Gene 467 (2010) 1–12

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Review

Mechanisms of genetically-based resistance to malaria

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ARTICLE INFO

Article history:
Accepted 13 July 2010
Available online 22 July 2010

Received by Leonardo Marino-Ramirez

Keywords:
Malaria
Hemoglobinopathy
Erythrocyte polymorphism
Natural resistance

ABSTRACT

Malaria remains one of the most prevalent parasitoses worldwide. About 350 to 500 million febrile episodes are observed yearly in African children alone and more than 1 million people die because of malaria each year. Multiple factors have hampered the effective control of this disease, some of which include the complex biology of the *Plasmodium* parasites, their high polymorphism and their increasingly high resistance to antimalarial drugs, mainly in endemic regions. The ancient interaction between malarial parasites and humans has led to the fixation in the population of several inherited alterations conferring protection against malaria. Some of the mechanisms underlying protection against this disease are described in this review for hemoglobin-inherited disorders (thalassemia, sickle-cell trait, HbC and HbE), erythrocyte polymorphisms (ovalocytosis and Duffy blood group), enzymopathies (G6PD deficiency and PK deficiency) and immunogenetic variants (HLA alleles, complement receptor 1, NOS2, tumor necrosis factor-α promoter and chromosome 5q31–q33 polymorphisms).

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1. Introduction

Malaria is a parasitic disease transmitted by Anopheline mosquitoes and is highly widespread throughout tropical and subtropical regions. The exact magnitude of the problem still remains unknown (Carvalho et al., 2002) since this disease is most commonly found in poor countries (Olumese, 2005) having less developed health systems and control strategies (Phillips, 2001). In these areas, the high rates of morbidity and mortality can be mainly attributable to the lack of access to effective treatment (Suh et al., 2004) and to the growing parasite resistance to antimalarial drugs such as chloroquine and pyrimethamine (Smith et al., 2002). According to the World Malaria Report 2008, published by the World Health Organization and UNICEF, 3.3 billion people living in 109 countries or territories were at risk of acquiring malaria by the end of 2006. It has been calculated that 250 million clinical episodes of malaria occur each year (mainly due to *Plasmodium falciparum* and *Plasmodium vivax* infections), of which more than 1 million people die (WHO and UNICEF, 2008).

The immune response induced in humans by infection caused by malarial parasites is complex and varies depending on the level of endemicity, epidemiological factors, genetic makeup, host age, parasite stage and parasite species. Repeated infection and continuous exposure are required to achieve clinical immunity (which reduces the risk of death from malaria and reduces the intensity of the clinical symptoms) and later anti-parasitic immunity (which directly reduces the numbers of parasites in an infected individual or inhibits parasite replication) (Mohan and Stevenson, 1998). Both innate and acquired immunity processes are invoked during the infection. Resistance involves genetically-based resistance mechanisms and cell-mediated immunological mechanisms, but also specific antibodies, which are able to reduce the severity of the symptoms and mortality are found among the main actors in the acquired immune response (Smith et al., 2002).

Innate immunity can be defined as being the host cells' ability to resist infection by the parasite, irrespective of their previous exposure to it (the review by Stevenson and Riley, 2004 gives detailed information about innate immunity against malaria). Resistance mechanisms have been described in both sporozoite entry to liver cells and erythrocyte invasion by merozoites (Yuthavong and Wilairat, 1993) (Fig. 1). Genetically-based resistance is involved in either altering erythrocyte invasion by merozoites, in lowering parasite

Abbreviations: 6PGL, 6-phosphogluconolactonase; CR1, Complement receptor-1; CSF, Granulocyte-macrophage colony-stimulating factor; DARC, Duffy antigen receptor for chemokines; DBL, Duffy binding like; DBP, Duffy binding protein; G6PD, Glucose-6-phosphate dehydrogenase; HBB, Hemoglobin beta gene; HbC, Hemoglobin C; HbE, Hemoglobin E; HbF, Fetal hemoglobin; HbH, Hemoglobin H; HbS, Hemoglobin S; HE, Hereditary elliptocytosis; HLA, Human leukocyte antigen; ICs, Immune complexes; MHC, Major histocompatibility complex; NADP, Nicotinamide adenine dinucleotide phosphate; NADPH, Reduced form of NADP; NO, Nitric oxide; NOS, NO synthase; NOS2, NO synthase 2; PBMCs, Peripheral blood mononuclear cells; PEP, Phosphoenolpyruvate; Pfbi, *P. falciparum* blood infection; PfEMP-1, *P. falciparum* erythrocyte membrane protein-1; Pfil1, *P. falciparum* infection level 1; PK, Pyruvate kinase; PMM, Prior mild malaria; PSM, Prior severe malaria; SAO, Southeast Asian ovalocytosis; SCD, Sickle-cell disease; SNP, Single nucleotide polymorphism; TNF, Tumor necrosis factor.

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0378-1119/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2010.07.008

growth or in impairing merozoite viability after being released from schizonts (Smith et al., 2002).

Some genetic mutations resulting in protection against parasitic infection are highly prevalent in regions where malaria is endemic. According to Haldane's malaria hypothesis (Haldane, 1949; Yuthavong and Wilairat, 1993; Smith et al., 2002; Duffy and Fried, 2006; Pasvol, 2006), this could result in a “balanced polymorphism” where the homozygote's hematological disadvantage is balanced by the resistance to malaria displayed by the heterozygote (Yuthavong and Wilairat, 1993; Agarwal et al., 2000; Smith et al., 2002; Akide-Ndunge et al., 2003).

Among the main alterations conferring resistance against malaria, various blood group polymorphisms such as hemoglobinopathies (including thalassemias, HbS, HbC and HbE), erythrocyte polymorphisms and immunogenetic variants, have been observed in different populations where malaria is prevalent (Yuthavong and Wilairat, 1993; Michon et al., 2001).

This review focused on mechanisms leading to genetically-based resistance against *Plasmodium* parasites.

2. Genetically-based resistance mechanisms

2.1. Hemoglobinopathies

Although hemoglobinopathies (hemoglobin structure and production disorders) confer resistance to malaria through immune mechanisms (Luzzi et al., 1991; Roberts and Williams, 2003; Williams et al., 2005c), they are also a major worldwide health problem. These disorders have a high carrier frequency, particularly in certain regions where malaria is endemic, and are characterized by having broad clinical and hematological phenotypic heterogeneity (Patrinos et al., 2005). Thalassemias and abnormal hemoglobins (which can be grouped together as hemoglobinopathies) (Weatherall, 2008), constitute the most common single-gene disorders (Yuthavong and Wilairat, 1993).

Although it is not always clear how erythrocyte abnormalities might confer protection against malaria, the overlapping geographical distribution displayed by malaria and hemoglobinopathies (such as thalassemias) led Haldane to propose his “malaria hypothesis” (Haldane, 1949; Duffy and Fried, 2006). The relatively high prevalence of heterozygous individuals for inherited erythrocyte diseases in areas where malaria is endemic seems to have been maintained by a balanced polymorphism in the human population (Haldane, 1949; Cooke et al., 2004; Richer and Chudley, 2005).

Distinct mechanisms conferring protection against severe and complicated malaria have been proposed for the different hemoglobinopathies (such as sickle-cell trait, beta thalassemia trait, homozygous HbH, HbAS), as will be detailed below (Weatherall, 1997; Ayi et al., 2004; Williams et al., 2005b). Among the most relevant mechanisms, reduced erythrocyte invasion by the parasite, decreased intra-erythrocytic parasite growth (Pasvol et al., 1978), enhanced phagocytosis of parasite-infected erythrocytes (Cappadoro et al., 1998; Ayi et al., 2004) and increased immune response against parasite-infected erythrocytes (Duffy and Fried, 2006) have all been described.

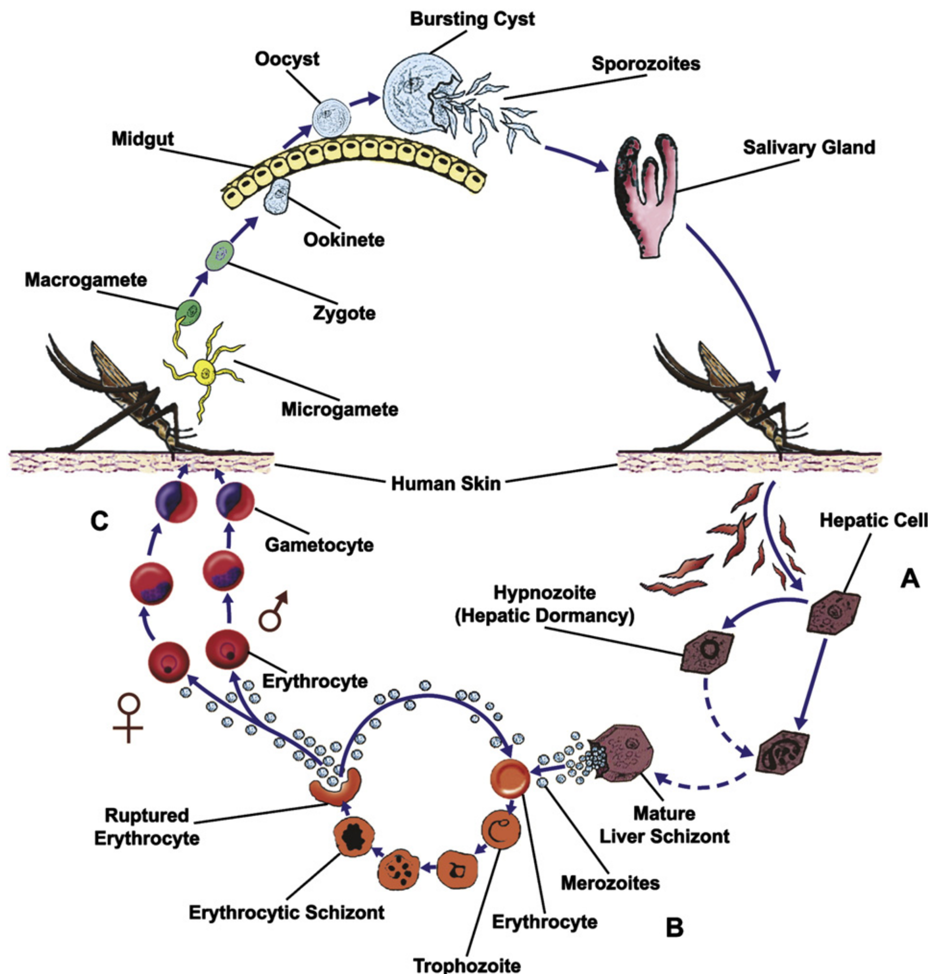

Fig. 1. Life cycle of the malaria parasite. The asexual stage pre-erythrocyte or hepatic (A) and erythrocyte (B) cycles as well as the sexual stage sporogonic (C) cycle are represented. (The hypnozoite form occurs only in *Plasmodium vivax* and *Plasmodium ovale*.)
Adapted from Oaks et al. (1991).

2.1.1. Thalassemias

Hemoglobin (tetramer constituted in adults by 2 α-globin and 2 β-globin chains) is the molecule responsible for providing and transporting oxygen to all tissues.

Among hemoglobin disorders, thalassemias refer to alterations in the synthesis of either globin chain (Allen et al., 1997; Vento et al., 2006). α-thalassemia displays an abnormal α-globin synthesis, while the amount of β-globin is diminished in β-thalassemia (Smith et al., 2002; Richer and Chudley, 2005). Thalassemias are widely distributed around the world (Fig. 2a) (Vento et al., 2006).

2.1.1.1. α-Thalassemia. α-Thalassemia is caused by decreased synthesis of an α-globin chain resulting from deletion of the duplicated α-globin genes located on chromosome 16 (16p13.3) (Yuthavong and Wilairat, 1993; Williams et al., 1996; Allen et al., 1997). The normal α-globin genotype is represented as αα/αα. There are two varieties of α-thalassemia: α^+ and α^0 thalassemia. Heterozygotes for α^+ thalassemia have lost one of the linked α-globin genes (−α/αα) and are clinically normal, whereas homozygotes (−α/−α) have mild anemia with reduced levels of hemoglobin in erythrocytes. α^0 thalassemia, in which both linked α globin genes are lost (−−/αα), is lethal in the homozygous state (−−/−−). Most of the studies where a link between α-thalassemia and malarial protection has been observed have reported the presence of the α^+ variety in the studied population (Williams et al., 1996; Weatherall, 1997).

