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Effective Recovery and Immunity to Virulent Malaria Following Red Cell Transfusion at Crisis[1] (38126)

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(Introduced by F. Stohlman, Jr.)

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The course of malaria infection and the ability to develop acquired host resistance to the parasite differs considerably among the various species of rodents. In this regard, *Plasmodium berghei* infection in mice has been carefully evaluated by Mercado and Coatney (1). Their studies revealed the presence of a 3–6 day prepatent period in which parasites were absent from the circulation. The ensuing patent period was characterized by a rapid increase in circulating parasites, lasting from 4–19 days, and by the development of a severe anemia. Although erythropoiesis was enhanced, as evidenced by a significant reticulocytosis, anemia was uncompensated as confirmed by low hematocrit values. The infection was fatal between days 8 and 29 and occurred approximately 24 hr after peak parasitemia. It is obvious from these data, that the mechanisms involved in acquired host resistance to *P. berghei* infection appear to be inadequate in the mouse.

In mice, *P. berghei* parasites have a definite prediliction for reticulocytes, as opposed to the most mature erythrocytic elements (2). Accordingly, it is of little surprise that mice undergoing enhanced erythropoieses, secondary to injection with phenylhydrazine (3) or antimouse erythrocyte rabbit serum (4), demonstrate an earlier and more marked parasitemia which parallels their reticulocytosis. Conversely, mice which are erythropoietically suppressed by red cell hypertransfusions are able to partially block subsequent *P. berghei* infection (5). Furthermore, if erythropoiesis is completely eliminated by red cell hypertransfusions coupled with X-irradiation, malarial infection does not ensue (loc. cit.).

The present investigation was initiated in an attempt to prolong the survival of *P. berghei*-infected mice. Repeated transfusions of red cells were given during peak parasitemia, just prior to the expected time of death. It was our feeling that such a maneuver would not only alleviate the consequences of severe anemia, but would also depress the numbers of available reticulocytes and hence parasitemia. This manipulation could, perhaps, allow for adequate time for development of host resistance and recovery from an otherwise lethal *P. berghei* infection. Information of this kind may bear significance not only from the implication of red cell transfusion as a possible therapeutic measure in malarial infection, but also from the standpoint of developing an organism which could be employed in future experimentation relative to acquired humoral and cell-mediated host resistance to the disease.

Materials and Methods. Thirty virgin female CD-1 mice (Charles River Labs.), 10–12 weeks of age, were given a single intraperitoneal injection of 15.0×10^6 red cells infected with *Plasmodium berghei* (NK/65 strain). At designated times thereafter, groups of five control and fifteen infected mice were bled from the tail vein. Red cell counts, hematocrit and hemoglobin concentration were determined electronically with an MK3 Haema-Counter (General Science Corp.). Percent parasitemia was determined on blood smears fixed in methanol and stained with Wright's and Giemsa's solutions. The percent reticulocytes were determined from blood stained with new methylene blue, in accord with the method of Brecher (6). Circulating parasites and re-

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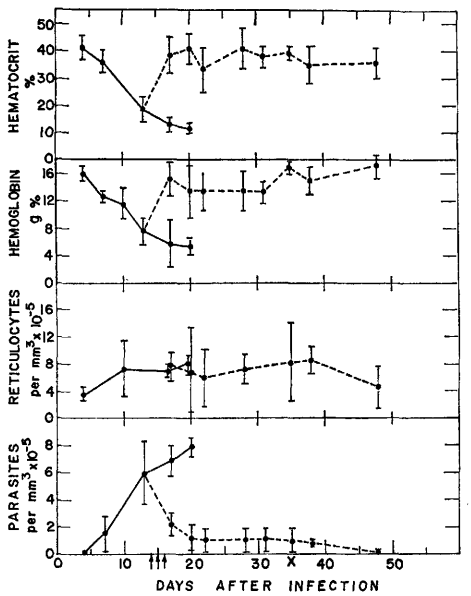

FIG. 1. Course of *Plasmodium berghei* infection in mice with and without red cell transfusion. (—) Infected mice. (– –) Infected mice given transfusions. (↑) designates transfusion. (×) designates reinfection with *P. berghei*. Each point represents the mean ± 1 S.D. of 8–15 animals.

ticulocytes were converted to absolute numbers/mm³ of blood. All parameters were expressed in terms of group mean ± 1 S.D. Fifteen additional *P. berghei*-infected mice were transfused intraperitoneally with approximately 1.0 ml of saline-washed homologous red cells on days 14, 15 and 16 of the infection as previously described for normal mice (7). On day 35 mice so transfused were reinfected with *P. berghei* as described above.

Results. The course of *P. berghei* infection in CD-1 mice is shown in Fig. 1. In approximately 90% of the animals, the infection was fatal within 24 days, with peak mortality occurring between days 18–20. All mice became severely anemic within 2 weeks, with the lowest values for hematocrit (11.7 ± 1.6%) and hemoglobin (5.2 ± 1.2g%) seen on day 20. A 2-fold reticulocytosis (7.3 ± 4.4 × 10^5/mm³) was apparent on day 10 and remained at this level during the course of infection. Parasitemia was pronounced by day 7 (1.5 ± 1.2 × 10^5/mm³) and progressed to peak levels on day 20 (7.9 ± 1.3 × 10^5/mm³).

