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REVIEW

Cyril Ruwende · Adrian Hill

Glucose-6-phosphate dehydrogenase deficiency and malaria

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Abstract Glucose-6-phosphate dehydrogenase (G6PD) is a cytoplasmic enzyme that is essential for a cell's capacity to withstand oxidant stress. G6PD deficiency is the commonest enzymopathy of humans, affecting over 400 million persons worldwide. The geographical correlation of its distribution with the historical endemicity of malaria suggests that 66PD deficiency has risen in frequency through natural selection by malaria. This is supported by data from in vitro studies that demonstrate impaired growth of *P. falciparum* parasites in G6PD-deficient erythrocytes. Attempts to confirm that G6PD deficiency is protective in field studies of malaria have yielded conflicting results, but recent results from large case control studies conducted in East and West Africa provide strong evidence that the most common African G6PD deficiency variant, G6PD A^{-}, is associated with a significant reduction in the risk of severe malaria for both G6PD female heterozygotes and male hemizygotes. The effect of female homozygotes on severe malaria remains unclear but can probably be assumed to be similar to that of comparably deficient male hemizygotes.

CYRIL RUWENDE
earned his doctorate from Oxford University, UK. He is now a Resident in Medicine at Johns Hopkins University, and concentrates on genetic susceptibility to infection.

ADRIAN HILL
also earned his medical degree from Oxford University. His interests relate to genetic susceptibility to infectious diseases and vaccine development.

C. Ruwende (✉)
Department of Medicine, Johns Hopkins Medical Institutions,
1830 East Monument Street, Baltimore, MD 21205, USA

A. Hill
Wellcome Trust Centre for Human Genetics,
University of Oxford, Windmill Road, Oxford OX 3 7BN, UK

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Abbreviations *G6PD* Glucose-6-phosphate dehydrogenase

G6PD

Glucose-6-phosphate dehydrogenase G6PD is a cytoplasmic, so-called 'house-keeping' enzyme that catalyses the first and rate-limiting step of the hexose monophosphate pathway for pentose phosphate synthesis. This pathway is an important source of NADPH that is required for several biosynthetic reactions and is essential for maintaining adequate intracellular levels of reduced forms of glutathione and other sulphydryl groups. By preserving and regenerating reduced forms of glutathione as well as promoting the stability of catalase, NADPH plays a major role in a cell's ability to withstand oxidant stress. The hexose monophosphate shunt catalysed by G6PD is also an important source of ribose which is essential for production of nucleotide coenzymes, the replication of nucleic acids and, therefore, cell division [1]. The biological importance of G6PD is underlined by the fact that this enzyme has been detected in virtually every cell type in all contemporary organisms [2].

Along with the loci encoding colour blindness and factor VIII, the G6PD gene is located in the telomeric region on the long arm (Xq28) of the X chromosome [3, 4]. The G6PD gene consists of 13 exons and spans approximately 18 kb [5]. The G6PD protein has a molecular weight of 59 kDa, and the active enzyme is composed variably of two or four identical 515 amino acid subunits [6].

Genetic polymorphism of G6PD

The G6PD locus is probably the most polymorphic locus in humans, with over 300 allelic variants known, at least

87 of which have reached polymorphic (i.e. >1%) frequencies [7, 8]. These variants have been characterised biochemically based primarily on their differing residual enzyme activities, electrophoretic mobility patterns and also on their physicochemical (thermostability, chromatographic behaviour) and kinetic (K_m for glucose-6 phosphate or NADPH, pH dependence and utilisation of substrate analogues) properties [7].

The G6PD variants are grouped into the following classes depending on the degree of enzyme deficiency and associated clinical symptoms:

– Class I: severely deficient associated with chronic non-spherocytic anaemia
– Class II: severely deficient, <10% residual enzyme activity
– Class III: moderately deficient, 10–60% enzyme activity
– Class IV: near normal or normal enzyme activity, 60–150% enzyme activity
– Class V: enzyme activity, >150%

To date, comparison of gene sequences encoding enzyme variants to that of the normal G6PD B gene has led to the identification of at least 34 different mutations [7]. These mutations are widely spread throughout the gene, being found in all exons except exon 3 and exon 13. All but one of these are point mutations associated with amino acid substitutions [2]. The exception is G6PD Sunderland, which is due to a 3-bp deletion and results in the loss of an isoleucine residue [9]. Interestingly, a substantial number of these variants are associated with variable forms of G6PD enzyme deficiency. The absence in the G6PD gene of larger deletions, or other mutations such as nonsense mutations or frameshift mutations that would completely abolish the function of the protein, suggests that complete absence of the G6PD enzyme is incompatible with life [2].

