Chemistry & Biology, Vol. 12, 1253–1254, December, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.chembiol.2005.12.001

Innovations

Biotechnology May Help Thwart Malaria

For millions of Africans, the bite of a mosquito is more than a nuisance; it means malaria. Malaria is caused by a parasite of the Plasmodium family, transmitted to humans through the bite of the *Anopheles* mosquito.

As the infected mosquito feeds, it injects a small amount of saliva as well as Plasmodium sporozoites, the "spore" of the Plasmodium. The sporozoites are carried by the bloodstream to the liver within minutes. The sporozoites infect the liver, where they rapidly asexually reproduce over 1 or 2 weeks. Some sporozoites transform into merozoites, which emerge to infect red blood cells, and divide, bursting apart the blood cells in a chain reaction, spewing out more merozoites. This stage, lasting 2 days, is characterized by chills, anemia, fever, and fatigue. The next mosquito that bites the infected person ingests the merozoites, which sexually reproduce in the mosquito's guts, producing sporozoites that migrate into its saliva glands. The parasites that remain in the liver can stay dormant for weeks or years until the next malaria attack.

The acute form of the disease kills an estimated one to three million people a year. The nonlethal form debilitates millions more. Small children and pregnant women are particularly vulnerable. Malaria is a low-profile disease in the developed world because it occurs mainly in impoverished countries. Malaria infections can be prevented by eliminating the mosquito vector, but in sub-Saharan Africa, a region plagued by everything from political instability to famines, the lack of basic services make any attempt to entirely eliminate the mosquito futile. Anopheles is increasingly resistant to cheap insecticides like DDT, and the lethal strain of *P. falciparum* is becoming resistant to common medications. The result is that despite all efforts, deaths from malaria have increased over the last 20 years. New biotechnology initiatives aim to reverse this trend.

Gin and Tonic, Anyone?

Quinine, extracted from the bark of the Peruvian Chinchona tree, was known to the indigenous population and was adopted by Spanish missionaries in the early seventeenth century as a malarial prophylactic. Quinine can lessen the severity of attacks, but only destroys the parasite in the blood. Chloroquine and sulphadoxine-pyrimethamine have been cheap, effective remedies but have been misused. As a result, resistant strains of the parasite have already rendered these formerly frontline drugs mostly useless.

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Currently, the most effective drug against malaria is artemisinin, first extracted in the 1970s by Chinese researchers from the Qinghao (*Artemisia annua*) or sweet wormwood plant. Currently, the World Health Organization (WHO) recommends an artemisinin-based combination therapy (ACT). Coartem, manufactured by Novartis under a WHO contract, is a three-dose Artemether-lumefantrine combination therapy. (Artekin, a cheaper ACT, is currently used in Cambodia, China, and Vietnam but is not yet approved for international use.) The ACT's high price of $1.20 to $2.40 a dose is due to the limited availability of artemisinin, which is extracted from an 18 month old plant. It is considered too expensive for wide use, compared to earlier drugs at 20 cents a dose.

"I'm a big supporter of artemisinin-based combination therapy," says Dr. Grant Dorsey, Assistant Adjunct Professor at UCSF, who has done years of clinical work in Uganda. "There are some questions. The artemisinins are the best drugs we have. They are the only class of drugs that produce no resistance, kill the asexual form of the parasite, and are extremely rapid acting." But as artemisinins are so short acting, they must be administered in a cocktail with a longer lived drug, he says. Moreover, they are not a panacea, as patients get reinfected. Dorsey advocated a "multifaceted approach" distributing insecticide treated bed nets, providing speedy diagnostics, intermittent preventative treatment in infants, and spraying residual insecticides inside houses.

Bioengineering Medicine from Wormwood

Because of the scarce availability of the drug and the fact that a fully synthetic route is complicated, a biotechnology approach seemed to be obvious. However, early bioengineered processes for the production of artemisinin yielded low concentrations of the substance.

A promising process was developed in the laboratory of Professor Jay Keasling at UC Berkeley and licensed royalty free to OneWorld-Health, a nonprofit drug company based in San Francisco. OneWorld-Health has received $42 million from the Gates Foundation to commercialize an affordable semisynthetic source of artemisinin. Keasling's startup, Amyris, (http://www.amyrisbiotech.com/), located in Emeryville, CA, is bioengineering *E. coli* to produce a precursor for artemisinin. The precursor is isolated from the soup of growth media and chemically modified to obtain artemisinin. The precursors that Amyris produces with their technology could be used for other drugs, like Taxol, extracted from the Pacific

Yew and in short supply for breast cancer treatments and isoprenoids for the flavor and fragrance industry, says Keasling. Once the process is optimized, OneWorldHealth will license the manufacture of the semisynthetic artemisinin to reduce the price of the combination therapy to below a dollar.

