Studies on Feline Haemobartonellosis

V. Role of the Spleen in Cats Infected with

Haemobartonella felis

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Abstract. The role of the spleen in feline haemobartonellosis was studied experimentally in 19 cats. The relationship between parasitemia and hematocrit (Ht) value, and the site of sequestration in 51Cr-labeled parasitized erythrocytes were mainly examined in both splenectomized and non-splenectomized cats. In splenectomized cats the Ht value showed no significant changes in the early stage of infection in spite of the appearance of Haemobartonella felis on erythrocytes. In cats with the spleen intact it showed remarkable changes corresponding to the appearance and disappearance of the organism on erythrocytes during the experimental period. The splenic radioactivity of infected cats was six times as high as that of normal cats even in the early stage of infection. It increased markedly according to an increase in number of organisms appearing on erythrocytes. The hepatic and pulmonary radioactivity of infected cats showed only a slight or moderate increase in the severe stage of infection. On the contrary, the radioactivity of the organs examined in splenectomized cats did not increase significantly in the early stage of infection, although the radioactivity of liver, lung and bone marrow in these cats was two to three times as high as that in control animals in the severe stage of infection. In addition, parasitemia persisted apparently for a longer time in splenectomized cats than in cats with the spleen intact. From these results it is assumed that parasitized erythrocytes in cats infected with H. felis may be sequestrated mainly by the spleen and that the organism may be removed from host cells, without destroying the same cells in the spleen.

It is known that splenomegaly is present in haemobartonellosis and is often the first and only readily apparent sign of latent infection [1]. The author observed in his previous studies that splenic enlargement changed with the progress of disease, and that in cats infected with Haemobartonella felis erythrocytes decreased rapidly in number following the appearance of H. felis on them, but that they tended to return soon to their previous level after the disappearance of the parasite from them [6, 7]. From those studies it was conjectured that parasitized erythrocytes in infected cats might have been sequestrated temporarily in the spleen. On the other hand, splenectomy is widely known as a potent activator of latent Haemobartonella infection, in which it induces severe hemolytic anemia leading frequently to death [1]. Thus the spleen seems to play an important role not only in suppressing Haemobartonella infection [10], but in stimulating the disease. The present study
was then carried out to clarify the role of the spleen in feline haemobartonellosis.

**Materials and Methods**

1. **Experimental animals**
   
   Nineteen healthy domestic cats ranging from one to five years of age were used for this study. Splenectomy was performed in 9 cats anesthetized with ketamine hydrochloride at least four weeks before the beginning of the experiment. During this period Giemsa-stained blood smears from these cats were examined microscopically for *H. felis* at three-day intervals. All the cats, except four serving as controls, were injected intraperitoneally with 1 ml of heparinized blood from a carrier cat of *H. felis*. For the purpose of finding the relationship of parasitemia and erythrocyte destruction, five of them (3 splenectomized and 2 unoperated cats) were examined for hematocrit and *H. felis* in blood smears every day for 60 days. Blood samples were collected from them by venesection of the auricular vein. The remaining 14 cats were used for the study on the site of sequestration of erythrocytes parasitized with *H. felis*.

2. **Observations**
   
   The examination for the site of sequestration of parasitized erythrocytes was carried out over a period from the 10th to 15th day (early stage of infection; group 1) and over a period from the 30th to 35th day (severe stage of infection; group 2) after inoculation. Each experimental group consisted of 2 splenectomized and 3 unoperated cats. Two splenectomized and 2 unoperated cats all of which were free from *H. felis* were also examined as controls. In addition, hematocrit determination, erythrocyte osmotic fragility test, direct Coombs test, and *Haemobartonella* body count were performed in each experimental animal over the same period by the methods mentioned in the previous studies [8]. The site of sequestration of erythrocytes in the experimental cats was determined by the following methods. From the jugular vein of a cat anesthetized with ketamine hydrochloride about 3 ml of blood was collected into a syringe containing 0.5 ml of acid citrate dextrose. The whole blood was incubated with 40 μCi Na$_2$tCrO$_4$ (Chromotop sodium; Daiichi Radioisotope Laboratories, Ltd.; specific activity, 227.5 mCi/mg$^{51}$Cr at 37°C for 30 minutes. Labeling was terminated by the addition of 50 mg of sodium ascorbate. Then exactly 5 ml of labeled blood was transfused into the jugular vein of the cat. The animals was sacrificed 24 hours after injection with the labeled blood by intravenous injection with sodium pentobarbital. The spleen, liver, kidneys, bone marrow, lungs and heart were removed immediately and weighed. The radioactivity of one gram of each tissue samples was determined in a well-type scintillation counter (Aloka, PS-9). Total organ radioactivity was calculated by multiplying specific activity (Cpm/g) by the weight of the organ. The bone-marrow radioactivity was determined by counting one gram of the marrow obtained from the femur. The total radioactivity of the injected erythrocytes was calculated from 20 μl of the labeled blood. Organ radioactivity was then expressed as per cent of the total injected activity.

