Malaria: Resurgence in Research Brightens Prospects

Americans tend to view malaria, if they even think about it at all, as a disease of the past, a once severe threat to life that has been eliminated by control of mosquitoes. In North America, Europe, and Northern Asia, it is largely a disease of the past, and the few cases of malaria that occur have usually been brought in from outside the region. In the tropical and subtropical areas of the world, however, malaria is still the single most severe health problem today. The World Health Organization (WHO) estimates that there are more than 100 million cases of malaria in the world each year that require treatment. In tropical Africa alone, malaria is responsible each year for the deaths of more than 1 million children under the age of 14. Some experts consider malaria to be the leading impediment to economic development in Africa.

Mosquito eradication programs and other antimalaria operations were viewed with great optimism after World War II. These programs have, in fact, been successful in temperate climates and were even responsible for some inroads in more tropical areas. But the programs have become entangled with administrative, organizational, personnel, operational, and financial difficulties. There have also been technical difficulties, such as (i) the development of resistance in mosquitoes to insecticides and in humans to antimalarial drugs, (ii) the behavioral patterns of some mosquitoes that prevent their contact with insecticides, and (iii) the physical impossibility of eliminating the breeding of mosquitoes in certain regions. The net result is that there has been little or no progress against malaria in tropical regions such as Africa, and a marked resurgence of the disease in countries such as India, Pakistan, and Sri Lanka.

It has become clear that methods other than simple mosquito eradication will be necessary to solve the malaria problem. The most frequently discussed need is for a vaccine against the *Plasmodium* species that cause malaria. But only a few scientists have been working in this area, and the development of the vaccine has been hindered principally by the lack of a suitable source of parasites from which such a vaccine could be prepared. Nearly as big a problem is the low immunogenicity of malaria parasites, which means that inducing immunity against them is difficult. Only recently has progress begun to be made on either problem, but that progress has attracted new workers to the field. Hence, with an increased number of investigators following up on the leads, the prospect of producing a vaccine against malaria has brightened considerably.

Outstanding among the developments in malaria research during the last quarter-century was the discovery of techniques to culture, for prolonged periods of time, *Plasmodium falciparum*, which is more widespread and more lethal then the other malaria parasites. This breakthrough was reported independently last year by William Trager and James Jensen of Rockefeller University and by J. David Haynes and his associates at the Walter Reed Army Institute of Research. Their accomplishment has been likened to that of John F. Enders and his colleagues at Harvard Medical School, who discovered a way to culture poliovirus in monkey kidney cells and thereby cleared the way for the rapid development of the polio vaccine.

Culture Asexual Forms

Both groups developed cultures of the asexual form (see glossary) of *Plasmodium* that grows in erythrocytes and produces merozoites. Their achievement consisted in finding a culture medium that contains the right combination of nutrients, gases, and buffers to keep the parasites alive. As simple as this may sound, it is a feat that has eluded scientists for some 65 years. Their success came primarily through the use of special culture media fortified with human blood serum; special buffers to maintain a proper *p*H; maintenance of an atmosphere high in carbon dioxide and low in oxygen, with oxidation inhibitors added to the medium; and the use of human erythrocytes which have been separated completely from contaminating white blood cells. With these techniques, it became possible to maintain the cultures indefinitely, compared to the former survival time of 2 to 4 days. Haynes concedes that the technique developed by Trager and Jensen is much better than his, and he has already adopted it for use in his own laboratory.

Trager, Jensen, Haynes, and several other investigators who have subsequently begun working with the culture system are now refining the technique so that larger quantities of parasites can be obtained. The investigators are also trying to find better ways to separate the parasites from the erythrocytes and to obtain other stages in the life cycle of the parasite.

