

Blood groups and malaria: fresh insights into pathogenesis and identification of targets for intervention

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Purpose of review

This review summarizes recent advances in our understanding of the interaction between malaria parasites and blood group antigens and discusses how the knowledge gleaned can be used to target the development of new antimalarial treatments and vaccines.

Recent findings

Studies of the interaction between *Plasmodium vivax* and the Duffy antigen provide the clearest example of the potential for basic research on blood groups and malaria to be translated into a vaccine that could have a major impact on global health. Progress is also being made in understanding the effects of other blood group antigens on malaria. After years of controversy, the effect of ABO blood groups on falciparum malaria has been clarified, with the non-O blood groups emerging as significant risk factors for life-threatening malaria, through the mechanism of enhanced rosette formation. The Knops blood group system may also influence malaria susceptibility, although conflicting results from different countries mean that further research is required. Unanswered questions remain about the interactions between malaria parasites and other blood group antigens, including the Gerbich, MNS and Rhesus systems.

Summary

The interplay between malaria parasites and blood group antigens remains a fascinating subject with potential to contribute to the development of new interventions to reduce the global burden of malaria.

Keywords

ABO, Duffy, Knops, plasmodium, virulence

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Introduction

Although the study of blood group antigens and malaria parasites is decades old, new advances continue to be made that profoundly influence our understanding of how malaria parasites interact with their human hosts. Because malaria parasites spend a substantial part of their life cycle invading red blood cells (RBCs) and growing within them (Fig. 1) [1], they have evolved specific receptor–ligand interactions to facilitate RBC binding, some of which involve blood group antigens. Variant RBCs with blood group polymorphisms or null phenotypes have been used to probe RBC–parasite interactions *in vitro*, and genetic epidemiological studies investigating the effect of blood group polymorphisms on malaria severity have been used to identify molecules and pathways that play a crucial role in life-threatening malaria. Recent advances in some of the major blood group systems affecting malaria are outlined below, followed

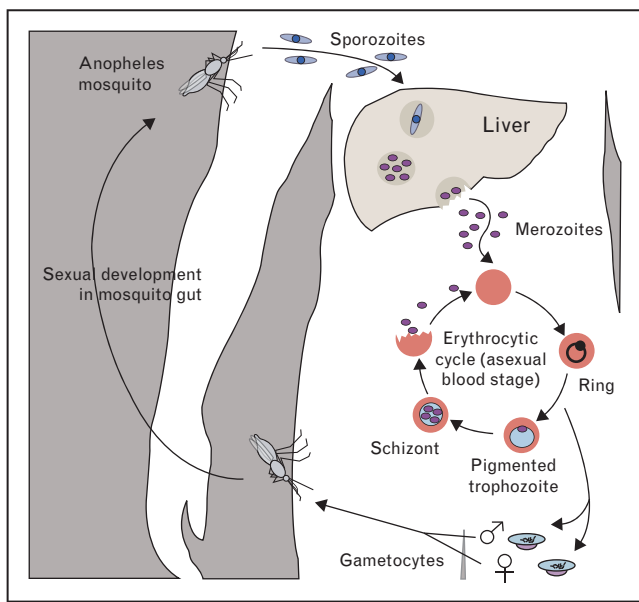
by important unanswered questions and associations that require further study.

The Duffy blood group

The Duffy blood group antigen provides the clearest example of a malaria resistance mechanism yet described. Its critical role in the invasion of RBC by both the simian parasite *Plasmodium knowlesi* and the related human parasite *P. vivax* was first demonstrated in the mid-1970s [2,3] and subsequently the role of the Duffy antigen in parasite invasion has been elucidated in considerable detail.

