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Antibody-Independent Immunity to Reinfection Malaria in B-Cell-Deficient Mice

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Immunity to "reinfection malaria" or "premunition" was studied in B-cell-deficient mice which had previously experienced acute malaria caused by the avirulent plasmodia *Plasmodium yoelii* or *P. chabaudi* or by the lethal *P. vinckei*. Such mice resisted challenge infection with large numbers of homologous parasites but differed in their capacity to resist challenge with heterologous species. Mice immune to *P. yoelii* resisted infection with *P. chabaudi* but developed acute-type, albeit nonlethal, infections when challenged with *P. vinckei*. Whereas mice immune to *P. chabaudi* resisted challenge with *P. vinckei* and vice versa, they developed fulminating malaria and died when infected with *P. yoelii*. The data suggest that immunity to reinfection malaria in B-cell-deficient mice, although antibody independent, is mediated by different mechanisms of resistance depending upon the plasmodial species used to initiate acute infection. Additional evidence supporting this concept was gained from preliminary experiments in which immunity to reinfection was measured by the ability of chronically infected mice to control endogenous parasites at low levels. B-cell-deficient mouse strains showed genotypic differences in their ability to develop immunity to reinfection with *P. yoelii*. In contrast, the same mouse strains uniformly developed immunity to reinfection with *P. chabaudi*. These findings suggest that different genetic loci control resistance to reinfection malaria caused by different species of plasmodia. Finally, B-cell-deficient mice acutely infected with lethal plasmodia, *P. vinckei* or *P. berghei*, died at the same time or earlier than similarly infected immunologically intact mice, indicating that "early death" in virulent malarial infections is an antibody-independent phenomenon.

Resistance to malaria is a complex phenomenon involving both innate and acquired immune responses (6, 14). Whereas the mechanisms involved in the resolution of acute infections or control of chronic disease or both remain to be determined, T cells are likely to be involved (2). Whether they function as helper cells in the production of protective antibodies or manifest their protective activity by some other means remains controversial.

Studies in our laboratory have utilized immunodeficient animals to investigate the role of T- and B-lymphocyte systems in resisting experimental malaria. Thus, acute *Plasmodium gallinaceum* infections of chickens (16) and acute *P. yoelii* infections of mice (17) were lethal if their respective hosts lacked either T or B cells. Similar findings have been reported previously by others (4, 22, 26). However, when these otherwise lethal infections were controlled in B-cell-deficient animals by short-term chemotherapy, the animals subsequently developed chronic low-grade malaria and resisted challenge infection with homologous parasites (10, 16, 19). These data suggested that, whereas acute malarial infections caused by these species of plasmodia were controlled by antibody-dependent mechanisms of immunity, resistance to "reinfection malaria" in B-cell-deficient hosts was mediated by antibody-independent mechanisms of immunity. After the termination of drug therapy, acute-type malaria did recur in both athymic nude mice (17) and thymectomized-irradiated chickens (Grun and Weidanz, unpublished data), indicating that immunity to reinfection malaria in chronically infected B-cell-deficient hosts was thymus dependent.

Subsequent studies with yet another murine plasmodial species, *P. chabaudi*, demonstrated that acute infections initiated with this parasite, in contrast to the above, were terminated spontaneously by antibody-independent mechanisms of immunity which were T-cell dependent (12). After the resolution of their acute infections, B-

cell-deficient mice developed chronic *P. chabaudi* malaria and resisted challenge infection with homologous parasites.

These findings showed that immunity to acute malaria caused by different plasmodial species could be differentiated from the need for B-cell-dependent mechanisms to participate in the termination of acute infection; i.e., acute *P. yoelii* infections required B-cell participation, whereas acute *P. chabaudi* malaria was controlled by B-cell-independent mechanisms of immunity. Chronic malaria in B-cell-deficient mice regardless of etiology was controlled by antibody-independent mechanisms of immunity.