In Africa G6PD is essentially a tri-allelic polymorphism (Table 1). G6PD B, the normal variant associated with normal or 100% enzyme activity, is the commonest allele, with frequencies of 60–80%. G6PD A which has 90% of the activity of G6PD B is the next commonest allele with frequency between 15–40%. The third allele which is the common deficiency allele in Africa is G6PD A$^-$. It is a class III variant with 12% enzyme activity, and it varies in frequency from 0% to 25% [7].

G6PD A$^-$ is unique in that it contains two mutations. The first at nucleotide 376, which on its own gives rise to G6PD A [10], is an adenine to guanine substitution that results in an asparagine to aspartate amino acid substitution while the second mutation, usually a guanine to adenine substitution at nucleotide 202, leads to a valine to methionine substitution [11]. Although the nucleotide 202 substitution accounts for at least 95% of the G6PD A$^-$ molecular variants in Africa, in a minority of individuals two other alternative second mutation sites have been identified at nucleotides 680 and 968 [11, 12].

Table 1 G6PD alleles in Africa and their enzyme activities

Alleles	Class	Enzyme activity	Frequency
G6PD B	IV	100%	0.60–0.80
G6PD A	IV	80%	0.15–0.40
G6PD A–	III	12%	0.00–0.25

G6PD deficiency

Frequency and distribution

G6PD deficiency is the commonest enzymopathy in man affecting over 400 million persons worldwide [13]. This disorder, which is caused by a multitude of the different structural allelic mutants of the G6PD gene referred to above, is found mainly in the tropical and sub-tropical regions of the world, with the highest rates, usually 5–30%, being found in Africa, Asia, the Middle East, the Mediterranean and Papua New Guinea [7]. Worldwide the frequency figures range from 62% in Kurdish Jews to 0.1% in Japan and northern Europe [14].

Clinical features

Clinical expression of G6PD deficiency is probably dependent on an interaction of the molecular properties of a given deficiency variant, exogenous factors and, possibly, additional genetic factors [7]. In unstressed normal cells G6PD activity is only 2% of total capacity [14], and therefore it is hardly surprising that most individuals with the more common class II and III G6PD deficiency variants are usually asymptomatic. Although there is no direct evidence to support this, it is likely that there is a correlation between the degree of enzyme deficiency and the propensity to develop clinical symptoms.

The most striking clinical syndrome associated with G6PD deficiency, acute haemolytic anaemia, occurs as a manifestation of this disorder on the mature red blood cell. On account of its long non-nucleated life-span and hence its impaired ability to generate adequate levels of NADPH and reduced forms of glutathione, the mature erythrocyte has a diminished reductive capacity to respond to oxidant stress. Uncompensated oxidant stress in the erythrocyte leads to oxidation of haemoglobin to methaemoglobin, heinz body formation and membrane damage [15]. In the extreme this leads to haemolysis while less severe oxident stress increases the deformability of the erythrocyte and probably enhances the likelihood that the stressed cell will be removed from circulation by the reticuloendothelial system [16, 17].

Acute haemolytic anaemia is therefore the most frequent clinical manifestation of G6PD deficiency. The haemolysis is precipitated most commonly by infections but can also occur after the ingestion of drugs and foodstuffs that contain oxidant components or in certain metabolic conditions such as diabetic ketoacidosis [18]. Agents with oxidant properties such as primaquine, sulphonamides, nitrofurantoin and several anti-inflam-

matory agents are the most common drugs associated with haemolysis [18]. Fava beans (*Vicia faba*) commonly ingested in the Mediterranean are the most well documented causative dietary agent and are associated with a well characterised condition, favism. Favism is associated with the severely deficient class II G6PD Mediterranean form and not the moderately deficient G6PD A– form that is common in Africa. Although all victims of favism are G6PD deficient, not all (only 25%) G6PD-deficient individuals develop favism after consumption of the fava beans [1, 7], suggesting that they may be other genetic or environmental factors involved in the pathogenesis of this condition.

