Susceptibility to malaria as a complex trait: big pressure from a tiny creature

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Malaria, which is a major infectious disease worldwide, is caused by the *Plasmodium* parasite, one of the longest-known parasites infecting humans. The malaria situation is complicated by the emergence of drug resistance and the lack of an effective vaccine. Genetic factors play a key role in disease susceptibility, progression and outcome. Interestingly, an increasing large number of polymorphisms associated with resistance and susceptibility in humans have been found in proteins from erythrocytes, the site of *Plasmodium* replication. Some of these deleterious alleles have been selected by direct genetic pressure from the parasite in endemic areas of malaria. A number of additional gene effects have been mapped both in humans and in mice using population studies and experimental models of malaria, respectively. These recent studies have started to reveal additional aspects of the complex host–parasite interactions.

Malaria is caused by the mosquito-borne hematoprotozoan parasites of the genus *Plasmodium*. It is endemic in more than 90 countries and, together with HIV, tuberculosis and diarrheal diseases, constitutes one of the major causes of death by infectious diseases worldwide (1,2). Between 300 and 500 million clinical cases of malaria are estimated to occur annually, with 1 million deaths each year. The vast majority of deaths occur among young children and pregnant women in Africa, especially in remote rural areas with poor access to health services (2). Major contributing factors to the severity of this disease are the widespread emergence of chloroquine/mefloquine resistance in *P. falciparum* as well as resistance to insecticides in the *Anopheles* vector (1). Other factors include civil disturbances, environmental and climatic changes, and increased mobility of populations (1). In addition, efforts to develop effective antimalarial vaccines have remained disappointingly unsuccessful, despite immense research efforts worldwide (3). A better understanding of the natural mechanisms of host defense against the *Plasmodium* parasite may provide new targets for therapeutic intervention in this disease. Such factors may manifest themselves as genetic determinants of susceptibility to infection in endemic areas of disease or during epidemics (4).

**GENETIC FACTORS INFLUENCING MALARIA INFECTION, MORBIDITY AND MORTALITY IN HUMANS**

Four species of *Plasmodium*, namely *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*, can infect humans. These species differ by the length of their life cycle, their erythrocyte preferences and their virulence potentiality. Almost all deaths worldwide are caused by *P. falciparum*, although occasionally patients die from rupture of an enlarged spleen during infection with other *Plasmodium* species (5). Even if severe malaria probably became endemic ~10,000 years ago (6), mild forms of the disease may have existed in humans throughout much of their evolutionary history. Owing to the length of this exposure time and because the infection had significant effects on morbidity and mortality before reproductive age, malaria has exerted a strong selective pressure on the human genome. Over the past 50 years, a large body of evidence has accumulated to indicate that genetic variants (summarized in Table 1) influence the onset, progression, severity of disease and ultimate outcome of malaria infection in humans. This genetic component is often complex and multigenic, and its analysis by genetic epidemiology, linkage and association studies, as well as by candidate gene testing, has revealed important three-way interactions between host genes, environment and the malaria parasite. In particular, the malarial parasite appears to have exerted positive heterozygote selection for retention of otherwise-deleterious and disease-associated polymorphisms affecting erythroid cells.

**Inherited erythrocyte polymorphisms**

The inherited disorders of hemoglobin, which include the hemoglobin variants and the thalassemias, are the most common type of monogenic diseases in man (7). Early observational studies noted the similarity in geographical

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distribution of hemoglobinopathies and *P. falciparum* malaria, suggesting that they could confer protection against this disease (8–10). Normal adult hemoglobin is composed of two pairs of distinct globin chains (α2β2; called hemoglobin A, HbA). In the variant hemoglobin called hemoglobin S (HbS), position 6 of the β-globin chain is mutated (β6Glu-Val). Although HbS homozygocity is associated with sickle cell anemia (reviewed in 11), individuals heterozygous for the HbS variant, especially young children, are strongly protected against severe malaria (12). The HbS allele seems not to prevent the infection per se, but is protective against death or severe disease (profound anemia and cerebral malaria), probably owing to impaired entry but is protective against death or severe disease (profound anemia and cerebral malaria), possibly owing to decreased brain sequestration of infected red cells (33). Another ovalocytosis condition caused by a deletion in the erythrocytic structural protein glycoporphin C has been reported in the Wosera region of Papua New Guinea (34). The association of this ovalocytosis with protection against malaria morbidity and mortality is interesting, but remains to be demonstrated.

