INFECTION AND IMMUNITY, Mar. 1984, p. 981–985
0019-9567/84/030981-05$02.00/0

Vol. 43, No. 3

Susceptibility of CXB Recombinant Inbred Mice to Murine Plasmodia

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Received 10 August 1983/Accepted 5 December 1983

The genetic control of susceptibility to two species of murine malarial parasites was investigated with recombinant inbred (RI) mouse strains as a model system. Initially, the nonlethal *Plasmodium yoelii* 17X strain was cloned by limiting dilution to minimize parasite variability. This cloned *P. yoelii* was used to infect C57BL and BALB/c mice, strains which are the progenitors of the CXB RI strains. Since these two strains displayed consistent differences in the kinetics of parasitemia, the seven CXB RI strains were compared for their susceptibility to the same parasite. The RI strains varied considerably when infected with *P. yoelii* and could be divided into susceptible and resistant groups based on mortality observed with this normally mild infection. This suggests a complex, multigenic inheritance determining susceptibility to this parasite. However, when the susceptible and resistant CXB RI mice were infected with another, unrelated plasmodial species, *Plasmodium chabaudi adami*, all the mice showed identical patterns of disease. Since susceptibility to different murine plasmodia does not cosegregate in the CXB RI mice, different mechanisms of resistance may be required for different plasmodial species.

One way of probing the processes which lead to successful defense against pathogens is to identify and study host genes that lead to resistance or susceptibility. It has been recognized that the genetic constitution of the host affects the outcome of infection by many different parasites, including plasmodia. Studies of naturally occurring genetic variations in human populations have revealed the importance of hemoglobin alterations, particularly the sickle cell trait (HbS), as well as glucose-6-phosphate dehydrogenase deficiencies in the evolution of resistance to malaria (14, 19; A. C. Allison, Contemp. Top. Immunobiol., in press). More recently, the observation that erythrocytes from individuals lacking either glycophorin A (15, 18, 20) or Duffy blood group substance (15, 16) are resistant to infection by particular plasmodial species has pointed to the importance of these substances in recognition or invasion of erythrocytes. Finally, the identification of other human populations, such as the Melanesians, which have ovalocytic erythrocytes resistant to *Plasmodium falciparum* should provide further information about the erythrocyte cytoskeleton in the process of infection (13).

Long-standing observations in the literature have also shown that different inbred mouse strains exhibit different susceptibilities to murine plasmodial species. Greenberg and colleagues (7) used the lethal parasite *Plasmodium berghei* to show characteristic differences in survival time after blood-stage infection. Their series of studies established a genetic basis of resistance to this parasite and suggested that this resistance was controlled by multiple genetic factors (6, 17). Later, Eugui and Allison (5) reported that A strain mice were highly susceptible to *Plasmodium chabaudi*, unlike B10.A mice. Pursuing this observation, Stevenson and colleagues (24) ascribed the difference in survival between these two strains to a single dominant gene which correlated with the ability of the resistant mice to induce an erythropoietic response after infection.

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We have used recombinant inbred (RI) strains as a model system to investigate the genetic control of susceptibility to malarial parasites. The CXB series of strains derived from an initial cross between BALB/c ByJ (designated C) and C57BL/6 ByJ (designated B) were compared with the two progenitor strains for their patterns of infection with cloned *Plasmodium yoelii* 17X, which infects reticulocytes. The individual RI strains varied considerably in their susceptibility to blood-stage infection with this species, particularly with respect to mortality induced by a normally mild infection. This suggests a complex, multigenic inheritance determining susceptibility to this species. However, when we infected susceptible and resistant CXB RI mice with another, unrelated parasite, *P. chabaudi adami*, all the mice showed identical patterns of disease. Since susceptibility to different plasmodia does not cosegregate in the CXB RI mice, it appears that different mechanisms of resistance are required for different plasmodial species.

MATERIALS AND METHODS

Experimental animals. Initial experiments were performed with C57BL and BALB/c mice. Subsequently, all experiments were performed with B, C, and their RI strains (2, 3). The RI strains, CXBD, CXBE, CXBG, CXBH, CXBI, CXBJ, and CXBK, and their progenitors, C and B, were originally provided by Donald Bailey (Jackson Laboratory, Bar Harbor, Maine) in 1980 and have been maintained in our animal colony. Age-matched male mice were used in all experiments.

Parasites. *P. yoelii* 17X was originally provided by John Finerty (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.). The parasites were maintained in the vapor phase of liquid nitrogen as a cryopreserved stabilate. *P. chabaudi adami* 556KA was kindly provided by David Wyler (National Institute of Allergy and Infectious Diseases, National Institutes of Health) and maintained as above.