They are looking for ways to eliminate the requirements for human erythrocytes and serum in the culture. These requirements could interfere with the mass culturing of parasites for the production of a vaccine, both because the supply is limited and because of the risk of contamination of the vaccine with human viruses. Nonetheless, these problems seem to be relatively minor in comparison to the initial hurdle of obtaining a long-term culture—particulary since use of the culture now makes it possible to study merozoite physiology and thus to learn growth requirements more precisely. And now that the culture technique has been developed, Trager argues, it should be possible within 3 to 5 years to demonstrate in animals whether a vaccine is practical.

It may be a major step from an animal vaccine to one for humans, however, because of the low immunogenicity of *Plasmodium*. The immunological response of a host to a *Plasmodium* infection is quite different from the response to a virus infection. Exposure to viruses generally produces lifelong immunity once the individual has recovered from the disease; vaccination with an attenuated virus or a killed-virus vaccine produces much the same type of immunity. This is not the case with *Plasmodium*. Children in Africa, for example, must contract malaria several times before they develop any immunity to *Plasmodium*, and even then many are still susceptible to low-grade infections. David J. Wyler of the National Institute of Allergy and Infectious Diseases (NIAID) suggests that this occurs because the immune systems of malaria victims may be suppressed by the release of a soluble substance by macrophages during an infection. It is also possible, although it has not yet been proved, that the parasite itself releases some substance that suppresses the immune system of the host.

Because of the low immunogenicity of the parasite, most investigators have generally found that it requires repeated exposure to *Plasmodium* antigens to produce immunity through vaccination. Alternatively, the antigens can be made

more immunogenic through the use of an adjuvant, a substance that stimulates the immune system nonspecifically.

The most commonly used adjuvant is Freund's complete adjuvant, a combination of heat-killed mycobacteria, an emulsifying agent, and mineral oil. A variant of this, called Freund's incomplete adjuvant, does not contain the mycobacteria. Both types of adjuvants have been used with *Plasmodium* antigens in attempts to stimulate immunity to malaria in animals. Freund's complete adjuvant cannot be used in humans, however, because it produces sterile abscesses and other ill effects.

Some scientists, for example, Jonas Salk of the Salk Institute, argue that it may be possible to use Freund's incomplete adjuvant in human vaccines, but there is strong resistance to this idea because other investigators have found that this formulation also produces abscesses. As promising as some of the vaccines appear, therefore, it may still require a considerable leap from animal to human vaccination because investigators have not yet found anything with efficacy comparable to Freund's complete adjuvant that is safe in humans.

Investigators have identified at least three distinct approaches toward making a vaccine. Each approach is targeted against a different stage in the *Plasmodium* life cycle, and each has its own particular set of advantages and problems.

Only one of the approaches has been tested in man. It involves development of immunity against sporozoites, the infective stage of *Plasmodium*. Much of the groundwork for this approach has been laid by Ruth Nussenzweig and her associates at the New York University Medical Center. Nussenzweig and others have found that x- or γ-irradiation of *Plasmodium*-infected mosquitoes attenuates the parasites so that they are still viable but do not cause disease. If the irradiated mosquitoes are then allowed to feed repeatedly on rodents, the rodents eventually develop immunity to the parasite.

Nussenzweig has also shown that repeated intravenous inoculation of purified attenuated sporozoites produces immunity in rodents. The immunity does not appear to be specific for either the strain or the species of the rodent parasite, as is the case with other forms of vaccination. The immunity lasts only for about 3 months, but it is apparently complete. No sporozoites are found, Nussenzweig says, when immunized animals are given a normally lethal dose of the parasite. Similar results have been obtained in mice by Richard Beaudoin of the Naval Medical Center in Bethesda, Maryland.

Nussenzweig has had some success in stimulating immunity in monkeys, although substantially greater quantities of parasites are required. David F. Clyde of the Louisiana State University Medical Center and Karl H. Rieckmann of the University of New Mexico have also been able to induce immunity to *P. falciparum* in a small number of human volunteers by allowing irradiated mosquitoes to feed on them repeatedly. The immunity induced in man is not strain-specific, but it is species-specific.

