Activation of Bovine Peripheral Blood Monocyte and Its Suppressive Effect on Parasitemia in Theileria sergenti Infected Calves

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ABSTRACT. Activation of bovine peripheral blood monocytes and its suppressive effect on parasite growth was examined in Theileria sergenti-infected calves by using a rosette assay that detects changes in Fc receptor expression and by luminol-dependent chemiluminescence response. Monocyte activation preceded the peak of parasitemia but was depressed parallelly with the growth of the parasites. When four calves were treated with prednisolone, three showed a good correlation between the suppression of monocyte activity and an increase of parasitemia. — KEY WORDS: Fc receptor, monocyte, Theileria sergenti.


Recent works of our group have shown that T cells [14, 15], NK cells [15] and monocytes [2] are activated during the course of Theileria sergenti (T. sergenti) infection in calves. Macrophages are thought to be effector cells to attack parasitized erythrocytes in rodent malaria [10]. In T. sergenti-infected calves, macrophage oxidative burst activity has been shown to increase with parasitemia (percentage of parasitized erythrocytes, PE) [2], and this activity was enhanced when the parasites as stimulant were treated with opsonins [12].

Although little is known about the role in T. sergenti infection of this macrophage activation, it seems reasonable to suppose that it includes increased expression of specific surface receptors for the Fc portion of immunoglobulin G. A significant increase of Fc receptor (FcR) expression was observed in monocytes from patients with Plasmodium falciparum infection [13]. In the present study, we monitored the activation of monocytes by rosette assay (FcR expression) and chemiluminescence (CL) response during the course of T. sergenti infection in calves. We also examined the relationship between the activity of monocytes and parasitemia in T. sergenti-infected calves treated with prednisolone (Pn).

MATERIALS AND METHODS

Experimental calves and inoculum: Two (No. 1 and 2), 3 to 6 month-old calves were splenectomized and inoculated subcutaneously with T. sergenti (Chitose stock)-infected blood. Four (Nos. 3 to 6), 6 to 12 month-old calves which had been splenectomized and inoculated subcutaneously with T. sergenti-infected blood were treated with Pn. All 4 calves showed a low level of PE (less than 1% in Nos. 3, 4 and 6, and 5% in No. 5) at the time of Pn administration. Each calf was injected with 100 mg/day of Pn intramuscularly for one week (No. 3), two weeks (No. 4) or 5 weeks (Nos. 5 & 6), respectively.

Blood samples were collected in citrate phosphate dextran as anticoagulant every week and used for hematological tests and as the source of monocytes. PE was assessed by examination of Giemsa-stained blood smears. Antibody against T. sergenti was detected by an indirect fluorescent antibody test.

Chemiluminescence (CL) response: CL of monocytes was measured with a bioluminescence reader (Biolumat LB 9500) at room temperature as described previously [2]. Briefly, monocytes from peripheral blood mononuclear cells (PBMC) were suspended in Hanks balanced salt solution (HBSS) without phenol red, and adjusted to 1×10⁶ cells/200 μl. Twenty μg of luminol were added to a 200 μl monocyte preparation and incubated for 10 min. When the background counts had fallen and become constant, 10 μl of each stimulant was added to the
vial and the CL response was monitored for 30 min. To test monocyte activity against specific and nonspecific stimulation, *T. sergenti* merozoites and zymosan (Shigma Co.) were used as stimulants, respectively. These stimulants were opsonized with either antibody-positive serum or with autologous sequentially collected serum. The method for opsonization has been described previously [2, 12].

**Rosette assay for Fc receptor expression:** PBMC, separated by the Ficoll-Conray method were suspended in HBSS containing 20% fetal calf serum, then placed in tissue-culture chamber slides (Lab-Tek 8 chamber, Nunk Co.) and incubated for 1 hr at 37°C. The resultant adherent cell monolayers, consisting of more than 95% monocytes after being washed with HBSS to remove non-adherent cells, were used for rosette assay for FcR expression as described previously [11]. Antibody sensitized red blood cells (RBC) were prepared by incubating 2% sheep RBC solution with rabbit anti-sheep RBC serum for 10 min at 37°C. The cells were then washed twice and resuspended in HBSS at a concentration of 1%. Sensitized RBC were added to each monocyte monolayer and allowed to settle at room temperature for 1 hr. The monolayers were then washed, fixed with 0.5% glutaraldehyde and stained with citrate-buffered Giemsa. Four hundred cells were counted per slide, and those with three or more RBC being adhered to were scored as rosette positive, which were then expressed as a percentage of the total monocyte population.

**RESULTS**

An increase of FcR expression in monocytes from *T. sergenti*-infected calves as evident by an increase in rosette positive cells, was observed when compared with that from normal calves (Fig. 1). PE and monocyte activity of two *T. sergenti*-inoculated calves (Nos. 1 and 2) were monitored by examining their blood smears, CL response and expression of FcR (Fig. 2a, b). PE were detected on day 10 post-inoculation (PI) and then gradually increased by day 30 PI, reaching a peak on day 35 PI. CL responses to zymosan increased after the infection and the highest cpm was observed around day 20-25 PI, which preceded the PE peak. In contrast, CL response to merozoites was relatively low until day 30 PI but rose to a peak between day 35-38 PI, which corresponds to that of PE peak. Before the parasite inoculation, expression of FcR in monocytes was less than 5%. However that percentage gradually increased during the course of the infection, reaching a peak around day 30 PI, just before the peak of PE. The monocyte activity, as determined by the CL response and the expression of