We now describe studies characterizing more fully acute malarial infections in B-cell-deficient mice caused by different species of plasmodia, as well as selected parameters of immunity induced in B-cell-deficient mice which had been immunized by acute infection. In addition, we have examined immunity to reinfection malaria or "premunition" (20) in B-cell-deficient mice which had previously experienced acute malaria resulting from infection with different species of murine plasmodia. To accomplish this, we have utilized two parameters of immunity to measure resistance in the immunized host: (i) the ability of the chronically infected B-cell-deficient mouse to control endogenous parasites at low levels, and (ii) the ability of B-cell-deficient mice which had previously experienced acute malaria to resist challenge with homologous as well as heterologous plasmodia. Data to be presented suggest that immunity to reinfection malaria, although antibody independent, may be mediated by different mechanisms of immunity depending upon the plasmodial species used to initiate acute infection.

MATERIALS AND METHODS

Mice. Male and female mice of the following strains were bred and housed under optimal conditions in our closed colony: C57BL/10, BALB/c, (BALB/c × C57BL/10)F_1, and (C57BL/10 × BALB/c)F_1. These animals were derived from C57BL/10 breeding pairs kindly supplied by Carole Long, Hahnemann University, and BALB/c parental stock originally purchased from the Institute for Cancer Research, Fox Chase, Pa.

In vivo suppression of B-cell development with anti-μ. Selected litters of mice were rendered B-cell deficient by a modification of methods described previously (17). Briefly, antiserum prepared in goats (anti-μ) against a purified mouse myeloma protein, MOPC 104E (μ,λ; Litton Bionetics), was heat inactivated, adsorbed with 5% washed mouse erythrocytes, sterilized by filtration, and stored at −20°C until used. Newborn mice were given daily injections of high-titered anti-μ for the first 2 or 3 days of life; thereafter, they were maintained on thrice-weekly injections for the duration of the experiments. Compared with immunologically intact animals, mice treated with anti-μ in this manner demonstrated severe B-cell deficiencies, but normal T-cell responsiveness (12). Although not an absolute indicator of the B-cell deficiency achieved by repeated treatment with anti-μ, good correlation was found between the B-cell-deficient status of injected mice and the continued presence of goat anti-μ combined with the absence of detectable mouse immunoglobulin M in the sera of such μ-suppressed animals (13, 26). For this reason, we performed gel diffusion analysis on the sera of selected experimental animals either before their use in or at the completion of each experiment to show that they contained goat anti-μ but lacked mouse immunoglobulin M, as described previously (12).

Malarial parasites. *P. yoelii* (17X), *P. berghei* (NYU-2), and *P. vinckei* (ATCC 30091) (all originally obtained from J. Finerty, National Institutes of Health), as well as *P. chabaudi adami* (556KA) (kindly provided by D. Wyler, National Institutes of Health), were maintained as cryopreserved stabilates of parasitized mouse blood. Experimental infections as described in the text were initiated with parasites taken during the first or second mouse passage of stabilate material. Parasitemias were determined by microscopic examination of thin blood films prepared from tail blood and stained with Giemsa stain.

Immunization of B-cell-deficient mice via active infection. Primary *P. yoelii* infections used to immunize B-cell-deficient mice were usually initiated by intravenous (i.v.) injections of 10^5 parasitized erythrocytes (pRBC). In several experiments, however, infection was accomplished by the intraperitoneal route with 10^5 to 10^6 pRBC. Beginning 8 to 18 days postinfection, when acute *P. yoelii* parasitemias surpassed 10%, mice were treated per os with clindamycin hydrochloride hydrate (Cleocin, The Upjohn Co.) four to six times over a 5- to 8-day period to arrest their infections (17–19). The drug was suspended in water immediately before use so that the desired daily dose (75 mg of active drug per kg of body weight) was contained in a volume of 25 μl. In several instances, chemotherapy was repeated 2 to 3 weeks after the completion of the original regimen. To determine whether protection against challenge infection could be attributed to the continued presence of the drug in host tissues, uninfected mice were also treated with the same clindamycin regimen and were later used as controls when the immunized mice were challenged with exogenous plasmodia. Chemotherapy alone, in the absence of the immunizing malarial infection, did not induce any observed protective response in these mice.

