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Host resistance to malaria: using mouse models to explore the host response

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Abstract Malaria is a disease that infects over 500 million people, causing at least 1 million deaths every year, with the majority occurring in developing countries. The current antimalarial arsenal is becoming dulled due to the rapid rate of resistance of the parasite. However, in populations living in malaria-endemic regions there are many examples of genetic-based resistance to the severe effects of the parasite *Plasmodium*. Defining the genetic factors behind host resistance has been an area of great scientific interest over the last few decades; this review summarizes the current knowledge of the genetic loci involved. Perhaps the lessons learned from the natural variation in both the human populations and experimental mouse models of infection may pave the way for novel resistance-proof antimalarials.

Background

Despite significant technical and medical advances in the past 100 years for controlling malaria infection, the prospect of eradicating the disease remains daunting. In part, this is due to the many socioeconomic factors faced by the countries most affected, but a major concern is also the problem of parasite drug resistance. All current antimalarial drugs target the parasite. The enormous capacity for genetic variation in the parasite in conjunction with the effect of the highly selective nature of drug treatment on the parasite's survival has resulted in the rapid development of drug-resistant strains to all currently used antimalarial agents. Chloroquine, once the frontline antimalarial drug, is now virtually useless in all malaria-endemic regions. Parasite strains have recently been isolated from Cambodia that display increasing resistance to artemisinin, a drug that has only recently been introduced into the antimalarial armamentarium (Dondorp et al. 2009). With the dramatic rise and spread of resistance against these parasite-directed drugs, a new approach is desperately needed.

With this in mind, this review instead focuses on host-protective mechanisms, particularly those with a genetic basis. Malaria has coexisted with humans for many millennia, and given the high mortality rate it causes, has had a profound impact on the human genome. A variety of polymorphisms and mutations that protect against infection and the symptoms of the disease have arisen in human populations living in malaria-endemic regions of the world. Many protective polymorphisms affect the red blood cell (RBC) in which the parasite resides during the symptomatic stages of the disease. The red cell sustains the greatest parasite mass for the longest period of time, and for the parasite *P. falciparum*, it is the target of an immune response that can clear the host of all parasites. It is therefore not surprising that a subversive battle has been fought between the host and parasite genomes for thousands of years, the battleground being the red cell; the outcome ultimately deciding the result of infection for the human host and the continuing cycle of the parasite.

The most severe and life-threatening manifestation of *Plasmodium falciparum* infection is cerebral malaria (CM). Human CM (HCM) mortality and morbidity in regions of malaria endemicity is particularly important in children under the age of 5, a population of patients who have not yet developed protective immunity against *Plasmodium*

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parasites. HCM is caused when infected erythrocytes are retained and become trapped in the body microvasculature, most notably capillaries and venules of the brain. This leads to an excessive, localized host-driven inflammatory response, resulting in hypoxia, loss of blood–brain barrier integrity, local hemorrhage, and additional complications. Symptoms include impaired consciousness with fever, generalized convulsions, and neurological abnormalities, and coma that lasts for 24–72 h, initially rousable and then unrousable. Additional complications of HCM include increased intracranial pressure, hemiplegia, encephalopathy, and acute renal failure with uremia; HCM is fatal in approximately 20% of patients (Mishra and Newton 2009). Treatment options are few and include high-dose quinine and/or artemisinin, as well as several adjunctive measures (antipyretics, microcirculatory flow, anticonvulsants, iron chelators) where efficacy remains debatable (Mishra and Newton 2009). The very high incidence of *Plasmodium* infection in populations living in regions of malaria endemicity (which is assumed to be constant during the wet season) and the fact that most HCM-associated deaths occur in young children have favored alteration of the human gene pool by malarial parasites.

Here we focus on what is known about genetic-based host resistance in both human populations and experimental mouse models of malaria and suggest how this research could be translated into novel antimalarial therapies that may avoid the problem of parasite resistance common to the current generation of therapeutics.