Transfusion of normal homologous erythrocytes into *P. berghei* infected mice on days 14, 15 and 16 increased survival to approximately 90%. The hematocrit and hemoglobin values were brought into the control range by day 17 and remained at these levels throughout the course of study (i.e. day 48). The absolute numbers of circulating reticulocytes were not significantly altered by transfusion. They remained elevated at approximately 6–8 × 10^5/mm³ of blood up to and including day 38 and gradually declined to control values by day 48. In contrast, the absolute numbers of circulating parasites decreased abruptly as a result of transfusion from 6.0 ± 2.3 × 10^5/mm³ on day 13 to 2.0 ± 1.5 × 10^5/mm³ on day 17. Thereafter, the number of circulating parasites gradually declined and were virtually eliminated by day 48.

When transfused mice were rechallenged with *P. berghei* on day 35, the circulating parasites did not increase above pre-challenge levels and were eliminated from the circulation as previously described. Although there was a slight depression in hematocrit and hemoglobin levels on day 38, the differences were not significant from control values.

Discussion. The course of *P. berghei* infection in CD-1 mice, described herein, is not significantly different from that previously reported by Mercado and Coatney (1). Of significance, however, is our finding that transfusion of homologous red cells into *P. berghei* infected mice dramatically enhances recovery to this otherwise lethal infection.

As shown in Fig. 1, *P. berghei* infected mice develop a severe anemia within 2–3 weeks, as evidenced by the precipitous decline in hematocrit and hemoglobin values. By day 10, a 2-fold reticulocytosis was noted, which remained at this level throughout the course of infection. Since there is no available data pertaining to the circulating life span of reticulocytes in malaria infected mice, a definitive statement regarding the kinetics of erythropoiesis is not possible. Based on our findings, however, it would appear that erythropoiesis did not keep pace with the rate of red cell destruction and hence the infected mice remained in a state of uncompensated hemolytic anemia. In this regard, it has previously been shown (8) that erythropoiesis in humans suffering from acute malaria, is temporarily inhibited for approximately 5–10 days after the onset of symptoms. Although the mechanism

for this inhibition is unclear, the inability of *P. berghei* infected mice to overcome the anemia, as shown in the present study, may perhaps be attributed to factors relating to impaired erythropoietin generation and/or stem cell resistance to erythropoietin.

Repeated transfusions of homologous red cells for the most part restored the erythroid status of *P. berghei* infected mice (Fig. 1). In spite of the return of hematocrit and hemoglobin to control levels, however, reticulocytes did not decrease in number, but persisted at the same 2-fold level throughout the course of infection. These data indicate that red cell destruction was still occurring in these animals, but anemia did not become manifest since the rate of erythropoiesis was fully compensatory subsequent to replacement of prior red cell deficits by transfusion. Although the early decline in absolute numbers of circulating parasites subsequent to transfusion of red cells, may in part reflect an initial dilution effect, the further decline in their numbers between days 16–22, would suggest the destruction and elimination of parasitized red cells. It is also possible, however, that transfusion of red cells into *P. berghei* infected mice may have depressed the rate of erythropoiesis and hence the numbers of newly available reticulocytes which have been shown to be preferentially invaded by this parasite (2). Accordingly, the persistent elevation in circulating levels of reticulocytes following transfusion may merely represent a class of cells with perhaps a longer circulating life span.

The present study clearly shows that repeated transfusions of red cells into *P. berghei* infected mice allows for development of host resistance and recovery to an otherwise lethal malarial infection. Although the mechanism whereby this is accomplished is at present unknown, it may perhaps relate to the alleviation of some of the severe consequences associated with acute hemolysis, thus allowing adequate time for the development of immunologic competence required to overcome the infection. Complete resistance is further exemplified by the inability of recovered mice to develop malaria upon reinfection with the parasite.

Summary. Mice infected with the malarial parasite, *P. berghei,* become severely anemic within 2–3 weeks, with approximately 10% survival noted by day 24. Transfusion of homologous erythrocytes into these mice on days 14, 15 and 16, completely restored peripheral erythroid parameters to control levels and increased survival to 90%. These data indicate that repeated red cell transfusions into *P. berghei* infected mice is an effective maneuver which allows for development of host resistance and recovery to an otherwise lethal infection. Complete immunity is further attested by the ability of recovered mice to block the development of malaria upon reinfection with the parasite.

1. Mercado, T. I., and Coatney, R. G., J. Parasitol. **37**, 479 (1951).

2. Zuckerman, A., J. Infect. Dis. **100**, 172 (1957).

3. Singer, I., J. Infect. Dis. **94**, 159 (1954).

4. Schwink T. N., Amer. J. Trop. Med. Hyg. **9**, 293 (1960).

5. Ladda, R., and Lalli, F., J. Parasitol. **52**, 383 (1966).

6. Brecher, G., Amer. J. Clin. Pathol. **19**, 895 (1949).

7. Morse, B. S., Rencricca, N. J., and Stohlman, F. Jr., Blood **35**, 761 (1970).

8. Trowell, H. C., *in* "Non-Infective Diseases in Africa", Edward Arnold Publishers, Ltd., London (1960).

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