α^+-thalassemia is responsible for a mild hemolytic state and is also associated with a certain degree of ineffective erythropoiesis, reduced erythrocyte survival, and therefore, an increased erythrocyte turnover with a relatively high proportion or circulating young erythrocytes (Weatherall, 1997). The α^+-thalassemia is the most frequent genetic disorder in the human population (Williams et al., 1996; Weatherall, 1997). Being very common in malaria-endemic regions it is considered to confer protection against clinical disease caused by severe forms of *P. falciparum* infection (Mockenhaupt et al., 2001, 2004b; Wambua et al., 2006).

Previous "in vitro" studies have shown that *Plasmodium*-infected α-thalassemic erythrocytes bind high levels of antibodies from malaria-endemic sera on their surface (which could be the optimum target for immune responses) (Luzzi et al., 1991; Yuthavong and Wilairat, 1993). Opsonization (Celada et al., 1982), complement-mediated lysis (Gabriel and Berzins, 1983), antibody-dependant cytotoxicity (Brown and Smalley, 1980) and inhibition of sequestration of infected erythrocytes (Udeinya et al., 1983) are the most frequent potential anti-parasite effector mechanisms which might be promoted by these antibodies.

More recent studies have shown that both polymorphism in the complement receptor-1 (CR1) gene leading to a reduced level of CR1 expression in erythrocyte (a polymorphism commonly found in regions of Papua New Guinea) and α-thalassemia are associated with CR1 deficiency. CR1-deficient erythrocytes have shown a marked reduction in their ability to form rosettes (pathogenic markers of severe malaria). CR1 polymorphisms and α-thalassemia independently confer protection against severe malaria; however, the mechanism responsible for reduced CR1 in α-thalassemic erythrocytes still remains unclear (Cockburn et al., 2004).

2.1.1.2. β-thalassemia. This disorder is produced by decreased synthesis of β-globin (Yuthavong and Wilairat, 1993) leading to a relative excess of α-globin chains. The β-globin gene is localized in chromosome 11p15.5 within the β-globin multigene cluster and is responsible for the synthesis of the hemoglobin's β-globin polypeptide (Das and Talukder, 2001). Such alteration promotes mechanical damage of erythrocyte precursors and their products as well as oxidative membrane destruction. Its heterozygous state is associated with extremely mild anemia and morphological changes in erythrocytes, causing ineffective erythropoiesis (Weatherall, 2000; Roberts and Williams, 2003), differently to its homozygote state which is associated with severe anemia during the second and third months of life; if it is not treated promptly with regular blood transfusions, it leads to death within the first two years of life (Weatherall, 2000).

The complete absence of β-globin synthesis is denoted as β^o and reduced synthesis levels as β^+. β-thalassemias result from more than 180 different mutations, either it be by point mutations or short nucleotide insertions or deletions (nonsense mutations) within the single β-globin gene (Yuthavong and Wilairat, 1993). As a result, there is a decrease in the output of β-globin chains, which ranges from a complete absence (β^0-thalassemia) or a partial absence (β^+-thalassemia) (Weatherall and Provan, 2000). Weatherall (2000) has suggested that disease severity is directly related to the degree of imbalance between α- and β-globin chains.

Several epidemiological studies have suggested that β-thalassemia confers protection against malaria. The findings of Pasvol et al. (1976) have suggested that a resistance mechanism against malaria during the first few months of life (particularly in β-thalassemia carriers) involves fetal hemoglobin (HbF) production declining more slowly than normal. A case–control study by Willcox et al. (1983) has presented strong evidence that both intracellular and extracellular determinants could play an important role in protecting β-thalassemic heterozygotes against lethal malarial infections. Brockelman et al. (1987) found that some erythrocytes from thalassemic Thai subjects inhibit the growth of *P. falciparum* "in vitro".

Other studies have revealed a protective effect against malaria in β-thalassemic children (Yuthavong and Wilairat, 1993). Luzzi et al. (1991) described significantly higher phagocytosis of infected β-thalassemic erythrocytes when compared to normal infected erythrocytes. Moreover, Smith et al. (2002) reported that antibodies present in sera collected from people living in malaria-endemic areas bound to β-thalassemic erythrocytes to a greater extent.

2.1.2. Sickle-cell trait

The term sickle-cell disease (SCD) or "sickle hemoglobin" refers to a group of symptomatic disorders associated with mutations on the *HBB* gene (Richer and Chudley, 2005), which produce the hemoglobin form known as Hemoglobin S (HbS). The homozygous form (HbSS) is fatal early in life without proper treatment. The heterozygous form (trait) displays the HbAS genotype (Richer and Chudley, 2005; Williams et al., 2005a) and encodes a mutant β-globin chain (β^S rather than normal β^A). The replacement of the β-globin's normal glutamic acid in position 6 in HbA for a valine in HbS induces hydrophobic spots, which facilitate HbS polymerization upon deoxygenation. Intra-erythrocytic HbS polymers lead to alterations in the shape of erythrocytes similar to a sickle.

SCD is one of the most prevalent erythrocyte alterations, mainly in malaria-endemic regions (Fig. 2a). Several studies have suggested that this is a protection mechanism against *P. falciparum* malaria. Allison (1964) has suggested that SCD is a protection mechanism against malarial mortality, possibly due to a selective advantage conferred by HbAS. Friedman (1978) has described mechanisms in which HbS-containing erythrocytes inhibit malarial parasite growth. He showed that HbS changes its nature in deoxygenating conditions and parasites become gravely affected, therefore showing that only erythrocyte mechanisms are sufficient for providing resistance "in vivo". He also showed that 90% of young parasites are eliminated in HbAS cell populations, of which only 60% are sickled or distorted and that the parasite contributes to conditions inducing sickling in its host cell.

Williams et al. (2005b) suggested in more recent studies that protection conferred by HbAS is remarkably specific to malaria; other studies have shown that heterozygous people carrying the sickle-cell trait (HbAS) are protected against severe malaria (up to >90% in some populations) (Friedman, 1978; Aidoo et al., 2002; Ackerman et al., 2005; Cabrera et al., 2005; Williams et al., 2005a; Duffy and Fried, 2006).

Different underlying mechanisms have been proposed over the years to explain why HbAS confer protection against severe malaria.

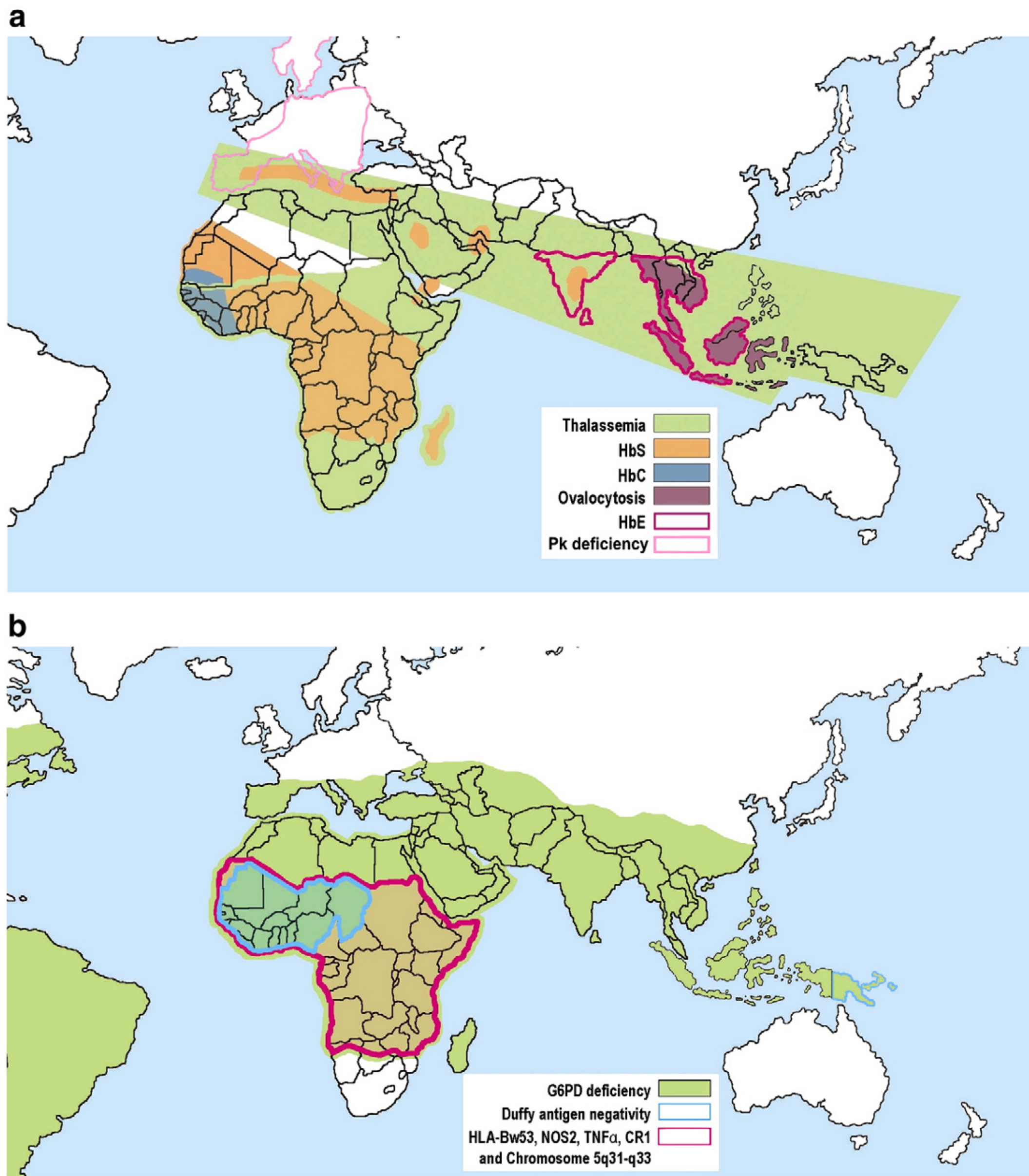

Fig. 2. Geographical distribution of hemoglobin-inherited disorders, erythrocyte polymorphisms and immunogenetic variants.

These include accelerated sickling of parasite-infected HbAS erythrocytes, low growth rates and parasite invasion in HbAS erythrocytes (in low oxygen conditions) and increased phagocytosis of infected HbAS erythrocytes (Pasvol et al., 1978; Weatherall, 1987; Abu-Zeid et al., 1991). However, more recent experimental data mainly support two of them. Firstly, it has been shown that intra-erythrocytic parasite growth is greatly inhibited by HbS polymerization when oxygen levels drop below 5%. Secondly, higher parasite-infected sickle erythrocyte phagocytosis by host immune cells has been observed when compared to infected normal erythrocytes (Smith et al., 2002; Akide-Ndunge et al., 2003; Roberts and Williams, 2003; Ayi et al., 2004; Cabrera et al., 2005).

It has been also shown that HbS erythrocytes infected by *P. falciparum* lower the surface expression of PfEMP-1, which results in a reduction of cytoadherence and thus protecton against severe malaria (Cholera et al., 2008).

2.1.3. Hemoglobin C (HbC)

Hemoglobin C (HbC) results from a point mutation leading to the replacement of glutamate by lysine in the β-globin's sixth amino-acid position (Agarwal et al., 2000; Fairhurst et al., 2005); it is almost exclusively present in western Africa (Modiano et al., 2001; Mockenhaupt et al., 2004a) and, although HbAC is asymptomatic, HbCC can produce mild hemolysis, splenomegally and gallstones (Mockenhaupt et al., 2004a) (Fig. 2a).

Several studies proposing possible immune mechanisms of protection against *P. falciparum* malaria have yielded contradictory results. Friedman et al. (1979) and Olson and Nagel (1986) have reported that this parasite does not proliferate in HbCC erythrocytes "in vitro" (while HbAC erythrocytes enable normal parasite growth) because the abnormal osmotic response of parasitized CC cells interferes with erythrocyte rupture needed for releasing infective merozoites. Likewise, Rihet et al. (2004) found in their recent study on malarial protection-inducing mechanisms that intra-erythrocytic parasite growth becomes reduced in individuals having HbC erythrocytes in both mild and severe malarial infection.