Natural resistance to malaria in human populations

Human genetics has long been associated with variation in susceptibility to numerous infectious diseases. Of all diseases that impact the human population, malaria has exerted the largest measurable level of selective pressure on our genome. Haldane (1949) was the first to recognize the importance of host genetics in malaria resistance when he postulated that the high rates of thalassemia in Mediterranean populations were due to a heterozygous advantage afforded to carriers. Since this initial observation, numerous other mutations that have an impact on the host resistance to malaria have been identified. These mutations target mainly red cell proteins, factors involved in the sequestration of parasites as well as immune response variants. These have often been initially suggested through coincidence of malarial endemicity and increased mutation frequency. However, in some cases a molecular basis for disease resistance has also been demonstrated, as shown in Table 1 and further detailed below.

Genetic epidemiological studies, linkage studies, and association studies in affected human populations have pointed to a complex genetic component of resistance/susceptibility to HCM, including a number of candidate genes such as erythrocyte polymorphisms (hemoglobinopathies) and genes involved in early inflammatory or innate immune response. These studies encompass a large body of literature that is not reviewed here. The reader is referred to excellent reviews on human host protection factors by Bongfen et al. (2009), Kwiatkowski and Luoni (2006), and Verra et al. (2009).

Recent genome-wide association approaches designed to discover novel loci that control malaria susceptibility have been disappointing, in part due to challenges in applying technologies developed for Caucasian genetic disease studies to Africans (Jallow et al. 2009). There is a large degree of genetic heterogeneity in African populations, making it more difficult to replicate the findings

Table 1 Examples of human polymorphisms associated with protection from malaria and the postulated mechanisms of resistance

Polymorphism/locus	Hypothesized mechanism	References
Sickle cell anemia	Early clearance of infected RBCs Reduced parasite growth and invasion	Ayi et al. 2004; Fortin et al. 2002; Friedman 1978
α-Thalassemia	Oxidative stress Immunological memory	Flint et al. 1986; Williams et al. 1996
β-Thalassemia	Early clearance of infected RBCs	Ayi et al. 2004
Glucose-6-phosphate-dehydrogenase deficiency	Reduced parasite growth	Reviewed in Ruwende and Hill 1998
Band 3 ovalocytosis	Reduced parasite invasion	Jarolim et al. 1991
Duffy negative antigen	Disruption of the GATA motif in the *Duffy* gene, resulting in absence of invasion by *P. vivax*	Tournamille et al. 1995
HLA class 1 antigen (HLA-Bw53)	Possibly destruction of liver-stage parasites by cytotoxic T cells	Hill et al. 1991
HLA class II haplotype (DRB1*1302-DQB1*0501)	Increased parasite clearance	Hill et al. 1991
TNF-α variants	Regulation of TNF-α, linked to both pathology and protection	McGuire et al. 1994

between regions. Future genome-wide association studies will require the concerted effort of large international collaborations and more tailored genetic variation detection technologies. While researchers undertake this effort, another way to overcome the limitations of human studies has been to use experimental mouse models of malaria.

Utilizing the mouse to model human susceptibility

The parasite causing malaria infection, *Plasmodium*, infects not only humans; several species of *Plasmodium* have the ability to cause malaria in animals, including rodents. The most commonly used to infect laboratory mice are *P. chabaudi*, *P. yoelii*, *P. berghei*, and *P. vinckei.* Mice are considered a comparable genetic model to humans: There is a high degree of genomic conservation (99%) (Pennacchio 2003), and it is well established that mice also exhibit natural differences in susceptibility to malarial infection (Greenberg et al. 1954). Murine models of malaria enable complex traits to be dissected in large crosses generated between resistant and susceptible strains, and allow the control of environmental factors such as parasite dose, strain and virulence, the route of infection, and nutritional intake (Fortin et al. 2002). Mice are also genetically tractable; the inbred strains of mice are genetically well defined and gene effects can be isolated through breeding. Their very large reproductive capacity is also advantageous, enabling studies of linkage analysis and positional cloning. The genome sequence of many inbred strains of mice is now available. In spite of recent criticism on the benefits mouse models provide in malaria studies (White et al. 2010), they have provided significant insight into host-protective mechanisms, as encapsulated in the robust responses to this article (Carvalho 2010; Hunt et al. 2010; Renia et al. 2010; Riley et al. 2010; Stevenson et al. 2010). As we discuss below, mouse malaria models in conjunction with applications in murine genetics provide a powerful and valuable way to discover novel mechanisms of host protection that may be amenable to the development of novel therapeutics.