Cloning of *P. yoelii*. Cloning of *P. yoelii* was achieved by using a limiting dilution technique similar to that described

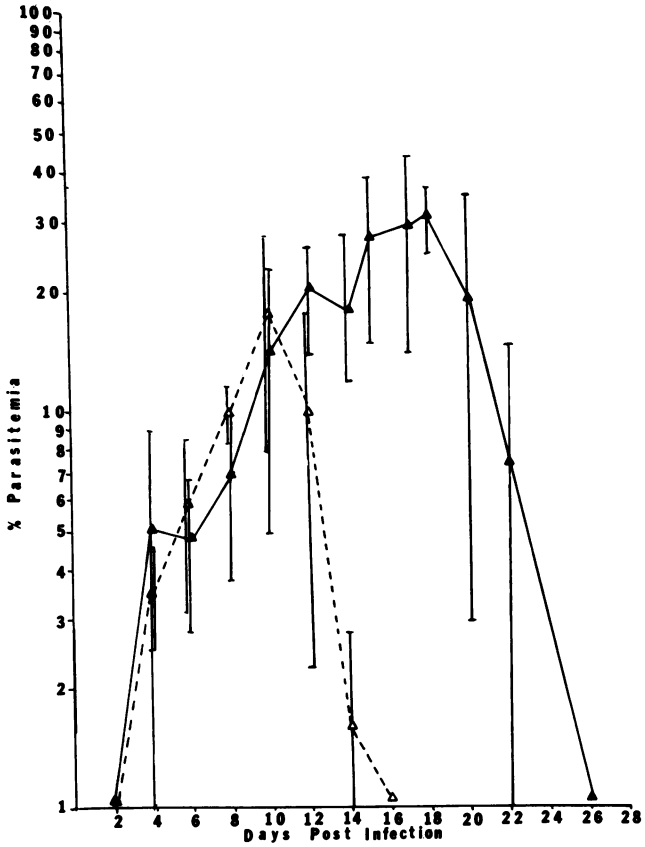

FIG. 1. Course of *P. yoelii* infection in BALB/c and C57BL mice. BALB/c (△) and C57BL male mice (▲) were infected i.p. with 10^6 parasitized erythrocytes on day 0. Points with brackets represent mean percentage of parasitemia ± standard deviations obtained from a group of five BALB/c and five C57BL mice.

by Walliker et al. (25). Blood from an infected mouse was collected in RPMI 1640 containing 10 U of heparin per ml. The blood was washed with RPMI 1640 and centrifuged at 1,600 rpm. The supernatant and the resulting buffy coat were removed and discarded. The blood cells were washed an additional two times and finally resuspended in medium. Subsequently, the erythrocytes were diluted to contain one parasitized erythrocyte per 0.2 ml. Recipient mice were injected intravenously with 3.4 mg of silica in phosphate-buffered saline 24 h before attempted infection (1). Beginning 7 days after injection, blood films were prepared from the tail vein and stained with Giemsa. Only 1 animal out of 10 developed patent parasitemia. Stabilate material was prepared from the infected blood of this mouse by standard methodology and was stored in the vapor phase of a liquid nitrogen refrigerator.

Experimental infections. Donor C male mice were infected intraperitoneally (i.p.) with thawed stabilate material. Once parasitemia exceeded 5%, the donor was bled via the retroorbital plexus into heparinized Hanks balanced salt solution. Parasitized erythrocytes were enumerated and adjusted to contain 5×10^6 parasitized erythrocytes per ml of diluent. Experimental animals were then injected i.p. with 0.2 ml of the inoculum. Parasitemias were estimated at subsequent intervals by counting infected erythrocytes in Giemsa-stained films of tail blood. A minimum of 200 erythrocytes was counted in each film.

RESULTS

Characterization of *P. yoelii* infection in BALB/c and C57BL mice. To compare the course of infection in two different strains of mice, age-matched BALB/c and C57BL male mice were infected with cloned *P. yoelii*. Parasitemias became patent by day 2 postinfection in both groups of mice and initially followed similar courses of infection (Fig. 1). Parasitemias in BALB/c mice reached peak values of about 18% on day 10 postinfection and began to decrease thereafter, becoming subpatent by day 16 postinfection. In contrast, parasitemias in the C57BL mice continued to rise, reaching peak values of 32% by day 18. The duration of infection in C57BL mice was considerably longer than in BALB/c mice, finally resolving by day 26 postinfection.