Contrasting with the above studies, Agarwal et al. (2000) have suggested that *P. falciparum* could infect and proliferate inside HbCC homozygotes, this being consistent with studies by Modiano et al. (2001) and Duffy and Fried (2006) who found that *P. falciparum*

parasites are able to efficiently replicate within homozygous HbC erythrocytes (HbCC) and that no protection is observed against mild malarial infections. However, agreeing with the former study, they did report protection against severe infection.

Other studies have shown that individuals homozygous for HbC display a reduced risk of having severe or non-severe infections by *P. falciparum* (Bradbury, 2001; Modiano et al., 2001; Mockenhaupt et al., 2004a,c; Min-Oo and Gros, 2005). Risk reduction in heterozygous individuals (HbAC) was 29%, while it reached 93% in homozygous individuals (HbCC) (Modiano et al., 2001).

Some authors have returned to the malaria hypothesis to explain HbC frequency in some populations; a hypothesis that proposes an explanation to why different regions of Africa, Middle East and Asia are kept in a state of balanced polymorphism due to the protective effect of the heterozygous state against severe malaria. However, epidemiological evidence has been contradictory for HbC (Agarwal et al., 2000). As mentioned above, the presence of HbC heterozygotes has not been shown to be correlated with reduced *P. falciparum* infection or density while HbCC homozygote individuals have shown possible protection against this parasite.

2.1.4. Hemoglobin E (HbE)

Hemoglobin E (HbE) is produced when the glutamic acid in position 26 of the β-globin chain is replaced by a lysine (Nagel et al., 1981; Chotivanich et al., 2002; Ohashi et al., 2004). It has been observed that erythrocytes from people having HbE show reduced plasticity and deformability “in vitro”, thus impairing merozoite growth and release (Bunyaratvej et al., 1992). Homozygous HbE erythrocytes (HbEE) are microcytic (having low mean corpuscular volume) at low hemoglobin concentration (Nagel et al., 1981). Such hemoglobinopathy is very common in Southeastern Asia (Chotivanich et al., 2002) (Fig. 2a).

It has been found that HbE confers protection against severe malarial episodes. Different mechanisms have been proposed (Nagel et al., 1981; Hutagalung et al., 1999, 2000; Agarwal et al., 2000; Chotivanich et al., 2002; Roberts and Williams, 2003; Ohashi et al., 2004): reduced erythrocyte invasion by merozoites, lower intra-erythrocytic parasite growth, enhanced phagocytosis of infected erythrocytes and increased probability of infection at an early age (mainly for *P. vivax*, which has been shown to confer a certain degree of protection against subsequent severe *P. falciparum* infection).

2.2. Erythrocyte polymorphisms

2.2.1. Ovalocytosis (band 3 variants)

Ovalocytosis is an uncommon variant of hereditary elliptocytosis (HE) belonging to the erythrocyte membrane inherited disorder group (Gallagher, 2005). It is caused by heterozygosity for a 27-base pair deletion in the gene encoding the erythrocyte membrane protein band 3 (*SLC4A1Δ27*) (Williams, 2006) on chromosome 17 (Jarolim et al., 1991). The deletion is in linkage disequilibrium with the “Memphis I” polymorphism (AAG → GAG; Lys56Glu) (Delaunay, 2007). Only the heterozygotic form is known, which is asymptomatic and endemic in Southeast Asia (Malaysia, Papua New Guinea, the Philippines, etc.) giving it the name of southeast Asian ovalocytosis (SAO) (Fig. 2a) (Gallagher, 2005).

The ovalocytic phenotype is caused by a structural polymorphism in band 3. It has been established that a 9-amino-acid deletion (SAO band 3) on the cytoplasmatic face close to the N-terminal region of the membrane domain (residues 400–408) is responsible for the unusual properties of ovalocytic erythrocytes (Wang, 1994; Bruce, 2006).

Some studies have suggested that ovalocytic erythrocytes are very rigid and relatively resistant to invasion by various strains of malarial parasites “in vitro” (Kidson et al., 1981; Mohandas et al., 1984). More recent studies have shown that this condition confers highly specific protection against cerebral malaria by maintaining similar parasitemia levels (Genton et al., 1995; Allen et al., 1999). It has also been suggested that reduced band 3 deletion frequency in patients suffering from cerebral malaria could be taken as an indicator of a selective advantage (Genton et al., 1995). A post-invasion protective mechanism against cerebral malaria has been suggested since SAO does not significantly alter parasitemia. Changes in the SAO-infected binding to endothelial receptors have been studied since cerebral malaria is mediated by increased cerebral endothelial cell cytoadherence by *P. falciparum*-infected erythrocytes. However, contrary to what one would expect, SAO-infected cells display increased binding to CD36 in flow conditions “in vitro” when compared to normal infected erythrocytes (Cortes et al., 2005). Although CD36 is considered to be the main receptor for infected-erythrocyte sequestration (Cooke et al., 1994; Newbold et al., 1997), its levels of expression in brain endothelial cells are low (Turner et al., 1994; Silamut et al., 1999). According to these results, the protective mechanism against cerebral malaria conferred by SAO might be via *P. falciparum*-infected erythrocyte sequestration in vascular beds high in CD36 levels, such as those found in non-vital organs and tissues like the skin and muscles.

2.2.2. Duffy antigen

The Duffy antigen, also known as DARC (Duffy antigen receptor for chemokines) is normally expressed on the surface of erythrocytes and also in other cell types (Michon et al., 2001; Cooke et al., 2004; Min-Oo and Gros, 2005). This blood group antigen is a 35–43 kDa transmembrane glycoprotein spanning the membrane 7 times. Its encoding genetic locus displays three alleles; *FY*A* (Fy^a) and *FY*B* (Fy^b) differ from each other by a SNP that involves shifting a glycine for an aspartic acid in residue 44 (G131A) (Iwamoto et al., 1996; Michon et al., 2001), while *FY*O* corresponds to a negative serological phenotype Fy(a−b−) (Hamblin and Di Rienzo, 2000). This antigen is an obligatory binding receptor for *P. vivax* (Michon et al., 2001; Cooke et al., 2004; Min-Oo and Gros, 2005); the interaction being mediated by the parasite's Duffy binding protein (DBP).

Lack of DARC expression in erythrocytes is mediated by a point mutation (T46C) in the GATA box of this gene's promoter (Michon et al., 2001). The expressed phenotype is Fy(a−b−) and leads to resistance to *P. vivax* infection (Hamblin and Di Rienzo, 2000; Tamasauskas et al., 2001; Weatherall et al., 2002). Duffy negativity is most common in Western Africa (Gelpi and King, 1976; King et al., 2002) and Papua New Guinea (Zimmerman et al., 1999), which explains why *P. vivax* is quite uncommon in these geographical regions (Cooke et al., 2004) (Fig. 2b). A recent study shows how the *FY* gene expression modulates the susceptibility of baboons to infection by *Hepatocystis* parasites, which cause a disease similar to malaria in these non-human primates. Fy protein expression depends on differences in the cis regulatory region of the coding gene, near to where point mutations that hamper *FY* transcription in human RBCs (conferring protection against vivax malaria) have been described (Tung et al., 2009).

An additional point mutation leading to an amino-acid shift from arginine to cysteine in position 89 of the DARC intra-cytoplasmatic domain has also been described (Tamasauskas et al., 2001). This allele has been found in 3.5% of the population and produces decreased levels of DARC on the erythrocyte surface, lower antigen expression and reduced ability to bind chemokines. It is therefore possible that this mutation might confer certain resistance to *P. vivax* infection.

In previous binding assays using various DBP recombinant fragments, the DBP region interacting with DARC has been mapped to the 320-aminoacid-long 5′ cysteine-rich region (named region II) (Chitnis and Miller, 1994). Site directed mutagenesis has allowed to pinpoint residues in DBP important for its interaction with the Duffy antigen (VanBuskirk et al., 2004). Several of the residues are basic and might interact with sulfated tyrosine 41 on the DARC protein since desulfation of this tyrosine results in the abolition of binding to DBP

(Hans et al., 2005). De Brevern et al. (2005) have drawn up and analyzed structural DARC models by using multiple prediction methods and biological data. Such studies have led them to suggesting that the sulfated tyrosine is located in an extended random coil. All these data allowed us to build a putative model in which the interaction with sulfated Tyrosine 41 is central and which makes use of the binding site as suggested by Singh et al. (2006) (Fig. 3). The model is quite consistent with the experimental data but suggests a secondary site that is more hydrophobic. This site could encompass the N-terminal region of DARC as suggested by Tournamille et al. (2005).

2.3. Enzymopathies

2.3.1. Glucose-6-phosphate dehydrogenase (G6PD) deficiency

G6PD is a cytoplasmic enzyme that catalyses the first step in the hexose monophosphate pathway leading to synthesis of pentose phosphate (Ruwende and Hill, 1998). It also catalyses conversion of nicotinamide adenine dinucleotide phosphate (NADP) to its reduced form (NADPH), thus protecting erythrocytes from oxidative damage (Frank, 2005). This enzyme has 515 amino acids and is only active in its dimeric form (Beutler, 1994); G6PD activity has been shown to be reduced in infected erythrocytes when compared to uninfected erythrocytes.

The G6PD encoding gene is localized in the telomeric region of the X chromosome's long arm (Xq28) and hence one of the two G6PD alleles present in females is subject to inactivation. This gene comprises 13 exons (Ruwende and Hill, 1998) and displays different mutations varying among different populations (Mehta et al., 2000). More than 400 different variants of this gene have been described to date (Beutler, 1994; Frank, 2005).

G6PD deficiency is one of the most common enzymopathies in humans (Ruwende et al., 1995); it was discovered when investigating why some people developed hemolytic anemia when they ingested the 8-aminoquinoline antimalarial drug primaquine (Beutler, 1994). G6PD-deficient erythrocytes are more susceptible to destruction by oxidative stress than normal erythrocytes due to the lower NADPH levels found in them; although clinical presentations include acute or chronic hemolytic anemia and neonatal hyperbilirubinemia, G6PD-deficiency can remain asymptomatic and is rarely mortal (Frank, 2005). The frequency of this deficiency in the population is very high in tropical and subtropical regions around the world and in the Middle East (Min-Oo and Gros, 2005), which agrees with malaria's geographical distribution (Frank, 2005) (Fig. 2b).

The molecular basis of G6PD deficiency in protection against malaria has been studied "in vitro" with somewhat conflicting results (Smith et al., 2002; Min-Oo and Gros, 2005). Some studies have found reduced intracellular parasite growth on G6PD-deficient erythrocytes (Roth et al., 1983; Miller et al., 1984), while no difference has been reported in other studies. Recent studies have proposed a higher susceptibility of parasitized G6PD-deficient erythrocytes (mainly ring-stage parasites) to be phagocytosed by monocytes as an additional mechanism (Cappadoro et al., 1998; Ayi et al., 2004).

The parasite's G6PD enzyme is different to that of humans because it is bifunctional, presenting 6-phosphogluconolactonase (6PGL) activity in the molecule's N-terminal region. Its expression in *P. falciparum* is an important factor for parasite survival within the host cell. However, Sodeinde et al. (2003), have reported that the abundance of parasite *Pf*G6PD-6PGL mRNA is low in G6PD-deficient children.

Previous studies (Roth et al., 1983; Cappadoro et al., 1998) have proposed that the resistance to *P. falciparum* infection conferred by G6PD deficiency in heterozygous females and hemizygous males is a multi-step process. First, parasite invasion to either G6PD-deficient or normal erythrocytes is similar. Secondly, G6PD levels coming from both the erythrocyte and the parasite are low, especially in G6PD-deficient erythrocytes, which makes these parasitized cells prone to suffering damage. Damaged cells are highly susceptible to phagocytosis. Thirdly, although the G6PD levels produced by the parasite during both trophozoite and schizont stages are high, infected erythrocytes are physically so different compared to non-infected ones by this time that they are good targets for phagocytes, regardless of their G6PD status. Fourthly, phagocytosis consequently occurs earlier in G6PD-deficient parasitized erythrocytes than in normal parasitized ones, thus limiting the parasitemia.

In contrast to findings by Ruwende et al. (1995), a later study by Guindo et al. showed that the A− form of G6PD deficiency confers protection against severe malaria in its uniform state (hemizygous males and homozygous females) but not in its mosaic state (heterozygous females) (Guindo et al., 2007). This finding is consistent with those protection mechanisms involving either enhanced phagocytosis or effects on the pathogenic consequences in the microcirculation of parasitized erythrocytes, since both are expected to operate preferentially on uniformly deficient erythrocytes.

