Human red blood cell polymorphisms and malaria

Thomas N Williams

Genetic factors are a major determinant of child survival in malaria endemic countries. Identifying which genes are involved and how they affect the malaria disease risk potentially offers a powerful mechanism through which to learn more about the host–parasite relationship. The past few years have seen significant progress towards achieving this goal for some of the best-known malaria resistance genes that determine the structure or function of red blood cells: Gerbich blood group antigen negativity; polymorphisms of the complement receptor genes (most notably CR1); Southeast Asian ovalocytosis; pyruvate kinase deficiency; haemoglobin E; the sickle cell trait; and α-thalassaemia are all examples. The challenge for the future must be to translate such advances into fresh approaches to the prevention and treatment of malaria.

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Introduction

Despite concerted efforts by the international community, malaria remains a vast public health problem throughout much of the tropical world. Upwards of half a billion episodes of clinical Plasmodium falciparum malaria occur each year [1] and result in the deaths of more than a million children in sub-Saharan Africa alone. Historically, this burden has resulted in the selection of hundreds, if not thousands, of genetic variants that confer some degree of protection against death from the disease [2–4]. The identification of such polymorphisms is not simply a matter of scientific stamp collecting: each has the potential to teach us something about the host–parasite relationship that could potentially inspire a new approach to the prevention and treatment of the disease.

The human red blood cell (RBC) is central to the malaria life cycle (Figure 1). Not only does it provide the parasite with food and shelter but most of the pathogenic features of the disease are related to interactions among malaria-infected red blood cells (iRBCs), uninfected RBCs and other tissues. It is perhaps not surprising, therefore, that many of the protective associations described to date relate to genes that affect RBC structure or function. Although many have helped us to understand more about malaria, so far this has resulted in few practical solutions. Recently, however, a number of studies have led to renewed hope that a genetic approach might yet result in novel approaches to achieving prevention and treatment. Such studies form the focus for this review (Table 1).

Malaria and the RBC

Whereas the RBC cycle is established by the release of a few thousand merozoites from the liver, the parasites number in their millions by the time they can be visualised in the peripheral blood, and have increased to billions as symptoms become apparent. This exponential multiplication is achieved through multiple rounds of division in RBCs, in addition to immune evasion through cytoadherence of iRBCs in deep capillaries. All of these steps are potentially sensitive to genetic variations in the host RBC that might interfere with these processes and affect disease outcome.

The RBC surface — invasion pathways

The process of merozoite invasion involves a complex sequence of events that includes attachment to the RBC surface, re-orientation, tight-junction formation and, finally, internalisation [5]. In the case of Plasmodium vivax, genetic studies were responsible for the elaboration of a crucial invasion pathway involving the RBC Duffy antigen and the P. vivax Duffy-binding protein, which is crucial for invasion [4] and is now the target for a vaccine-based solution to P. vivax disease [5*]. Although P. falciparum does not deploy Duffy, and can invade using a number of different pathways [6], the Duffy story sets a precedent for the belief that understanding these pathways might lead to similar interventions. A genetic approach has recently led to the identification of one pathway that might be important in some populations. The Gerbich-negative blood group (Ge−), which results from the deletion of exon 3 in the gene encoding glycoporphin C (GYPCex3), has long been implicated in malaria resistance because it is found at very high frequencies in malaria-endemic regions of Papua New Guinea [7]. It has now been shown that in vitro, GYPCex3 confers protection against a subset of parasites that use an invasion pathway which involves the P. falciparum merozoite erythrocyte-binding antigen 140 (EBA-140) [8] and...
Further work has shown that GYPΔex3 is also a significant cause of ovalocytosis [10–12], leading to the alternative suggestion that this mutation might result in protection from malaria through a mechanism it shares in common with other causes of that condition (see below). Nevertheless, GYPΔex3 does not affect either the prevalance or the density of symptomless parasitaemia in subjects randomly sampled from the community [10,11]. The results of future studies investigating the role of GYPΔex3 in clinical and fatal malaria are therefore awaited with considerable interest.

