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Susceptibility of Different Strains of Mice to Hepatic Infection with *Plasmodium berghei*

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Despite the low susceptibility of BALB/c mice to hepatic infection by *Plasmodium berghei*, this animal model is routinely used to investigate the basic biology of the malaria parasite and to test vaccines and the immune response against exoerythrocytic (EE) stages derived from sporozoites. A murine model in which a large number of EE parasites are established would be useful for furthering such investigations. Therefore, we assayed six mouse strains for susceptibility to erythrocytic and hepatic infections. The administration of 50 sporozoites by intravenous inoculation was sufficient to establish erythrocytic infections in five of five C57BL/6 mice compared with 10,000 sporozoites required to infect 100% of BALB/c mice. To assay for hepatic infections, mice received an intravenous inoculum of 10^6 sporozoites, and liver sections for light microscopy and histology were obtained at 29 and 44 h postinoculation. EE parasites were visualized by immunofluorescence, using an antibody to a *P. falciparum* heat shock protein. The mean number of EE parasites per 100 cm^2 for C57BL/6 and A/J strains was significantly higher than that for BALB/c (2,190 ± 260, 88 ± 38, and 6 ± 2, respectively). The proportion of inoculated sporozoites transforming into liver schizonts was 8.2% in C57BL/6 and <1% in C3H/HeJ, DBA/1, and Swiss CD-1/ICR mice. Nonspecific inflammatory infiltrates around EE parasites were less prevalent in liver sections from C57BL/6 mice than in those from BALB/c mice, which contributed to the decrease in developing EE stages in BALB/c mice. These data indicate that the C57BL/6-*P. berghei* system is preferable for investigating the biology and immunology of liver stage parasites.

The exoerythrocytic (EE) stage of the malaria parasite in the host liver is the stage between the sporozoite, which originates from the bite of an infected anopheline mosquito, and the erythrocytic stage, which results from the release of merozoites from hepatocytes. The EE forms of *Plasmodium* spp. were the last to be discovered and remain the least known (19, 23) due to the inherent difficulties of experimentally manipulating a vital organ such as the liver and to the small size and sparse distribution of these parasites. Consequently, animal models of experimental malaria were pursued (6). *Thamnomys surdaster*, a tree rat, is the natural host of *Plasmodium berghei*, but it is not amenable to routine use in the laboratory. Therefore, these rats have been replaced by more manageable strains of mice.

Inbred strains of mice are used to study the basic biology of the malaria parasite, test vaccines, and investigate the immune mechanisms they elicit. Data gathered from different mouse strains concerning the irradiated-attenuated sporozoite vaccine (4, 15) were useful for designing vaccine trials in humans (2, 3, 7–9, 17). Previous studies have shown that BALB/c mice have a low susceptibility to *P. berghei* hepatic infection; only 0.01% of inoculated sporozoites infect the liver (13). This low susceptibility was correlated with the ability of BALB/c mice to elicit a cellular inflammatory response against the EE stages of *P. berghei*. This innate inflammatory response began eliminating developing EE stages as early as 24 h post-sporozoite inoculation (13, 20).

Because the innate response in BALB/c mice could interfere with the study of the cellular protective mechanisms involved in sporozoite-induced immunity, the BALB/c-*P. berghei* system is considered a relatively poor model for addressing immunological issues (13). A mouse strain that is more permissive for EE infection would be useful in evaluating the cellular protective mechanisms involved in sporozoite-induced immunity by allowing quantitation of liver stage parasites. We evaluated six strains of mice (C57BL/6, A/J, BALB/c, C3H/HeJ, DBA/1, and Swiss CD-1/ICR) for susceptibility to *P. berghei* (ANKA clone) sporozoites. Susceptibility was measured by the ability of sporozoites to invade and develop into microscopically detectable hepatic schizonts in the liver and by the onset of cellular responses against these EE stages. The data suggest that the C57BL/6-*P. berghei* system provides an optimal model for investigating the liver stages. This model could also be utilized to study the parasites that result in the host liver following immunization with live, irradiated-attenuated sporozoites, as well as the cellular protective mechanisms they elicit against the EE stages derived from sporozoite challenge.

