INFECTION AND IMMUNITY, Oct. 1982, p. 80–88
0019-9567/82/100080-09$02.00/0

Vol. 38, No. 1

Murine Malaria: Genetic Control of Resistance to *Plasmodium chabaudi*

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Received 31 March 1982/Accepted 16 June 1982

Strain variation in the level of resistance to malaria was investigated in inbred strains of mice after infection with *Plasmodium chabaudi*. When infected intraperitoneally with 10^6 *P. chabaudi*-parasitized erythrocytes, mice of 11 inbred strains could be separated into two groups by using survival time as the criterion; C57BL/6J, C57L/J, DBA/2J, CBA/J, and B10.A/SgSn mice were found to be resistant to *P. chabaudi*, whereas A/J, DBA/1J, BALB/c, C3H/HeJ, AKR/J, and SJL/J mice were susceptible. An examination of F_1 hybrids revealed that resistance was dominant over susceptibility. A segregation analysis of backcross and F_2 progeny derived from susceptible A/J and resistant B10.A/SgSn parental mice suggested that host resistance in this strain combination was genetically controlled by a single, dominant, non-*H-2*-linked gene. Inheritance of resistance was autosomal, but expression of the trait was influenced by the sex of the host, female mice being more resistant than male mice. Phenotypic expression of the resistance gene was apparent within 6 days of infection as a significant difference between resistant and susceptible mice in the level of parasitemia. A preliminary analysis of the mechanism of resistance showed that compared with susceptible A/J mice, resistant B10.A/SgSn hosts had an augmented erythropoietic response during the course of malaria, as well as phenylhydrazine-induced anemia. These results suggest that the ability to replace destroyed erythrocytes quickly and efficiently may determine host survival after infection with *P. chabaudi*.

Differences among inbred strains of mice in response to infection with various rodent malaria species have been observed. More than 25 years ago, Greenberg and co-workers (9) showed that the times until death of various inbred strains of mice after infection with *Plasmodium berghei* varied significantly. Although infection with this species of rodent malarial parasite is eventually lethal in all mice, resistant strains, such as C57BL mice, survive almost twice as long as susceptible mice, such as DBA/2. In contrast, infection with *Plasmodium chabaudi* results in death only in some strains of mice, whereas other strains are resistant and recover from the infection; for example, strain A/J (A) mice are susceptible, whereas strain C57BL mice are resistant (7, 8).

It has also been shown that resistance to *P. berghei* as assessed by time until death is under genetic control (16). A genetic analysis of the responses of backcross and F_1 progeny derived from C57BL and DBA/2 parental mice showed that resistance is due to inheritance of a single gene or a closely linked set of genes, at least in this strain combination. When the model of genetically determined resistance to infection with *P. berghei* is used, analyses of the early events leading to host resistance to malaria as measured by a characteristic as complex as death may be difficult to interpret. However, infection with *P. chabaudi*, which results in death only in susceptible animals, may provide a useful model to determine the mechanism of host response in resistant animals which leads to recovery and subsequent survival of the infected animals. Therefore, we examined the strain variation in resistance to infection with *P. chabaudi* among inbred mice and analyzed the mode of inheritance by segregation analysis of backcross and F_2 progeny derived from one combination of resistant (strain B10.A/SgSn [B10.A]) and susceptible (strain A) progenitors.

MATERIALS AND METHODS

Mice. Age- and sex-matched mice 8 to 12 weeks old were used in all experiments. B10.A, A, F_1 hybrid (B10.A × A, A × DBA/2J, and A × BALB/c), F_2, and backcross mice were bred in our laboratory. The following inbred strains were purchased from the Jackson Laboratory, Bar Harbor, Maine: C3H/HeJ, SJL/J, AKR/J, DBA/1J, CBA/J, C57BL/6J, C57L/J,

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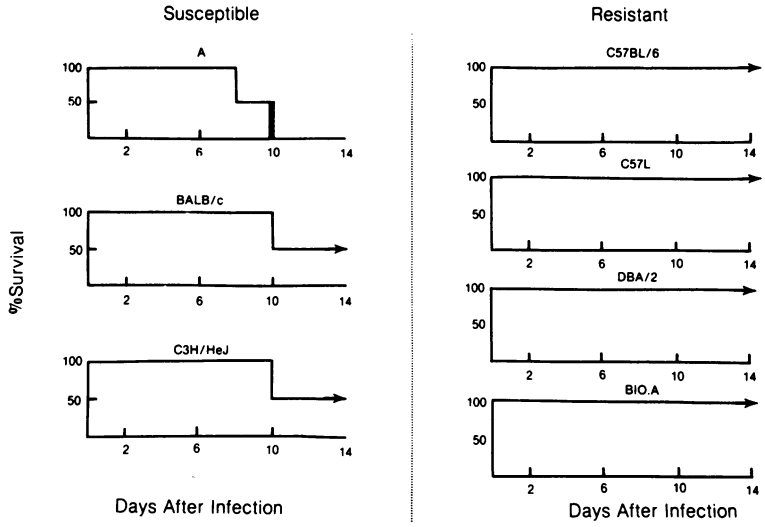