Similarly, mice were protected against *P. vinckei* infection by chloroquine therapy during acute parasitemia. Chloroquine diphosphate (Sigma Chemical Co.) was administered to the mice in their drinking water (125 mg of chloroquine per liter of water) for 5 consecutive days beginning on day 7 after i.v. infection with 10^5 *P. vinckei*. One intraperitoneal injection of 0.8 mg of chloroquine diphosphate was given on day 9 postinfection. Immunity to *P. vinckei* was tested in these mice by i.v. challenge with 5×10^5 homologous plasmodia 36 days after the initiation of the primary infection.

P. chabaudi infections were initiated by the i.v. injection of 10^5 or 10^6 pRBC, as indicated in the text, and the mice were allowed to resolve their acute malaria spontaneously.

TABLE 1. Acute malaria in B-cell-deficient versus immunologically intact mice[a]

Infecting species of *Plasmodium*	Immunological status of host	No. of mice	Acute malarial infection		
			Peak parasitemia		Mean day of death
			Mean (%)	Day	
P. yoelii	Intact	9	27 ± 18	11	Nonlethal
	B-cell deficient	13	63 ± 13	25	26 ± 7.5
P. berghei	Intact	6	62 ± 10	15	17 ± 6.0
	B-cell deficient	7	49 ± 6	7	9 ± 2.5
P. chabaudi	Intact	11	14 ± 6.3	6	Nonlethal
	B-cell deficient	11	13 ± 4.5	7	Nonlethal
P. vinckei	Intact	6	71 ± 9	8	9 ± 0.5
	B-cell deficient	6	63 ± 12	8	9 ± 0.5

[a] Groups of (BALB/c × C57BL/10)F_1 and (C57BL/10 × BALB/c)F_1 mice aged 6 to 10 weeks were infected i.v. with 10^6 pRBC.

RESULTS

Acute malarial infections in B-cell-deficient mice. Selected characteristics of acute malaria caused by the infection of B-cell-deficient mice with various plasmodia are summarized in Table 1. Whereas *P. yoelii* infections were nonlethal in immunologically intact mice, they were uniformly lethal in B-cell-deficient mice of the same strain. Parasitemias became patent at the same time (day 3) and followed similar courses in both types of mice. However, parasitemias in intact mice began to decrease after day 11 postinfection, but continued to increase in B-cell-deficient mice until these animals died.

In contrast, infections with *P. vinckei* followed the same course in both B-cell-deficient and normal mice. Parasitemias exceeded 60% in both types of mice by day 8 postinfection, and all of the mice died within 24 h thereafter.

A somewhat different pattern of disease was observed when both deficient and intact mice were infected with the lethal parasite *P. berghei*. Early on, the kinetics of infection was similar in both types of mice. However, whereas all B-cell-deficient mice had died by day 14, most immunologically intact mice were still alive at this time (Table 1). By day 25, the remaining animals had died.

Acute infection with *P. chabaudi* followed similar kinetics in both immunologically intact mice and B-cell-deficient mice. Peak parasitemias occurred 6 to 7 days after infection. None of the infected mice died.

