THE INNATE RESISTANCE OF THALASSAEMIA TO MALARIA: A REVIEW OF THE EVIDENCE AND POSSIBLE MECHANISMS

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SYNOPSIS

Many population, clinical and experimental studies have been done to verify the hypothesis that individuals with the thalassaemia trait are innately more resistant to malaria, thereby confering upon themselves a selective advantage in the presence of malaria endemicity. Such studies have not yet been conclusive. Nevertheless, how this resistance can come about is the subject of many other studies. The possible mechanisms that can operate include oxidant stress exerted by the thalassaemic erythrocyte on the oxidant-sensitive asexual intra-erythrocytic forms of the parasite, enhanced cell-mediated host responses against the infected thalassaemic red cell and effects of associated vitamin deficiencies and dietary interations.

INTRODUCTION

A high incidence of thalassaemic trait is found in many geographical regions. A high frequency of these traits in Singapore, about 2·3% each for alpha and beta thalassaemia, (1, 2) results from the composition of the island's main races that had originated from southern China, the Malay Peninsula and the Indian sub-continent. These regions and many others are also endemic for malaria, including some, like Singapore, which until recently, were endemic (3). Such an observation viz. that thalassaemic and malarious areas coincide led to the proposal that the malaria parasite, as a human evolutionary agent, has selected for the high frequencies of genes for thalassaemia (4). Much work in terms of population, clinical and experimental studies have been done to examine the association between thalassaemia and malaria.

This article reviews such studies, particularly newer data that have accumulated. Two questions are addressed:

1. What is the evidence to show that the high gene frequencies of thalassaemia result from natural selection by malaria?
2. How does the thalassaemic trait confer resistance to malaria?

EVIDENCE FOR THE RESISTANCE OF THALASSAEMIA TO MALARIA

1. Population studies:

Geographical correlations done in Greece, Cyprus, Malta, the Sudan and New Guinea to examine the association between the distribution of past or present malaria and the frequency of beta-thalassaemia trait have not yielded clear-cut results, unlike the more definite correlation between malaria and HbS or glucose-6-phosphate dehydrogenase (G6PD) deficiency (5). Hence, beta-thalassaemia has been found to occur in both malarious and non-malarious regions of these countries. It has been suggested (5) that the high frequency of the beta-thalassaemia gene in a population with haemoglobinopathies like HbS or HbE can never be achieved because the heterozygotes of genes for the latter are fitter and tend to replace those with beta-thalassaemia genes. HbS and HbE have also been associated with resistance to malaria (6-8).

'Micro-mapping' of thalassaemia genes at first sight yields more persuasive data.In Sardinia, a distinct correlation has been found between the frequency of malaria and beta-thalassaemia and there is a strong inverse correlation between beta-thalassaemia trait and altitude, where altitude is taken to be a measure of malaria morbidity (9). However, the validity has been questioned and the distribution of genetic traits of beta-thalassaemia (as well as G6PD deficiency) in Sardinia can also be explained, using historical analysis, by gene drift instead of gene selection (10). In Papua New Guinea, most of the individuals living in a lowland region were heterozygotes or homozygotes for the deletion form of alpha-thalassaemia, but those living in a highland area were normal; there were preliminary geographical and linguistic analyses done which suggested that the prevalence of this form of thalassaemia was related to altitude and therefore, as in the Sardinian study, to malaria (11).

Data from such population studies are therefore very indirect. But one notes from the theoretical consideration, that so many different mutations at 2 different loci of 2 different chromosomes have yielded large numbers of thalassaemic heterozygotes of a relatively homogenous phenotype in geographically distant malarious areas, is itself persuasive of the view that the thalassaemia trait protects against malaria (12).

2. Clinical studies:

There were some surveys done in Thailand where individuals from malaria-endemic areas were tested for the presence of thalassaemia and haemoglobinopathies as well as for malaria positivity and morbidity. While there was a significant correlation between HbE incidence and malaria, there were no correlation found for alpha- and beta-thalassaemia (13, 14).