MATERIALS AND METHODS

Animals. Female 6-week-old mice of the following strains were used in these experiments: C57BL/6, BALB/c, DBA/1, and Swiss CD-1/ICR (Charles River Laboratories, Wilmington, Mass.) and A/J and C3H/HeJ (Jackson Laboratories, Bar Harbor, Maine). The animals were cared for and used strictly in accordance with the Public Health Service guidelines (Committee on Care and Use of Laboratory Animals, National Institute of Health, Bethesda, Md.).

Sporozoites. *P. berghei* sporozoites (ANKA clone) were obtained from laboratory-reared female *Anopheles stephensi* mosquitoes maintained at 18°C for 18 to 30 days after feeding on parasitemic Swiss CD-1/ICR mice. Mosquitoes were separated into abdomen and head/thorax. Heads and thoraxes were triturated with a mortar and pestle and suspended in medium

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199 containing 5% mouse serum (Rockland Co., Gilbertsville, Pa.). The sporozoites were isolated from this suspension by using a biphasic gradient with Renografin 60 (Squibb Diagnostics, New Brunswick, N.J.), as previously described (16). Sporozoites isolated from the same batch of mosquitoes were inoculated into all strains of mice on the same day to control for biological variability in sporozoite preparations (21). To ensure that inoculated sporozoites were viable following the isolation procedure, they were stained with a vital dye containing fluorescein diacetate (50 mg/ml in acetone) and ethidium bromide (20 μg/ml in phosphate-buffered saline; Sigma Chemical Co., St. Louis, Mo.) as previously described (11, 18) and counted in a hemocytometer. The viability of sporozoites ranged from 90 to 100%.

Determination of susceptibility of different strains of mice to sporozoite-induced blood infection. The infectivity of the viable, isolated sporozoites was estimated by determining the minimal number of sporozoites, inoculated intravenously, necessary to produce an infection in 100% of the mice per strain, as previously described (12). Five mice per strain were each inoculated with either 50, 500, 5000, 10,000, or 20,000 sporozoites. Beginning on day 3 postinoculation, blood smears were made on each of the mice, and the smears were stained with Giemsa (1).

Liver resections and detection of EE forms. Nine C57BL/6 and BALB/c mice and six A/J, C3H/HeJ, DBA/1, and Swiss CD-1/ICR mice were each inoculated intravenously with 10^6 sporozoites. This larger sporozoite inoculum was used to facilitate finding the EE forms in the livers of mice. Livers from six mice of each strain were removed at 29 h postinoculation. The three remaining livers from C57BL/6 and BALB/c mice were removed at 44 h postinoculation. Liver samples were either embedded in Tissue-Tek O.C.T. medium (Miles, Inc., Elkhart, Ind.) and frozen in liquid nitrogen or fixed immediately in 10% buffered formalin, passed through graded alcohols and xylene, embedded in paraffin, and sectioned. Sections, 4 μm thick, were stained with Giemsa (1) counterstained with Evans blue or by the immunofluorescence assay, using rabbit polyclonal antisera against the *P. falciparum* heat shock protein 70 (kindly provided by N. Kumar, The Johns Hopkins University), which cross-reacts with the heat shock protein of *P. berghei*. To avoid counting the same EE schizont more than once in sequential liver sections, every 10th slide was stained and examined. The distance between two sections examined was thus 40 μm, the diameter of a mature *P. berghei* schizont at 48 h.

Evaluation of the number of EE forms. Slides of tissue sections were placed on the microscope stage and overlaid with a plastic sheet with a window measuring 0.25 cm^2. This window was superimposed on the section, and all parasites within this area were counted. A total of 400 areas in each mouse liver [(400 × 0.25 cm^2)/area = 100 cm^2], per strain, were screened. The mean number of EE stages in 100 cm^2 in individual mice was used to determine the mean EE stages per strain ± the standard deviation. The number of EE stages in whole liver was calculated by dividing the volume of mouse liver (~2.6 cm^3) by the volume of a liver section (1 cm^2 × 4 μm). The resultant value was multiplied by the mean number of EE stages in 100 cm^2 to give a total number of EE stages per liver: (volume of liver/0.0004 μm) × (mean EE/100 cm^2).

