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REVIEW ARTICLE
How Malaria Has Affected the Human Genome and What Human Genetics Can Teach Us about Malaria

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Malaria is a major killer of children worldwide and the strongest known force for evolutionary selection in the recent history of the human genome. The past decade has seen growing evidence of ethnic differences in susceptibility to malaria and of the diverse genetic adaptations to malaria that have arisen in different populations: epidemiological confirmation of the hypotheses that G6PD deficiency, α^+ thalassemia, and hemoglobin C protect against malaria mortality; the application of novel haplotype-based techniques demonstrating that malaria-protective genes have been subject to recent positive selection; the first genetic linkage maps of resistance to malaria in experimental murine models; and a growing number of reported associations with resistance and susceptibility to human malaria, particularly in genes involved in immunity, inflammation, and cell adhesion. The challenge for the next decade is to build the global epidemiological infrastructure required for statistically robust genomewide association analysis, as a way of discovering novel mechanisms of protective immunity that can be used in the development of an effective malaria vaccine.

Introduction

One of the most important causes of child mortality worldwide is the malaria parasite *Plasmodium falciparum,* which annually kills >1 million children in Africa alone. This death toll is only one aspect of the global burden of malaria. *P. falciparum* is estimated to cause about half a billion episodes of disease each year (Snow et al. 2005), and there are hundreds of millions of cases due to other parasite species—*Plasmodium vivax, Plasmodium malariae,* and *Plasmodium ovale.* In regions of high malaria transmission, every member of the community might be chronically infected (Trape et al. 1994), and, in one Gambian village, it was found that about one-quarter of all children with severe complications of the disease were admitted to the hospital during the first 10 years of life (Snow et al. 1997).

When malaria's effect on child mortality is considered—and it was probably even greater before antimalarial drugs and other control measures were introduced—it is not surprising that malaria is the strongest known selective pressure in the recent history of the human genome. Malaria is the evolutionary driving force behind sickle-cell disease, thalassemia, glucose-6-phosphatase deficiency, and other erythrocyte defects that together comprise the most common Mendelian diseases of humankind. HbS, the allele that gives rise to sickle hemoglobin, is regarded as the classic paradigm of balanced polymorphisms in human populations (Feng et al. 2004; Hedrick 2004). It is a variant of the *HBB* gene (which encodes β-globin) that has arisen independently in different locations and is maintained at ~10% frequency in many malaria-endemic regions (Flint et al. 1998). HbS homozygotes suffer sickle-cell disease, but heterozygotes have a 10-fold reduced risk of severe malaria (Hill et al. 1991; Ackerman et al. 2005).

What is remarkable is the range of erythrocyte variants, apart from HbS, that have resulted from evolutionary selection by malaria. They include other variants of the *HBB* gene—namely, HbC and HbE (Agarwal et al. 2000; Modiano et al. 2001*b*; Chotivanich et al. 2002; Ohashi et al. 2004); regulatory defects of *HBA* and *HBB,* which cause α and β thalassemia (Flint et al. 1986; Williams et al. 1996; Allen et al. 1997), variation in the structural protein SLC4A1, which causes ovalocytosis (Foo et al. 1992; Genton et al. 1995; Allen et al. 1999); variation in the chemokine receptor *FY,* which causes the Duffy-negative blood group (Miller et al. 1976; Chitnis and Miller 1994; Tournamille et al. 1995; Hamblin and Di Rienzo 2000); and polymorphisms of the red-cell enzyme gene *G6PD,* which causes glucose-6-phosphate dehydrogenase deficiency (Bienzle et al. 1972; Ganczakowski et al. 1995; Ruwende and Hill 1998; Tishkoff et al. 2001; Sabeti et al. 2002*b*).

This is probably only the tip of the iceberg. Surpris-

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ingly little is currently known about the effects of malaria on the evolution of the human immune system, possibly because the phenotypic consequences are more subtle than those of the classic erythrocyte variants; for example, alteration of a splenic dendritic cell receptor is not as easy to visualize as a sickling red cell. However, the last few years have seen a rapid growth in the number of reported genetic associations with susceptibility and resistance to malaria, many of which involve immune system and inflammatory genes.

The purpose of this review is to provide an overview of what is currently known about genetic resistance to malaria and to highlight directions that are likely to see major advances in the next few years.

Evolutionary Selection by Malaria

Evolutionary selection by malaria (reviewed by Flint et al. [1998] and Tishkoff and Williams [2002]) is remarkable in two respects. First, the selective pressure is very strong; this is evident from the fact that the HbS allele has risen to high frequencies in malaria-exposed populations despite the fatal consequences for homozygotes. Second, different populations have developed independent evolutionary responses to malaria, and this is seen at both the global and the local levels. The most striking example is the *HBB* gene, in which three different coding SNPs confer protection against malaria: *Glu6Val* (HbS), *Glu6Lys* (HbC), and *Glu26Lys* (HbE). The HbS allele is common in Africa but rare in Southeast Asia, whereas the opposite is true for the HbE allele. However, a more complex picture emerges at the local level, exemplified by the Dogon people of Mali, who have a much lower frequency of the HbS allele than do most other West African groups and instead have a high frequency of the HbC allele (Agarwal et al. 2000). A further level of complexity is that, within Africa, the HbS allele is found in four distinct haplotypes (Chebloune et al. 1988; Nagel and Ranney 1990; Lapoumeroulie et al. 1992), a finding that has been generally interpreted to imply that the same mutation has arisen independently in four different Africa populations, although it has been pointed out that there may be other explanations (Flint et al. 1998). The different geographic distributions of α thalassemia, G6PD deficiency, ovalocytosis, and the Duffy-negative blood group are further examples of the general principle that different populations have evolved different genetic variants to protect against malaria.

The fact that different malaria-resistance alleles have arisen in different places suggests that a great deal of evolutionary selection by malaria has happened relatively recently in human history and certainly since humans started to migrate out of Africa. This is supported by analyses of recent positive selection in the human genome. Haplotype analysis and statistical modeling of an African malaria-resistance allele at the *G6PD* locus suggests an origin within the last 10,000 years or so (Tishkoff et al. 2001), whereas analysis of the Southeast Asian HbE allele suggests that it originated within the last 5,000 years (Ohashi et al. 2004). Studies of *G6PD* and *CD40L* malaria-resistance alleles in West Africa that made use of the long-range haplotype test are also consistent with recent positive selection (Sabeti et al. 2002*b*).

Another line of evidence comes from population-genetics analysis of malaria parasites. Malaria parasites existed long before humans—there are different *Plasmodium* species that infect birds, lizards, rodents, and other primates—but what geneticists would like to know is the timepoint at which the unusually virulent species *P. falciparum* began to expand in human populations. This has been addressed by a variety of approaches, with conflicting results (reviewed by Conway [2003] and Hartl [2004]), but the most persuasive evidence comes from a detailed analysis of 100 mtDNA sequences sampled from around the world (Joy et al. 2003). This suggests that some forms of *P. falciparum* may have existed 100,000 years ago, but that the African malaria parasite population suddenly increased ~10,000 years ago and subsequently spread to other regions. This observation, together with analysis of the speciation of human malaria vectors by polytene chromosome analysis (Coluzzi 1999; Coluzzi et al. 2002), is consistent with the hypothesis that the emergence of *P. falciparum* as a major human pathogen coincides with the beginnings of agriculture, when human populations started to form resident communities that allowed the establishment of a substantial reservoir of infection.