***P. yoelii* infection in CXB RI mice.** Since reproducible differences in the course of infection were observed with these two inbred strains, we next investigated infections in the RI mice derived from the two progenitors to establish whether the infection patterns would segregate among the RI strains. Characterization of the course of *P. yoelii* infection was extended to include the two progenitors, C and B, and

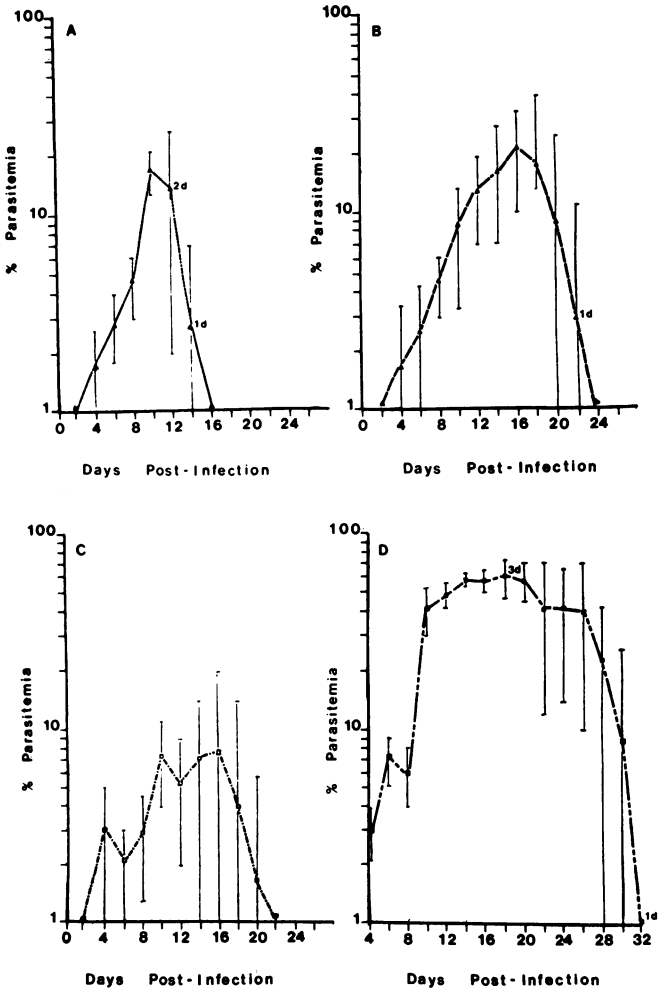

FIG. 2. Course of *P. yoelii* infection in the progenitors and two CXB RI strains. Each point represents the mean percentage of parasitemia ± standard deviations in mice infected with 10^6 *P. yoelii* parasitized erythrocytes. All groups consisted of 10 6-month-old male mice, except CXBJ, which had 7 mice. The panels depict strains C (A), B (B), CXBH (C), and CXBJ (D). d, Death.

TABLE 1. Characteristics of *P. yoelii* infection in the CXB RI strains and their progenitors[a]

Strain	% Peak parasitemia (± SD)	Day of peak parasitemia (± SD)	Duration of infection[b] (± SD)	No. of deaths/ no. infected
Progenitor strains				
C	17.0 ± 4.3	10.4 ± 0.89	14.4 ± 0.89	3/10
B	22.0 ± 11.7	15.6 ± 2.5	20.7 ± 1.7	1/10
RI strains				
CXBG	18.0 ± 8.0	13.2 ± 1.9	18.2 ± 2.2	0/10
CXBH	7.1 ± 13.4	11.8 ± 2.6	17.0 ± 1.9	0/10
CXBK	39.0 ± 18.0	15.5 ± 2.8	23.0 ± 3.5	2/10
CXBE	25.0 ± 18.8	18.0 ± 0	23.0 ± 3.7	2/10
CXBI	35.0 ± 24.0	16.6 ± 3.3	22.5 ± 3.0	3/10
CXBJ	60.0 ± 13.2	16.2 ± 2.1	28.0 ± 3.5	4/7

[a] Groups of 10 6-month-old male mice were injected i.p. with 10^6 *P. yoelii* 17X parasitized erythrocytes. The CXBJ group consisted of only seven mice.

[b] Mean day on which parasitemia became subpatent. Fifty oil immersion fields were searched before the blood smear was considered negative and subpatent.

all seven of the RI strains derived from these progenitors. Parasitemias in all animals became patent by day 2 postinfection, and the progenitor strains C and B (Fig. 2A and B, respectively) exhibited kinetics of parasitemia similar to those presented in Fig. 1.