The mean number of EE stages per 100 cm^2 at 29 h was compared between strains by using a one-way analysis of variance and Tukey's test using the EpiXact program from the EGRET epidemiological data analysis package.

Hepatic histopathological characterization. In a separate experiment, BALB/c and C57BL/6 mice were inoculated with 10^6 sporozoites, and controls were inoculated with salivary gland lysates from uninfected mosquitoes. Animals were sacrificed and livers were harvested at 19, 24, 29, 34, 39, and 44 h postinoculation, using three infected and two control mice per strain for each time point. To ensure an unbiased histopathology characterization, livers were delivered to personnel in a separate laboratory (Pathology Laboratory Services) for fixing, sectioning, mounting, and staining and coding. The 1-cm^2 section per slide was examined, and the ratio of the types of histopathology was determined by using the following criteria: inflammatory cell aggregates and scattered foci versus granulomatous-like microabscesses versus well-defined granulomas. All slides were examined and scores were compiled before the code was broken.

TABLE 1. Number of sporozoites necessary to cause parasitemia in six different strains of mice

No. of sporozoites	No. of infected mice/no. of mice challenged[a]					
	C57BL/6	A/J	BALB/c	C3H/HeJ	DBA/1	CD-1/ICR
50	5/5	0/5	0/5	0/5	0/5	0/5
500	5/5	0/5	0/5	0/5	0/5	0/5
5,000	5/5	5/5	2/5	0/5	0/5	0/5
10,000	5/5	5/5	5/5	2/5	1/5	1/5
20,000	5/5	5/5	5/5	5/5	5/5	5/5

[a] There were five mice per strain per sporozoite inoculum dose; infection was determined by recording the onset of parasitemia in Giemsa-stained blood smears beginning on day 3 postinoculation.

RESULTS

Susceptibility of different mouse strains to *P. berghei* infection. The number of sporozoites needed to initiate a blood infection in 100% of the mice in each strain is given in Table 1. The C57BL/6 mice were the easiest strain to infect, requiring only 50 sporozoites per animal (Table 1), followed by A/J mice, requiring 5,000; BALB/c mice, requiring 10,000; and C3H/HeJ, DBA/1, and Swiss CD-1/ICR mice, requiring 20,000 sporozoites.

The susceptibility of mice to liver infections with *P. berghei* was determined by counting the number of EE parasites in the liver at 29 and 44 h postinoculation with sporozoites. Data for the 29-h time points are presented in Table 2. Each mouse strain exhibited a different susceptibility to *P. berghei*, with the most susceptible mouse strain being C57BL/6 (2,190 ± 260 EE per 100 cm^2; $P = 0.0001$), followed by A/J (88 ± 38 EE per 100 cm^2) and BALB/c (6 ± 2 EE per 100 cm^2). There were no

TABLE 2. Numbers of *P. berghei* EE stages in different strains of mice inoculated with 10^6 sporozoites

Strain	No. of animals	No. of EE/100 cm^2 at 29 h (mean ± SD)	% Hepatic infection[a]
C57BL/6	9	2,190 ± 260[b]	8.21
A/J	6	88 ± 38	0.33
BALB/c	9	6 ± 2[b]	0.02
C3H/HeJ	6	5 ± 2	0.02
DBA/1	6	4 ± 1	0.01
CD-1/ICR	6	1 ± 0	0.01

[a] (Number of EE stages in the whole liver/number of sporozoites inoculated) × 100%. Number of EE stages/liver = (volume in liver/0.0004 μm) × (mean EE/100 cm^2).

[b] The number of EE stages at 44 h in C57BL/6 mice was 1,150 ± 200 per 100 cm^2 and that in BALB/c mice was 0.5 ± 0.01 EE per 100 cm^2 ($P = 0.001$) versus the number obtained at 29 h by the one-way analysis of variance and Tukey's test.

