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Susceptibility to Experimental Cerebral Malaria Induced by *Plasmodium berghei* ANKA in Inbred Mouse Strains Recently Derived from Wild Stock

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The neurological syndrome caused by *Plasmodium berghei* ANKA in rodents partially mimics the human disease. Several rodent models of cerebral malaria (CM) exist for the study of the mechanisms that cause the disease. However, since common laboratory mouse strains have limited gene pools, the role of their phenotypic variations causing CM is restricted. This constitutes an obstacle for efficient genetic analysis relating to the pathogenesis of malaria. Most common laboratory mouse strains are susceptible to CM, and the same major histocompatibility complex (MHC) haplotype may exhibit different levels of susceptibility. We analyzed the influence of the MHC haplotype on overcoming CM by using MHC congenic mice with C57BL/10 and C3H backgrounds. No correlation was found between MHC molecules and the development of CM. New wild-derived mouse strains with wide genetic polymorphisms were then used to find new models of resistance to CM. Six of the twelve strains tested were resistant to CM. For two of them, F_1 progeny and backcrosses performed with the reference strain C57BL/6 showed a high level of heterogeneity in the number and characteristics of the genetic factors associated with resistance to CM.

Cerebral malaria (CM) is an immunophysiopathological process caused by *Plasmodium falciparum* in humans and *Plasmodium berghei* ANKA in rodents. CM causes over 2 million deaths per year, mainly in young children. The clinical features of CM are well documented, but many aspects of its pathogenesis remain unclear. A number of parameters influence the severity of CM, including the genotype (22), the transmission level of the parasite, and the age (2), immune status (6), and genetic factors (8, 12, 17) of the host. However, the exact roles that these factors play are still not understood.

In the past, several attempts have been made to develop rodent models to study the mechanisms that lead to cerebral disease in patients with malaria. These include efforts in rats (19), golden hamsters (26), and mice (16, 25, 26) infected with either *P. berghei* ANKA or K173. *P. berghei* ANKA, isolated from Thicket rats (*Grammomys surdaster*) (30), can induce a neurological syndrome that partially mimics human CM in susceptible rodent laboratory strains. The symptoms associated with CM in current mouse models include respiratory distress syndrome, decreased body temperature, and neurological manifestations characterized by ataxia, paralysis (mono-, hemi-, and tetraplegia), and coma, followed by death (10). Even though these models do not exhibit all of the features of the human syndrome (28, 31), they were used to show that the sequestration of leukocytes (21) and parasitized erythrocytes (10) in the small vessels of the brain (24) and endothelial-cell damage (20, 29) are involved in pathogenesis. The histopathological changes involved in both human and murine CM are characterized by loss of vascular cell integrity, tissue edema, and congestion of microvessels with parasitized erythrocytes and/or mononuclear cells (18). Sequestration has been attributed to the expression of receptors such as ICAM-1 and CD36 at the surface of endothelial cells (3, 13, 23, 27). Proinflammatory cytokines, such as tumor necrosis factor alpha, play a significant role in sequestration by modulating the expression of these adhesion molecules (7, 15).

The commonly used inbred laboratory strains of mice are derived from a very small pool of ancestors which therefore have limited genetic polymorphism. Thus, studies of the genetic factors favoring the development of CM with these strains are very much constrained. To overcome this, inbred strains were recently derived from wild-trapped genitors from various taxons (9). This led to the development of novel experimental models of malaria with extensive genetic diversity.

In this study, we first analyzed the susceptibility to CM of several inbred laboratory strains and major histocompatibility complex (MHC) congenic strains of C57BL/10 and C3H background to evaluate the role of the MHC haplotype. We subsequently tested the behavior of 12 inbred strains recently established from wild progenitors belonging to several species of the *Mus* genus infected with *P. berghei* ANKA. Six strains were found to be highly resistant to CM. We determined the dominant-recessive nature of the genetic factor(s) associated with CM resistance in three strains by determining the phenotype of the F_1 progeny and backcrosses performed with the reference strain, C57BL/6. These new wild-derived mouse strains may be used as models to map the genetic factors

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TABLE 1. Distribution of the different strains used in this study among the different species and subspecies of the *Mus* genus

Mus sp.	Strain(s)
M. musculus	
musculus	MAI/Pas, MBT/Pas, and PWK/Pas
domesticus	38CH/Pas, BIK/g/Pas, WMP/Pas, and WLA/Pas
castaneus	CAST/Ei/Pas
M. spretus	SEG/Pas, SPRET/Ei/Pas, and STF/Pas
M. spicilegus	ZYD/Pas

involved in the physiopathological mechanisms associated with resistance or susceptibility to experimental fatal malaria.

MATERIALS AND METHODS

Mice. Eight-week-old BALB/c, DBA/2, 129/Sv, and C3H mice were purchased from Charles River (Saint Aubin-les-Elbeuf, France) and Harlan Sprague-Dawley (Gannat, France). All C57BL/10 and C3H congenic for different *H-2* haplotypes (B10, B10.A, B10.D2, B10.M, B10.WB, B10.BR, B10.G, B10.RIII, B10.S, B10.PL, B10.SM C3H.B10, C3H.NB, and C3H.Q) were kindly provided by Marika Pla (INSERM U93, Hôpital Saint Louis, Paris, France).