These findings are not just of historical interest, they may also be of practical value in the search for novel malaria-resistance loci. One of the major challenges confronting genomewide association analysis is determining how to select the most-efficient set of markers to analyze. In the case of malaria, we are particularly interested in alleles that show evidence of recent positive selection in regions where malaria is endemic. *P. falciparum* seems to have emerged as a powerful selective force subsequent to the divergence of African, Asian, and European populations, so an obvious starting point for genomewide analysis of malaria-resistance loci is to screen alleles that show large differences in frequency between major population groups.

Complex Genetic Basis of Resistance to Malaria

The genetic basis of resistance to malaria is complex at several levels. It is likely that many different genes are involved and that they interact with environmental variables and with parasite genetic factors. Here, we consider some further complexities of studying genetic resistance to malaria—namely, the range of phenotypes

involved, the practical difficulties of studying families, and the remarkable geographic and ethnic heterogeneity of malaria-resistance factors.

Phenotypes

Susceptibility to and resistance to malaria can be measured in several ways. Usually, they are studied in regions with high levels of malaria transmission. When everyone is repeatedly bitten by infected mosquitoes, many children and adults are likely to have parasitemia (parasites in the blood), children may have two or three episodes of malaria fever each year, and a small minority (e.g., 1%) of malaria-fever episodes lead to severe malaria (i.e., death or life-threatening complications due to malaria). Different genetic factors may determine the risk of an exposed person for developing parasitemia, the risk of a parasitemic person for becoming ill with malaria fever, and the risk of a person with malaria fever for developing severe malaria. Parasitemia and fever can be regarded as quantitative phenotypes that are ascertained by repeated measurements within the community, whereas severe malaria is a qualitative phenotype that is typically ascertained in hospital-based studies. In principle, studies of severe malaria would be expected to detect genetic factors at each stage in the causal chain of disease progression.

Severe malaria is the phenotype that matters most to vaccine developers and to those interested in evolutionary selection. Severe malaria is composed of a number of subphenotypes that may occur alone or in combination. In African children, these are cerebral malaria, severe malarial anemia, and respiratory distress (Marsh et al. 1995). When we recruit 100 children with severe malaria who are in the hospital to a study, we are effectively identifying those, of perhaps 10,000 children who live in the hospital's catchment area, with the lowest levels of protection, and this tends to enrich the sample for strong genetic effects. For example, HbS heterozygotes have an ~2-fold reduction in the risk of malaria-fever episodes but a 10-fold reduction in the risk of severe malaria (Hill et al. 1991).

Family Studies

It is difficult to get an accurate measure of the familial component of resistance to severe malaria. Cases of severe malaria are relatively easy to identify in hospitals, but the transient nature of the illness makes it difficult to ascertain the affected status of relatives, particularly in communities that lack detailed medical records and in which there are other infectious causes of child mortality that can be confused with malaria. By careful questioning of families of affected individuals, a recent study in Mali estimated a sibling risk (λ_s) of 2.5 for cerebral malaria and 4.9 for severe malarial anemia (Ranque et al. 2005). This, of course, does not distinguish genetic effects from those of shared environment.

To do an accurate family study of malaria, it is necessary to perform detailed longitudinal analysis, which, in practice, means studying a relatively small number of individuals, such as a set of twins or a village of a few hundred people. Such studies lack power to investigate severe malaria, which is a relatively rare event, but they can evaluate quantitative traits such as the level of parasitemia or frequency of malaria-fever episodes. The fact that the level of parasitemia and the frequency of malaria fever both decline markedly with age must be factored into the analysis. A longitudinal study of Gambian twins showed that susceptibility to malaria-fever episodes is determined partly by genetic factors (Jepson et al. 1995), with linkage to the major histocompatibility complex (MHC) region on chromosome 6 (Jepson et al. 1997). A series of longitudinal family studies of parasitemia have been performed in Cameroon and Burkina Faso. An initial segregation analysis suggested the involvement of a major gene that controls blood infection levels (Abel et al. 1992), and this hypothesis seemed to be supported by a somewhat bimodal distribution of parasite density levels among pregnant women, who are particularly susceptible to malaria (Cot et al. 1993). However, subsequent studies indicate that complex genetic factors are involved (Garcia et al. 1998*a*; Rihet et al. 1998*a*) and that there is linkage to the MHC and the 5q31-33 region (Garcia et al. 1998*b*; Rihet et al. 1998*b*; Flori et al. 2003). A longitudinal family analysis in Sri Lanka concluded that there were consistent individual differences in susceptibility to clinical malaria episodes, of which about one-half appeared to have a genetic basis (Mackinnon et al. 2000).

It is also possible to estimate the genetic component of individual variation in immunological responses. A twin study in Liberia found evidence of heritability in antimalarial antibody responses that did not appear to be determined by HLA class II genes (Sjoberg et al. 1992). Familial segregation analysis of immunological responses to malaria antigens in Papua New Guinea has suggested that Mendelian effects might govern specific antigen responses, but the overall picture is complex (Stirnadel et al. 1999*a*, 2000*a*, 2000*b*).

Ethnic Differences

One of the most striking examples of differential disease susceptibility among human populations is the complete resistance of most of the population of sub-Saharan Africa to *P. vivax* infection, whereas all other human populations are vulnerable to this species of malaria parasite. This resistance is due to a SNP in the *FY* gene that results in the Duffy blood group–negative phenotype (Miller et al. 1976; Tournamille et al. 1995); the

precise protective mechanism is discussed below (see the "Malaria and the Red Cell" section). Thus, whereas *P. vivax* infection is common in Asia and South America and used to be widely distributed throughout Europe, it is extremely rare in most of sub-Saharan Africa, although all the right environmental conditions exist for it to be transmitted there.

Striking differences in resistance to malaria have also been observed among ethnic groups who live in the same area. The Tharu people, who inhabit the malarious Terai region of Nepal, have a much lower prevalence of malaria than do other ethnic groups in the same region (Terrenato et al. 1988), and this may possibly be explained by the extremely high frequency of α thalassemia in the Tharu population (Modiano et al. 1991).

The Fulani people are traditionally nomadic pastoral people who are found across West Africa, often settled in close proximity to other ethnic groups. Studies in Burkina Faso (Modiano et al. 1996) and, more recently, in Mali (Dolo et al. 2005) have documented a significantly lower prevalence of malaria parasitemia and fewer clinical attacks of malaria among the Fulani than among other ethnic groups who live in neighboring villages. The Fulani have a distinctive culture, but detailed epidemiological investigations indicate that their resistance to malaria arises primarily from genetic factors. Importantly, it has also been observed that the Fulani have high levels of antimalarial antibodies (Modiano et al. 1998, 1999) and a low frequency of protective globin variants and other classic malaria-resistance factors (Modiano et al. 2001*a*). There is therefore much interest in discovering the genetic factors that determine the high antibody responses seen in the Fulani; the possible role of the *IL4* gene is discussed below (see the "Antibody Response" section) (Luoni et al. 2001; Farouk et al. 2005).