Vaccinating against a Devious Foe

"*Plasmodium falciparum* kills more children in the world than any other infectious agent," says Dr. Stephen Hoffman, CEO and founder of Sanaria (http://www.sanaria.com/), a Rockville, MD vaccine company. "Malaria is curable with current drugs if caught early, but the problem is getting diagnosed in time." It is also a matter of the intensity of transmission. Just one parasite injected by a mosquito is enough to get infected. Hoffman says, "In the areas of Africa where we work, during malaria season, every child is bitten by a mosquito every day and sometimes two or three times a day. The chance of getting infected is 100% every day." Thus, the ideal remedy is a vaccine, Hoffman concludes.

But the complicated lifecycle of the parasite makes developing effective vaccines a challenge. *P. falciparum* expresses numerous antigens on its surface over its life cycle, complicating antibody recognition. Once it is inside the liver cells, the body's immune system does not detect the parasite. Natural immunity to malaria is partial and short lived, leading to reoccurrences or new infections. Even so, there are around 50 vaccine candidates under development worldwide, several in clinical trials. Conservative estimates are that they are at least 10 years away from a worldwide roll out.

Nevertheless, in October 2005, the Gates Foundation gave $107 million to the PATH Malaria Vaccine Initiative (MVI) the organization the Gates Foundation had established to collaborate with GlaxoSmithKline Biologicals (GSK) to do further development and testing on GSK's recombinant vaccine RTS,S/AS02A. This vaccine is currently the candidate closest to actual use for infants and children between 1 and 4 years old. RTS,S, which has been under development for 15 years, fuses part of the *P. falciparum* circumsporozoite protein with the hepatitis B surface antigen molecule RTS,S. "We don't know what the effective vaccines are, and in my opinion, we are looking to use them in conjunction with other therapies," says Dr. Filip Dubovsky, Vice President of Scientific Affairs at MVI. "We now have pretty clear proof that we've had a scientific breakthrough in that we can impact disease, infection, and severe disease using a single antigen. In our case, our vaccine has one out of the 5200 proteins that malaria carries. We can impact those clinical disease patterns for 18 months."

However, this vaccine offers only partial protection. In 2004, a proof-of-concept study was carried out under the aegis of the Manhiça Health Research Centre (CISM) on a group of 1442 children in Mozambique ages 1 to 4 years. "In the 18 months of the study, RTS,S reduced clinical disease by 35% and severe disease by 48%," Dubovsky says. "We thought there was a 29% lower rate of infection in the vaccinated group versus the control group. The impact over 6 months was 45%."

Other vaccine approaches include Transmission Blocking Vaccines being developed at the National Institute of Allergy and Infectious Disease, which attempt to prevent feeding mosquitoes from passing infection, and the LSA-1 Liver Stage Antigen that the Walter Reed Army Institute of Research is pairing with GSK's adjuvant, AS02A. GenVec (http://www.genvec.com/), a Maryland biotech company, is working with MVI and the US Naval Medical Research Center to develop a multicomponent vaccine that uses a human cold virus to carry genes for up to five antigens (CSP, SSP2, LSA1, MSP1, and AMA1) from different stages of the parasite's life cycle in one vaccine.

Help Wanted: Sharp-Eyed Mosquito Dissectors with a Steady Hand

A unique strategy is being pursued by Sanaria based on research showing that short-lived immunity can be induced by vaccination with the sporozoites found in the saliva of infected mosquitos. Sanaria's vaccine is aimed at preventing the parasite not from entering the body but from leaving the liver into the bloodstream. Their approach is distinct in another way: "We are using an attenuated whole parasite, meaning weakened, live parasite," says Hoffman. "It is the sporozoite, which is the stage which is inoculated by the mosquitos. And everyone else is using a recombinant, modern approach, meaning recombinant protein or recombinant virus."

Sanaria's vaccine is based upon a classic technique of vaccine production like the Sabin polio vaccine and the vaccine for the flu. Research conducted in the 1970s showed that weakened sporozoites could confer immunity. This was not further developed into a clinical vaccine because of the technical difficulties in obtaining, stabilizing, and storing a sufficient quantity of weakened sporozoites, Hoffman says. But biotechnology advances within the last 3 years have provided the company with the tools to develop assays of purity, potency, and safety as well as improved understanding of the molecular biology of the parasite and the means to efficiently culture it.

Sanaria irradiates infected mosquitoes to attenuate the parasite and then manually dissects out the mosquitoes' salivary glands to get the sporozoites—one mosquito at a time by hand. "We have such high production per mosquito," says Hoffman. "Our dissectors are so efficient that the way we think about it is what a chip factory looks like in Southeast Asia. Even producing 60 million doses a year won't take more than a hundred people." Sanaria is funded by the Gates Foundation, NIH, and the US Army. OneWorldHealth is providing regulatory support.

If vivisection of mosquitoes can be done on a mass scale, then modernizing an old vaccine technique may be a viable strategy. "Can we bring to bear modern bioengineering to produce the quantities of these sporozoites under aseptic conditions, extract them from the mosquitoes, purify them, preserve them in a bottle, and demonstrate that we can inject them with a needle and syringe?" Hoffman asks. "Because if we can do all of that, we have a real vaccine with us right now."

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