P. chabaudi infections share a number of features that are typical of *P. falciparum* in humans. These include symptoms such as anemia, splenomegaly, and hepatomegaly. Sequestration of infected erythrocytes in the liver and spleen is observed, as are the development of analogous blood-stage antigens and the suppression of B- and T-cell responses (although cerebral disease is not a feature of this species) (Hernandez-Valladares et al. 2005; Min-Oo et al. 2007b; Weatherall et al. 2002). Importantly, *P. chabaudi* has also been used to recapitulate the protective effects of a number of human polymorphisms in conjunction with strains of mice carrying the equivalent mutations. However, *P. chabaudi* cannot be used as a model for cerebral malaria.

Although no animal model accurately mimics all aspects of CM in humans, infection of mice with *Plasmodium berghei* ANKA (PbA) reproduces many of its characteristics. Experimental cerebral malaria (ECM) induced by infection with PbA is defined by the appearance of neurological symptoms, including ataxia, hind limb paralysis, deviation of the head, and coma, with death occurring between 6 and 10 days following infection. Death by ECM occurs with low levels of parasitemia, typically less than 20% parasitized erythrocytes depending on the mouse strains used (Hunt et al. 2006). Conversely, mouse strains naturally resistant to ECM (such as BALB/c mice) develop hyperparasitemia and severe anemia, with death occurring about 3 weeks after infection. Similar to what is observed in humans, sequestration in the brain microvasculature of PbA-infected mice has been documented in some studies (de Souza et al. 2010; Francischetti 2008; Sanni et al. 2004; Stevenson et al. 2010). Similarly, a correlation between sequestered parasite biomass and disease severity has also been demonstrated (Amante et al. 2007; White et al. 2010). Other ECM features shared by HCM patients and PbA-infected mice include reversal of symptoms, platelet activation, and coagulopathy (Cox and McConkey 2010; Francischetti 2008). Studies in the ECM model have provided important clues for understanding the role of the immune response in HCM, which is difficult to study in human subjects for ethical reasons (de Souza et al. 2010). In addition, although controversial, research on adjunctive therapy in the ECM model has driven clinical research studies in human subjects (de Souza et al. 2010; White et al. 2010) (http://www.controlled-trials.com/mrct/search.html).

There have been two types of genetic analysis conducted in mouse models of malaria. The first is the reverse genetics approach in which mouse stocks bearing targeted mutations in candidate genes have been assessed for modulation of *Plasmodium* species. The second is the forward genetics approach in which inbred mouse strains showing different degrees of susceptibility to *Plasmodium* species have been analyzed to map the major gene effects regulating the differential response. Studies from both approaches are reviewed below.

Genetics of host resistance to *P. chabaudi* and *P. yoelii*

Infection of any one strain of mouse with *P. chabaudi* leads to a reproducible infection and a high rate of concordance between parasite growth kinetics and outcome to infection. However, there is considerable variability in both the rate of increase of parasites in circulation and the outcome of infection between inbred mouse strains. Of the inbred mouse strains, C57BL/6, BALB/c, DBA/1, C57BL/10, SPRET/Ei, and 129/SvJ are resistant when infected with

P. chabaudi, as shown by survival and decreased parasitemia. SJL, C3H/He, NC/Jic, DBA/2, CBA/J, SM/J, MOLF/Ei, and A/J are all susceptible strains and exhibit higher parasitemia at the peak of infection and a high mortality rate, with death usually occurring the second week after infection (Fortin et al. 2002; Hernandez-Valladares et al. 2005). This implies that host genetic differences underlie susceptibility to malarial infection. The genetic basis is likely to be complex, and although not fully elucidated, several quantitative trait loci (QTLs) that determine resistance or susceptibility to *P. chabaudi* infection (*Char* loci) have been identified. Phenotypes analyzed include parasitemia and associated kinetics and survival. At present, ten loci associated with the control of parasitemia in *P. chabaudi*, six loci linked to cerebral malaria in *P. berghei* infection, and one locus associated with susceptibility to *P. yoelii* infection have been reported (Table 2). The genetics of *P. berghei* infection are discussed in the next section.

