The Search for a Malaria Vaccine

The goal is in sight—a vaccine against malaria looks feasible at last, giving researchers a new weapon to eradicate this ancient disease

Parasitology, a long neglected field, is now becoming a hot research area. Investigators who are familiar with the field say it is becoming attractive to others because its problems are approachable with the new methods of molecular biology and because the problems are scientifically interesting as well as medically important. An estimated 3 billion people suffer from parasitic diseases. Reflecting this new interest in parasitology, the MacArthur Foundation recently announced that it is starting a $20-million research program. Science *will be reporting on the most recent developments in parasitology in a series of Research News articles, beginning with the following story on malaria.*

Research on malaria has been nothing if not humbling. Despite nearly a hundred years of attempts at eradicating the disease, it still threatens 2 billion people, which is nearly one-third of the world's population, afflicts about 300 million people a year, and causes 2 million to 4 million deaths. Yet ever since the parasite and its mosquito vector were identified at the end of the last century, researchers have predicted that victory over malaria was just around the corner.

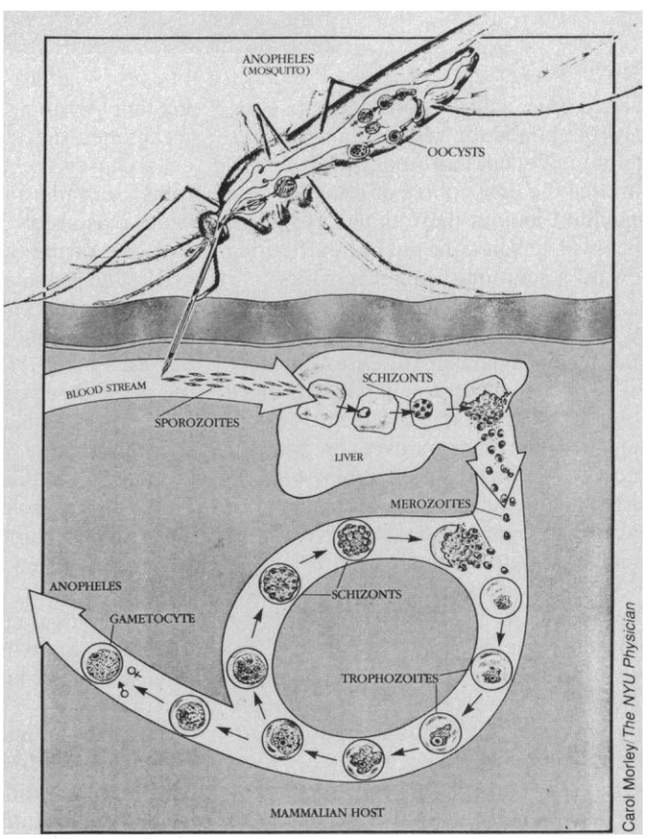
But now, investigators say, they have overcome their naïvete about the disease and they truly understand what they are up against. And they have the powerful techniques of molecular biology at their disposal, enabling them to clone genes of the malaria parasite, identify how the parasite interacts with its host, and discover how to foil those interactions. Researchers may not be able to wipe out malaria in the next few decades, but they should be able to develop a vaccine that will make inroads against the disease, especially when combined with drug treatment and insecticides.

Funding agencies agree. The U.S. Agency for International Development (AID) just decided to double its funds for research on a malaria vaccine—from $11.9 million to $22.7 million for the years 1983 to 1985. The National Institute of Allergy and Infectious Diseases (NIAID) is spending $5.4 million on malaria vaccine studies, which is one-fifth of the $27 million earmarked for tropical disease research. The total spent on malaria vaccine research in 1984 from government, international, and philanthropic institutions is $17 million, which is a substantial amount for any area of parasitology—a chronically underfunded discipline.