Malaria and the Red Cell

Erythrocyte Surface

Many important things happen at the erythrocyte surface in malaria (table 1). The parasite binds to erythrocyte surface molecules as the first stage in a complex and marvelous series of events—still poorly understood—that gets the parasite into the erythrocyte without destroying it (Sibley 2004). Once inside the erythrocyte, the parasite manufactures a set of proteins that it sends to the cell surface (Kyes et al. 2001). Some of these parasite-derived erythrocyte-membrane proteins bind to endothelial adhesion molecules and thereby cause parasitized erythrocytes to sequester in small blood vessels; this is thought to be a strategy for immune evasion, since it prevents the parasites from having to circulate through the spleen. But these parasite-derived molecules on the erythrocyte surface are themselves targets for immunological attack, which they counter with an extraordinary capacity for antigenic variation. We discuss in the "Cytoadherence, a Major Factor in Malaria Pathogenesis" section how human genetic variation influences endothelial cytoadherence; here, we focus on how it affects erythrocyte invasion.

The study of human genetics uncovered a key step in the molecular process of erythrocyte invasion by *P. vivax*. The Duffy antigen, encoded by the *FY* gene, is a chemokine receptor that is expressed in various cell types. The Duffy antigen is expressed in erythrocytes in most populations (but not in sub-Saharan Africa) that have a promoter SNP that disrupts a binding site for the erythroid transcription factor GATA-1 (Tournamille et al. 1995). This completely prevents *P. vivax* from invading erythrocytes, and it accounts for the remarkable absence of *P. vivax* in parts of Africa in which other species of malaria parasite are extremely common (Miller et al. 1976). It was this genetic discovery that led to the discovery of the *P. vivax* Duffy-binding protein, a parasite molecule that is critical for erythrocyte invasion by *P. vivax* (Chitnis and Miller 1994) and is now undergoing clinical trials as a candidate agent for a vaccine against this species of parasite (Yazdani et al. 2004). The African Duffy-negative allele, denoted "FY*O," has the highest F_{ST} value observed in humans and has other features that are strongly suggestive of recent positive selection (Hamblin and Di Rienzo 2000; Hamblin et al. 2002). There is further support for selective pressure at this locus, from the observation of an entirely independent *FY* polymorphism, which has emerged in Papua New Guinea, that decreases Duffy-antigen expression and acts to reduce *P. vivax* invasion efficiency (Zimmerman et al. 1999; Michon et al. 2001). The apparent strength of selection at the *FY* locus is somewhat puzzling for malariologists, since *P. vivax* infection is not generally lethal, and it has even been proposed that *P. vivax* infection may protect against the much more lethal parasite *P. falciparum* (Williams et al. 1996).

The study of human genetic polymorphisms has also been informative about how *P. falciparum* invades erythrocytes, but, in contrast to *P. vivax*, the available data suggest multiple invasion pathways with considerable redundancy (Hadley et al. 1987). A lot of attention has focused on *GYPA, GYPB,* and *GYPC,* the genes encoding glycophorins A, B, and C, respectively.

Various blood groups are determined by the erythrocyte-membrane sialoglycoproteins glycophorin A and B, and genetic deficiency of glycophorin A or B expression makes erythrocytes relatively resistant to invasion by *P. falciparum* (Facer 1983). Specific sialic-acid residues on the glycophorin A molecule are recognized by a Duffy-binding–like domain of *P. falciparum* erythrocyte-binding antigen 175 (Orlandi et al. 1992; Mayor et al. 2005). Sequence analysis shows evidence of strong evolutionary

Table 1

Common Erythrocyte Variants That Affect Resistance to Malaria

Gene	Protein	Function	Reported Genetic Associations with Malaria
FY	Duffy antigen	Chemokine receptor	FY*O allele completely protects against *P. vivax* infection.
G6PD	Glucose-6-phosphatase dehydogenase	Enzyme that protects against oxidative stress	G6PD deficiency protects against severe malaria.
GYPA	Glycophorin A	Sialoglycoprotein	GYPA-deficient erythrocytes are resistant to invasion by *P. falciparum*.
GYPB	Glycophorin B	Sialoglycoprotein	GYPB-deficient erythrocytes are resistant to invasion by *P. falciparum*.
GYPC	Glycophorin C	Sialoglycoprotein	GYPC-deficient erythrocytes are resistant to invasion by *P. falciparum*.
HBA	α-Globin	Component of hemoglobin	α^{+} Thalassemia protects against severe malaria but appears to enhance mild malaria episodes in some environments.
HBB	β-Globin	Component of hemoglobin	HbS and HbC alleles protect against severe malaria. HbE allele reduces parasite invasion.
HP	Haptoglobin	Hemoglobin-binding protein present in plasma (not erythrocyte)	Haptoglobin 1-1 genotype is associated with susceptibility to severe malaria in Sudan and Ghana.
SCL4A1	CD233, erythrocyte band 3 protein	Chloride/bicarbonate exchanger	Deletion causes ovalocytosis but protects against cerebral malaria.

selection, not only for *GYPA* and *GYPB* in the human host (Baum et al. 2002; Wang et al. 2003), but also for EBA-175 in the *P. falciparum* parasite, which implies an ongoing evolutionary struggle between the parasite ligand and the host receptor (Wang et al. 2003).

Glycophorin C is a minor component of the erythrocyte membrane that serves as a receptor for the *P. falciparum* erythrocyte-binding antigen 140 (EBA140). The Gerbich-negative blood group, caused by a deletion of *GYPC* exon 3, is common in coastal areas of Papua New Guinea and results in reduced invasion by *P. falciparum* (Maier et al. 2003). Epidemiological studies indicate that this *GYPC* deletion does not alter the prevalence or density of asymptomatic malaria infection, but so far there has been no study of how it affects the clinical severity of infection (Patel et al. 2001, 2004).

Another erythrocyte-membrane protein that has been implicated in malaria resistance is an anion exchanger known as "band 3 protein," encoded by *SLC4A1*. A 27-bp deletion in this gene results in a form of ovalocytosis that is common in parts of Southeast Asia. It appears to be protective both against malaria infection (Cattani et al. 1987; Foo et al. 1992) and against cerebral malaria (Genton et al. 1995; Allen et al. 1999). The mechanism of protection is not yet known. It may relate to the involvement of band 3 protein in endothelial cytoadherence or to some inhibitory effect on parasite invasion or growth.

Structural Variation of Globin Genes

Erythrocytes are essentially bags filled with hemoglobin, and the malaria parasite has developed a lifestyle that is hugely dependent on its hemoglobin environment. Alterations in hemoglobin may affect the biochemical and cellular machinery of parasite development, and they may affect the ability of the spleen and other immune mechanisms to recognize parasites, by affecting the morphology, mechanical properties, or surface structure of the parasitized erythrocyte. The biological importance of these dependencies is highlighted by the huge selective pressure that malaria has exerted on the structure and regulation of α globin (encoded by the identical *HBA1* and *HBA2* genes) and β globin (encoded by *HBB*) that together comprise the tetrameric protein backbone of adult hemoglobin.

HBB has three different coding SNPs that each confer resistance against malaria and that have risen to high frequency in different populations. The HbS allele is a glutamic acid→valine substitution at codon 6 of the β globin chain, HbC is a glutamine→lysine substitution at codon 6, and HbE allele is a glutamic acid→lysine substitution at codon 26. The corresponding proteins are known as "hemoglobin S" (or "sickle hemoglobin"), "hemoglobin C," and "hemoglobin E."

