Malarial parasite pathogenesis and drug targets

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Abstract

This report highlights recent insights into malarial parasite pathogenesis that are relevant for new antimalarial drug discovery.

Introduction and context

Of the 160 or more species of *Plasmodium*, five are known to infect humans, one zoonotically. Each species lives in at least two hosts and proceeds through at least 12 distinct stages of differentiation, and formally, each human parasite causes a different disease. Simply elucidating the biology of human malarial parasites is thus a huge undertaking, particularly since we can easily culture only a handful of the distinct stages. Translating what has been learned in the laboratory (largely via *Plasmodium falciparum* blood-stage culture) into insights relevant for understanding malaria pathogenesis is difficult and limited in scope. Using that information to then develop new drugs active against drug-resistant *P. falciparum*, as well as *P. vivax* infections, is arguably the greatest challenge in modern infectious disease.

On the bright side, we now have more genome sequences for *Plasmodium* species than any other eukaryotic genus, and along with advances in other tools (transfection, microscopy, animal models, and inexpensive drug-susceptibility assays) and the cellular models of pathogenesis that can now be developed with these tools, this bodes well for new therapeutic advances [1–3].

Pathogenesis

Malarial pathogenesis is still not fully understood [4,5]. Hepatosplenomegaly, thrombocytopenia and anemia usually occur as malaria develops, particularly in children. Increasing cyclic fever, recurrent headaches,

fatigue, nausea, and musculoskeletal pain are other clinical signs. Malarial deaths follow from coma, kidney failure, or other complications. Hyper-reactive malarial splenomegaly arises from an aberrant immune response to malarial parasites and can be exacerbated via co-infection with schistosomes or HIV [6,7]. Placental malaria and cerebral malaria in children are not fully understood and each presents very serious additional clinical challenges. The cellular and molecular causes of this wide array of clinical symptoms are complex. Laboratory and/or animal models to determine these causes, against which candidate drugs can be conveniently screened, are severely limited, both in number and in scope.

The initial site of parasite invasion is the skin, when sporozoites are injected via a mosquito blood meal. These quickly localize to the liver, invade hepatocytes, differentiate, and divide. Very little is known about liver stages, but new tools and approaches, particularly intravital imaging techniques and recombinant parasites expressing green fluorescent protein (GFP), are rapidly defining additional key concepts [8]. Sequestration and egress from the liver vary for different *Plasmodium* sp., but for *P. falciparum*, new merozoites emerge within approximately 2 weeks as large clusters called merozoites. Free merozoites are then disseminated in the blood, where a multitude of cellular and molecular events that occur upon red blood cell invasion have been documented and characterized in great detail by many laboratories.

Major recent advances

Important complementary information on the consequences of hematological changes associated with malaria comes from biochemical and genetic studies that have recently begun to define the underlying molecular causes of resistance to severe malaria [9–12]. Various hemoglobinopathies have been linked to resistance to malaria for some time, but defining the cellular and molecular mechanisms for these phenomena has proven elusive. Recently, however, one common theme that seems to be emerging is that vascular adhesion and rosetting defects exist for infected red blood cells (iRBCs) from malaria patients who also carry sickle cell or thalassemia traits [9,10]. This then re-emphasizes that cellular adhesion phenomena are key to malarial pathogenesis. Also, recently [13], the major receptor for adhesion of iRBCs to the placenta during maternal malaria was defined. It should be possible to extend these and other advances to develop potent new assays for drug screening.

Almost all known antimalarial drugs target the intraerythrocyte stages of *P. falciparum* parasite development, yet there are at least two other human stages (intrahepatocyte and blood gametocyte) that in theory could also be specifically targeted. There is confusion regarding whether known drugs [for example, chloroquine (CQ), primaquine (PQ), and artemisinin (ART)] target multiple stages, and there is also disagreement regarding how effectively new and existing drugs act versus all important species (for example, *P. falciparum* versus *P. vivax*). These issues are due in part to our inability to culture all stages effectively and the fact that *P. vivax* cannot be conveniently cultured at all without constant addition of fresh reticulocytes. Thus, currently, we have no way of expeditiously screening for drugs that would be effective versus multiple stages of all human malarias. Also, as mentioned above, assays for various layers of cellular pathogenesis are still in their infancy. This leads us to take-home message 1: Designing versatile pharmacophores or combination therapies with multiple antimalarial uses depends crucially on fundamental advances in cell culture and assay design that so far have proven elusive.