Independently, two groups published papers in 1997 that first identified loci controlling parasitemia during *P. chabaudi* malarial infection (Foote et al. 1997; Fortin et al. 1997). *Char1* and *char2* were mapped to chromosomes 9 and 8, respectively, by Foote et al. using linkage analyses of the F2 generation of crosses between two susceptible strains, SJL and C3H/He, and the resistant strain C57BL/6 (Foote et al. 1997). These loci controlled survival and peak parasitemia. At the same time, Fortin et al. mapped the same *char2* locus controlling parasitemia using both backcrossing and intercrossing between A/J (susceptible) and C57BL/6 (Fortin et al. 1997). A further study analyzing a NC/Jic and 129/SvJ cross found that parasitemia during *P. yoelii* infection mapped to a locus on chromosome 9, at the same location as *char1*, suggesting that this may represent a ubiquitous locus (Ohno et al. 2001). Additional crosses between C57BL/6 and C3H/HeJ also found a linkage peak over *char2*. Further studies of subcongenic lines for *char2* have uncovered considerable complexity to this locus, with at least two loci mapping to a similar region (Lin et al. 2006).

A separate locus, *char3*, was identified on chromosome 17 from a [C3H/Hex C57BL/6]F2 cross (Burt et al. 1999). Unlike *char1* and *2*, *char3* was mapped using the time of peak parasitemia rather than the outcome to infection or the magnitude of peak parasitemia. It was hypothesized that *char3* controls the rate of parasite clearance. This locus was most closely linked to markers in the mouse major histocompatibility complex (H-2), which had previously been shown to be linked to resistance to malarial infection in mice (Wunderlich et al. 1988). C57BL/6 animals carrying a donor *char3* interval, however, were considerably more resistant to death from *P. chabaudi* infection than the otherwise resistant C57BL/6, a result that was not predicted from the original cross, indicating that there may be epistatic interactions at work.

The disadvantage of the methods used to identify *char1–3* is that the chromosomal regions mapped were

Table 2 Genetic loci linked to murine malaria resistance and the method used to identify them, as well as the associated phenotype

Murine parasite	Locus	Cross	Phenotype	Location	LOD or χ^2	Reference
P. chabaudi	*Char1*	Backcross and/or intercross	Parasitemia	9	6.6/9.1	Foote et al. 1997
	Char2		Parasitemia	8	8.83	Foote et al. 1997; Fortin et al. 1997
	Char3		Parasitemia	17	5.0	Burt et al. 1999
	Char4	Recombinant congenic strains	Parasitemia	3	6.57	Fortin et al. 2001
	Char5	Advanced intercross lines	Parasitemia	5	2.16	Hernandez-Valladares et al. 2004a
	Char6		Parasitemia	5	2.16	Hernandez-Valladares et al. 2004a
	Char7		Parasitemia	17	5.75	Hernandez-Valladares et al. 2004a
	Char8		Parasitemia	11	1.9	Hernandez-Valladares et al. 2004b
	Char9	Recombinant congenic strains	Parasitemia	10	4.74	Min-Oo et al. 2007b
	Char10			9	7.24	Min-Oo et al. 2010
P. berghei	[a]	Backcross and/or intercross	Survival	18	$\chi^2 = 30.1$	Nagayasu et al. 2002
	Berr1		Survival	1	$\chi^2 = 18.98$	Bagot et al. 2002
	Berr2		Survival	11	$\chi^2 = 16.51$	Bagot et al. 2002
	cmsc		Survival	17	$\chi^2 = 26.18$	Ohno and Nishimura 2004
	Berr 3	Intercross	Parasitemia	9	4.9	Campino et al. 2005
	Berr 4	Intercross	Parasitemia	14	3.42	Campino et al. 2005
	Berr5	Intercross	Survival	19	4.69	Berghout et al. 2009
P. yoelii	*Pymr*	Backcross	Parasitemia	9	4.4	Ohno et al. 2001