FIG. 1. Resistance of inbred mouse strains to infection with *P. chabaudi*. Groups of six mice per inbred strain were injected intraperitoneally with 10^6 parasitized erythrocytes, and the course of infection was followed.

and DBA/2J. BALB/c mice were purchased from Canadian Breeders, St. Constant, Quebec, Canada.

Parasite. *P. chabaudi* was a kind gift from Pierre Viens, Université de Montréal, Montreal, Quebec, Canada. This parasite was maintained by weekly passage in (B10.A × A)F_1 male mice. After 12 passages, the *P. chabaudi* preparation was discarded, and a fresh inoculum prepared from frozen stock cultures was stored at −40°C until it was used. For passage or infection of experimental mice, heparinized blood was collected from groups of infected F_1 animals and pooled. Total erythrocyte counts and parasitemia (percentage of 200 Wright-stained erythrocytes infected) were determined for duplicate samples. Erythrocytes diluted in sterile phosphate-buffered saline were adjusted to the desired concentration of parasitized erythrocytes and injected intraperitoneally into passage or experimental mice. For passages, infection was initiated with a dose of 10^6 to 10^7 parasitized erythrocytes.

Determination of parasitemia. The course of experimental infections was monitored every third day by examining Wright-stained thin blood smears. Parasitemias of individual mice were determined by counting a minimum of 200 erythrocytes per blood sample. Parasitemia is expressed as the mean percentage of erythrocytes infected from groups of 3 to 10 mice.

Determination of erythrocyte characteristics. The number of erythrocytes, hematocrit, and amount of hemoglobin were determined by standard procedures (1) for individual samples of blood from normal or infected mice. Erythrocyte characteristics are presented as the mean values for blood samples from groups of four to eight mice. Reticulocyte counts were determined by counting a minimum of 500 erythrocytes on blood smears stained with New Methylene Blue (A.J.P. Scientific, Clifton, N.J.). Reticulocytosis is expressed as the mean percentage of reticulocytes in blood samples from groups of four to six normal or treated mice. Anemia was induced chemically in mice by intraperitoneal injection of 3.2 mg of phenylhydrazine hydrochloride (Fisher Scientific, Montreal, Canada) diluted in phosphate-buffered saline and adjusted to pH 7.0. The 50% lethal dose was calculated by the method of Reed and Muench (20) and was determined after injection of doses of phenylhydrazine-hydrochloride ranging from 0.8 to 8.0 mg into groups of four mice. Mice were observed daily for 10 days, and mortality was recorded.

RESULTS

Strain survey of resistance and susceptibility to *P. chabaudi*. The level of resistance to infection with the murine malarial species *P. chabaudi* was examined in various inbred strains of mice. After intraperitoneal infection with a typing dose of 10^6 parasitized erythrocytes, the following two categories of resistance were apparent: resistant and susceptible. When survival of 100% of the infected animals at day 14 was used to identify resistant strains, strain B10.A, DBA/2J, C57BL/6J, and C57L/J mice were characterized as resistant (Fig. 1). In fact, resistant mice continued to survive indefinitely beyond day 14. In contrast, strain A, BALB/c, and C3H/HeJ mice were characterized as susceptible. Infection with *P. chabaudi* was lethal to 100% of strain A mice and 50% of BALB/c and C3H/HeJ mice within 10 days.

In general, murine malaria due to infection with *P. chabaudi* is not lethal in certain inbred strains of mice (8). However, after prolonged passage in mice (>16 passages), a virulent form of the parasite developed. Infection with 10^6 parasitized erythrocytes containing this substrain of *P. chabaudi* resulted in the death of