TABLE 3. Histopathological characterization of liver sections of immunologically naive mice following inoculation of *P. berghei* sporozoites

Mouse strain[a]	Histopathology[b] at given time postinoculation of sporozoites						
	19 h	24 h	29 h	34 h	39 h	44 h	48 h
BALB/c	—	11:0:0	5:9:0	4:7:2	1:4:6	0:2:7	1:2:10
Controls	—	—	—	—	—	—	—
C57BL/6	—	—	—	—	2:0:0	2:3:0	1:7:0
Controls	—	—	—	—	—	—	—

[a] BALB/c and C57BL/6 control mice received tail vein inoculations of salivary gland lysates from uninfected mosquitoes.

[b] Values represent a ratio between the following histopathology observed in 75 sections (1-cm^2 area) in BALB/c and C57BL/6 mice and 50 sections in control mice per time point: (inflammatory cell aggregates and scattered foci: granulomatous-like microabscesses:well-defined granulomas). —, little or no histopathology observed.

significant differences between the numbers of EE stages observed in A/J, BALB/c, C3H/HeJ, and DBA/1 strains ($P \geq 0.05$). Approximately 8.2% of the sporozoites inoculated invaded and developed into EE stages in C57BL/6 mice, whereas only 0.33% developed in A/J mice and less than 0.02% developed in BALB/c mice. C3H/HeJ, DBA/1, and Swiss CD-1/ICR mice exhibited very low susceptibilities to sporozoite infection [(1 to 5) ± 2 EE per 100 cm^2], and less than 0.02% of the sporozoites inoculated developed into EE stages.

Histopathology observed in BALB/c and C57BL/6 mouse livers. Histopathological alterations in the livers of C57BL/6 and BALB/c mice were assessed at 19, 24, 29, 34, 39, 44, and 48 h postinoculation with sporozoites. The onset of inflammatory cells surrounding *P. berghei* in BALB/c mice was observed at 24 h (Table 3). Granulomatous-like microabscesses were seen as early as 39 h, while small, scattered foci of mononuclear cells were seen from 24 to 48 h. By 44 h, these abscesses developed into small granulomas. The granulomas increased in number and size from 44 to 48 h postinoculation, with an accompanying significant decrease in the number of EE stages observed (e.g., at 29 h, 6 EE per 100 cm^2 were observed in BALB/c mice, but at 44 h only 0.5 EE per 100 cm^2 was observed; $P = 0.001$) (Table 2). In contrast, no significant inflammatory response was observed in the livers of C57BL/6 mice through 39 h post-sporozoite inoculation (Table 3). At 39 h, few foci of inflammatory cell aggregates were observed; these increased in size and number as the infection progressed from 39 to 48 h. The number of EE stages observed at 44 h post-sporozoite inoculation was not as drastically reduced (e.g., 2,190 EE per cm^2 at 29 h and 1,150 EE per cm^2 at 44 h) as in BALB/c mice. No significant histopathology was observed in control mice that received tail vein inoculations of salivary gland lysates.

DISCUSSION

The susceptibility of different strains of mice to infection with *Plasmodium* sporozoites has previously been reported to be mediated by the numbers of sporozoites required to cause a blood infection in 100% of the recipients (12, 14, 21). Furthermore, it has been shown that the differences in susceptibility among mouse strains are independent of the ability of the parasite to invade and undergo schizogony in erythrocytes. There appeared to be no difference between C57BL/6 and BALB/c mice in the early invasion of erythrocytes by parasites following their release from hepatocytes, indicating that erythrocyte (reticulocyte) susceptibility to *P. berghei* at an early time point was not strain related (22).

As part of our laboratory's research into the role of the hepatic stages of irradiated sporozoites in eliciting protection, we were interested in expanding upon the results of previous investigations on the susceptibility of different strains of mice to sporozoites. Our aim was to identify a model system characterized by a high level of *P. berghei* parasites in hepatocytes.

The susceptibility of mouse strains routinely used in the laboratory to hepatic infection with sporozoites has not been previously performed in a study utilizing a large population of mice and screening a large number of histological samples of liver. We present here a quantitative analysis of the susceptibility of six strains of laboratory mice to hepatic infection with *P. berghei* sporozoites. As expected, strains of mice inoculated with 10^6 sporozoites exhibited differences in the mean number of EE parasites observed in liver sections. C57BL/6 strain mice were the most susceptible, followed by A/J, BALB/c, C3H/HeJ, DBA/1, and Swiss CD-1/ICR mice. The degree of hepatic susceptibility to sporozoites in mice correlated with the order of the onset of parasitemia.