The HbS allele is found across a large part of sub-Saharan African as well as parts of the Middle East. It has the distinction of being one of the first human genetic variants to be associated with a specific molecular defect (Pauling et al. 1949). Hemoglobin S tends to polymerize at low oxygen concentrations, which causes the erythrocyte to deform into a sickle-like shape (Brittenham et al. 1985). HbS homozygotes have sickle-cell disease, a debilitating and often fatal disorder caused by these red-cell deformities. The heterozygous state (denoted "HbAS") is not generally associated with any clinical abnormality and confers ~10-fold increase in protection from life-threatening forms of malaria, with a lesser degree of protection against milder forms of the disease (Allison 1954; Gilles et al. 1967; Hill et al. 1991; Allen et al. 1992; Stirnadel et al. 1999*b*; Sokhna et al. 2000; Ackerman et al. 2005). It is still not known precisely how HbAS protects against malaria. Two plausible mechanisms, which are not mutually exclusive, are suppression of parasite growth in red cells (Pasvol et al. 1978) and enhanced splenic clearance of parasitized erythrocytes (Shear et al. 1993). A study of Kenyan children found that the protective effect of HbAS against malaria increased from 20% to 56% between the ages of 2 and 10 years, which implies that it enhances or acts in synergy with the acquired immune response (Williams et al. 2005*a*). The trade-off between risks and benefits acts to maintain the HbS polymorphism at allele frequencies of ~10% in many parts of Africa, despite the lethal consequences for homozygotes, which provides the most striking known example of heterozygote advantage in human genetics.

Hemoglobin C is found in several parts of West Africa, although less commonly than is HbS. It results in a much-less-damaging clinical phenotype than sickle-cell disease: homozygotes have a relatively mild hemolytic anemia, and heterozygotes do not experience a significant reduction in hemoglobin levels (Diallo et al. 2004). Both heterozygotes and homozygotes of HbC are protected against severe malaria (Agarwal et al. 2000; Mockenhaupt et al. 2004*a*; Rihet et al. 2004), but the protective effect appears to be substantially greater in homozygotes (Modiano et al. 2001*b*). It has been proposed that the protective effect of HbC may operate by increasing immune clearance of infected erythrocytes. This is based on observations of reduced parasite cytoadherence, abnormal PfEMP1 expression, clustering of erythrocyte band 3 protein, and altered surface topography of the erythrocyte membrane in the presence of hemoglobin C (Arie et al. 2005; Fairhurst et al. 2005; Tokumasu et al. 2005).

Hemoglobin E is common in Southeast Asia, with carrier rates of 50% in some places, and analysis of haplotype structure suggests that the mutation is relatively recent and has risen rapidly in allele frequency (Ohashi et al. 2004). Homozygotes generally have symptom-

less anemia. Although it has not been epidemiologically proven that HbE protects against severe malaria, this is assumed to be the case, and it has been observed that erythrocytes from HbE-heterozygous individuals are relatively resistant to invasion by *P. falciparum* (Chotivanich et al. 2002).

Regulatory Variation of Globin Genes

The thalassemias are the most common Mendelian diseases of humans and constitute a major global health problem (Weatherall and Clegg 2001). They comprise a group of clinical disorders that result from defective production of α- or β-globin chains, which arise from deletions or other disruptions of the globin gene clusters on chromosomes 11 and 16. There is a broad spectrum of clinical phenotypes, reflecting the range of different genetic variants that exist and given greater complexity by the fact that α-globin is produced by two identical genes, *HBA1* and *HBA2*. Broadly speaking, homozygous thalassemia results in severe disease or is fatal, whereas heterozygotes are healthy apart from mild anemia. An exception to this general rule arises when either the *HBA1* or the *HBA2* gene, but not both, is disrupted, so that some α-globin production is possible. This condition is known as "α^+ thalassemia," and α^+ thalassemia homozygotes are only mildly anemic.

Over half a century ago, J. B. S. Haldane proposed balanced polymorphism as the explanation for why thalassemia had risen to high frequencies—approaching fixation—in certain populations (Haldane 1949). He argued that heterozygotes might be protected against some important disease, and malaria was the obvious candidate, since the global distribution of thalassemia encompasses the major malarious regions of Africa and Asia and Mediterranean regions where malaria was once common. There are many other circumstantial lines of evidence to support this concept; for example, the Tharu people have both a much higher allele frequency of α thalassemia, of ~0.8, and a much lower incidence of malarial illness than do other ethnic groups that inhabit the same region of Nepal (Modiano et al. 1991). Arguably the strongest population-genetic evidence comes from a detailed survey in Melanesia that showed that the frequency of α^+ thalassemia varied according to both altitude and latitude in a manner that was highly correlated with malaria endemicity, whereas haplotypic analysis seemed to rule out the possibility that this could have arisen because of founder effects (Flint et al. 1986).

Although the population-genetic evidence seems overwhelming, it is only relatively recently that direct evidence that thalassemia protects against malaria has emerged, and the case is still not absolutely clear-cut. A study of Kenyan children found that both heterozygous and homozygous α^+ thalassemia was protective against severe malaria (Williams et al. 2005*b*), whereas a study of Ghanaian children found that heterozygotes were protected (Mockenhaupt et al. 2004*b*). In Papua New Guinea, the risk of severe malaria was found to be reduced by 60% in children who were homozygous for α^+ thalassemia and to a lesser degree in heterozygotes, but the result did not seem to be malaria-specific, since a protective effect was also observed for other childhood infections (Allen et al. 1997).

The protective mechanism of thalassemia is unknown. Flow-cytometry studies in vitro have shown that erythrocytes with the α thalassemia phenotype show reduced parasite growth (Pattanapanyasat et al. 1999) and increased binding of antibodies from malaria-immune sera (Williams et al. 2002). Enhanced splenic clearance of malaria-infected cells is a further possibility but is difficult to test in vivo. However, much more complex explanations are also possible, as illustrated by findings of data from Vanuatu which are unusual in two respects: first, both *P. falciparum* and *P. vivax* infection are common, but severe malaria is remarkably uncommon in this population; and, second, young children with α^+ thalassemia have a significantly higher incidence of malaria than do nonthalassemic children (Williams et al. 1996). This latter result is exactly the opposite of what Williams et al. (1996) set out to prove, but they came up with a possible explanation that was based on the further observation that *P. vivax* infection, which does not cause severe disease, is acquired at an earlier age in this population than is *P. falciparum* infection. The proposal is that, in this particular epidemiological scenario, α^+ thalassemia may enhance early exposure to *P. vivax* infection, thereby in some way protecting against severe disease from later exposure to *P. falciparum*.

Oxidative Stress

Malaria parasites need to break down hemoglobin to make room to grow, quite apart from any nutritional benefit they may derive from this. This process releases by-products that are potentially toxic—particularly iron, which is a source of oxidative stress.

An important form of defense against oxidative stress within the erythrocyte is production of the electron donor nicotinamide adenine dinucleotide phosphate by the enzyme glucose-6-phosphate dehydrogenase (G6PD), encoded by *G6PD* on chromosome X. There are many different variants of *G6PD,* and those that markedly compromise enzyme activity result in hemolytic anemia. The geographical distribution of G6PD deficiency is consistent with evolutionary selection by malaria (Ganczakowski et al. 1995), and analysis of haplotypic structure at the *G6PD* locus supports the hypothesis of recent positive selection (Tishkoff et al. 2001; Sabeti et al. 2002*b*). Deficient G6PD enzyme activity has been shown

to correlate with protection against severe malaria in Nigerian children (Gilles et al. 1967). A study of >2,000 Gambian and Kenyan children found that the common African form of G6PD deficiency (G6PD A^-) is associated with ~50% reduced risk of severe malaria in female heterozygotes and in male hemizygotes (Ruwende et al. 1995). Reduced parasite replication in G6PD-deficient erythrocytes is thought to be the mechanism of protection (Luzzatto et al. 1969), but the parasite appears to counter this by manufacturing G6PD itself (Usanga and Luzzatto 1985).

