Hereditary red cell disorders and malaria resistance

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Human malaria is attributed to five species of the *Plasmodium* parasite (this includes the discovery that *P. knowlesi* infects humans[1]). The most virulent form of the disease is caused by *P. falciparum*, which continues to have a major impact on human populations: a third of the world's population live in malaria endemic areas and upwards of a million individuals succumb to the disease each year, predominantly children in sub-Saharan Africa.[2] Historically, the impact was more profound and the current confinement of endemic malaria to the equatorial and sub-tropical regions is, in evolutionary terms, a very recent phenomenon. The discovery that pyruvate kinase (PK) deficient human erythrocytes are resistant to malaria has again highlighted the host-parasite relationship.[3,4] This paper provides an evolutionary perspective of the interactions between *P. falciparum* and the human erythrocyte in the context of these developments.

The effects of P. falciparum on the human genome

Malaria was a global health risk up to the last century when vector control programs eradicated malaria from most of the developed world. By way of example, the distribution of malaria in 1900 extended from Scandinavia in the north to Argentina and South Africa in the south.[5] This historically extensive distribution of the disease, as well as its exceptional virulence, led researchers to explore the relationship between malaria resistance and hereditary conditions ranging from erythrocyte disorders[6] to skin color.[7] The association between β-thalassemia and malaria was first proposed by JBS Haldane in 1949[8] and became known as the "malaria hypothesis". Subsequently, varying degrees of evidence emerged that demonstrated a relative resistance to malaria by numerous hereditary red cell disorders including hemoglobinopathies, membrane protein disorders and enzyme deficiencies, as well as blood group polymorphisms. A list of the conditions and genes that protect against malaria is provided in Table 1.

Pyruvate kinase deficiency and malaria

The issue of whether PK deficiency is protective against malaria has been considered in numerous reviews of hereditary erythrocyte disorders.[6,9] The association had previously been demonstrated in the murine model,[10] but the first direct evidence for this phenomenon in humans was demonstrated by two groups independently (Kodjo *et al.*[3], 24 April 2008, N Engl J Med and Durand and Coetzer[4], 6 May 2008, Haematologica). The Canadian group[3] demonstrated the protective effect of the 1269A splicing and 823delG frameshift mutations in the *PKLR* gene in homozygous PK deficient patients *in vitro*. They also presented evidence that the resistance conferred by the mutations was due to decreased parasite invasion in the homozygote and that parasitized PK-deficient erythrocytes from homozygous and heterozygous subjects were more vulnerable to phagocytosis than control cells. Similarly, our group[4] demonstrated that the most common mutation in the *PKLR* gene, a 1529A point mutation resulting in an Arg510Gln change in the enzyme, conferred protection against the parasite *in vitro* in the homozygote. We hypothesized that the mechanism was related to the intracellular depletion of ATP and subsequent effects on membrane proteins. Interestingly, in both publications, there was a mild, but not statistically significant decrease, in the invasion/growth of the parasite in heterozygous versus control erythrocytes, although the sample numbers were too small to provide a definitive answer.

Co-evolution of humans and malaria

There is a considerable body of evidence that demonstrates a co-evolutionary relationship between P. falciparum and humans.[6,9] This relationship is common to most host-pathogen interactions and typically leads to an "arms race". The host evolves new genetic determinants, which decrease susceptibility to infection, while the pathogen in turn evolves virulence factors, resulting in ongoing adaptations in host and pathogen fitness. The relationship between human hereditary red cell disorders and the malaria parasite is similar, although in some situations a typical "arms race" interplay may not hold true, since compensatory adaptations in the parasite have not always been identified (Figure 1). Using the sickle cell trait as an example, malaria has selected for the abnormal β-hemoglobin gene (HbS) in the host, but so far there have been no counteractive adaptations identified in the parasite. In addition, the disorder decreases the fitness of the uninfected host. This trade-off in a population leads to a balanced polymorphism[8] and explains the high allele frequencies of disorders like sickle cell disease, thalassemia and G6PD deficiency. In each instance, it is the degree of the selective advantage provided by the abnormal allele, and the degree to which the fitness of the heterozygote and/or homozygote is decreased that determine the allele frequency of a particular mutation. There is extensive evidence from epidemiological and clinical studies,[11] as well as *in vitro* experiments,[12] to show that malaria has selected for the HbS mutation. Similar data exist for several inherited red cell conditions and polymorphisms,[6,9] however, the picture is less clear with PK deficiency.

