Molecular Aspects of Host-Parasite Interactions in Malaria: A Brief Review

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Introduction

From the point of view of the human host, the purpose of host-parasite interactions is to identify the differences between the two and how these might be exploited, for the selective benefit of the host. Malaria organism is a highly successful parasite with very sophisticated mechanisms for survival in the host's hostile environment. However, with the recent availability of the genomes of the human host, the parasite (3D7 clone of Plasmodium falciparum)[1] and the vector (Anopheles gambiae)[2, 3], the stage is well set for obtaining a greatly improved understanding of all relevant interactions. This should yield new drugs, vaccines and other tools for the control of malaria. This subject is vast, for, in addition to the genomes, it covers the relevant transcriptomes and proteomes[4]. Thus, this article is restricted to three (3) illustrative topics: P. falciparum genome structure, its merozoite surface protein -1 (PfMSP-1) and its glucose 6-phosphate dehydrogenase (PfG6PD).

P. falciparum genome structure

Ploidy. Unlike in the human host, the parasite exists in nature in the haploid state throughout its life except for the zygote produced by fertilization of the female gametocyte by the male gamete in the mosquitoe midgut.

All the blood stages which account for all the human pathology in malaria are haploid parasites. Any issues relating to this difference in ploidy status are likely to be taken care of by the results of the ongoing International HapMap Project (**http://genome.gov/10005336**), which seeks to develop haplotype map of the human genome.

Codon usage

One of the peculiarities of P. falciparum is that it is extremely A-T rich. In its genomic DNA, Adenine + Thymine together make up 80%. Other species are not that A-T rich. For example, the monkey parasite P. knowlesi is only 62% A-T-rich, similar to that of the host [5] [6]. Genes from these sub-human parasites are sometimes easier to clone and express as soluble proteins are therefore used to get some idea of gene expression in Plasmodia where P. falciparum poses specific experimental difficulties[7, 8].

Extra-chromosomal (mitochondrial and plastid) DNA

The 23- megabase nuclear genome of P. falciparum is distributed among 14 chromosomes and encode about 5,300 genes [1]. The mitochondrial genome is 6 kilobases long and is arranged as polydisperse linear concatemers. These mtDNA concatemers replicate by a mechanism similar to that in certain bacteriophages and plasmids[9] another potential opportunity for selective disruption of parasite metabolism, without affecting the host. The small size of the P. falciparum mtDNA genome and the small number of copies (20) per cell, are consistent with the lack of a functional Krebs cycle and electron transport chain in malaria parasites. Thus, like in its red cell host, glucose metabolism in the malaria parasite is limited to glycolysis. The pyruvate generated by this is converted to lactate, a major source of the metabolic (lactic) acidosis seen in clinical malaria. Although somewhat controversial, during fertilization in primates including man, all the mitochondria in the zygote are thought to come from the mother, as the mitochondria in the middle piece and tail of the spermatozoon are thought not to enter the ovum[10]. It is not clear whether an analogous situation occurs in malaria parasites, although one study already suggests this may be so, with implications for parasite drug-resistance studies[11].

The plastid DNA (plDNA) genome is 35 kilobases long and is a covalently-closed circular DNA. Its DNA sequence data indicate that it is of plant origin, being similar to plastids in plants. Among others, it

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carries genes for 25 tRNAs which have unique features, including the presence of an intron in one of them[12]. It also encodes the elongation factor Tu (EF-Tu)[13] and carries the sufB gene, analogous to a bacterial operon associated with iron metabolism[14] and so may be important in the production of malaria pigment. EF-Tu an elongation (of protein chains during synthesis) factor that is sensitive to some antibiotics. On balance, protein synthesis in malaria parasites resembles the mechanisms in plants in some aspects, bacteria in some others and resembles mammals in yet other ways. Thus, gentamycin has no effect at all on parasites. In fact it is added to the culture medium in continuous cultures[15]. Yet, rifampicin apparently does inhibit P.vivax growth even if only partially[16]. By contrast, macrolide antibiotics (erythromycin and azithromycin) and tetracyclines (tetracycline and doxycycline) are useful, at least when combined with other drugs, in treating multidrug-resistant falciparum malaria.