More recent work done by Willcox and colleagues in Liberia reveals a somewhat different picture. A case control study of clinical malaria in a heterogenous population from a northern town showed that the number of cases with beta-thalassaemia trait was significantly less than that of the general population; when a stratified analysis was done, there was a lower weighted relative risk for beta-thalassaemia heterozygotes (15). In another study of subjects from two northern rural holoendemic areas each with a homogenous population group, *Plasmodium falciparum* prevalence rates in normal children were similar to those with beta-thalassaemia traits; however, the density of parasitaemia was significantly lower among children under 5 years with the trait compared to those who were normal; furthermore, there was an overall lower estimate of relative risk for *P. Falciparum* in children with the trait (16). In yet another study, in a central Liberian town, though there was a lower rate of malaria infection in subjects with beta-thalassaemia compared to those without, it was not statistically significant (17). It would appear therefore that to determine if there is an actual relative resistance, the choice of parameters used to measure malaria infection and the type of population studies can lead to different conclusions.

3. Experimental studies:

Attempts to provide more direct evidence could be made when it became possible to culture continuously in vitro, *P. Falciparum* in human erythrocytes (18, 19). It was then a matter of propagating the parasite in thalassaemic red blood cells (RBCs) and comparing multiplication rates with that in normal RBCs. But the results have not been clear cut. The earliest study (20) showed that under usual culture conditions, multiplication rates in red cells of alpha- and beta-thalassaemia heterozygotes were the same as in normal RBCs. Subsequent work of the same nature also suggested this (21, 22). However, these negative results may be artefactual. The culture medium used in these studies certainly does not reflect in vivo conditions. For example, the RPMI-1640 medium which is used in msot studies contains ten times more reduced glutathione (GSH), a potent reducing agent (23), compared to normal plasma (24); assuming that the malaria parasite is oxidant-sensitive, a concept which will be discussed at length below, it may be relatively well protected in vitro compared to in vivo. Indeed, when RPMI-1640 depleted of GSH was used, parasite growth in thalassaemia RBCs was inhibited (20).

Such in vitro studies have been confined to *P. Falciparum*. Similar work with *P. vivax*, which can also be considered the other human evolutionary agent, has not been forthcoming in site of claims that it is now possible to culture it in vitro (25, 26).

Hence all the data accumulated thus far remains suggestive that thalassaemic heterozygotes can be protected against malaria but this hypothesis has not been established with certainty. In spite of this, many workers have proceeded to examine the mechanisms that we possibly render thalassaemic heterozygotes resistant to malaria.

POSSIBLE MECHANISMS THAT MAY PROTECT THALASSAEMIC HETEROZYGOTES FROM MALARIA

1. Susceptibility of malaria parasites and thalassaemic RBCs to oxidant damage:

Malaria parasites in their erythrocytic stage are microaerophilic and are susceptible to oxidant stress. Allison and Eugui (27) have documented data up to 1982 that substantiate this. Most of these are of the effects of oxidants on human and animal malaria parasites at the erythrocytic stage: oxidants like

polyamine oxidase, polyamines, tumour-necrosis factor, phenylhydrazine and alloxan have been found to decrease multiplication rates or cause degeneration of the parasites. In addition, t-butylhydroperoxide, which liberates superoxide (O_2^-), when injected to mice infected with rodent malaria parasites, caused a decrease in parasitaemia and produced distorted forms in the red cells. A subsequent in vitro study showed that ^{14}C-isoleucine incorporation of parasitized RCSs, which measures anabolic activity, was inhibited by t-butylhydroperoxide; this in vitro work was done with *P. Falciparum* (29). More recent lines of evidence further suggest that reactive O_2 intermediates kill malaria parasites. In vitro introduction of hydrogen peroxide into mouse RBCs infected with *P. yoelli* and *P. berghei* killed the parasites; in vivo injection of H_2O_2 reduced *P. yoelii* parasitaemia (29). Also, *P. yoelii* was killed in vitro when exposed to systems generating H_2O_2 and O_2^-, but killing was prevented when catalase was also introduced (30). Further work with *P. falciparum* yielded somewhat similar results (31).

On balance, it is noted that the malaria parasite may have the capacity to mount protective responses in the face of oxidant stress. When mouse RBCs were infected with *P. vinckeii,* there was a marked increase in GSH together with the activities of enzymes that lead to its formation (32). Subsequent work with *P. berghei* showed that the GSH content of the cytoplasm of the infected RBC was not raised but that of the parasite was; also, a parasite glutathione reductase (GR), which catalyses the reduction of oxidised glutathione, was detected (33). Furthermore, *P. falciparum* has been found to possess its unique G6PD (34). However, to what extent the malaria parasite can tolerate additional oxidant stress is not known.