Antibody-independent immunity to recurring malaria in B-cell-deficient mice. When otherwise lethal *P. yoelii* infections in B-cell-deficient mice were treated with a dosage regimen of clindamycin sufficient to reduce their parasitemias to subpatent levels, the mice subsequently developed chronic low-grade malaria of long-lasting duration, with parasitemias ≤1.0%. Such mice were also resistant to exogenous challenge infection with homologous parasites administered 11 weeks after the initiation of acute infection (Table 2). Interestingly, B-cell-deficient mice whose

TABLE 2. Resistance of *P. yoelii*-immune B-cell-deficient mice[a] to homologous challenge infection

Strain	No. of mice	*P yoelii* challenge infection		Days after primary infection	Resistance to challenge infection (no. resistant/no. challenged)[b]
		Inoculum	Route		
(BALB/c × C57BL/10)F_1	7	1×10^6	i.p.	71	7/7
(BALB/c × C57BL/10)F_1	6	1×10^6	i.v.	78	6/6
(BALB/c × C57BL/10)F_1	6	4×10^7	i.v.	78	6/6
BALB/c	6	1×10^6	i.p.	77	6/6

[a] Sex and age-matched mice 8 to 18 weeks old were treated with clindamycin (75 mg/kg of body weight) four to five times during a 6-day period beginning 15 days after intraperitoneal (i.p.) infection with 10^5 pRBC or 13 days after i.v. infection with the same inoculum. In several experiments, drug treatment was repeated several weeks after the first regimen.

[b] Mice resistant to challenge infection controlled parasitemias ≤1% during the 20-day period after the injection of exogenous parasites. Challenge infection of clindamycin-treated naive B-cell-deficient control mice was lethal in all instances.

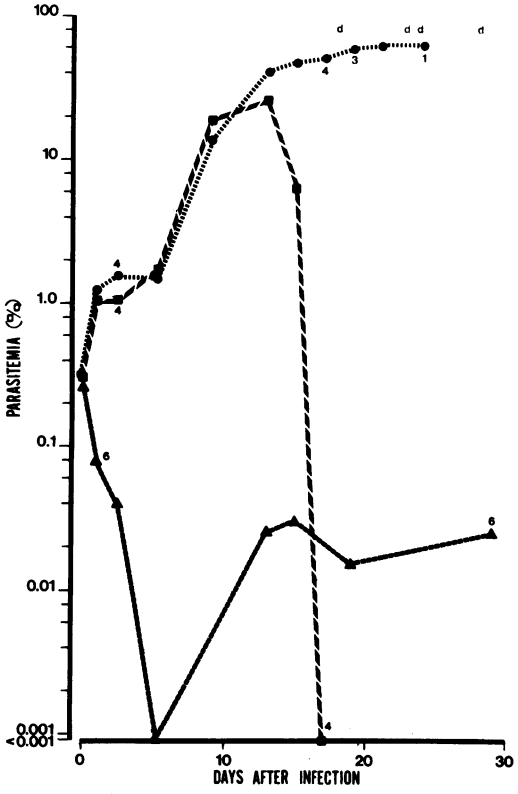

FIG. 1. Clearance of exogenously introduced *P. yoelii* from the blood of 6-month-old male (BALB/c × C57BL/10)F_1 hybrid B-cell-deficient mice immunized 78 days previously with homologous parasites (▲). Naive B-cell-deficient (●) and immunologically intact (■) mice which had been pretreated with clindamycin served as controls. All mice were challenged i.v. with 4×10^7 *P. yoelii* pRBC. Data points represent the mean parasitemia of the number of mice indicated. d, Death.

acute *P. yoelii* infections had seemingly been sterilized by chemotherapy died with fulminating malaria when subsequently challenged with *P. yoelii*, suggesting that the continued presence of parasites in host tissue was essential to maintain immunity.

To determine the strength of this reinfection immunity in B-cell-deficient mice, animals chronically infected with *P. yoelii* were injected with enough *P. yoelii* to yield an immediate patent parasitemia of 0.25%. B-cell-deficient mice which had not been infected with *P. yoelii* previously, as well as naive immunologically intact mice, received the same inoculum and served as controls. Immune B-cell-deficient mice cleared the parasites from their blood over a period of several days (Fig. 1). In contrast, control mice developed typical acute *P. yoelii* infections which followed predictable courses; i.e., B-cell-deficient mice developed fulminating malaria and died, whereas naive immunologically intact mice resolved their acute infections.

