





The Worldwide War on Malaria

Decades of work to develop a vaccine against malaria, and there is still no way to prevent this complex killer. With new approaches, however, excited researchers claim solutions may be in sight.

By JENNIFER BOETH DONOVAN

Adrian Hill was in Farafenni, a town in the West African nation of The Gambia, meeting last winter with the local field staff working on a trial of his malaria vaccine. Most greeted him with grins, recalls Hill, but Modou Jallow's face was uncharacteristically long and his eyes sad. "I had a bad weekend," he said softly.

Jallow's two-year-old son had died of malaria the day before. Yet there he was, back at work fighting the disease, checking on some of the 372 adults who had been vaccinated in the first large-scale trial of a novel approach to vaccine design. Losing children to malaria is an everyday event in The Gambia. Parents mourn; life goes on.

A new generation of potential malaria vaccines, such as the one developed by Hill, comes too late to save Jallow's little boy. But the HHMI international research scholar believes that the "prime-boost" malaria vaccine he and colleagues at Oxford University in England developed might save many others. He is one of several HHMI international research scholars working to develop vaccines and drugs against malaria.

Caused by a parasite spread by the bite of an infected mosquito, malaria kills more than 1 million people a year, most of them children in sub-Saharan Africa. Only tuberculosis and AIDS take a greater toll. Half a billion people are infected by malaria annually, so most do survive, though many of them still suffer years later from the anemia and developmental disorders caused by severe malaria infection.

There are drugs that prevent malaria temporarily—long enough to be useful to travelers but not for residents of endemic regions. Such drugs include doxycycline, mefloquine and Malarone, but they are too costly for those in poor countries and can have serious side effects.

Quinine sulfate, chloroquine, Malarone and mefloquine can be used to treat some strains of malaria, such as the one caused by *Plasmodium vivax*, which broke out unexpectedly in northern Virginia in 2002, but *Plasmodium falciparum*, the

A scanning electron micrograph of a female *Anopheles* mosquito, one of several species of mosquito that transmit malaria.

EYE OF SCIENCE/PHOTO RESEARCHERS, INC.

most lethal malaria parasite, has developed resistance to most of these drugs. Even where antimalarial drugs are still effective, they are prohibitively expensive for malaria-prone developing nations. One treatment can cost more than \$5, and children in Africa—where the per capita expenditure for all medicines averages less than \$5 per year—often contract four or five malaria infections a year, says Monica Parise, a researcher with the Centers for Disease Control and Prevention's Malaria Epidemiology Branch.

So some scientists are focusing on preventive measures, including vaccines, insecticides, mosquito netting and public education, while others work to develop new treatments to replace drugs to which *P. falciparum* has become resistant.

"Malaria is a complex problem that must be addressed in many different ways," says Thomas E. Wellem, acting chief of the Laboratory of Malaria and Vector Research at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland. "The more approaches we use to learn the secrets of the parasites, the better it will be for the future of malaria control."

A Vaccine in Five Years?

Hill hopes to fill a major gap: the lack of effective vaccines. "We have some drugs that work, but we don't have vaccines," he says.

Researchers around the world have been trying to create malaria vaccines for decades. They've come up with a few promising candidates, but none have proved effective enough. *P. falciparum* is a formidable opponent. It has a complex life cycle, changing character and virulence as it moves from an infected female *Anopheles gambiae* mosquito to the liver of the human host, and from there into the bloodstream and back into another mosquito. *P. falciparum* also is clever at mutating to evade a vaccine's antibodies. "It's almost as if the antigen taunts the immune system," Hill explains, "saying 'take a good look at me, because the next time you see me I'm going to be entirely different.'"



P. falciparum is clever at mutating to evade a vaccine's antibodies. "It's almost as if the antigen taunts the immune system," Hill explains, "saying 'take a good look at me, because the next time you see me I'm going to be entirely different.'"