Much data have also accumulated to suggest that thalassaemic RBCs are in a state of oxidant stress and may therefore exert additional oxidant damage to asexual forms of *Plasmodium* that develop in the RBCs. There are several mechanisms that may operate in the thalassaemic RBC to generate O_2 radicals. The excess alpha or beta haemoglobin chains found in thalassaemia can undergo autoxidation (35), a process which produces O_2^-(36) and therefore, H_2O_2, OH^- and singlet oxygen. The response to these radicals would be the increased activity of O_2 radical scavenger enzymes present in the RBC, i.e., superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px). Indeed, SOD activity has been found to be increased in RBCs of beta-thalassaemia minor (37), beta-thalassaemia major (38) and HbH (39); catalase activity was also shown to be raised in beta-thalassaemia minor (37, 40) and GHS-Px activity was found to be increased in alpha-thalassaemia (41). Furthermore, GR was shown to be decreased in RBCs of alpha- and beta-thalassaemia (42).

Thalassaemics may also lack the protective antioxidant action of vitamin E (43). Serum levels in those with beta-thalassaemia major and minor are lower compared to normal individuals. (44, 45) Vitamin E deficiency can render mice resistant to *P. berghei* infection, probably through the premature lysis of the infected RBCs; vitamin supplementation restores susceptibility to parasitization (46).

Othan than the direct killing of indwelling malaria parasites by O_2 radicals in the thalassaemic RBCs, other possible mechanisms of resistance resulting from the oxidant-stressed state of such RBCs include membrane changes of the RBC, which among other effects, lead to cation leakage (47); K^+ leak has been associated with death of the intra-cellular parasite (20).

Another important consideration is the concept that the malaria parasite can induce oxidant stress in the infected red cell. Thus in the case of the thalassaemic RBC which is already loaded with O_2 radicals, the invasion of such a red cell by the malaria parasite, itself already vulnerable to the radicals and yet producing more of such intermediates, can be viewed to be a suicidal act. There is much to suggest this, although the data do not form a very coherent picture. A decrease in activities of erythrocyte SOD and GSH-Px was found in mice after infection with *P. berghei* and there was an increase in blood concentration of malonyldialdehyde, a lipid peroxidation product (48). These indicate that parasitization induces oxidation which depletes the activities of the scavenger enzymes. However, another study showed that mouse RBC SOD activity was actually increased after parasitization by *P. berghei* (49). There is also evidence that in mouse erythrocytes infected with *P. berghei,* H_2O_2 is generated (50). This observation was extended to *P Falciparum* infecting human RBCs in vitro (51). Finally, patients infected with *P. vivax,* in spite of normal activities of GSH-Px and G6PD, displayed a decrease in erythrocyte GSH content and GR activity both of which normalised after treatment (52); this suggests that RBCs of *P. vivax*-infected individuals could have exhausted their GSH supply in the presence of oxidant stress imposed by the parasities.

There has been only one study thus far which examines the interaction of thalassaemic RBCs, *P. Falciparum* and oxidant damage (20). Incubation of *P. Falciparum* in RBCs of individuals with alpha- and beta-thalassaemia traits under high O_2 environment diminished multiplication rates of the parasite; addition of certain redox catalysts like riboflavin and menadione also caused multiplication decreases but addition of vitamin E restored multiplication rates to normal levels. Though the in vitro test conditions are highly unphysiological, these experiments are the most important to date that advance the concept that it is oxidant damage which mediates resistance of thalassaemic erythrocytes of *P. Falciparum.*

2. Increased vulnerability of parasitized thalassaemic RBCs to cell-mediated damage:

Cell-mediated immunity to asexual erythrocytic forms of *Plasmodium* is effected by macrophages that, after binding to parasitized RBCs in the red pulp of the spleen or post-capillary venules, discharge O_2^-, the parasites, being vulnerable to O_2^-, then degenerate (53). Since uninfected thalassaemic RBCs are by themselves already susceptible to phagocytosis (54, 55), it is conceivable that thalassaemia and cell-mediated immunity can act in synergism to bring about resistance to malaria.

