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

J. Alexandra Rowe, D. Herbert Opi, and Thomas N. Williams

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, FYa and FYb, which encode respectively the Fya and Fyb 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, pigment-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 pigment-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 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].

**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, FyA*null*, which was associated with 50% lower Fya 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 from the micro- nemes 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
scientific interest. Two recent studies have been particularly informative. First, Grimberg et al. [18] used recombinant proteins based on PvDBPII (rPvDBPII) to generate antibodies that inhibited binding of rPvDBPII to DARC. Further, these antibodies bound to native PvDBPII 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 PvDBPII to DARC and *P. vivax* infections [19]. Both studies, therefore, suggest that a vaccine based on PvDBPII 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...
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 Fry et al. [31**] 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), Mc\(b\)/Mc\(a\) (KN3/KN6) and Sl1/Sl2 (KN4/KN7), as well as the Yk\(^a\) (KN5), Sl3 (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 malarious 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 Sl2 and Mc\(b\) (Table 1) [47,66,67]. The S/L SNP is under positive selection [68*], and it has been hypothesized that Sl2 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
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 *Sl2* and *McC* 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 *Sl2* or *McC* alleles and protection from severe malaria [66]. In contrast, a study in western Kenya found that children with the *Sl2*/Sl2 genotype were at reduced risk of cerebral malaria (OR 0.17, 95% CI 0.04–0.72, \( P = 0.02 \)) compared with children with *Sl1/Sl1* [67]. A recent study of a Gambian population found no significant effect of the *Sl2 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, *GYP C*) 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 Jk\(^a\) 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.

### Acknowledgements

We are grateful to Monica Arman for Figs 1 and 3 and to James Beeson and Brendan Crabb for Fig. 2. We are also grateful to our colleagues in Edinburgh, Mali, Kenya and the United States for discussions on this topic. Our work is funded by the Wellcome Trust (grant no. WT084226 to J.A.R. and WT076934 to T.N.W.).

## 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).


This seminal paper shows that antibodies directed towards PvDBPII inhibit binding of Plasmodium vivax to red blood cells. This study provides the best evidence to date that naturally acquired antibodies to Plasmodium vivax specific binding inhibitory antibodies confer protection from blood-stage malaria.

This paper, simultaneously published in the same volume of *PLoS Medicine* with Ref. [11*], also provides detailed epidemiological data that challenges the benign view of *Plasmodium vivax* malaria.


This seminal paper shows that antibodies directed towards POdBPI contributes to malaria resistance in Cameroon. This paper suggests that in days gone by, before effective treatments were available, *P. vivax* may well have been associated with considerable mortality, an important consideration when interpreting the current distribution of DARC negativity.


Case-control and family-based association studies that show that non-O alleles are risk factors for severe malaria across multiple sub-Saharan countries and suggest a possible parent of origin effect.


blood groups and malaria

Rowe et al.