For class I G6PD deficiency variants the formed enzyme is functionally so poor that the red cell life-span is shortened even in the absence of stress, and hence class I variants are associated with a chronic non-spherocytic hemolytic anaemia [7] with affected individuals typically having mild to moderate anaemia and splenomegaly. The disadvantage of the chronic anaemia probably outweighs any survival advantage afforded by these class I mutations, and not surprisingly most of these mutations arise sporadically and are not usually propagated in populations [7]. Interestingly, most of the mutations that give rise to these class I variants are clustered near the carboxyl terminus of the G6PD protein [13].

Another serious clinical effect of G6PD deficiency is icterus neonatorum or neonatal jaundice, which in severe cases can lead to permanent neurological damage or death. Increased red blood cell destruction accounts for some of the hyperbilirubinaemia observed in this syndrome, but it is likely that severe enzyme deficiency in the hepatocyte may impair the catabolism of bilirubin and thus also contribute to the development of jaundice [7].

X-chromosome activation and G6PD deficiency

The G6PD gene is on the X chromosome and hence one of the two G6PD alleles present in females is subject to inactivation. Variable X-chromosome inactivation means that expression of G6PD deficiency differs markedly among female heterozygotes as their red blood cell populations are variable mosaics of deficient and normal cells [19]. This phenomenon affects all somatic cells in the body such that G6PD phenotypes have been successfully used in the past to determine the clonal origins of certain tumours and embryonal tissues in such female G6PD heterozygotes [19–22].

The G6PD deficiency and malaria hypothesis

Epidemiological evidence

Although several different hypotheses have been advanced to explain why G6PD deficiency has been selected for in different populations [1, 23]. The striking geographical correlation between the distribution of these polymorphic deficiency variants with areas with historical endemicity of *P. falciparum* malaria suggests that disorder has risen in frequency through natural selection by malaria. The geographical distribution of G6PD deficiency can not be attributable solely to gene flow. Indeed, the presence of many diverse G6PD variants that have arisen independently and reached polymorphic frequencies in geographically disparate areas [7] further supports the occurrence of natural selection of this disorder. This hypothesis is further supported by the results of micromapping studies within relatively restricted geographical areas such as Kenya [24], Papua New Guinea [25], Greece [26] and Sardinia [27] that have demonstrated a similarly remarkable geographical correlation between altitude and the distribution of G6PD deficiency with the lower altitude (<1000 m) areas, known to have more intense malaria transmission, being clearly associated with higher frequencies for G6PD deficiency.

In vitro evidence

Reports from early field studies that *P. falciparum* and *P. vivax* parasites preferentially invade younger red blood cells that have relatively higher G6PD activity [28, 29], as well as the observation that in the presence of normal and deficient erythrocytes malaria parasites preferentially develop in the normal cells [30], led investigators to propose that G6PD-deficient erythrocytes confer protection against malaria by inhibiting erythrocyte invasion or intracellular development of the malaria parasite [31, 32]. Since then there have been several independent studies in the literature reporting impaired growth of *P. falciparum* in G6PD-deficient erythrocytes [33, 34], although in some studies this was only observed when cultures were subjected to oxidative stress [32]. Furthermore, there are data that indicate that in heterozygous females, who as a consequence of variable X-chromosome inactivation have different proportions of normal and deficient cells, the degree of parasite growth inhibition is proportional to the percentage of deficient cells present [35].

Although there is growth inhibition in G6PD-deficient erythrocytes, it is now clear that after a few growth cycles the parasite can overcome the inhibition [36], and it had been suggested that the parasite achieved this by producing its own G6PD enzyme [37, 38]. An ingenious mechanism (based on the premise that expression of parasite G6PD enzyme is determined by G6PD genotype of the host erythrocyte) was put forward [38] as a possible mechanism to account for the results of a previous study that had indicated that G6PD deficiency protection against malaria was the sole prerogative of female heterozygotes [39]. Hence in uniformly deficient red blood cells such as those found in deficient hemizygous males or deficient hemizygous females the parasite's own induced G6PD enzyme would compensate for the lack of the host's enzyme. However, in female heterozygotes,

who necessarily have mixed populations of deficient and non-deficient erythrocytes, parasite adaptation would be compromised, and thus the parasite growth and multiplication impaired by the parasites need to repeatedly switch on and off its own enzyme as it moved from deficient to non-deficient host red blood cell. While confirming the phenomenon of adaptation, subsequent studies have found that the parasite G6PD levels do not appear to be affected by the host red cell genotype [40–42].