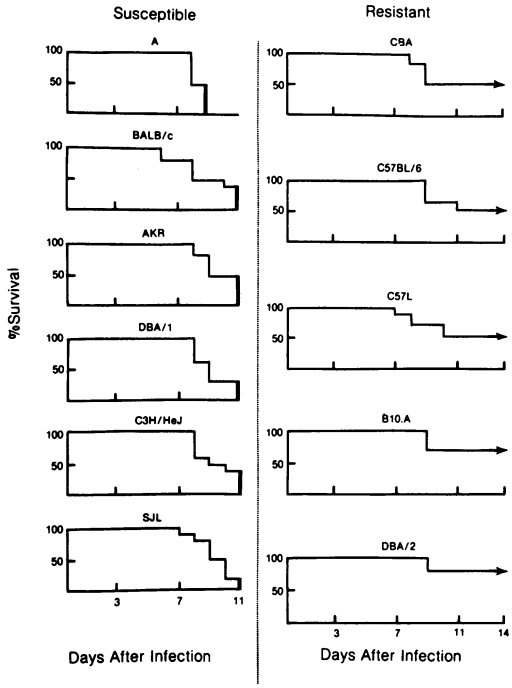

FIG. 2. Resistance of inbred mouse strains to infection with *P. chabaudi* (virulent). Groups of 10 mice per inbred strain were injected intraperitoneally with 10^6 erythrocytes parasitized with *P. chabaudi* made virulent by prolonged passage in mice. The percentage of surviving mice and the time until death were determined for each strain.

some animals even in the strains characterized in the experiment described above as resistant. Despite the increased virulence of the typing strain, mouse strains could be divided into two nonoverlapping categories by using the criterion of 100% lethality to identify susceptible strains. This highly virulent variant of *P. chabaudi* was used only for the experiments reported in Fig. 2. The strain distribution pattern of resistance and susceptibility was concordant with the results of the experiment described above. Strain B10.A, DBA/2J, C57BL/6J, and C57L/J mice were again typed as resistant. CBA/J mice were also found to be resistant. The inbred strains of mice which were characterized as susceptible were strains A, BALB/c, C3H/HeJ, DBA/1J, AKR/J, and SJL/J. Despite the virulence of the typing strain of *P. chabaudi*, a clear difference between resistant and susceptible mice was evident; by day 11, 100% of the susceptible mice had succumbed to infection.

Hybrid and backcross analysis of resistance to *P. chabaudi*. The results of the strain survey suggested that the level of resistance to infection with *P. chabaudi* was dependent upon the genetic background of the host. Furthermore, the *H-2* complex did not appear to be important in determining the level of resistance since strain A mice ($H\text{-}2^a$) were highly susceptible, whereas H-2 congenic strain B10.A mice ($H\text{-}2^a$) were resistant. To determine the mode of inheritance of resistance to malaria caused by *P. chabaudi*, the level of resistance in F_1 hybrids was examined. Two F_1 hybrid combinations resulting from crosses between susceptible strain A mice and resistant strain B10.A or DBA/2J mice were studied. In each case, 100% of the (B10.A × A)F_1 or (A × DBA/2J)F_1 mice were resistant to infection with 10^6 parasitized erythrocytes injected intraperitoneally (Table 1). In a manner similar to the resistant parents, 100% of the animals of both F_1 hybrids were alive at day 14 and continued to survive beyond this time, whereas 100% of the parental strain A mice succumbed to the infection by day 10. An analysis of the level of resistance in (A × BALB/c)F_1 progeny derived from two susceptible parents revealed a low level of resistance which was similar to that observed in animals of the parental strains. These data suggest that the level of resistance to infection with *P. chabaudi* is a dominant trait.

To analyze further the genetic control of the level of resistance to infection with *P. chabaudi*, a segregation analysis of the trait was undertaken, using backcross and F_2 animals. Highly susceptible strain A mice and very resistant strain B10.A mice were chosen as the parental strains. Using a typing dose of approximately 10^6 to 10^7 erythrocytes parasitized with *P. chabaudi*, we examined the level of resistance of 530 parental strain A, parental strain B10.A, hybrid (B10.A × A)F_1, backcross (F_1 × B10.A and F_1 × A), and F_2 animals (Fig. 3). During this

TABLE 1. Genetic control of resistance to malaria in F_1 hybrids

Mouse strain[a]	No. surviving/ total no.	Mean survival time (days)[b]
A	0/6	8.4 ± 0.2
B10.A	6/6	>14
(B10.A × A)F_1	8/8	>14
A	0/9	9.7 ± 0.4
DBA/2	10/10	>14
(A × DBA/2)F_1	10/10	>14
A	0/6	8.3 ± 0.3
BALB/c	4/8	8.6 ± 0.5
(A × BALB/c)F_1	2/5	8.3 ± 0.3

[a] Five to ten mice per group were infected intraperitoneally with 10^6 parasitized erythrocytes, calculated as described in the text.

[b] Infected mice died at the times indicated or survived for more than 14 days. Results are expressed as means ± standard errors of the mean.