Haptoglobin, encoded by *HP,* is not an erythrocyte protein but is mentioned here because it is a hemoglobin-binding protein that is present in plasma. It could act to defend the malaria-infected individual in several ways: by trapping free hemoglobin, it helps to prevent hemoglobin-induced oxidative tissue damage; it has been shown to inhibit the development of malaria parasites in vitro (Imrie et al. 2004); and it appears to reduce parasite load, as determined by murine gene knockout studies (Hunt et al. 2001). The haptoglobin 1-1 genotype, characterized by protein electrophoresis, has been associated with susceptibility to severe *P. falciparum* malaria (Elagib et al. 1998; Quaye et al. 2000), although a DNA-based study of haptoglobin polymorphisms in the Gambia failed to detect such an association (Aucan et al. 2002)

Cytoadherence, a Major Factor in Malaria Pathogenesis

A critical event in the pathogenesis of severe malaria is the sequestration of *P. falciparum*–infected erythrocytes in small blood vessels (Taylor et al. 2004). A range of receptor-ligand interactions causes parasitized erythrocytes to stick to endothelium, platelets, and other erythrocytes; this is thought to be an immune-evasion strategy that allows the parasite to stay within the vascular compartment but to avoid circulating through the spleen. On the parasite side, the major ligand is *P. falciparum* erythrocyte-membrane protein-1 (PfEMP-1)—encoded by a gene family called "*var*" because each parasite contains many different copies of the gene—and, by switching expression between the copies, it is able to cause a remarkable degree of antigenic variation (Kyes et al. 2001). On the host side, a range of different adhesion molecules expressed on endothelium, platelets, macrophages, and other erythrocytes serves as binding receptors for different forms of PfEMP-1.

A number of associations have been reported between severe malaria and polymorphisms of host receptors for cytoadherence by *P. falciparum*–infected erythrocytes (table 2). When the same polymorphism has been tested in different geographical locations, the results have been variable—not simply failure to replicate but, in some cases, the association of the same polymorphism with susceptibility to severe malaria in one study and with resistance in another. As with all genetic association studies, it is possible that these results are statistical artifacts that will ultimately be resolved by larger sample sizes and by finer-scale genetic mapping of these loci. However, in this particular area of genetic analysis, it is not out of the question that the functional consequences of a single polymorphism could vary between locations and might even vary over time at a single location. As outlined above, the biological phenomenon of parasite cytoadherence to endothelium and to other cells is driven by the parasite, not by the host. The parasite varies its pattern of sequestration in different organs by constantly switching between different forms of PfEMP-1 that bind to different host receptors (and different parts of the same receptor) in a promiscuous and opportunistic manner (Roberts et al. 1992).

Many isolates of *P. falciparum* bind to endothelium via the CD36 antigen (Barnwell et al. 1989). Encoded by *CD36,* this is a receptor for a range of different molecules, including thrombospondin and long-chain fatty acids, and is expressed by platelets and dendritic cells as well as endothelium. It is a molecule of considerable interest to malaria immunologists because, as well as being a mediator of parasite sequestration, CD36 acts to bind parasitized erythrocytes to dendritic cells, an event that seems to incapacitate the dendritic cell when it comes to presenting parasite antigens (Urban et al. 1999, 2001). Several *CD36* polymorphisms have been described in malarious regions (Aitman et al. 2000; Omi et al. 2003), but the results of disease-association studies are confusing. A study of both Gambian and Kenyan case-control samples found that homozygotes for a nonsense polymorphism, the $CD36^+$1264G allele, were susceptible to cerebral malaria (Aitman et al. 2000), but a study of the same allele in Kenya alone found that heterozygosity was associated with protection against severe malaria (Pain et al. 2001). In Thailand, a dinucleotide repeat sequence in intron 3, implicated in alternative splicing, has been associated with protection against cerebral malaria (Omi et al. 2003).

Some isolates of *P. falciparum* bind strongly to endothelium via intercellular adhesion molecule-1 (Berendt et al. 1989). This is encoded by *ICAM1,* and its normal function is to serve as an endothelial- and immune-cell adhesion receptor for integrin-expressing leukocytes. A polymorphism in the N-terminal domain, present at high frequencies in African populations, acts to reduce binding (Fernandez-Reyes et al. 1997). A study in Gabon found that the low-binding allele was associated with reduced susceptibility to severe malaria (Kun et al. 1999), but a study in Kenya found increased susceptibility (Fernandez-Reyes et al. 1997), and another in the Gambia found no significant effect (Bellamy et al. 1998*a*).

Table 2

Host Molecules That Mediate Cytoadherence by *P. falciparum*–Infected Erythrocytes and That Have Been Reported to Show Association with Resistance or Susceptibility to Malaria

Gene	Protein	Interaction with Parasitized Erythrocyte[a]	Reported Genetic Associations with Malaria
CD36	CD36 antigen, thrombospondin receptor	PE-binding receptor on endothelium and dendritic cells	*CD36* polymorphisms show variable associations with severe malaria in the Gambia, Kenya, and Thailand.
CR1	CR1, complement receptor 1	PE-binding receptor on erythrocytes	*CR1* polymorphisms show variable associations with severe malaria in the Gambia, Thailand, and Papua New Guinea.
ICAM1	CD54, intercellular adhesion molecule-1	PE-binding receptor on endothelium	*ICAM1* polymorphisms show variable associations with severe malaria in Kenya, Gabon, and the Gambia.
PECAM1	CD31, platelet-endothelial cell–adhesion molecule	PE-binding receptor on endothelium	*PECAM1* polymorphisms show variable associations with severe malaria in Thailand, Kenya, and Papua New Guinea.

[a] PE = parasitized erythrocyte.

A survey of different ethnic groups in India found a number of novel *ICAM1* variants that merit analysis in disease-association studies (Sengupta et al. 2004).

Another endothelial-binding receptor for *P. falciparum* is the platelet–endothelial cell adhesion molecule, encoded by *PECAM1* (Treutiger et al. 1997). A common coding variant (Leu→Val at codon 125) was analyzed in case-control studies of severe malaria in Papua New Guinea and Kenya, but no significant association was identified (Casals-Pascual et al. 2001). A study from Thailand has reported a *PECAM1* haplotype that is more common in cerebral malaria than in other forms of severe malaria (Kikuchi et al. 2001).

Parasite sequestration is not due only to endothelial binding. Some *P. falciparum* isolates show a phenomenon known as rosetting, where a parasitized erythrocyte binds to other erythrocytes. One mechanism for rosetting is through PfEMP-1 binding to erythrocyte–complement receptor 1, encoded by *CR1* (Rowe et al. 1997). Up to 80% of the population of a malarious region of Papua New Guinea have erythrocyte CR1 deficiency, which has been associated both with polymorphisms in the *CR1* gene and with α^+ thalassemia, which is also common in this population (Cockburn et al. 2004). In the same study, it was found that both *CR1* polymorphisms and α thalassemia were independently associated with resistance to severe malaria. However, studies of *CR1* polymorphisms in the Gambia found no evidence of association with disease severity (Bellamy et al. 1998*a*; Zimmerman et al. 2003). In Thailand, an RFLP that is associated with reduced expression of CR1 on erythrocytes has been associated (in homozygotes) with susceptibility to severe malaria (Nagayasu et al. 2001).