Eight- to twelve-week-old males and females from the wild-derived inbred (between 20 and 50 brother/sister crosses) strains of different taxons (*Mus musculus musculus*, *M. musculus domesticus*, *M. musculus castaneus*, *M. spretus*, and *M. spicilegus*) were used in this study (Table 1). The nomenclature of these strains is given in Appendix 1. All of these strains were developed at the Institut Pasteur except for SPRET/Ei and CAST/Ei, which were purchased from the Jackson Laboratory (Bar Harbor, Maine). All of the strains are maintained at the Institut Pasteur. Eight-week-old C57BL/6JRj females were obtained from Janvier (Le Genest-St.-Isle, France). The F_1 progeny of the wild-strain-derived mice and the C57BL/6 mice were produced in our laboratory animal facility and were designated as follows: (wild-type strain $\times$ B6)F_1 for a cross between a wild-type male and a C57BL/6 female and (B6 $\times$ wild-derived strain)F_1 for a cross between a wild-type female and a C57BL/6 male.

Parasites. Blood-stage samples of *P. berghei* ANKA clone 1.49L were kindly provided by D. Walliker (Institute of Genetics, Edinburgh, United Kingdom) and were maintained in C57BL/6J mice. This clone was selected for its great capacity to induce CM (1). The parasite was conserved as stabilates of 10^7 parasitized red blood cells (pRBCs) stored in liquid nitrogen in Alsever's solution containing 10% glycerol.

Induction of CM. Infection was initiated by intraperitoneal injection of 10^6 pRBCs. In "susceptible" strains, CM resulted in a neurological syndrome characterized by clinical signs including ataxia, paralysis (mono-, hemi-, para- or tetraplegia), deviation of the head, convulsions, and coma, followed by death. In most common laboratory strains of mice, neurological signs developed when between 5 and 15% of the red blood cells were parasitized, depending on the host strain. In resistant mouse strains death generally occurred during the third or fourth week postinfection due to anemia caused by hyperparasitemia (70 to 90% of pRBCs). Characterization of histopathological events associated with CM was also performed by determining the presence of adherent leukocytes in cerebral microvessels. For this, brains were collected from the mice developing CM and fixed overnight in phosphate-buffered saline (PBS) containing 4% paraformaldehyde (pH 7.4), and 10-μm frozen sections were stained with hematoxylin and eosin. The integrity of the blood-brain barrier was ascertained by the Evans Blue leakage method (29).

Determination of parasitemia. Blood samples were daily taken from the tail veins of infected mice during the first week of infection and every 2 days thereafter. Blood cells (10^7 to 10^9 cells) were fixed in 1 ml of 0.25% glutaraldehyde (G5882; Sigma, Saint Quentin Fallavier, France) in PBS (pH 7.4) and stored at 4°C before being stained. Erythrocytes infected with *P. berghei* ANKA were stained as previously described (14). Briefly, 10^6 to 10^8 fixed red blood cells were stained for 1 h at 37°C in the dark with a solution of 1 μM Hoechst 33258 (B2883; Sigma) in PBS. Red blood cells were analyzed by cytofluorometry using a FACStar plus cytofluorometer (Becton Dickinson, Grenoble, France) equipped with a Coherent Innova 90 laser tuned to UV excitation (351 nm, 200 mW). A 424DF44 filter was selected as the emission filter for the blue Hoechst fluorescence. Files were analyzed by using Cellquest 3.2 software. Red blood cells were carefully gated by light scatter (forward scatter and side scatter). For each sample, 10,000 events were acquired and recorded. The percentage of pRBCs was determined on the basis of the positive blue fluorescence of infected erythrocytes.

Statistical analysis. Survival curves were compared by the Logrank (Mantel-Cox) Test using Statview 4.5 software. Student's *t* test was used to compare parasitemia and survival between different groups. Values are expressed as the analysis of several individuals $\pm$ the standard deviation. A P value of <0.05 was considered significant and a P value of ≤ 0.01 was considered highly significant.

RESULTS AND DISCUSSION

CM is thought to develop as a result of multifactorial disturbances induced during the complex and multiple interactions between a virulent plasmodial strain and a host with a susceptible genetic background.

Most common laboratory strains of mice, except DBA/2 (O. Gorgette et al., unpublished data), are susceptible to the neurological disease associated with *P. berghei* ANKA infection. The phenotype of susceptibility to neuropathology is defined by clinical status, death, and parasitemia. In susceptible mice, infection with *P. berghei* ANKA blood-stage parasites leads to a neurological syndrome characterized by clinical signs, including ataxia, hyperventilation, convulsion, paralysis, deviation of the head, and a decrease in body temperature. Susceptible mice died within 6 to 10 days after being challenged with 10^6 pRBCs from frozen stabilates of infected C57BL/6 blood. Compared to CM^- mice, histopathological studies performed on CM^+ mice showed typical pictures of sequestrated leucocytes and parasites around the brain endothelial cells (Fig. 1A). Loss of microvascular integrity was also observed in the same group of mice by Evans Blue leakage (Fig. 1B). CM-resistant mice exhibited similar parasitemia without any noticeable signs of CM and died 15 days later from anemia due to hyperparasitemia. Common laboratory strains of mice exhibited various degrees of susceptibility to CM (Fig. 2A) which can be divided into three categories: highly susceptible to the neurological syndrome, with an incidence of CM ranging from 60 to 100% (C57BL/6 and 129/Sv strains); weakly susceptible, with an incidence of CM ranging between 20 and 60% (BALB/c and C3H); and one resistant strain, DBA/2.

The resistance of the DBA/2 strain to CM has been associated with the presence of mammary tumor virus 7 (MTV-7), an integrated mouse MTV (Gorgette et al., unpublished). This MTV targets all T cells expressing Vβ6, Vβ7, Vβ8.1, and Vβ9 segments and has a deleterious superantigenic effect. By deleting the $V\beta8^+$ T-cell subpopulation that is involved in the pathogenesis of CM, MTV-7 prevents the development of CM in $H\text{-}2^d$ mice (5).