The Duffy antigen receptor for chemokines

Duffy blood group is determined by two co-dominant alleles, *FY*1* and *FY*2*, which encode respectively the Fy^a and Fy^b blood group antigens. The population expression of these antigens is regionally specific and

Figure 1 Life cycle of *Plasmodium falciparum*

When an infected female *Anopheles* mosquito takes a blood meal, sporozoite forms of *Plasmodium falciparum* are injected into the human skin. The sporozoites migrate into the bloodstream and then invade liver cells. The parasite grows and divides within liver cells for 8–10 days, then daughter cells, called merozoites, are released from the liver into the bloodstream, where they rapidly invade red blood cells (RBCs). Merozoites subsequently develop into ring, pigmented-trophozoite, and schizont stage parasites within the infected RBC. *P. falciparum*-infected erythrocytes express parasite-derived adhesion molecules on their surface, resulting in sequestration of pigmented-trophozoite and schizont stage-infected RBCs in the microvasculature. The asexual intraerythrocytic cycle lasts 48 h and is completed by the formation and release of new merozoites that will re-invade uninfected RBCs. It is during this asexual bloodstream cycle that the clinical symptoms of malaria (fever, chills, impaired consciousness, etc.) occur. During the asexual cycle, some of the infected RBCs develop into male and female sexual stages called gametocytes that are available to be taken up by feeding female mosquitoes. The gametocytes are fertilized and undergo further development in the mosquito, resulting in the presence of sporozoites in the mosquito's salivary glands ready to infect another human host. Reproduced with permission from [1].

is determined by mutations affecting these alleles that give rise to four major phenotypes: Fy(a+b+), Fy(a+b-), Fy(a-b+) and Fy(a-b-) [4]. In the absence of an obvious disadvantage of Duffy negativity on RBC, the precise role of the Duffy antigen has been something of a mystery. However, it has now been shown that Duffy binds a wide range of pro-inflammatory chemokines, leading to the hypothesis that it may have an important role in modulating their concentrations in plasma [5] and to the new name, Duffy antigen receptor for chemokines (DARC). Further interest in DARC has been fuelled by its potential role in asthma [6], in susceptibility and survival from HIV [7**] and as a determinant of peripheral blood neutrophil counts [8]. Nevertheless, our focus here will be on recent developments regarding the involvement of DARC in malaria biology.

Duffy antigen receptor for chemokines negativity supports the importance of *Plasmodium vivax* malaria

The Fy(a-b-) Duffy negative phenotype resulting from a GATA-1 mutation in the promoter region of the *DARC* gene [9] has reached fixation in much of west and central Africa and accounts for the absence of *P. vivax* malaria from the region [3]. This conclusion is reinforced by a recent survey conducted using sensitive PCR-based typing methods, which found no occurrences of *P. vivax* malaria in nine African malaria-endemic countries [10]. Given the widespread perception of *P. vivax* malaria as a 'benign' disease, the reason that DARC negativity has reached such high frequencies has been the subject of some speculation. However, two recent studies conducted in Indonesia [11**] and in Papua New Guinea (PNG) [12**], have challenged this perception and suggest that *P. vivax* might well have exerted a selective pressure, particularly in the era prior to the development of effective treatments.

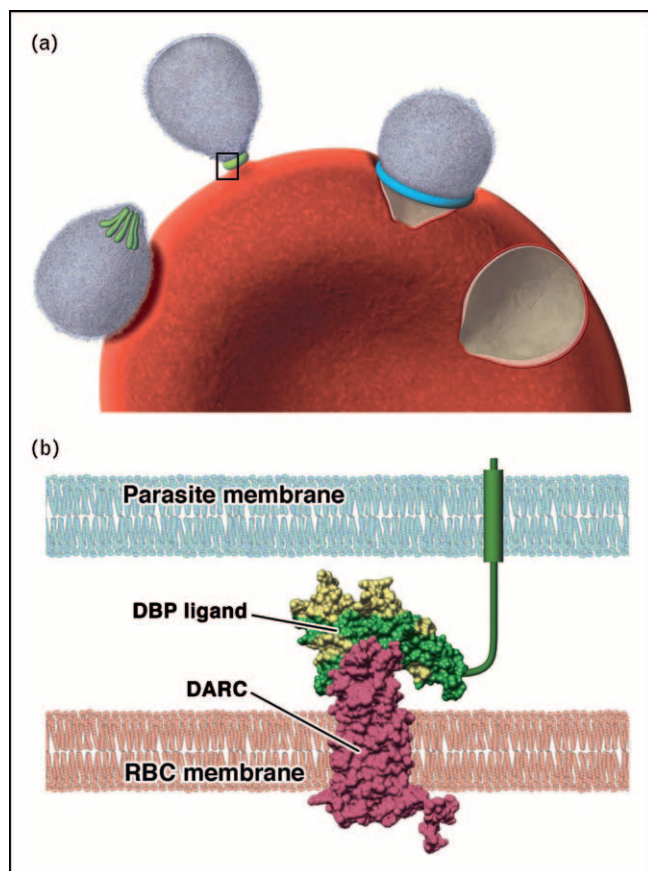
Duffy antigen receptor for chemokines and *Plasmodium vivax* infection

