How Do Hemoglobins S and C Result in Malaria Protection?

Thomas N. Williams1,2,3,4

1Kenya Medical Research Institute–Wellcome Trust Programme, Kilifi, Kenya; 2Nuffield Department of Medicine and 3Department of Paediatrics, University of Oxford, United Kingdom; and 4INDEPTH Network, Accra, Ghana

The search for a link between genetic factors and human diseases is at the core of a modern multibillion-dollar industry. Nevertheless, although the pages of countless high-impact journals are packed every week with data reporting genetic associations for an entire alphabet of diseases, disappointingly few have delivered on the promise to revolutionize treatment. The association between the sickle cell trait and human malaria is no exception—despite being one of the earliest and still one of the most clear-cut disease-association genes described, the one that is used as the preeminent example of genetic selection in schools and colleges throughout the world, the precise mechanisms by which the trait exerts its protective advantage remain elusive. It is in this light that studies such as the one reported by Tan et al [1] in this edition of the Journal are so important.

Hemoglobins S and C (HbS and HbC, respectively) are 2 structurally variant forms of normal adult hemoglobin (HbA) that result from separate mutations in the β-globin gene. The protective effect of HbS against death from Plasmodium falciparum malaria was first suspected > 60 years ago when Beet [2], and then Allison [3, 4], highlighted the remarkable coincidence in the geospatial distributions of these 2 important conditions. Similar evidence subsequently emerged for HbC [5, 6]. In the years that have followed, overwhelming evidence for clinical protection against P. falciparum malaria by both HbS and HbC has been provided by multiple studies (summarized in references [7, 8]).

In the case of HbC, protection is greatest in homozygous subjects with HbCC [9–11], but the situation regarding HbS is less clear-cut. Homozygous subjects with HbSS suffer from sickle cell disease, a condition that in Africa has traditionally been associated with very high rates of child mortality [12]. Malaria has commonly been viewed as a major cause of this early mortality [13, 14], but recent data suggest that the situation is much more complicated [15]. Children with HbSS may actually be more resistant to malaria infection than children with HbAS, but when they do become infected mortality appears to be extremely high, with the net result that positive selection for HbAS is counterbalanced by the negative selection for HbSS. Overall, both HbAS and HbCC are associated with a >90% reduction in the risk of severe and fatal malaria [11, 16], although important differences may exist with regard to the clinical spectra of severe malaria against which each protects [17].

Although the stories of HbS and HbC have provided fascinating insights into evolutionary biology, a clear understanding of their malaria-protective mechanisms will be required before these lessons will be of any practical importance. In this regard, HbS has been studied more extensively than HbC, although broadly similar hypotheses have developed for both. These hypotheses fall into 3 main categories. Early work suggested that both erythrocytes containing HbS and erythrocytes containing HbC might be less supportive of P. falciparum growth and multiplication than normal red cells under low oxygen tension [18–21]. Recently, data have also accumulated to suggest that both HbS and HbC might attenuate the pathophysiological consequences of P. falciparum by reducing the display of the parasite-encoded protein P. falciparum erythrocyte membrane protein-1 (PFEMP1) on the surface of the malaria-infected erythrocyte [22, 23]. The implication of these data is that P. falciparum–infected red blood cells that contain either HbS or HbC will cytoadhere less efficiently to capillary endothelium, a process that has been implicated in both the pathogenesis of severe malaria and in the evasion of parasite-infected red blood cells from immunological removal. Finally, parasite-infected HbS- and HbC-containing erythrocytes may also be removed more rapidly from circulation. Regarding this hypothesis, most studies have focused on HbS. There is good evidence that in common with G6PD deficiency and β-thalassemia, ring-stage parasite-infected HbAS red blood cells are
removed more efficiently from the circulation by monocyte-mediated opsonic phagocytosis that is precipitated by a series of oxidative events including increased denaturation of hemoglobin, membrane binding of hemichromes and free iron, aggregation of band 3, antibody binding, and the deposition of complement C3c fragments [24]. Moreover, parasite-infected HbAS red blood cells also sickle under physiological conditions in vitro [18, 25, 26], a process that may result in their premature destruction by the spleen [18, 27]. However, in addition to these innate mechanisms there is also evidence that immune mechanisms might be involved, and it is this hypothesis that is the focus of Tan et al’s [1] study.