Field studies

It has long been recognised that definitive answers on the malaria/G6PD hypothesis have to come from field studies. Such studies have needed to answer several important questions. Firstly, is G6PD deficiency protective against malaria, and if so, is it protective against uncomplicated mild malaria, severe malaria or both? Secondly, if the disorder is protective, what is the extent of protection, and are all the different male and female deficiency genotypes afforded similar protection? Answers to these questions would provide a basis for understanding of the mechanism of protection of this disorder against malaria in addition to clarifying the evolutionary mechanisms responsible for the high prevalence of this genetic disorder in most tropical and sub-tropical populations.

The literature on previous G6PD deficiency/malaria field studies is full of conflicting reports [28–30, 39, 43–47], summarised in Table 2. The most widely quoted field study was carried out by Luzzatto's group in Nigeria in the early 1970s [39]. The frequencies of the G6PD A males and G6PD A^{-}/B females were lower in those children with malaria, suggesting that these genotypes are protective against the disease. Their observation that the mildly deficient G6PD A males and only G6PD A^{-}/B heterozygotes and not G6PDA^{-}/A heterozygotes with comparable G6PD activities, nor the more markedly deficient hemizygous males and homozygous females, are protected against malaria appears to indicate that *the degree* of enzyme deficiency per se is not involved the mechanism of protection against in G6PD deficiency.

Unlike the sickle cell trait, whose protective effect in malaria has been relatively straightforward to prove in vitro and field studies, the proposed protective role of G6PD deficiency in malaria has proved difficult to verify. There are several reasons that may explain this. In contrast to sickle haemoglobin, G6PD follows a sex-linked rather than an autosomal inheritance pattern, and males therefore have distinctly different genotypes from females. In addition, there is considerable genetic heterogeneity associated with G6PD deficiency, and in some populations more than one deficiency variant is present. More pertinently, it is known that fitness of the deficien-

Table 2 Summary of some field studies on G6PD deficiency and malaria

Investigator	*n*	Comment	Population
Studies supporting protective role of G6PD deficiency			
Alison and Clyde [28]	532	Lower parasite rates and densities in deficient male children; reduced levels similar to children with sickle trait for both indices	East Africa
Gilles et al. [43]	100	Reduced frequency of both deficient males and females in cases with very high parasite counts (>100,000@mm3) compared to controls	Nigeria
Luzzatto et al. [30]	–	Greater rates of parasitisation of non-deficient erythrocytes in mixed erythrocyte populations of female heterozygotes with acute malaria	Nigeria
Bienzle et al. [39]	702	Reduced frequency of female heterozygotes only in paediatric cases; reduced parasite densities in same group	Nigeria
Butler [44]	277	Lower incidence of malaria in nonimmune deficient adult African-American males compared to their non-deficient counterparts	Vietnam
Kar et al. [45]	708	Significantly reduced parasitisation rates in deficient males and females	India
Studies indicating no protective role of G6PD deficiency			
Kruatrachue et al. [29]	203	Increased malaria in deficient males (1–3 years old) compared to non-deficient males in same age group	Thailand
Powell and Brewer [47]	16	No difference in parasitisation and parasitaemia levels in experimentally infected deficient and non-deficient nonimmune adult African-American male prison inmates	USA
Martin et al. [46]	68	No protection against cerebral malaria for any of the deficiency genotypes	Nigeria