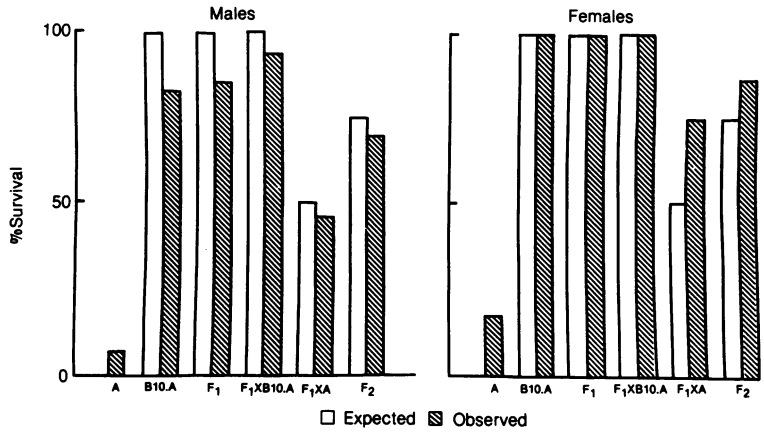

FIG. 3. Segregation analysis of resistance to *P. chabaudi* in male and female mice. Groups of 10 to 100 male or female mice belonging to strains A and B10.A and their F_1, F_2, and backcross progeny were injected intraperitoneally with 10^6 to 10^7 parasitized erythrocytes. The outcome of infection (either death or recovery) was followed in individual animals (expected or observed percentage of survival for genetic control by a single, dominant gene[s]).

investigation, it became apparent that the sex of the host influenced the outcome of the infection. For example, 100% (15 of 15) of female strain B10.A mice and 100% (11 of 11) of female F_1 mice survived the infection, whereas only 83% (67 of 80) of male B10.A mice and 86% (43 of 50) of male F_1 animals survived. Since the sex influence on survival after infection with *P. chabaudi* was statistically significant in the animals of the $F_1 \times A$ backcross generation ($\chi^2 = 4.0$; $P < 0.025$), we performed a genetic analysis on separate groups of male and female mice. Using the criterion of survival at day 14 as a measure of resistance, we obtained the following results: in groups of male mice (expressed as number of animals surviving/total number of animals), 94% (32/35) of $F_1 \times$ B10.A, 46% (29/62) of $F_1 \times$ A, and 70% (43/57) of F_2 animals were resistant, whereas in groups of female mice 100% (10/10) of B10.A $\times F_1$, 75% (24/32) of A $\times F_1$, and 87% (36/41) of F_2 animals were resistant. These results are consistent with the hypothesis that the major determinant of resistance to infection with *P. chabaudi* in B10.A mice is a single, dominant gene (or group of closely linked genes) which is not expressed in strain A animals. Furthermore, the expression of this gene is influenced by the sex of the host. The increased resistance of females is not due to a sex-linked gene because in F_1 hybrids derived from a resistant parent and a susceptible parent, the level of resistance in the mother did not influence the resistance of the F_1 offspring.

TABLE 2. Resistance to malaria: dose response

Dose[a]	Mouse strain	No. surviving/ total no.	Mean survival time (days)[b]
10^4	A	0/3	12.7 ± 0.3
	B10.A	3/3	>14
10^5	A	0/3	10.0 ± 0.6
	B10.A	3/3	>14
10^6	A	0/4	9.5 ± 0.9
	B10.A	4/4	>14
10^7	A	0/4	7.7 ± 0.5
	B10.A	4/4	>14
10^8	A	0/4	6.0 ± 0.4
	B10.A	4/4	>14
10^9	A	0/6	5.5 ± 0.2
	B10.A	5/6	>14

[a] Male mice were injected intraperitoneally with different doses of parasitized erythrocytes, calculated as described in the text.

[b] Infected mice died at the times indicated or survived for more than 14 days. Results are expressed as means ± standard errors of the mean.