**Other erythrocyte polymorphisms**

Glucose-6-phosphate dehydrogenase (G6PD) catalyses the first step of the hexose monophosphate pathway and plays a critical role in the metabolism of glucose and in maintaining the balance of reduced/oxidized states of glutathione. G6PD enzyme deficiency is the most common enzymopathy of humans, affecting an estimated 400 million people. Many mutations reducing G6PD activity have been associated with protection against malaria infection, and may have evolved by natural selection (6,35,36). These data supported the existence of genetic factors determin-

### Segregation and genetic linkage analyses

A comparative survey was performed on three sympatric West African ethnic groups, Fulani, Mossi and Rimaibe, living under the same conditions of hyperendemic malaria transmission in Burkina Faso. Parasitological, clinical and immunological investigations showed consistent interethnic differences in *P. falciparum* infection rates, malaria morbidity and prevalence, and levels of antibodies to various *P. falciparum* antigens. These data supported the existence of genetic factors determining interethic differences in susceptibility to malaria (43). Recent comparison of gene frequencies for HbS, HbC, α-thalassemia, G6PD deficiency (A-allele) and HLA-

### Table 1. Major genes, proteins and loci influencing response to malaria in humans

<table>
<thead>
<tr>
<th>Type of study</th>
<th>Condition</th>
<th>Associated genes/molecules</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographical distribution*</td>
<td>β-Thalassemia</td>
<td>β-Globin</td>
<td>(7,20)</td>
</tr>
<tr>
<td></td>
<td>HbS</td>
<td></td>
<td>(12)</td>
</tr>
<tr>
<td></td>
<td>Hbc</td>
<td></td>
<td>(17)</td>
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<tr>
<td></td>
<td>Hbe</td>
<td></td>
<td>(18)</td>
</tr>
<tr>
<td></td>
<td>α-Thalassemia</td>
<td>α-Globin</td>
<td>(21,23)</td>
</tr>
<tr>
<td></td>
<td>Southern Asian ovalocytosis</td>
<td>Band 3</td>
<td>(33)</td>
</tr>
<tr>
<td>Hemolysis episodes</td>
<td>G6PD</td>
<td></td>
<td>(35)</td>
</tr>
<tr>
<td></td>
<td>DARC</td>
<td></td>
<td>(19)</td>
</tr>
<tr>
<td>Linkage analysis</td>
<td>5q31-q33</td>
<td></td>
<td>(48)</td>
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<tr>
<td>Association studies</td>
<td>TNF</td>
<td></td>
<td>(60,62,63)</td>
</tr>
<tr>
<td></td>
<td>HLA-A, HLA-DR</td>
<td></td>
<td>(64)</td>
</tr>
<tr>
<td></td>
<td>ICAM-1</td>
<td></td>
<td>(65,66)</td>
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<tr>
<td></td>
<td>NOS type 2</td>
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<td>(69,70)</td>
</tr>
<tr>
<td></td>
<td>CD36</td>
<td></td>
<td>(76,77)</td>
</tr>
</tbody>
</table>

*Correlation with malaria endemicity.*
A segregation analysis of blood parasite densities performed on Cameroonian families provided evidence for the presence of a major recessive gene controlling the degree of infection in human malaria (46). An independent segregation analysis of blood parasitemia was performed on families from the tropical rain forest of southern Cameroon. The results of this later study were consistent with the existence of complex genetic factors controlling blood infection levels, and also showed a dramatic influence of age and a putative major gene controlling the trait (47). A complex mode of inheritance for a major gene controlling blood parasitemia and influenced by age was also suggested by independent segregation analyses conducted in Burkina Faso (48).

