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Resistance to *Plasmodium chabaudi* in B10 Mice: Influence of the *H-2* Complex and Testosterone

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Resistance to *Plasmodium chabaudi* has been examined in different inbred mouse strains bearing identical *H-2* haplotypes on different genetic backgrounds as well as in *H-2*-congenic mouse strains on B10 background. Resistance is expressed in terms of percent survival after a challenge with 10^6 *P. chabaudi*-infected erythrocytes. We can show that murine resistance to *P. chabaudi* is under complex polygenic control involving a non-*H-2* gene(s) as well as genes in both *I-A* and *I-E* subregions of the *H-2* complex. Our data indicate in particular that malaria protective antigens can be presented in context with $I\text{-}A^b$ molecules but not in context with $I\text{-}A^k$ molecules. Resistance controlled by $I\text{-}A^b$ does not become apparent when $I\text{-}E^k$ molecules are coincidentally expressed. Moreover, testosterone abrogates $I\text{-}A^b$-controlled resistance to *P. chabaudi*.

Parasitic protozoans of the genus *Plasmodium* cause malaria in humans and other vertebrates. The parasites asexually develop in the erythrocytes of the host (for a review, see reference 4). The severity of *Plasmodium* infections varies largely with the parasite-host combination, and considerable variations exist even in a given host infected with a given *Plasmodium* species (3, 6–8, 11). Recently, first attempts have been undertaken to identify genes in mice which control resistance and susceptibility to *Plasmodium* infections. The results are still conflicting. Some authors claim that resistance is controlled by one dominant autosomal gene (39–41), presumably located on chromosome 1 (2). Other authors, however, state that resistance is under polygenic control (15) as in other protozoan infections in mice (42, 44).

In this context, two aspects are of particular interest. First, an important role in the immune response in general is played by the major histocompatibility complex, which discriminates between self and nonself (23). The murine major histocompatibility complex, the *H-2* complex (25), has been demonstrated to influence the susceptibility to different types of infections caused, for example, by herpes simplex virus type 1, cytomegalovirus, *Listeria* monocytogenes, and *Leishmania donovani* (for a review, see reference 23). A possible influence of *H-2* genes on malaria infections has not yet been unequivocally investigated to date (18). Second, some information is available that murine resistance to *Plasmodium* is sex dependent in that female mice are more resistant than male mice. This is ascribed to a superior erythropoietic system in female mice (40). However, it is also conceivable that resistance is under a more direct control of sex hormones. This prompted us to examine the role of *H-2* and the male sex hormone testosterone in resistance and susceptibility to *Plasmodium chabaudi* in mice.

MATERIALS AND METHODS

Parasite infection. Blood infections with *P. chabaudi* were passaged weekly in NMRI mice (48). Parasitemia was monitored in Giemsa-stained blood smears. Erythrocytes were counted in a Neubauer chamber.

Mouse strains. The following mouse strains were bred in our own colony under specific pathogen-free conditions: C57BL/10, B10.D2, B10.BR, B10.A, B10.A(3R), B10.A(4R), B10.M, B10.RIII, BALB/c, and DBA/2J. F. Figueroa and J. Klein (Max-Planck Institut für Immungenetik, Tübingen, Federal Republic of Germany) kindly provided mice of the following strains: C57BL/10, B10.A, B10.A(3R), B10.A(4R), B10.RIII, and B10.OH. BALB/b mice were delivered from Olac Laboratories (Bicester, England), and B10.A mice were delivered from Jackson Laboratory (Bar Harbor, Maine).

Resistance and susceptibility. All mice were challenged with 10^6 *P. chabaudi*-parasitized erythrocytes obtained from NMRI mice. Resistance was expressed in terms of percent survival and time until death, i.e., mean survival of succumbing mice (40).

Castration. C57BL/10 males were castrated at 3 to 4 weeks of age. Mice were anesthetized, and testes were pulled off through a scrotal incision. The ductuli efferentes were transected by electrocautery, and testes and epididymis were removed (33, 38).

Testosterone treatment. Castrated and female C57BL/10 mice were treated with Testoviron-Depot-10 (Schering Corp., Berlin, Federal Republic of Germany). Treatment started at age 3 to 4 weeks. Mice received 1.5 mg of testosterone per week in two subcutaneous injections for 3 weeks and, subsequently, 2.1 mg of testosterone per week in two injections for the following 4 weeks (33).