The story of malaria research until now has been one of great hopes and dashed expectations. It began just before the turn of the century when researchers discovered that malaria was not caused by bad air (Italian for bad air is *mala aria*) or decay or filth but by a single celled creature, a protozoan, that is carried by *Anopheles* mosquitoes. Eradication of the disease sounded simple—just get rid of the mosquitoes. Ronald Ross, who discovered the mosquito vector, thought that malaria could be eradicated from Freetown, Sierra Leone, if people would just sweep up the puddles where the mosquitoes bred. But even today malaria is endemic in Freetown.

By the end of World War II, scientists recognized that the pesticide DDT was extremely effective against the *Anopheles* mosquito and that the drug chloroquine killed *Plasmodium*, the malaria parasite. So, in 1957, the World Health Organization (WHO) launched an ambitious and expensive program to use DDT and drugs to rid the world of malaria. At first, the WHO attempt, which cost about $6 billion, seemed effective. By the mid-1960's, malaria was eliminated or nearly gone from 80 percent of the target areas. But then the picture began to change. The mosquitoes began developing resistance to DDT and the protozoa became resistant to the drugs. Malaria came back with a vengeance in many tropical areas. In Sri Lanka, for example, the WHO effort had caused a drop in malaria cases from an estimated 1 million to only 17 by 1963, 5 of which were imported from other countries. Twelve years later, 600,000 cases were reported and the actual number is thought to have been four times higher.

In retrospect, says James Erickson, who directs AID's malaria program, the health scientists were terribly naïve. They should have realized, he says, that "the project was doomed from the start. It had too narrow a focus. As scientists, we're all to blame." Louis Miller of NIAID adds that the researchers should have suspected that chloroquine resistance, at least, was going to occur. In the late 1950's, he says, Indian researchers

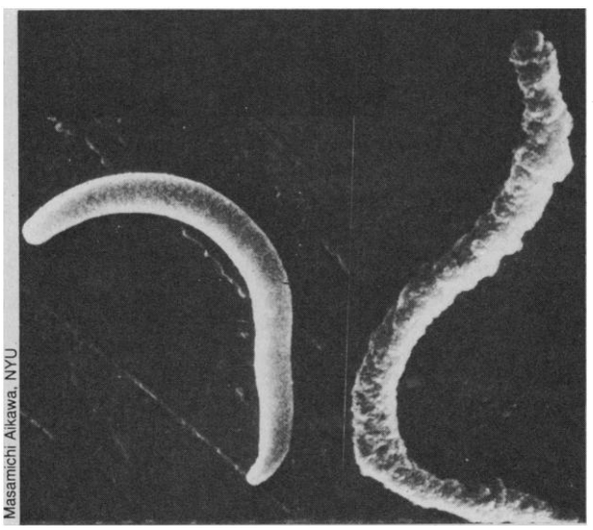


Life cycle of the malaria parasite

Injected into the bloodstream by a mosquito, the parasites quickly move to the liver, change their form, and then enter the blood again, where they hide in red blood cells and multiply.

showed that *Plasmodium* species that infect mice can become resistant to the drug. However, Miller remarks, "everyone thought that was a peculiarity of the mouse model."

Now, with the bitter experience of the unsuccessful WHO effort behind them, some investigators say their greatest fear is that the malaria parasites will become resistant to a vaccine as well, changing its antigens to elude the vaccine-induced antibodies. But researchers hope to find antigens to vaccinate against that are so crucial to the survival of the parasite that it literally cannot vary them. Even if such a goal is unobtainable, says Miller, it still may take some time for the parasite to develop resistance to the vaccine and during that time new research may lead to other ways to control the disease. "It [the hoped-for vaccine] may be like chloroquine, but that's not bad. It gets us through the next couple of decades. One shouldn't look at these things as though they will be solutions but as contributing toward a solution," Miller remarks.



Antibody to sporozoites removes their surface

Like a snake shedding its skin, the sporozoite (left) loses its surface coat (right) when it is exposed to antibodies to the major coat protein.