To localize gene(s) controlling blood parasitemia in human, a sib-pair linkage analysis was performed on nine families from a southern Cameroonian village (49). Five candidate chromosomal regions were investigated in this study: 6p21 (HLA-TNF region), 2q13–q21 (containing genes coding for IL-1z and b), 14q11 (locus coding for the z chain of the T cell antigen receptor), 7q35 (gene cluster for the T-cell receptor) and 5q31–q33 [locus containing several immune-related molecules, including IL-4, IL-9 and IL-13, and controlling infection levels by Schistosoma mansoni (50)]. Linkage was found with the 5q31–q33 region, suggesting that this locus was critical in the control of different parasite infections (49). Independent sib-pair linkage analyses performed on sibs from 34 families of an urban population of Burkina Faso also established linkage between blood infection levels and chromosome 5q31–q33 (51). Since then, the 5q31–33 chromosomal region has also been associated with other traits such as atopy and asthma (52,53).

A longitudinal survey of malaria morbidity performed in a cohort of twin children from Gambia suggested that host genetic factors could influence susceptibility to malaria-induced fever (54). In addition, further studies in this cohort, as well as in other populations, have suggested genetic control of cell-mediated immune response and level of IgG antibody to various P. falciparum antigens (55–58). Familial patterns of inheritance of immune response against merozoite surface proteins were also studied in an highly malaria-endemic area of Papua New Guinea (59). In this population, preliminary variance analysis indicated familial aggregation of both humoral and cellular immune responses against RESA and MSP2 antigens, but no significant association between HLA class I or II alleles was found. Together, these results suggest that the human immune response to P. falciparum antigens is, at least in part, genetically regulated.

**Association studies and candidate-based approach**

In a large case–control study of Gambian children, a functional polymorphism at position −308 in the tumor necrosis factor (TNF-α) promoter (the TNF2 allele) has been shown to be associated with autoimmunity and high TNF-α production. Homozygosity for TNF2 was shown to carry a 7-fold increased risk of death from cerebral malaria. Although the TNF2 allele was in linkage disequilibrium with several neighboring HLA alleles, the association seemed to be independent of HLA class I and class II variation. The maintenance of the TNF2 allele in The Gambia suggested that the increased risk of cerebral malaria in homozygotes could be counterbalanced by some unknown biological advantage (60). In vitro experiments showed that the TNF2 allele was a much stronger transcriptional activator than the common allele (TNF1), suggesting that the −308 polymorphism was functional and had direct effects on TNF-α gene regulation (61). In the same Gambian population, it was also observed that severely anemic children with P. falciparum infection had low plasma TNF-α levels—a condition associated with the presence of a second polymorphism in the TNF-α promoter (TNF-238A) (62). Finally, a third polymorphism (TNF-376A) in the TNF-α promoter region was discovered through systematic DNA footprinting (63). This latter polymorphism was shown to differentially affect the recruitment of the transcription factor OCT-1 to the TNF promoter in vitro, modulating gene expression in human monocytes. In large case–control studies of West African and East African populations, the TNF-376A genotype was found in ~5% of Africans and was associated with a 4-fold increased susceptibility to cerebral malaria (63).

In West African children, a large case–control study of P. falciparum infection has shown that an HLA class I antigen (HLA-B53) and an HLA class II haplotype (DRB1*1302-DQB1*0501) are independently associated with protection from severe malaria (14). Further studies showed that HLA-B53-restricted cytotoxic T lymphocytes could recognize a conserved peptide from the parasite liver-stage-specific antigen 1 (LSA-1), providing a possible molecular basis for this HLA-disease association (64).

