Trop. Med., 28 (4), 279–284, December, 1986

Activation of Host Defense Mechanism against Murine Malaria by Homologous Erythrocyte Inoculation

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Abstract: It is difficult to estimate the involvement of the spleen function of destroying self-erythrocytes in host defense against malaria by using the procedure of splenectomy. Therefore, an attempt was made to activate only the above function in ICR mice by inoculating homologous or heterogenous erythrocytes before *Plasmodium berghei* infection. Only the mice treated with homologous erythrocytes showed delayed growth of parasitaemia and survived significantly longer than the mice treated with heterogenous erythrocytes or the control mice treated with culture medium. The spleen enlargement was also conspicuous only in the former mice in contrast to the mice treated with heterogenous erythrocytes which did not produce distinguishable effect.

Key words: *Plasmodium berghei*, Host defense, Spleen

INTRODUCTION

The spleen has been known to play an important role in malaria. Many experiments using rodent or simian malaria and the splenectomy at the porper time revealed the defense mechanism by the spleen in the course of infection (Conrad and Dennis, 1968; Barker and Powers, 1971; Wyler *et al.*, 1977; Oster *et al.*, 1980; Quinn and Wyler, 1980; Playfair, 1982; Waki *et al.*, 1985). These results suggested that the spleen fuction such as destruction of blood cells might undertake main part of host defense against malaria in the early course of infection until it might be soon replaced by immunodefense function. In the present work we attempted to activate the mouse spleen function of destroying self-erythrocytes by inoculating homologous erythrocytes before *Plasmodium berghei* infection in order to clarify the involvement of this function in host defense against malaria.

Received for Publication, October 20, 1986
Contribution No.1818 from the Institute of Tropical Medicine, Nagasaki University
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Materials and Methods

Parasite: *Plasmodium berghei* NK 65 (from Prof. M. Suzuki, Department of Parasitology, Gunma University School of Medicine) was used. Parasitized erythrocytes for the experimental infection were collected from an infected mouse with about 10% parasitaemia.

Mice: ICR female mice 5−6 weeks old were used.

Erythrocytes:

1) Homologous erythrocytes ICR mouse blood was collected in RPMI 1640 medium containing 10 U/ml heparin sodium and washed three times in RPMI 1640 by centrifugation at 800 × *g* for 5 min. After each centrifugation the buffy coat was aspirated off to remove other blood cell components than erythrocytes. Subsequently the packed erythrocytes were suspended in equal volume of RPMI 1640 medium and denaturated by incubation in a water-bath at 45℃ for 30 min.

2) Heterogenous erythrocytes Rabbit blood was prepared in the same manner except the denaturation step.

Erythrocytes inoculation: Three groups of mice:−the group A, B and C, each of which consisted of ten mice, were prepared. Each mouse of the group A was daily inoculated intraperitoneally with 0.5 ml of homologous erythrocytes suspension for three successive days. The group B and C were treated with heterogenous erythrocytes and RPMI 1640 medium, respectively in the same manner.

Spleen rate: Three mice from each group were sacrificed 24 hr after the final erythrocyte injection and body weight (BW) and spleen weight (SW) were measured. The same measurements were done whenever mice died after *P. berghei* infection. The spleen rate was expressed in the following formula. Spleen rate=SW/BW × 100.

Experimental infection: Each mouse was intraperitoneally inoculated with 10^7 parasitized erythrocytes 24 hr after the final erythrocyte injection. Subsequently, parasitaemias were daily counted on Giemsa stained films of tail blood. When a mouse died, the survival days and the spleen rate were recorded.

Results

As shown in Table 1, the inoculation of homologous erythrocytes induced conspicuous spleen enlargement in the group A mice, while that of heterogenous erythrocytes in the group B caused slight enlargement compared with that of RPMI 1640 medium in the group C. This result corresponded well with the result of survival days (Fig. 1.). The mean survival days (16.2 ± 1.2) in the group A were significantly longer than those in the group B (9.0 ± 3.3) and in the group C (7.8 ± 2.2). Furthermore, the spleen rate of the group A mice on their death was remarkably higher than other groups (Fig. 2).