After the termination of their acute *P. chabaudi* infections, B-cell-deficient mice, in contrast to immunologically intact mice, developed chronic low-grade malaria. Parasitemias in B-cell-deficient mice ranged between 0.001 and <0.1% for a 3-week period after the resolution of acute malaria (Fig. 2), at which time the animals were used in a specificity experiment described below. Other experiments showed that chronic *P. chabaudi* infections persisted for periods of up to 450 days. Such mice were resistant to challenge infection with homologous parasites.

To determine whether B-cell-deficient mice could be protected against reinfection with *P. vinckei*, mice were infected with 10^5 pRBC and treated with chloroquine for 5 consecutive days beginning on day 7 postinfection. Treated mice remained free of patent infection for 24 days after completion of chemotherapy. The mice were challenged at that time with 5×10^5 *P. vinckei*. Twenty-two of 26 B-cell-deficient mice immunized in this manner developed low-grade *P. vinckei* parasitemias (<2%) after challenge and recovered. Parasitemias ranging from 5 to 50% were observed in the remaining four ani-

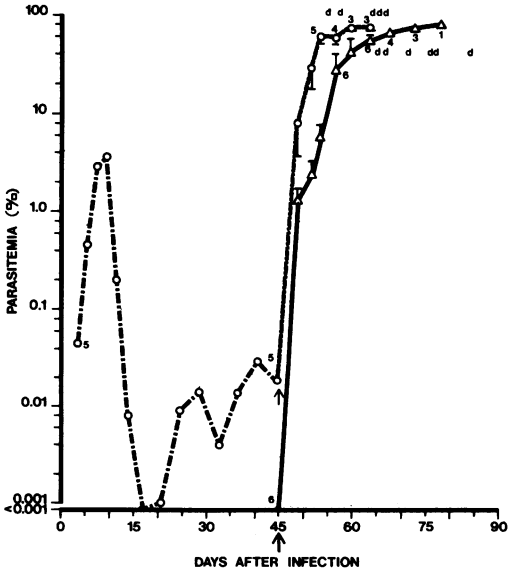

FIG. 2. Course of *P. yoelii* malaria in 3-month-old (BALB/c × C57BL/10)F_1 B-cell-deficient mice immunized 45 days previously with *P. chabaudi* (○). Naive B-cell-deficient mice (△) served as controls. All mice were challenged i.v. with 10^6 *P. yoelii* pRBC at the time indicated by the arrow. Data points represent the mean parasitemia of the number of mice indicated. d, Death.

mals. One of these immunized B-cell-deficient mice succumbed to the *P. vinckei*, as did all of the naive control mice.

Effect of mouse genotype on induction of antibody-independent immunity to malaria in B-cell-deficient mice. Mice of several strains as well as their F_1 hybrids were rendered B-cell deficient as described above and tested subsequently for their ability to develop immunity to reinfection with endogenous parasites after the resolution of their acute infections. Groups of B-cell-deficient mice of the same genotype were infected with either *P. yoelii* or *P. chabaudi* since these parasites were known to cause chronic malaria in B-cell-deficient mice of certain strains. The use of *P. yoelii* necessitated that acute infections in B-cell-deficient mice be controlled by treatment with one or two regimens of clindamycin HCl. The mice were then observed for recrudescent fulminating malaria. Mice which controlled their remaining endogenous parasites at low levels (≤2%) were designated as being immune to reinfection. B-cell-deficient mice infected with *P. chabaudi* did not require chemotherapy to limit their acute infections, which terminated spontaneously. The mice were observed subsequently for their ability to control their chronic parasitemias at low levels (≤2%).

The data in Table 3 show that the ability of B-cell-deficient mice to develop antibody-independent immunity to recurrent malaria was dependent upon both the genotype of the mouse and the infecting species of plasmodia. Whereas a varied but significant portion of B-cell-deficient mice from several mouse strains was immunized by drug-limited *P. yoelii* infections, none of 25 C57BL/10 mice developed antibody-independent immunity to *P. yoelii*. In contrast, most B-cell-deficient mice, regardless of genotype, controlled their chronic *P. chabaudi* parasitemias. As the sex of the animals did not significantly alter the kinetics of these infections (data not shown), results were analyzed without consideration of this factor. However, in any given experiment, groups were matched with respect to age and sex.