A possible role of polymorphic variants in proteins involved in adhesion of parasitized red blood cells (PRBC) to the vascular endothelium have also been conducted. Systematic sequencing of the ligand-binding N-terminal domain of the ICAM-1 gene in Kenya identified a single polymorphism (Kili allele) present at high frequency (65). The ICAMKili allele was associated with severe clinical malaria in Kenyan children (65) but with mild malaria in Gabonese children (66). A third study in The Gambia showed no effect of the ICAMKili allele on the outcome of P. falciparum infection (67). Functional studies have been conducted in vitro to verify a possible role of ICAMKili in malaria pathology, particularly in sequestration of PRBC in the brain. Static adhesion assays of PRBC to transfected COS cells have shown no significant difference between the two ICAM alleles, despite the fact that antibody-mediated studies had suggested a difference in their structure (65). More recent in vitro studies have shown that ICAMKili differentially binds to diverse P. falciparum parasite sub-strains (68). In addition, it was observed that the Kilifi allele has less affinity for the T-cell receptor LFA-1 (leukocyte function-associated antigen 1) compared with the normal allele and could not bind fibrinogen (68).

Conflicting results have emerged from the study of another candidate gene, nitric oxide synthase, (NOS), with both positive and negative association with severe malaria being reported. In Gabon, a single polymorphism of the NOS type 2 (NOS2) promoter region has been associated with protection from severe malarial anemia (G-954C, also called Lambarené allele) (69), while in The Gambia, a shorter form of a CCTTT microsatellite repeat located 2.5 kb 5' to the NOS2 transcriptional start site has been associated with susceptibility to fatal malaria (70). In Tanzania, neither polymorphism was found to
be associated with susceptibility to severe malaria (71). Possible functional roles of these allelic variants on transcriptional activity have still to be demonstrated, and would be required to allow better understanding of their potential involvement in malaria physiopathology.

CD36 is a major receptor for PRBC in humans. This receptor has been associated with the phenomenon of sequestration and suppression of dendritic cell-mediated T-cell stimulation (72,73), as well as with the clearance of non-opsonized PRBC by monocytes and macrophages (74) and with the platelet-mediated clumping of \textit{P. falciparum} PRBC (75). Systematic sequencing of the \textit{CD36} gene has shown the presence of many polymorphisms in the African population (76,77). Some of these polymorphisms have been associated with susceptibility to severe malaria (76), while at least one other was associated with protection from severe disease (78). The high frequency of polymorphisms in \textit{CD36} is interesting and could reflect regional parasite-host adaptations or the consequence of selective pressure by factors unrelated to malaria.

\textbf{Other levels of complexity in population studies}

In contrast to studies mentioned above (21,23) that suggest a protective role of thalassemia against malaria, a paradoxically high incidence of uncomplicated \textit{P. vivax} infection in young \(\alpha\)-thalassemic children has been reported in Vanuatu (22). Authors have proposed that early susceptibility to uncomplicated \textit{P. vivax} infection may lead to limited cross-species immunity sufficient to reduce the risk of subsequent severe \textit{P. falciparum} infection (22). This study exemplifies one of the difficulties in deciphering gene effects regulating susceptibility to infectious diseases in populations from endemic areas of disease where more than one antigenically related parasite may coexist. Genetic heterogeneity, variability in expressivity and penetrance of susceptibility/resistance genes, and differences in parasite-encoded virulence determinants (affecting disease severity) are additional parameters that further complicate genetic studies in humans. These may explain the lack of reproducibility or contradictory results obtained in association studies performed in distinct populations.

\textbf{MOUSE MODEL OF MALARIA INFECTION}

Complex genetic traits such as host response to malaria infection can be dissected in genetically well-defined inbred recombinant inbred and recombinant congenic strains of mice in which single-gene effects may either have naturally segregated or have been experimentally isolated by breeding. These genes can then be localized in linkage studies, and, in certain cases, can be identified by transcription mapping and positional cloning (79–83). In addition, variable parameters of infection such as virulence status of the parasite, dose and route of introduction can be standardized in an experimental mouse model, facilitating identification of possible genetic effects. Furthermore, candidate genes can be tested directly for their role in physiopathology by the creation of null alleles through gene targeting. Finally, the relevance of loci mapped in the mouse as potential candidates for disease susceptibility in humans can subsequently be tested in association studies in endemic areas of disease.