Location = chromosomal location

[a] Locus not designated by authors

large and contained several hundred candidate genes due to low rates of recombination in the generation of F2 crosses (Darvasi and Soller 1995). The more recent use of congenic animals, recombinant congenic strains, and especially advanced intercross lines has sought to overcome these problems and has led to additional *char* loci being reported, and in one case identification of the underlying gene. Recombinant congenic strains (Mullerova and Hozak 2004) were used to identify a new malaria susceptibility locus on chromosome 3, *char4*. Fortin et al. (2001) developed a set of 33 recombinant congenic strains between the susceptible A/J (A) and resistant C57BL/6 (B). The strain AcB55 was shown to be highly resistant due to markedly reduced peak parasitemia, despite having susceptibility alleles at the *char1* and *char2* loci. Linkage analysis of an F2 cross (parents AcB55 and A/J) mapped the peak parasitemia determinant to *char4* (located on chromosome 3). Sequencing of candidate genes in this locus showed that resistance in the AcB55 recombinant congenic mouse was due to a loss-of-function mutation in the pyruvate kinase gene (Min-Oo et al. 2003). This led to the proposal that pyruvate kinase deficiency results in malaria resistance due to the primary defect causing hemolytic anemia that in turn results in an increased compensatory rate in erythropoiesis (Min-Oo et al. 2004). This study showed the power of the genetic approach, in particular using recombinant congenic lines to identify new mechanisms involved in the host response to malaria. Recently, polymorphisms in the human pyruvate kinase gene have been associated with protection against *P. falciparum* (Ayi et al. 2008).

Identification of additional *char* loci has been aided by the development of advanced intercross lines (AILs). AILs are produced by randomly and sequentially intercrossing a population generated from two initial inbred lines for at least eight generations, increasing the probability of recombination and providing a greater mapping resolution (Darvasi and Soller 1995). Hernandez-Valladares et al. used an F11 AIL from A/J and C57BL/6 parents to identify two significant host-response QTLs on chromosome 5, *char5* and *char6*, as well as a locus on chromosome 11 (*char8*), and confirmed the QTL already identified on chromosome 8 (*char2*) (Hernandez-Valladares et al. 2004a, b). They also resolved the previously named *char3* locus on chromosome 17 into two loci, *char3* and *char7*.

Recently, a ninth and a tenth locus associated with malarial resistance were identified on chromosomes 10 and 9, respectively. *Char9* was mapped to chromosome 10 (LOD = 4.74) using an intercross of parents AcB55 and A/J that identified *char4* (Min-Oo et al. 2007a). *Char9* regulates the blood-stage replication of *P. chabaudi* independently of the pyruvate kinase gene originally found by these authors. Using gene expression analysis, they have identified two Vanin genes, *Vnn1* and *Vnn3*, as the likely candidates responsible for the resistance associated with *char9*. *Char10* has recently been identified on chromosome 9 (LOD = 7.24) in [AcB62 × CBA-pk]F2; this locus modulates severity of malaria in a context of pyruvate kinase deficiency (Min-Oo et al. 2010). In addition, studies in mouse mutants have revealed further host genes associated with outcomes to *P. chabaudi* infection. Genes implicated in host resistance include immune system processes and RBC structural proteins (Table 3).

Genetics of host resistance to *P. berghei ANKA (PbA)*

Inbred strains of mice exhibit different responses to *Plasmodium berghei* ANKA (PbA), with susceptible strains such as C57BL/6 (B6) or CBA developing an acute cerebral syndrome within 6–7 days characterized by ataxia, paraplegia, seizures, and coma leading to uniform lethality by day 8–10 postinfection. On the other hand, resistant mice, including the BALB/c and wild-derived WLA/Pas strains, do not develop neurological symptoms/ECM but go on to succumb 2–4 weeks postinfection due to severe anemia caused by high levels of blood parasitemia. Using either the appearance of ECM or time of death following PbA infection as phenotypic markers of susceptibility, QTL mapping studies by whole-genome scanning in mice have detected a number of chromosomal regions regulating