Although the link between DARC negativity and *P. vivax* resistance was originally made in Africa, much of the subsequent work has been undertaken in areas where *P. vivax* remains a significant clinical problem. Of particular interest are studies conducted by a group working in PNG. In the late 1990s, they identified a small number of heterozygotes for a new Duffy negative allele, *Fy*A^{null}*, which was associated with 50% lower Fy^a expression [13]. Subsequent studies have confirmed that such persons are half as likely to be infected with *P. vivax* at cross-sectional survey, are significantly less likely to suffer from clinical *P. vivax* infections, and, if they are infected, parasite densities in them are significantly lower than those achieved in their normal counterparts [14*], observations that confirm the importance of DARC expression in *P. vivax* infections.

The mechanism of RBC invasion of *Plasmodium vivax* merozoites

Recognizing the importance of DARC to *P. vivax* transmission has led to a detailed understanding of the molecular mechanisms by which *P. vivax* invades RBC (Fig. 2) [15]. In recent years, it has been shown that this involves a complex, multistep process, one of which is critically dependent on a specific molecular interaction between DARC, expressed on the RBC surface, and the *P. vivax* Duffy-binding protein (PvDBP) secreted by the micronemes of the *P. vivax* merozoite (reviewed by Chitnis and Sharma [16]). The binding site for PvDBP maps to a 35 amino acid sequence at the N-terminal extracellular region of DARC, whereas the receptor-binding domain lies in cysteine-rich region II of the PvDBP (PvDBPII) [17]. Because this interaction is so critical to the invasion of *P. vivax* into RBC, it has been the focus of considerable

Figure 2 The role of Duffy antigen receptor for chemokines in *Plasmodium vivax* invasion



(a) The *Plasmodium vivax* Duffy binding protein (PvDBP) is located in the micronemes of the merozoite (green). After attachment of the merozoite to the red blood cell (RBC) (reticulocyte) surface, the merozoite re-orientates, so that its apical end is in contact with the RBC membrane. DBP is then released and a tight junction (blue) is formed between the merozoite and the RBC membrane. The tight junction moves from the apical to posterior pole as the merozoite invades the RBC, propelled by an actin-myosin motor. The RBC membrane is resealed once invasion is complete. The entire process from initial attachment to completed invasion takes approximately 1 min. (b) A model of the binding of the PvDBP to the Duffy antigen receptor for chemokines (DARC) [inset from (a)]. Amino acid residues in PvDBP that are conserved are green and polymorphic yellow. Antibodies are predicted to bind to a polymorphic region of the DBP that is separate from, but may overlap with, the DARC-binding site. Reproduced with permission from [15].

scientific interest. Two recent studies have been particularly informative. First, Grimberg *et al.* [18^{••}] used recombinant proteins based on PvDBP_{II} (rPvDBP_{II}) to generate antibodies that inhibited binding of rPvDBP_{II} to DARC. Further, these antibodies bound to native PvDBP_{II} and reduced the ex-vivo invasion of *P. vivax* parasites into DARC-positive RBC. More recently, the same group has found a strong negative correlation between the presence of natural antibodies that inhibit the binding of PvDBP_{II} to DARC and *P. vivax* infections [19^{••}]. Both studies, therefore, suggest that a vaccine based on PvDBP_{II} might well prove

successful, and progress toward this aim is ongoing [16]. Nevertheless, although PvDBP is semiconserved, genetic variability is greatest at the receptor-binding domain, which may complicate both the design and testing of a DBP vaccine [20[•],21].