Specificity of antibody-independent immunity to malaria in chronically infected B-cell-deficient mice. To determine the specificity of antibody-independent immunity in B-cell-deficient mice chronically infected with *P. yoelii*, groups of mice were challenged with heterologous species of plasmodia. Such B-cell-deficient mice immunized against *P. yoelii* resisted infection with *P. chabaudi* and *P. berghei* (Table 4). Six of seven chronically infected B-cell-deficient mice challenged with heterologous *P. vinckei* developed full-blown malaria with peak parasitemias of between 27 and 67%. Note, only one of these six B-cell-deficient mice died. The remaining five B-cell-deficient mice immunized against *P. yoelii* resolved their active *P. vinckei* challenge infections spontaneously, as did the seventh mouse which had developed low-grade infection upon challenge.

TABLE 3. Influence of mouse genotype on induction of immunity to reinfection malaria in B-cell-deficient mice

Immunizing infection	No. of mice developing recurrent malaria after termination of acute disease/no. of animals studied (%)[a]		
	C57BL/10	(BALB/c × C57BL/10)F_1[b]	BALB/c
P. yoelii[c]	25/25 (100)	30/93 (35)	43/54 (80)
P chabaudi[d]	2/21 (10)	0/98 (0)	1/37 (3)

[a] Mice were designated as having recurrent malaria if their parasitemias exceeded levels of 2% after the termination of acute disease.

[b] (C57BL/10 × BALB/c)F_1 mice gave similar results (31% developed recurrent *P. yoelii* malaria, but none developed recurrent *P. chabaudi* malaria).

[c] B-cell-deficient mice were immunized by treating acute *P. yoelii* infections with clindamycin to suppress patent parasitemia.

[d] B-cell-deficient mice were immunized by means of acute *P. chabaudi* infections which were allowed to terminate spontaneously.

Previously, we had reported that B-cell-deficient mice chronically infected with *P. chabaudi* were resistant to challenge infection with *P. vinckei* but were susceptible to infection with either *P. berghei* or *P. yoelii* (12). To determine whether the presence of a chronic *P. chabaudi* infection in B-cell-deficient mice was capable of influencing the course of a superimposed *P. yoelii* infection, B-cell-deficient mice with chronic *P. chabaudi* malaria, as well as naive B-cell-deficient mice, were challenged with *P. yoelii*. *P. yoelii* infections in nonimmune B-cell-deficient mice, as well as in B-cell-deficient mice immune to *P. chabaudi*, were fulminating and lethal (Fig. 2). The kinetics of the infections was similar regardless of the host's prior experience with *P. chabaudi*, indicating that mice immune to *P. chabaudi* were totally lacking immunity to *P. yoelii*.

Restricted specificity of antibody-independent immunity to challenge infection with heterologous plasmodia was also observed in B-cell-deficient mice which had been rescued from otherwise lethal *P. vinckei* infection by means of chemotherapy with chloroquine. Such immunized mice resisted homologous *P. vinckei* parasites as well as challenge with *P. chabaudi* but developed fulminating malaria and died when infected with either *P. yoelii* or *P. berghei* (Table 5).