\textbf{Mouse pathology}

Four species of rodent malaria parasites are currently being used in laboratories. These species are \textit{P. chabaudi}, \textit{P. yoelli}, \textit{P. berghei} and \textit{P. vinickei}. There are two subspecies, of \textit{P. chabaudi}: \textit{chabaudi chabaudi} and \textit{chabaudi adami}. \textit{P. chabaudi chabaudi} is the most commonly used parasite subspecies, and many strains exist such as 54X, 864VD, AS and 96V. Experimental infections are performed by direct injection of PRBC into a naive host, either intra-peritoneally or intravenously. The physiopathology associated with blood-stage infection of mice with \textit{P. chabaudi}, while not identical to human malaria, shares many similarities, which makes it a useful model to study the human disease (84,85). Many of the blood-stage antigens expressed by \textit{P. chabaudi} are similar to those of \textit{P. falciparum}, and blood-stage parasites usually replicate in mature red cells, although reticulocytes can also be infected. Replication in red cells leads to hemolysis, thrombosis in the small vessels, ischemia, and necrosis of surrounding tissues. \textit{P. chabaudi} AS undergoes sequestration in the spleen and liver, but not in the brain as does the human parasite (86). Anemia is very severe and progresses rapidly. Intense erythropagocytosis of infected and normal red blood cells occurring in dramatically enlarged spleens (splenomegaly). The liver also becomes enlarged with Kupffer cells loaded with hemozoin (also called malaria pigment). Renal damage is similar to that observed in humans with possible nephritic syndrome and glomerulonephritis, and, as with \textit{P. falciparum} infection, severe hypoglycemia due to hyperinsulinism may be observed. The presence and severity of pathological manifestations are clearly dependent upon the genetic background of the mouse (87,88). In non-permissive or resistant strains, there is rapid blood-stage replication of the parasite during the first 6–8 days, followed by a curative phase, with inflammatory, immune and erythropoietic responses which clear the parasites by the third to fourth week post infection. In susceptible strains, the parasite replicates in an uncontrolled fashion, ultimately leading to death of the animals by day 10–13 (reviewed in 89). Although \textit{P. chabaudi} does not cause cerebral malaria (CM), a mouse model for CM has recently been developed using \textit{P. berghei} ANKA as the infectious agent (90,91). \textit{P. berghei}-induced CM displays a wide spectrum of physiopathological features, including deviation of the head, decrease of body temperature, hyperventilation, ataxia, convulsion, paralysis, coma and death (90). Both \textit{P. chabaudi} and \textit{P. berghei} infection models have recently been successful used for genetic studies (see below).

The effect of many naturally occurring or experimentally induced mutations on susceptibility to malaria has been investigated over the years. Studies in nude mice (92) and SCID mice (93) as well as CD4\(^+\) T-cell-depleted mice (94,95) have established a key role for CD4\(^+\) T cells in resistance to infection. CD4\(^+\) T cells that mediate macrophage activation and delayed-type hypersensitivity responses (Th1 cells) predominate during the early phase of infection, followed by the appearance during the late phase of another type of CD4\(^+\) T cells (Th2 cells) that are capable of providing help for
maturation of B cells to plasma cells and for antibody production (96). Expression of resistance requires intact T-cell and macrophage function and is eliminated by splenectomy prior to infection (92,97). Infection of TNF receptor-null mice demonstrated that these mice can produce systemic IL-12 early in blood-stage infection and clear the parasites by 4 weeks of infection, suggesting that TNF is not required for the development of normal Th1 response (98). Nitric oxide has also been shown to be neither necessary nor sufficient for resolution of P. chabaudi malaria (99). Studies involving other cytokine knockout mice have indicated that whereas the absence of IL-4 does not affect recovery from primary P. chabaudi infection (100), abrogation of IL-10, a potent mediator of inflammatory response, causes a high mortality rate (101). Not only the presence of the IL-10, but also the timing of its boost, has been shown to be important in the resolution of infection, since an early high level of IL-10 has also been associated with lethality (85). More recent studies in mouse mutants bearing null mutations have established that granulocyte–macrophage colony-stimulating factor (GM-CSF) (102) interferon-γ (IFN-γ) (103), IL-12 (104) and the SR-A scavenger receptor (105) are all required for resistance to infection.