The parasitaemia in the group A became detectable one day later than in other groups and its growth rate was slightly slower (Fig. 3). The suppression of parasitaemia growth appeared at the level of about 15% parasitaemia in the group B and C in contrast to about 3% in the group A.

Table 1. Spleen enlargement in ICR mice after erythrocytes inoculation

Experimental Group	Spleen Rate
Group A	2.31±0.47
Group B	1.03±0.10
Group C	0.82±0.08

Group A: 0.5 ml of homologous erythrocytes suspension was injected intraperitoneally for three successive days. Group B and C: 0.5 ml of rabbit erythrocytes suspension and RPMI 1640 medium were injected in the same manner, respectively. Spleen rate (= spleen weight/body weight × 100) was examined 24h after the final injection.

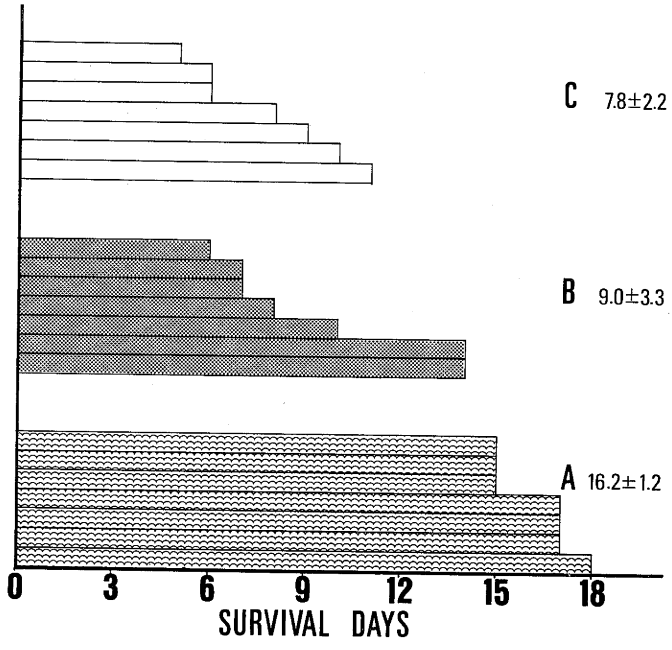

Fig. 1. Survival days of each mouse after *P. berghei* infection. A: group A, B: Group B, C: group C. Explanation of each group is shown in the legend to Table 1. A bar represents survival days of each mouse. Numerals indicated in A, B and C show respective mean survival days ± SE.

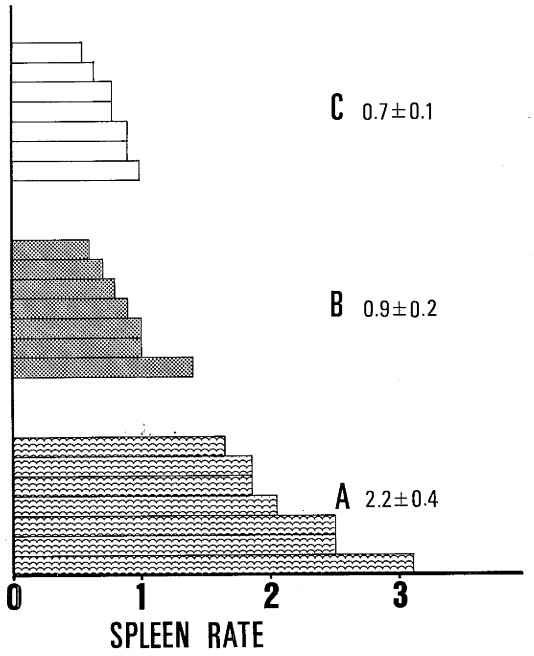

Fig. 2. Spleen rate of each mouse on its death. A bar represents spleen rate of each mouse. Numerals indicated in A, B and C show respective mean spleen rate ± SE. Explanation of A, B and C is shown in Fig 1.