These results suggest that susceptibility to CM depends upon the genetic background of the host. These data also imply that *H-2* haplotypes are not important in determining the level of susceptibility since different strains of mice with the same $H\text{-}2^d$ haplotype (B10.D2, BALB/c, and DBA/2) presented different patterns of susceptibility to CM.

Since the MHC haplotype has been suggested to be involved in the mechanisms of resistance to CM in humans (11), we analyzed the incidence of CM in several *H-2* congenic C57BL/10 (B10) and C3H strains infected with *P. berghei* ANKA. All congenic B10 strains tested were susceptible to the neuropathology, although the percentage of animals developing the syndrome varied (Fig. 2B). This confirms that the immunopathology associated with *P. berghei* ANKA infection in mice is

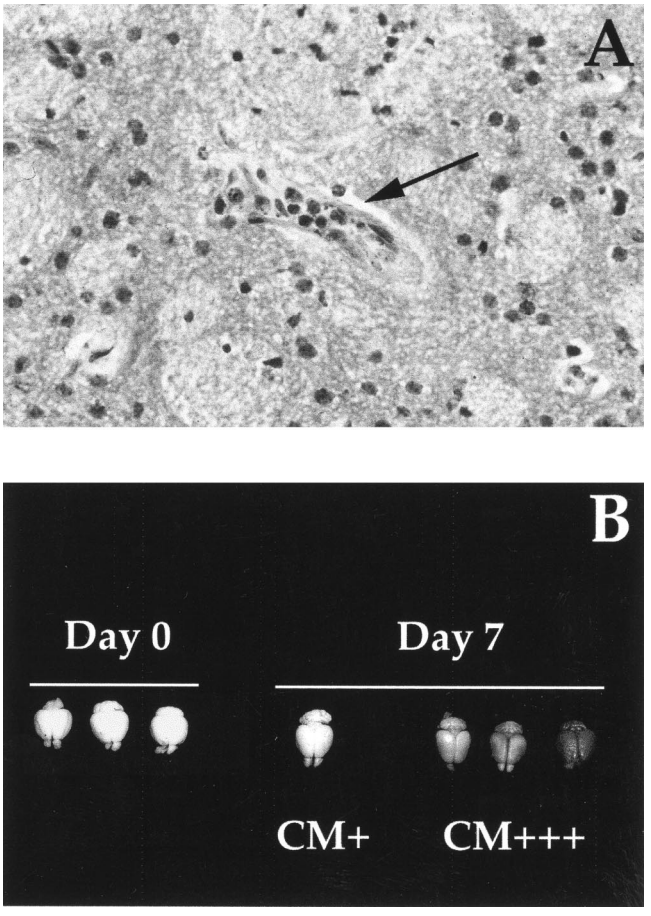

FIG. 1. (A) Histopathological studies show typical microvessels with adherent leukocytes (arrow). (B) Evans Blue leakage analysis was performed for control, CM^+, and CM^{+++} C57BL/6 mice infected *P. berghei* ANKA. CM^+ and CM^{+++} nomenclature was used to define degrees of severity of the neurological syndrome. As exemplified in the picture, CM^+ mice showed mild symptoms, whereas CM^{+++} mice were at the latest stage of the disease.

under a non-*H-2*-linked genetic control. The same results were obtained for *H-2* congenic animals in a C3H background (Fig. 2C). From these data, we conclude that the *H-2* haplotype is not sufficient to determine susceptibility or resistance to pathogenesis during experimental malaria; this, in turn, suggests a genetic control by genes located elsewhere in the mouse genome.

As all laboratory strains are derived from the same limited number of ancestors, we infected 12 independent wild-derived mouse strains belonging to *M. musculus musculus*, *M. musculus domesticus*, *M. musculus castaneus*, *M. spretus*, and *M. spicilegus* that constitute a wide genetic polymorphism pool with *P. berghei* ANKA and tested their susceptibility to CM. The ancestors of *M. musculus castaneus* were trapped in Asia, those of *M. musculus domesticus* were trapped in Western Europe, and those of *M. musculus musculus* were trapped in Eastern Europe. The ancestors of ZYD, the only inbred strain of *M. spicilegus*, were also trapped in Eastern Europe. Ancestors of *M. spretus* were from the Iberian peninsula and northern Africa (4). C57BL/6 mice were used as control for all of the wild-derived strains tested. For this prototype CM-susceptible strain, the immunopathology associated with *P. berghei* ANKA infection appeared at day 7.4 ± 0.5 with a low level of parasitemia (14.3% ± 3.7%). Three strains derived from independent progenitors of the *M. musculus musculus* subspecies were tested: MAI, MBT, and PWK. We found that 89% of MAI mice, 95% of MBT mice, and 67% of PWK mice developed CM in the same time frame as C57BL/6 mice ($P = 0.26$, df 3, $\chi^2 = 3.99$) (Fig. 3). The level of parasitemia in the MAI strain was lower than in C57BL/6 mice when the neuropathology appeared (6.2% ± 1.1% versus 14.3% ± 3.7%, $P = 0.006$, df 11, $t = -4.78$), whereas in MBT and PWK mice, the parasite levels in the blood reached 23.2% ± 8.1% and 25.1% ± 10.2%, respectively. Four *M. musculus domesticus* strains (38CH,