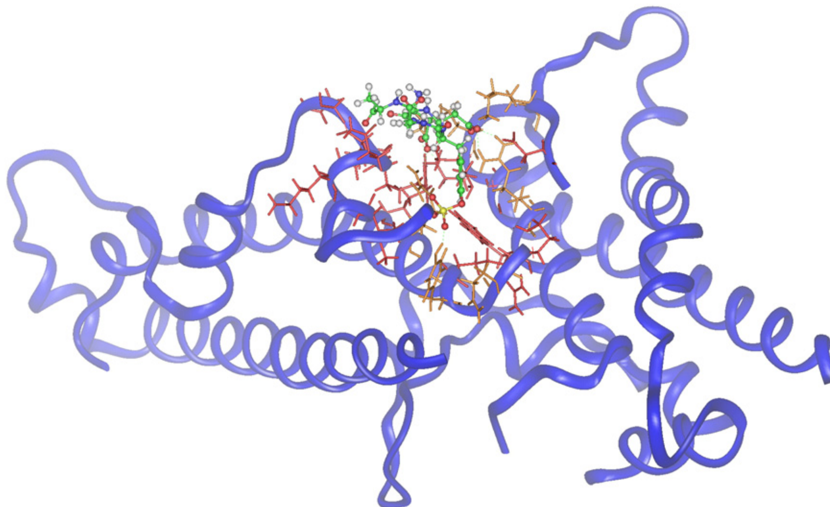

Fig. 3. Putative model of the *Plasmodium knowlesi* Duffy binding-like domain and a hexapeptide encompassing the Duffy antigen sulfated tyrosine (D-G-D-Y(SO3)-G-A) (aa 38–42). The binding domain was modeled starting from PDB2c6j (Singh et al., 2006). The peptide was constructed using the putative cationic binding site (Singh et al., 2006) to start building the peptide with the sulfated tyrosine. Conjugated gradient minimization and molecular dynamics were used to dock the peptide in an energetically favorable position. DBL secondary structure is shown as a blue ribbon, the peptide is shown as ball and sticks and the residues involved in the binding interaction are shown as sticks. Residues in orange correspond to those defined by mutational analyses (VanBuskirk et al., 2004) as being essential for binding.

2.3.2. Pyruvate kinase deficiency

Pyruvate kinase (PK) is a glycolytic enzyme which catalyses the irreversible conversion of phosphoenolpyruvate (PEP) to pyruvate. The PK-catalyzed reaction constitutes an important step in producing ATP in the glycolytic route. PK plays an important role in erythrocyte metabolism since these cells depend on ATP produced by glycolysis for maintaining cellular integrity and functionality. It has been found that PK activity becomes greatly increased in infected erythrocytes (Min-Oo et al., 2003). PK has also been recognized as a promising target for new drugs against *P. falciparum*, since the parasite's intra-erythrocytic life-cycle is highly dependent on glucose for obtaining energy (Chan and Sim, 2005; Zanella et al., 2007).

PK deficiency is the most frequent enzymatic abnormality of the glycolytic route commonly causing hereditary non-spherocytic hemolytic anemia. It has been found that the most frequent mutations (1529A and 1456T) are distributed according to ethnic and regional components. Such deficiency presents worldwide distribution, but is more prevalent in Caucasian populations (Fig. 2a) (Zanella et al., 2007).

Previous "in vivo" studies in mice (Min-Oo et al., 2003) have shown that PK deficiency confers protection to homozygote mice against infection with *Plasmodium chabaudi* parasites, thus suggesting a similar protective effect of PK deficiency in humans. Such protective effect has only been observed in humans until very recently. Durand and Coetzer (2008) evidenced that PK deficient human erythrocytes are resistant to "in vitro" malaria infection and suggested the existence of a driving force maintaining the polymorphic frequency of the abnormal allele. Ayi et al. (2008) reported a protective effect of PK deficiency against *P. falciparum* infection and replication inside human erythrocytes, possibly associated to an invasion defect of erythrocytes in subjects bearing the homozygous mutation and to a preferential macrophage clearance of ring-infected erythrocytes evidenced both in homozygous and heterozygous individuals.

The possible mechanisms by which such deficiency interferes with *Plasmodium*'s DNA replication in erythrocytes was suggested by Min-Oo et al. (2003). In their study, they showed that membrane rigidity affects parasite invasion and that the altered membrane properties result in shortening of the erythrocytes' half-life, regardless of whether they are infected or not. The reduction of the erythrocyte's half-life is due to increased phagocytosis and a great abundance of metabolic intermediates and oxidative species that generate a hostile intracellular environment. Also an altered reticulocyte: mature erythrocyte ratio may interfere with parasite replication and impairment of the parasite's intra-erythrocytic glucose metabolism.

2.4. Immunogenetic variants

2.4.1. HLA-Bw53/HLA-DRB1*1302-DQB1*0501

The human major histocompatibility complex (MHC) is one of the most important components of the immune system, being formed by multiple polymorphic genes (Babbitt et al., 1985; Buus et al., 1986; Jardetzky et al., 1990; Falk et al., 1991; Matsumura et al., 1992). It has been subdivided into three main groups (Class I, II and III). The first two classes are membrane-bound molecules able to activate T-lymphocytes to initiate or enhance an acquired immune response (Katz et al., 1973; Dausset, 1981; Bjorkman et al., 1987; Rothbard and Taylor, 1988; Fremont et al., 1992; Brown et al., 1993; Stern et al., 1994; Pieters, 1997), while class III genes encode soluble proteins such as the complement cascade proteins and some cytokines and heat shock proteins. MHC in humans is denoted as human leukocyte antigen (HLA) for its predominant expression in these cells (Abbas, 2003). Multiple different human class II loci have been identified. These have been named DP, DM, DO, DN, DQ and DR, and exhibit different degrees of allelic polymorphism (Hardy et al., 1986; Bjorkman and Parham, 1990; Mason and Parham, 1998).

Susceptibility to malaria has been shown in people having certain HLA class I and class II alleles (Weatherall et al., 2002). Studies carried out by Miller (1994) and Jarolim et al. (1991) have revealed that the HLA-DR system could play an important role in protection against malaria. Moreover, Hill et al. (1991, 1992) showed that carriers of the class I HLA antigen HLA-Bw53 (frequently occurring in sub-Saharan Africa) were protected against severe malaria and that there was a 14.7% reduction in cases of severe anemia and 16.1% reduction of cerebral malaria. These findings are supported by the data reported by Gilbert et al. (1998) and Wilkinson and Pasvol (1997). The class II HLA haplotype, DRB1*1302-DQB1*0501, is associated with reduced susceptibility to severe malaria in the population of Gambia in western Africa (Fig. 2b). This supports the hypothesis that MHC polymorphism is maintained by altering susceptibility to infectious pathogens.

Wilkinson and Pasvol (1997) suggested that protection against cerebral malaria and severe anemia are associated with the class I HLA antigen HLA-B53 and protection against anemia alone is conferred by the class II haplotype DRB1*1302-DQB1*0501; however, these data have still to be confirmed regarding other ethnic groups. Nevertheless, studies carried out by Modiano et al. (1996) on 3 west-African ethnic groups exposed to the same transmission conditions revealed that the differences obtained regarding susceptibility to the disease and parasitemia among the groups analyzed in such study could have been related to unknown genetic factors, possibly related to the humoral immune response and not to genetic resistance to malaria.

2.4.2. Complement receptor 1 (CR1) polymorphism

Complement receptor 1 (CR1, also called CD35) is a 190—280 kDa protein (Thathy et al., 2005), localized on erythrocyte surface, which belongs to the erythrocyte complement-regulatory protein group and is responsible for complement-regulatory properties on erythrocyte surface (Stoute, 2005). In the erythrocyte, this protein is responsible for removing immune complexes (ICs) from the circulation by binding to ICs containing C3b (molecule which can bind to the cell surface to facilitate macrophage phagocytosis) (Reinagel et al., 1997).

Some studies have suggested that CR1 is the *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) receptor and that the PfEMP-1 interaction with CR1 could be implicated in rosette formation (a phenomenon related to severe malaria in which erythrocytes infected by *P. falciparum* late stage parasites bind to uninfected erythrocytes "in vitro") (Rowe et al., 1997; Thathy et al., 2005; Williams, 2006). The CR1 encoding gene exhibits a number of size variants that might be the result of unequal gene crossover. These polymorphic variants have been related to different levels of CR1 expression on erythrocytes. Two antigens belonging to the Knops blood group, named Swain-langley (Sl) and McCoy (McC), have been mapped to the CR1 protein. Phenotypic absence of these antigens (correlated to low-CR1 copy numbers on the erythrocyte) is conferred by the *Sl2* and *McC*^{b} allelic variants commonly found in Africa (Stoute, 2005; Thathy et al., 2005). Since high levels of CR1 on the erythrocyte surface increase rosetting, and rosetting is associated with severe malaria, one would expect lower CR1 levels to confer protection against the severe form of the disease. However, unexpected and contradictory results have been obtained. Severe malaria was thus associated with low-level CR1 genotype and high-level genotype with rosette formation in a study carried out by Nagayasu et al. (2001). Thathy et al. (2005) have shown that low-CR1 phenotypes confer protection against cerebral malaria but not against malaria-associated anemia in Kenyan children. On the contrary, a study carried out by Zimmerman et al. (2003) in the Gambian population, it was reported that no association was observed between CR1 alleles and protection against severe malaria phenotypes.

2.4.3. Nitric oxide synthase 2 (NOS2) promoter polymorphism

The nitric oxide synthase 2 (NOS2) enzyme produces nitric oxide (NO) (free radical mediating several physiological processes in

immune-regulation) (Hobbs et al., 2002), which is implicated in innate immunity against malaria. The NOS2 encoding gene is localized in chromosome 17 and consists of 26 exons, its transcription site beginning in exon 2 and its stop codon in exon 26 (Chartrain et al., 1994; Coia et al., 2005).

Even though the molecular mechanisms responsible for protection against severe malaria remain elusive, some studies have suggested that polymorphism in the NOS2 gene promoter region does increase NO production and could thus be an antimalarial resistance mechanism. Perkins et al. (1999) measured NO production and NOS (NO synthase) activity in peripheral blood mononuclear cells (PBMCs) from Gabonese children having a history of prior mild malaria (PMM) or prior severe malaria (PSM) caused by *P. falciparum*. The study showed that the PMM group had significantly higher levels of NOS activity in isolated PBMCs, high NO production and NOS activity in cultured PBMCs (Kun et al., 2001). On the contrary, a study by Kremsner et al. (1996) revealed increased NO levels in plasma from patients suffering from severe *P. falciparum* malaria, high levels of NO production being associated with the disease's severity.

Kun et al. (1998) showed that a new NOS2 promoter mutation was restricted to highly malaria-endemic areas (Fig. 2b) and that this mutation was probably involved in antimalarial resistance, mainly in children, partly depending on their innate immune response.

In a recent study assessing the relation between NOS2 promoter SNPs and haplotypes with malaria severity in Tanzanian children, no consistent associations were found. Authors conclude that this cause–effect relationship might be more complex than previously thought (Levesque et al., 2010).

2.4.4. Tumor necrosis factor-α promoter polymorphism

Tumor necrosis factor-α (TNF-α) is a cytokine presenting a broad range of pro-inflammatory activities. It is mainly produced by monocytes and macrophages, even though it can also be produced in lesser amounts by other types of cells, such as T- and B-cells (Wilson et al., 1997). It plays an important role in inflammation and is relevant for autoimmune and infectious diseases (Bayley et al., 2004). It also acts in several cell systems, regulating the expression of adhesion molecules (Tchinda et al., 2007).

Several studies have suggested that TNF-α is the main mediator of secondary complications accompanying severe malaria caused by *P. falciparum* and that it could become fatal among people suffering from cerebral malaria, severe anemia, lactic acidosis and hypoglycemia (Odeh, 2001). Likewise, several experimental studies have demonstrated this cytokine's role in pathogenic malarial anemia, including severe disruption of erythropoiesis and erythroid cell suppression and proliferation (Tchinda et al., 2007).