The course of infection varied considerably among the different RI strains. The infection patterns of two RI strains, CXBH and CXBJ, illustrated in Fig. 2C and D, respectively, depict extremes of high and low parasitemias found in the RI mice. The CXBH strain consistently demonstrated low levels of parasitemia, generally remaining under 10%. The duration of infection was similar to that in C57BL/6. On the other hand, the CXBJ strain exhibited a dramatic early rise in parasitemia, reaching values of 50% by day 10 postinfection; subsequently, the parasitemias increased further, attaining peak values averaging 60%. The severity of infection in the CXBJ mice is indicated by the fact that in this experiment four out of seven animals succumbed to the infection.

Selected characteristics of acute malarial infection in RI strains are compared in Table 1. Although none of these RI mice displayed characteristics identical to those of the progenitors, the day of peak parasitemia and the duration of infection more closely resembled those of the B strain. Inspection of cumulative mortality data (Table 2) revealed that the RI strains could be divided into susceptible and resistant groups based on the lethality of infection with cloned *P. yoelii*. The three resistant RI strains demonstrated mortalities of 0 to 18%, and no deaths occurred in two of these strains. In contrast, susceptible RI strains showed mortalities ranging between 45 and 80%. In fact, 37 of the 68 test animals constituting the four susceptible strains succumbed to infection. These data show that some RI mice were more susceptible to *P. yoelii* infection than either progenitor strain.

Susceptibility of CXB RI strains to an unrelated plasmodial species. Since differences were observed among strains in susceptibility to infection with *P. yoelii*, we next determined whether similar differences in susceptibility would be observed with an unrelated species, *P. chabaudi adami*. The susceptible CXBJ and resistant CXBH, as well as the two progenitors, B and C, were infected with 10^6 parasitized erythrocytes. Acute infection with *P. chabaudi adami* followed similar kinetics in all four strains of mice (Fig. 3). No deaths were observed. These findings contrast markedly with those observed with *P. yoelii*.

DISCUSSION

Recent evidence has identified genes which affect host resistance with a variety of infectious agents (23). Mapping of these genes and determining their modes of action is one approach to defining specific resistance mechanisms. Some of these genetic studies have been facilitated by the existence of RI strains (3). The RI strains are useful because the parental genes have recombined and become fixed at each locus; consequently, these strains differ from each other at many genetic loci. Since information obtained with these strains is cumulative, the RI mice may be used for linkage analysis. For example, the Lsh gene which affects resistance of mice to *Leishmania donovani* has been mapped to chromosome 1 with RI strains (4). Moreover, this genetic locus also appears to control host resistance to *Salmonella typhimurium* (21).

In the present study, we used the CXB RI strains to examine genetic control of susceptibility to murine malaria initiated with *P. yoelii*, since in preliminary studies consistent differences were found in the kinetics of parasitemia of BALB/c and C57BL mice. The *P. yoelii* 17X strain was first cloned by limiting dilution to minimize genetic variability in the inoculum. This cloned *P. yoelii* was then tested for its patterns of infection in the two CXB progenitor strains, and these patterns were compared with those of the seven CXB RI strains. The patterns of infection in some of the RI strains

TABLE 2. Cumulative mortality data in CXB RI strains and their progenitors infected with *P. yoelii*

Mouse strain	No. of expt	No. of deaths/ no. of animals	% Deaths
Progenitor			
C	4	5/20	25
B	4	2/23	7
Resistant RI			
CXBG	3	0/18	0
CXBH	4	0/24	0
CXBK	3	3/17	18
Susceptible RI			
CXBD	2	8/10	80
CXBE	3	9/19	53
CXBI	3	9/20	45
CXBJ	4	11/19	58

