PERSPECTIVES

The missing pieces

Nine experts give their opinion on the 'known unknowns' in malaria research.

BRENDAN S. CRABB & JAMES G. BEESON
Unravel natural immunity

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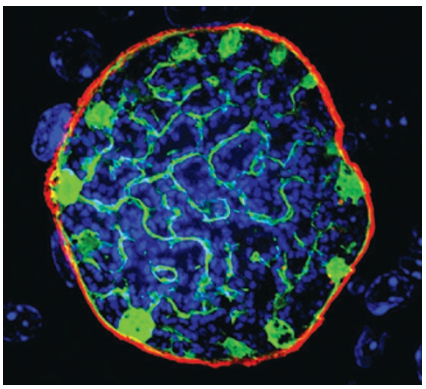
Parasite development goes through several distinct stages in both the human host and mosquito (see 'One parasite — many hiding places', page S17). After repeated infections with *Plasmodium falciparum* or *P. vivax*, the two main causes of malaria, people do eventually develop effective immunity that prevents symptomatic and severe illness and controls the blood-stage infection. This observation has long provided a strong rationale for malaria vaccine development, yet we know remarkably little of how malaria immunity works in naturally exposed individuals. Our limited knowledge of both the key molecular targets and the specific immunological mechanisms has severely constrained vaccine development. What we do know is that protective immune responses predominantly act against the blood stages of *Plasmodium* parasites and have multiple targets and effector mechanisms. Although immune responses also develop against liver-stage parasites and transmissible forms of the parasite (gametocytes), even less is known about their nature and relevance.

Malaria research needs to establish the relative significance of the many known or predicted antigens. It should focus on defining the mechanisms that clear or prevent infection, and should be complemented by studies into how malaria immunity is acquired and maintained as well as how the parasite evades the immune response. To help achieve these goals, government and private funding agencies must build research capacity in malaria-endemic countries, which will help promote greater linkages between immunology research, population studies and clinical trials. Understanding the basis of human immunity will be key to developing long-lasting malaria vaccines, and will also enable us to identify populations at highest risk and to monitor those populations in malaria elimination programmes over time.



A malaria parasite in the liver stage of its complex lifecycle.

ROGERIO AMINO & ROBERT MÉNARD
Identify the critical antigens

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The symptoms of malaria arise only after *Plasmodium* has left the hepatocytes (liver cells) and infected red blood cells — or erythrocytes. Therefore, an important goal is to develop an efficient vaccine against the pre-erythrocytic (PE) stages of the parasite — the sporozoites injected into the skin by the mosquito and the parasite forms that develop inside hepatocytes. Live PE parasites attenuated by irradiation or gene inactivation are known to provide solid protection against infection; however, their use as vaccines for humans in endemic areas faces major technical and logistical limitations (see 'The take-home lesson', page S24). The subunit vaccines now in clinical trials, which are based on sporozoite antigens, have shown limited efficacy. Clearly, other vaccine candidates must be identified. The biggest research need is to identify the right antigens among the thousands expressed by PE parasite stages.

Studies of the attenuated parasite vaccine in rodents indicate the crucial protective role of a certain type of immune cell, $CD8^+$ T cells, which detect and destroy hepatocytes infected by the malaria parasite. A functional assay that exploits this ability of $CD8^+$ T cells would help identify such antigens. Recent technical developments, such as transcriptomics and proteomics to identify PE antigens, expression cloning procedures to catalogue the many antigens found, and new imaging technologies, make possible systematic screens to find antigens capable of eliciting protective immunity.

ANDY WATERS
Focus on the ookinete

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Female *Anopheles* mosquitoes transmit the malaria parasite. In the mosquito gut, male and female gametocytes fuse to create a zygote, which develops into an ookinete. The ookinete crosses the mosquito midgut wall and implants, where it continues to develop. A successful ookinete has overcome tremendous odds to survive, and it exists in a hostile environment, surrounded by human blood cells that are being enzymatically digested. It is here in the mosquito gut that the *Plasmodium* parasite suffers the greatest proportional loss of numbers of any stage of its lifecycle, making it an interesting target for blocking transmission.

In 1911, Ronald Ross (who won a Nobel prize for his work linking malaria transmission to the *Anopheles* mosquito) recognized that, in many endemic settings, malaria dies out if transmission rates drop below a certain level. Although transmission is dropping in Africa, mainly thanks to insecticide-treated bed nets, we need additional measures to accelerate that trend. An increased understanding of ookinete biology would help this effort, including the identification of virulence factors critical to its survival, and the discovery of elements that could stimulate an innate immune response in the mosquito. Logically, targeting the weakest link in the Plasmodium lifecycle will be the best way to develop new vaccines, drugs

SEATTLE BIOMED

or vector-control methods that will further reduce the passage of the parasite through the mosquito and hasten its extinction.