***Plasmodium vivax* infections in Duffy antigen receptor for chemokine-negative patients**

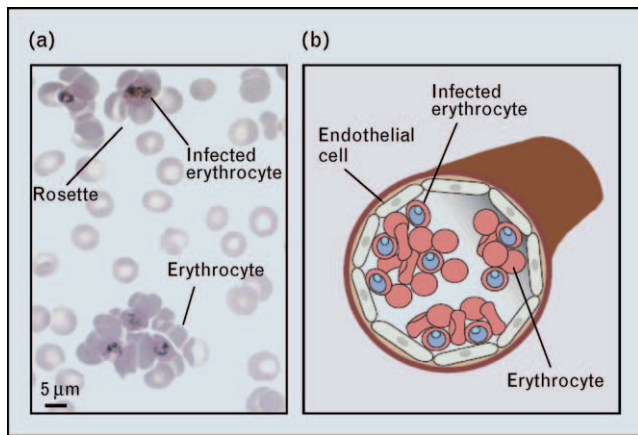
Although the evolution of DARC negativity in PNG suggests human adaptation to *P. vivax*, a number of recent reports suggest that the parasite might also be adapting to its human host. First, in western Kenya, Ryan *et al.* [22] identified parasites with the characteristics of *P. vivax* both in *Anopheles* mosquitoes and a number of DARC-negative humans. More recently, *P. vivax* parasites have also been identified in a small number of DARC-negative people in the Brazilian Amazon [23,24]. Although these reports suggest, therefore, that *P. vivax* might be able to escape its dependence on DARC and use alternative receptors for invasion, they involved very few participants and the relevance of this finding to the development of a *P. vivax* vaccine remains to be seen.

The ABO blood group

ABO and malaria have both been studied for over 100 years, and there are numerous papers on the effects of ABO blood group on various forms of malaria from multiple countries, many coming to contradictory conclusions (covered in some recent reviews [25,26]). Remarkably, until recently, there has been no clear answer to the crucial and obvious question: does ABO blood group affect susceptibility to life-threatening malaria? Preliminary evidence suggested that blood group A might be detrimental [27,28] and group O protective [29]; however, a definitive case-control study taking into account other, potentially confounding, malaria risk factors such as hemoglobin variants was lacking. This need has now been met by two recent studies agreeing in their conclusions that blood group O confers resistance to severe malaria [30^{••},31^{••}].

ABO blood group, parasite rosetting and malaria susceptibility

Rosetting is characterized by the binding of *P. falciparum*-infected RBCs to uninfected RBCs to form clusters of cells that are thought to contribute to the pathology of falciparum malaria by obstructing blood flow in small blood vessels [1,32] (Fig. 3). The rosetting phenotype varies between parasite isolates and correlates with severe falciparum malaria in sub-Saharan Africa (reviewed in [1]). Previous work had shown that rosetting parasites form larger, stronger rosettes in non-O blood groups (A, B or AB) than in group O RBCs [33,34]. Furthermore, the percentage of infected RBCs forming rosettes is significantly lower in fresh clinical isolates

Figure 3 *Plasmodium falciparum* rosetting

(a) Rosetting in a *Plasmodium falciparum* in-vitro culture, observed after preparation of a Giemsa-stained thin smear and light microscopy. (b). Schematic representation of *P. falciparum* rosette formation in the microvasculature. Rosetting infected red blood cells (RBCs) are thought to have the ability to bind simultaneously to microvascular endothelial cells and uninfected RBCs, resulting in obstruction to blood flow in microvessels contributing to pathological effects such as hypoxia and acidosis. Adapted with permission from [1].