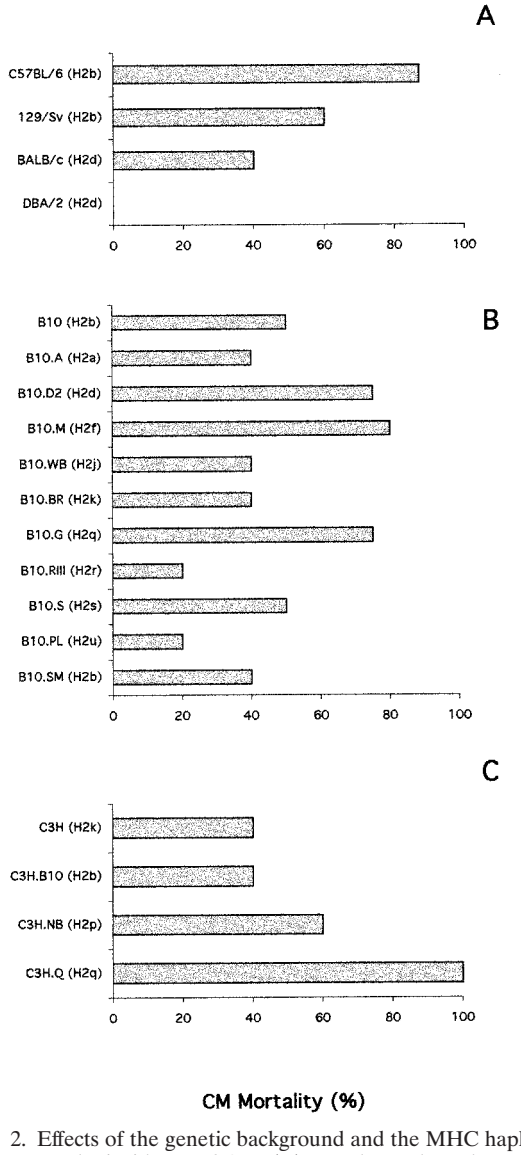

FIG. 2. Effects of the genetic background and the MHC haplotype of the host on the incidence of CM. (A) Bar charts show the percentage of mortality from CM of some common strains of laboratory mice: C57BL/6 ($n = 19$), O129/Sv ($n = 12$), BALB/c ($n = 25$), and DBA/2 ($n = 25$). (B) CM mortality was also studied for MHC congenic C57BL/10 strains (5 to 41 mice per strain). (C) The same study was repeated on MHC congenic C3H strains (5 to 16 mice per strain).

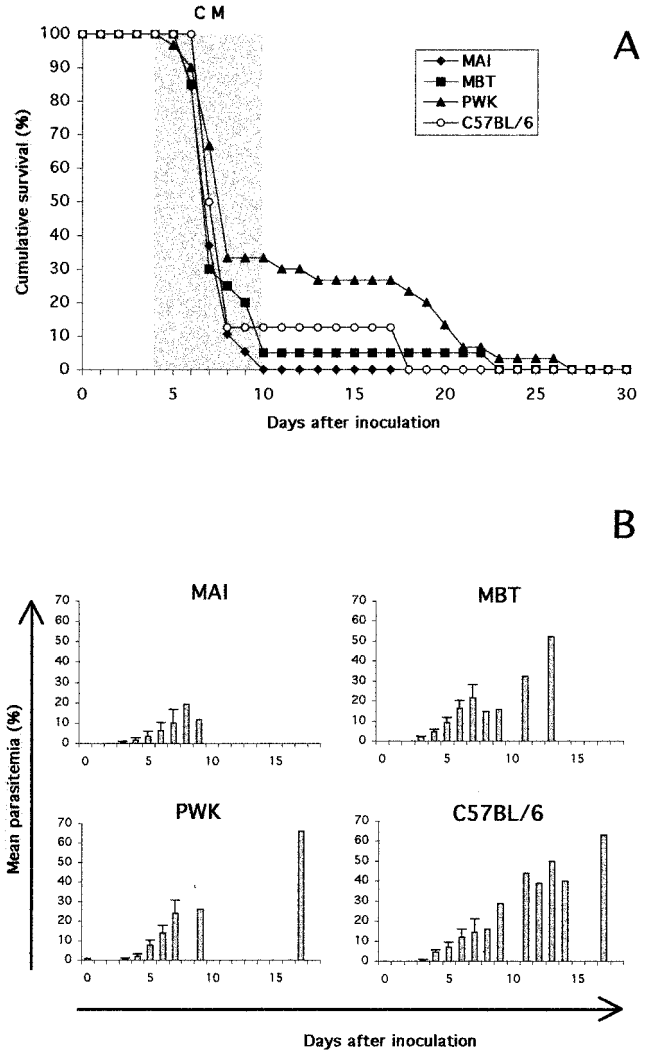

FIG. 3. Determination of CM susceptibility for three strains belonging to *M. musculus musculus*. (A) Survival curves for strains MAI (◆; 10 females and 9 males), MBT (■; 10 females and 10 males), and PWK (▲; 14 females and 16 males). The shaded portion represents the time window of mortality from CM for the strains tested. (B) Parasitemia was determined for strains MAI (five males and four females), MBT (five females and five males), and PWK (eight females and six males). Bar charts represent the mean percentage of parasitemia ± the standard deviation. The survival curve (○) and parasitemia of C57BL/6 are included as a reference.