A malaria vaccine, when it comes, will be a first. There has never been a vaccine against a parasitic disease. Developing a vaccine against malaria is far more complex than making a vaccine against smallpox or other viruses or bacteria because the malaria parasite is so much larger and because it has a complex life cycle that seems particularly evolved to elude its host's immune system.

A malaria infection begins when a person is bitten by a female *Anopheles* mosquito carrying one of the four *Plasmodium* species that infect humans. (There are more than 100 *Plasmodium* species, which infect birds, reptiles, rodents, and nonhuman primates.) A form of the malaria parasite, called sporozoites, enters the person's blood from the mosquito's saliva and, within half an hour, each sporozoite invades a liver cell where it presumably is safe from the body's defenses. There each sporozoite starts to divide, forming a structure in the liver cell known as a schizont. Each schizont eventually contains thousands of a second form of the parasite called merozoites. The schizonts rupture and spill the merozoites into the person's bloodstream. There, within 20 seconds, they invade red blood cells and multiply again asexually. The red cells burst open and the merozoites quickly take up residence in other red cells and multiply again. This cycle continues until the person dies of anemia, kidney failure, or brain damage, or until the disease is brought under control by the person's immune system or by drugs. In the meantime, the rupturing of the blood cells causes the typical malarial symptoms of chills and fever, although the exact reason for this is not known.

A few of the merozoites in the blood cells differentiate into sexual forms, called gametocytes, which are ingested by *Anopheles* mosquitoes along with the person's blood. The sexual forms break out of the blood cells when they enter the mosquito's stomach. After a series of events, starting with fertilization, thousands of threadlike sporozoites are produced and go from the mosquito's stomach to its salivary glands, ready to enter a new host when the mosquito takes its next blood meal.

Despite this complicated life cycle, immunity to malaria is possible. The disease usually kills young children below the age of 5. Those who survive have developed immunity. "By age 5 or 6 in Africa, people don't die of malaria infections," says Miller. "Sometimes they have a considerable infection and yet they are not sick. An adult in Africa may have a low fever once a year and get better in a day or so." Mothers pass on immunity to their babies with their breast milk; blood from immune persons can protect children against the symptoms of malaria. Yet immune people living in areas where malaria is endemic frequently carry small numbers of malaria parasites in their blood for the rest of their lives, thereby serving as a source of the disease. Even though they may not get very sick from malaria, many of those who carry the parasite are by no means well. Their immune systems are generally suppressed, making them more susceptible to other diseases.

Each stage of the *Plasmodium* life cycle—sporozoite, merozoite, and gametocyte—is antigenically distinct, and researchers are attempting to make vaccines against each of them. They expect that, eventually, people may be given a combination vaccine that will immunize them against all three forms or, at least, against the sporozoites and merozoites. But, for now, the work on a sporozoite vaccine is most advanced.

At first, when the WHO eradication program began to fail and researchers began thinking seriously about producing a malaria vaccine, the conventional wisdom was that a sporozoite vaccine could never be effective by itself. The sporozoites are present in the bloodstream for only about half an hour, investigators reasoned, and a mosquito ejects only a small number of them—a few thousand—when it bites. Moreover, if even one sporozoite escapes the attack by the host's immune system, it will go to the liver and proliferate, creating a malaria infection. Thus, it is frequently argued, a sporozoite vaccine would either have to be 100 percent effective or it would have to be combined with a merozoite vaccine anyway.

This assumption, says Victor Nussenzweig of New York University (NYU) Medical Center, is likely to be incorrect. He and Ruth Nussenzweig head a research team that has shown over and over again in animals that a sporozoite vaccine can give complete protection against malaria. Eleven years ago, it was even demonstrated that immunity against sporozoites can protect humans against malaria. In that study, Harry Most and Jerome Vanderberg of NYU and David Clyde and Vincent McCarthy of the University of Maryland irradiated whole mosquitoes, thus attenuating the sporozoites that the mosquitoes carried. For several weeks, they allowed these mosquitoes to bite volunteers in a Maryland prison. Then they let mosquitoes

carrying viable sporozoites bite the volunteers. The volunteers were protected, indicating that they had built up complete immunity to the sporozoites. The immunity only lasted 3 to 6 months, however.