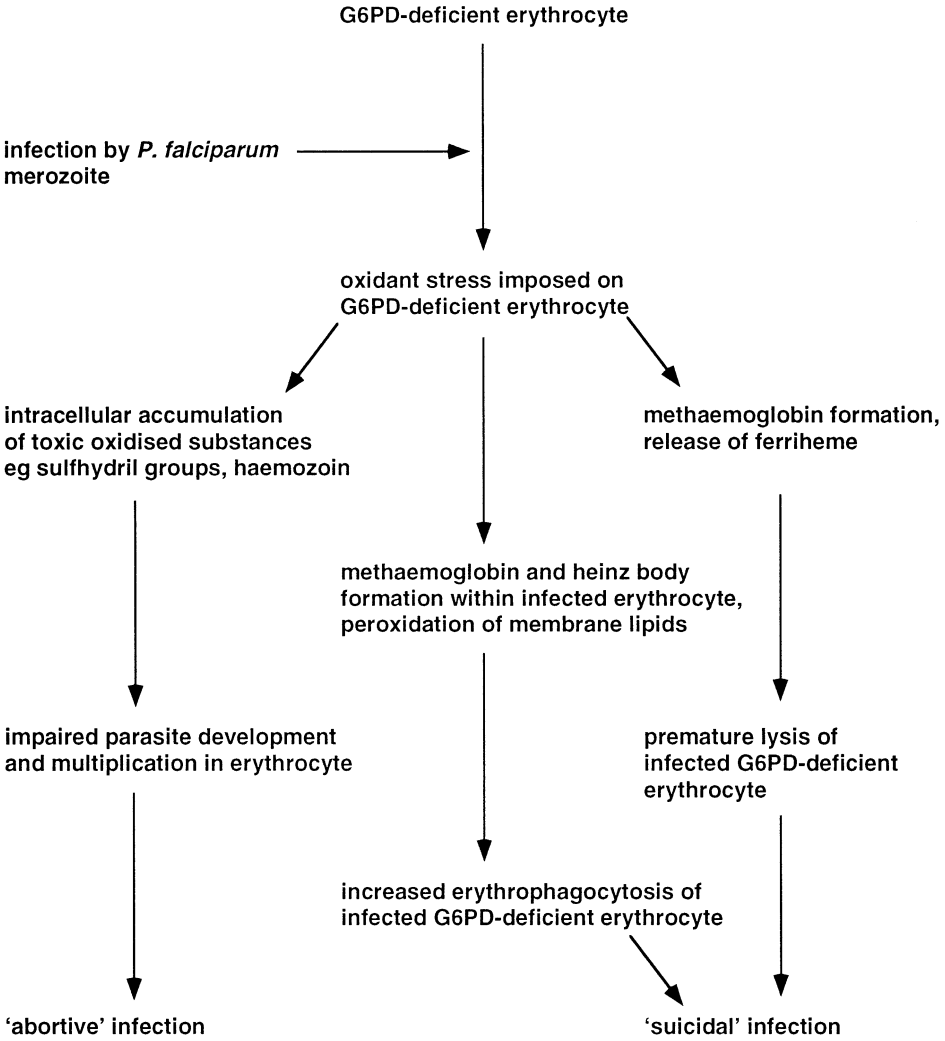

Fig. 1 Possible protective mechanisms of G6PD deficiency against severe malaria

cy phenotype is decreased significantly only under a limited number of specific circumstances and therefore on average very little. Lastly, G6PD deficiency may interact with other genetic factors and population specific non-malaria environmental factors, such as diet, to modify the net fitness of the carrier [48].

In retrospect it would have been surprising if a significant effect of G6PD deficiency had been clearly demonstrated in some of the major field studies carried out in the past. Invariably these clinical studies attempted to define the various genotypes from phenotypic measurements of enzyme levels, electrophoretic mobility and cytochemical staining patterns [39, 43, 46]. This is difficult because overlapping levels of enzyme activity are seen among genotypes, related partly to variable inactivation of X chromosomes in female heterozygotes and partly to altered rates of erythrocyte turnover in acute malaria [7]. Furthermore, several of these studies were studying parasite densities in cases of the more common clinical condition, mainly mild or uncomplicated malaria [39, 46]. Recently it has been found that comparison of genotype frequencies in children with the relatively rare condition of complicated or severe malaria [49, 50] with those of matched controls seems to offer a more sensitive measure of the effect of malaria resistance alleles [51, 52].