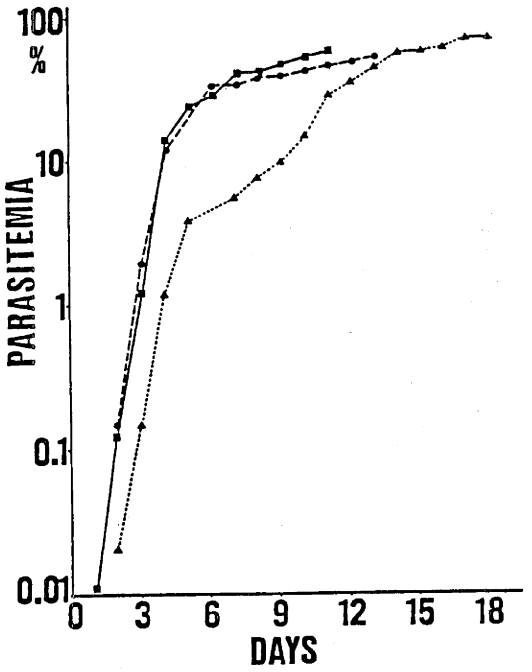

Fig. 3. Parasitaemia growth of each group of mice. Group A: ▲·····▲, Group B: ●---●, Group C: ■—■. Explanation of each group is shown in Table 1.

Discussion

Inoculation of homologous erythrocytes into ICR mice caused more effective enlargement of the spleen and induced significant prolongation of the survival time after lethal infection by *P. berghei*. Heterogenous rabbit erythrocytes were not able to produce distinguishable effect to mice. One of the reasons may be due to the intraperitoneal route of erythrocytes injection, for most of the heterogenous erythrocytes are probably captured or destroyed before they reach the spleen.

Intravenous inoculation of parasitized erythrocytes with *P. berghei* produced immediate growth of the parasitaemia (Waki *et al.*, 1985; Nakazawa, unpublished data), while intraperitoneal inoculation required a lag phase before parasitaemia development (Barker and Powers, 1971; Oster *et al.*, 1980; Quinn and Wyler, 1980). This suggests that only the parasitized erythrocytes at the young stage possibly enter the blood stream while those at the mature stage are caught before entering the blood stream. In the present work the parasitaemia showed about ten fold increase a day in the early course of infection and there was a delay for one day in the group A to reach the same level of parasitaemia compared with other groups. Therefore, it was supposed that 9/10 of the parasitized erythrocytes which successfully entered the blood stream are killed by the activated function in the group A mice. In contrast to activation of the destructive system against self-erythrocytes, activation of the phagocytic system by inoculation of heterogenous erythrocytes may have no effect on the splenomegaly and subsequent suppression of parasitaemia growth, because there are no difference between the group B and group C.

Suppression of parasitaemia growth which appeared from around five days after infection in the group B and C in the present work was generally seen in both murine (Barker and Powers, 1971; Oster *et al.*, 1980; Quinn and Wyler, 1980; Waki *et al.*, 1985) and simian malaria (Wyler *et al.*, 1977) but this effect was diminished by the splenectomy. These resuts suggested that the suppressive effects by the spleen were induced in experimental animals without any treatment when the parasitaemia reached to a certain level. Of course, these effects are considered as the total function of the spleen. If intraperitoneal inoculation of homologous erythrocytes in the present work activated only the spleen function of destroying self-erythrocytes, this function would be considered to be involved in host defense and promote the total spleen function.

Further investigation using intravenous inoculation in combination with splenectomy is required to give the definite conclusion.

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同系赤血球接種によるマウスのマラリアに対する抵抗性の増強

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脾臓を中心とした自己赤血球破壊作用がマラリアに対する宿主の防御機構にいかに関係しているかは，全機能を失わせる脾摘を用いた実験系では推定し難い．そこでこの作用のみを特異的に増強する目的で ICR マウスを用いて，同系の赤血球浮遊液を腹腔内に接種した．対照マウス群には異種の家兎赤血球浮遊液および浮遊用の培養液のみを接種した．上記接種の後に *Plasmodium berghei* の致死感染を行いその後の経過を観察した．同系赤血球処理を受けたマウス群のみが，血中の感染赤血球の出現およびその後の感染赤血球数増加の遅れを示し，結果的に有意の生存日数の延長を示した．これに一致して同マウス群のみが，赤血球処理後およびマラリア感染による死亡時の調べで，著名な脾の腫大を示した．一方異種赤血球処理マウス群は，培養液のみの接種群との間に有意の差を認めず，上述のマラリア防御にかかわる効果を生じ得なかった．

熱帯医学　第28巻　第4号　279−284頁，1986年12月