BIK/g, WMP, and WLA) were also infected with *P. berghei* ANKA (Fig. 3). Of these strains, only WMP was susceptible to CM. This strain displayed an incidence of 70%, a delay in the appearance of the neurological syndrome (6.1 ± 1.4 days), and a level of parasitemia (11.5% ± 5.0%) similar to those for C57BL/6 (i.e., $P = 0.26$, df 1, and $\chi^2 = 4.92$ and $P = 0.33$, DDL = 10, and $t = 1.01$). The other three strains (38CH, BIK/g, and WLA) presented a strong resistant phenotype to CM but died as a result of hyperparasitemias that peaked at 60.6% ± 18.6% at day 18.9 ± 5.8 for WLA, at 36.5% ± 19.9% at day 16.3 ± 3.3 for 38CH, and at 43.4% ± 18.7% at day 25.7 ± 10.6 for BIK/g. Of the *M. musculus castaneus* subspecies (Fig. 5), CAST/Ei was the only strain studied that exhibited very pronounced neurological signs before death and in which CM appeared later (8.25 ± 0.6 days) than in C57BL/6 mice ($P = 0.01$, df 1, $\chi^2 = 6.39$). Nevertheless, the CAST/Ei mice that escaped from the neurological syndrome died at day 11.4 ± 1.5 from a high anemia with a pRBC percentage of 31.6% ± 7.8%. All *M. spretus* mouse strains tested (SEG, SPRET/Ei, and STF) were

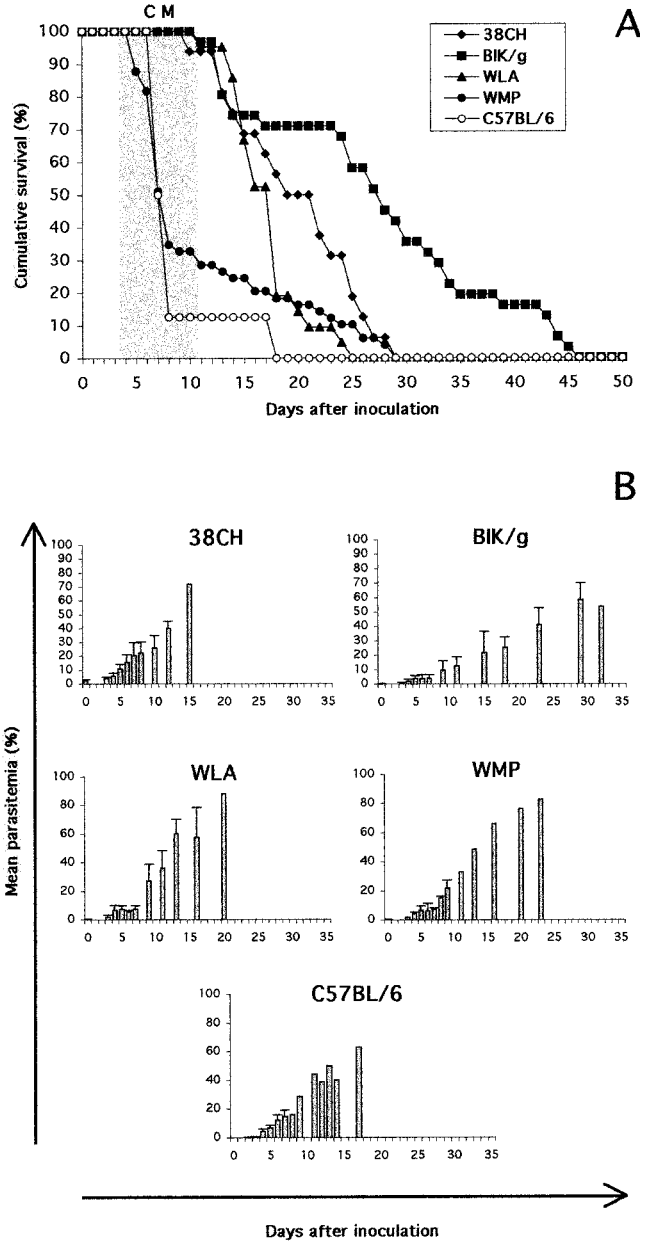

FIG. 4. Determination of CM susceptibility for four *M. musculus domesticus* strains. (A) Survival curves were constructed for strains 38CH (◆) (4 females and 12 males), BIK/g (■; 14 females and 17 males), WLA (▲; 11 females and 10 males), and WMP (●; 27 females and 22 males). The shaded portion represents the time window of mortality from CM, which occurred only in WMP and C57BL/6 strains. (B) Parasitemia was determined for strains 38CH (five males), BIK/g (five females and three males), WLA (five females and five males), and WMP (five females and five males). Bar charts represent the mean percentage of parasitemia ± the standard deviation. The survival curve (○) and parasitemia of C57BL/6 are included as a reference.

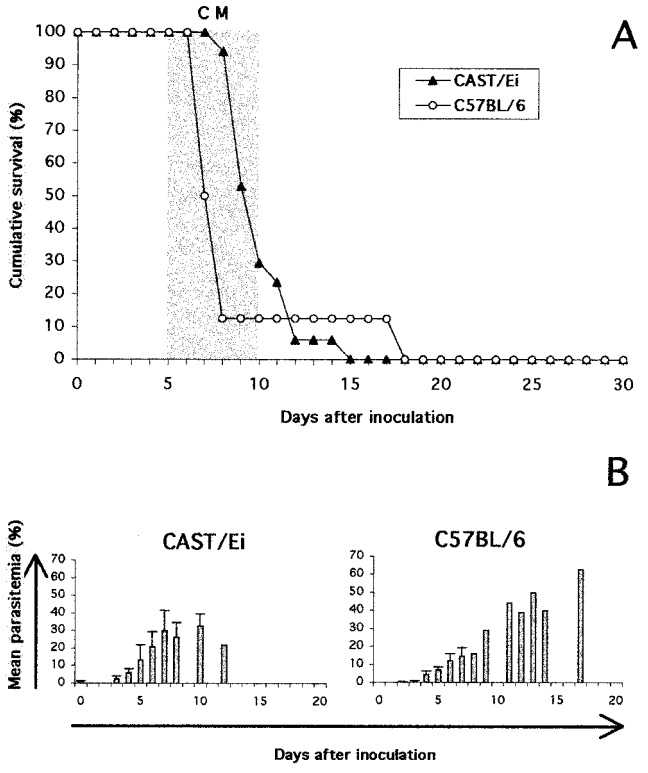

FIG. 5. Study of CM susceptibility of *M. musculus castaneus* (CAST/Ei). (A) Survival curve (▲) and parasitemia (B) were determined with 17 female mice. The shaded portion represents the time window of mortality from CM. Bar charts represent the mean percentage of parasitemia ± the standard deviation. The survival curve (○) and parasitemia of C57BL/6 mice are included as reference.