Has pyruvate kinase deficiency been selected for by malaria?

When one excludes the founder effect in Amish populations, the highest frequencies of the PK allele are

Table 1. Hereditary human erythrocyte disorders and polymorphisms that protect against *P. falciparum* malaria. Evidence that these disorders and polymorphisms protect against malaria has come from *in vitro* and/or clinical and/or epidemiological data. For items with an asterisk (*), there is only *in vitro* evidence available.

Condition	*Protein conferring a protective effect*
Hemoglobinopathies	
Sickle cell trait	Hb S
Alpha thalassemia	α-Hb
Beta thalassemia	β-Hb
Hemoglobin C	Hb C
Hemoglobin E	Hb E
Red cell membrane proteins	
Hereditary spherocytosis*	Spectrin, band 3, protein 4.2
Hereditary elliptocytosis*	Spectrin, protein 4.1
Hereditary pyropoikilocytosis*	Spectrin
South-east Asian ovalocytosis	Band 3
Blood group O	Glycosyl tansferase
Other blood group antigens	Glycophorin A, B and C
Complement receptor	CR-1
Red cell enzymes	
G6PD deficiency	Glucose-6-phosphate dehydrogenase
PK deficiency	Erythrocyte pyruvate kinase

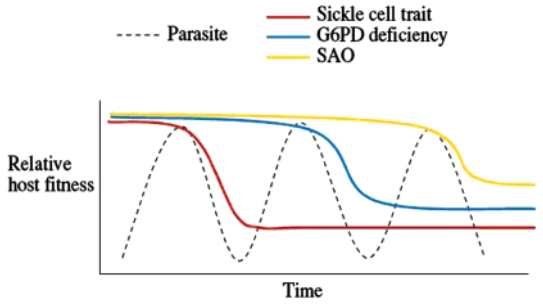

Figure 1. The human-malaria co-evolutionary relationship. This figure conceptualizes the adaptive nature of the human-malaria relationship. New protective mechanisms against malaria have constantly evolved in humans, and here three examples are provided: a hemoglobinopathy (sickle cell trait), a membrane disorder (south-east Asian ovalocytosis, SAO) and an enzyme deficiency (G6PD deficiency). Once a new resistance mechanism has evolved in humans, the parasite's fitness decreases in that particular group of individuals, but this may be temporary if the parasite develops a counteractive mechanism. The selective advantage to survive episodes of malaria has a variable negative effect on the fitness of the host. The process repeats itself, selecting for a new protective mechanism in the susceptible host each time. The timeline reflects the iterative process and does not represent the sequential evolution of mutations. Colored lines indicate changes to the host's relative fitness as a new resistance mechanism arises. The dashed line represents the fluctuation in the virulence and reproductive success of *P. falciparum* in response to protective mutations in the host.

found in parts of Europe and Asia with a prevalence ranging from 1% to 3.6%.[13,14] The question arises whether malaria was responsible for maintaining this frequency or whether the ~180 mutations resulting in PK deficiency are simply the product of random variation or other population genetic phenomena such as drift or migration.

Historically, endemic malaria was present in regions of the world, which have the highest prevalence of PK deficiency.[5] A noteworthy exception is Africa, where the prevalence of PK deficiency is not known, although the perception exists that the disease is rare in this region. However, this may reflect a lack of testing rather than a lack of the disease. Another possibility is that negative epistasis[15] has played a role in the distribution of the PK allele. This has been demonstrated for the sickle cell trait and α^+-thalassemia where the co-occurrence of these two conditions in the same individual cancel out the malaria-protective effect afforded by each mutation individually. This may apply equally to the abnormal PK allele, whereby the high prevalence of HbS might have diminished the frequency of PK deficiency. If the marked *in vitro* protective effect of homozygosity for PK deficiency against malaria translates into the field (and the murine model data[10] suggest that it will), the argument that malaria has maintained the polymorphic frequency of the abnormal allele becomes more plausible. In addition, the large number of *PKLR* mutations *per se* also indicates that these have been maintained by a selective force. PK deficiency is an extremely heterogeneous condition. Most of the clinical phenotypes, including the phenotype of the most common 1529A mutation, are mild or moderate in severity[14] and only a minority of patients is transfusion dependant. This suggests that the reproductive cost of PK deficiency was not limiting.