Table 3 Single-gene defects associated with response to *P. chabaudi*

Gene ID	Gene name	Knockout effect on *P. chabaudi* infection	Reference
TLR9	Toll-like receptor 9	Partially resistant	Hisaeda et al. 2008
Taut	Taurine transporter	Decrease survival	Delic et al. 2010
MIF	Macrophage migration inhibitory factor	Increased survival	McDevitt et al. 2006
ANK-1	Ankyrin-1	Heterozygous mice show increased survival	Rank et al. 2009
CD24/HSA	CD24/Heat stable antigen	Increased parasitemia	Nielsen et al. 1997
Spna1	Spectrin alpha-1	Increased survival	Shear et al. 1991
Csf2	Colony-stimulating factor 2 (granulocyte-macrophage)	Decreased survival	Riopel et al. 2001
Il15	Interlukin-15	Delayed parasite clearance	Ing et al. 2005

response to PbA infection. The genetic determinants regulating differential susceptibility of C57BL/6 J (very susceptible) and DBA/2 J (less susceptible) to PbA infection were studied in informative F1 and F2 mice. Resistance was found to be inherited in a recessive fashion and linked to two markers in the central portion of chromosome 18 (*D18Mit123* and *D18Mit202*) (Nagayasu et al. 2002). The high degree of resistance to PbA infection of wild-derived mice was studied using informative backcross and F2 progeny between C57BL/6 J (susceptible) and wild-derived WLA/Pas mice (highly resistant). In such crosses, resistance to ECM was found to be dominant and under complex genetic control. Two loci, designated *Berr1* and *Berr2*, that control the appearance of ECM were initially mapped in backcross animals to chromosomes 1 (*D1Mit221* near transforming growth factor β2, *Tgfb2*) and 11 (*D11Mit338*), respectively (Bagot et al. 2002). In a second study using a [C57BL/6 J X WLA] F2 cross, the same group showed that time of survival following PbA infection was controlled by *Berr1* (LOD = 6.4) and by an additional locus on the distal portion of Chr 9 (LOD = 4.9; *D9Mit18*), which they designated *Berr3*. An additional weaker linkage on Chr 14 (*Berr4*; *D4Mit27*; LOD = 3.42) was also detected in this study (Campino et al. 2005). Finally, the *Cmsc* locus mapping to the H-2 region of chromosome 17 was found to affect susceptibility in progeny of CBA mice crossed with the resistant DBA/2 strain (Ohno and Nishimura 2004). In all cases, the detected QTLs span large chromosomal segments, and although there are a number of attractive positional candidates within their confidence intervals, the causative genes underlying their effect on survival time in *P. berghei*-infected animals have not been identified.

More recently, Berghout et al. (2009) used survival time to estimate susceptibility to PbA infection in an F2 cross ($n = 257$), and identified linkage to chromosome 19 [*Berr5*, LOD = 4.69] that controls, in part, the differential response between resistant BALB/c and susceptible C57BL/6 progenitors. BALB/c alleles convey increased survival through the cerebral phase of infection but have no quantitative effect on parasitemia during the later, anemic phase. The *Berr5* locus colocalizes with three other immune loci, including *Trl-4* (tuberculosis resistance), *Tsiq2* (T-cell secretion of IL-4), and *Eae19* (experimental allergic encephalitis 19), suggesting the possibility of a common genetic effect underlying these phenotypes. Potential positional candidates include the family of *Ifit1–3* (interferon inducible protein with tetratricopeptide repeats 1–3), whose expression is differentially regulated in response to infection in a parental haplotype-specific fashion (Berghout et al. 2009).

Current evidence suggests that ECM in mice infected with PbA is caused in part by robust but detrimental localized inflammation in response to parasitized erythrocytes trapped in the brain microvasculature (Lamb et al. 2006; van der Heyde et al. 2006). Transcript profiling experiments have documented a substantial proinflammatory response in situ in infected tissues that is more pronounced in susceptible mice (Delahaye et al. 2006; Lovegrove et al. 2007; Sexton et al. 2004). Studies in mouse mutants have clearly demonstrated that early proinflammatory responses, including Th1 polarization of the immune response, are a central component of the host-driven pathogenesis in ECM, and that mutations in genes from this pathway are protective against PbA-induced ECM (Bongfen et al. 2009). Such studies are summarized in Table 4 and further detailed below.