![Fig. 1. Rosette assay for Fc receptor expression on monocytes from a calf infected with T. sergenti (× 1,200) Left: Rosette negative (Before inoculation of T. sergenti) Right: Rosette positive (After T. sergenti infection).](image-url)
MONOCYTE ACTIVATION IN *T. SERGENTI* INFECTED CALVES

Fig. 2. Comparison of parasitemia (percentage of parasitized erythrocytes, PE), CL responses and expression of Fc receptor (FcR) in calves infected with *T. sergenti* a, b: Responses after *T. sergenti*-infection (a, No. 1 calf; b, No. 2 calf) c: Responses in *T. sergenti*-infected calf (No. 3) after prednisolone (Pn) treatment for one week. For CL assay, opsonized zymosan (●) and opsonized merozoits (●) were used as stimulants.

Fig. 3. Comparison of parasitemia (PE), CL responses and antibody (Ab) titer in *T. sergenti*-infected calves after prednisolone (Pn) treatment (—). Each calf was inoculated with 100 mg/day of prednisolone for two weeks (a, No. 4) or five weeks (b, No. 5; c, No. 6). For CL assay, opsonized zymosan (●) and opsonized merozoits (●) were used as stimulants.

FcR, increased before the peak of parasitemia and then decreased with the grow of the parasites.

To examine whether suppression of cellular immunity by Pn administration will increase PE or not, three calves with low level of PE (less than 1% in Nos. 4 and 6, and 5% in No. 5) were treated with Pn for 2 to 5 weeks, and PE and CL response were monitored (Fig. 3). During Pn treatment in calves Nos. 4 and 5, PE gradually increased. Before Pn administration, CL responses against both zymosan and merozoits were relatively high in calves Nos. 4 and 5, but these activities immediately decreased after the Pn treatment (Fig. 3a, b). Antibody titers also decreased to a low level in calves Nos. 4 and 6 (Fig. 3a, c). Calf No. 6 showed low CL response and low PE before Pn treatment but the former increased slightly and the latter gradually increased during the course of the Pn treatment. (Fig. 3c). A
relatively good correlation between the suppression of monocyte activity and an increase of PE in calves Nos. 4 and 5 was observed. During Pn treatment, an increase in CL response was noted for day 30 followed by an increase in parasitemia as shown in Fig 3b, c. We also examined the FcR expression on monocytes after Pn treatment (Fig. 2c). In calf No. 3, CL response decreased after Pn treatment and the PE increased correspondingly. However, no significant alteration of the expression of FcR was noted.

**DISCUSSION**

We had previously reported the activation of monocytes in *T. sergenti*-infected calves by CL response [2]. This CL response was enhanced when the stimulant was treated with whole antibody molecule but not with papain-treated Fab portion of the antibody [12]. This result leads us to the idea that the expression of FcR may increase during *T. sergenti* infection. In the present experiment, a significant increase of FcR expression in the monocytes from the infected calves was observed but the peak of the activity determined by CL response and FcR expression differed from each other as seen in Fig. 2, suggesting different activation processes might be present in CL response and FcR expression.

Activation of monocytes may be caused by gamma interferon or other lymphokines released from activated T cells. Activated monocytes or macrophages may attack to parasitized RBC. The phagocytic activity of macrophage is believed to contribute to the clearance of parasitized RBC in liver and spleen during malaria infection [10]. Alternatively, activated macrophages may release factors toxic to the malarial parasite such as superoxide anion which may cause death to the intraerythrocytic parasite.

Immunosuppression of monocyte activity during malaria infection has been reported [1, 7]. Monocyte activation has been shown to initially increase in acute *Plasmodium knowlesi* infection and then decrease parallelly with a drop in NADPH oxidase activity [7]. Furthermore, cytotoxicity of splenic macrophages is enhanced during the early phase of infection with *Plasmodium berghei* and inhibited in the latter phase of the disease [9]. In *T. segenit* infection in calves, a similar phenomenon was observed in our previous [2] and present studies, that is the monocyte activities (CL response and FcR expression) increased with the appearance of the parasites and decreased parallelly with the increase of PE. We have also previously found immunosuppressive factors in sera from *T. sergenti*-infected calves [15]. Thus, the decrease of the monocyte activity in the infected calves (Fig. 2a, b) may be caused by the immunosuppressive effect of the parasites.

Glucocorticoid treatment is known to cause an increase of PE in *T. sergenti*-infected cattle by interfering with the function of macrophages and T cells such as the release of IL-1, and IL-2, and the actions of gamma interferon on macrophages [5, 6, 8]. We speculated that glucocorticoid treatment may directly or indirectly suppress macrophage activity thus resulting in an increase of PE. Among the four *T. sergenti*-infected calves treated with Pn, three of them showed a good correlation between the suppression of monocyte activity and an increase of PE (Fig. 2c, 3a, b). One calf (No. 6 in Fig. 3c) showed an increase of PE without any change in monocyte activity because of the low level of CL response before Pn treatment. Although the importance of T cells in *Plasmodium chabaudi adami* blood-stage immunity was reported in the mouse malaria system [3, 4], the mechanism of T cells in killing parasites *in vivo* is still unknown. T cell could be activated by monocytes which phagocytize and process parasitized RBC, and subsequently display parasite antigen on their surface. For studying *T. sergenti* blood-stage immunity, we should therefore focus on the activation mechanisms of T cells and macrophages.

**REFERENCES**