Phenotypic expression of genetically controlled resistance to malaria. (i) Dose response. The death of susceptible animals early in the course of infection with *P. chabaudi* suggested that the mechanism of genetically determined resistance was phenotypically expressed in the early stages of malaria. To define the phenotypic expression of the major genetic determinant responsible for the difference in survival between strain B10.A and A mice, we first examined survival after infection with a range of doses of parasitized erythrocytes. Table 2 shows that susceptible strain A mice succumbed to infection at all doses of parasitized erythrocytes examined (10^4 to 10^9 cells). Furthermore, as the infective dose was increased, the length of the mean survival time

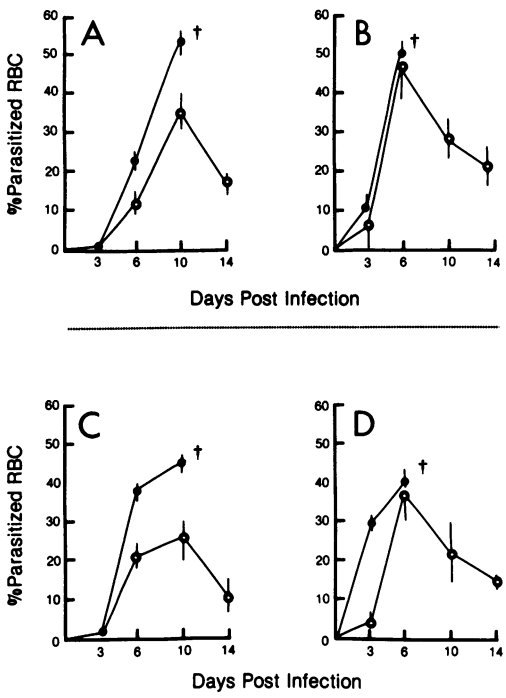

FIG. 4. Levels of early parasitemia in resistant and susceptible mouse strains. Groups of five male mice of susceptible strain A (●) or resistant strain B10.A (○ [A and B]) or strain DBA/2J (○ [C and D]) were infected intraperitoneally with 10^6 (A and C) or 10^8 (B and D) parasitized erythrocytes. At different times after infection, the percentages of parasitized erythrocytes (RBC) were determined on blood smears as described in the text. The data are expressed as mean ± standard error of the mean. The daggers represent death. Similar results were obtained with female mice.

decreased from approximately 13 days after infection with 10^4 parasitized erythrocytes to less than 6 days after infection with 10^9 parasitized erythrocytes. In contrast, B10.A mice were 100% resistant at all doses except the highest dose used (10^9 parasitized erythrocytes). At this dose, one of six B10.A mice was dead at day 6. Therefore, the 50% lethal doses for susceptible strain A and resistant B10.A mice were $<10^4$ and $>10^9$ parasitized erythrocytes, respectively.

(ii) Parasitemia. As a second measure of the phenotypic expression of resistance to malaria, the levels of parasitemia after infection with a low dose (10^6 cells) and a high dose (10^8 cells) of parasitized erythrocytes were examined in susceptible and resistant animals (Fig. 4). Through 14 days after infection, a distinct difference in the pattern of parasitemia between susceptible strain A mice and resistant B10.A mice was discernible. The level of parasitemia in strain A mice reached a peak of approximately 40 to 50% after infection with either dose. When this critical parasitemia level was reached, the infection terminated in the death of 100% of the susceptible strain A mice. Similar to the results of the dose-response study, both the time until death and the prepatent period of the infection decreased after infection with 10^8 parasitized erythrocytes.

In resistant strain B10.A mice, the level of peak parasitemia was dependent upon the dose of parasitized erythrocytes used. At the low dose, the percentage of parasitized erythrocytes peaked at approximately 35% in B10.A mice at 10 days, compared with 52% in strain A mice. By 14 days after infection, the level of parasitemia in B10.A mice was decreasing. In contrast, at the high dose the level of peak parasitemia approached the 50% level characteristic of susceptible strain A mice. However, even at the high dose, resistant B10.A mice survived the level of parasitemia that was lethal to strain A mice, and the percentage of parasitized erythrocytes was decreasing by 10 to 14 days after infection. In a second study, in which DBA/2J mice were used as the resistant strain, the results observed with B10.A mice were confirmed. Resistant DBA/2J mice survived infection with 10^8 parasitized erythrocytes despite reaching a parasitemia level which was lethal to 100% of susceptible strain A mice. The results of the dose-response and parasitemia studies suggest that the mechanism of genetically determined resistance to infection with *P. chabaudi* is apparent in resistant strains of mice early in the course of infection. Moreover, the phenotypic expression of high resistance as measured by survival is apparently not dependent upon either the dose of the infective inoculum or the level of peak parasitemia. Resistant mice survive infec-

TABLE 3. Erythrocyte characteristics of strain A and B10.A mice

Mouse strain	Erythrocytes (10^6 cells/mm^3)	Mean hematocrit (%)	Mean hemoglobin (g/100 ml of blood)	MCV (μm^3)[a]	MCH (μg)[a]	MCHC (%)[a]
A (n = 8)	9.9 ± 0.1	53.0 ± 0.7	17.3 ± 0.1	44.0 ± 0.4	17.3 ± 0.2	40.0 ± 0.2
B10.A (n = 8)	9.1 ± 0.3	53.3 ± 1.3	15.2 ± 0.2	43.0 ± 0.4	11.7 ± 0.4	39.7 ± 0.9

[a] MCV, Mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration (1).

