind and mediate uptake of modified low density lipoproteins (LDL), such as acetylated LDL (AcLAL) (Terpstra 1997). However, the range of ligands recognized by SR-A is wide, including lipopolysaccharide (LPS) and lipoeichonic acid. This broad ligand specificity has suggested that SR-A may play a role in wide range of macrophage-associated physiological and pathophysiological processes. In the present study, we focused on the possible role of macrophages in the control of primary infection with *B. microti* using SR-knock out mice (Suzuki et al. 1997).

MATERIALS AND METHODS

Mice

Mice deficient in type I and II SR-A (SRKO mice) were produced by disruption of exon 4 of the SR-A gene, which encodes the α-helical coiled coil domain, essential for the formation of functional trimeric receptors, by homologous recombination, these mice were bred onto a genetic background of 129 × ICR, and mice were cross bred on to an identical 129/ICR background.

Female SRKO mice were obtained from CSK Research Park (Gotenba, Japan) and were used at 8 to 15 weeks of age.

Parasites Seldom

Babesia microti was kindly provided by Prof. A. O. Heydorn of the Institute of Parasitology and Tropical Veterinary Medicine, Free University of Berlin,Germany. SRKO mouse were inoculated intraperitoneally with 1×10^7 *B. microti* parasites. Parasitemia was daily monitored by counting parasitized erythrocytes of Giemsa-stained mouse blood smears, everyday. Packed cell volume was monitored every 4 days after infection.

Preparation of spleen cells

Infected and uninfected control mice were killed under anesthesia, and spleens were removed aseptically, minced with scissor, and squeezed between two frosted glass slides. The cell suspension was filtered through the mesh (#200) to removed tissue fragments. Contaminated erythrocytes were lyzed with 0.83% ammonium chloride, washed with phosphate buffered saline (PBS, pH7.4) and resuspended to concentration of 5×10^6 cells/ml in PBS.

Flow cytometric analysis of T cell subset

Spleen cells which suspended in PBS containing 2.0% fetal bovine serum (FBS: BIO WHITTAKER Walkersville, Maryland) were incubated with FITC-conjugated anti-Thy1.2, FITC-conjugated anti-CD4, PE-conjugated anti-CD8, PE-conjugated anti-B220 or PE-conjugated NK1.1 mAb for 30min on ice. After washing with PBS, cell samples were analyzed by flow cytometry (EPICS XL, COULTER CORPORATION, Miami, Florida).

Peritoneal macrophage

Mouse peritoneal macrophages were taken by washing the peritoneal cavity with 5 ml of sterile PBS, pH 7.4. The cells were centrifuged once and counted total cell number. At some experiment cells were resuspended to adjust cell concentration in DMEM (GIBCO BRL, Grand Island, New York) containing 10% heat-inactivated FBS. Some animals were infected with *B. miroti* for10 days before harvesting the macrophages. The cells were seeded on 15-mm round glass coverslips on 24-well plate at a density of 1×10^6 cells/well.

Phagocytosis

Phagocytosis was assayed by overlaying the peritoneal macrophage with 0.1ml of parasitized erythrocytes, suspended to 2×10^8 cells/ml. Coverslips were

incubated for 45 min at 37°C, 5% CO_2 in air. They were rinsed in PBS, fixed in methanol for staining and quantification. After being stained with May-Grunwald solution for 10 min, and with Giemsa solution for 25 min, coverslips were washed with distilled water. The percentage of ingested red blood cells was determined by counting 200 macrophage/coverslip.

Parasitized erythrocytes

Parasitized erythrocytes were obtained from SR+/+ mice 10 days after infection. They were washed three times with DMEM and resuspended to 2×10^8 cells/ml.

Statistics

Data were analyzed statistically by Student's t-test.

RESULTS

Fluctuations of parasitemias and packed cell volumes in SR+/+ and SR-/- mice that had been inoculated intraperitoneally with 1×10^7 *B. microti* are shown in Figures 1 and 2. SR-/- mice developed acute infection registering 47.0% peak parasitemia on 10 days after infection, while SR+/+ mice developed registering 52.9% peak parasitemia on 10days. Recover of parasitemia was similar for both strains of mice during 30 days after infection. However, the packed cell volumes of SR+/+ mice at 16 -30 days after infection were significantly lower than those of

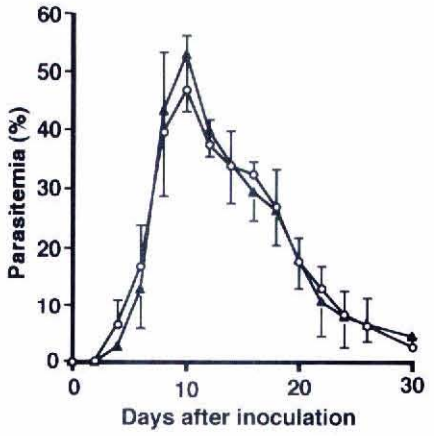

Fig. 1 Infection course of *Babesia microti* in SR+/+ mice (○) and SR-/- mice (▲). Mean parasitemia were obtained from groups of four mice. Data represents the mean ± SD.

