Knocking out malaria

By Tim Fulmer, Senior Writer

Developing subunit vaccines against *Plasmodium falciparum* is challenging because the malaria parasite expresses a variety of antigenic proteins over the course of its complex lifecycle. Work by a group at **Albert Einstein College of Medicine** and **New York University School of Medicine** now suggests a strategy that could take antigen choice out of the equation by using attenuated *P. falciparum* strains as the basis for whole-organism vaccines.[1]

A key challenge is to prove that the strategy generates strains sufficiently attenuated to be safe in humans.

The life cycle of the *Plasmodium* parasite has three distinct stages. During the pre-erythrocytic or liver stage, sporozoites migrate from the site of a mosquito blood meal to the host's liver, where they differentiate within liver cells into merozoites, which are released into the blood again.

In the erythrocytic or blood stage, the merozoites invade erythrocytes, which eventually rupture and release new merozoites as well as gametocytes into circulation. The latter are then transmitted to a mosquito during feeding.

The third stage occurs in the gut of the mosquito, where the gametocytes form a zygote, closing the developmental cycle that ultimately leads to the production of thousands of new sporozoites (*see* **Figure 1, "The malaria parasite life cycle and antimalarial vaccine strategies"**).

A pre-erythrocytic malaria vaccine should generate antibodies against sporozoites and thus prevent infection from progressing beyond the asymptomatic liver stage to the clinically symptomatic blood stage.

An erythrocytic vaccine would be expected to produce antibodies against merozoites and thus potentially limit clinical manifestations of disease.

Finally, a sexual-stage or transmission-blocking vaccine would generate antibodies against gametocytes. These antibodies would be taken up by the mosquito during its blood meal and would prevent zygote formation in the insect's gut.

The most advanced malaria vaccine is RTS,S/AS02, a subunit vaccine from **GlaxoSmithKline plc** that targets the pre-erythrocytic stage. RTS,S/AS02, in Phase II testing, consists of a single *P. falciparum* antigen, circumsporozoite protein, which is fused to a surface antigen

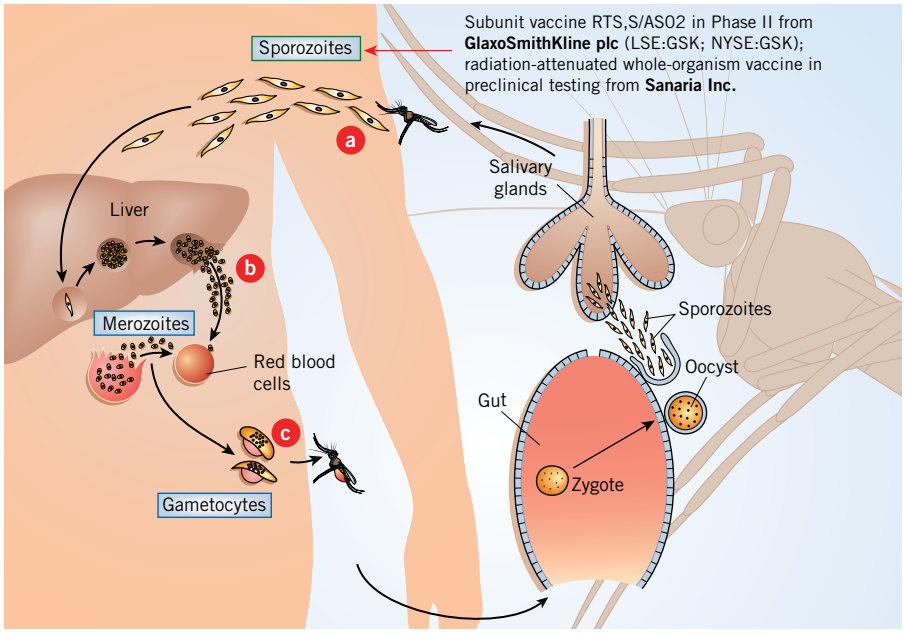

Figure 1. The malaria parasite life cycle and antimalarial vaccine strategies. Three distinct stages of the parasite's life cycle are potential targets for both subunit and whole-organism vaccines.