Patients with cerebral malaria display increased TNF-α levels. Thus, two TNF-α promoter polymorphisms seem to be independently associated with cerebral malaria in infant populations from Gambia and Kenya (Africa) (Fig. 2b) (Odeh, 2001): children who were homozygous for TNF-308A presented increased susceptibility to cerebral malaria (Knight et al., 1999) and, in the same population, TNF-376A alleles were associated with increased susceptibility to developing cerebral malaria (Kwiatkowski, 2000). Studies related to a homozygous TNF-α variant have shown an increased risk of contracting cerebral malaria independently of their HLA alleles (Wilson et al., 1997). The immune-pathogenic mechanisms implicated in severe malaria are being studied; however, TNF-α seems to be very important in them (Tchinda et al., 2007).

2.4.5. Chromosome 5q31–q33

The 5q31–q33 region on chromosome 5 contains many genes with important functions regulating the immune response against pathogens such as granulocyte-macrophage colony-stimulating factor (CSF2), IL-3, IL-4. IL-5, IL-9, immune regulatory factor 1 (IRF1) and colony-stimulating factor 1 receptor (CSF-1R) (Garcia et al., 1998).

Some studies have tried to determine the importance of this region and its implication in controlling the immune response against the malarial parasite. Flori et al. (2003) used sib-pair linkage and association analysis to identify the "*pfbi*" (*P. falciparum* blood infection) locus and Rihet et al. (1998) used linkage analysis to identify the "*Pfil1*" (*P. falciparum* infection level 1) locus on chromosome 5q31–q33 that controls blood infection levels. The former studies and that by Garcia et al. (1998) on a population from Cameroon (Africa) (Fig. 2b), showed that the genes localized in this chromosome region could play a central role in controlling blood infection levels and could be essential in understanding mechanisms protecting humans against *P. falciparum* malaria. However, the genes involved in this process have not yet been clearly identified (Flori et al., 2003).

3. Conclusions

Malarial parasites have coevolved together with the human host for thousands of years (Jongwutiwes et al., 2005; Kwiatkowski, 2005), which have led them to constitute an important driving evolutionary force behind common erythrocyte variants (Kwiatkowski, 2000), such as thalassemia, sickle-cell disease, HbC, HbE, gluose-6-phosphate deficiency and other erythrocyte anomalies (Koella and Boete, 2003; Kwiatkowski, 2005). Host–parasite interactions have led to a host's relative resistance to the parasite and parasite strain-specific susceptibility or virulence (Boots and Haraguchi, 1999).

As has been shown in this review, the molecular bases of hemoglobinopathies vary greatly. However, some common characteristics can be found in support of the malaria hypothesis, such as the coincidence between the global distribution of hemoglobinopathies with that of malaria as well as the increase in various regionally specific mutations during the last 5000 years despite their different molecular origins, which points toward a common cause (Flint et al., 1998).

The different hemoglobinopathy-related alleles could be related to the beginnings of human colonization in different areas of the world, since activities such as agriculture, trade and fishing would have led to the parasite becoming dispersed to different geographical areas and thus to the fixation of new alleles related to such hemoglobinopathies in the population (reviewed by Flint et al., 1998; Kwiatkowski, 2005).

Some studies have shown that malaria-mediated evolutionary selection has involved 2 main aspects: strong selective pressure (e.g. in the case of the higher frequency HbS allele found in malaria-exposed populations) and independent evolutionary responses developed by different populations both at a global and local level. The best example at a global level could be given by the *HBB* gene in which 3 different SNPs (HbS, HbC and HbE) have been shown to confer protection against malaria because the mutations produced affect hemoglobin functionality (as mentioned above). At a local level it has been observed that some of the Dogon people from Mali present a very low frequency of the HbS allele, in contrast to other African groups, and a high frequency of the HbC allele frequency (Agarwal et al., 2000). However, four different HbS haplotypes have been found in Africa (Chebloune et al., 1988; Nagel and Ranney, 1990; Lapoumeroulie et al., 1992), which leads one to think that they could have had an independent origin from 4 different African populations (Kwiatkowski, 2005).

As different mechanisms conferring protection against malaria are widely found among different populations, it is plausible to suppose that populations have evolved and developed different genetic variants which are related to resistance to the disease. This, in turn, could imply that the maintenance of these alleles in the population has been due to the effects of positive selection arising from relatively recent human history (i.e. when human populations were migrating from Africa).

Even though the above could explain why mutations conferring malarial protection are highly variable and are maintained in the population, the association between malarial transmission and the occurrence of mutations is not always straightforward. An example can be given by some populations where no malaria has been found but hemoglobinopathies occur with relatively high frequency (i.e. Polynesia), while there are other malarial regions around the world where hemoglobinopathies are not common (i.e. South-America). The foregoing could be explained by the presence of random fluctuations in the genetic frequency of some hemoglobinopathies (i.e. α^+-thalassemia). It has been observed that in regions where there is no malaria negative selection does not act to eliminate the relatively benign mutation (hemoglobinopathy) from a particular population (such regions lack of the parasite's selective pressure for raising and maintaining high frequencies).

Several research groups have also shown that mutations conferring protection against malaria differ among different populations. However, studies carried out on the Pacific Island of Vanuatu (Ganczakowski et al., 1995) have revealed that similar mutation patterns are found between some neighboring islands, including those where malaria has not been found. This strange pattern fits in with current opinions about how and when humans colonized these islands since colonization took place in two stages (according to anthropological studies). The first stage took place during the Pleistocene age (>29,000 years ago) in New Guinea–Australia and the second began during the Holocene age (<3500 years ago) in the Bismarck Archipelago in islands of the far western Pacific. It has thus been suggested that the time during which man has inhabited these islands is proportional to the diversity and distribution of both mosquito and malaria (Lum et al., 2004), bearing in mind that each island was colonized by a small number of people thereby disturbing gene frequencies and that some groups of colonists could have had high allele frequencies while others had low ones. This has happened with α^+-thalassemia where it has been observed that such populations could present a variation in the frequencies of this disorder.

Most of the traits conferring protection against malaria have not been studied in depth. However, they do present important common characteristics such as the presence of mutations, genetic traits associated with malaria's geographical distribution, inhibition of parasite growth and increased phagocytosis (Roberts and Williams, 2003).

Although *P. falciparum* and *P. vivax*, the two most prevalent malarial parasite species co-exist in most parts of the world, distinctive parasite localization associated with specific host genetically-based protective mechanisms has been observed. *P. vivax* is not very common in western Africa as most of the population is negative for the Duffy blood group (Miller et al., 1976; Chitnis and Miller, 1994); a similar behavior can be observed on the northern coasts of South-America where there are descendants of slave populations brought from western Africa carrying *P. vivax* resistance with them. By contrast, *P. falciparum* presents a different geographical distribution (mainly in tropical areas of western Africa) that correlates with the distribution of some genetically-based resistance mechanisms (such as hemoglobinopathies, erythrocyte polymorphism, HLA alleles and NOS2 promoter polymorphism) presenting a protection-inducing effect against this parasite species in both severe and non-severe cases of malaria and in homozygotic or heterozygotic individuals (Hill et al., 1991, 1992; Allen et al., 1997; Burgner et al., 1998; Kun et al., 1998; Stirnadel et al., 1999).

The distinct geographical localization of the genetically-based alterations conferring protection against malaria has led to proposing that the various human populations have been co-evolving with the malarial parasites rather evolving differently. This co-evolutionary process has resulted in mutual benefits for both organisms; on one hand, it confers relative protection against parasite infection to the host and, on the other hand, it limits parasite virulence allowing it to remain within the host for longer periods of time.

Acknowledgments

This paper is dedicated to Aminta Perez de Gutierrez for encouraging us in our scientific careers. We would like to thank Jason Garry for translating the manuscript and Maria Cristina Rueda for creating the figures. We would also like to thank Professor Manuel Elkin Patarroyo for his invaluable comments and suggestions. Special thanks are given to the ECOS-Nord Colombian-French agreement (Project No. 00PFC29S03). This work was supported by the "Instituto Colombiano para el Desarrollo de la Ciencia 'Francisco Jose de Caldas' (Colciencias)", contract RC-2008.

References

Abbas, A.K., 2003. Cellular and Molecular Immunology, 5th ed. Saunders, Philadelphia.

Abu-Zeid, Y.A., Abdulhadi, N.H., Hviid, L., Theander, T.G., Saeed, B.O., Jepsen, S., Jensen, J.B., Bayoumi, R.A., 1991. Lymphoproliferative responses to *Plasmodium falciparum* antigens in children with and without the sickle cell trait. Scand. J. Immunol. 34, 237–242.

Ackerman, H., Usen, S., Jallow, M., Sisay-Joof, F., Pinder, M., Kwiatkowski, D.P., 2005. A comparison of case–control and family-based association methods: the example of sickle-cell and malaria. Ann. Hum. Genet. 69, 559–565.

Agarwal, A., Guindo, A., Cissoko, Y., Taylor, J.G., Coulibaly, D., Kone, A., Kayentao, K., Djimde, A., Plowe, C.V., Doumbo, O., Wellems, T.E., Diallo, D., 2000. Hemoglobin C associated with protection from severe malaria in the Dogon of Mali, a West African population with a low prevalence of hemoglobin S. Blood 96, 2358–2363.

Aidoo, M., Terlouw, D.J., Kolczak, M.S., McElroy, P.D., ter Kuile, F.O., Kariuki, S., Nahlen, B. L., Lal, A.A., Udhayakumar, V., 2002. Protective effects of the sickle cell gene against malaria morbidity and mortality. Lancet 359, 1311–1312.

Akide-Ndunge, O.B., Ayi, K., Arese, P., 2003. The Haldane malaria hypothesis: facts, artifacts, and a prophecy. Redox Rep. 8, 311–316.

Allen, S.J., O'Donnell, A., Alexander, N.D., Alpers, M.P., Peto, T.E., Clegg, J.B., Weatherall, D.J., 1997. Alpha+-Thalassemia protects children against disease caused by other infections as well as malaria. Proc. Natl. Acad. Sci. U. S. A. 94, 14736–14741.

Allen, S.J., O'Donnell, A., Alexander, N.D., Mgone, C.S., Peto, T.E., Clegg, J.B., Alpers, M.P., Weatherall, D.J., 1999. Prevention of cerebral malaria in children in Papua New Guinea by southeast Asian ovalocytosis band 3. Am. J. Trop. Med. Hyg. 60, 1056–1060.

Allison, A.C., 1964. Polymorphism and natural selection in human populations. Cold Spring Harb. Symp. Quant. Biol. 29, 137–149.

Ayi, K., Turrini, F., Piga, A., Arese, P., 2004. Enhanced phagocytosis of ring-parasitized mutant erythrocytes: a common mechanism that may explain protection against falciparum malaria in sickle trait and beta-thalassemia trait. Blood 104, 3364–3371.

Ayi, K., Min-Oo, G., Serghides, L., Crockett, M., Kirby-Allen, M., Quirt, I., Gros, P., Kain, K. C., 2008. Pyruvate kinase deficiency and malaria. N. Engl. J. Med. 358, 1805–1810.

Babbitt, B.P., Allen, P.M., Matsueda, G., Haber, E., Unanue, E.R., 1985. Binding of immunogenic peptides to Ia histocompatibility molecules. Nature 317, 359–361.

Bayley, J.P., Ottenhoff, T.H., Verweij, C.L., 2004. Is there a future for TNF promoter polymorphisms? Genes Immun. 5, 315–329.

Beutler, E., 1994. G6PD deficiency. Blood 84, 3613–3636.

Bjorkman, P.J., Parham, P., 1990. Structure, function, and diversity of class I major histocompatibility complex molecules. Annu. Rev. Biochem. 59, 253–288.

Bjorkman, P.J., Saper, M.A., Samraoui, B., Bennett, W.S., Strominger, J.L., Wiley, D.C., 1987. Structure of the human class I histocompatibility antigen, HLA-A2. Nature 329, 506–512.

Boots, M., Haraguchi, Y., 1999. The evolution of costly resistance in host–parasite systems. Am. Nat. 153, 359–370.

Bradbury, J., 2001. Haemoglobin C protection against malaria. Lancet 358, 1702.

Brockelman, C.R., Wongsattayanont, B., Tan-ariya, P., Fucharoen, S., 1987. Thalassemic erythrocytes inhibit in vitro growth of *Plasmodium falciparum*. J. Clin. Microbiol. 25, 56–60.

Brown, J., Smalley, M.E., 1980. Specific antibody-dependent cellular cytotoxicity in human malaria. Clin. Exp. Immunol. 41, 423–429.