ELIZABETH A. WINZELER
Don't ignore vivax

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Malaria caused by *P. vivax* is more widespread than that caused by *P. falciparum* — it endangers up to 40% of the world's population. Indeed, of the two parasites, most scientists agree that the malaria caused by *P. vivax* will be the more difficult to control and eliminate: infections are trickier to detect; the parasite can hide as a hypnozoite (a dormant form) in the liver and cause relapses years after initial infection; and there are fewer tools available to study *P. vivax*.

The only licensed drug that is able to eliminate *P. vivax* hypnozoites is primaquine. This drug needs to be given at low doses over 2 weeks because of its potentially lethal toxicity to individuals who are deficient in the metabolic enzyme glucose-6-phosphate dehydrogenase. Parasite resistance to primaquine is suspected but, within an endemic region, there is currently no accepted method to distinguish relapses caused by drug failure from those caused by reinfection. Furthermore, *ex-vivo* assays cannot accurately assess resistance because primaquine becomes active only after the liver metabolizes it.

Although it is widely agreed that we need a new drug to replace primaquine, there are no established anti-hypnozoite targets, nor are there accepted screening methods that do not involve non-human primates. These are crucial gaps for researchers to address.

MATS WAHLGREN
Tackle severe malaria

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Improvements in supportive care, including adjunct drugs, could lower the fatality rate for the 10 million or so individuals who develop severe malaria each year.

The critical factor in malaria pathogenesis is the obstruction of blood-flow that results when parasitized red blood cells block the human microvasculature and cause inflammation. Indeed, in patients with cerebral malaria, the level of vascular obstruction correlates directly with the depth of coma. For severe *P. falciparum* malaria cases, death rates remain high in spite of the availability of anti-parasitic drugs (artesunate — derived from artemisinin), intravenous fluids and state-of-the-art intensive care. No adjunctive treatment has been shown to be of benefit in severe malaria and there are few clinical data regarding the optimal supportive care of patients in the early stages of their hospitalization.

In patients with severe malaria, the majority of deaths occur within the first 48 hours of hospitalization. It is therefore crucial for the physician to be able to 'unstick' the infected cells and restore blood-flow without delay. Research that helps us understand the molecular details of this process is vital because it will allow for the development of novel anti-adhesive and anti-inflammatory strategies. Questions such as 'how does the parasitized red blood cell bind in the human microvasculature' and 'how do these cells block the capillaries and cause inflammation' need to be answered.

DAVID A. FIDOCK
Define resistance

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Recent evidence for the emergence of *P. falciparum* parasite resistance to derivatives of the antimalarial artemisinin suggests that in a few short years we may be faced with the loss of this vital drug (see 'Holding out for reinforcements', page S16). Despite intense efforts to discover and develop new antimalarial agents, no suitable alternative is ready to replace artemisinin. How can the research community best help, at a time of shrinking science budgets? Understanding the biological features of artemisinin tolerance or resistance is key to defining molecular markers to monitor its spread, and to developing therapeutic strategies that effectively treat drug-resistant strains. This will require an exceptional level of sharing of reagents, technologies and knowledge, as the genetic and molecular basis of decreased parasite susceptibility to this drug is likely to be particularly complex.

More research is also required to define how artemisinins work, how this translates into parasite death, and what metabolic pathways enable parasites to withstand drug action. Such investigations will involve the application of next-generation sequencing, genetic association studies to define candidate loci, faster methods of genetic manipulation of *P. falciparum*, and metabolomics. Research into mechanisms of resistance also needs to extend to the partner drugs used in artemisinin-based combination therapies and to the new chemical entities that are entering clinical trials.

The recent reductions in malaria deaths are very encouraging, but these gains are fragile. We cannot allow malaria to resurge.

SOLOMON NWAKA
Harness local knowledge

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About 80% of the populations of Africa, Asia and Latin America rely on local traditional medicines to meet their primary healthcare needs. In some instances, these local solutions have spurred innovation that has transcended national borders to save millions of lives around the world. Take artemisinin, for example. Now the mainstay of malaria control, this drug was derived from centuries-old traditional Chinese medicine. But there is a significant knowledge gap on how best to tap into local knowledge. Research and guidelines are needed to: inform and develop sample collection methods; generate and evaluate data on the efficacy, safety and quality of traditional medicines; and to help us understand how these approaches work.

Several discoveries based on traditional African medicines are also in our hands, but these are in desperate need of translation into usable products. This is true not only for treatment and control of malaria, but for a variety of other diseases.

Our work with the African Network for Drugs and Diagnostics Innovation (ANDI) and by other groups suggest that what is needed are coordinated mechanisms and investment to support the translation of local knowledge into affordable, safe and accessible products. Sustainable solutions can be realized only if appropriate regulatory and policy frameworks are established to guide research, development, production and use of such medicines. ■

Elizabeth A. Winzeler and Mats Wahlgren declare conflicts of interest: go.nature.com/spwwfg