[**a**] At the asymptomatic pre-erythrocytic stage, an infected mosquito takes a blood meal and injects *Plasmodium falciparum* sporozoites into the blood stream of the human host. The sporozoites soon reach the liver, where they infect hepatocytes and undergo asexual reproduction to produce merozoites. A pre-erythrocytic malaria vaccine would generate antibodies against sporozoites.

[**b**] The erythrocytic or blood stage begins when the infected hepatocytes rupture to release merozoites into the blood stream. The merozoites then infect red blood cells (erythrocytes). During this stage, clinical symptoms occur including fever, anemia and loss of renal function. An erythrocytic vaccine would produce antibodies against merozoites.

[**c**] The final, sexual stage begins when a proportion of the merozoites develop into male and female gametocytes, which are taken up by a feeding mosquito. The gametocytes combine in the gut of the insect to form a zygote and, ultimately, develop into a new crop of infective sporozoites that collect in the salivary glands prior to injection into the host. A sexual-stage or transmission-blocking vaccine would generate antibodies against gametocytes. (Figure based on Figure 1 in ref. 9.)

from HBV to enhance immunogenicity. The vaccine is delivered with an AS02 adjuvant.

In trials in sub-Saharan Africa, the vaccine was safe and immunogenic in adults and children.[2,3]

A variety of other subunit vaccines target the pre-erythrocytic or erythrocytic stages of the parasite.[4] Researchers have also considered attenuated whole-organism vaccines.[5]

In a paper in *Nature Medicine*, the researchers hypothesized that knocking out purine nucleoside phosphorylase (PNP), an enzyme that plays a role in the purine recycling and salvaging pathways, could lower *Plasmodium* viability without completely killing the organism.

Unlike their mammalian hosts, *Plasmodia* cannot synthesize purine molecules, which are required for DNA and RNA synthesis. Consequently, the parasites have evolved a pathway to salvage and recycle purines and thus ensure survival under conditions of minimal purine availability in the host.

In mice infected with *P. yoelii*—a murine model of malaria infection—knockout of the *PNP* gene in *P. yoelii* lowered blood-stage rates of parasite growth, decreased host parasitemia (infected red blood cells) and increased host survival compared with what was seen in mice infected by wild-type *P. yoelii*.

The *PNP*-deficient strain also had impaired development in the mosquito compared with that of the wild-type strain.

Importantly, mice that cleared the attenuated *PNP* knockout strain were completely protected against subsequent challenge with i.v. wild-type *P. yoelii*–infected erythrocytes.

The authors wrote that their study suggests "a strategy for the development of attenuated nontransmissible metabolic mutants as blood-stage vaccine strains."

Weak but not weak enough

A key question is whether the PNP knockout strategy could produce a strain sufficiently attenuated for safe use as a human malaria vaccine.

"Immunization with the attenuated blood stages depends on first establishing a blood-stage infection that the host is capable of clearing without succumbing to any features of clinical malaria," said Chris Janse, head of the Leiden Malaria Research Group at the **Leiden University Medical Center**. "The most important concern for protection mediated by attenuated blood-stage parasites is that these parasites themselves should not result in clinical symptoms, pathology or severe disease."

"The researchers present an elegant and sound strategy for producing genetically attenuated blood-stage malaria parasites," said Stephen Hoffman, CEO and CSO of vaccine developer **Sanaria Inc.**, who nevertheless thinks the Albert Einstein and NYU team should study the knockout strategy in monkey models.

"Infection with the attenuated strain nevertheless results in parasitemia levels of around 20% in mice—which would certainly not count as attenuated in humans," he said. "Parasitemia levels greater than even 0.1% in subjects previously unexposed to malaria can result in clinical disease."

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Kami Kim, corresponding author on the paper and professor of medicine, microbiology and immunology at Albert Einstein, agreed. She told *SciBX* she is looking at "whether we can make a human malaria *Plasmodium falciparum PNP* knockout strain that is attenuated but protects in a primate model."

