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# Proposed Mechanisms Responsible for Resistance to Severe *Plasmodium falciparum* Malaria in Sickle Cell Trait Carriers

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**ABSTRACT**

Sickle cell trait is a benign condition characterized by the inheritance of a normal haemoglobin gene from one parent and an abnormal, mutated beta-globin gene from the other parent, resulting in a haemoglobin genotype of $Hb^A/Hb^S$ (AS). Sickle cell trait (HbAS) provides a survival advantage against malaria fatality over people with normal haemoglobin (HbAA) in regions where malaria is endemic, as it is said to provide partial protection against severe malaria, especially those caused by *Plasmodium falciparum*. This is evidenced by the fact that the geographical distribution of the sickle cell trait overlaps with past and present distribution of *P. falciparum* malaria in Africa. More than six decades after the protective effects of the sickle trait was first described, there have been numerous clear and convincing pieces of evidence with regard to associations between HbAS and protection against malaria; a number of credible non-mutually-exclusive protective mechanisms have been proposed over the last half-century. Early studies found that the biochemical and physical properties of HbAS erythrocytes led to decreased intraerythrocytic growth, impaired rosette formation, decreased erythrocyte invasion, reduced vascular cytoadherence and increased sickling of infected erythrocytes. However, recent evidence suggests that protection by HbAS may be also mediated, at least in part, by acquired immunity via increased lymphoproliferative response. Similarly, relative protection against uncomplicated malaria compared with HbAA individuals has been shown to increase with age in HbAS children. In addition, some studies have found more robust humoral immune responses against certain *P. falciparum* antigens in HbAS individuals.

Keywords: mechanisms, resistance, plasmodium falciparum, malaria, sickle cell trait

## INTRODUCTION

Malaria, especially that caused by *Plasmodium falciparum* remains a major cause of morbidity and mortality in sub-Saharan Africa, despite the availability of numerous interventions [1]. This has consequently resulted in exertion of extraordinary evolutionary pressure on the human genome and appears to have selected for multiple genetic polymorphisms that provide protection against severe disease [2-4]. As noted by Gong *et al.* [5] and Tantawy [6],

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The high prevalence of HbS in sub-Saharan Africa and some other tropical areas is generally thought to be because of the survival advantage conferred by its heterozygous form [7-15], referred to as the sickle cell trait (HbAS) [16]. The sickle cell trait has been shown to offer 70-90% protection against severe malaria and 50% protection against uncomplicated malaria compared with individuals not carrying the sickle haemoglobin gene (HbAA) [17-18]. After more than 60 years since the protective effects of sickle trait were first described, several protective mechanisms have been postulated, some of which include reduced erythrocyte invasion by the parasite, decreased intra-erythrocytic parasite growth, enhanced phagocytosis of parasite-infected erythrocytes, increased immune response against parasite-infected erythrocytes, weakened cytoadherence, induction of heme-oxygenase-1, decreased *P. falciparum* Erythrocyte Membrane Protein 1 (PfEMP1) expression, etc [19-21]). Despite this array of proposed mechanisms of sickle trait protection against severe malaria infection in HbAS individuals, the biological mechanism underlying the protection against severe malaria is still not yet completely elucidated [19]. While there have been clear and convincing pieces of evidence with regard to associations between HbAS and protection against malaria, data from clinical studies aiming to identify the mechanism(s) of protection have been less consistent. Older studies found a lower prevalence of parasitaemia in HbAS individuals irrespective of symptoms suggesting HbAS exerts protection against the establishment of parasitaemia, however, multiple other reports failed to identify an association between HbAS and the prevalence of asymptomatic parasitaemia [21]. This review will therefore focus on providing valuable insight into these proposed sickle trait protective mechanisms, both older and recent ones, as well as highlight the host-parasite relationship and the role of the host immune system in protection against malaria.