**Results**

1. **Relations between parasitemia and Ht value**
   
   The Ht value in the unoperated cats showed increase and decrease repeatedly according to the disappearance and appearance of *H. felis* on erythrocytes during the experimental period. In the splenectomized cats, on the contrary, it showed no significant variations in spite of the appearance of *H. felis* on erythrocytes in the early stage of infection, although it showed a rapid decrease in the period of severe parasitemia. In addition, the recovery of the decreased Ht value in the splenectomized cats was slower than that in the unoperated cats. Two of the splenectomized cats died of severe hemolytic anemia on the 36th and 42nd day, respectively, after inoculation. The unoperated cats recovered from anemia after the termination of the experiment. On the other hand, parasitemia continued over a period of 1 to 16 days, or 7 days on the average, in the splenectomized cats, and over a period of 1 to 10 days, or 3.5 days on the average, in the unoperated cats. These results are shown in Fig. 1.

2. **Erythrocyte osmotic fragility and direct Coombs tests**
   
   After splenectomy, the osmotic fragility of erythrocytes decreased markedly in all
the cats examined; that is, the average of hemolysis in 0.55% saline concentration was 19.3% before splenectomy and 1.4% after splenectomy. It increased markedly following the appearance of \textit{H. felis} on erythrocytes in both splenectomized and unoperated cats (Fig. 2).

The direct Coombs test was positive in all the cats of group 2 and negative in all those of group 1.

3. Organ distribution of \textsuperscript{51}Cr-labeled erythrocytes

Splenectomized cats: In the cats of group 2 mean total liver and bone marrow radioactivity was two times and mean total lung activity three times as high as that in the control cats. All the organ examined in group 1 failed to show any significant increase in radioactivity, as compared with those in the control group.

Unoperated cats: (a) group 1: Mean total splenic radioactivity was about six times as high as that in the normal control cats, and two times as high as that of the liver. No other organs showed any significant change. (b) group 2: Mean total splenic radioactivity was about twelve times as high as that in the normal control cats and three times as high as that of the liver. Mean total liver radioactivity was a little higher than, and mean total lung radioactivity two times as high as, that in the normal control cats. The kidneys, heart and bone marrow demonstrated no changes.

Total splenic radioactivity in the cats infected with \textit{H. felis} increased markedly corresponding to high percentage of paratized erythrocytes in the labeled blood. Any other organ of the unoperated cats did not show such corresponding increase in radioactivity. These results are shown in
Table 1 and Fig. 2.

Discussion

The site of sequestration of erythrocytes with naturally occurring or experimentally induced erythrocyte abnormalities in man and animals has been studied by a number of investigators [2, 4–5, 11]. As a result, it was demonstrated that erythrocytes only slightly injured passed through the liver and were sequestered mainly by the spleen, and that the hepatic sequestration was in proportion to the severity of injury of erythrocytes.

In the present studies, $^{51}$Cr-labeled parasitized erythrocytes in unsplenectomized cats concentrated markedly in the spleen even in the early stage of infection. Such concentration was intensified with an increase in number of H. felis organisms on erythrocytes. In any other organ there were no significant concentrations of labeled cells in the early stage of infection, as compared with control animals. These cells, however, increased slightly or moderately in number in both liver and lungs in the severe stage of infection. In contrast to the observations on cats with the intact spleen, the total radioactivity of liver, bone marrow or lungs in splenectomized cats was
about two to three times as high as that of controls in the severe stage, although there was no significant increase in radioactivity in the early stage of infection.

These results seem to be consistent with the observations that the hematocrit value in the cats with the intact spleen showed a rapid increase and decrease repeatedly corresponding to the disappearance and appearance of organisms on erythrocytes during the experimental period, and that the hematocrit value in the splenectomized cats, on the contrary, remained unchanged in spite of the appearance of organisms on erythrocytes in the early stage of infection, although it decreased intensively in the severe stage. These results indicate that the hematocrit value in the splenectomized cats in the early stage of infection might be unchangeable a reason why no parasitized cells were sequestrated by any specific organ in that stage.

The increase in severity of injuries of the cells, as indicated by an increase in osmotic fragility and a positive direct Coombs test, might lead to an increase in compensative sequestration and destruction of the cells by the liver, lungs and bone marrow in the severe stage of infection.

Moreover, parasitemia in infection with *H. felis* persisted apparently for a longer time in the splenectomized cats than in those with the intact spleen. This result may also verify the supposition that the parasitized erythrocyte of the cat infected with *H. felis* will be sequestrated mainly by the spleen.

Crosby [5] presented evidence that iron particles were pitted from siderocytes by the spleen and suggested that the other inclusions of erythrocytes might be removed in the same way. Schnitzer et al. [8] carried out an ultrastructural study on the spleen of a rhesus monkey infected with *Plasmodium knowlesi* and found that pitting of parasites did occur in the spleen. The as-
association of *Haemobartonella* with the host erythrocyte seemed to be so tenacious and so stable in heavily parasitized blood that no organisms were free in the plasma [9, 12]. A sudden total loss of organisms from erythrocytes, however, was also observed. The site and mechanism of such detachment of organisms from the host cells are yet unknown. In this respect, it is assumed from the results of the present study that the spleen of the cat infected with *H. felis* may have an action to remove the organism from its host cell by pitting or some other unknown mechanism.

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References