resistant to CM (Fig. 6). In these mice, death associated with anemia, severe ataxia, and cachexia occurred at day 10.4 ± 3 for STF mice, at day 11.3 ± 3.8 for SPRET/Ei mice, and at day 13.8 ± 7.5 for SEG mice. Strains SEG and SPRET/Ei had low levels of parasitemia (13.3% ± 9.1% and 16.3% ± 7.0%, respectively) compared to strain STF (33.9% ± 10.9%). Finally, 60 to 70% of ZYD mice, belonging to the *M. spicilegus* species, died from CM at day 7.1 ± 1.3 with only 9.7% ± 1.3% pRBCs (Fig. 7). In this mouse strain, the neurological syndrome was very transient because the clinical signs only appeared between 0.5 and 2 h before death.

CM-susceptible mouse strains were found in all of the taxons studied except *M. spretus.* The delay before the appearance of CM in susceptible strains was quite similar to that in C57BL/6 mice except for strain CAST/Ei, which developed CM 1 day later. This delay may be due to the high level of parasitemia required to develop the neurological syndrome. These results suggest that the level of parasitemia needed to develop the neurological pathology is controlled genetically.

To determine the mode of inheritance of the genetic factors conferring resistance to CM caused by *P. berghei* ANKA, we analyzed the phenotype of five groups of F_1 hybrids resulting from crosses between the susceptible C57BL/6 strain and the resistant strains 38CH, BIK/g, and WLA (Fig. 8). (38CH × B6)F_1 cross progeny were susceptible to CM, with an incidence of 81% (n = 78 mice). The delay in CM appearance was comparable to C57BL/6 mice (6.9 ± 0.8 days, P = 0.2, df 1, χ^2=1.61). F_1 progeny derived from BIK/g × B6 and B6 × BIK/g crosses were also susceptible to CM, with incidences of 69 and 53%, respectively. These data suggest that susceptibility to CM in these mice is a dominant trait and that the sex does not influence the outcome of the disease. Surprisingly, in contrast to 38CH and BIK/g, F_1 progeny resulting from WLA × B6 and B6 × WLA crosses were highly resistant to CM. (WLA × B6)F_1 mouse crosses died with clear signs of anemia due to hyperparasitemia reaching 85.6% ± 9.8%, at day 22.9 ± 5.0, and F_1 mice resulting from B6 × WLA crosses died at day 17.6 ± 1.6, with hyperparasitemia reaching 85.6% ± 9.1%.

Finally, segregation analysis of the genetic determinants was performed on two backcross progenies. In the first experiment (38CH × B6)F_1 mice, recognized as susceptible to CM, were

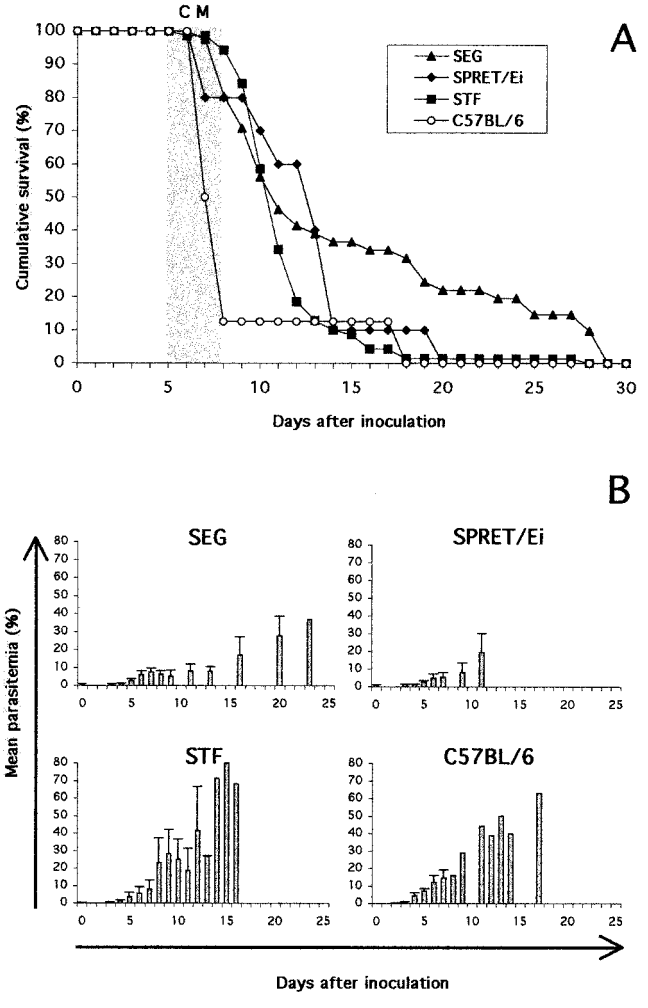

FIG. 6. CM susceptibility of three strains belonging to *M. spretus*. (A) Survival curves were determined for strains SEG (▲; 20 females and 10 males), SPRET/Ei (◆; 7 females and 3 males), and STF (■; 39 females and 31 males). (B) Parasitemia was determined for strains SEG (5 females and 3 males), SPRET/Ei (4 females and 5 males), and STF (9 females and 13 males). Bar charts represent the mean percentage of parasitemia ± the standard deviation. The survival curve (○) and parasitemia of C57BL/6 mice are included as reference. The shaded portion represents the time window of mortality from CM for C57BL/6 strain.