TABLE 4. Effect of malaria infection on erythrocyte characteristics of strain A and B10.A mice

Mouse strain	Dose of *P. chabaudi*[a]	Parasitemia (%)[b]	Erythrocytes (10^6 cells/mm^3)[b]	Hematocrit (%)[b]	No. of parasitized erythrocytes (10^6 cells/mm^3)[b]
A			9.9 ± 0.07[c]	53.0 ± 0.7[c]	
B10.A			9.1 ± 0.3	53.3 ± 1.3	
A	10^6	56.6 ± 2.1[c,d]	4.6 ± 0.03	20.7 ± 7.2[e]	2.61 ± 0.02[c,f]
B10.A	10^6	35.8 ± 2.7[d]	9.3 ± 0.27	43.7 ± 1.7[e]	3.33 ± 0.24[f]
A	10^9	50.3 ± 1.7	5.6 ± 0.45	26.8 ± 2.2[f]	2.81 ± 0.22[f]
B10.A	10^9	46.8 ± 1.2	4.7 ± 0.07	23.4 ± 0.4[f]	2.17 ± 0.03[f]

[a] Number of parasitized erythrocytes injected intraperitoneally as described in the text. Each group contained six to eight male mice.

[b] Determined on day 7 after infection with 10^6 parasitized erythrocytes or on day 4 after infection with 10^9 parasitized erythrocytes.

[c] Mean ± standard error of the mean.

[d] Significant ($P < 0.005$).

[e] Significant ($P < 0.01$).

[f] Not significant ($P < 0.10$).

tion with a high dose of parasitized erythrocytes despite reaching a level of parasitemia which kills susceptible animals.

(iii) Erythrocyte characteristics and response to anemia in resistant and susceptible strains. Since anemia is one of the major symptoms which develop during malaria, differences between strain A and B10.A mice in the level of resistance to *P. chabaudi* could be due to differences in host response to anemia. Therefore, we examined several characteristics of the erythrocyte systems of normal strain A and B10.A mice, including the number of erythrocytes, hematocrit, and volume of hemoglobin (Table 3). We found no differences in the characteristics of the erythrocyte systems when we compared normal adult animals of the two strains. The values determined for numbers of erythrocytes, percent hematocrit, and hemoglobin volume are in agreement with previously published values for normal, adult strain A and B10.A mice (4).

After infection with *P. chabaudi*, however, there were significant differences in the erythrocyte characteristics of strain A and B10.A mice (Table 4). It is interesting that in resistant B10.A hosts, these differences, like the differences in the level of peak parasitemia (Fig. 4), were dependent upon the infecting dose. At a dose of 10^6 parasitized erythrocytes, there was a significant difference in the level of parasitemia, as well as significant differences in the number of erythrocytes and the percent hematocrit, between resistant B10.A and susceptible strain A mice. There was a decrease in the hematocrit of strain A mice of approximately 60%, compared with a 20% decrease in B10.A mice at 7 days after infection with 10^6 parasitized erythrocytes. At a high dose (10^9 parasitized erythrocytes) of *P. chabaudi*, there were decreases of approximately 50% in the numbers of erythrocytes and in the hematocrits of both strain A and B10.A mice within 4 days of infection. There was no significant difference between resistant and susceptible animals in the level of peak parasitemia after infection with 10^9 parasitized erythrocytes, yet, as noted above, the survival values for resistant and susceptible mice infected with this dose were markedly different (Table 2). There was an inverse correlation between the level of parasitemia and the percent hematocrit; a high level of parasitemia was associated with a low hematocrit. However, a comparison of the absolute number of infected erythrocytes in resistant B10.A and susceptible strain A hosts at either dose of *P. chabaudi* showed that there were 2×10^6 to 3×10^6 parasitized erythrocytes in both mouse strains. Thus, these results suggest that a superior erythropoietic response to anemia in B10.A hosts might be related to resistance to malaria.