As discussed by the authors of the current study [1], an acquired immunological component to the malaria-protective effects of HbS and HbC has been suggested by multiple previous studies. For example, in a cohort study conducted on the coast of Kenya, we found that HbAS had no effect on the incidence of uncomplicated clinical malaria among very young children, but that clinical immunity—protection against symptomatic malaria disease—developed significantly more rapidly during the course of childhood among subjects with HbAS than it did in those with HbAA [28]. This observation, which echoed those of several previous investigators [29–31], suggested to us that the protection afforded by HbAS might not only be innate, but it might also include an acquired immune component. A number of biologically plausible mechanisms have been suggested to explain how improved immunity might be driven. First, the chain of events that begins with oxidant damage to ring-stage infected red blood cells is thought to end in the exposure of P. falciparum–altered host band-3 protein [32], a process that appears to be accelerated in HbS-containing red blood cells [24]. It is at least possible that this protein is a target for accelerated acquisition of specific antibodies that might account for an immunological component to the protection afforded by HbS [33]. Alternatively, it is also possible that innate protective processes might alter the dynamics of parasite infection in subtle ways that result in a more generalized upregulation of the malaria-specific immune response. For example, by controlling parasite densities during the course of P. falciparum infections, innate mechanisms might paradoxically increase the duration of individual infections, to result in greater overall exposure of the host to P. falciparum–specific antigens [34]. Finally, it has also been suggested that HbS might improve nonspecific immunity to malaria, an example being the immune tolerance to cerebral malaria reported in recent studies in an HbS-expressing transgenic mouse model [35].

The above-mentioned research notwithstanding, surprisingly few studies have been conducted to date that have investigated the immunology of HbS or HbC. Seroepidemiological studies have come to inconsistent conclusions. For example, higher total or malaria-specific antibody concentrations have been reported in both HbAS and HbAC compared with HbAA in children in some studies [36–40], but not in others [29, 31, 41]. However, in general, the studies conducted to date have focused on a limited number of antibody responses to a narrow range of P. falciparum antigens.

In the current study, Tan et al [1] used cutting-edge technology to investigate antibody responses to a very wide range of P. falciparum proteins expressed during the whole lifecycle of the parasite in the human host. The authors used a protein microarray displaying a total of 491 antigens to compare both the magnitude and the breadth of P. falciparum–specific immunoglobulin G (IgG) responses in Malian children with HbAS, HbAC, and HbAA in cross-sectional surveys conducted both before and after an annual malaria transmission season. They found no significant differences in either the proportion of antigens recognized or in the magnitude of those responses. This was true for antigens expressed at all stages in the parasite lifecycle, including those expressed on the surface of the infected erythrocyte such as PfEMP1, RIFIN, STEVOR, and SURFIN proteins.

On the face of it, the current study appears to reject enhanced immunity as a mechanism for malaria protection by HbS or HbC. However, although the study adds useful and important data to a subject that has not been adequately studied, it does not lay the immune hypothesis completely to rest for a number of reasons. First, although the authors studied a large number of responses, the numbers of HbAS or HbAC subjects included (n = 15 and n = 20, respectively) were too small for the study to be considered definitive. Moreover, the combination of small numbers of subjects and a large number of comparisons made their search for significant differences a pretty tall order. Finally, as the authors acknowledge, their findings do not exclude the possibility that HbAS or HbAC might enhance immunity to antigens not included in their assays (including altered band 3 protein), that the 3-dimensional configuration of proteins on their array might not represent their antigenic state in nature, or that responses that they did not measure (such as IgG subclass or non-IgG responses) might still be enhanced. Nevertheless, the study does provide a nice example of how state-of-the-art technology can provide a new approach to an old question, and would be well worth repeating in larger cohorts, in this or other settings, and extending to the investigation of other malaria-protective traits.

The story behind HbS, HbC, and malaria is a long one, and the literature is full of apparently contradictory hypotheses. It seems most likely that the actual mechanisms by which these conditions confer their malaria protection in nature will be much more complicated than at first believed and may actually represent the sum of many of the processes described above. Nevertheless, dissecting the interactions between these conditions remains an important exercise that may yet help us come to a more complete understanding of the biology of malaria than we already have. With the development of new technologies such as those used in the current
study, the opportunities for improving our understanding of these and other host-parasite interactions have never been better.

**Note**

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**References**