Brown, J.H., Jardetzky, T.S., Gorga, J.C., Stern, L.J., Urban, R.G., Strominger, J.L., Wiley, D.C., 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature 364, 33–39.

Bruce, L., 2006. Mutations in band 3 and cation leaky red cells. Blood Cells Mol. Dis. 36, 331–336.

Bunyaratvej, A., Butthep, P., Sae-Ung, N., Fucharoen, S., Yuthavong, Y., 1992. Reduced deformability of thalassemic erythrocytes and erythrocytes with abnormal hemoglobins and relation with susceptibility to *Plasmodium falciparum* invasion. Blood 79, 2460–2463.

Burgner, D., Xu, W., Rockett, K., Gravenor, M., Charles, I.G., Hill, A.V., Kwiatkowski, D., 1998. Inducible nitric oxide synthase polymorphism and fatal cerebral malaria. Lancet 352, 1193–1194.

Buus, S., Sette, A., Colon, S.M., Jenis, D.M., Grey, H.M., 1986. Isolation and characterization of antigen-Ia complexes involved in T cell recognition. Cell 47, 1071–1077.

Cabrera, G., Cot, M., Migot-Nabias, F., Kremsner, P.G., Deloron, P., Luty, A.J., 2005. The sickle cell trait is associated with enhanced immunoglobulin G antibody responses to *Plasmodium falciparum* variant surface antigens. J. Infect. Dis. 191, 1631–1638.

Cappadoro, M., Giribaldi, G., O'Brien, E., Turrini, F., Mannu, F., Ulliers, D., Simula, G., Luzzatto, L., Arese, P., 1998. Early phagocytosis of glucose-6-phosphate dehydrogenase (G6PD)-deficient erythrocytes parasitized by *Plasmodium falciparum* may explain malaria protection in G6PD deficiency. Blood 92, 2527–2534.

Carvalho, L.J., Daniel-Ribeiro, C.T., Goto, H., 2002. Malaria vaccine: candidate antigens, mechanisms, constraints and prospects. Scand. J. Immunol. 56, 327–343.

Celada, A., Cruchaud, A., Perrin, L.H., 1982. Opsonic activity of human immune serum on in vitro phagocytosis of *Plasmodium falciparum* infected red blood cells by monocytes. Clin. Exp. Immunol. 47, 635–644.

Chan, M., Sim, T.S., 2005. Functional analysis, overexpression, and kinetic characterization of pyruvate kinase from *Plasmodium falciparum*. Biochem. Biophys. Res. Commun. 326, 188–196.

Chartrain, N.A., Geller, D.A., Koty, P.P., Sitrin, N.F., Nussler, A.K., Hoffman, E.P., Billiar, T.R., Hutchinson, N.I., Mudgett, J.S., 1994. Molecular cloning, structure, and chromosomal localization of the human inducible nitric oxide synthase gene. J. Biol. Chem. 269, 6765–6772.

Chebloune, Y., Pagnier, J., Trabuchet, G., Faure, C., Verdier, G., Labie, D., Nigon, V., 1988. Structural analysis of the 5′ flanking region of the beta-globin gene in African sickle cell anemia patients: further evidence for three origins of the sickle cell mutation in Africa. Proc. Natl. Acad. Sci. U. S. A. 85, 4431–4435.

Chitnis, C.E., Miller, L.H., 1994. Identification of the erythrocyte binding domains of *Plasmodium vivax* and *Plasmodium knowlesi* proteins involved in erythrocyte invasion. J. Exp. Med. 180, 497–506.

Cholera, R., Brittain, N.J., Gillrie, M.R., Lopera-Mesa, T.M., Diakite, S.A., Arie, T., Krause, M.A., Guindo, A., Tubman, A., Fujioka, H., Diallo, D.A., Doumbo, O.K., Ho, M., Wellems, T.E., Fairhurst, R.M., 2008. Impaired cytoadherence of *Plasmodium falciparum*-infected erythrocytes containing sickle hemoglobin. Proc. Natl. Acad. Sci. U. S. A. 105, 991–996.

Chotivanich, K., Udomsangpetch, R., Pattanapanyasat, K., Chierakul, W., Simpson, J., Looareesuwan, S., White, N., 2002. Hemoglobin E: a balanced polymorphism protective against high parasitemias and thus severe *P falciparum* malaria. Blood 100, 1172–1176.

Cockburn, I.A., Mackinnon, M.J., O'Donnell, A., Allen, S.J., Moulds, J.M., Baisor, M., Bockarie, M., Reeder, J.C., Rowe, J.A., 2004. A human complement receptor 1 polymorphism that reduces *Plasmodium falciparum* rosetting confers protection against severe malaria. Proc. Natl. Acad. Sci. U. S. A. 101, 272–277.

Coia, V., Juliger, S., Mordmuller, B., Kreidenweis, A., Stroh, A.L., Ortega, C., Vindigni, A., Dengjel, J., Lukyanov, D.V., Destro-Bisol, G., Fedorov, A., Podgornaya, O.I., Kun, J.F., 2005. Analysis of polymorphic sites in the promoter of the nitric oxide synthase 2 gene. Biochem. Biophys. Res. Commun. 335, 1123–1131.

Cooke, B.M., Berendt, A.R., Craig, A.G., MacGregor, J., Newbold, C.I., Nash, G.B., 1994. Rolling and stationary cytoadhesion of red blood cells parasitized by *Plasmodium falciparum*: separate roles for ICAM-1, CD36 and thrombospondin. Br. J. Haematol. 87, 162–170.

Cooke, B.M., Mohandas, N., Coppel, R.L., 2004. Malaria and the red blood cell membrane. Semin. Hematol. 41, 173–188.

Cortes, A., Mellombo, M., Mgone, C.S., Beck, H.P., Reeder, J.C., Cooke, B.M., 2005. Adhesion of *Plasmodium falciparum*-infected red blood cells to CD36 under flow is enhanced by the cerebral malaria-protective trait South-East Asian ovalocytosis. Mol. Biochem. Parasitol. 142, 252–257.

Das, S.K., Talukder, G., 2001. A review on the origin and spread of deleterious mutants of the beta-globin gene in Indian populations. Homo 52, 93–109.

Dausset, J., 1981. The major histocompatibility complex in man. Science 213, 1469–1474.

de Brevern, A.G., Wong, H., Tournamille, C., Colin, Y., Le Van Kim, C., Etchebest, C., 2005. A structural model of a seven-transmembrane helix receptor: the Duffy antigen/receptor for chemokine (DARC). Biochim. Biophys. Acta 1724, 288–306.

Delaunay, J., 2007. The molecular basis of hereditary red cell membrane disorders. Blood Rev. 21, 1–20.

Duffy, P.E., Fried, M., 2006. Red blood cells that do and red blood cells that don't: how to resist a persistent parasite. Trends Parasitol. 22, 99–101.

Durand, P.M., Coetzer, T.L., 2008. Pyruvate kinase deficiency protects against malaria in humans. Haematologica 93, 939–940.

Fairhurst, R.M., Baruch, D.I., Brittain, N.J., Ostera, G.R., Wallach, J.S., Hoang, H.L., Hayton, K., Guindo, A., Makobongo, M.O., Schwartz, O.M., Tounkara, A., Doumbo, O.K., Diallo, D.A., Fujioka, H., Ho, M., Wellems, T.E., 2005. Abnormal display of PfEMP-1 on erythrocytes carrying haemoglobin C may protect against malaria. Nature 435, 1117–1121.

Falk, K., Rotzschke, O., Stevanovic, S., Jung, G., Rammensee, H.G., 1991. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. Nature 351, 290–296.

Flint, J., Harding, R.M., Boyce, A.J., Clegg, J.B., 1998. The population genetics of the haemoglobinopathies. Baillieres Clin. Haematol. 11, 1–51.

Flori, L., Kumulungui, B., Aucan, C., Esnault, C., Traore, A.S., Fumoux, F., Rihet, P., 2003. Linkage and association between *Plasmodium falciparum* blood infection levels and chromosome 5q31–q33. Genes Immun. 4, 265–268.

Frank, J.E., 2005. Diagnosis and management of G6PD deficiency. Am. Fam. Physician 72, 1277–1282.

Fremont, D.H., Matsumura, M., Stura, E.A., Peterson, P.A., Wilson, I.A., 1992. Crystal structures of two viral peptides in complex with murine MHC class I H-2Kb. Science 257, 919–927.

Friedman, M.J., 1978. Erythrocytic mechanism of sickle cell resistance to malaria. Proc. Natl. Acad. Sci. U. S. A. 75, 1994–1997.

Friedman, M.J., Roth, E.F., Nagel, R.L., Trager, W., 1979. The role of hemoglobins C, S, and Nbalt in the inhibition of malaria parasite development *in vitro*. Am. J. Trop. Med. Hyg. 28, 777–780.

Gabriel, J., Berzins, K., 1983. Specific lysis of *Plasmodium yoelii* infected mouse erythrocytes with antibody and complement. Clin. Exp. Immunol. 52, 129–134.

Gallagher, P.G., 2005. Red cell membrane disorders. Hematology Am. Soc. Hematol. Educ. Program 1, 13–18.

Ganczakowski, M., Bowden, D.K., Maitland, K., Williams, T.N., O'Shaughnessy, D., Viji, J., Lucassen, A., Clegg, J.B., Weatherall, D.J., 1995. Thalassaemia in Vanuatu, south-west Pacific: frequency and haematological phenotypes of young children. Br. J. Haematol. 89, 485–495.

Garcia, A., Marquet, S., Bucheton, B., Hillaire, D., Cot, M., Fievet, N., Dessein, A.J., Abel, L., 1998. Linkage analysis of blood *Plasmodium falciparum* levels: interest of the 5q31–q33 chromosome region. Am. J. Trop. Med. Hyg. 58, 705–709.

Gelpi, A.P., King, M.C., 1976. Association of Duffy blood groups with the sickle cell trait. Hum. Genet. 32, 65–68.

Genton, B., al-Yaman, F., Mgone, C.S., Alexander, N., Paniu, M.M., Alpers, M.P., Mokela, D., 1995. Ovalocytosis and cerebral malaria. Nature 378, 564–565.

Gilbert, S.C., Plebanski, M., Gupta, S., Morris, J., Cox, M., Aidoo, M., Kwiatkowski, D., Greenwood, B.M., Whittle, H.C., Hill, A.V., 1998. Association of malaria parasite population structure, HLA, and immunological antagonism. Science 279, 1173–1177.

Guindo, A., Fairhurst, R.M., Doumbo, O.K., Wellems, T.E., Diallo, D.A., 2007. X-linked G6PD deficiency protects hemizygous males but not heterozygous females against severe malaria. PLoS Med. 4, 0516–0522.

Haldane, J.B.S., 1949. The rate of mutation of human genes. Hereditas Suppl. 35, 267–273.

Hamblin, M.T., Di Rienzo, A., 2000. Detection of the signature of natural selection in humans: evidence from the Duffy blood group locus. Am. J. Hum. Genet. 66, 1669–1679.

Hans, D., Pattnaik, P., Bhattacharyya, A., Shakri, A.R., Yazdani, S.S., Sharma, M., Choe, H., Farzan, M., Chitnis, C.E., 2005. Mapping binding residues in the *Plasmodium vivax* domain that binds Duffy antigen during red cell invasion. Mol. Microbiol. 55, 1423–1434.

Hardy, D.A., Bell, J.I., Long, E.O., Lindsten, T., McDevitt, H.O., 1986. Mapping of the class II region of the human major histocompatibility complex by pulsed-field gel electrophoresis. Nature 323, 453–455.

Hill, A.V., Allsopp, C.E., Kwiatkowski, D., Anstey, N.M., Twumasi, P., Rowe, P.A., Bennett, S., Brewster, D., McMichael, A.J., Greenwood, B.M., 1991. Common west African HLA antigens are associated with protection from severe malaria. Nature 352, 595–600.

Hill, A.V., Elvin, J., Willis, A.C., Aidoo, M., Allsopp, C.E., Gotch, F.M., Gao, X.M., Takiguchi, M., Greenwood, B.M., Townsend, A.R., et al., 1992. Molecular analysis of the association of HLA-B53 and resistance to severe malaria. Nature 360, 434–439.