Currently, there is no definitive answer to the question whether the abnormal PK allele was selected for by malaria, however, the following aspects will provide further insights. Firstly, clinical case control studies will establish whether the protective effect of PK deficiency *in vitro* translates into the field. These findings may parallel those from other studies, which demonstrated that the selective advantage afforded individuals protection from severe life-threatening complications of malaria and did not necessarily decrease their susceptibility to infection. Secondly, it is important to confirm that the emergence of the common mutations in *PKLR* coincided with an increase in *P. falciparum* virulence, as is the case with most other protective red cell disorders and polymorphisms.[16] It can then be determined whether the frequency of the abnormal PK allele is compatible with positive selection. Such investigations were performed for G6PD deficiency[17] and hemoglobin E[18] mutations and the findings provided strong evidence that the expansion of these genes into the relevant populations was due to the selective pressure of malaria.

Conclusions

At the moment, the *in vitro* data and the *in vivo* murine model strongly indicate that PK deficiency evolved as a protective response to malaria, however, this hypothesis remains to be confirmed. Clinical studies have been initiated to establish whether this phenomenon translates into the field. Nevertheless, the *in vitro* findings themselves are a significant step forward in the fight against malaria since a clarification of the mechanism by which PK deficiency confers resistance may lead to a greater understanding of malaria pathogenesis and potential therapeutic strategies.

Key words: malaria, pyruvate kinase deficiency.

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Familial erythrocytosis: molecular links to red blood cell control

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Familial or hereditary erythrocytosis is a rare disorder of red cell production that can be inherited in either an autosomal dominant or recessive fashion. It is characterized by an absolute increase in red cell mass with elevated hematocrit and hemoglobin levels. In contrast to the acquired myeloproliferative disorder of polycythemia vera, the erythrocytosis is not accompanied by increased numbers of white cells and platelets. Familial erythrocytosis can exhibit a spectrum of erythropoietin (EPO) levels, which reflects the diverse genetic origins of this disorder. Erythrocytosis can be further classified as either primary or secondary depending on whether the defect is intrinsic or extrinsic to the erythroid progenitor cells. In primary erythrocytosis the serum EPO level is subnormal and the erythroid progenitors are hypersenstive to EPO, which suggests the defect lies in the EPO-induced signaling pathway. Secondary erythrocytosis is associated with inappropriately normal or raised serum EPO levels indicating a defect in the control of EPO synthesis by the oxygen-sensing pathway. Both reflect our current understanding of how red blood cell mass is regulated. This perspective focuses on primary and secondary familial erythrocytoses, as currently recognized by Online Mendelian Inheritance in Man (OMIM; available online at URL http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim). For other causes of erythrocytoses, including those arising from high oxygen affinity hemoglobins, the reader is referred to an excellent review by Hodges *et al.*[1]

In adults, EPO is synthesized predominantly in the kidney, although it should be recognized that liver can be a secondary source.[1] *EPO* gene transcription is regulated by a transcriptional mechanism that is more generally employed in the cellular response to changes in oxygen tension, to be described later. EPO then exerts its influence by binding to a specific receptor, the EPO receptor (EPOR), on the cell surface of erythroid progenitor cells, resulting in erythroid proliferation, differentiation and inhibition of apoptosis. The EPOR homodimerizes in the presence of EPO and autophosphorylation of the Janus tyrosine kinase 2 (JAK2) occurs.[2] Once JAK2 is activated, specific EPOR tyrosines are phosphorylated and form docking sites for adaptor molecules such as Grb2, the signal transducer and activator of transcription 5 (STAT5) and phosphatidylinositol 3-kinase (PI3-K) (Figure 1). Activated STAT5 forms dimers and translocates into the nucleus where it induces transcription of genes involved in proliferation and cell survival. PI3-K, via activated Akt, also induces the expression of several anti-apoptotic proteins, such as Bcl-2 and Bcl$_x$, to prolong cell survival.[2] Furthermore, activation of the Ras/extracellular-signal-regulated kinase mitogen-activated pro-