Even if a sporozoite vaccine does not give complete protection, it may still be valuable, Victor Nussenzweig argues. "I have strong reservations about the idea that a vaccine is useless if even one parasite escapes," he remarks. A partially effective vaccine should at least diminish the severity of the disease in children who have not developed any immunity to malaria, according to Nussenzweig. "There is indirect evidence that the disease is more severe when the vector is better—when the mosquito has more sporozoites in its salivary glands. If we diminish the inoculum [with a partially effective sporozoite vaccine] there should be a profound impact on the disease in children. And if you consider adults who already have some immunity, you may find that if you diminish the inoculum they will not get the disease."

Until recently, however, it was all but impossible to make a sporozoite vaccine. The problem was getting the sporozoites. The only way was to dissect salivary glands from anesthesized mosquitoes—a tedious and time-consuming operation. A technician has to work for hours to get only 10 to 100 million sporozoites. This problem has now been circumvented by investigators using the powerful techniques of molecular biology.

First, the NYU group produced a monoclonal antibody to the surface of the sporozoites of a mouse malaria. This antibody protected the mice against infection by sporozoites and zeroed in on a protein that seems to sheathe the surface of the mouse malaria sporozoite. Then the Nussenzweigs and their colleagues made similar monoclonal antibodies that identified similar proteins on the surfaces of sporozoites from a species of monkey malaria and from two species of human malaria—*P. falciparum* and *P. vivax*. When they mixed these antibodies with the sporozoites, the surface of the parasites peeled off like a skin and the parasites were no longer infectious.

The next step was to clone the gene coding for this surface antigen. Last year, G. Nigel Godson and Joan Ellis of NYU cloned the gene for the antigen from monkey malaria sporozoites. This year Vincenzo Enea, Ruth Nussenzweig and their colleagues cloned the gene from the human malaria *P. falciparum* and Miller and Thomas McCutchan of NIAID and their colleagues at Walter Reed Army Institute of Research cloned the gene from a different strain of *P. falciparum* (*Science*, 10 August, p. 607).

All of these surface proteins studied so far have short repeated sequences of amino acids. About 40 percent of the molecule consists of these repeats, indicating that it might be possible to synthesize just the repeated segment and immunize with it. "If there is any good candidate for a synthetic vaccine, it is this one," Victor Nussenzweig says. In addition, the sporozoite antibody seems effective against different strains of the same species but not against different species of the parasite.

At the same time as work continues on a sporozoite vaccine, investigators are attempting to determine which proteins of the malaria merozoite would be useful targets for a vaccine. The difficulty is that, unlike sporozoites, which have a single coat protein, the merozoites are antigenically so diverse that researchers suspect they may constantly vary the proteins on their surfaces. For example, it was shown in the 1960's that monkeys with chronic relapsing malaria infections have antigenically distinct populations of merozoites in their blood with each relapse. "Up until now, there is no defined

Coordinating the Effort

About 19 years ago, when it was clear that the World Health Organization's plan to eradicate malaria with DDT and drugs was running into trouble, the Agency for International Development (AID) decided that what was needed was a vaccine. AID administrators went to various government organizations to ask about the possibility of developing a malaria vaccine, but, says James Erickson of AID, the consensus was that it could not be done. But AID decided to go ahead anyway and built what it calls a "malaria research network"—a collaborative group of 17 universities and other research institutions that are working under contract to get a vaccine ready for testing. It is the same sort of research that goes on in a drug company, Erickson says. The AID puts out contracts for specific research objectives and coordinates the entire project.