Phenylhydrazine treatment. Mice of strains C57BL/10 and B10.A at age 10 to 12 weeks were intraperitoneally injected with 0.08 mg of phenylhydrazine per g of mouse weight (30, 43). Reticulocytes were evaluated in Giemsa-stained blood smears.

Metabolic labeling. *P. chabaudi*-infected erythrocytes were isolated from NMRI mice as described previously (46, 47). Cells (2.5×10^8/ml) were cultivated in phosphate-buffered saline supplemented with 113 mM glucose and 3% fetal calf serum (GIBCO Laboratories, Karlsruhe, Federal Republic of Germany) at 25°C under 7% CO_2, 3% O_2, and 90% N_2. The cells were incubated with [^{14}C]isoleucine (specific activity, 337 Ci/mmol; Amersham Corp., Braunschweig, Federal Republic of Germany) in the presence and absence of testosterone for 3 h.

In vitro proliferation of T lymphocytes. Female mice of

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TABLE 1. Resistance to *P. chabaudi* infections in female mice with *b* and *d H-2* haplotypes on different genetic backgrounds

Mouse strain	*H-2* haplotype	No. tested	Surviving mice (%)	Time until death (no. of days ± SD)
C57BL/10	*b*	110	79	10.7 ± 1.7
BALB/b	*b*	23	15	11.6 ± 3.9
B10.D2	*d*	30	77	11.4 ± 3.3
BALB/c	*d*	24	25	12.2 ± 3.1
DBA/2J	*d*	18	38	10.2 ± 1.9

TABLE 2. Resistance to *P. chabaudi* in female B10 mice with unrelated *H-2* haplotypes

Mouse strain	*H-2* haplotype	No. tested	Surviving mice (%)	Time until death (no. of days ± SD)
C57BL/10	*b*	110	79	10.7 ± 1.7
B10.D2	*d*	30	77	11.4 ± 3.3
B10.M	*f*	12	33	12.3 ± 2.3
B10.BR	*k*	17	29	11.6 ± 4.5
B10.RIII	*r*	16	81	11.7 ± 3.1

strains C57BL/10, B10.D2, B10.A(3R), and B10.A(4R) were immunized at age 10 to 12 weeks by subcutaneous injection of 200 μl of antigen at the base of the tail. The antigen consisted of 10^8 *P. chabaudi*-infected erythrocytes which were freeze-thawed in liquid nitrogen three times before being emulsified in an equal volume of Freund complete adjuvant. After 7 days, spleens were aseptically removed, and splenic T lymphocytes were enriched by the nylon-wool procedure (20). T-cell-enriched lymphocytes were seeded in microwell plates with 96 round-bottom wells (Nunc, Wiesbaden, Federal Republic of Germany) at a concentration of 8×10^4 per well in 0.2 ml of RPMI 1640 supplemented with 10 μg of kanamycin per ml, 10 μg of tylosin (Nunc) per ml, 2×10^{-5} M mercaptoethanol, and 10% fetal calf serum. The lymphocytes were cultivated in a humidified atmosphere of 5% CO_2 at 37°C. The cells were stimulated for 4 days with one of the following: 2 μg of concanavalin A, 10^6 *P. chabaudi*-infected erythrocytes, 10^6 noninfected erythrocytes, 10^6 free parasites, or 10^6 ghosts isolated from infected erythrocytes. During the last 24 h of culture, the cells were pulsed with 0.5 μCi of [^{3}H]thymidine (specific activity, 2 Ci/mmol) per well before being harvested with a cell harvester (Nunc) and counted in a liquid scintillation counter (model 8000; Berthold, Wildbad, Federal Republic of Germany).

Preparation of malaria antigens. Erythrocytes were isolated from noninfected NMRI mice as described previously (46). *P. chabaudi*-infected erythrocytes were isolated from infected NMRI mice, and free parasites and plasma membranes of erythrocytes in the form of ghosts were prepared by the procedure detailed previously (46, 47).

RESULTS

Non-*H-2* gene(s). To examine resistance of different mouse strains to *P. chabaudi*, we challenged mice with 10^6 *P. chabaudi*-infected erythrocytes and determined percent survival. Mouse strains were termed resistant if more than 70% of the mice survived the infection for at least 23 days. Strains were susceptible if more than 60% of the mice succumbed to the infection.