For example, mice deficient in interferon-γ (van der Heyde et al. 1997) and its downstream target, the key transcriptional activator STAT1 (P. Gros, unpublished), are resistant to PbA-induced ECM. In the same type of analysis, it has been shown that the absence of functional lymphotoxin A (Togbe et al. 2008), Fas (Ohno et al. 2005), platelet factor 4 (Srivastava et al. 2008), and the complement component C5a (Patel et al. 2008) protects against ECM. Likewise, mutations in *Tnfr1b* (Piguet et al. 2002), *Ccr5* (Belnoue et al. 2003), *Cxcr3* (Campanella et al. 2008), *CD14* (Oakley et al. 2009), *CD8* (Oakley et al. 2009), and *CD40* (Piguet et al. 2001) increase survival times following infection with PbA. Finally, mutations in members of the IRF family of transcriptional regulators, IRF1 (Senaldi et al. 1999) and IRF8 (Gros et al., unpublished), can also protect against ECM. In this case, protection appears concomitant with impaired production of IL12p40, and/or alterations in the number and type of natural killer (NK) cells and myeloid cells, including monocytes and CD11c + dendritic cells produced in these mice (P. Gros et al., unpublished). These observations support the hypothesis of a central role for immune-mediated pathology in ECM, with NK cells, T cells, platelets, and myeloid cells, including resident macrophages and dendritic cells, implicated in early inflammatory response and tissue injury.

Future approaches to discovery of novel genes that provide resistance to infection

Through the aforementioned mouse studies and earlier epidemiological studies on human populations, it can be seen that the genetic susceptibility to malaria and the host response to the infection is very complex. The genetic differences exhibited between mouse strains, as well as the polymorphisms identified in human populations, most likely control or contribute to a wide range of processes that influence the outcome of a malarial infection, including erythrocytic contributions and the immune response. So far most of the known human polymorphisms have limited

Table 4 Single-gene defects associated with response to *P. berghei*

Gene ID	Gene name	Knockout effect on *P. berghei* infection	Reference
Immune system processes			
Ccr2	Chemokine (C-C motif) receptor 2	None	Belnoue et al. 2003
Cd1	CD1 antigen	Increased survival	Hansen et al. 2003
Cd8	CD8 antigen	Increased survival	Oakley et al. 2009
Cd14	CD14 antigen	Increased survival	Oakley et al. 2009
Cd40/Cd40 l	CD40 antigen/ligand	Increased survival	Piguet et al. 2001
Fas	Fas^{lpr} (hypomorph)	Increased survival	Ohno et al. 2005
Gpx1	Glutathione peroxidase	None	Potter et al. 2005
Hc	Hemolytic complement, C5	Increased survival	Patel et al. 2008
Icam1	Intracellular adhesion factor 1	Increased survival	Li et al. 2003
Ifng	Interferon γ	Increased survival	Amani et al. 2000; Evans et al. 2006; Yanez et al. 1996
Il1r	Interleukin 1 receptor	None	Reimer et al. 2010
Il4	Interleukin 4	Decreased parasitemia	Saeftel et al. 2004
Irf1	Interferon responsive factor 1	Increased survival	Senaldi et al. 1999
Lta	Lymphotoxin A	Increased survival	Engwerda et al. 2002; Hansen et al. 2004; Piguet et al. 2002; Stoelcker et al. 2002; Togbe et al. 2008; Togbe et al. 2007
Ltbr	Lymphotoxin B receptor	Increased survival	Togbe et al. 2008
Myd88	Myeloid differentiation primary response	Increased survival (controversial)	Coban et al. 2007; Togbe et al. 2007
Nlrp3	Nod-like receptor 3	CM delay	Reimer et al. (2010)
Nod1/2	Nod-like receptor 1/ 2	None	Finney et al. 2009
Selp	P-selectin	Increased survival	Combes et al. 2004
Tirap	Toll-interleukin 1 receptor domain containing adaptor	None	Coban et al. 2007
Tlr1-7	Toll-like receptor 1-7	None	Coban et al. 2007; Togbe et al. 2007
Tlr9	Toll-like receptor 9	Increased survival (controversial)	Coban et al. 2007; Togbe et al. 2007
Tnf	Tumor necrosis factor α	None	Engwerda et al. 2002; Hansen et al. 2004; Togbe et al. 2008
Response to stimulus			
Ccr5	Chemokine (C-C) receptor 5	Increased survival	Belnoue et al. 2003
Hmox1	Heme oxygenase 1	Decreased survival in CM-R BALB/c	Pamplona et al. 2007
Plat/Plau	Plasminogen activator, tissue/urokinase	None	Piguet et al. 2000
Socs1	Suppressor of cytokine signaling 1	Increased survival	Bullen et al. 2003
Regulation of biological processes			
Casp1	Caspase 1	None	Reimer et al. 2010
Cxcr3	Chemokine (C-X-C motif) receptor 3	Increased survival	Campanella et al. 2008; Miu et al. 2008; van den Steen et al. 2008
Mmp9	Matrix metallopeptidase 9	None	van den Steen et al. 2008
Msr1	Macrophage scavenger receptor 1	None	Cunha-Rodrigues et al. 2006
Nos3	Nitric oxide synthase 2, inducible	None	Gramaglia et al. 2006
Pla2g4a	Phospholipase A2, group IVA	None	Ishikawa et al. 2004
Socs2	Suppressor of cytokine signaling 2	None	Bullen et al. 2003
Other (cellular processes, growth, etc.)			
Abca1	ATP-binding cassette, A1	Increased survival	Combes et al. 2005
Asc	PYD/CARD domain containing	None	Reimer et al. 2010
Ttpa	Alpha tocopherol transfer protein	Increased survival	Herbas et al. 2010
Clnc1	Chloride channel 1	None	Huber et al. 2004