Two long flow charts are posted on the wall of Erickson's small corner office, showing the steps to a vaccine. It is, Erickson says, "a management nightmare." The NIH and Walter Reed are cooperating with the project now, as are WHO, the Food and Drug Administration, the Centers for Disease Control, the Defense Department, the Rockefeller Foundation, and various drug companies. Erickson has set up ad hoc committees that meet frequently to discuss the progress of the research. The committees representing funding sources, for example, meet twice a year. The committee on clinical trials meets every 2 months, and the researchers get together at least twice a year.

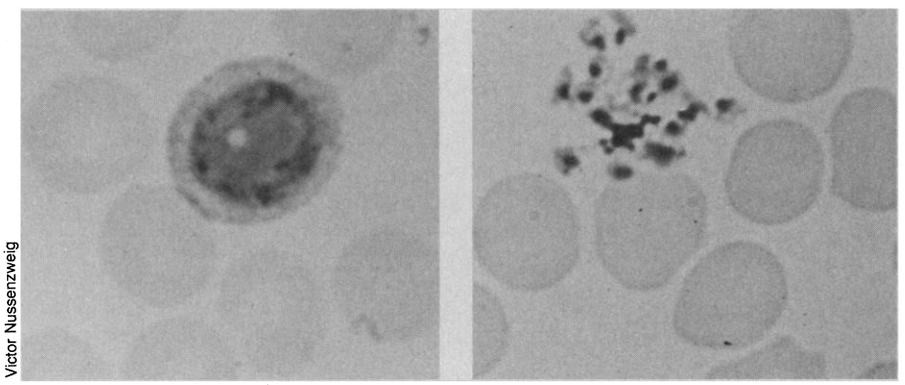
Erickson, who is already looking for FDA approval of the vaccine, requires the researchers under contract to AID to follow FDA guidelines which were established with drug companies in mind. If these guidelines are not adhered to, the FDA may not accept the work when the data are submitted for a new drug license. For example, the investigators must sign their data books each day, in ink—a practice few, if any, would have followed otherwise.

And just to be sure the researchers are properly motivated, Erickson tries to make sure they have actually seen malaria. "Eight years ago, when we had ten major scientists under contract [there are now 30], I found out that only two had ever seen a clinical case of malaria," Erickson remarks. "If they don't know what the disease is like, how can I instill in them a sense of urgency? Malaria is horrible to see, so I pushed to get people in the field. They come back a whole lot more enthusiastic about doing and completing the work."

The flow charts show that 45 projects are planned, 20 of which are completed. AID plans to begin phase one clinical trials of a sporozoite vaccine in about 2 years and to have phase two and phase three trials in about 5 years. "There's no question that we will have a vaccine," Erickson says. "The only questions are how to expedite it, how to get through the last steps of basic science and into the developing countries where the vaccine is so desperately needed."—**G.K.**

candidate for a merozoite vaccine," says Miller. "We first have to identify a surface antigen which is not variable and then we must show that antibodies to this antigen prevent parasite development."

But immunity against merozoites is possible—many people living in areas where malaria is endemic do eventually become immune. The problem, though, is that it may take repeated infections over a period of years to become immune to the repertoire of merozoite antigens. If this is so, Miller points out, it may be as difficult to develop a merozoite vaccine as it is to develop one against influenza. Investigators are hoping, though, that the situation is not this bleak. So far, according to Erickson, about 200 antigens have been isolated from merozoites or infected red blood cells and these are being evaluated to see whether any is suitable for use in developing a merozoite vaccine.



Victor Nussenzweig

Parasites grow in red blood cells

Sequestered in red blood cells, the merozoites multiply (left) until finally the cells burst, releasing the malaria parasites (right), which then enter other red blood cells. This cycle causes chills and fever.

Anthony Holder and Robert R. Freeman of Wellcome Research Laboratories in Beckenham, England, for example, have been working for 5 years with a large surface antigen that they identified on the surface of merozoites from a mouse malaria strain. When they immunize mice with this protein, the animals are protected against an infection with injected merozoites. Now Holder and Freeman are attempting to clone the gene for the corresponding antigen on the surface of merozoites from human malaria strains and to determine, among other things, whether the antigen varies during an infection.