Khan and Vanderberg (13) reported that the susceptibility of *P. yoelii* is inversely related to the innate cellular response directed against the EE stages of the parasite. They reported that the BALB/c response to *P. berghei* can be observed within the liver 4 h post-sporozoite inoculation and develops into massive histopathological changes by 24 h after infection. Such histopathological changes around the EE stages of *P. yoelii* in naive mice do not occur until the rupture of mature schizonts (~44 h). Thus, BALB/c mice are 2,000 times more susceptible to sporozoites of *P. yoelii* than to sporozoites of *P. berghei*. No notable liver inflammation was observed when naive BALB/c mice were inoculated with a very high dose (12×10^6) of *P. yoelii* sporozoites (10). The nonspecific immune response to *P. berghei* observed in BALB/c mice is probably a consequence of the poor adaptation of the parasite to the mouse host (21). Thus, the BALB/c-*P. berghei* system has an inherent drawback as a model for sporozoite immunization studies, as the cellular response in these animals could obscure immune-mediated effector mechanisms (13).

Therefore, we explored the possibility of finding an alternative system that would yield large numbers of EE stages without the induction of a cellular response in the liver. We observed that an innate cellular response is not directed against EE stages of *P. berghei* until 36 h in C57BL/6 mice, as opposed to 24 h as previously reported in the traditionally utilized BALB/c-*P. berghei* system. Consequently, more EE stages develop to full maturity in C57BL/6 mice, making this a superior mouse model with which to investigate antimalarial drugs against these stages, the fate of irradiated sporozoites in hepatocytes, the antigens that irradiated parasites express, and the cellular effector mechanisms involved in protection of *P. berghei*-irradiated, sporozoite-immunized mice.

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REFERENCES

1. **Bray, R. S., and P. C. C. Garnham.** 1962. The Giemsa-colophonium method for staining protozoa in tissue sections. Indian J. Malariol. **16**:153–155.