Hobbs, M.R., Udhayakumar, V., Levesque, M.C., Booth, J., Roberts, J.M., Tkachuk, A.N., Pole, A., Coon, H., Kariuki, S., Nahlen, B.L., Mwaikambo, E.D., Lal, A.L., Granger, D.L., Anstey, N.M., Weinberg, J.B., 2002. A new NOS2 promoter polymorphism associated with increased nitric oxide production and protection from severe malaria in Tanzanian and Kenyan children. Lancet 360, 1468–1475.

Hutagalung, R., Wilairatana, P., Looareesuwan, S., Brittenham, G.M., Aikawa, M., Gordeuk, V.R., 1999. Influence of hemoglobin E trait on the severity of Falciparum malaria. J. Infect. Dis. 179, 283–286.

Hutagalung, R., Wilairatana, P., Looareesuwan, S., Brittenham, G.M., Gordeuk, V.R., 2000. Influence of hemoglobin E trait on the antimalarial effect of artemisinin derivatives. J. Infect. Dis. 181, 1513–1516.

Iwamoto, S., Li, J., Sugimoto, N., Okuda, H., Kajii, E., 1996. Characterization of the Duffy gene promoter: evidence for tissue-specific abolishment of expression in Fy(a−b−) of black individuals. Biochem. Biophys. Res. Commun. 222, 852–859.

Jardetzky, T.S., Gorga, J.C., Busch, R., Rothbard, J., Strominger, J.L., Wiley, D.C., 1990. Peptide binding to HLA-DR1: a peptide with most residues substituted to alanine retains MHC binding. EMBO J. 9, 1797–1803.

Jarolim, P., Palek, J., Amato, D., Hassan, K., Sapak, P., Nurse, G.T., Rubin, H.L., Zhai, S., Sahr, K.E., Liu, S.C., 1991. Deletion in erythrocyte band 3 gene in malaria-resistant Southeast Asian ovalocytosis. Proc. Natl. Acad. Sci. U. S. A. 88, 11022–11026.

Jongwutiwes, S., Putaporntip, C., Iwasaki, T., Ferreira, M.U., Kanbara, H., Hughes, A.L., 2005. Mitochondrial genome sequences support ancient population expansion in *Plasmodium vivax*. Mol. Biol. Evol. 22, 1733–1739.

Katz, D.H., Hamaoka, T., Dorf, M.E., Benacerraf, B., 1973. Cell interactions between histoincompatible T and B lymphocytes. The H-2 gene complex determines successful physiologic lymphocyte interactions. Proc. Natl. Acad. Sci. U. S. A. 70, 2624–2628.

Kidson, C., Lamont, G., Saul, A., Nurse, G.T., 1981. Ovalocytic erythrocytes from Melanesians are resistant to invasion by malaria parasites in culture. Proc. Natl. Acad. Sci. U. S. A. 78, 5829–5832.

King, C.L., Malhotra, I., Wamachi, A., Kioko, J., Mungai, P., Wahab, S.A., Koech, D., Zimmerman, P., Ouma, J., Kazura, J.W., 2002. Acquired immune responses to *Plasmodium falciparum* merozoite surface protein-1 in the human fetus. J. Immunol. 168, 356–364.

Knight, J.C., Udalova, I., Hill, A.V., Greenwood, B.M., Peshu, N., Marsh, K., Kwiatkowski, D., 1999. A polymorphism that affects OCT-1 binding to the TNF promoter region is associated with severe malaria. Nat. Genet. 22, 145–150.

Koella, J.C., Boete, C., 2003. A model for the coevolution of immunity and immune evasion in vector-borne diseases with implications for the epidemiology of malaria. Am. Nat. 161, 698–707.

Kremsner, P.G., Winkler, S., Wildling, E., Prada, J., Bienzle, U., Graninger, W., Nussler, A. K., 1996. High plasma levels of nitrogen oxides are associated with severe disease and correlate with rapid parasitological and clinical cure in *Plasmodium falciparum* malaria. Trans. R. Soc. Trop. Med. Hyg. 90, 44–47.

Kun, J.F., Mordmuller, B., Lell, B., Lehman, L.G., Luckner, D., Kremsner, P.G., 1998. Polymorphism in promoter region of inducible nitric oxide synthase gene and protection against malaria. Lancet 351, 265–266.

Kun, J.F., Mordmuller, B., Perkins, D.J., May, J., Mercereau-Puijalon, O., Alpers, M., Weinberg, J.B., Kremsner, P.G., 2001. Nitric oxide synthase 2(Lambarene) (G-954C),

increased nitric oxide production, and protection against malaria. J. Infect. Dis. 184, 330–336.

Kwiatkowski, D., 2000. Genetic susceptibility to malaria getting complex. Curr. Opin. Genet. Dev. 10, 320–324.

Kwiatkowski, D.P., 2005. How malaria has affected the human genome and what human genetics can teach us about malaria. Am. J. Hum. Genet. 77, 171–192.

Lapoumeroulie, C., Dunda, O., Ducrocq, R., Trabuchet, G., Mony-Lobe, M., Bodo, J.M., Carnevale, P., Labie, D., Elion, J., Krishnamoorthy, R., 1992. A novel sickle cell mutation of yet another origin in Africa: the Cameroon type. Hum. Genet. 89, 333–337.

Levesque, M.C., Hobbs, M.R., O'Loughlin, C.W., Chancellor, J.A., Chen, Y., Tkachuk, A.N., Booth, J., Patch, K.B., Allgood, S., Pole, A.R., Fernandez, C.A., Mwaikambo, E.D., Mutabingwa, T.K., Fried, M., Sorensen, B., Duffy, P.E., Granger, D.L., Anstey, N.M., Weinberg, J.B., 2010. Malaria severity and human nitric oxide synthase type 2 (NOS2) promoter haplotypes. Hum. Genet. 127, 163–182.

Lum, J.K., Kaneko, A., Tanabe, K., Takahashi, N., Bjorkman, A., Kobayakawa, T., 2004. Malaria dispersal among islands: human mediated *Plasmodium falciparum* gene flow in Vanuatu, Melanesia. Acta Trop. 90, 181–185.

Luzzi, G.A., Merry, A.H., Newbold, C.I., Marsh, K., Pasvol, G., Weatherall, D.J., 1991. Surface antigen expression on *Plasmodium falciparum*-infected erythrocytes is modified in alpha- and beta-thalassemia. J. Exp. Med. 173, 785–791.

Mason, P.M., Parham, P., 1998. HLA class I region sequences, 1998. Tissue Antigens 51, 417–466.

Matsumura, M., Fremont, D.H., Peterson, P.A., Wilson, I.A., 1992. Emerging principles for the recognition of peptide antigens by MHC class I molecules. Science 257, 927–934.

Mehta, A., Mason, P.J., Vulliamy, T.J., 2000. Glucose-6-phosphate dehydrogenase deficiency. Baillieres Best Pract. Res. Clin. Haematol. 13, 21–38.

Michon, P., Woolley, I., Wood, E.M., Kastens, W., Zimmerman, P.A., Adams, J.H., 2001. Duffy-null promoter heterozygosity reduces DARC expression and abrogates adhesion of the *P. vivax* ligand required for blood-stage infection. FEBS Lett. 495, 111–114.

Miller, L.H., 1994. Impact of malaria on genetic polymorphism and genetic diseases in Africans and African Americans. Proc. Natl. Acad. Sci. U. S. A. 91, 2415–2419.

Miller, L.H., Mason, S.J., Clyde, D.F., McGinniss, M.H., 1976. The resistance factor to *Plasmodium vivax* in blacks. The Duffy-blood-group genotype, FyFy. N. Engl. J. Med. 295, 302–304.

Miller, J., Golenser, J., Spira, D.T., Kosower, N.S., 1984. *Plasmodium falciparum*: thiol status and growth in normal and glucose-6-phosphate dehydrogenase deficient human erythrocytes. Exp. Parasitol. 57, 239–247.

Min-Oo, G., Gros, P., 2005. Erythrocyte variants and the nature of their malaria protective effect. Cell. Microbiol. 7, 753–763.

Min-Oo, G., Fortin, A., Tam, M.F., Nantel, A., Stevenson, M.M., Gros, P., 2003. Pyruvate kinase deficiency in mice protects against malaria. Nat. Genet. 35, 357–362.

Mockenhaupt, F.P., May, J., Bergqvist, Y., Meyer, C.G., Falusi, A.G., Bienzle, U., 2001. Evidence for a reduced effect of chloroquine against *Plasmodium falciparum* in alpha-thalassaemic children. Trop. Med. Int. Health 6, 102–107.

Mockenhaupt, F.P., Ehrhardt, S., Cramer, J.P., Otchwemah, R.N., Anemana, S.D., Goltz, K., Mylius, F., Dietz, E., Eggelte, T.A., Bienzle, U., 2004a. Hemoglobin C and resistance to severe malaria in Ghanaian children. J. Infect. Dis. 190, 1006–1009.

Mockenhaupt, F.P., Ehrhardt, S., Gellert, S., Otchwemah, R.N., Dietz, E., Anemana, S.D., Bienzle, U., 2004b. Alpha(+)-thalassemia protects African children from severe malaria. Blood 104, 2003–2006.

Mockenhaupt, F.P., Ehrhardt, S., Otchwemah, R., Eggelte, T.A., Anemana, S.D., Stark, K., Bienzle, U., Kohne, E., 2004c. Limited influence of haemoglobin variants on *Plasmodium falciparum* msp1 and msp2 alleles in symptomatic malaria. Trans. R. Soc. Trop. Med. Hyg. 98, 302–310.

Modiano, D., Petrarca, V., Sirima, B.S., Nebie, I., Diallo, D., Esposito, F., Coluzzi, M., 1996. Different response to *Plasmodium falciparum* malaria in west African sympatric ethnic groups. Proc. Natl. Acad. Sci. U. S. A. 93, 13206–13211.

Modiano, D., Luoni, G., Sirima, B.S., Simpore, J., Verra, F., Konate, A., Rastrelli, E., Olivieri, A., Calissano, C., Paganotti, G.M., D'Urbano, L., Sanou, I., Sawadogo, A., Modiano, G., Coluzzi, M., 2001. Haemoglobin C protects against clinical *Plasmodium falciparum* malaria. Nature 414, 305–308.

Mohan, K., Stevenson, M.M., 1998. Acquired immunity to asexual blood stages. In: Sherman, I.W. (Ed.), Malaria: Parasite Biology, Pathogenesis and Protection. ASM press, Washington, D. C, pp. 467–493.

Mohandas, N., Lie-Injo, L.E., Friedman, M., Mak, J.W., 1984. Rigid membranes of Malayan ovalocytes: a likely genetic barrier against malaria. Blood 63, 1385–1392.

Nagayasu, E., Ito, M., Akaki, M., Nakano, Y., Kimura, M., Looareesuwan, S., Aikawa, M., 2001. CR1 density polymorphism on erythrocytes of falciparum malaria patients in Thailand. Am. J. Trop. Med. Hyg. 64, 1–5.

Nagel, R.L., Ranney, H.M., 1990. Genetic epidemiology of structural mutations of the beta-globin gene. Semin. Hematol. 27, 342–359.

Nagel, R.L., Raventos-Suarez, C., Fabry, M.E., Tanowitz, H., Sicard, D., Labie, D., 1981. Impairment of the growth of *Plasmodium falciparum* in HbEE erythrocytes. J. Clin. Invest. 68, 303–305.

Newbold, C., Warn, P., Black, G., Berendt, A., Craig, A., Snow, B., Msobo, M., Peshu, N., Marsh, K., 1997. Receptor-specific adhesion and clinical disease in *Plasmodium falciparum*. Am. J. Trop. Med. Hyg. 57, 389–398.

Oaks, S.C., Mitchell, V.S., Pearson, G.W., Carpenter, C.C.J., 1991. Malaria: Obstacles and Opportunities.

Odeh, M., 2001. The role of tumour necrosis factor-alpha in the pathogenesis of complicated falciparum malaria. Cytokine 14, 11–18.

Ohashi, J., Naka, I., Patarapotikul, J., Hananantachai, H., Brittenham, G., Looareesuwan, S., Clark, A.G., Tokunaga, K., 2004. Extended linkage disequilibrium surrounding the hemoglobin E variant due to malarial selection. Am. J. Hum. Genet. 74, 1198–1208.

Olson, J.A., Nagel, R.L., 1986. Synchronized cultures of *P falciparum* in abnormal red cells: the mechanism of the inhibition of growth in HbCC cells. Blood 67, 997–1001.