**Strain variations in susceptibility to malaria infection**

When measured by either the extent of blood-stage parasite replication or overall mortality, inbred mouse strains differ markedly in the degree of susceptibility to *P. chabaudi* infection. Using survival as an indicator of susceptibility, the inbred strains B10.A, CBA, C57BL/6, C57L and DBA/2 are resistant (R) while the strains A/J, BALB/c, AKR, DBA/1, C3H/HeJ, SJL and 129/ICR are susceptible (S) (88,106,107). Susceptibility is also associated with a higher percentage of PRBC, with the strains 129/Sv and A/J showing 55–75% PRBC at the peak of infection, while C57BL/6 and DBA/2 show only 20–45% (88). Generally, F1 hybrids between R and S strains are more resistant to infection than either parent, suggesting that resistance is dominant and suggesting complementation of additional parental susceptibility loci in the hybrid. Finally, male mice are generally more susceptible than females with respect to peak parasitemia and survival (88,107,108). Independent studies with *P. yoelii* have classified 129/Sv as resistant to infection, while the NC/Jic strain was found to be susceptible (109), as measured by survival time. Studies in the *P. berghei* ANKA infection model have also identified strain variations in susceptibility assessed phenotypically by the appearance of CM-associated neurological manifestations (90): the strains C57BL/6 and 129/Sv are highly susceptible, with manifestation of CM-induced phenotypes seen in 60–100% of animals; the strains BALB/c and C3H are intermediate, with 20–60% incidence; while the DBA/2 strain is highly resistant to CM. The susceptibility and resistance of C57BL/6 and DBA/2 to *P. berghei* ANKA infection, were independently verified by using survival at day 14 as a phenotypic marker of susceptibility (91).

The marked interstrain difference in susceptibility to *Plasmodium* infection in inbred strains of mice suggested that a genetic approach could be used to identify key host defense mechanisms against this infection.

**Genetic linkage studies: identification of Char loci**

The physiological, immunological and genetic basis of differential strains susceptibility to malaria has been studied extensively in A/J and C57BL/6J (B6) strains infected with *P. chabaudi* (reviewed in 89). Susceptibility in A/J is associated with high blood-stage parasite replication, poor reticuloctysis and uniform death following infection, as opposed to B6 mice, which limit parasite replication, mount robust erythropoietic response and survive infection. Resistance in B6 mice is also associated with activation of CD4+ Th1 cells (88) and production of IFN-γ while a Th2-type response is seen in A mice, with production of IL-4, IL-5 and IL-10 (110,111). The genetic control of the A/J versus B6 interstrain difference was initially studied in a set of 16 AXB/BXA recombinant inbred strains (RIS), using peak parasitemia and overall survival as phenotypic traits. Each AXB/BXA strain contain a 50:50 ratio of A:B6 genomic DNA fixed as chimeric chromosomes with distinct combination of parental haplotypes (112). Such strains can rapidly deciper single gene effects, including mapping of major genes. Studies in AXB/BXA strains (i) revealed that the genetic control of peak parasitemia was complex, with multiple genes involved, and (ii) suggested that some of the genetic determinants affecting mortality may have segregated from those controlling blood parasitemia (89).