Until recently, malaria vaccines were all based on antibodies, proteins produced by B cells in the immune system to neutralize the antigens or proteins that cause disease. Hill's vaccine is different. It is designed to rev up the immune system's production of T cells that target and kill infected cells. It is called "prime-boost" because immunization is a two-step process: First, a fragment of *P. falciparum* DNA "primes" the immune system to recognize the malaria antigen; then a virus—modified so that it can't replicate and cause illness (called modified vaccinia virus Ankara, or MVA)—boosts production of T cells. These in turn attack and destroy the malaria parasite at an early stage, in the liver, before it can burst free to flood the bloodstream and produce the high fevers and other life-threatening effects of malaria.

"At first, we thought this approach wouldn't work," Hill recalls. In studies in mice, the DNA alone provided no protection, and the MVA by itself protected less than 20 percent of the time. MVA followed by DNA did not work very well either, but—"amazingly," says Hill—DNA followed by MVA produced 100 percent protection. "We all remember that day," he remarks.

Only three years after the groundbreaking mouse studies, Hill and colleagues moved into human safety studies, or phase I trials. At Oxford, the group recruited volunteers who were willing to be vaccinated and then bitten by mosquitoes infected with a treatable form of malaria. The results looked promising, so his team moved on to phase I trials in The Gambia, collaborating with the United Kingdom's Medical Research Council unit based there.

In 2002, the first large-scale trial began. Working with tribal chiefs and village leaders, Hill and co-investigators Vasee Moorthy and Kalifa Bojang found 372 adult villagers from rural parts of the country who volunteered to be vaccinated. The trial was randomized and blinded, so half of the volunteers received a placebo—in this case rabies vaccine. Rabies vaccine is much needed in The Gambia but is prohibitively expensive. Even if the placebo hadn't offered benefits, Hill thinks his group would have had no trouble getting volunteers. "Malaria is such a devastating part of their lives that both the people and the government of The Gambia are eager to assess potential new malaria vaccines," he explains. "Happily, neither the test vaccine nor the rabies placebo caused any significant adverse side effects."

If the adult trials are a success, the next step would be a phase II trial testing the vaccine's efficacy in 500 to 1,000 children. Here too, Hill doesn't anticipate any



Adrian Hill's prime-boost vaccine is being tested with high hopes in The Gambia. Baba Tam Bah (facing page), a Gambian government nurse, vaccinates a study participant.

difficulty finding parents willing to have their children immunized with the experimental vaccine. "If you've got something that works in adults, you're going to get lots of volunteers," he says.

The final stage, Hill continues, would be a phase III clinical trial in more than 1,000 infants, which would take another two years. All told, he estimates the earliest any vaccine could become generally available is around 2008. But even getting there that fast would require more resources, he says.

Different Paths, Same Goal

Regina Rabinovich, a pediatrician and epidemiologist who headed the \$50 million Malaria Vaccine Initiative at the Program for Appropriate Technology in Health in Rockville, Maryland, funded by the Bill and

Melinda Gates Foundation, thinks Hill's work is important. "He has been uniquely able to move his concepts into successful clinical trials," says Rabinovich, who recently moved to the Gates Foundation as director of its infectious diseases program. "Whether or not the prime-boost approach proves itself, we're really interested in his viral vectors, the modified viruses used to boost T-cell production."

Hill is 1 of 12 HHMI international research scholars working on different aspects of malaria. Louis Schofield of the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia, is developing a vaccine based on glycosylphosphatidylinositol (GPI), a toxin responsible for the intense fevers of malaria. In August 2002, Schofield and colleagues Peter H. Seeberger and Michael C. Hewitt at the Massachusetts Institute of Technology reported success in tests of an anti-GPI vaccine in mice.

"We are a long way from clinical trials with a vaccine," says

Melanesians Provide Malaria Clues

Just as a car can approach a city using different roads, *Plasmodium falciparum* can invade a red blood cell through several portals, says Alan F. Cowman, an HHMI international research scholar at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia.