## Overview of the sickle cell trait

Sickle cell trait (SCT) is a benign condition in which an individual has one abnormal allele of the haemoglobin beta gene [22]. In other words, sickle cell trait is characterized by the inheritance of a normal haemoglobin gene (Haemoglobin A) from one parent and an abnormal, mutated beta globin gene, the sickle haemoglobin gene (Haemoglobin S) from the other parent, hence, they are said to be heterozygous [23]. Sickle cell trait is not a mild form of sickle cell disease; having sickle cell trait simply implies that the individual carries a single sickle haemoglobin gene [24-29] and can pass this gene along to their children [30]. Normal adult blood contains three types of haemoglobin, viz: Haemoglobin A (Hb-A), a small quantity of Haemoglobin $A_2$ (Hb-$A_2$) and Haemoglobin F (Hb-F) [22-]. Each haemoglobin type consists of four globin chains attached to four heme molecules to form a tetramer. The globin chains are alpha, beta, gamma and delta. The beta globin chain consists of 146amino acids and has a molecular weight of 15,126 [22]. Haemoglobin S (HbS) arises from a mutation substituting thymine for adenine in the sixth codon of the beta-chain gene, GAG to GTG. This causes the coding of valine instead of glutamate in position 6 of the Hb beta chain. The resulting Hb has the physical properties of forming polymers under conditions of deoxygenation. It also exhibits changes in solubility and molecular stability [31]. The heterozygous inheritance of an abnormal allele (HbS) from one parent with a normal one (HbS) from the other parent results in a haemoglobin genotype of $Hb^A/Hb^S$ (HbAS) with the individual having a symptomless carrier state or "trait" [32]. Carriers of the sickle cell trait with a normal alpha-globin chain have approximately 40% haemoglobin S (HbS) in their erythrocytes, with the rest being haemoglobin A (HbA), hence, they are mostly asymptomatic, except in rare cases [23]. Sickle cell trait (HbAS) has been hypothesized to provide a survival advantage against malaria fatality over people with normal haemoglobin (HbAA) in regions where malaria is endemic, as it is said to provide partial protection against severe malaria, especially those caused by *Plasmodium falciparum* [33]. This is a prime example of natural selection, evidenced by the fact that the geographical distribution of the sickle cell trait coincides with the present or past distribution of *Plasmodium falciparum* malaria in Africa, hence, the protection conferred by SCT on malaria is believed to be responsible for the sustenance of the disorders in this region of the world [34].

## Biochemical mechanisms of sickle cell trait protection against severe *P. falciparum* malaria

### Sickling of circulating infected erythrocytes

In the 1970s, two groups of researchers showed that parasitized HbAS red cells tend to sickle at a staggering two to eight times more than non-parasitized cells [19]. Luzzatto and his colleagues incubated erythrocytes of HbAS Nigerian children infected with *P. falciparum* and found that parasitized erythrocytes sickled much more readily under low oxygen tension than uninfected erythrocytes [20]. Afterwards, Roth and his associates demonstrated that increased sickling was limited to HbAS erythrocytes containing small plasmodium (ring) forms. Although extensive HbS polymerization was also observed in erythrocytes containing trophozoites and schizonts, sickling was precluded probably by the large inclusions [35]. The enhanced HbS polymerization in parasitized HbAS RBCs is likely due to the increased oxygen consumption that accompanies the robust metabolic activity of the intracellular parasite [20,35]. However, parasitization could also enhance polymer formation by lowering intracellular pH or by increasing intracellular haemoglobin concentration. The resultant increased sickling of parasitized red blood cells

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in HbAS individuals promotes enhanced phagocytosis and clearance of circulating parasitized RBCs and, therefore, results in reduced parasitaemia in HbAS individuals compared to that in HbAA individuals [21]. These simple but elegant experiments suggested that rapid clearance and destruction of circulating parasitized RBCs protect HbAS persons from severe infestation.

**Reduced intra-erythrocytic growth and oxidant damage of *P. falciparum in* HbAS red blood cells**

Under normoxic conditions, the invasion, growth, and multiplication of *P. falciparum* in HbAS erythrocytes were the same as that of HbAA erythrocytes. However, under conditions of low oxygen tensions, *P. falciparum* ring-stage parasites did not mature to trophozoites and schizonts in HbAS red blood cells [36-37]. It was hypothesized that the impairment in parasite growth could result from specific intra-erythrocytic conditions of HbAS red blood cells, such as low intracellular potassium, high concentrations of haemoglobin or osmotic shrinkage of the red blood cell causing an inhospitable environment for parasites [21]. Alternatively, the impairment in parasite growth could be the result of polymer-induced RBC dehydration [20]. Similarly, multiple studies also found that oxidant damage is a mechanism common to HbAS RBCs, as well as thalassemic and Glucose-6-phosphate dehydrogenase (G6PD)-deficient RBCs in mediating resistance to malaria [38]. It is thought that when these RBCs are parasitized, they are subjected to additional oxidative stress because of the enhanced generation of reactive oxygen species (ROS) by both the parasite and the mutant RBC. However, it is yet to be elucidated whether the enhanced oxidant stress of these mutant RBCs impacts more on impairing parasite growth or enhancing uptake by phagocytes [20].