Therefore, differences in the responses of the erythrocyte systems of strain B10.A and A mice were defined further by inducing anemia with the chemical agent phenylhydrazine. Groups of five mice of both strains were injected intraperitoneally with doses of phenylhydrazine hydrochloride ranging from 0.8 to 8.0 mg/ml, and the 50% lethal dose for each strain was determined. A significant difference in 50% lethal dose between the strains was observed (4.0 mg for strain A versus 5.0 mg for B10.A mice). In addition, production of reticulocytes in phenylhydrazine-treated mice not only was greater but also occurred sooner in B10.A mice than in similarly treated strain A mice (Fig. 5). Within 3 days of injection of phenylhydrazine (3.2 mg intraperitoneally), there were twice as many reticulocytes in the blood of B10.A mice as in the blood of

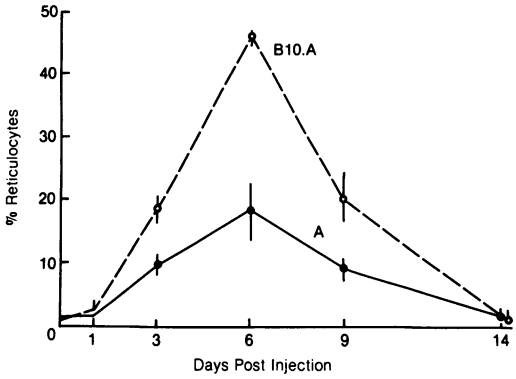

FIG. 5. Course of the reticulocytosis in phenylhydrazine-treated mice. Groups of five strain A (●) or strain B10.A (○) mice were injected intraperitoneally with 3.2 mg of phenylhydrazine. The percentage of reticulocytes was determined on New Methylene Blue-stained blood smears. The data are expressed as mean ± standard error of the mean.

strain A mice. This twofold difference represented an 18-fold increase in the level of 1% reticulocytes found in both normal strain B10.A and normal strain A mice. The peak of reticulocytosis was apparent in both strains at day 6. Although approximately the same number of erythrocytes were detected in strain B10.A and A mice (6.4 ± 0.52 versus 6.0 ± 1.12 cells), there were almost three times as many reticulocytes in the blood of strain B10.A hosts. Thus, the responses of the erythrocyte systems to equal stresses of anemia differed in malaria-resistant B10.A and susceptible strain A mice. The difference was apparent both in the tempo of the response and in the level of the response; the response of resistant B10.A mice was both quicker and greater.

DISCUSSION

Although genetically determined differences in survival time after infection with *P. berghei* are apparent among inbred strains of mice, infection with this species of murine *Plasmodium* is eventually lethal to all animals (9, 16). Infection with another murine malaria species, *P. chabaudi*, which we used in this study, results in a much clearer distinction between susceptible and resistant mice. Studies by Eugui and Allison (7, 8) have demonstrated that certain strains of mice, such as strain A, are susceptible, reaching a level of fulminant parasitemia and succumbing to infection within 10 days, whereas other strains, such as C57BL, CBA, and B10.A, exhibit transient parasitemia, followed by recovery.

We extended the observation of differences in resistance to malaria by examining survival after infection with *P. chabaudi* in 11 inbred strains of mice. The level of resistance was found to be genetically controlled by a single, dominant, non-*H-2*-linked gene (or by a group of closely linked genes) by a genetic analysis of the progeny derived from the strain pair combination of susceptible strain A mice and *H-2* congenic strain B10.A on the resistant C57BL/10 background. The genetic locus is apparently autosomal, but female mice were more resistant than male mice. For example, among (B10.A × A)F_1 × A backcross mice, 46% (29 of 62) of the male mice survived infection, whereas 75% (24 of 32) of the female mice survived. The expected survival based on the hypothesis of genetic control by a single, dominant gene was 50% in the susceptible backcross population. Increased resistance of female animals compared with male animals has been described in several murine parasite systems, including *P. berghei* (9), *Trypanosoma rhodesiense* (10), *Nematospiroides dubius* (5), and *Trypanosoma cruzi* (24). However, the basis of sex-related resistance remains unknown.

An examination of percent survival and time until death apparent among inbred strains of mice (Fig. 1 and 2) reveals a spectrum of responses. For example, infection with *P. chabaudi* was 100% lethal in strain A mice, 50% lethal in C3H/HeJ mice, and, except for sex differences, nonlethal in B10.A mice. In addition, there were significant differences in mean survival time ($P < 0.005$) among the various strains. Strain A mice survived 7.6 ± 0.2 days, whereas C3H/HeJ mice survived 9.7 ± 0.6 days after infection. We intentionally used the most susceptible strain (A) and one of the most resistant strains (B10.A) of the spectrum for the segregation analysis of genetic control of resistance to malaria. In this strain combination, the difference at a single genetic locus is the most likely explanation for the ratios of resistant to susceptible progeny obtained in backcross and F_2 generations. However, formal proof of this hypothesis will require further backcrossing of individual animals of the segregating populations and testing of the progeny to determine whether the genetic factor continues segregating as a single gene. The fact that mice of several strains which are all classified as susceptible by our criteria exhibited a spectrum of mean survival times (SLJ/J > C3H/HeJ > DBA/1J > AKR/J > BALB/c > A) suggests that other genes, probably of lesser influence, also contribute to the overall level of resistance to *P. chabaudi* in mice. However, it was clearly advantageous to analyze the extreme ends of the spectrum (strains A and B10.A) since strain A mice seem to possess no resistant alleles at the other putative genetic loci regulating the varying degrees