TABLE 4. Resistance of *P. yoelii*-immune B-cell-deficient mice to challenge infection with heterologous plasmodia

Challenge infection in *P. yoelii*-immune B-cell-deficient mice[a]	No. of mice	Susceptibility to challenge infection		
		Peak parasitemia		No. died/no. challenged
		Mean (%)	Range	
P. berghei	9	2.75	0.02–21	0/9
P. chabaudi	9	0.067	<0.005–0.25	0/9
P. vinckei	7	40.5	0.40–67	1/7

[a] B-cell-deficient (BALB/c × C57BL/10)F_1 mice were 12 to 18 weeks old at the time of primary infection. Clindamycin (75 mg/kg) was administered four times during a 6-day period beginning day 13 after i.v. infection with 10^5 *P. yoelii*. Two additional doses of clindamycin were given on days 38 and 42 postinfection. All mice were challenged with 10^6 pRBC of the designated species 78 days after initiation of the immunizing *P. yoelii* infection.

DISCUSSION

The investigation described herein represents an extension of previous studies in our laboratory aimed at identifying resistance mechanisms capable of protecting the host against acute malarial infection. In the present study, we have examined immunity to reinfection malaria in B-cell-deficient hosts which had previously experienced acute malaria. Perhaps the most significant finding of the present study was the observation that B-cell-deficient mice which had experienced acute malarial infections caused by different plasmodial species displayed different patterns of resistance to subsequent challenge infections with heterologous species. Thus, B-cell-deficient mice which had been protected by chemotherapy against the lethal effects of acute infection with either *P. yoelii* or *P. vinckei* developed acute-type malaria when challenged with the heterologous parasite. Such mice were resistant to homologous challenge infection as well as challenge infection with yet another parasite, *P. chabaudi* (Table 6). In contrast, B-cell-deficient mice immune to *P. chabaudi* while resistant to *P. vinckei* remained fully suceptible to *P. yoelii*. These results indicate that immunity to reinfection malaria in B-cell-deficient mice can be mediated by qualitatively different mechanisms of resistance activated by a particular plasmodial species. Whether challenge infections with *P. chabaudi* in B-cell-deficient mice with chronic malaria are controlled by identical or different mechanisms of resistance depending upon the plasmodial species used to initiate acute infection remains to be determined. *P. chabaudi* may prove to be more susceptible than other plasmodia to resistance mechanisms activated by either *P. yoelii* or *P. vinckei*.

In addition to being antibody independent, immunity to reinfection malaria in B-cell-deficient hosts requires the participation of T cells since athymic nude mice infected with either *P. yoelii* or *P. chabaudi* were unable to maintain chronic low-grade malarial infections. T cells may function in certain infections by activating macrophages or natural killer cells to become effector cells producing oxygen-derived radicals

TABLE 5. Susceptibility of *P. vinckei*-immune B-cell-deficient mice to challenge infection with heterologous plasmodia

Challenge infection in *P. vinckei*-immune B-cell-deficient mice[a]	No. of mice	Mouse strain	Susceptibility to challenge infection (No. died/no. challenged)
P. chabaudi	7	(BALB/c × C57BL/10)F_1	0/7[b]
P. yoelii	4	(BALB/c × C57BL/10)F_1	4/4[c]
P. yoelii	4	(C57BL/10 × BALB/c)F_1	4/4[c]
P. berghei	7	(C57BL/10 × BALB/c)F_1	7/7[c]

[a] B-cell-deficient male mice, 2 to 5 months of age, were immunized by drug treatment of a primary *P. vinckei* infection. Beginning on day 7 after i.v. infection with 10^5 *P. vinckei* pRBC, mice were treated for 5 days with chloroquine (125 mg/liter of drinking water). Immunity to *P. vinckei* was tested by i.v. challenge with 5×10^5 homologous plasmodia 36 days after initiation of primary infection. Immune mice were challenged with heterologous plasmodia 17 days later. Mice were challenged i.v. with 10^6 pRBC on day 53 after initial infection with *P. vinckei*.

[b] Parasitemias did not exceed 0.05% in challenged *P. vinckei*-immune animals.

[c] Infection kinetics was similar in both *P. vinckei*-immune and previously uninfected mice.