Females of mouse strains with identical *H-2* haplotypes on different non-*H-2* backgrounds exhibited different levels of resistance to *P. chabaudi* (Table 1). Mice with a BALB or DBA background were susceptible, while strains with B10 background were resistant. This indicates that resistance and susceptibility are controlled by a non-*H-2* gene(s), which confirms previously presented data (2, 15, 39–41).

***H-2* complex.** The results described above prompted us to evaluate the possible influence of the *H-2* complex on resistance to *P. chabaudi* in female B10 mice. Table 2 shows different levels of resistance in B10 strains carrying some major different unrelated *H-2* haplotypes. Resistant strains were those with the *b*, *d*, and *r H-2* haplotypes, in which about 80% of the mice survived the challenge infection. Mouse strains with the *H-2* haplotypes *f* and *k* were susceptible. It is obvious that genes in the *H-2* complex influence resistance and susceptibility to *P. chabaudi*.

Table 3 shows attempts to assign those *H-2* genes influencing resistance to the different (sub)regions of the *H-2* complex. Mouse strains with the "resistant" $H\text{-}2^b$ and $H\text{-}2^d$ haplotypes and the "susceptible" $H\text{-}2^k$ haplotype are compared with mouse strains carrying different recombinant *d/k* and *b/k H-2* haplotypes. Mice remained resistant or susceptible when recombinations occurred in the *D* region. For instance, the B10.OH strain ($K^D\ I^D\ D^k$) was resistant to *P. chabaudi*, as was the B10.D2 strain ($K^D\ I^D\ D^D$). Conversely, B10.A mice ($K^k\ I^k\ D^D$) were susceptible, as were B10.BR mice ($K^k\ I^k\ D^k$). This indicates that the *D* region of the *H-2* complex is not involved in the control of resistance to *P. chabaudi*. Also, those class I molecules which are encoded by the *K* region are apparently not involved. For instance, B10.A(3R) mice which express K^b were susceptible, as were those strains which express K^k, such as B10.BR and B10.A.

Resistance and susceptibility to *P. chabaudi* are obviously controlled by genes in the *I* region involving both the *I-A* and the *I-E* subregions. Mouse strains were susceptible when $I\text{-}E^k$ was expressed, as in B10.BR, B10.A, and B10.A(3R), regardless of coincidental expression of $I\text{-}A^k$ or $I\text{-}A^b$ (Table 3). On the other hand, B10.A(4R) and C57BL/10 mice, which do not express *I-E* genes at all, were susceptible and resistant, respectively (Table 3). The relevant difference between these two strains is in the *I-A* region: B10.A(4R) mice possess $I\text{-}A^k$, while C57BL/10 mice express $I\text{-}A^b$.

Genes in the *I* region encode class II molecules which play a dominant role in antigen presentation to T cells (22, 23). It is therefore possible that antigen presentation and/or T cells are defective with respect to malaria antigens in *P. chabaudi*-susceptible mice. To test this, mice from two resistant strains, C57BL/10 and B10.D2, and from two susceptible strains, B10.A and B10.A(4R), were immunized with *P. chabaudi*-infected erythrocytes. After 7 days, T-cell-enriched lymphocytes were isolated from spleens and their responses to different crude malaria antigens were measured by in vitro proliferation assay. T-cell-enriched lymphocytes from all mouse strains responded strongly to the T-cell mitogen concanavalin A but did not significantly respond to noninfected erythrocytes (Table 4). In contrast, infected erythrocytes induced significant responses in the T-cell-enriched lymphocytes as well as isolated host cell plasma membranes and isolated free parasites did (Table 4). This indicates that there is at least no general defect in antigen presentation and/or in the ability of T cells to be stimulated in *P. chabaudi*-susceptible strains.