Malaria and the Immune System

The genetic interaction between malaria and the immune system is potentially of huge practical interest, for two distinct reasons. First, although there is a vast literature on immunological responses to malaria in humans and in experimental model systems, there is still surprisingly little concrete evidence about precisely which immunological responses are causal mechanisms of protective immunity in naturally exposed populations, and this is a fundamental roadblock in the development of an effective malaria vaccine. One way to establish causality is to obtain clear-cut evidence that functional variation in a specific immune gene affects the clinical outcome of infection. Second, just as the selective pressure of malaria on the erythrocyte has led to common hematological disorders, such as sickle-cell disease and thalassemia, it is possible that we might learn a great deal about mechanisms of chronic immunological and inflammatory disorders if we had a better understanding of the selective pressure that malaria has exerted on the immune system.

Here, we consider a number of immune gene associations with malaria resistance and susceptibility that have emerged over the past 15 years (table 3). The same caveats, discussed above for adhesion molecules, apply here. Few of these associations have been tested in several different studies and, when this has been done, the results have been variable. Thus, much of this section should be regarded as indicative of ongoing research activity rather than definitive results. However, it is important to bear in mind that there potentially are biological reasons why the same immune gene polymorphism might have different consequences in different malarious regions. For example, HLA associations might vary according to the local prevalence of critical parasite-antigen polymorphisms. And associations with inflammatory cytokines and other immune genes may be affected by regional differences in the intensity of malaria transmission, which have complex consequences for the development of acquired immunity and the pattern of severe disease (Snow et al. 1997). These are issues that may have far-reaching implications for vaccine efficacy, and, to tackle them robustly, we need much larger sample sizes and more fine-grained genetic association maps than we have at present.

Antigen Recognition

In malaria, the opportunities for antigen presentation are limited by the fact that erythrocytes do not express MHC molecules. Liver cells, however, express MHC class I molecules and therefore provide a potential target for cytotoxic T cell (CTL) responses during the first phase of malaria infection, when parasites replicate in the liver prior to invading erythrocytes. *HLA-B* is an exceptionally polymorphic gene that encodes an MHC class I heavy chain that, together with β2 microglobulin, makes up the *HLA-B* antigen–presentation complex. The *HLA-B53* allele is extremely common in West Africa, compared with other parts of the world, and is associated with a significantly reduced risk of severe malaria in Gambian children (Hill et al. 1991). In view of the fact that *HLA-B* is expressed by liver cells but not by erythrocytes, this genetic association implies that liver-stage parasites provide a significant target for naturally acquired protective immunity. This has boosted efforts to develop a liver-stage malaria vaccine, and it has been proposed that T cell epitope targets for malaria-vaccine development may be obtained by analyzing the peptides that bind to HLA-B53 (Hill et al. 1992).

But when a polymorphic parasite antigen interacts with a polymorphic host antigen–presenting system, there are many opportunities for complexity. For example, within a single fragment of the *P. falciparum* circumsporozoite protein (CSP), it has been observed that the Gambian parasite population has two different variants (cp26 and

Table 3

Immune Genes Reported to Be Associated with Different Malaria Phenotypes

Gene	Protein	Function	Reported Genetic Associations with Malaria
FCGR2A	CD32, low affinity receptor for Fc fragment of IgG	Clearance of antigen-antibody complexes	Association with severe malaria in the Gambia
HLA-B	HLA-B, a component of MHC class I	Antigen presentation that leads to cytotoxic T cells	HLA-B53 association with severe malaria in the Gambia
HLA-DR	HLA-DR, a component of MHC class II	Antigen presentation that leads to antibody production	HLA-DRB1 association with severe malaria in the Gambia
IFNAR1	Interferon α receptor component	Cytokine receptor	Association with severe malaria in the Gambia
IFNG	Interferon γ	Cytokine with antiparasitic and proinflammatory properties	Weak associations with severe malaria in the Gambia
IFNGR1	Interferon γ receptor component	Cytokine receptor	Association with severe malaria in Mandinka people of the Gambia
IL1A/IL1B	Interleukin-1α and -1β	Proinflammatory cytokines	Marginal associations with severe malaria in the Gambia
IL10	Interleukin-10	Anti-inflammatory cytokine	Haplotypic association with severe malaria in the Gambia
IL12B	Interleukin-12 β subunit	Promotes development of Th1 cells	Association with severe malaria in Tanzania
IL4	Interleukin-4	Promotes antibody-producing B cells	Association with antimalarial antibody levels in Fulani people of Burkina Faso
MBL2	Mannose-binding protein	Activates classic complement	Association with severe malaria in Gabon
NOS2A	Inducible NO synthase	Generates NO, a free radical	Various associations with severe malaria in Gabon, the Gambia, and Tanzania
TNF	Tumor necrosis factor	Cytokine with antiparasitic and proinflammatory properties	Various associations with severe malaria and reinfection risk in the Gambia, Kenya, Gabon, and Sri Lanka
TNFSF5	CD40 ligand	T cell–B cell interactions leading to immunoglobulin class switching	Association with severe malaria in the Gambia

cp29) that bind to *HLA-B53*, and these two variants are found together in the same individual more frequently than expected by chance. Although both cp26 and cp29 are effective targets for CTLs at the individual level, they appear to show immunological antagonism such that each acts to suppress the CTL response to the other, and it has been postulated that this is an immune-evasion strategy on the part of the parasite (Gilbert et al. 1998).

HLA-DRB1 encodes an HLA class II β chain that, together with an α chain, comprise the *HLA-DR* antigen–presenting complex. This is expressed in B lymphocytes, dendritic cells, and macrophages and is crucial for antibody production. *HLA DRB1*1302-DQB1*0501* has been associated with resistance to severe malaria in Gambian children (Hill et al. 1991), and the incidence of malaria-fever episodes in Gambian children is reported to show an overall association with distribution of MHC class II haplotypes (Bennett et al. 1993).

The Antibody Response

Interleukin-4, encoded by *IL4,* is produced by activated T cells and promotes proliferation and differentiation of antibody-producing B cells. A study of the Fulani of Burkina Faso, who have both fewer malaria attacks and higher levels of antimalarial antibodies than do neighboring ethnic groups, found that the *IL4*-524 T allele was associated with elevated antibody levels against malaria antigens, which raises the possibility that this might be a factor in increased resistance to malaria (Luoni et al. 2001).

CD40 ligand, encoded by the X chromosome gene *TNFSF5,* is expressed in T cells and binds to CD40 in B cells, which acts to regulate immunoglobulin class switching and other aspects of B cell function. In a Gambian case-control study, the *TNFSF5*-726C allele was associated with protection against severe malaria (Sabeti et al. 2002*a*), and long-range haplotype analysis of this allele suggests that it has recently undergone positive evolutionary selection (Sabeti et al. 2002*b*).

Many leukocytes express receptors for the Fc portion of IgG, which are used to engage and remove antigen-antibody complexes. A His→Arg substitution at codon 131 of *FCGR2A,* which encodes low-affinity IIa receptor for the Fc fragment of IgG, results in failure to bind to IgG2 and has been associated with protection against high levels of *P. falciparum* parasitemia in Kenya (Shi et al. 2001). Follow-up studies in Thailand and the Gambia found that homozygotes for the 131His genotype are susceptible to cerebral malaria (Omi et al. 2002; Cooke et al. 2003). In the Thai study, the *FCGR2A* association involved interaction with an *FCGR3B* gene polymorphism.