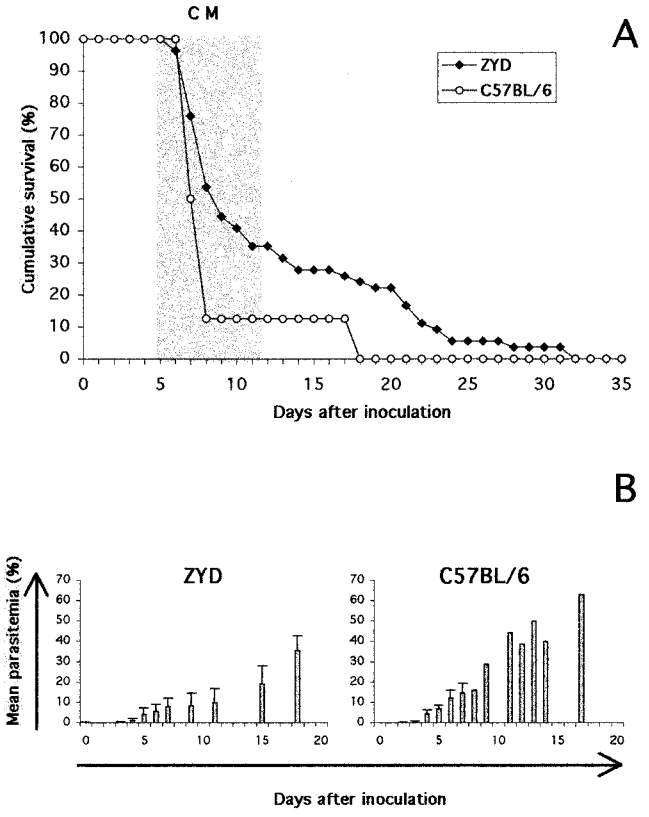

FIG. 7. CM susceptibility of ZYD (*M. spicilegus*). (A) The survival curve (▲) and parasitemia (B) were determined with 17 females. The shaded portion in panel A represents the time window of mortality from CM. Bar charts represent the mean percentage of parasitemia ± the standard deviation. The survival curve (○) and parasitemia of C57BL/6 mice are included as reference.

crossed with resistant mice of the 38CH strain. The offspring were challenged under experimental conditions identical to those described above. In this case, 38 of 44 mice (i.e., 72.6 to 94.8%) appeared to be susceptible to CM (Fig. 9). These data suggest that several dominant genetic factors of C57BL/6 origin and conferring susceptibility to CM are segregating in this cross.

In a second experiment, we found that 102 of 208 mice (42.0 to 56.0%) in the (WLA × B6)F_1 × B6 backcross population died from a neurological syndrome appearing at day 8.8 ± 2.6. This observation is compatible with the hypothesis that a single dominant factor, carried by strain WLA, can confer CM resistance to the progeny.

Whereas most of the laboratory strains of mice were susceptible to CM, 6 of the 12 wild-derived strains tested were resistant (38CH, BIK/g, WLA, SEG, STF, and SPRET/Ei). These resistant strains belong to two taxons (*M. musculus domesticus* and *M. spretus)*. Nevertheless, there is a clear distinction between these two taxons in terms of resistance to hyperparasitemia: *M. spretus* mice succumb to *P. berghei* ANKA infection earlier than do *M. musculus domesticus* mice. It is noteworthy that *M. spretus* mice, strains SEG and SPRET/Ei, develop anemia when there are low levels of the parasite in the blood.

We extended our observations by investigating the recessive or dominance characteristics of the genetic factors involved in resistance to CM by using *M. musculus domesticus* strains. The examination of the phenotypic expression and the percentage of mortality from CM among the progeny derived from crossing C57BL/6 with the three resistant *M. musculus domesticus* strains revealed a single dominant, polymorphic, and autosome-resistant gene transmitted by the WLA strain. Work is in