derived from group O than in non-O patients [35]. It appears that this is because the A and B antigens are receptors for rosetting on uninfected RBCs [36], being bound by a parasite protein called PfEMP1 which is expressed on the surface of infected RBCs [37]. Rosettes still form in group O RBCs (albeit smaller and weaker than in non-O RBC) through the involvement of other RBC molecules which act as alternative receptors for rosetting (see complement receptor 1 and the Knops blood group system below).

Thus, it was reasoned that if rosetting contributes directly to the pathogenesis of severe malaria and is reduced in blood group O RBCs, then group O individuals should be protected against life-threatening malaria. This hypothesis has recently been confirmed through a case-control study conducted in Mali, west Africa, in which the odds ratio (OR) for severe malaria in blood group O versus non-O participants was 0.34 [95% confidence interval (CI) 0.19–0.61, $P < 0.0005$] [30**]. Furthermore, a significant interaction was found between parasite rosette frequency and host ABO blood group, supporting the hypothesis that protection was mediated by reduced rosette formation in group O RBC. This work illustrates how a study of blood group polymorphisms can yield insights into malaria pathogenesis; in this case, providing strong support for the theory that rosetting is important in the pathogenesis of severe malaria.

ABO genotypes and malaria susceptibility

A second recent study on ABO and malaria in sub-Saharan Africa used case-control and family-based

association methods to show that non-O alleles are associated with an increased risk of severe malaria (OR 1.18, 95% CI 1.11–1.26, $P < 0.0005$ for all data pooled). The study by Fry *et al.* [31**], which examined ABO genotypes [based on four single-nucleotide polymorphisms (SNPs) in the ABO glycosyltransferase gene], rather than serological phenotypes, involved almost 4000 cases of severe malaria from Kenya, The Gambia and Malawi. Using blood group phenotypes inferred from SNP haplotypes, individuals with blood groups A or AB were found to be at particular risk of severe malaria [OR 1.33 (95% CI 1.13–1.56, $P = 0.00065$) and OR 1.59 (95% CI 1.15–2.21, $P = 0.006$) respectively]. Another recent study on a Gambian population confirmed that non-O alleles increase risk of severe malaria (OR 1.26, 95% CI 1.11–1.44, $P = 0.0005$) [38**]. By comparison, when the results of Rowe *et al.* [30**] are presented in a similar form, the Mali study showed an OR of 2.94 (95% CI 1.64–5.26, $P < 0.0005$) for severe malaria in non-O versus O blood groups. The exact magnitude of the protective effect of group O may vary between populations due to regional differences in the prevalence of other malaria-resistance genes, varying levels of malaria transmission and population immunity, and possibly differences in pathogenic mechanisms.

The study of Fry *et al.* [31**] also identified a possible 'parent of origin effect' in which non-O alleles inherited from the mother led to a greater risk of severe malaria than non-O alleles inherited from the father. Although the explanation for this remains unclear, a genomic imprinting effect has been suggested.

Implications of studies on ABO and malaria susceptibility

Taken together, the study by Rowe *et al.* [30**], with its focus on pathogenic mechanisms, and that by Fry *et al.* [31**], with its focus on genetic mechanisms, provide strong evidence that individuals with non-O blood groups are at increased risk of severe malaria. Furthermore, the data obtained support the hypothesis that malaria parasite rosetting plays a direct role in the pathogenesis of severe malaria and provide extra impetus for research exploring the potential for rosette-disrupting drugs [39,40] or vaccines [41] as interventions against life-threatening malaria [1]. These results could also have implications for the use of blood transfusions in severe malaria. It is possible that transfusion of non-O blood could promote rosetting; therefore, for those severe malaria patients requiring blood transfusion, it might be preferable to use group O blood whenever possible.