However, Kim added, "because humans regulate levels of purines in the blood quite tightly, we predict that a *PNP* knockout strain in the human parasite *Plasmodium falciparum* will be more attenuated than in the rodent malaria species."

Testing this prediction could prove difficult in preclinical models because it is unclear whether attenuated malaria strains with lower growth rates in those models will also demonstrate less virulence and disease severity in humans, Janse told *SciBX*.

"No appropriate nonhuman primate model exists to study *P. falciparum* virulence and therefore the analysis of virulence is only possible through experimental human infection," he said.

Stefan Kappe, associate member of the **Seattle Biomedical Research Institute**, suggested that the researchers might want to develop additional mutant strains that are more attenuated than those in the paper and that don't result in high parasitemia.

Again, Kim agreed. She said her group "will also attempt to enhance the attenuation by deleting another gene in the purine pathway. The idea is that this new strain would still be protective but less likely to cause symptomatic disease. Also, a strain with more than one gene deletion should be safer because the multiple deletions should make it less capable of regaining virulence."

A full characterization of the mouse immune response to the attenuated strain is also important, said Timothy Wells, CSO of **Medicines for Malaria Venture**, a not-for-profit organization that supports public-private partnerships in malaria research.

Following infection by the attenuated strain, it would be ideal if the host organism shifted away from a cell-mediated, macrophage-driven immune response to a primarily antibody-driven response with increased IL-4-mediated B cell activation and enhanced production of antibodies against the parasite, Wells said.

Such immunological characterization studies are already planned for the attenuated strain in the *Nature Medicine* paper, Kim noted.

Liver before blood

Some researchers working on attenuated malaria vaccines have preferred to focus upstream of the blood stage of the parasite's life cycle.

Sanaria is developing a radiation-attenuated *P. falciparum* sporozoite vaccine.[6]

"Our approach has been to focus on the pre-erythrocytic or liver stage of the parasite's life cycle rather than the erythrocytic stage, as is done in the *Nature Medicine* paper," said Hoffman. "We've found that irradiated malaria sporozoites are capable of invading hepatocytes and expressing antigenic proteins but cannot replicate there. There is thus no blood-stage parasitemia. Nonetheless, the radiation-attenuated strain can elicit both an antibody and a killer T cell response in the host."

The company plans to start a U.S. trial of a vaccine based on the radiation-attenuated strain in 1H09. The trial is expected to enroll about 100 healthy volunteers. Endpoints will be safety and prevention of malaria infection.

Kappe and colleagues also are focusing on the pre-erythrocytic sporozoite stage, but they are using genetic knockout in place of irradiation to produce attenuated strains. In mouse models of malaria, their strategy produced attenuated *P. yoelii* and *P. berghei* strains that did not cause blood-stage infection but nevertheless protected against challenge by wild-type infectious sporozoites.[7,8]

"We have recently engineered the first *P. falciparum* attenuated strains and will go into clinical trials in U.S. volunteers by spring of next year," Kappe told *SciBX*.

Kim agreed that a liver-stage vaccine is appealing because it targets the parasite before it causes parasitemia. Nevertheless, even a single parasite escaping the liver may be enough to cause infection of erythrocytes and clinical disease. Thus, liver-stage vaccines need to be exceedingly efficient to ensure all liver-stage parasites are killed, she said.

Moreover, in areas with high endemic rates of disease, many adults have detectable but asymptomatic parasitemia. In such areas, said Kim, a blood-stage vaccine could help protect children and others at risk from severe clinical disease, even while allowing asymptomatic, subclinical disease to persist.

At the end of the day, "responses against a broad range of antigens may be beneficial and so combinations of vaccines may provide better coverage and efficacy," said Stephen Todryk, a senior lecturer at **Northumbria University**. Todryk and colleagues are exploring the use of viral vector vaccines against malaria.

Patenting and licensing status of the findings described in the *Nature Medicine* article has not been disclosed by the authors.

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