Parasitemia and reticulocytosis. The different percentages of survival and mortality among the different strains may reflect differences in the outcome of overt infections, courses of infections, and/or the efficiencies of the erythropoietic systems. The first possibility can be clearly excluded,

TABLE 3. Resistance to *P. chabaudi* in female B10 mice with recombinant *H-2* haplotypes

Mouse strain	*H-2* haplotype	Allele at *H-2* locus [a]:							No. tested	Surviving mice (%)	Time until death (no. of days ± SD)
		K	A_β	A_α	E_β	E_α	*S*	*D*			
C57BL/10	*b*	*b*	*b*	*b*	(*b*)	(*b*)	*b*	*b*	110	79	10.7 ± 1.7
B10.D2	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	30	77	11.4 ± 3.3
B10.OH	*o2*	*d*	*d*	*d*	*d*	*d*	*d*	*k*	8	75	16.5 ± 3.5
B10.BR	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	17	29	11.6 ± 4.5
B10.A	*a*	*k*	*k*	*k*	*k*	*k*	*d*	*d*	51	12	11.2 ± 2.2
B10.A(3R)	*i3*	*b*	*b*	*b*	*b*	*k*	*d*	*d*	38	29	11.8 ± 3.3
B10.A(4R)	*h4*	*k*	*k*	*k*	(*k*)	(*b*)	*b*	*b*	23	22	10.4 ± 2.3

[a] Designations as described by Klein et al. (24).

since all challenged mice were visibly sick on days 8 to 12 postinfection (p.i.), indicating that both surviving and succumbing mice had patent and even fulminant *P. chabaudi* infections at about the same time in all strains. Incidentally, the surviving mice remained alive and well even beyond day 23 p.i.

To detect possible different courses of the infections, parasitemias were followed up in the resistant B10 strain and the susceptible B10.A strain. However, the *P. chabaudi* infections took about the same course in the surviving and succumbing mice of both resistant and susceptible mouse strains (Fig. 1 and 2). Parasitized erythrocytes appeared in the peripheral blood on about day 4 p.i., and maximum parasitemia occurred on approximately day 8 p.i. In surviving mice, maximum parasitemia was always followed by a second, lower parasitemia peak occurring on approximately day 16 p.i. on the average. Thereafter, parasitized erythrocytes disappeared from the peripheral blood. In some animals, however, they reappeared at a very low frequency on about day 30 p.i. before they finally disappeared.

Finally, mice of the susceptible B10.A strain did not appear to have a less effective erythropoietic system than mice of the resistant B10 strain. Indeed, these two strains did not significantly differ in their erythropoietic response to phenylhydrazine-induced anemia (Fig. 3). In both strains, maximum reticulocytosis, about 40 to 45%, occurred on day 6 after phenylhydrazine treatment.

Testosterone. In contrast to its effect in female mice, the *H-2* complex had no influence on resistance to *P. chabaudi* in male mice. Indeed, male mice of all inbred strains examined proved susceptible, i.e., their survival rate was less than 40% (Table 5). Likewise, resistance was not *H-2* dependent when assessed in terms of time until death (Table 5).

An influence of sex on mouse resistance to *Plasmodium* infection has been repeatedly ascribed to a superior erythropoietic system in female versus male mice. However, there was no significant difference when the erythropoietic response to phenylhydrazine-induced anemia was compared between male and female C57BL/10 mice (Fig. 3). Maximum reticulocytosis (about 40%) occurred on day 6 post-phenylhydrazine injection in both female and male mice.

Another reason for the sex-dependent resistance and susceptibility might be that the expression of genes controlling resistance are modulated by sex hormones such as testosterone. Indeed, castration of male B10 mice entailed a dramatic increase in survival; about 78% of the castrated mice survived the challenge infection (Table 6). Castrated mice, however, became susceptible again when treated with testosterone (Table 6). Also, female mice were able to be converted from resistant to susceptible by testosterone treatment (Table 6). It is noteworthy that the effect of testosterone is obviously mediated by the host, since parasite growth within erythrocytes, measured as incorporation of [^{14}C]isoleucine in *P. chabaudi*-infected erythrocytes in vitro, was not affected (Fig. 4).

DISCUSSION

The present study shows that female mice exhibit different strain-dependent levels of resistance to blood stage infections of *P. chabaudi*, manifested in different percentages of survival. This resistance is obviously controlled by a non-*H-2* gene(s), is influenced by genes of the *H-2* complex (cf. also reference 31), and is depressed by testosterone. Thus, our data support the view that murine resistance to *P. chabaudi* is under not unigenic (2, 39–41) but rather complex polygenic control (15) and even involves delicate hormonal modulation. The latter in particular may explain the observed heterogeneity of survival and mortality within a genetically homogenous inbred strain. However, the reason that polygenic control becomes apparent only in terms of percent survival and mortality but not in terms of time until death, which appears to be identical in the succumbing mice of most strains, remains unknown.

