Changes of Serum Hemolytic Activity and the Number of Reticulocytes in Canine Babesia gibsoni-Infection

Takafumi ONISHI* and Sayoko SUZUKI
University of Osaka Prefecture, College of Agriculture, Department of Veterinary Internal Medicine, Sakai, Osaka 591, Japan
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ABSTRACT. When the serum hemolytic activity in Babesia gibsoni-infected dogs was determined with self red blood cells from the infected animals, the decrease in the activity is paralleled with the increase in the number of reticulocytes. The activity determined with red blood cells from phenylhydrazine-induced anemia also decreased parallel with the increase in the number of reticulocytes. These results suggest that the rapid decrease in the serum hemolytic activity after reaching the peak is due to the increase in reticulocytes, which are probably unsusceptible to the hemolytic factor(s).—Key words: Babesia gibsoni, reticulocyte, serum hemolytic activity.


In the previous study we have reported that the decrease of hematocrit value in Babesia gibsoni-infected dogs was accompanied with the increase in serum hemolytic activity [1]. This suggests that progressive anemia of the disease is even if at least partly, due to the hemolytic factor(s), which appears in blood concomitantly with an initiation of parasitemia after the infection [1, 2]. However, after 16th day of the infection, the hemolytic activity decreased rapidly when determined with self red blood cells from the infected animals, whereas the activity decreased gradually when determined with nonself red blood cells from uninfected animals [2]. Preliminary experiments implied that the rapid decrease in serum hemolytic activity with self red blood cells is due to the appearance of resistant cells, possibly of reticulocytes, in blood. In the present study, therefore, we investigated the correlation between the increase in the number of reticulocytes and the decrease in serum hemolytic activity during B. gibsoni-infection in dogs. In addition, to examine the effect of the reticulocytes on the serum hemolytic activity, the activity was determined with the red blood cells from the dogs of phenylhydrazine-induced anemia.

Experimental infection with B. gibsoni in dogs and the assays for serum hemolytic activity, hematocrit value and parasitemia were done as described previously [1]. The number of reticulocytes were counted in 1,000 red blood cells in thin blood smears stained with Brilliant Kresyblue and then with Giemsa solution. The cells were calculated as percentage in total number of red blood cells.

Figures 1-a and -b show the changes in hematocrit value and parasitemia and those in serum hemolytic activities and the number of reticulocytes after experimental infection with B. gibsoni in dogs, respectively. As we reported previously [1], the serum hemolytic activity determined with self red blood cells increased gradually and then decreased rapidly after reaching the maximum, whereas the activity determined with nonself red blood cells increased parallelly but decreased more gradually than that with self red blood cells (Fig. 1-b). To see whether this rapid decrease in the hemolytic activity with self red blood cells is due to with the changes in red blood cell composition, the number of reticulocytes was determined during the course of the anemia. The number of reticulocytes in B. gibsoni-infected dog increased, though it delayed the increase in both serum hemolytic activities determined with self and nonself red blood cells, and then decreased gradually (Fig. 1-b). The period of continuously high level in the number of reticulocytes corresponds to that of the rapid decrease in serum hemolytic activity with self red blood cells, suggesting that the marked decrease in the activity is due to the increase in reticulocytes in assay system.

To examine further the correlation between the de-
Fig. 2. Serum hemolytic activity of B. gibsoni-infected dog determined with red blood cells from phenylhydrazine-induced anemia. The anemia was induced in two dogs as described in the text and the red blood cells were prepared from them on the day indicated. One of two replicate experiments, which gave very similar results, was shown in the figure. The arrows indicate the day of injection of the drug. The sera were pooled from each two of B. gibsoni-infected and uninfected dogs and stored until used. The hemolytic activity was determined as described previously [2]. Symbols: O, hemolytic activity of the serum of B. gibsoni-infected dog determined with red blood cells from the drug-induced anemia; ●, the activity of uninfected animal determined with red blood cells from the anemia; △, the number of reticulocytes and ■, hematocrit value.

crease in serum hemolytic activity and the increase in the number of reticulocytes, the serum hemolytic activity of B. gibsoni-infected dogs was determined with red blood cells from the animal of phenylhydrazine-induced anemia. The anemia was induced by subcutaneous injection of phenylhydrazine at a daily dose of 7.5 mg per kg of body weight until a marked anemia (25% or less in hematocrit value) was induced. The serum used for the assay was obtained from dogs 15 days after B. gibsoni-infection and stored at −40°C. Figure 2 shows that the decrease in serum hemolytic activity determined with the red blood cells prepared from the drug-induced anemia was parallele with the increase in the number of reticulocytes. When the injection of the drug was terminated and the animal recovered from the anemia, the activity returned to the same level before the induction of the anemia. The hemolytic activity in the serum of uninfected animal determined with red blood cells from the drug-induced anemia remained very low throughout the period tested (Fig. 2). Similarly, the hemolytic activity in the serum of the animals of the phenylhydrazine-induced anemia determined with red blood cells from uninfected animals was very low and remained almost on the same level throughout the experiment (Data not shown). This indicates that anemia in dogs does not always cause an increase in the serum hemolytic activity.

The present results show that the rapid decrease in serum hemolytic activity after about two weeks of B. gibsoni-infection is probably due to the increase in the number of reticulocytes in blood, which are seen to be unsusceptible to the serum hemolytic factor(s). At present, the significance of the increased serum hemolytic activity in canine B. gibsoni-infection is not yet clearly defined. However, the finding that progressive anemia in the infection is accompanied with the increase in serum hemolytic activity suggests that the hemolytic factor(s) plays, even if it is at least a part, a role in the induction of the anemia. Accordingly, the decrease in the serum hemolytic activity followed by the increase in the number of reticulocytes is considered to be a way of resistance of the host to the infection by producing the unsusceptible cells to the hemolytic factor(s), although these immatures cells are physiologically less functional than the matured cells.

REFERENCES