Cowman thinks he's found a new route of the malaria parasite. Cued by an unusually high frequency of malaria resistance and very mild cases among the Melanesian people of Papua New Guinea, Cowman and his colleagues have identified an important point of entry on red blood cells that could ultimately serve as a vaccine target. Defects in this portal may explain the relatively healthy Melanesians who live in an area where malaria is otherwise quite common.

That entry point is glycophorin C, which accounts for a mere 4 percent of the glycoproteins that dot the red blood cell's surface. About half of the Papuan Melanesians are missing part of glycophorin C, and evidence suggests that they do not suffer severe malaria. In 1984, Michael J. Tanner, at the University of Bristol, showed that cells lacking glycophorin C admit fewer parasites, but he didn't zero in on the significance of his findings. "Our results are now explained," Tanner says, "by the presence of a distinct invasion pathway elucidated in the paper by the Cowman group" (published in the January 2003 issue of *Nature Medicine*).

With fewer parasites

About half of the Melanesians in Papua New Guinea (see children below) are unusually resistant to malaria. Australia's Alan Cowman has figured out why.

entering red blood cells, the critical mass necessary to trigger a full-fledged siege of chills and fever isn't reached. "The parasites still invade, but the person is unlikely to get as sick," says Cowman. The mutation in the *glycophorin C* gene may also deform the red blood cell membrane, which may contribute to dampening parasite invasion.

Cowman and his colleagues identified a molecule that participates in the bonding between the malaria parasite and its host. First, the researchers identified a protein they named EBA140 (for erythrocyte-binding antigen) from *P. falciparum*. Then, using normal parasites as well as mutants they constructed that lack EBA140, and red blood cells that have or lack glycophorin C, they mixed

combinations of surface proteins from the parasite and host cells to reveal the important relationships. Results were clear: Parasites lacking EBA140 could not latch onto glycophorin C, and parasites with EBA140 could not attach to proteins from red blood cells that lack glycophorin C. That is, the pathogen's EBA140 must bind with the red blood cell's glycophorin C to gain entry to the cell.

Blocking glycophorin C, then, might prevent or slow infection, at little or no physical cost. Though glycophorin C normally helps hold the cell's outer membrane to its inner skeleton, other glycoproteins do the same, so its loss is not harmful.

The discovery of the role of glycophorin C in malaria explains why Papuan Melanesians have an easy time with the

disease. "Their mutation is an advantage," says Cowman. Over time, the mutation accumulated in the population as malaria weeded out those who lacked it. Now, the discovery may benefit countless others elsewhere. "The work done by Dr. Cowman and his colleagues could help develop a vaccine that would stop the parasite from spreading from one red cell to another," says Brian Greenwood, director of the Malaria Centre at the London School of Hygiene and Tropical Medicine.

—RICKI LEWIS



Schofield. “The vaccine has to be put through preclinical testing in monkeys.” Daniel E. Goldberg, an HHMI investigator at Washington University in St. Louis, whose own research focuses on the biochemistry of malaria infection in humans, calls Schofield’s published findings on the GPI toxin “elegant and important.” Goldberg explains: “He actually synthesized this complex carbohydrate molecule to show that the activity seen was due to the GPI, then he demonstrated the central role of the GPI toxin in *P. falciparum* pathogenesis, suggesting that this molecule would be a good vaccine target.” Schofield’s approach is also controversial, says the Gates Foundation’s Rabinovich, because essentially it treats the fever, which is itself deadly, but does not attack the underlying cause.

Meanwhile, Chetan Chitnis, an HHMI international research scholar at the International Centre for Genetic Engineering and Biotechnology in New Delhi, India, is doing extremely promising malaria research, according to Rabinovich. Using *Plasmodium knowlesi*—a malaria parasite that infects monkeys—as a model, Chitnis is studying the invasion pathways that *P. knowlesi* uses to infect red blood cells. *P. falciparum* and *P. vivax*—malaria parasites that infect humans—use similar invasion pathways. Understanding the molecular interactions that mediate these pathways has enabled Chitnis to develop a vaccine for *P. vivax* that blocks invasion of red blood cells. He expects to begin phase I clinical trials by the end of 2003.