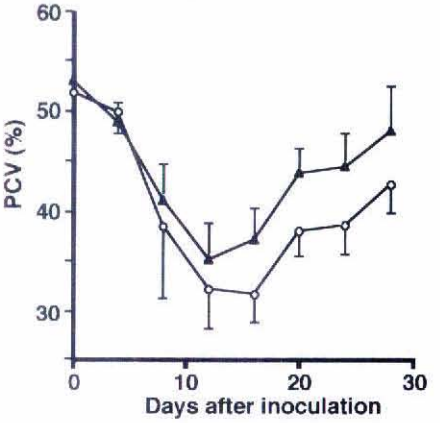

Fig. 2 Infection course of *Babesia microti* in SR+/+ mice (○) and SR-/- mice (▲). Mean PCV were obtained from groups of eight mice. Data represents the mean ± SD. *Student t-test $P<0.05$.

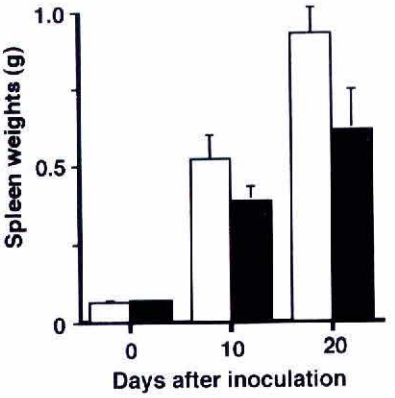

Fig. 3 Spleen weights in SR+/+ mice (□) and SR-/- mice (■) during infection course of *Babesia microti*. Data represents the mean ± SE.

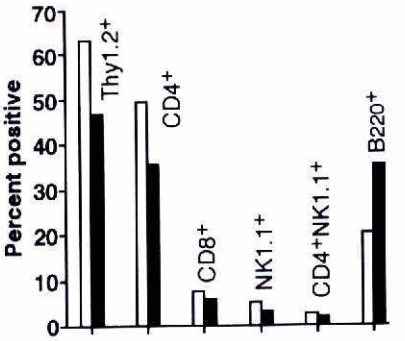

Fig. 4 Phenotypic distribution of spleen cells in SR+/+ mice (□) and SR-/- mice (■). Cell surface expression of the indicated markers was determined by flow cytometer.

SR-/- mice. Spleen weights of SR+/+ mice were higher than those of SR-/- mice at 20 days after infection, as show Figure 3. Phenotypic compositions of spleen cells in non-infected SR+/+ and -/- mice are shown in Figure 4. Percentage of CD4+ T cells from SR+/+ mice was higher than that from SR-/-, while percentage of B220 positive B cells from SR+/+ mice was lower than that from SR-/-. Total cell number and spleen weight were not different between two strains. Figures 5 and 6 show the rates of splenic lymphocyte subsets after infection, as examined by flowcytometer. Percentages of B220+ positive B cells at 10 days after infection were 35.9% and 38.8% from SR+/+ and SR-/- mice, respectively. They became

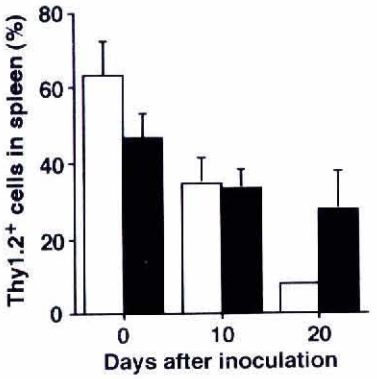

Fig. 5 Infection course of Thy1.2+ cell population in SR+/+ mice (□) and SR-/- mice (■) during infection course of *Babesia microti*. Data represents the mean ± SD.

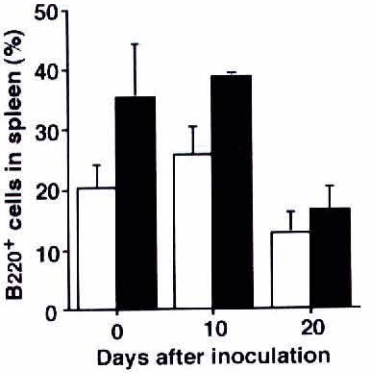

Fig. 6 Infection course of B220+ cell population in SR+/+ mice (□) and SR-/- mice (■) during infection course of *Babesia microti*. Data represents the mean ± SD.