The Proinflammatory Response

Tumor necrosis factor is encoded by the *TNF* gene located in the MHC class III region, flanked by the MHC class I and II regions. It is a proinflammatory cytokine that is critical for innate immunity against malaria parasites but has also been implicated in the pathogenesis of severe malaria (reviewed by Kwiatkowski [1995] and Clark et al. [2004]). Two longitudinal family studies, in the Gambia and Burkina Faso, have identified linkage between the MHC region and susceptibility to malaria-fever episodes, and *TNF* is at the center of the linkage peaks (Jepson et al. 1997; Flori et al. 2003). Several TNF-promoter polymorphisms have been independently associated with severe malaria. Gambian children who are homozygous for the *TNF*-308A allele have been observed to have increased susceptibility to cerebral malaria (McGuire et al. 1994), and, in Gabon, it was found that those who carried this allele were more likely to encounter symptomatic reinfections with *P. falciparum* (Meyer et al. 2002). A study in Sri Lanka found that the carriers of the *TNF*-308A allele had increased risk of severe infectious diseases in general (Wattavidanage et al. 1999), whereas a study in Kenya found an increase in infant mortality and malaria morbidity (Aidoo et al. 2001). *TNF*-376A confers allele-specific binding of the transcription factor OCT-1 and has been associated with susceptibility to cerebral malaria (Knight et al. 1999), whereas the *TNF*-238A allele has been associated with susceptibility to severe malarial anemia (McGuire et al. 1999). A longitudinal study in Burkina Faso suggests that several different TNF-promoter SNPs are involved in the regulation of parasite density (Flori et al. 2005). The functional role of *TNF*-308 and other *TNF* polymorphisms remains open to question (Abraham and Kroeger 1999; Knight et al. 2003; Bayley et al. 2004), but the surrounding MHC class III region has many other interesting immunological genes and complex patterns of linkage disequilibrium (Ackerman et al. 2003). Thus, although *TNF* is unquestionably an important mediator of both immunity and pathogenesis for malaria, it remains possible that the observed genetic associations with *TNF* polymorphisms arise from functional variation in neighboring genes.

Inducible nitric oxide (NO) synthase, encoded by *NOS2A,* generates NO. This is a free radical, with antiparasitic properties, but it also has a potential immunosuppressive role and has been proposed as a factor in cerebral malaria because of its role in neurotransmission (reviewed by Clark and Rockett [1996]). The *NOS2A*-954C allele has been associated with elevated NO synthase activity in cells from Gabonese individuals, and, in that population, it has been associated with protection from severe malaria and resistance to reinfection (Kun et al. 1998, 2001), but studies in the Gambia and Tan-

zania failed to detect such a disease association (Levesque et al. 1999; Burgner et al. 2003). The *NOS2A*-1173T allele—which appears, on the basis of measurements in urine and plasma, to be associated with high NO production in Tanzanian children—is associated with protection from malarial illness in Tanzania and from severe malarial anemia in Kenya (Hobbs et al. 2002), but no protective effect against severe malaria was detected in the Gambia (Burgner et al. 2003). In Gambian children, an *NOS2A* microsatellite polymorphism has been associated with susceptibility to fatal malaria (Burgner et al. 1998), and a haplotype uniquely defined by the *NOS2A*-1659T allele was associated with cerebral malaria by both the transmission/disequilibrium test (TDT) and case-control analysis (Burgner et al. 2003).

Interferon-γ, encoded by *IFNG,* is a key immunological mediator that is believed to play both a protective and a pathological role in malaria (Stevenson and Riley 2004). Analysis of SNPs in the region of *IFNG* and the neighboring *IL22* gene found several weak associations with severe malaria in Gambian children but no clear-cut effect (Koch et al. 2005). A study of *IFNGR1,* which encodes the ligand-binding α chain of the interferon-γ receptor, found that in Mandinka, the major Gambian ethnic group, heterozygotes for the IFNGR1-*56* polymorphism were protected against cerebral malaria (Koch et al. 2002). Reporter-gene analysis suggests that the minor allele acts to reduce levels of *IFNGR1* gene expression (Juliger et al. 2003).

IFNAR1 encodes interferon α receptor 1, a type I membrane protein that forms one of the two chains of a receptor for interferons α and β. In a murine malaria model, it has been observed that interferonα inhibits parasite development within erythrocytes (Vigario et al. 2001). A Gambian case-control study found two *IFNAR1* SNPs that were associated with protection against severe malaria, and a resistance haplotype was identified (Aucan et al. 2003).

IL12B encodes a subunit of interleukin-12, a cytokine produced by activated macrophages that is essential for the development of Th1 cells. Homozygotes for an *IL12B*-promoter polymorphism were found to have decreased NO production when measured in blood samples, and this genotype has been associated with a fatal outcome in cerebral malaria in Tanzanian but not in Kenyan children (Morahan et al. 2002)

The interleukin-1 family of cytokines, produced mainly by macrophages, are important mediators of the inflammatory response to infection and of fever. In a Gambian case-control study, a SNP in *IL1A* (encoding interleukin-1α) and another in *IL1B* (encoding interleukin-1β) showed a marginal association with susceptibility to malaria (Walley et al. 2004).

Interleukin-10, encoded by *IL10,* is a crucial anti-inflammatory cytokine. Several lines of evidence indicate that IL10 is protective against severe malaria and that IL10 production is genetically determined. An analysis of *IL10* SNPs in Gambian children found a common haplotype that was strongly associated with protection against severe malaria by case-control analysis but not by TDT analysis of the same population (Wilson et al. 2005). Since the case-control analysis was ethnically matched, this raises the question of whether *IL10* associations with severe malaria might be confounded by fetal survival rates or other sources of transmission bias, since genetic variation at the *IL10* locus has been implicated as a determinant of fertility (Westendorp et al. 2001).

Other Serum Factors

MBL2 encodes a serum mannose-binding lectin (MBL) protein that recognizes mannose and N-acetylglucosamine on bacterial pathogens and can activate the classic complement pathway. *MBL2* polymorphisms have been associated with susceptibility to various infectious diseases. A study in Gabon found that children with severe malaria had low serum MBL levels compared with those of children with mild malaria and that mutations in codons 54 and 57 of *MBL2* (which lead to low protein levels) were present at a higher frequency in those with severe malaria (Luty et al. 1998). However, a study in the Gambia failed to replicate this association with severe malaria (Bellamy et al. 1998*b*).

Differences in Resistance to Infection among Inbred Mouse Strains

Inbred strains of mice show marked and consistent differences in their response to malaria infection (Greenberg et al. 1954; Greenberg and Kendrick 1959; Rest 1982; Stevenson et al. 1982; Stevenson and Skamene 1985). Put simply, some mouse strains are more resistant to malaria than are others, but the details are somewhat more complex, since mice that are relatively resistant to one parasite strain may be relatively susceptible to a different parasite strain. For example, some strains of mice are resistant to nonlethal strains but highly susceptible to lethal strains of *Plasmodium yoelii,* whereas other mouse strains show the opposite pattern (Sayles and Wassom 1988). This confirms that both host and parasite genes—and the specific way in which they are combined—are important determinants of resistance or susceptibility to malaria.