Murine resistance to *P. chabaudi* differs from the well-known phenomenon of innate resistance described for human pathogenic *Plasmodium* species (for reviews, see refer-

TABLE 4. In vitro proliferation of T-cell-enriched lymphocytes from spleens of B10 mice immunized against *P. chabaudi*

T cell source (no. of mice)	Stimulation by [a]:				
	ConA	niE	iE	iG	Pa
C57BL/10 (4)	113 ± 19	3.8 ± 0.7	5.2 ± 2.0	3.5 ± 1.0	1.0 ± 0.1
B10.D2 (4)	51 ± 15	0.6 ± 0.0	8.4 ± 1.4	2.7 ± 0.8	9.6 ± 1.3
B10.A (6)	106 ± 30	1.0 ± 0.1	22 ± 7	23 ± 8	36 ± 14
B10.A(4R) (3)	54 ± 16	1.3 ± 0.5	19 ± 5	9.7 ± 3.6	16 ± 8

[a] ConA, Concanavalin A; niE, noninfected erythrocytes; iE, infected erythrocytes; iG, ghosts isolated from infected erythrocytes; Pa, parasites isolated from infected erythrocytes. Values are stimulation index ± standard error.

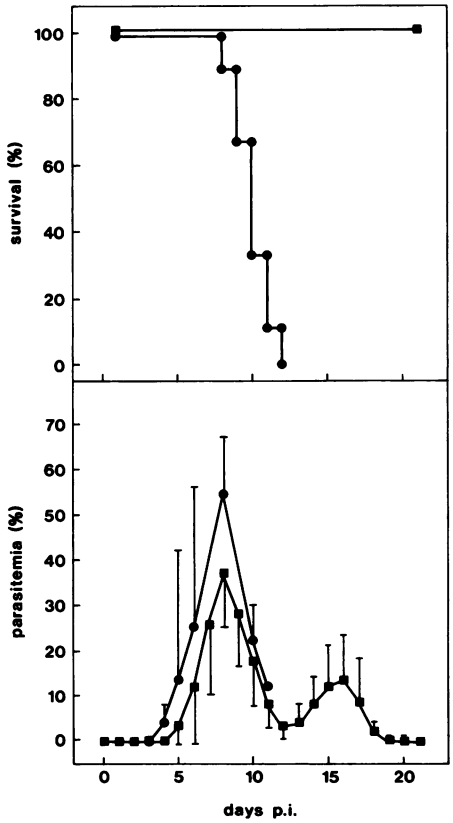

FIG. 1. *P. chabaudi* infections in C57BL/10 mice. Mice were challenged with 10^6 *P. chabaudi*-infected erythrocytes, and percent survival and parasitemia were evaluated for 12 surviving (■) and 9 succumbing mice (●), respectively. Means ± standard deviations are given.

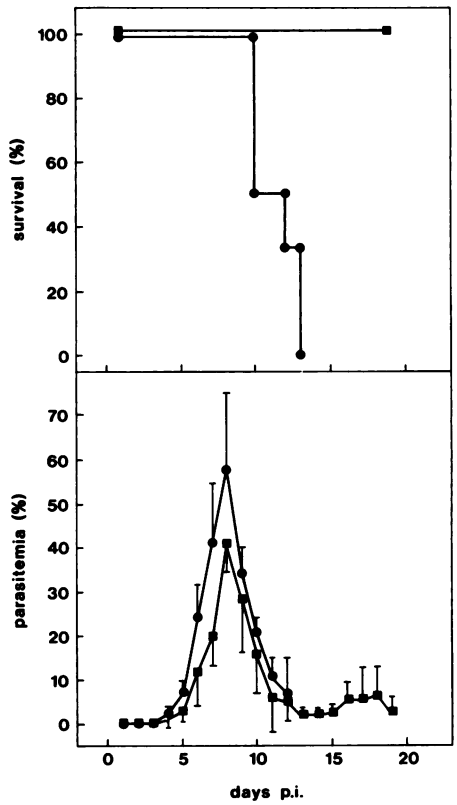

FIG. 2. *P. chabaudi* infections in female B10.A mice. Percent survival and parasitemia were evaluated for 6 surviving (■) and 6 succumbing mice (●), respectively. Means ± standard deviations are given.