Individual loci controlling the A/J versus B6 interstrain difference have been recently mapped as quantitative trait loci (QTL), and designated Char loci (for ‘chabaudi resistance’). A whole-genome scan conducted in a subset of 94 (AXB6)XA backcross mice showing extreme phenotypes with respect to peak parasitemia after *P. chabaudi chabaudi* AS intraperitoneal infection suggested linkage to central chromosome 8. This linkage was further validated in a population of (AXB6)F2 mice, with a combined maximum LOD score of 4.3 (Char2; see below) (107). Independently, Foote et al. (106) conducted similar linkage studies in (SJLXB6)F2 and in (C3HXB6)F2 female mice using an intravenous infection model with *P. chabaudi adami* DS. Two loci, on central chromosome 8 (Char2; LOD = 6.0) and distal chromosome 9 (Char1; LOD = 11.5) were mapped and associated with survival to infection in both crosses. The chromosome 9 locus was also linked to the control of peak parasitemia in both crosses (LOD = 9.1 and 6.6, respectively), while the chromosome 8 locus was found to control peak parasitemia in the C3H-derived cross only (LOD = 8.8) (see Table 2 for a summary of linkage data). Subsequently, a linkage to the major histocompatibility locus (H-2) on chromosome 17 (LOD = 5.0) was noted on the day following the peak of parasitemia in *P. chabaudi adami* DS-infected (C3HXB6)F2 female animals, and was given the appellation Char3 (113). Re-analysis of the AXB/BXA data set verified the importance of Char1 and Char2, since RIS showing A/J haplotypes at these loci were the most susceptible to infection as measured by peak parasitemia and by survival (89). The Char1 locus (designated Pymr in that study) also came up positive (LOD = 3.3) in a whole-genome scan for the identification of loci controlling levels of blood parasitemia 5 days following infection with *P. yoelii*, and using an informative backcross derived from susceptible NC/Jic and resistant 129/Sv parents. In this cross, Char1 alleles were also found to influence death before day 7 post infection, and
survival after day 14 (109). Linkage studies in F2 mice derived from B6 and DBA/2 strains, respectively susceptible and resistant to experimental severe malaria induced by *P. berghei* ANKA (as measured by survival after day 14 post infection) have mapped a major gene effect on the central portion of chromosome 18 (D18Mit123, 31 cM; $\chi^2 = 30.1$); In these studies, an additional suggestive linkage was also noted in the *Char2* region on Chromosome 8 (91). Taken together, these studies have demonstrated the particular importance of the *Char1* and *Char2* loci for regulation of blood-stage parasite replication and overall host response and survival to infection with different *Plasmodium* species. It will be of interest to determine whether or not the corresponding synthetic chromosomal regions in humans are associated with susceptibility to malaria in different populations and in different endemic areas of disease worldwide. Likewise, the identification by positional cloning of the genes underlying those QTL may reveal new important host-response mechanisms against malaria infection, including possible new therapeutic targets for pharmacological intervention. However, this step might not be straightforward, since the *Char1*/2 QTL intervals range in size from 15 to 30 cM. The difficulties in going from QTL to positional candidate genes, including the identification of quantitative trait nucleotides (QTN) have been reviewed comprehensively by Flint and Mott (114). Attractive candidate genes map under the *Char2* QTL interval: they include several erythrocyte structural proteins, as well as IL-15 and the class A scavenger receptor (SR-AI/SR-AII) (107). Interestingly, sequence variations in SR-AI/SR-AII of A and B6 mice that affect recognition by specific antibodies have been reported (115,116), while loss-of-function mutations at this locus impair resistance to bacterial and viral infections (117). Likewise, the *Char1* region on chromosome 9 contains genes involved in red blood cell physiology and iron metabolism such as transferrin and haptoglobin (106).