Plasmodium chabaudi *Infection: A Model of How the Parasite Population Is Controlled*

P. chabaudi infection is controlled and cleared by some inbred strains of mice (e.g., C57BL/6J, C57L/J, DBA/2J, CBA/J, and B10.A/SgSn) much more effectively than by

others (e.g., A/J, DBA/1J, BALB/c, C3H/HeJ, AKR/J, and SJL/J) (Stevenson et al. 1982). At least some of these interstrain differences show classic Mendelian patterns of segregation in crossbreeding experiments and that show resistance is generally dominant over susceptibility. When susceptible A/J and resistant C57BL mice were crossbred, it was found that the degree of splenic enlargement after infection was genetically linked to the ability to suppress parasitemia (Stevenson and Skamene 1985).

Genomewide linkage screens have been performed after crossbreeding susceptible mice (C3H, SJL, or A/J) with resistant mice (C57BL/6J). A locus on chromosome 9 (Char1) determines death or survival (Foote et al. 1997). A well-characterized locus on chromosome 8 (Char2) determines the control of parasite density (Foote et al. 1997; Fortin et al. 1997; Burt et al. 2002). A locus in the MHC region of chromosome 17 (Char3) influences parasite clearance rates at the time immediately after peak parasitemia (Burt et al. 1999).

A further resistance locus (Char4) was identified after deriving recombinant congenic strains from susceptible A/J and resistant C57BL/6J mice. The Char4 locus maps to a small congenic B6 fragment on chromosome 3 (Fortin et al. 2001). Sequencing of candidate genes across this region has identified a plausible functional mutation—namely, a loss-of-function coding variant of the pyruvate kinase gene (*Pklr*). In uninfected animals, this mutation causes hemolytic anemia that is compensated by constitutive reticulocytosis and splenomegaly (Min-Oo et al. 2003, 2004). It is possible that the malaria-protective effect is a direct consequence of impaired viability and increasing splenic clearance of host erythrocytes.

An advanced intercross line population derived from susceptible A/J and resistant C57BL/6J mice was used to identify QTLs for control of parasitemia (Hernandez-Valladares et al. 2004*a*). Of particular interest was a novel QTL (Char 8) in the chromosome 11 region that is homologous to the human 5q31-q33 region discussed above, which is rich in Th2 cytokine genes (Hernandez-Valladares et al. 2004*b*).

Plasmodium berghei *ANKA Infection: A Model of Inflammatory Pathology*

The ANKA strain of *P. berghei* causes more-severe pathology than do most other experimental murine parasites. A curious feature *of P. berghei* ANKA (PBA) infection in some inbred mouse strains (e.g., A/J and C57BL/6) is that 100% of the mice die after 5–8 d, with cerebral hemorrhages as a terminal event (Rest 1982). Cloned lines of PBA differ in their tendency to cause these cerebral changes, which indicates that pathology is determined by a specific combination of host and parasite genotype (Amani et al. 1998).

PBA-induced cerebral pathology is not a reliable model of human cerebral malaria. In particular, parasite sequestration in cerebral capillaries, a hallmark of human cerebral malaria (Taylor et al. 2004), is notable by its absence in the PBA experimental model. The pathological features of PBA in susceptible mouse strains include mononuclear cell adhesion to endothelium, which is absent in human cerebral malaria, together with hemorrhage and cerebral endothelial cell damage, with breakdown of the blood-brain barrier and cerebral edema (Thumwood et al. 1988; Neill and Hunt 1992; Neill et al. 1993). However, PBA has provided an interesting experimental system in which to study how immunopathological processes are affected by different interventions (e.g., Grau et al. [1987*a*, 1987*b*, 1989], Kremsner et al. [1991], Hunt et al. [1993], Engwerda et al. [2002], and Schofield et al. [2002]).

To search for genetic factors that determine PBA-induced cerebral pathology, one approach has been to enlarge the pool of genetic diversity by deriving new inbred mouse strains from wild-mouse populations (Bagot et al. 2002*b*). When a resistant wild-derived inbred strain (WLA) was crossed with a susceptible laboratory strain (C57BL/6J), all of the F_1 progeny and 97% of the F_2 progeny displayed resistance. A genomewide screen, performed after backcrossing the resistant wild strain onto the susceptible laboratory strain, found that resistance was linked to loci on chromosome 1 (Berr1) and chromosome 11 (Berr2) (Bagot et al. 2002*a*).

Recent analysis of the F_2 progeny of WLA and C57BL/6 strains has revealed a fascinating combinatorial effect: it seems that the WLA allele at the Berr1 locus confers resistance to early death from cerebral pathology, whereas the C57Bl/6 allele at a locus on chromosome 9 (Berr3) increases the ability of the mouse to clear the infection. Thus, the progeny have greater resistance to malaria than either of the parental strains (Campino et al. 2005).

A study that crossbred susceptible C57BL/6 mice with resistant DBA/2 mice identified a major resistance locus on chromosome 18 (Nagayasu et al. 2002). Another study that crossed susceptible CBA mice with resistant DBA/2 mice identified a susceptibility locus at the MHC region on chromosome 17 (Ohno and Nishimura 2004).

Conclusions

As geneticists brace themselves to perform genomewide association analysis of common diseases—a task that is going to require massive investment both in epidemiological infrastructure and in genotyping technology—malaria stands out as a target for which this approach is feasible and potentially of huge importance for disease prevention. In terms of feasibility, malaria is the most powerful known force for recent selection of human genetic variants, so malaria-protective polymorphisms are

likely to be at high frequencies in affected populations, and, if recently selected, they may also show strong linkage disequilibrium with neighboring genetic markers.

In terms of practical importance for disease prevention, genetic studies of malaria have already yielded results: the observation that Duffy antigen–negative individuals are resistant to infection with *P. vivax* was the starting point of a chain of molecular discovery that led to a candidate vaccine against *P. vivax* that is now undergoing trials. A major impetus for researchers working in this area is the hope that large-scale genomic epidemiology will be a way of getting at basic questions that decades of immunological research have failed to resolve, such as how infected individuals clear parasites from the bloodstream or why malaria causes cerebral complications in some people but not others. The holy grail of this field is to discover novel molecular pathways for protective immunity that will provide critical insights for the development of a vaccine to reduce the massive global burden of disease due to *P. falciparum*.

Malaria research groups across the world have collected DNA samples and detailed clinical data from thousands of individuals with severe malaria, as well as from parents and population controls. Until recently, the resource has been fragmented, with different groups pursuing relatively small studies of their own samples, but there is a growing impetus to link these independent studies to form a global infrastructure for genomic epidemiology of malaria. One such initiative is Malaria Genomic Epidemiology Network (MalariaGEN), which brings together research groups in 15 different malaria-endemic countries. Large-scale epidemiological studies are needed for the sample sizes required to detect modest effects while testing perhaps a million SNP markers (Risch 2000). And once the genome-screening phase has been completed, data from different populations are needed to analyze gene-environment interactions and are needed because haplotypic diversity provides the means to dissect functional polymorphisms from nonfunctional genetic markers.

Finally, it should not be forgotten that, as recently as a century ago, malaria was found in parts of Europe and North America, in addition to its current distribution across most of Africa and large parts of Asia and South America. Sickle-cell disease and thalassemia are two classic examples of how the historical effects of malaria have left an imprint on the pattern of disease in contemporary populations. It remains an open question as to whether any of the immunological, inflammatory, and other chronic diseases that are found in modern societies are, in part, due to the evolutionary pressure that malaria exerted on our ancestors.

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Web Resource

The URL for data presented herein is as follows:

MalariaGEN, http://www.malariagen.net/

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