Olumese, P., 2005. Epidemiology and surveillance: changing the global picture of malaria—myth or reality? Acta Trop. 95, 265–269.

Pasvol, G., 2006. Does alpha+-thalassaemia protect against malaria? PLoS Med. 3, 0591–0592.

Pasvol, G., Weatherall, D.J., Wilson, R.J., Smith, D.H., Gilles, H.M., 1976. Fetal haemoglobin and malaria. Lancet 1, 1269–1272.

Pasvol, G., Weatherall, D.J., Wilson, R.J., 1978. Cellular mechanism for the protective effect of haemoglobin S against *P. falciparum* malaria. Nature 274, 701–703.

Patrinos, G.P., Kollia, P., Papadakis, M.N., 2005. Molecular diagnosis of inherited disorders: lessons from hemoglobinopathies. Hum. Mutat. 26, 399–412.

Perkins, D.J., Kremsner, P.G., Schmid, D., Misukonis, M.A., Kelly, M.A., Weinberg, J.B., 1999. Blood mononuclear cell nitric oxide production and plasma cytokine levels in healthy gabonese children with prior mild or severe malaria. Infect. Immun. 67, 4977–4981.

Phillips, R.S., 2001. Current status of malaria and potential for control. Clin. Microbiol. Rev. 14, 208–226.

Pieters, J., 1997. MHC class II restricted antigen presentation. Curr. Opin. Immunol. 9, 89–96.

Reinagel, M.L., Gezen, M., Ferguson, P.J., Kuhn, S., Martin, E.N., Taylor, R.P., 1997. The primate erythrocyte complement receptor (CR1) as a privileged site: binding of immunoglobulin G to erythrocyte CR1 does not target erythrocytes for phagocytosis. Blood 89, 1068–1077.

Richer, J., Chudley, A.E., 2005. The hemoglobinopathies and malaria. Clin. Genet. 68, 332–336.

Rihet, P., Traore, Y., Abel, L., Aucan, C., Traore-Leroux, T., Fumoux, F., 1998. Malaria in humans: *Plasmodium falciparum* blood infection levels are linked to chromosome 5q31–q33. Am. J. Hum. Genet. 63, 498–505.

Rihet, P., Flori, L., Tall, F., Traore, A.S., Fumoux, F., 2004. Hemoglobin C is associated with reduced *Plasmodium falciparum* parasitemia and low risk of mild malaria attack. Hum. Mol. Genet. 13, 1–6.

Roberts, D.J., Williams, T.N., 2003. Haemoglobinopathies and resistance to malaria. Redox Rep. 8, 304–310.

Roth Jr., E.F., Raventos-Suarez, C., Rinaldi, A., Nagel, R.L., 1983. Glucose-6-phosphate dehydrogenase deficiency inhibits *in vitro* growth of *Plasmodium falciparum*. Proc. Natl. Acad. Sci. U. S. A. 80, 298–299.

Rothbard, J.B., Taylor, W.R., 1988. A sequence pattern common to T cell epitopes. EMBO J. 7, 93–100.

Rowe, J.A., Moulds, J.M., Newbold, C.I., Miller, L.H.P., 1997. *falciparum* rosetting mediated by a parasite-variant erythrocyte membrane protein and complement-receptor 1. Nature 388, 292–295.

Ruwende, C., Hill, A., 1998. Glucose-6-phosphate dehydrogenase deficiency and malaria. J. Mol. Med. 76, 581–588.

Ruwende, C., Khoo, S.C., Snow, R.W., Yates, S.N., Kwiatkowski, D., Gupta, S., Warn, P., Allsopp, C.E., Gilbert, S.C., Peschu, N., et al., 1995. Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. Nature 376, 246–249.

Silamut, K., Phu, N.H., Whitty, C., Turner, G.D., Louwrier, K., Mai, N.T., Simpson, J.A., Hien, T.T., White, N.J., 1999. A quantitative analysis of the microvascular sequestration of malaria parasites in the human brain. Am. J. Pathol. 155, 395–410.

Singh, S.K., Hora, R., Belrhali, H., Chitnis, C.E., Sharma, A., 2006. Structural basis for Duffy recognition by the malaria parasite Duffy-binding-like domain. Nature 439, 741–744.

Smith, T.G., Ayi, K., Serghides, L., McAllister, C.D., Kain, K.C., 2002. Innate immunity to malaria caused by *Plasmodium falciparum*. Clin. Invest. Med. 25, 262–272.

Sodeinde, O., Clarke, J.L., Vulliamy, T.J., Luzzatto, L., Mason, P.J., 2003. Expression of *Plasmodium falciparum* G6PD-6PGL in laboratory parasites and in patient isolates in G6PD-deficient and normal Nigerian children. Br. J. Haematol. 122, 662–668.

Stern, L.J., Brown, J.H., Jardetzky, T.S., Gorga, J.C., Urban, R.G., Strominger, J.L., Wiley, D.C., 1994. Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. Nature 368, 215–221.

Stevenson, M.M., Riley, E.M., 2004. Innate immunity to malaria. Nat. Rev. Immunol. 4, 169–180.

Stirnadel, H.A., Stockle, M., Felger, I., Smith, T., Tanner, M., Beck, H.P., 1999. Malaria infection and morbidity in infants in relation to genetic polymorphisms in Tanzania. Trop. Med. Int. Health 4, 187–193.

Stoute, J.A., 2005. Complement-regulatory proteins in severe malaria: too little or too much of a good thing? Trends Parasitol. 21, 218–223.

Suh, K.N., Kain, K.C., Keystone, J.S., 2004. Malaria. Cmaj 170, 1693–1702.

Tamasauskas, D., Powell, V., Saksela, K., Yazdanbakhsh, K., 2001. A homologous naturally occurring mutation in Duffy and CCR5 leading to reduced receptor expression. Blood 97, 3651–3654.

Tchinda, V.H., Tadem, A.D., Tako, E.A., Tene, G., Fogako, J., Nyonglema, P., Sama, G., Zhou, A., Leke, R.G., 2007. Severe malaria in Cameroonian children: correlation between plasma levels of three soluble inducible adhesion molecules and TNF-alpha. Acta Trop. 102, 20–28.

Thathy, V., Moulds, J.M., Guyah, B., Otieno, W., Stoute, J.A., 2005. Complement receptor 1 polymorphisms associated with resistance to severe malaria in Kenya. Malar. J. 4, 54.

Tournamille, C., Filipe, A., Badaut, C., Riottot, M.M., Longacre, S., Cartron, J.P., Le Van Kim, C., Colin, Y., 2005. Fine mapping of the Duffy antigen binding site for the *Plasmodium vivax* Duffy-binding protein. Mol. Biochem. Parasitol. 144, 100–103.

Tung, J., Primus, A., Bouley, A.J., Severson, T.F., Alberts, S.C., Wray, G.A., 2009. Evolution of a malaria resistance gene in wild primates. Nature 460, 388–391.

Turner, G.D., Morrison, H., Jones, M., Davis, T.M., Looareesuwan, S., Buley, I.D., Gatter, K.C., Newbold, C.I., Pukritayakamee, S., Nagachinta, B., et al., 1994. An immunohistochemical study of the pathology of fatal malaria. Evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. Am. J. Pathol. 145, 1057–1069.
Udeinya, I.J., Miller, L.H., McGregor, I.A., Jensen, J.B., 1983. *Plasmodium falciparum* strain-specific antibody blocks binding of infected erythrocytes to amelanotic melanoma cells. Nature 303, 429–431.
VanBuskirk, K.M., Sevova, E., Adams, J.H., 2004. Conserved residues in the *Plasmodium vivax* Duffy-binding protein ligand domain are critical for erythrocyte receptor recognition. Proc. Natl. Acad. Sci. U. S. A. 101, 15754–15759.
Vento, S., Cainelli, F., Cesario, F., 2006. Infections and thalassaemia. Lancet Infect. Dis. 6, 226–233.
Wambua, S., Mwangi, T.W., Kortok, M., Uyoga, S.M., Macharia, A.W., Mwacharo, J.K., Weatherall, D.J., Snow, R.W., Marsh, K., Williams, T.N., 2006. The effect of alpha+-thalassaemia on the incidence of malaria and other diseases in children living on the coast of Kenya. PLoS Med. 3, 0643–0651.
Wang, D.N., 1994. Band 3 protein: structure, flexibility and function. FEBS Lett. 346, 26–31.
Weatherall, D.J., 1987. Common genetic disorders of the red cell and the 'malaria hypothesis'. Ann. Trop. Med. Parasitol. 81, 539–548.
Weatherall, D.J., 1997. Thalassaemia and malaria, revisited. Ann. Trop. Med. Parasitol. 91, 885–890.
Weatherall, D.J., 2000. Single gene disorders or complex traits: lessons from the thalassaemias and other monogenic diseases. Bmj 321, 1117–1120.
Weatherall, D.J., 2008. Genetic variation and susceptibility to infection: the red cell and malaria. Br. J. Haematol. 141, 276–286.
Weatherall, D.J., Provan, A.B., 2000. Red cells I: inherited anaemias. Lancet 355, 1169–1175.
Weatherall, D.J., Miller, L.H., Baruch, D.I., Marsh, K., Doumbo, O.K., Casals-Pascual, C., Roberts, D.J., 2002. Malaria and the red cell. Hematology Am. Soc. Hematol. Educ. Program 1, 35–57.
WHO and UNICEF, 2008. World Malaria Report 2008.
Wilkinson, R.J., Pasvol, G., 1997. Host resistance to malaria runs into swampy water. Trends Microbiol. 5, 213–215.
Willcox, M., Bjorkman, A., Brohult, J., 1983. Falciparum malaria and beta-thalassaemia trait in northern Liberia. Ann. Trop. Med. Parasitol. 77, 335–347.
Williams, T.N., 2006. Red blood cell defects and malaria. Mol. Biochem. Parasitol. 149, 121–127.
Williams, T.N., Maitland, K., Bennett, S., Ganczakowski, M., Peto, T.E., Newbold, C.I., Bowden, D.K., Weatherall, D.J., Clegg, J.B., 1996. High incidence of malaria in alpha-thalassaemic children. Nature 383, 522–525.
Williams, T.N., Mwangi, T.W., Roberts, D.J., Alexander, N.D., Weatherall, D.J., Wambua, S., Kortok, M., Snow, R.W., Marsh, K., 2005c. An immune basis for malaria protection by the sickle cell trait. PLoS Med. 2, e128.
Williams, T.N., Mwangi, T.W., Wambua, S., Alexander, N.D., Kortok, M., Snow, R.W., Marsh, K., 2005a. Sickle cell trait and the risk of *Plasmodium falciparum* malaria and other childhood diseases. J. Infect. Dis. 192, 178–186.
Williams, T.N., Mwangi, T.W., Wambua, S., Peto, T.E., Weatherall, D.J., Gupta, S., Recker, M., Penman, B.S., Uyoga, S., Macharia, A., Mwacharo, J.K., Snow, R.W., Marsh, K., 2005b. Negative epistasis between the malaria-protective effects of alpha+-thalassemia and the sickle cell trait. Nat. Genet. 37, 1253–1257.
Wilson, A.G., Symons, J.A., McDowell, T.L., McDevitt, H.O., Duff, G.W., 1997. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc. Natl. Acad. Sci. U. S. A. 94, 3195–3199.
Yuthavong, Y., Wilairat, P., 1993. Protection against malaria by thalassaemia and haemoglobin variants. Parasitol. Today 9, 241–245.
Zanella, A., Fermo, E., Bianchi, P., Chiarelli, L.R., Valentini, G., 2007. Pyruvate kinase deficiency: the genotype–phenotype association. Blood Rev. 21, 217–231.
Zimmerman, P.A., Woolley, I., Masinde, G.L., Miller, S.M., McNamara, D.T., Hazlett, F., Mgone, C.S., Alpers, M.P., Genton, B., Boatin, B.A., Kazura, J.W., 1999. Emergence of FY*A(null) in a *Plasmodium vivax*-endemic region of Papua New Guinea. Proc. Natl. Acad. Sci. U. S. A. 96, 13973–13977.
Zimmerman, P.A., Fitness, J., Moulds, J.M., McNamara, D.T., Kasehagen, L.J., Rowe, J.A., Hill, A.V., 2003. CR1 Knops blood group alleles are not associated with severe malaria in the Gambia. Genes Immun. 4, 368–373.