Although linkage studies provide valuable entry points for gene discovery, the small fraction of phenotypic variance explained by each locus together with the relatively large genetic interval of the mapped QTL (25–30 cM) impedes identification by positional cloning. To circumvent these issues, we have implemented a strategy taking advantage of a novel set of 37 reciprocal recombinant congenic mouse strains (RCS) recently derived in our laboratory from A/J and B6 parental strains (118). RCS sets have recently been used as a powerful tool to study complex traits such as susceptibility to cancer, infections and metabolic diseases (119–123). The AcB/BcA RCS set (15 AcB and 22 BcA strains) was derived by systematic inbreeding of double-backcross (N3) animals, each strain containing a small amount (12.5%) of DNA from one parent fixed as a set of discrete congenic segments on the background (85.7%) of the other parent. The AcB/BcA set has been genotyped for 625 informative markers (average spacing 2.6 cM), and the position of all congenic fragments has been established (118). Individual resistance/susceptibility loci contributing to a complex trait may have segregated in individual RCS and can be studied in isolation—both for gene mapping/identification experiments and for elucidating unigenic contributions to the overall phenotype. Importantly, the relatively small size of the congenic segments fixed in individual RCS facilitates the search and testing of candidate genes. Phenotyping 13 AcB and 5 BcA strains (10 males and 10 females) for malaria (blood-stage replication, peak parasitemia and mortality) has confirmed a good correlation between resistance/susceptibility and *Char1* and *Char2* haplotypes (89). However, one of the AcB strains, AcB55, was highly resistant to infection despite 83% susceptible A/J genomic composition, including fixed susceptibility alleles at *Char1* and *Char2* (124). Early onset of parasite clearance in AcB55 is associated with low peak parasitemia (20–25% PRBC) and absence of mortality. Linkage analysis in 200 mice from an informative (AcB55 × A)F2 population genotyped for segregation of congenic B6 chromosomal segments, and using peak parasitemia as a quantitative trait, located a new and very strong B6-derived resistance locus on chromosome 3 (LOD = 6.57, $P < 0.0000003$, 17% of phenotypic variance) that we designated *Char4* (124). *Char4* maps to a small ~6 cM congenic B6 fragment in AcB55 that is syntenic with the human 4q21–q25 region. Therefore, studies in RCS have not only validated the important role of *Char1* and *Char2*, but also enabled the mapping of novel resistance loci. The positional cloning of these and other susceptibility loci in the mouse promises to provide novel insight into host defenses against malaria infection.

**CONCLUSIONS**

The malarial parasite has been endemic in many areas of the world for a long period of time, during which it has coexisted with human populations. This situation has created direct selective pressure from the parasite on its host, resulting in retention in the gene pool of otherwise-deleterious alleles at certain human genes (namely sickle cell anemia, thalassemias and others). The combined analysis of such mutations in humans, together with additional linkage and association studies with candidate loci, has started to unveil the complexity of the interaction between the parasite and its host that controls initial infection, disease progression, pathological manifesta-

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**Table 2. Linkage mapping for malaria susceptibility in mice**

<table>
<thead>
<tr>
<th>Chromosomal location</th>
<th>Locus</th>
<th>Host strain combination</th>
<th><em>Plasmodium</em> sp.</th>
<th>Refs</th>
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<tr>
<td>Distal chromosome 9</td>
<td>Char1</td>
<td>(C3H × B6)F2</td>
<td><em>chabaudi</em> adami DS</td>
<td>(106)</td>
</tr>
<tr>
<td></td>
<td>Pymr</td>
<td>(C57BL/6j × C3H)F2</td>
<td><em>yoelii</em> 17XL</td>
<td>(109)</td>
</tr>
<tr>
<td>Central chromosome 8</td>
<td>Char2</td>
<td>(A × B6)A</td>
<td><em>chabaudi</em> adami AS</td>
<td>(107)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(A × B6)F2</td>
<td><em>chabaudi</em> AS</td>
<td>(106)</td>
</tr>
<tr>
<td></td>
<td>Char3</td>
<td>(DBA/2 × B6)F2</td>
<td><em>bergheri</em> ANKA</td>
<td>(91)*</td>
</tr>
<tr>
<td></td>
<td>Char4</td>
<td>(AcB55 × A)F2</td>
<td><em>chabaudi</em> adami DS</td>
<td>(113)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(AcB55 × A)F2</td>
<td><em>chabaudi</em> AS</td>
<td>(118)</td>
</tr>
<tr>
<td>Central chromosome 18</td>
<td></td>
<td>(DBA/2 × B6)F2</td>
<td><em>bergheri</em> ANKA</td>
<td>(91)</td>
</tr>
</tbody>
</table>

*Only suggestive linkage was detected in this study.*
tions and ultimate outcome. Parallel genetic studies in mouse models of disease have begun to catalog novel loci that may regulate host-parasite interactions in previously unsuspected ways.

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REFERENCES

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