ences 9 and 26). Innate resistance is due to different genetically controlled disorders manifested in defects in host erythrocytes, such as anomalies in hemoglobin (9, 26), absence of glycophorin (34–36) or Duffy blood group antigens (27–29), deficiencies in glucose-6-phosphate dehydrogenase (26, 37), or ovalocytic forms of erythrocytes (21). Such host cell defects normally prevent parasitemia or permit only a delayed outcome of parasitemias which are clinically not overt. However, similar defects in erythrocytes do not occur in the inbred mouse strains we have investigated here, since *P. chabaudi* obviously causes fulminant blood stage infections in both resistant and susceptible strains. In addition, parasitemias even take about the same course in the resistant B10 strain and the susceptible B10.A strain. Thus, murine resistance is due to genetically determined mechanisms which help to overcome fully developed infections, entailing a clearance of parasitized erythrocytes from the peripheral blood. It is unlikely that such a clearance is due primarily to a superior erythropoietic system in resistant versus susceptible strains, since chemically induced erythropoiesis takes about the same course in resistant B10 mice as in susceptible B10.A mice. Rather, the clearance signals the development of protective immune mechanisms.

Indeed, it is consistent with this view that mice which have resisted a *P. chabaudi* infection have acquired immunity against homologous rechallenge as shown previously (45). Moreover, it is consistent that resistance depends on the *I* region and obviously not on the *K* and *D* regions of the *H-2* complex, as shown by our studies with intra-*H-2* recombinant mouse strains on B10 background. However, this does not necessarily mean that the *K* and *D* loci are not important for the defense against those *Plasmodium* species which invade preferentially reticulocytes, for example, *P. yoelii*. Indeed, it has been shown in *P. yoelii* infections that reticulocytes express increased levels of *K* and *D* antigens (19).

The influence of the *I* region appears to be quite complex, involving genes of both *I-A* and *I-E* subregions. For instance, mice are resistant when only $I\text{-}A^b$ molecules are expressed. However, when $I\text{-}E^k$ is coincidentally expressed with $I\text{-}A^b$ or when only $I\text{-}A^k$ is expressed, mice are susceptible. Our data indicate that susceptible strains are not defective in their

TABLE 5. Susceptibility to *P. chabaudi* in male *H-2*-congenic B10 mice

Mouse strain	*H-2* haplotype	No. tested	Surviving mice (%)	Time until death (no. of days ± SD)
C57BL/10	*b*	53	8	11.3 ± 2.8
B10.D2	*d*	40	38	11.1 ± 2.8
B10.M	*f*	15	13	12.6 ± 2.5
B10.RIII	*r*	24	17	12.9 ± 1.6
B10.A	*a*	49	2	10.8 ± 1.9
B10.OH	*o2*	13	0	12.7 ± 1.7
B10.A(4R)	*h4*	56	0	11.7 ± 2.5
B10.A(3R)	*i3*	16	0	11.3 ± 1.4
B10.BR	*k*	27	0	11.4 ± 3.2

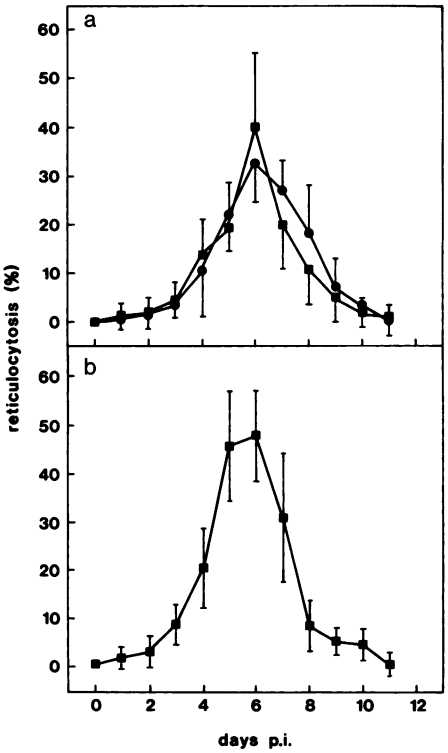

FIG. 3. Phenylhydrazine-induced reticulocytosis in C57BL/10 and B10.A mice. (a) Means ± standard deviations from 12 male (■) and 12 female (●) C57BL/10 mice. (b) Means ± standard deviations from 14 female B10.A mice.