On the bright side, genome and proteome data tell us that malarial parasites express a plethora of unique potential molecular targets. Laboratory studies tell us that many of these are essential to pathogenesis. Some new target categories are well conserved across *Plasmodium* sp. and they include unusual ion channels [14], proteases [15], kinases [16], and metabolic pathways [17]. Since for decades these target categories have been the focus of more commercially lucrative drug design for

treating heart disease and cancer epidemics in Western societies, it is possible in theory to piggy back on those efforts to very rapidly define additional novel antimalarial pharmacophores [18–20].

Take-home message 2: Coordination between academic and pharmaceutical industry communities presents a unique and very potent opportunity for rapid advances in antimalarial drug discovery, particularly design of 'off-the-shelf' combination therapies.

Importantly, many of these new molecular targets have recently been localized to unique parasite organelles, including the acidocalcisome [21], vestigial mitochondrion [22], apicoplast [23], and digestive vacuole (DV) [24,25]. These and other organelles play critical roles in various aspects of cellular pathogenesis. Most of these organelles are just beginning to be explored in molecular detail, but it is already clear that they harbor a cornucopia of unique enzymes and metabolic pathways that in theory provide excellent drug targets.

Take-home message 3: Unique parasite organelles harbor many excellent drug targets that remain to be capitalized upon.

The DV has been, and remains, of primary interest since one unique molecular target within the DV has exquisitely important properties. Namely, free heme released from catabolized hemoglobin (Hb) is a principle target (but presumably not the only target [26]) for several established, as well as new, classes of drugs [27,28]. Mutation or alternate expression of drug targets are major routes to drug resistance. But because released heme is a cofactor made by the host, it cannot be mutated by the parasite. Since there is a fixed amount of Hb within the RBC, since the parasite must metabolize Hb in order to survive, and since the parasite cannot enzymatically degrade heme, the target cannot be alternately expressed. Also, common heme-targeted antimalarial compounds such as CQ are not broken down by the parasite. Thus the most common and potent routes to drug resistance are inaccessible, which presumably is why resistance to the quintessential heme-targeted antimalarial, CQ, took so very long to evolve on a large scale even in the presence of massive selective pressure. The CQ resistance (CQR) mechanism is therefore unique. Elucidating the CQR mechanism is beginning to define additional routes to quinoline pharmacophore modification that will provide inexpensive, stable, and effective drugs [29,30].

Take-home message 4: In finding new targets in unique organelles, we should not forget that elucidating

resistance to known targets provides equally potent paths to drug design in some cases.

Future directions

Promising advances in vaccine science notwithstanding, new antimalarial drug therapies are desperately needed. Yet relevant biochemistry, pathogenesis research, and translational research remain underfunded. It remains to be seen how efficiently X-omics will compete with more traditional medicinal chemistry and pharmacology in defining effective new treatments for malaria. To this author, there is more than a pressing need for both approaches. Novel drug target mining is (and must remain) an ongoing endeavor for the medium to long term, but with millions of deaths annually, rapid definition of inexpensive existing drugs and drug combinations as well as discovery of new, useful, and inexpensive modifications of existing field-proven pharmacophores must be accelerated.

Abbreviations

CQ, chloroquine; CQR, chloroquine resistance; DV, digestive vacuole; Hb, hemoglobin; iRBC, red blood cell infected with *Plasmodium falciparum* malaria; RBC, red blood cell.

Competing interests

The author declares that he has no competing interests related to the concepts and ideas expressed in this article.

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