TABLE 6. Specificity of antibody-independent immunity to malaria

Immunizing parasite	Outcome of challenge infection with:			
	P. yoelii	*P. chabaudi*	*P. berghei*	*P. vinckei*
P. yoelii	Resistant	Resistant	Resistant	Acute-type infections[a]
P. chabaudi	Acute-type infection and death	Resistant	Acute-type infection and death	Resistant
P. vinckei	Acute-type infection and death	Resistant	Acute-type infection and death	Resistant

[a] Mice demonstrate significant parasitemia but survive challenge infection.

capable of killing asexual forms of certain plasmodial species, as recently suggested by several groups of investigators (3, 5, 8). The present findings indicate that the activation of additional mechanisms of resistance may also be required.

Why B-cell-deficient mice immune to *P. yoelii* survived challenge infection with *P. vinckei* even though such mice developed acute-type *P. vinckei* infection is uncertain. One might speculate that mice with chronic *P. yoelii* infections are anemic at the time of challenge, and when further stressed by the superimposed *P. vinckei*, they accelerate erythropoiesis, resulting in a reticulocytosis which serves to control the *P. vinckei* infection. For example, the induction of anemia and compensatory reticulocytosis in mice by either repeated bleeding or the injection of phenylhydrazine has been shown to protect mice from death during acute *P. vinckei* infection, and although *P. vinckei* were observed to invade reticulocytes, the parasites failed to develop into schizonts in this abnormal host cell (24).

Whereas the genetic basis for developing antibody-independent immunity to reinfection malaria awaits future analysis, our results suggest that the genotype of B-cell-deficient mice determines their ability to prevent recurrent malaria. This was most evident in the case of *P. yoelii*, where significant immunity was induced only in F_1 hybrid mice. In contrast, B-cell-deficient mice regardless of genotype readily developed immunity to reinfection with *P. chabaudi*, indicating that different genetic loci control resistance during chronic malarial infections caused by different species of plasmodia. Other investigators, using different strains of immunologically intact mice, have reported differences in susceptibility to infection with another subspecies of *P. chabaudi* (9, 21). Future studies with additional mouse strains made B-cell deficient may reveal similar genotypic differences in susceptibility and provide a basis for defining the antibody-independent mechanisms involved.

Several reports in the literature have suggested that T cells play an essential role in pathological events leading to "early death" in virulent malarial infections (15, 23, 25). Finley et al. (11) have recently reported that a significant proportion of euthymic mice developed a rapidly fatal neurological syndrome early in the course of infection with *P. berghei*. In contrast, athymic nude mice failed to demonstrate neurological signs when infected with the same parasite and died late during the course of disease. Whether this was due to a helper role of T cells in the production of antibody capable of mediating pathology or to a direct effector function of a select subset of T lymphocyte has not been determined. The results of the present study show that B-cell-deficient mice infected with either *P. vinckei* or *P. berghei* died at the same time or earlier than similarly infected immunologically intact mice. This finding suggests that T cells participate directly as effector cells in producing pathology responsible for causing early death in virulent malarial infections.

In conclusion, these studies represent an ongoing effort to gain insight into mechanisms of resistance induced by infection with hemoprotozoan parasites. Whereas a protective role for antibody has been established in malaria caused by *P. falciparum* (6, 7), the identity and significance of other resistance mechanisms responsible for coping with this disease remain purely speculative. However, it is not unlikely that additional mechanisms of resistance do contribute to immunity in the malarious human host. In fact, it has recently been reported (J. Jensen, M. Baland, and M. Akood, Annu. Meet. Am. Soc. Trop. Med. Hyg., 31st, 8 Nov. 1982) that the sera of certain Sudanese living in areas of hyperendemic malaria were inhibitory to the in vitro growth of *P. falciparum*, despite the absence of antibody activity as measured by immunofluorescence. We hope that the use of immunodeficient animal models in future studies will aid in identifying and characterizing mechanisms of resistance which protect the host against malaria. Although it is recognized that the results of such experiments may not be directly applicable to human malaria, they may provide a basis for

the eventual development of future immunizing agents to be used against this disease.

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