**Ovalocytosis**

As implied above, ovalocytosis, a condition that can result from a number of different genetic lesions, has been considered a strong malaria-protective candidate for some time. Studies conducted in Papua New Guinea have now confirmed that the commonest form, Southeast Asian ovalocytosis (SAO), which is caused by heterozygosity for a 27-base pair deletion in the gene that encodes the RBC membrane protein band 3 (SLC4A1Δ27), is strongly protective against severe malaria and that this protection is highly specific to the cerebral form of the disease [13,14]. Two recent observations now provide some possible explanations. First, Cortes et al. [15] found that SAO was associated with marked resistance to invasion by many, but not all, of a range of different parasite isolates in vitro, raising the possibility that protection might result from resistance to a subset of parasites that cause severe disease. They also found a marked increase in the capacity for *P. falciparum*-infected SAO RBCs to adhere to the endothelial receptor CD36 [16], suggesting that adherence to this receptor, which is not expressed on vascular endothelium in the brain [17], might act as a decoy in order to reduce neurovascular binding through other receptors. Interestingly, in these experiments SAO RBCs were relatively refractory to

**Table 1**

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**RBC enzymes**

| Glucose-6-phosphate dehydrogenase deficiency | [29,31,33,34**] |

**Haemoglobinopathies**

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parasite lines that had the capacity to bind to ICAM-1, the main candidate receptor for the cytoadherence of iRBCs in the brain [18]: an observation that might support their earlier hypothesis. Nevertheless, in common with Ge, SAO has no apparent effect on the prevalence or density of parasite carriage in symptomless individuals [10,13,14]. Whereas these tantalising studies therefore provide some fascinating insights, the mechanism by which ovalocytosis confers protection requires confirmation through further studies.

RBC complement receptors
The role of the RBC membrane complement regulatory proteins, particularly complement receptor 1 (CR1;CD35), in the pathophysiology of malaria has been the subject of a number of studies. The most common polymorphisms of the CR1 gene in Caucasian and Pacific populations correlate with the quantitative expression of RBC CR1 [19], whereas those found in Africa affect its structure and determine the Knops family of blood group antigens [20]. There are several reasons to suspect that CR1 might be important. First, in common with other malaria protective genes, the frequencies of many of the CR1 polymorphisms are high in a number of malaria-endemic areas [21]. Second, CR1 is the major RBC receptor for mediating rosetting — a P. falciparum virulence phenotype in which uninfected RBCs adhere to iRBCs to form clumps in vitro [22] — which has been associated with severe malaria in a number of studies in Africa [23**]. Recent work supports some involvement of CR1 in the pathophysiology of severe falciparum malaria. For example, Cockburn et al. [24**] showed that, polymorphisms that result in low CR1 expression are extremely common in malaria-endemic regions of Papua New Guinea and are associated with protection from severe forms of the disease. Similarly, in Western Kenya Thathy et al. [25] found a protective relationship between the Swain-Langley Knops blood group, determined by the Sl2 allele, and cerebral malaria. Nevertheless, the link between CR1 and severe malaria is not straightforward. In Thathy’s study, a trend towards susceptibility was seen for a second Knops antigen, McCoy McCb [25*]. Moreover, apparently contradictory results have been reported in other populations, including an association between low CR1 expression and severe malaria in Thai adults [26], and no effect of either the Sl2 or McCb alleles in a study in the Gambia [27]. These confusing observations demonstrate how difficult it can be to tease out the mechanisms of some of these malaria protective associations: the role of CR1 will not be fully resolved without further studies in diverse populations.

RBC enzymes
The malaria-protective effect of the X-linked condition glucose-6-phosphate dehydrogenase (G6PD) deficiency has been suspected for many years on the basis of its high prevalence and diverse genetic origins in malaria endemic communities [28]. New support for this hypothesis has been provided by a recent haplotypic analysis of the mutant A-allele and Med alleles of the G6PD gene, which suggests that each has evolved independently within the last 10,000 years at a rate too rapid to be explained by random genetic drift [29]. That this observation is explained by malaria selection is supported by consistent evidence of clinical protection in a range of settings [30–32]; however, the mechanism of protection has remained uncertain. Compelling evidence now suggests that it involves the early phagocytosis of iRBCs [33]. Ring-stage infected G6PD-deficient RBCs are phagocytosed more than twice as efficiently as infected normal cells through a mechanism that appears to involve the deposition of IgG and complement on their surface [33]. It now appears that this protective mechanism, which might result from accelerated oxidative membrane damage as a result of the impaired anti-oxidant defence in deficient cells, might also apply to other malaria-protective polymorphisms, including some of the haemoglobinopathies (see below) [34**]. Recent work, involving segregation-analysis in cross-bred mice, has identified a functional mutation affecting the production of a second RBC enzyme, pyruvate kinase, as a major determinant of resistance to the murine parasite P. chabaudi [35]. The results of further studies, aimed at determining whether polymorphisms in Pklr also protect against malaria in humans and, if so, whether they involve a similar mechanism, are awaited.