Robin Anders and David Kemp of the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia, are studying the structure of another protein, the S (for soluble) antigen, of merozoites, which differs a great deal from strain to strain. When people become immune to malaria, Anders says, they have antibodies to the S antigen in their blood. Whether or not it may eventually be possible to find a region of the S antigen that is constant and to vaccinate against it, Anders says, "We think the S antigen will tell us how antigenic diversity is generated because it varies so much."

Peter Perlmann, Hedvig Perlmann, Klavs Berzins, and Birgitta Wahlin of the University of Stockholm and the Karolinska Institute are studying still another antigen. This one is on the surface of red blood cells infected with merozoites. Antibodies to this protein prevent merozoites from entering the red blood cells in vitro. Moreover, says Wahlin, people who are immune to malaria have antibodies to this protein in their serum.

Finally, there are attempts under way to begin developing a vaccine against gametocytes. Such a vaccine would be of no benefit to the infected person, whose disease and symptoms are caused by the asexual red blood cell forms of the parasite, but it would prevent the transmission of the disease. Any gametocyte vaccine, Miller points out, would most likely be combined with a sporozoite or merozoite vaccine so the person would get some benefit from being vaccinated. So far, the gametocyte antigens that have been identified seem quite variable, but the work on this form of the parasite is just beginning.

Erickson argues, however, that a gametocyte vaccine would be unlikely ever to come into general use because it would be extremely difficult to prove to a drug licensing agency such as the FDA that it is efficacious. To show such a vaccine works, it would be necessary to demonstrate that the population of mosquitoes carrying the malaria parasite drops as a result of the vaccine. But, Erickson remarks, the number of parasites in mosquitoes "may *wildly* fluctuate from time to time and from place to place. You may see 10 percent of the mosquitoes in an area carrying them one year, 90 percent the next, and 2 percent the year after. We haven't the foggiest idea why this happens." So it would be quite a task to prove that the number of parasites dropped because of a vaccine. Because efficacy would be so difficult to demonstrate, the large drug companies have shown little interest in work on a gametocyte vaccine and AID does not fund gametocyte vaccine research.

If a vaccine against at least the sporozoite and merozoite forms of the malaria parasite is developed—and almost everyone expects field trials of a sporozoite vaccine, at least, to begin within a few years—a final hurdle will be to find a good way of delivering it. It may be quite difficult to vaccinate in areas of Africa and India, for example, if the vaccine must be refrigerated and then injected with a sterile hypodermic needle. But Bernard Moss and Geoffery L. Smith at the NIAID believe that it may be possible to deliver a malaria vaccine in a vaccinia virus—which is the way smallpox vaccine is delivered. This would mean that the vaccine would not have to be refrigerated, would cost only pennies per dose, and would be administered by scratching the skin.

Moss and Smith are using the methods of genetic engineering to introduce new genes into vaccinia viruses and have already put the hepatitis B surface antigen gene, a herpes gene, and influenza genes into vaccinia. These engineered vaccinia viruses protect animals against hepatitis B, herpes, and influenza infections. Now Moss and Smith are working with the gene for the sporozoite surface protein that was cloned by the Nussenzweigs and their colleagues. They have put the gene into vaccinia viruses, have demonstrated that the viruses express the protein, and that when they vaccinate rabbits with the altered vaccinia, the rabbits make antibodies to the sporozoite protein.

Now the hope is that a malaria vaccine will give the eradication effort a new chance. No one thinks it will be a panacea—the parasites could very well become resistant to a vaccine just as they became resistant to drugs. But it will be a new weapon which, combined with sanitation, drugs, and insecticides, could give the world what Erickson calls "a DDT-like chance." It may mean that the first big payoff from molecular biology is a fresh opportunity to wipe out an ancient disease—GINA KOLATA