overall capacity to present malaria antigens and/or in the responsiveness of their T cells to malaria antigens. However, it is possible that susceptible mice are not able to present and/or recognize those malaria antigens that are relevant for protective immunity. For instance, the protective malaria antigen(s) is obviously presented in context with $I\text{-}A^b$ molecules but not in context with $I\text{-}A^k$ molecules. The latter might be not suited for that, for example, because of an unfavorable structure and/or conformation (for a review, see reference 23). Moreover, protective antigens presented in context with $I\text{-}A^b$ may be down-regulated by $I\text{-}E^k$-controlled reactions. Evidence that $I\text{-}A^b$ molecules are especially appropriate for presenting malaria antigens has also been provided recently by other authors (5, 10). It was shown that only $I\text{-}A^b$-expressing mice and not those expressing $I\text{-}A^k$ are capable of mounting an effective antibody response after immunization with repeats of the tetrapeptide Asn-Ala-Asn-Pro. These $(NANP)_n$ repeats are the immunodominant repetitive sequences of the circumsporozoite protein of *P. falciparum*, which have been recently tested as vaccine immunogens in human volunteers (1, 14).

TABLE 6. Effects of castration and testosterone on susceptibility and resistance to *P. chabaudi* infections in C57BL/10 mice

Sex and treatment	No. tested	Surviving mice (%)	Time until death (no. of days ± SD)
Male	26	0	9.7 ± 2.0
Male, castration	24	78	10 ± 2
Male, castration + testosterone	15	0	9.1 ± 1.6
Female	28	76	9.6 ± 1.0
Female, testosterone	15	0	12.3 ± 2.6

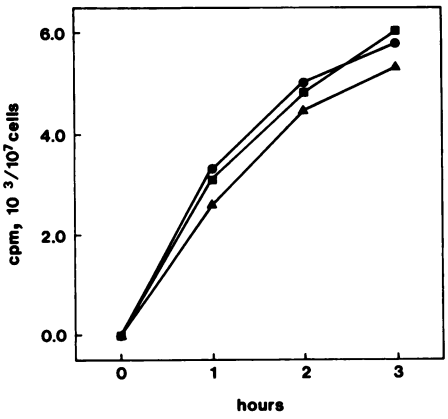

FIG. 4. Incorporation of [^{14}C]isoleucine in *P. chabaudi*-infected erythrocytes. Erythrocytes were isolated and incubated in vitro without testosterone (●) or in the presence of 0.45 μg (■) or 4.5 μg (▲) of testosterone per ml.

Furthermore, our data show that testosterone abrogates the $I\text{-}A^b$-controlled resistance to *P. chabaudi*. Male B10 mice which are susceptible become resistant by castration and are normally reconverted to susceptibility by testosterone treatment. In addition, testosterone renders the resistant female B10 mice susceptible to *P. chabaudi*. The mechanisms by which testosterone exerts its abrogative action are totally unknown. We can exclude only a direct effect of testosterone on parasites, i.e., testosterone does not accelerate growth and multiplication of parasites within erythrocytes. In this context, three observations which point to linkages between the *H-2* complex and testosterone are worth mentioning. First, the metabolism of testosterone and, more generally, androgen metabolism in mice are under polygenic control, but the major role is played by the *Hom-1* locus, presumably linked to the *H-2* complex (for a review, see reference 22). Second, male mice possess a sex-limited protein, designated Slp antigen, which is expressed by the *S* region of the *H-2* complex and which is under control of testosterone (12, 13, 32, 33). Third, the susceptible female B10.A mice are known to possess higher levels of endogenous testosterone than female mice of the resistant B10 strain (16, 17, 22).

Whatever the malaria-relevant 'testosterone target' may be, the fact per se that testosterone negatively interferes with the development of protective immune mechanisms against malaria parasites has an important implication for the vaccine and vaccination trials.

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