The haemoglobinopathies
The haemoglobinopathies, disorders of haemoglobin structure and production, were the first genetically determined conditions to be implicated in malaria protection almost 60 years ago [36]. The population frequencies of many of these conditions correlate strongly with the historic incidence of malaria (Figure 2) [37], and several have been shown to protect against various manifestations of clinical P. falciparum malaria through a range of different approaches [38]. Nevertheless, there is still no consensus regarding the mechanisms involved.

The haemoglobinopathies fall into two broad categories: those associated with the production of structurally variant forms of haemoglobin (including haemoglobins S, C or E), and those caused by the reduced production of normal forms of either the α-globin or β-globin components of haemoglobin — the α-thalassaemias and β-thalassaemias respectively. The clinical effects of these conditions are highly variable. In their carrier forms, all are benign with few, if any, adverse effects. In their homozygous state some, such as HbS (HbSS), also known as sickle cell disease) and β-thalassaemia, cause severe sequelae and a reduced life-expectancy, some, such as HbC (HbCC) and HbE (HbEE), are associated with mild clinical effects including haemolysis and splenomegaly, whereas others, such as homozygous α-thalassaemia, are clinically silent.
The structural haemoglobin variants HbC, HbE and HbS

The malaria-protective effect of many of the structural haemoglobin variants is well supported by population genetic data; however, clinical evidence has been slow to accumulate. Early studies of HbC were not particularly convincing [39–43]; however, more recent studies support a marked effect, particularly against rigorously defined severe disease [44,45,46]. Whereas, to some extent, these inconsistencies might be explained by design issues, it might also be true that, until recently, investigators have been following the wrong hypothesis. In contrast to HbS, in which selection for the carrier state has occurred at the cost of the loss of homozygotes from sickle cell disease, it now seems likely that the selective advantage of HbC is greatest in homozygotes (HbCC). In a very large study conducted in Burkino Faso, Modiano et al. [45] found that, although significant, the protective effect of HbAC was only 30% as compared to >90% in HbCC. A homozygous advantage for HbC is consistent with observations from two smaller studies which reported no episodes of severe malaria in HbCC subjects [44,46] and, although both studies lacked the power to draw definitive conclusions, these observations are in agreement with in vitro experiments in HbAC RBCs show little effect, but the major effects of HbC is seen only in experiments conducted in HbCC cells.

Whereas it is possible that the protective effect of HbC relates to the reduced ability of *P. falciparum* parasites to grow and multiply in variant RBCs [47–50], this is only partially supported by in vivo data on parasite densities [45,46,51]. A plausible alternative mechanism is offered by recent in vitro studies relating HbC to the reduced expression of the major parasite-encoded RBC adhesion protein *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), an effect that is most marked in homozygotes [48,52]. As iRBC cytoadherence has been associated with the pathogenesis of severe malaria, this observation could explain how HbC might influence the incidence of severe disease, yet be indiscernible against less severe outcomes.

Further support for the protective effect of HbE is provided by the observation that both the prevalence and severity of complications were reduced in adult carriers with malaria, admitted to hospital in Thailand [53]. Moreover, a recent genetic analysis suggests that in one Thai population, HbE β26Glu–Lys, the most frequent variant in Southeast Asia, has reached its current frequency in less than 5000 years, a rate that could never have been achieved without some form of positive selection [54]. Like HbC, the homozygous state of HbE is relatively benign, and thus it remains possible that selection might favour both heterozygotes and homozygotes — a question that is yet to be answered through further studies.