The largest field study on G6PD deficiency and malaria was carried out in two malaria endemic regions in East and West Africa and measured the frequencies of the G6PD A^-, G6PD A and G6PD B genotypes in over 2000 DNA samples collected from children under 10 years [53]. This was the first and certainly the largest field study to use precise molecular techniques to unequivocally define G6PD genotypes in an investigation of the proposed protective effect of a G6PD deficiency allele, such as G6PD A^-, against malaria. There was no significant heterogeneity in odds ratios between the two populations studied, and the results of both studies demonstrated that the frequencies of both female heterozygotes and male hemizygotes are lower in the children with severe malaria than in controls. While female heterozygotes were significantly protected against both se-

vere malaria (46%) and mild malaria (41%), male hemizygotes were only significantly protected against severe malaria (58%). Unfortunately, female G6PD A$^-$ homozygotes were too rare to allow measurement of the susceptibility of this genotype to severe malaria. A possible protective effect of the G6PD A genotype, with 85% of normal enzyme activity, was sought by comparing the frequencies of this genotype between the clinical groups in which an effect was perhaps most likely to be detectable, i.e. males with severe malaria to control males. This did not demonstrate any significant differences in the frequency of this genotype between these groups. Heterozygosity for haemoglobin S was strongly protective against both severe and mild malaria in both areas, but no clear evidence of interaction between G6PD and haemoglobin S genotypes was observed, although the power of the study to detect this was low.

The data from this African case-control study strongly suggest that the G6PD A$^-$ allele is associated with substantial resistance to severe malaria in hemizygous males as well as in heterozygous females. Its results concur with in vitro studies showing impaired growth of *P. falciparum* in enzyme-deficient erythrocytes [33] and suggest that the degree of enzyme deficiency is central to the protective mechanism of G6PD deficiency against malaria. There are several mechanisms which might explain this phenomenon at the molecular level (Fig. 1). The most likely attributes protection to reduced multiplication in deficient erythrocytes, probably as a result of the intracellular accumulation of toxic oxidized substances such as disulphide glutathione and haemozoin [17, 34, 54]. However, there is some evidence that infected deficient erythrocytes are more susceptible to haemolysis as a result of increased methaemoglobin and release of ferriheme, a known cytolytic agent [55], and that in addition, they are more readily phagocytosed by cells of the reticuloendothelial system as a result of the erythrocyte changes (i.e. methaemoglobin and heinz body formation, membrane damage) that are associated with the oxidant stress imposed by parasite infection [17].

The degree of protection associated with G6PD deficiency reported by Ruwende et al. [51, 56] is less than that afforded to carriers of sickle haemoglobin but equal to or greater than that associated with some HLA variants and thalassaemias. The African A$^-$ variant has a higher level of enzyme activity than the most prevalent Mediterranean and some Asian types of G6PD deficiency, and the associated disorder is milder [7]. Therefore if the degree of enzyme deficiency is important for the mechanism of protection, it is likely that protection against malaria conferred by G6PD deficiency is at least as great in many non-African populations with variants associated with greater G6PD enzyme deficiency.

The main clinical complications associated with G6PD deficiency today are acute haemolysis and neonatal jaundice [7]. Individuals with more severe enzyme deficiency such as male hemizygotes and deficient female homozygotes appear more susceptible to all of these manifestations. Since the estimate of the degree of malaria resistance afforded to female heterozygotes is little different from that of male hemizygotes, the overall fitness of female heterozygotes is likely to be greater than that of hemizygotes and homozygotes. This situation constitutes a balanced polymorphism [57] and perhaps explains the observed rarity of populations in which frequencies of G6PD deficiency are in excess of 50% [7].

Most studies addressing the malaria G6PD question have involved the more common *P. falciparum* malaria, and hence there is a paucity data on the role of G6PD deficiency in non-falciparum malaria. One study in Nigeria reported a lower than expected frequency of female heterozygotes amongst 33 girls with *P. malariae* associated nephrotic syndrome [1]. Another study by Kar et al. [45] in northern India reported protection in female heterozygotes and male hemizygotes against both *P. falciparum* and *P. vivax* malaria.

There are still several issues that have not been addressed on the malaria G6PD hypothesis. Data on the role of enzyme-deficient female homozygotes as well as that of the numerous other known G6PD deficiency variants on severe malaria are still needed. It will also be interesting to pursue these current data with in vitro work to assess the possible effects of G6PD deficiency on important host-parasite phenomena such as sequestration, rosetting and macrophage release of key cytokines such as tumor necrosis factor, interleukin-4, and interferon-γ in response to parasite infection. Furthermore elucidation of the precise biochemical pathways with which oxidative stress in deficient erythrocytes interferes with parasite growth may provide additional impetus for efforts to modify existing or design novel chemotherapeutic and possibly immunotherapeutic agents that more effectively target essential enzymes and cofactors in the parasite growth process within the infected erythrocyte.

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