Table 4 continued

Gene ID	Gene name	Knockout effect on *P. berghei* infection	Reference
Cybb	Cytochrome b-245, B-polypeptide	None	Potter et al. 2005
Hdc	Histidine decarboxylase	Increased survival	Beghdadi et al. 2008
Hp	Haptoglobin	None	Hunt et al. 2001
Lgals3	Galectin 3	Increased survival	Oakley et al. 2009
P2xp7	P2X purinoceptor 7	None	Reimer et al. 2010
Pfx	Platelet factor 4	Increased survival	Srivastava et al. 2008
Plaur	Plasminogen activator, urokinase receptor	CM delay	Piguet et al. 2000
Prf1	Perforin 1	Increased survival	Potter et al. 2006
Smpd	Acid sphingomyelinase	Decreased parasitemia	Brand et al. 2008
Trif	Toll-like receptor adaptor molecule	None	Coban et al. 2007

therapeutic potential. While the mapping techniques mentioned above have successfully identified over 15 regions of the genome in mice associated with host resistance, each containing potentially novel genes, most studies have been unsuccessful in identifying these causative genes. In order to utilize the work of these studies and unlock their therapeutic potential, we need to know the actual genes involved with resistance, not simply the chromosomal region where they map.

There is significant promise in the discovery of novel host gene variants that protect against malaria infection. In stark contrast to the problem of parasite resistance to current antimalarial drugs, the development of parasite resistance against the protective polymorphisms found in humans is (virtually) nonexistent [with perhaps one recently discovered exception involving *P. vivax* and *Duffy* in Madagascar (Menard et al. 2010)]. This is because the basis for the protective effect lies within the host, often modulating the environment of the host erythrocyte. These host cell perturbations affect elements fundamental to the parasite's growth and development. Importantly, protection is governed by the host genome, not the parasite, and thus the parasite is unable to easily develop means to circumvent these changes. This renders such host-protective polymorphisms effectively "parasite-resistance proof." Drugs that mimic such protective mutations or target other host processes essential for parasite survival may similarly avoid parasite drug-resistance problems.

To progress from the genetic loci already identified into such "parasite-resistance proof" antimalarials, new avenues of research need to be explored. The development of genetic data sets tailored for the heterogeneous African populations will allow future genome-wide association studies to be more successful. As our understanding of the host–parasite interactions and kinetics increases, our ability to narrow down large intervals through candidate gene analysis will dramatically improve. The generation of mice with single-point mutations displaying resistance to malaria, developed through mutagenesis screens, will potentially offer a faster route of gene identification than traditional genetic mapping. The resultant possibility of therapies that could be developed will be entirely novel: they will be targeting the host rather than the parasite.

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