Blood group O occurs in approximately 40–80% of the population in different parts of Africa (reviewed by [26]). If group O protects against life-threatening malaria,

why then is the frequency of O not higher in malarious countries? It seems likely that other balancing selection pressures need to be considered. For example, cholera and other diarrhoeal diseases that may be substantial causes of death in sub-Saharan Africa are more common or more severe in group O individuals [42,43]. Population frequencies of blood group O may, therefore, be determined by the regionally specific selection pressures, with *P. falciparum* playing an important but not exclusive role.

The Knops blood group

The Knops (KN) blood group system consists of nine antigens: the antithetical pairs Kn^a/Kn^b (KN1/KN2), McC^a/McC^b (KN3/KN6) and S11/S12 (KN4/KN7), as well as the Yk^a (KN5), S13 (KN8) and KCAM (KN9) antigens [44,45]. These antigens are located on the complement receptor 1 (CR1) molecule [46–48]. CR1 is a RBC membrane glycoprotein that is important for the removal of immune complexes coated with activated complement components (C3b/C4b) and for the control of complement-activating enzymes [49].

The Helgeson phenotype, parasite rosetting and malaria susceptibility

A role for CR1 in malaria was first suggested by Rowe *et al.* [50], who screened a panel of RBCs with null blood group phenotypes for their ability to form rosettes with *P. falciparum*-infected RBCs. It was found that Helgeson RBCs (the null phenotype for the Knops system) showed greatly reduced rosetting. Furthermore, soluble CR1 protein inhibited rosetting [50] and a mAb specific for the C3b-binding site on CR1 reduced rosetting in both laboratory strains and field isolates [51].

Following the same reasoning as that described above for blood group O, it was hypothesized that if the Helgeson phenotype reduces rosetting and rosetting contributes to the development of life-threatening malaria, then individuals with the Helgeson phenotype should be protected from severe disease. A high frequency of the Helgeson phenotype had been reported in PNG [52], therefore, the association between CR1 levels and malaria susceptibility was examined there. Previous work had shown that the expression of CR1 on RBCs of healthy individuals can vary in the range of 50–1200 molecules per cell [53], and that Helgeson phenotype RBCs usually have fewer than 100 molecules per RBC [54]. In Caucasian, Asian and Melanesian populations, the variation in RBC CR1 levels is known to be genetically determined and is associated with SNPs in intron 27 and exon 22 of the CR1 gene giving low (L) and high (H) expression alleles [53,55,56]. These genetic markers were used to examine the relationship between CR1 levels and malaria susceptibility in a case–control study in a highly malar-

ious region of PNG. Low CR1 levels were found to be extremely common (~80% of the population had <200 CR1 molecules per RBC) and the L allele conferred significant protection against severe malaria in heterozygotes (OR 0.33, 95% CI 0.14–0.77, $P=0.01$) [56]. LL homozygotes showed a trend towards protection, but this was not statistically significant.

A conflicting result was obtained from a case–control study in Thailand, which showed that LL homozygotes are at increased risk of severe malaria (OR 2.74, 95% CI 1.33–5.66) [57]. Rosetting is not associated with severe malaria in this region (where severe malaria differs in demographic and clinical features compared with sub-Saharan Africa) (discussed in [1]). A second study in Thailand found no effect of the intron 27 SNP on susceptibility to severe or cerebral malaria [58]. A range of other SNPs within the CR1 coding sequence [55,59] also had no association with malaria susceptibility [58]; however, a promoter polymorphism associated with low CR1 expression was a significant risk factor for cerebral malaria [58].

A possible explanation for some of the discrepancies between studies was provided by a recent study from India, which reported that the effects of RBC CR1 expression level vary depending on malaria endemicity. In a population with low/epidemic malaria transmission, there was a significant correlation between low CR1 expression and severe malaria; however, in a higher transmission area, high CR1 levels were associated with disease [60••]. It may be that different pathogenic mechanisms are operating in regions of varying endemicity and in different disease states, with rosetting being an important factor in some circumstances, whereas other factors such as ability to remove immune-complexes could be important in others [61•,62•].

