Hematological Findings and Antibody Responses in Syrian Hamster (Mesocricetus auratus) Infected with Babesia microti

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ABSTRACT. Hematological findings during the course of infection and the antibody response in Syrian hamsters infected with Babesia microti were examined. A macrocytic hypochromic anemia with an increase of the reticulocyte count was detected as a rise in the parasitized erythrocyte rate. White blood cell counts also remarkably increased with the increases of both neutrophils and active-shaped monocytes, and thus they particularly play an important role in eliminating the parasite. In Western blotting with the sera from the hamsters infected with B. microti, a 38 kDa protozoan antigen reacted to the early-term sera, and additionally 28, 32, and 34 kDa antigens also reacted to the medium- and latter-term, and convalescent sera. These antigens were immunodominant and the antibodies against these antigens had also important roles for inhibition of this parasite.

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KEY WORDS: Babesia microti, hematological findings, Syrian hamster.

Babesiosis is caused by the protozoan blood parasites of the genus Babesia which induce pyrexia, hemolytic anemia, and defluxion of hemoglobinuria. The disease is widespread in both domestic and wild animals throughout the world and is an important infectious disease in veterinary clinics [6]. The parasites divide and multiply in erythrocytes of the host animal, and cause the destruction of blood cells as a cardinal symptom of babesiosis [11, 20, 21]. Babesia microti is well known as a causative protozoan in rodent babesiosis and its final hosts are Peromyscus leucopus and infrequently Microtus pennsylvanicus in U.S.A. [6, 9]. This protozoan is also the causative agent of human babesiosis [8, 10, 19], an emerging tick-borne zoonosis, which has been frequently recognized in the northeastern and upper midwestern U.S.A., and also in Japan [12]. On the other hand, it is reported that Syrian hamsters show high susceptibility to B. microti infection and commonly used for maintenance of its agent in the laboratories [2, 4, 5, 7, 13, 15]. Nevertheless, there are few reports on hematological findings in the hamsters experimentally infected with B. microti [2]. This study deals with hematological findings, especially white blood cell parameters as immunocompetent cells, and also antibody responses in Syrian hamsters infected with B. microti.

Twenty female Syrian hamsters, 5-week-old, were purchased from Saitama Experimental Animals Supply Co., Ltd. (Saitama, Japan). The hamsters were divided into two groups, B. microti infected and non-infected (control) groups.

B. microti AJ strain, isolated from a human patient at Harvard University, U.S.A. and maintained by blood passages with Syrian hamsters in Nippon Veterinary and Animal Science University, was used.

B. microti was inoculated interperitoneally with $1 \times 10^7$ parasitized erythrocytes (PE) per head collected from the serially inoculated Syrian hamsters.

Parasitemia in peripheral circulation was monitored throughout the observation period by examining a thin blood smear from a drop of blood collected from a hamster at the tip of tail and its percentage was determined by counting the infected cells in at least 1,000 erythrocytes per stained smear. Red blood cell (RBC, /µl) count, packed cell volume (PCV, %), hemoglobin (Hb, g/dl), and white blood cell (WBC, /µl) count were estimated in the blood of the hamsters by using Celltac (Nihon Kohden, Japan). Mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin concentration (MCHC, %), mean corpuscular hemoglobin (MCH, pg) were also calculated from the above hematological values. Reticulocytes and granulocytes, lymphocytes and monocytes were counted by the observation of blood smears under a microscope.

For examination of antibody response, the same volume of serum samples were collected and pooled from the 10 Syrian hamsters belonging to the B. microti infected group during the following terms, and were designated as the early-term (0–13 days post infection (PI)), medium-term (14–30 days PI), latter-term (31–90 days PI), and convalescent (over 91 days PI) samples.