**Impaired rosette formation**

Another mechanism that has been shown to alter severe disease progression in carriers of the sickle trait is Impaired rosette formation [39]. According to Gong *et al.* [21], microcirculatory obstruction observed in cerebral malaria is thought to result from rosette formation (binding of *P falciparum*-infected erythrocytes to uninfected erythrocytes). However, in *P falciparum*-infected HbAS erythrocytes, rosette formation was found to be impaired under deoxygenated conditions, either as a result of increased sickling of these cells in deoxygenated conditions or reduced expression of erythrocyte surface adherence proteins [40]. The impaired rosette formation and the consequential decrease in microcirculatory obstruction are presumed to be contributory factors to the protective action of HbAS against cerebral malaria.

**Reduced vascular cytoadherence**

Parasitized erythrocytes express one of a family of parasite-encoded *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) molecules on the erythrocyte surface, with which they adhere to endothelial cells in the microvasculature, a process known as cytoadherence [41]. PfEMP-1, which is expressed on knob-like protrusions on the surface of infected RBCs, serves as the parasite's primary ligand for mediating endothelial adherence, binding primarily to CD36 (Cluster of Differentiation 36) which is the main cytoadherence receptor on the surface of host endothelial cells and monocytes, or to ICAM-1 (Intercellular Adhesion Molecule 1) a cell surface glycoprotein which is typically expressed on endothelial cells in the brain (Gong *et al.*[21]. This cytoadherence enables the sequestration of ring-containing RBCs deep within postcapillary beds to avoid splenic clearance, and also leads to endothelial activation and associated inflammation in the brain and other organs, events that are important in the progression to severe malaria [20, 41]. . This reduced vascular cytoadherence by HbAS RBCs likely leads to increased splenic clearance, and may in part explain lower parasite densities and a lower incidence of severe malaria in HbAS individuals [21].

**Immunological mechanisms of sickle cell trait protection against severe *P. falciparum* malaria**

These include enhanced phagocytosis of parasitized HbAS red cells, and enhanced acquired immune response.

**Enhanced phagocytosis of parasitized HbAS red blood cells**

Scientists have found evidence for enhanced innate immunity in HbAS subjects [38-47]. HbAS (as well as beta-thalassemia and Glucose-6-phosphate dehydrogenas-deficient) RBCs containing ring forms (but not trophozoites) were more prone to uptake by macrophages than normal or alpha-thalassemia RBCs, indicating that the innate immune system may play a role in protection against *P. falciparum* in HbAS individuals [21]. This improved phagocytosis may be a result of a combination of hemichrome formation, aggregates of band 3, and deposition of autologous IgG immunoglobulin and complement (C3) on RBCs (i.e., increased opsonin presentation) [21-38]. These opsonins, which are thought to be involved in the removal of senescent red blood cells, were found to be significantly higher in infected HbAS red blood cells compared to HbAA red blood cells, although they were first discovered to be elevated in G6PD deficiency, a red blood cell enzyme deficiency that is also protective against malaria [18]. Similarly, clearance of erythrocytes with exposed phosphatidylserine, a surface maker for damaged erythrocytes, by monocytes was found to be accelerated in parasitized HbAS RBCs than in HbAA cells [42-47]. Consequently, the enhanced opsonization and clearance of parasitized HbAS RBCs by the spleen in HbAS individuals may contribute to their enhanced antigen presentation and earlier acquisition of immunity, in comparison to their HbAA counterparts [21].

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**Enhanced acquired immune response**

There is epidemiologic evidence of an increase in protection against malaria with age in HbAS children [18]. According to population studies, the protective effect of HbAS rises with age, implying that there is an acquired component of protection. In a cross-sectional study of children with malaria in Nigeria, researchers discovered that HbAS children had considerably lower mean parasite density than HbAA children in those aged two to four years old, but not in those aged less than two years old [18,21]. Similarly, in Kenyan children, the protection provided by HbAS against symptomatic malaria rose from 20% in children under the age of two to 56% by the age of 10 [18]. Investigators have proposed several immune bases for acquired protection with the most plausible ones being increased lymphoproliferative response (improved cell-mediated response) and Increased levels of immunoglobulin-G (IgG) in HbAS compared to HbAA children, but a significant difference has not consistently been found between HbAA and HbAS adults [21]. The table below is a summary of the biochemical and immunological mechanisms of sickle cell trait protection against severe malaria.



## CONCLUSION

Malaria parasites have coevolved together with the human host for thousands of years, which has led them to constitute an important driving evolutionary force behind common erythrocyte variants including the sickle cell trait. Resolving the relative importance of these apparently non-mutually-exclusive mechanisms of resistance to *P. falciparum* by HbAS individuals will provide fresh insights into the complex interrelationship between the genome of the host and that of threatening microbial pathogens. The challenge for the future must be to convert these advances into fresh approaches to the prevention and treatment of malaria, especially in the tropical world.

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