

## Erythropoietin Production in Virulent Malaria

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Erythropoietin, the hormone responsible for stimulating erythrocyte production, was shown to increase significantly in the serum of mice during virulent malaria infection. Although erythropoiesis was enhanced, it did not keep pace with the rate of erythrocyte destruction; hence all *Plasmodium berghei*-infected mice quickly succumbed to the deleterious consequences of severe uncompensated hemolytic anemia. Since this apparently inadequate rate of erythropoiesis is not attributed to impaired erythropoietin generation, mechanisms relating to (i) hemopoietic stem-cell resistance to endogenous erythropoietin, (ii) deficits in numbers of hemopoietic stem cells, and/or (iii) ineffective erythropoiesis are of interest.

Anemia is a major consequence in the pathology of malaria, as observed in humans (6, 12, 22) and in rodents (5, 19, 27). Anemia associated with malarial infection is attributed to the destruction of parasitized erythrocytes as a consequence of parasitic schizogony, and to the elimination of parasitized and nonparasitized erythrocytes in concert with immune and phagocytic mechanisms (20, 25, 26, 30). However, since the maintenance of normal circulating erythrocyte values in mammals is governed by the precise homeostatic balance between erythropoiesis and erythrocyte destruction (11, 24), it is conceivable that depressed and/or enhanced but inadequate rates of erythropoiesis may likewise be a contributing factor in the anemia of malarial infection. Suggestive evidence for the former possibility derives from studies which show that humans, during acute primary *Plasmodium vivax* and *Plasmodium falciparum* infection have decreased numbers of marrow erythroid precursors (21, 24) and an impairment on the part of these cells to incorporate radioiron in vitro (23). Conversely, although it has been shown that erythropoiesis in rodents is enhanced in medullary (4) and ectopic (18) sites in response to the attendant anemia and hypoxemia of malarial infection, it is clear that erythrocyte production does not keep pace with the rate of erythrocyte destruction. Hence, infected animals quickly succumb to the deleterious consequences of severe uncompensated anemia. In view of the above, it is impossible to make a definitive statement concerning the dynamics of erythropoiesis during malarial infection at the present time. However, based on the data reported herein, the impairment does not seem to be related to inadequate erythropoi-

etin (EP) generation since *P. berghei*-infected mice do, in fact, elaborate significant quantities of EP into their serum during the progression of infection and ensuing anemia.

### MATERIALS AND METHODS

Female CD-1 mice, 10 to 12 weeks of age, were injected intraperitoneally with  $15.0 \times 10^6$  *P. berghei* (NK/65 strain)-infected erythrocytes. At designated times thereafter, groups of 6 control and 12 infected mice were bled by cardiac puncture for determination of erythroid indexes, parasitemia, and serum EP titers. Erythrocyte enumeration and percentage of hematocrit were measured electronically with an MK 3 Haema-counter (General Sciences Corp.). The percentage of parasitemia was determined from blood smears stained with Wright and Giemsa solutions. The percentage of reticulocytes was monitored from slides of blood stained with new methylene blue. Serum EP levels were assayed in adult female CD-1 mice, after hypertransfusion on days 0 and 1 with 1 ml of saline-washed homologous erythrocytes (adjusted to a 70% hematocrit). This procedure inhibits endogenous EP production and thus serves as a sensitive EP assay system. On days 4 and 5, groups of eight mice were injected subcutaneously with 0.2 ml of isotonic saline, standard doses of EP, or pooled serum from *P. berghei*-infected mice. On day 6, each mouse received an intravenous injection of  $0.2 \mu\text{Ci}$  of  $^{59}\text{FeCl}_3$ . After 18 h, blood was collected by cardiac puncture and radioactivity measurements were made on 0.5-ml saline-washed samples in a well-type scintillation counter (Tracer Laboratories). The percentage of 18-h  $^{59}\text{Fe}$  incorporation into newly formed erythrocytes was based on an estimated blood volume of 8% of body weight for hypertransfused mice. EP levels in mouse serum were converted to units per milliliter from the EP log dose-response curve established for the assay. All values were expressed in terms of group means  $\pm 1$  standard error.

## RESULTS

Erythroid parameters, parasitemia, and serum EP levels of mice during the course of *P. berghei* infection are shown in Table 1. Malaria-infected mice became progressively anemic, with the lowest values for erythrocytes ( $1.69 \pm 0.31/\text{mm}^3 \times 10^6$ ) and percentage of hematocrit ( $11.05 \pm 0.73$ ) seen on day 18. Parasitemia was evidenced on day 7 ( $2.83 \pm 0.24/\text{mm}^3 \times 10^5$ ) and remained at approximately these levels throughout. A reticulocytosis ( $3.83 \pm 0.66/\text{mm}^3 \times 10^5$ ) was noted on day 7 and persisted at this almost twofold level during the entire infection. Serum EP levels in normal control mice were nondetectable, but in malaria-infected animals rose from  $0.32 \pm 0.06$  U/ml on day 7 to a peak value of  $7.75 \pm 0.61$  U/ml on day 14. On day 18, the EP levels in these mice declined to  $4.75 \pm 0.33$  U/ml.