Merozoite surface protein -1 (MSP-1)

Active immunisation approaches (natural and recombinant proteins)

In several rodent and primate experimental models of malaria, MSP-1 has been shown to be a good target for development as a candidate blood stage vaccine[17]. It is particularly attractive as a target because it is the only blood stage form of the parasite that comes into direct contact with antibody without the presence of host cell molecules in the red cell membrane or elsewhere. MSP-1 is processed (cleaved) in 2 proteolytic steps, the second of which occurs and goes to completion only during successful red cell invasion. This leaves a 19 kilodalton fragment attached to the merozoite surface which enters the red cell with the parasite. The bulk of the protein is released into the surrounding supernatant. Antibodies binding to the C-terminus of MSP-1 inhibit the second processing step as well as red cell invasion i.e. they are inhibitory antibodies. Other antibodies which bind to the C-terminus or elsewhere in the molecule block this inhibition i.e. they are blocking antibodies. Recently, it has been shown that these antibody responses also occur in natural human infections with P. falciparum [18]. Using a combination of site-directed mutagenesis and PEPSCAN, recombinant MSP-1(19) bearing amino acid substitutions in various combinations have been prepared and evaluated. Some of these recombinant (mutant) MSP-1 have been identified which bind inhibitory but not blocking antibodies. These mutants may prove more suitable for development as MSP-1-based vaccines than the naturally-occurring molecules [19].

Passive immunisation approaches (diabodies and chimeric molecules)

The fact that passive immunisation succeeded in the past, suggests that immunoglobulin-based therapies are potentially useful. A novel approach has been the generation of anti-MSP-1(19) single chain diabody and chimeric molecules of IgG1 and IgA1. Although these molecules caused merozoite phagocytosis in-vitro they did not protect against an in-vivo parasite challenge [20]. This indicates that the Fc fragment is essential for immunoglobulin-based protection in-vivo, at least with regard to the particular MSP-1 epitope studied.

Glucose 6-phosphate dehydrogenase (G6PD)

Although the epidemiological evidence for protection against severe malaria conferred by G6PD-deficiency has been compelling for a long time [21, 22] and the cellular mechanisms fairly well-established [23-25], studies on the structure and function of the parasite's own G6PD gene has gained momentum only relatively recently, with the availability of the results of the genome sequencing projects on the human host and the parasite. Basically, it has been shown that P. falciparum G6PD molecule is much larger (approximately twice the size) than the human enzyme [26] [27]. More recently it has been shown that parasite has a novel bifunctional enzyme, containing enzymes for the first two steps of the hexose monophosphate shunt on the same molecule [7], just like the ones for folate metabolism. Considering the nanometre or Angstrom unit distances in which molecules interact, this bifunctional enzyme arrangement may be metabolically more efficient than the situation in the human host where the two enzyme activities lie on different molecules. In addition to the foregoing, the parasite enzyme has a unique insert between the two enzyme domains. In cloning experiments, excising portions of this insert led to loss or reduction of enzyme activity. This indicates another possible target for drugs or vaccines [8].

The foregoing insights were gained through the use of *P. Berghei*, which proved easier to clone than *P. falciparum*, probably because of the very high A-T richness of the *P. falciparum* genome. Relatively little is known about the pattern of expression of *P. falciparum* G6PD at different stages of the erythrocytic cycle or when growing in host cells of various genotypes, particularly G6PD-deficient red

cells. What evidence there is indicates that the expression of parasite G6PD is greater during the ring (young) red cell stage than in the more mature trophozoites and schizonts. This is what one would expect, since in the oxygen-rich environment of the red cell, oxidant stress is probably greater for the small (ring-form parasite than for the trophozoites and shizonts which occupy a larger proportion of the red cell. But, when growing in G6PD-deficient children with natural infections, the findings were counter-intuitive [28]. Logically, one would have expected that more expression of the parasite gene in G6PD-deficient hosts than normal. However, this finding is fully consistent with the malaria-G6PD hypothesis in that protection is conferred on G6PD-deficient individuals. Nevertheless, it must be noted that the foregoing experiments measured messenger RNA (mRNA) for P. falciparum only, without measuring the protein production. Transcription of DNA to mRNA does not prove translation into protein. Thus, experiments measuring parasite G6PD protein are still required.

In summary, molecular approaches to the study of host-parasite interactions are yielding new potential targets for drugs, vaccines and other methods of intervention.

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