Knops antigens and malaria susceptibility in sub-Saharan Africa

The effect of the Knops blood group system in sub-Saharan Africa is not well understood. An initial study in The Gambia reported no significant association between the CR1 low expression allele (L) and susceptibility to severe malaria [63]; however, it was later realized that the L allele does not correlate with RBC CR1 expression level in African populations [64,65].

One remarkable feature of CR1 in Africa is the occurrence at high frequency of the Knops antigens S12 and McC^b (Table 1) [47,66,67]. The *S12* SNP is under positive selection [68•], and it has been hypothesized that *S12* may offer a survival advantage in a malaria-endemic setting. In-vitro studies showed that adhesion to the malaria parasite rosette-forming protein PfEMP1 expressed in COS-7 cells was impaired in RBCs

Table 1 Percentage of the Knops blood group system *S11/S12* and *McC^a/McC^b* genotypes in various populations

Population (<i>n</i>) [reference]	<i>S11/S11</i>	<i>S11/S12</i>	<i>S12/S12</i>	<i>McC^a/McC^a</i>	<i>McC^a/McC^b</i>	<i>McC^b/McC^b</i>
Mali (99) [47]	9	30	61	50	40	10
The Gambia (853) [66]	5	30	65	38	47	15
Western Kenya (460) [67]	10	44	45	48	45	7
Caucasian Americans (100) [66]	99	1	0	100	0	0
Asian Americans (99) [66]	95	4	1	96	4	0
Hispanic Americans (100) [66]	94	6	0	95	5	0

displaying the *Sl*:1,2 [previously called *Sl(a-)*] phenotype [50]. This implies that *Sl*:1,2 RBCs will show reduced rosetting with *P. falciparum*-infected RBCs; however, this has not yet been demonstrated. The effect of the *S12* and *McC^b* alleles on malaria susceptibility has been examined in three studies to date, with conflicting results. A case-control study in The Gambia reported no significant association between the *S12* or *McC^b* alleles and protection from severe malaria [66]. In contrast, a study in western Kenya found that children with the *S12/S12* genotype were at reduced risk of cerebral malaria (OR 0.17, 95% CI 0.04–0.72, $P=0.02$) compared with children with *S11/S11* [67]. A recent study of a Gambian population found no significant effect of the *S12* SNP (rs17047661) on malaria susceptibility [38**].

The differences between these studies may be explained by ethnic differences, study design, varying pathogenic mechanisms in different areas relating to transmission intensity, levels of immunity, or interactions with other malaria-resistance genes. These contradictory reports demonstrate that the role of CR1 in malaria pathogenesis is not yet clearly understood and that more studies involving larger samples and more diverse populations are needed.

Other blood groups and malaria: unanswered questions

The Gerbich negative phenotype (caused by a deletion in the gene encoding Glycophorin C, *GYPC*) is common in malarious regions of PNG [69], and Glycophorin C is an RBC receptor for *P. falciparum* invasion [70,71]. Although it is plausible, therefore, to suggest that in PNG, Gerbich negativity may have been selected to its current frequencies through a survival advantage against severe malaria, this has not been formally tested. Glycophorin B is also an invasion receptor [72*], and again, the effect of Glycophorin B negativity (the S-s-U- phenotype, found almost exclusively in people of African ancestry) on malaria susceptibility is unknown. Similarly, although other blood group antigens that occur at high frequency in malaria-endemic areas, such as the *Js^a* and *V* antigens of the KEL and RH blood group systems, respectively [73], might play a role in malaria resistance, this has not been formally investigated.

Conclusion

Thus, although recent advances in understanding interactions between malaria parasites and the Duffy, ABO and Knops systems illustrate that much has already been learned, it is clear that many intriguing possibilities remain to be explored. Future studies of blood group antigens will continue to provide valuable insights into human malaria.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 527–528).

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