There are more data to support the protective advantage of HbS than for any other polymorphism. Nevertheless, although heterozygotes (HbAS) have consistently been shown to enjoy >90% protection against severe and lethal malaria [55,56], they are not protected from symptomless parasitaemia [38,55], and the mechanism of protection remains controversial. To some extent, it almost certainly relates to the premature removal of iRBCs through an innate mechanism resembling that seen in G6PD deficiency [34**]. However, recent evidence from one population shows that HbAS protection increases with age.
The thalassaemias

The thalassaemias provide a further example of how difficult it has been to pinpoint definitive malaria protective mechanisms. Despite compelling evidence from population genetics [37], support from clinical studies has lagged behind. In keeping with many of the best-characterised malaria protective genes, \( \alpha \)-thalassaemia has no effect on the prevalence of symptomless parasitaemia [38]. Moreover, few data suggest that it protects against mild clinical disease [60,61,62]. Consistent evidence for protection by \( \alpha \)-thalassaemias against severe and fatal malaria has been shown in a number of populations, despite their having no apparent effect on parasite densities in individuals that do go on to develop the disease [62,63–65]. Unlike HbAS, which protects against all forms of the disease, the effect of \( \alpha \)-thalassaemia appears to be relatively specific to severe malaria anaemia [62,64]. Relevant to this is the observation that \( \alpha \)-thalassaemia is associated with reduced expression of CR1, an effect that appears to be independent of other genetic determinants of CR1 production [24]. It remains to be seen whether these new findings are relevant to the mechanisms of malaria protection.

Finally, one further observation might explain why some of the studies relating to malaria-protective alleles have been difficult to reproduce in multiple populations. A series of cohort studies, conducted in coastal Kenya, highlighted the epistatic interaction between HbAS and \( \alpha \)-thalassaemia, showing that the protective effects of each of these are lost when these conditions are inherited together [66]. Interactions of this sort are unpredictable and, if common, could make the hunt for both protective alleles and their mechanisms even more difficult.

Conclusions

This short review provides a flavour of some fascinating recent developments that relate to the malaria protective polymorphisms of RBCs. It can be seen that malaria has had a profound effect on the genetic makeup of many tropical populations, and that studies that aim to understand how such polymorphisms affect malaria risk continue to provide important clues regarding the host–parasite relationship. The challenge for the future will be to convert these lessons into practical advances in the prevention and treatment of the disease.

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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


2. Mackinnon MJ, Mwangi TW, Snow RW, Marsh K, Williams TN: Heritability of malaria in Africa. Plos Med 2005, 2:e340. These authors used family-pedigree based genetic variance component analysis to show that, as a whole, genetic factors account for a substantial proportion of the variability that is seen between children with regard to their risk of developing clinical P. falciparum malaria.


5. Arevalo-Herrera M, Castellanos A, Yazdani SS, Shakir AR, Chitnis CE, Dominik R, Herrera S: Immunogenicity and protective efficacy of recombinant vaccine based on the receptor-binding domain of the Plasmodium vivax Duffy binding protein in Aotus monkeys. Am J Trop Med Hyg 2005, 73:25-31. These authors report on the immunogenicity of a new vaccine aimed at protecting against P. vivax malaria that was developed as a result of genetic studies which showed that Duffy blood group negativity is completely protective against disease caused by this parasite.


15. Cortes A, Benet A, Cooke BM, Barnwell JW, Reeder JC: Ability of
This study shows that, in vitro, there is marked variability in the ability of different lines of *P. falciparum* parasites to invade ovocytotic red blood cells, bringing into question the mechanisms that might explain such differences.

This study shows that under conditions of flow, *P. falciparum*-infected ovocytotic RBCs adhere significantly more strongly than normal RBCs to the endothelial receptor CD36. This raises the possibility that ovocytosis protects from cerebral malaria by distracting parasites from adhesion in the brain, where CD36 is not expressed.


This study provides further support for the conclusion that HbC protects from severe malaria but does not protect from infection per se. The paper includes interesting comparative data for HbS.


This study builds on earlier work from the same group suggesting that the protective effect of HbC might result from the failure of such cells to support the display of PIEMP1 on the RBC surface.


This study provides what is probably the most comprehensive clinical description of the effect of sickle cell trait on malaria and other diseases that has been published to date.


This paper illustrates that the protective effect of sickle cell trait increases with age, providing evidence that the mechanism might not simply be innate but might also include an immunological component.


This study provides evidence that the protective effect of the sickle cell trait might include an immunological component related to the accelerated acquisition of responses to PIEMPs.


The authors explore the protective effect of alpha-thalassaemia against malaria and other diseases. They conclude that the effect of thalassaemia is specific to malaria, but that protection is limited to the anaemic form of the disease.


This study provides a fascinating example of a biological interaction between two genes that result in the sickle cell trait and alpha-thalassaemia. Individually, each results in a marked degree of malaria resistance, which is cancelled out when both genes are inherited together. This might explain the distribution of these conditions.