B. microti antigen (BMag) was prepared according to the methods of Saeki and Ishii [14] and Sugimoto et al. [16] with modification. Briefly, blood samples infected with B. microti were obtained by cardiac puncture of the animals which were washed with 0.01 M phosphate buffered saline (PBS, pH7.2), and theuffy coat was removed. A pellet of erythrocytes was lysed with 5 times the volume of 0.1% saponin, and the lysate was centrifuged at 10,000 g for 30 min, and the precipitate was resuspended in PBS. Solutions of 40% and 60% (vol/vol) Percoll (Amersham Pharmacia Biotech, U.K.) were prepared in PBS containing 5 mM EDTA (PBS-EDTA). The erythrocyte lysate was gently layered over the top of a discontinuous density gradient of 40% and 60% Percoll solutions in a tube for ultracentrifugation. After centrif-
Fig. 1. Parasitemia (A), red blood cell (RBC) count (B), packed cell volume (PCV) (C), hemoglobin (D), reticulocyte count (E), mean corpuscular volume (MCV) (F), mean corpuscular hemoglobin concentration (MCHC) (G), white blood cell (WBC) count (H), neutrophil count (I), and monocyte count (J) estimation in the blood of Babesia microti infected Syrian hamsters. Values are expressed as the mean ± Standard deviation (SD) (n=10)  ○: Infection group, ●: Control group.
ugation at 45,000 g for 20 min, the visible band at the interface of 40% and 60% Percoll solutions was collected, diluted with PBS-EDTA buffer, and washed 3 times at 10,000 g for 10 min in a microcentrifuge tube. The parasites resuspended in PBS were sonicated with the ultrasonicator (TAITEC VP-15S, Saitama, Japan) at set 5 for 5 min and stored at −80°C until use.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were performed in accordance with the methods of Weber and Osborn [19] and Towbin et al. [18]. Briefly, the BMag was separated by SDS-PAGE and transferred onto nitrocellulose membrane (Amersham Pharmacia Biotech). After treated with skim milk (Wako Pure Chemical Industries, Osaka, Japan) to block non-specific binding of the antibody, the membrane was reacted to Syrian hamster’s sera as the first antibody and horseradish peroxidase-conjugated rabbit anti-hamster IgG (Rockland, PA, U.S.A.) as the second antibody. The antigen-antibody reaction was detected by an ECL Western Blotting Detection System (Amersham Pharmacia Biotech).

In the Syrian hamsters infected with B. microti, the PE rate began to rise about 10 days PI, then it remained at the level of 30–40%, thereafter it suddenly descend for 30–45 days PI, and finally the parasites became impossible to be detected in the peripheral blood smears about 3 months PI (Fig. 1A). A remarked decrease in the RBC count (Fig. 1B), PCV value (Fig. 1C), and Hb concentration (Fig. 1D) and an increase in the reticulocyte count (Fig. 1E) were also seen accompanied by a rise in the PE rate. A increase in MCV and a decrease in MCHC (Figs. 1F and 1G) indicated macrocytic hypochromic anemia, which was reported to be the typical anemia in babesiosis [2, 15].

In leucocyte findings, total WBC counts increased remarkably with the rise of PE rate and the decrease in RBC counts (Fig. 1H). This clinical sign was mainly due to the increase of neutrophils and monocytes (Figs. 11 and 1J). Benach et al. [2] reported that a remarked increase of WBC count in a B. microti infected hamster is due to an increase of metamyelocytes or immature neutrophils, and that the parasites were phagocytized by neutrophils rather than monocytes. However, in this study, both mature and immature neutrophils increased, and additionally active-shaped monocytes remarkably increased (Fig. 2). It is well known that the nonspecific phagocytosis by monocytes cause anemia in B. rodhaini infection, in which both infected and non-infected erythrocytes are ingested [14]. Bautista and Kreier [1] showed that the phagocytosis of erythrocytes by macrophages was more enhanced during B. microti infection than in the non-infected state, inhibiting the growth of the parasites in vitro. In general, positive feedback mechanism by dendritic cell and monocyte/macrophages which phagocytized pathogens, in addition to the activity of interleukin-12 and interferon-gamma (IFN-γ), produced by them, promote the proliferation and activation of monocyte/macrophage. Active monocytes induced by IFN-γ exclude the pathogens by nitrogen peroxide and active oxygen produced by them. Moreover, they transform into dendritic cells and play an important role as cells presenting antigens to CD4+ T lymphotic cell. These Th1 immunoreactions are important for host defense system [3]. Our findings and the previous reports suggest that monocytes including macrophages play an important role same as neutrophils for exclusion of B. microti by phagocytosis and as antigen presenting cell to T lymphotic cell in the hamsters infected with this parasite.

On the other hand, from SDS-PAGE with BMag, it was confirmed that 38 kDa antigen was the most immunodominant in B. microti infection (Fig. 3). In the Western blotting analyses with early-, medium-, latter-term and convalescent sera from the Syrian hamsters infected with B. microti, the 38 kDa protein reacted to the early-term, while 28, 32 and 34 kDa antigens reacted to the medium-, latter-term, and convalescent sera (Fig. 3). The latter 3 antigens of BMag
were hardly observed in SDS-PAGE with Coomassie brilliant blue stain but they seemed to be also immunodominant against the host because they were strongly recognized in Western blotting. Parasites were hardly detected in the blood smear at convalescence, but all the antigens, 28, 32, 34 and 38 kDa BMag, were recognized by Western blotting with the convalescent serum. From these results, these antigens were immunodominant in the Syrian hamsters infected with *B. microti* and the antibodies against these antigens had important roles for inhibition of this parasite.

*B. microti* parasitizing Syrian hamsters was destroyed by phagocytosis and activities of neutrophils and monocytes and treated with specific antibodies. It was shown that these mechanisms improved parasitemia and other hematological findings in convalescence, although *B. microti* persistently infected the Syrian hamsters.

REFERENCES