of resistance within the malaria-susceptible group of strains. Therefore, using this strain combination, one might be able to analyze a single mechanism of resistance without the interference of the other gene products.

An examination of the phenotypic expression of the malaria resistance gene revealed (i) that death of susceptible animals was not dependent upon the dose of the infective inoculum and (ii) that the level of parasitemia in susceptible strain A mice showed significant differences in the percentage of parasitized erythrocytes as early as 6 days after infection with 10^6 parasitized erythrocytes compared with the levels in two resistant strains, B10.A and DBA/2. When the parasitemia reached a fulminant level (approximately 50%), all strain A mice succumbed to the infection. However, in resistant strains the level of parasitemia was moderate and transient at a low infective dose (10^6 parasitized erythrocytes), whereas at a high dose (10^9 parasitized erythrocytes) resistant animals recovered and survived the infection despite fulminant parasitemia. Since superior levels of resistance in mice surviving infection with *P. chabaudi* are apparent as soon as 6 days after infection, we can hypothesize that genetically determined resistance to malaria in mice is another example of innate natural resistance. Natural resistance in mice has been described recently for a variety of pathogens, including bacteria, viruses, and parasites (23). Based on studies with other *Plasmodium* species, the phenotypic expression of genetically determined resistance to *P. chabaudi* that is apparent early in the course of the disease is most probably independent of the development of specific immunity (2, 11, 13, 19, 21, 26). However, an evaluation of the course of malaria in T- and B-cell-deprived animals will be required in our system to support the hypothesis of natural resistance to *P. chabaudi*.

NK cell activity, which is also genetically determined (15) and is considered to be a mechanism of natural resistance against tumors in vivo (14), has been implicated as a mechanism for control of parasitemia during malaria. Increased NK cell activity during malaria has been observed in both murine and human systems (7, 8, 12, 17). The conclusions of studies with mice were based on a positive correlation between high NK cell activity and a high level of resistance to *P. chabaudi* in certain inbred strains (7, 8). However, as Eugui and Allison (8) point out, backcross linkage studies are necessary to determine whether the two traits segregate together or independently. In a separate report, we will present evidence for dissociation of resistance to malaria and high NK cell activity (manuscript in preparation).

Alternatively, the mechanism of resistance may be related to differences in host responses to anemia. It has been suggested that if a host can replace destroyed erythrocytes quickly, the host recovers and the parasite is eliminated by more specific immune antiparasitic mechanisms (22). Our observations of differences between resistant B10.A and susceptible strain A mice in the numbers of erythrocytes during infection and during the course of reticulocytosis after treatment with phenylhydrazine suggest that resistant strains of mice have a superior erythropoietic system. Thus, these results suggest that the course and severity of anemia during malaria are strain dependent. Similar observations have been reported in a study which followed the course of *P. berghei* infections in various inbred mouse strains (6). Generally, the rate of erythrocyte loss was found to be mouse strain specific and to correlate inversely with the mean survival time.

In our mouse system, the tempo and quantity of reticulocyte production during chemically induced anemia were superior in resistant B10.A animals. Agents such as phenylhydrazine or corticosteroids, which alter the production of reticulocytes, have been found to influence the outcome of infections with various *Plasmodium* species, including *P. chabaudi* (3, 18, 25, 26). For example, stimulation of reticulocytosis by phenylhydrazine resulted in a diminished level of parasitemia after infection with *P. chabaudi*, which lacks a preference for reticulocytes (18). Resistant mice, such as strain B10.A, may survive by rapidly producing large numbers of young erythrocytes or reticulocytes which replace mature blood cells destroyed by the parasite yet are not infected by the parasite. Thus, the net outcome of the massive destruction of erythrocytes which occurs during the course of infection may aid the host by simultaneous destruction of parasites and stimulation of reticulocyte production. Further investigation will be required to determine whether there is a cause-and-effect relationship between the level of host resistance to malaria and host response to anemia. Our murine model of a major genetic determinant of resistance to infection with *P. chabaudi* should prove to be a useful tool for this analysis.

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