2. **Clyde, D. F., V. McCarthy, R. M. Miller, and W. E. Woodward.** 1975. Immunization of man against falciparum and vivax malaria by use of attenuated sporozoites. Am. J. Trop. Med. Hyg. **54:**397–401.
3. **Clyde, D. F., V. McCarthy, and J. P. Vanderberg.** 1973. Immunization of man against sporozoite induced falciparum malaria. Am. J. Med. Sci. **266:**169–177.
4. **Cochrane, A. H., R. S. Nussenzweig, and E. H. Nardin.** 1980. Immunization against sporozoites, p. 166. *In* J. P. Kreier (ed.), Malaria, vol. 3. Academic Press, Inc., New York.
5. **Coosemans, M., M. Wery, E. Van Marck, and G. Timperman.** 1981. Studies on the infectivity on *Plasmodium berghei* sporozoites in experimental hosts. Ann. Soc. Belge Med. Trop. **61:**349–368.
6. **Cox, F. E. G.** 1988. Major animal models in malaria research, p. 1503–1544. *In* W. H. Wernsdorfer and I. A. McGregor (ed.), Malaria. Principles and practice of malariology, Vol. 2. Churchill Livingstone, Edinburgh.
7. **Edelman, R., S. L. Hoffman, J. R. Davis, M. Beier, M. B. Sztein, G. Losonsky, D. A. Herrington, H. A. Eddy, M. R. Hollingdale, D. M. Gordon, and D. F. Clyde.** 1993. Long-term persistence of sterile immunity in a volunteer immunized with X-irradiated *Plasmodium falciparum* sporozoites. J. Infect. Dis. **168:**1066–1070.
8. **Ferreira, A., L. Schofield, V. Enea, H. Schellekens, P. van der Meide, W. E. Collins, R. S. Nussenzweig, and V. Nussenzweig.** 1986. Inhibition of development of exoerythrocytic forms of malaria parasites by gamma-interferon. Science **232:**881–884.
9. **Herrington, D. A., D. F. Clyde, G. Losonsky, M. Cortesia, J. R. Murphy, J. R. Davis, S. Baqar, A. M. Felix, E. P. Heimer, D. Gillessen, E. H. Nardin, R. S. Nussenzweig, V. Nussenzweig, and M. M. Levine.** 1987. Safety and immunogenicity in man of a synthetic peptide malaria vaccine against *Plasmodium falciparum* sporozoites. Nature (London) **328:**257–259.
10. **Hoffman, S. L., D. Isenbarger, G. W. Long, M. Sedegah, A. Szarfman, L. Waters, M. R. Hollingdale, P. H. van der Meide, D. S. Finbloom, and W. R. Ballou.** 1989. Sporozoite vaccine induces genetically restricted T cell elimination of malaria from hepatocytes. Science **244:**1078–1081.
11. **Jackson, R. R., M. G. Pappas, and B. D. Hansen.** 1985. Fluorogenic substrate detection of viable intracellular and extracellular pathogenic protozoa. Science **227:**435–437.
12. **Jaffe, R. I., G. H. Lowell, and D. M. Gordon.** 1990. Differences in susceptibility among mouse strains to infection with *Plasmodium berghei* (ANKA clone) sporozoites and its relationship to protection by gamma-irradiated sporozoites. Am. J. Trop. Med. Hyg. **42:**309–313.
13. **Khan, Z. M., and J. P. Vanderberg.** 1991. Role of host cellular response in differential susceptibility of nonimmunized BALB/c mice to *Plasmodium yoelii* sporozoites. Infect. Immun. **59:**2529–2534.
14. **Mackey, L. J., A. Hochman, C. H. June, C. E. Contreras, and P. H. Lambert.** 1980. Immunopathological aspects of *Plasmodium berghei* infection in five strains of mice. II. Immunopathology of cerebral and other tissue lesions during the infection. Clin. Exp. Immunol. **42:**412–420.
15. **Nussenzweig, R. S., J. P. Vanderberg, H. Most, and C. Orton.** 1967. Protective immunity produced by the injection of X-irradiated sporozoites of *Plasmodium berghei*. Nature (London) **216:**160–162.
16. **Pacheco, N. D., C. P. A. Strome, F. Mitchell, M. P. Bawden, and R. L. Beaudoin.** 1979. Rapid, large-scale isolation of *Plasmodium berghei* sporozoites from infected mosquitoes. J. Parasitol. **65:**414–417.
17. **Rieckmann, K. H., P. E. Carson, R. L. Beaudoin, J. Cassells, and K. W. Sell.** 1974. Sporozoite induced immunity in man against an Ethiopian strain of *Plasmodium falciparum*. Trans. R. Soc. Trop. Med. Hyg. **68:**258–259.
18. **Seguin, M. C., W. R. Ballou, and C. A. Nacy.** 1989. Interactions of *Plasmodium berghei* sporozoites and murine Kupffer cells *in vitro*. J. Immunol. **143:**1716–1722.
19. **Shortt, H. E., N. H. Fairly, G. Covell, P. G. Shute, and P. C. C. Garnham.** 1951. The pre-erythrocytic stage of *Plasmodium falciparum*. Trans. R. Soc. Trop. Med. Hyg. **44:**405–419.
20. **Vanderberg, J. P., Z. M. Khan, and M. J. Stewart.** 1993. Induction of hepatic inflamatory response by *Plasmodium berghei* sporozoites protects BALB/c mice against challenge with *Plasmodium yoelii* sporozoites. J. Parasitol. **79:**763–767.
21. **Vanderberg, J. P., R. S. Nussensweig, and H. Most.** 1968. Further studies on the *Plasmodium berghei-Anopheles stephensi*-rodent system of mammalian malaria. J. Parasitol. **54:**1009–1016.
22. **Winger, L. A., and R. E. Sinden.** 1992. Immunoprotection in mice susceptible to waning memory against the pre-erythrocytic stages of malaria after validated immunization with irradiated sporozoites of *Plasmodium berghei*. Parasitol. Res. **78:**427–432.
23. **Yoeli, M., and H. Most.** 1965. Studies on sporozoite-induced infection of rodent malaria. I. The pre-erythrocytic tissue stage of *Plasmodium berghei*. Am. J. Trop. Med. Hyg. **14:**700–714.