## DISCUSSION

In accord with the data reported herein (Table 1), it is apparent that the rate of erythrocyte destruction in mice infected with *P. berghei* exceeded the rate of erythropoiesis. Although erythropoiesis in these mice was, in fact, enhanced, as shown by the nearly twofold reticulocytosis throughout the course of infection, a definitive statement regarding the precise kinetics of erythrocyte production is not possible, since the circulating life span of reticulocytes in malaria-infected mice is not available. In view of previous reports (17, 29, 31), which indicate that reticulocytes are preferentially invaded by *P. berghei* parasites, the meager reticulocyte response reported herein may reflect an underestimation of the extent of erythropoiesis. Furthermore, based on clinical studies (15), it would appear that erythropoiesis during malarial infection is inadequate since the expected rise in peripheral reticulocytosis

during the ensuing anemia did not occur until adequate antimalarial treatment had been administered. In any case, the rate of erythropoiesis in *P. berghei*-infected mice was less than the rate of excessive erythrocyte destruction.

Anemia is a common occurrence in many chronic infectious disorders (2, 3, 9, 28). In this regard it has been clearly shown that affected individuals elaborate significantly lower amounts of EP into their serum than those with iron deficiency or primary hematopoietic diseases with the same degree of anemia (28). In contrast, although the mechanism for the insufficient erythropoietic response in *P. berghei*-infected mice, as reported herein, is unclear, it does not appear to be attributed to impaired EP generation in response to their progressive development of anemia. In this regard, an inverse relationship between degrees of anemia and serum EP levels was noted, with serum EP levels peaking on day 14. These values are consistent with those previously reported (16) for normal Swiss mice in response to hemorrhage, and accordingly represent what one might expect to find in normal mice with a similar degree of erythropoietic stimulation. Insofar as the kidney is the major site of EP generation in mammals (8), the lower serum EP titers on day 18 might reflect renal damage, which has been shown to be a serious consequence of hemolysis (13, 14), a situation which may exist during the terminal stages of malaria infection. Although we showed that the endogenous levels of serum EP are elevated in malaria-infected mice, it is conceivable that even higher levels could perhaps further enhance erythropoiesis. In this regard, it has previously been shown (1, 7) that human suffering from certain types of anemia are capable of responding erythropoietically if their already high endogenous levels of EP are elevated even further. Accordingly, it would appear that the hemopoi-

TABLE 1. Erythroid parameters, parasitemia, and serum EP levels in mice during the course of *P. berghei* infection

Group	Day	Erythrocytes/ $\text{mm}^3 \times 10^{-6}$ ( $\pm 1 \text{ SE}^a$ )	Hematocrit (%) ( $\pm 1 \text{ SE}$ )	Parasites/ $\text{mm}^3$ $\times 10^{-5}$ ( $\pm 1 \text{ SE}$ )	Reticulocytes/ $\text{mm}^3 \times 10^{-5}$ ( $\pm 1 \text{ SE}$ )	EP (U/ml) ( $\pm 1 \text{ SE}$ )
Control	7-18 (pooled)	$9.08 \pm 0.55$	$39.96 \pm 1.10$		$2.40 \pm 0.54$	ND <sup>b</sup>
Infected	7	$7.31 \pm 0.72$	$35.13 \pm 0.74$	$2.83 \pm 0.24$	$3.38 \pm 0.66$	$0.32 \pm 0.06$
	11	$3.77 \pm 0.18$	$19.58 \pm 0.70$	$2.66 \pm 0.13$	$4.07 \pm 0.48$	$0.88 \pm 0.15$
	14	$2.51 \pm 0.33$	$15.18 \pm 1.34$	$3.39 \pm 0.35$	$3.83 \pm 0.34$	$7.75 \pm 0.61$
	18	$1.69 \pm 0.31$	$11.05 \pm 0.73$	$3.98 \pm 0.96$	$5.06 \pm 1.16$	$4.75 \pm 0.33$

<sup>a</sup> SE, Standard error.

<sup>b</sup> ND, Nondetectable.

etic stem cells in these patients were refractory to EP but were capable of responding at a higher threshold. It is also possible that impaired erythropoiesis in *P. berghei*-infected mice might reflect deficits in stem cell numbers and/or ineffective erythropoiesis.

It is noteworthy that information relative to erythrokinetics and restoration of normal erythroid parameters in malaria-infected animals may have some bearing on their immune status and hence survival. In this regard, we have shown (10) that transfusion of erythrocytes into *P. berghei*-infected mice is an effective maneuver that increases survival to greater than 90% in this otherwise fatal disease.

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