“His fundamental scientific rationale is sound, and he has shown the ability to take a protein made on the benchtop and translate it into a real product of sufficient quality to test in humans,” says Rabinovich. “To say that is not easy is an understatement.”

Alan Cowman, an HHMI international research scholar also at Australia’s Walter and Eliza Hall Institute of Medical Research, studied a population with uncommon resistance to malaria and identified an important pathway—used by *P. falciparum* to infect red blood cells—that might be exploited to prevent or slow infection (see sidebar).

The work of another Australian research scholar, Magdalena Plebanski, at the Austin Research Institute in Melbourne, focuses on dendritic cells—key players in the immune response because they activate pathogen-fighting T cells. Plebanski has shown that blood-stage malaria parasites impair the ability of dendritic cells to mature, thereby suppressing some of the T cells that could protect against the disease. However, when she injected mice with immature dendritic cells that had been altered by interaction with parasite-infected whole blood cells, the animals developed 80–100 percent immunity after one injection. Four months later—a long time in mice—protective immunity had not diminished, and the procedure did not make the mice sick.

Plebanski has developed a method to target dendritic cells in vivo with a novel carrier-adjuvant—a substance added to a vaccine to take it to the right place in the body and to boost the immune response—that elicits similarly high and long-lasting levels of protection against lethal blood-stage malaria. “Some vaccines are carriers, and others are adjuvants. Good vaccines aim to be both,” she explains.

Although the United States has been and continues to be a major contributor to malaria research, the enormity of the problem and the importance of the solution mandate an international effort, says Jill Conley, director of HHMI’s international program. “History has shown that no single country has the answer,” she observes.

Prevent it. Treat it. **Protect** people from mosquito bites. Kill the mosquitoes. **Transform** them into harmless nuisances through genetic engineering. There are **myriad** potential approaches to combating malaria, and a great many are **being tried**.

A Portfolio of Approaches

Prevent it. Treat it. Protect people from mosquito bites. Kill the mosquitoes. Transform them into harmless nuisances through genetic engineering. There are myriad potential approaches to combating malaria, and a great many are being tried.

Washington University’s Goldberg believes that’s a good thing. He also endorses the private-public partnerships that have been forming to take on malaria. “They’re the only way to go. Industry will not take the lead because malaria is not a financially rewarding venture for them,” he explains, “and public funding is not adequate by itself to take things forward.”

In addition to the Gates-funded Malaria Vaccine Initiative, there is the Multilateral Initiative on Malaria, whose players include the Wellcome Trust, the National Institutes of Health and HHMI; Roll Back Malaria, a global partnership of the World Health Organization, the United Nations and the World Bank; and the Medicines for Malaria Venture, a private-public partnership underwritten by the Gates Foundation, ExxonMobil Corporation, the Global Forum for Health Research, the International Federation of Pharmaceutical Manufacturers Associations and others. In November 2002, pharmaceutical company GlaxoSmithKline awarded \$1.5 million in community development grants to combat malaria in seven African nations.

Meanwhile, two international consortia of scientists published the completed genomes of *P. falciparum* (*Nature*, October 3, 2002) and *Anopheles gambiae* (*Science*, October 4, 2002). Many scientists hailed the work as a breakthrough in the war on malaria, predicting that data mined from the parasite, mosquito and human genomes will yield new and effective drugs, insecticides and vaccines. One new antimalarial, fosmidomycin, already has been developed in Germany by using *P. falciparum* genome data, and preliminary results of animal tests have been promising.

Hill still pins his hopes on prime-boost vaccines. “If the genome helps,” he says, “that’s really bad news!” Come again? “That means we’d have to wait another 25 years for an effective vaccine,” Hill explains. “With anything that comes out of the genome, we’d be starting at square one, with unfamiliar, untested genes and antigens. There are several candidate vaccines in trials right now that can be made at reasonable cost and that could be useful well before then, if the resources are provided for vaccine development. A useful malaria vaccine is needed by this generation’s children, not their grandchildren.” **H**