Glomerular filtration rate and plasma solutes in BALB/c mice infected with *Plasmodium berghei*

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Abstract. Immune-complex glomerular nephritis (ICGN) is known to develop during malarial infections, but little is known of its impact on renal function. A total of 24 male BALB/c mice were infected with *Plasmodium berghei*, and measurements of the glomerular filtration rate (GFR), parasitemia, and plasma solute concentrations were made on days 0, 7, 14, and 19 post-infection. Identical observations were made on 24 uninfected controls. The GFR declined progressively in infected mice from a mean of 201 ± 2.1 (day 0) to 51 ± 3 μl/min (day 19), whereas parasitemia rose to 47% ± 4.2% infected erythrocytes. In controls, the GFR remained unchanged, averaging 205 ± 3.4 μl/min. Plasma osmolality rose in infected mice (326 ± 1 vs 310 ± 0.6 mosmol/kg in controls) as a result of increased chloride (123 ± 0.7 vs 117 ± 0.6 mEq/l) and urea (17.8 ± 2.8 vs 9.3 ± 0.7 mM/l) levels. The data reveal a substantial deterioration of renal function during the course of a malarial infection that is short of outright renal failure.

Immune-complex glomerular nephritis (ICGN) is documented as being part of the pathology of malaria (Aikawa et al. 1980). Murine malarias caused by *Plasmodium berghei* and *P. yoelii* have been valuable experimental models for the study of such nephritis and of other aspects of malarial pathology (Yoeli and Hargreaves 1974). Research on the histological and ultrastructural aspects of the glomerular lesions (Boonpucknavig et al. 1972; Ehrich and Voller 1972; George et al. 1976; Poels et al. 1977; Suzuki 1974; Weise et al. 1973) has been the focus of most reports. Garnham (1980) noted few controlled studies of the impact of ICGN on renal function and body-fluid metabolism in either laboratory animals or humans infected with malaria and, although numerous observations relating the renal function appear in the literature, we found only four papers specifically dealing with the subject. Keeler et al. (1960) described disturbance in the electrolyte excretion of rats infected with *P. berghei*. Using albino mice infected with *P. berghei*, Miller et al. (1968) reported increased blood urea nitrogen and decreased excretion and phenolsulfonephthalein by day 7 of the infection, and these indicators became more abnormal as the infection progressed. Sesta et al. (1968) indicated that plasma urea and hemoglobin in the urine increased but blood creatinine remained normal in hamsters that had been infected with *P. berghei* for ≥7 days. Recently, Hioki and Ohtomo (1989) showed that plasma urea rose in *P. berghei*-infected male BALB/c mice during the final 2 days of a 7-day infection.

In the present study we used the single-injection method for measuring renal clearance (Haines and McKenna 1988) to evaluate glomerular function in BALB/c mice infected with *P. berghei*. The data show that the glomerular filtration rate declines progressively throughout the infection.

**Materials and methods**

A total of 48 male BALB/c mice were used in two identical 2 × 4 factorial experiments. Each experiment comparing determinations in controls vs infected animals on days 0, 7, 14, and 19 post-infection involved 24 animals. Determinations of the glomerular filtration rate (GFR), plasma osmolality, and concentrations of urea and chloride ions were done on day 0 and on each of the above-mentioned days post-infection. On any day, plasma from three control and three infected mice was processed. Data from the two experiments were combined after initial analysis had shown that there were no statistical differences between the two. Results were analyzed using two-way analysis of variance, model 1 (Sokal and Rohlf 1969). *Plasmodium berghei* was obtained from the American Type Culture Collection. Infection was induced by intraperitoneal injection of 10 million infected erythrocytes in 0.2 ml Hanks' balanced salt solution.

The GFR was determined using a single-injection method described elsewhere (Haines and McKenna 1988). At the beginning of a clearance determination (time zero), 40 μl of 0.03 μCi/μl tritiated methoxy-insulin (New England Nuclear 086L) was injected into a lateral caudal vein. Samples of blood were collected from the contralateral caudal vein, which was punctured with a sterile, pointed blade (Bard-Parker 11) at 1, 2, 4, 6, 12, 24, 50, and 70 min.
post-injection. Known volumes (5-10 μl) of plasma were dissolved in 5 ml of liquid scintillation fluid (Beckman HP) and counted (Beckman LS-133). The GFR was calculated as the quotient of tracer activity (counts per minute), divided by the area under the tracer curve (counts per minute per microliter x minute). For details, see Haines and McKenna (1988).

Following a period of at least 15 h, during which radioactive inulin was completely excreted, all six animals were decapitated and trunk blood was collected in polystyrene weighing dishes (73 mm², 25 mm deep) that had previously been rinsed with ammonium heparin (Lancer, 1000 IU/ml). Blood smears were made (fixed in methanol, stained with Giemsa) for determinations of parasitemia (percentage of parasitized erythrocytes). Plasma analyses were carried out as follows: enzymatic color determination of urea nitrogen, using Sigma kit 640 and a Spectronic 20 colorimeter; chloride ions, by coulombmetric titration (Aminco-Cotlove chloride titrator); and osmolality, by vapor pressure osmometry (Wescor model 5133).

Results

GFR and parasitemia

GFR and parasitemia were inversely related (Fig. 1). The GFR of infected and control mice on day 0 was 201 ± 2.1 and 196 ± 1.7 μl/min, respectively. Over the subsequent 19 days, it declined to 51 ± 3 μl/min in infected mice, whereas in control animals it averaged 205 ± 3.4 μl/min. The rate of decline among the infected mice was 7.9 μl/min per day. Analysis of variance showed that the GFR of the two groups differed from day 7 onward (P < 0.01); the overall average (days 0-19) for controls was 190 ± 2.8 μl/min and that for infected mice was 124 ± 5.8 μl/min (P < 0.01). Parasitemia rose progressively with the duration of infection, averaging 47% ± 4.2% on day 18.

Plasma solutes

Infected mice showed disturbances in plasma osmolality and in concentrations of chloride and urea. By day 14, plasma osmolality had risen to 332 mosmol/kg in infected mice as compared with 304 mosmol/kg in controls (P < 0.01, Table 1), reaching a value of 343 mosmol/kg on day 19. Plasma chloride increased parallel with osmolality (Table 1), also showing a difference between experimental and control values on day 14 of the infection (P < 0.02). The plasma urea concentration had also significantly elevated by day 14 in infected mice (P < 0.05), being almost 3 times that measured in control animals.

Discussion

The GFR declined linearly as parasitemia fulminated, with the former declining by 75% and the latter rising