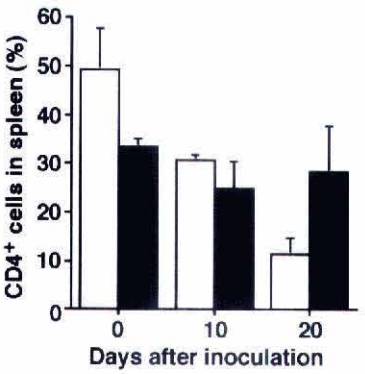

Fig. 7 Infection course of $CD4^+$ cell population in SR+/+ mice (□) and SR-/- mice (■) during infection course of *Babesia microti*. Data represents the mean ± SD.

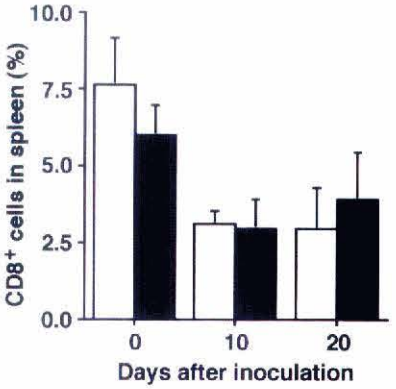

Fig. 8 Infection course of $CD8^+$ cell population in SR+/+ mice(□) and SR-/- mice (■) during infection course of *Babesia microti*. Data represents the mean ± SD.

lower than half of 10 days in both SR+/+ and SR-/-mice at 20 days after infection. There were no significant differences in the rate of B220 positive B cells between SR+/+ and SR-/- mice. Percentage of $Thy1.2^+$ positive T cells reduced percent change from 63.2% to 7.9% at 20 days after infection in SR+/+ mice. The reduction was less drastic from 46.6% in SR-/- mice to 16.5%. This period corresponded to the time when splenomegary occurred in SR+/+ mice. As show in Figures 7 and 8, fluctuations of $CD8^+$ positive T cell were less evident in both strains; changed from 7.6% and 6.0% to 2.9 and 3.9% at 20 days after infection in SR+/+ and SR-/- mice, respectively. There were no significant differences in the rate of $CD8^+$ positive T cells between them. On the other hands, the rates of $CD4^+$ positive T cells changed from 49.4% to 11.5% at 20 days after infection in SR+/+ mice and 33.5% to 28.3% in SR-/-mice. The percentages of $NK1.1^+$ cell and $NK1.1^+$ $CD4^+$ cells did not differ both strains (data not shown). As shown in Table 1, peritoneal macrophages obtained from infected mice at 10 days after infection or from non-infected SR+/+ mice ingested 26.0% or 20.3% erythrocytes infected with *B. microti*. In SR-/- mice, percents of peritoneal macrophages that ingested the infected erythrocytes were 16.8% and 15.0% when obtained from 10 days after infection and from non-infected mice. The rates of peritoneal macrophages that ingested infected erythrocytes were significantly higher in SR+/+ mice than those from SR-/- mice.

DISCUSSION

The importance of cell-mediated immunity and IFN-γ in intercellular protozoa infection has been reported (Shear et al. 1989; Sally et al. 1990; Sedegah

et al. 1994). It has been shown that $CD4^+$ T-cell plays a very important role in host immune response to protozoan infection (Süss et al. 1988; Podoba et al. 1991; Waki et al 1992). Mice treated with anti-CD4 mAb fail to control *B. microti* infection. However, the mechanism mediating the primary infection with *B. microti* has not been fully understood. The role of macrophage for the resolution of acute primary infection with *B. microti* was investigated in this study. The development of mice deficient in type I and type II Scavenger receptors, through disruption of exon 4 of the SR-A gene, has provided a method for investigation. Fluctuations of parasitemia were not different between SR-/- and SR+/+ mice after *B. microti* infection. Based on this result, scavenger receptor seems not to play an important role for exclusion of parasitized erythrocytes with *B. microti*. However, the packed cell volume of SR+/+ mice at 16-30 days after infection were significantly lower than those of SR-/- mice, and spleen weights of SR+/+ mice were heavier than those of SR-/- mice at 20 days after infection. Interestingly, the peritoneal macrophages from SR+/+ mice were more phagocytic than those from SR-/- mice in vitro. This suggests that scavenger receptor mediates uptake of both of infected and non-infected erythrocytes, although the phagocytic activity of macrophages against *B. microti*-infected erythrocytes in the present study is lower than that reported for the uptake of modified-LDL. In the same time, flow cytometric studies revealed that the percentage of lymphocytes in spleen from SR+/+ mice was lower than that from SR-/- mice after *B. microti* infection.

Based on our results, we conclude that scavenger receptor does not directly play an role in protection against *B. microti* infection. However, scavenger receptor, as a binding factor of macrophage, presumably enhance immune responses and induce macrophage for elimination of parasitized and damaged erythrocyte.

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