3. Elevation and persistance of HbF in infancy and early childhood of thalassaemia heteroxygotes:

Innate resistance to human malaria in endemic areas, to protect effectively, must operate during infancy and early childhood, the phase in life where passive immunity declines, acquired immunity has not developed and mortality from malaria is highest. Those who are beta-thalassaemia heterozygotes have elevated HbF compared to normal individuals; also, HbF declines at a slower rate, persisting into the second year of life (56). In vitro experiments have shown that while RBCs containing HbF do not inhibit merozoite invasion by *P. Falciparum,* subsequent development in the red cell is retarded (57, 58); this retardation is due to the presence of HbF itself (59). Again, oxidant damage may also mediate the

resistance (20). However, since HbF decline is not slow in alpha-thalassaemia, the protection exerted by HbF is probably not too important.

4. Intra-erythrocytic iron depletion in thalassaemia:

Nurse (60) on drawing from observations that iron supplementation of iron-deficient individuals induced flare-ups of malaria and arguing that there is a depletion of intra-cellular iron arising from lessened haem breakdown in thalassaemic RBCs, postulated that depletion of iron pool within thalassaemic RBCs deprives iron from the parasite and this then forms the basis of resistance. However, recent work casts doubts on this hypothesis. In vitro work with *P. Falciparum* suggests that iron is actually obtained from exo-erythrocytic uptake rather than from intra-erythrocytic haemoglobin breakdown which would in theory provide a more abundant source of iron (61, 62).

5. Interacting nutritional deficiencies:

Folic acid deficiency is a well-known accompaniment of the thalassaemia trait (63, 64). A recent in vitro study shows that such a deficiency can prevent the completion of schizogony of *P. Falciparum* (65).

Vitamin E deficiency in thalassaemia occurs and its possible effect on resistance of malaria has been discussed. Riboflavin deficiency, while not a direct result of the thalassaemic state, is apt to occur in areas of malnutrition coinciding with the thalassaemia belt. Animal experiments (66) and clinical studies (67) have indicated that this deficiency is also associated with resistance to malaria; the possible mechanism may be the decreased activity of flavin-dependant GR of the parasite, of RBC, or of both, leading to decreased RBC GSH content.

6. Dietary interactions:

Ingestion of fava beans is common among those living in the Mediterranean basin. While there is evidence for the in vitro interaction of fava bean extract with G6PD deficient RBC which results in inhibition of *P. Falciparum* development (68), there is also another study which shows that sera of individuals who have ingested fava inhibit multiplication rates of *P. Falciparum* in thalassaemic RBCs in vitro (51). Again, the mechanism of such inhibition is related to oxidant stress, since fava beans contain substances, e.g., isouramil, that behave as oxidants.

7. Low red cell indices:

Reduced mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration, occurring in thalassaemic states, can conceivably prevent schizogony on account of the relative depletion of cytoplasmic contents (12). Furthermore, since Plasmodium may derive most of its iron requirements from haemoglobin, low MCH in this respect may also be related to malaria resistance (60), but this is not likely since, as discussed earlier. The parasite more probably obtains iron exo-erythrocytically.

8. Associated pyridoxine phosphate oxidase activity in thalassaemia:

This enzyme, which catalyses the conversion of pyridoxine phosphate to pyridoxal phosphate, is defective in heterozygotes of alpha- and beta-thalassaemia (42, 69). This may be related to malaria resistance since low activity of pyridoxal-kinase, which catalyses the conversion of pyridoxine to pyridoxine phosphate, has been associated with protection of Nigerians from severe falciparum malaria (70).

CONCLUSION

It is seen therefore that the theoretical considerations for the view that thalassaemia trait protects against malaria is attractive, but the evidence is still tenuous primarily because each approach that has examined the issue has its own deficiencies. In any case, if thalassaemia actually confers resistance to malaria, there are many mechanisms that can possibly operate and it is readily apparent that such factors can act synergistically.

Some of the mechanisms reviewed are not unique to thalassaemia. The concept of oxidant susceptibility of *Plasmodium* unifies the reported resistance of HbF, HbE, G6PD deficiency as well as thalassaemia; low MCH may operate the resistance of HbC, HbE and thalassaemic traits.

The human malaria parasite spends the longest period of its cycle in the erythrocyte which affords it protection from host responses and which provides a rich pool of nutrients. This erythrocyte-multiplication stage is also the most damaging to the host and the latter can be viewed to develop many obstacles to overcome its vulnerability to the parasite. Hence, not only are high frequencies of genes for thalassaemia and certain haemoglobinopathies and red cell deficiencies found in certain endemic areas, genes for other 'variants' like Duffy-negative blood group and Melanesian ovalocytosis are also found in high frequencies in other malarious regions (71, 72).

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