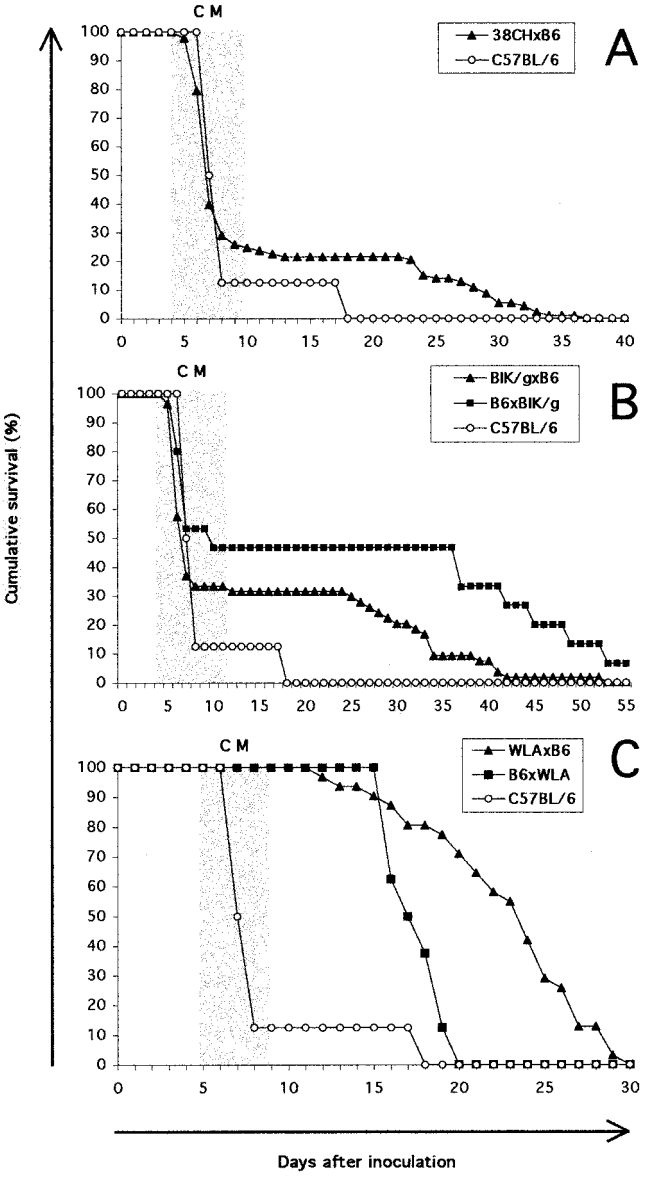

FIG. 8. Susceptibility to CM exhibited by (CM-resistant strain × C57BL/6)F_1 progenies. Survival curves for F_1 crosses with C57BL/6 were performed with strains 38CH (A), BIK/g (B), and WLA (C). A cross between a male from a resistant strain and a female C57BL/6 mouse is indicated by a triangle, and the opposite cross is indicated by a square. Experiments were performed with 45 females and 48 males for (38CH × B6)F_1, 26 females and 28 males for (BIK/g × B6)F_1, 7 females and 8 males for (B6 × BIK/g)F_1, 13 females and 18 males for (WLA × B6)F_1, and 8 females for (B6 × WLA)F_1. The survival curve (○) of C57BL/6 mice is included as reference. The shaded portion represents the time window of mortality from CM.

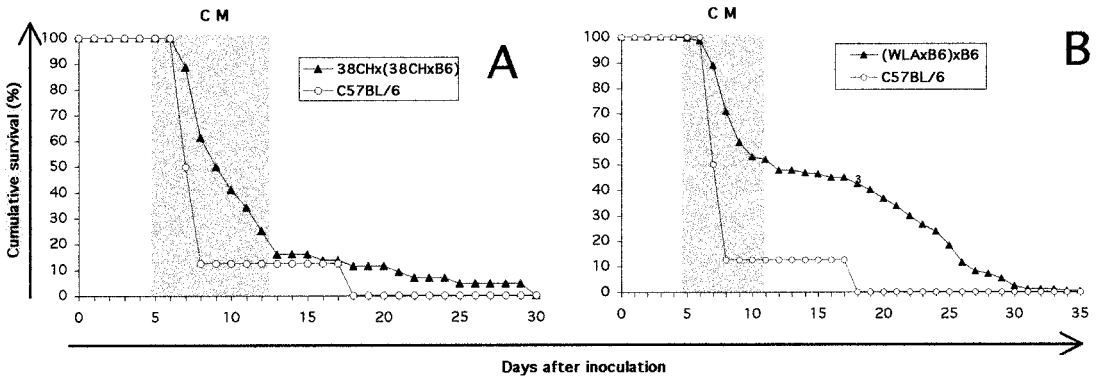

FIG. 9. CM susceptibility of mice resulting from (38CH × B6)F_1 progeny backcrossed with 38CH mice (A) and from (WLA × B6)F_1 progeny backcrossed with C57BL/6 mice (B). The survival curves for these mice are represented by triangles. The survival curve (○) and parasitemia of C57BL/6 mice are included as a reference. The shaded portions represent death from CM.

progress to map this gene and to detect additional minor genes implied in the resistance.

These models of experimental murine CM with mouse strains derived from wild strains provide excellent tools for analyzing the physiological pathways involved in disease processes and for identifying candidate loci involved in resistance and susceptibility to fatal syndromes during malaria. Thus, F_2 progeny and backcrosses are being used to map the genetic factors associated with resistance to CM (S. Bagot et al., unpublished data) and to hyperparasitemia (S. Campino et al., unpublished data).

APPENDIX

The nomenclature for mouse inbred strains was defined according to the official nomenclature (15a), except for strains 38CH/Pas, BIK/g/Pas, STF/Pas, and ZYD/Pas. These four strains were derived from strains 38CH, BIK/g, STF, and ZYD developed in the laboratory of François Bonhomme (CNRS UMR5000 "Génome, Populations, Interactions," Université des Sciences et Techniques du Languedoc, Montpellier, France). The ancestors of the two strains belonging to *M. musculus domesticus*, 38CH and BIK/g, were originally trapped in Chiarello, Italy, and in Kefar Galim, Israel, respectively, in 1982. 38CH exhibits the particularity of having 2N = 38 chromosomes. The STF strain (*M. spretus*) was initiated in 1984 with mice from Fonduck Djedid, Tunisia, and the ZYD strain (*M. spicilegus)* was derived from mice trapped in Yugoslavia in 1985.

In the text, mouse strain designations were as follows: MAI = MAI/Pas, MBT = MBT/Pas, PWK = PWK/Pas, 38CH = 38CH/Pas, BIK/g = BIK/g/Pas, WMP = WMP/Pas, WLA = WLA/Pas, CAST/Ei = CAST/Ei/Pas, SEG = SEG/Pas, SPRET/Ei = SPRET/Ei/Pas, STF = STF/Pas, ZYD = ZYD/Pas, and C57BL/6 = C57BL/6JRj.

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