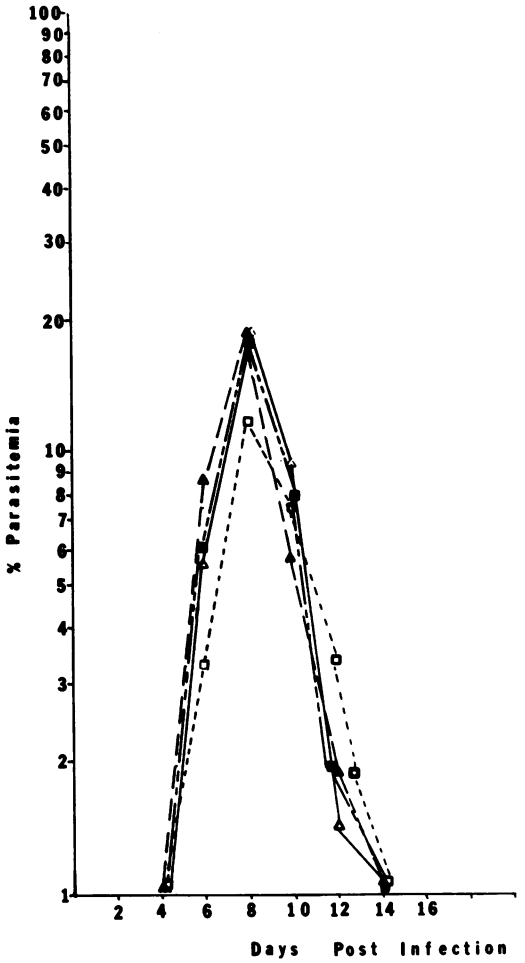

FIG. 3. Course of *P. chabaudi* infection in C, B, CXBH, and CXBJ mice. Each point represents the mean percentage of parasitemia derived from five mice. C (△), B (▲), CXBH (□), and CXBJ (■) mice were infected i.p. with 10^6 parasitized erythrocytes on day 0.

differed markedly from those seen with the parentals. For convenience, we have divided RI mice into susceptible and resistant groups based on mortality resulting from this infection, as summarized in Table 2. For example, CXBD mice demonstrated mortality of 80% and thus were very susceptible. In contrast, other RI strains showed low levels of parasitemia and low mortality and were highly resistant. The CXBH strain, as an example, was more resistant than either parent with regard to these parameters. Thus, susceptibility to infection in the RI strains did not follow the patterns of infection observed in either of the parental strains. This makes it difficult to map genes controlling these patterns by using information already accumulated on the CXB RI strains. These results also provide preliminary evidence for a complex multigenic mode of inheritance of susceptibility to this parasite. These findings are compatible with the results of previous experiments by Greenberg and colleagues using the lethal *P. berghei* (7, 17). Such complex modes of inheritance may be explained by the fact that the malarial parasite interfaces with the host at many points, and numerous host genes are then able to influence this interaction. For example, as summarized above, numerous host genes have been identified which influence infection by certain primate *Plasmodium* species.

With regard to mechanisms of resistance to *P. yoelii* in mice, several lines of evidence have shown that the production of serum antibody is required for the resolution of infection (11). Infection of B-cell-deficient mice results in fulminating, fatal infections by this normally nonlethal parasite (22, 26). CBA/N mice, which are deficient in certain B-cell subsets, are also very susceptible to this parasite (10, 12). Although we have not yet identified the mechanisms which are responsible for the enhanced susceptibility observed with some of the CXB RI strains, it is possible that certain of these mechanisms may be associated with the humoral immune response and the production of protective antibodies. When serum from CXBJ mice was used to specifically immunoprecipitate [^{35}S]methionine-labeled plasmodial antigens, these mice appeared to have lower levels of serum antibodies to *P. yoelii* antigens than the parentals did when displayed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (data not shown). Future studies with sera derived from susceptible versus resistant RI mice may prove useful in identifying plasmodial antigens as well as antibody isotypes or idiotypes which are required for protection.

Recent evidence suggests that resistance to different malarial parasites or to different stages of a single parasite species may be modulated by different mechanisms. In contrast to the virulence of *P. yoelii* in B-cell-deficient mice, these immunodeficient mice are capable of resolving acute infections with *P. chabaudi adami*, displaying the same kinetics as normal mice (8, 9). Therefore, we tested the susceptibility of CXB RI mice and their progenitors to infection with *P. chabaudi adami*. Strains that were susceptible or resistant to *P. yoelii*, as well as the C and B progenitors, all showed similar patterns of infection with this unrelated parasite species. These results clearly show that susceptibility to *P. chabaudi adami* does not cosegregate with susceptibility to *P. yoelii* in CXB RI mice. These genetic experiments support the earlier studies with immunodeficient mice by demonstrating that different parasites are resisted by different mechanisms. The observation that different mechanisms of resistance are required for different murine plasmodial species may also be relevant to infection of human populations by different *Plasmodium* species. The possible requirement for the activation of different mechanisms of resistance must be considered in any potential vaccination program aimed at preventing infection by a particular *Plasmodium* species.

ACKNOWLEDGMENTS

We thank Tom Daly for assistance with the cloning procedure, Akhil Vaidya for reading the manuscript, and Alice Smith for expert secretarial assistance.

This work was partially supported by Public Health Service grant no. R01AI 16153, from the National Institute of Allergy and Infectious Diseases, and by the UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases.

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