Immunity Must Be Complete

Despite these successes, there still are several problems with this method. It is crucial that the immunity produced by vaccination is complete. If even one sporozoite escapes immune detection, it can lodge in the liver and cause a fatal infection. The immunization provides no protection against the other stages of the *Plasmodium* life cycle, and therefore the immunized individual is still susceptible to infections carried through the blood. And the immunity produced by sporozoite inoculations is short-lived, so that frequent vaccinations would be required if the technique were used in humans.

A further obstacle is that only very limited quantities of sporozoites can be obtained from the salivary glands of infected mosquitoes. It may be possible to grow large quantities of sporozoites, says Louis H. Miller of NIAID, but it would require major achievements in cultivation in vitro. Such a development will probably be more difficult than the achievement of Trager, Jensen, and Haynes.

The second approach has been to vaccinate with merozoites. Most studies associated with this method have been performed with *P. knowlesi* in rhesus monkeys, but the work has been hindered by a rather unusual difficulty. In 1965, K. Neil Brown and his associates at the Na-

A Malaria Primer

Malaria is caused by more than 50 different species of the protozoa *Plasmodium*. Only four species attack humans: *P. falciparum, P. vivax*, and less commonly, *P. malariae* and *P. ovale*. The rest attack several hundred other animal hosts. Of the two most common types of clinical malaria, *vivax* is the milder. It is characterized by periodic chills and fever, an enlarged spleen, anemia, severe abdominal pain and headaches, and extreme lethargy. If not treated, vivax malaria may subside spontaneously in 10 to 30 days, only to recur at a later date. The fatality rate is low, but the disease is extremely debilitating and lowers the patient's resistance to other diseases. Falciparum malaria produces these symptoms and others, including edema of the brain and lungs and blockage of kidney activity. The fatality rate for falciparum malaria is high if the disease is not treated promptly.

Plasmodium species have a complex life cycle. They are usually transmitted to man by the bite of an infected female *Anopheles* mosquito. When such a mosquito bites, its saliva, which contains the *Plasmodium* at the sporozoite stage of its life cycle, is injected into the bloodstream of the victim. The sporozoites quickly enter the liver, where they divide and develop into multinucleated forms known as schizonts. Within 6 to 12 days, the schizonts split apart and release into the bloodstream the form known as merozoites. The merozoites invade the host's erythrocytes, where they grow and divide to form more schizonts. These schizonts also split apart, destroying the erythrocytes and releasing more merozoites into the bloodstream to repeat the cycle. The major symptoms of malaria are associated with the rupture of the schizonts.

Some of the asexual merozoites in the victim's bloodstream develop into male and female forms known as gametocytes. If at this stage the victim is bitten by a mosquito, the gametocytes enter the mosquito's stomach where they become free male and female gametes, and fertilization occurs. The fertilized gamete passes to the outside of the stomach lining, where it develops into an oocyst containing many sporozoites. When the sporozoites mature, they migrate to the salivary glands, there to begin the complete cycle all over again.—T.H.M.

Malaria Drugs: New Ones Are Available, but Little Used

Despite the rancor raised in the United States by the Vietnam War, it produced at least one beneficial side effect. As a direct result of this country's involvement in Southeast Asia, the United States Army established a large program to find and develop new drugs to combat the drug-resistant strains of malaria which decimated combat divisions in that conflict. Today, the Army has virtually the only program in the world for developing antimalaria drugs, and, as a federal program, it is second in size only to the National Cancer Institute's program for identifying new anticancer agents, despite NCI's much larger budget. Unfortunately, however, the drugs developed in the program have seen little use since the end of the Vietnam War.

The drug most commonly associated with malaria, of course, is quinine, which was the drug of choice for more than 100 years—not because it was a great drug, but because it was the only one available. It was supplanted during World War II by quinacrine, which in turn was supplanted by chloroquine and primaquine, the current drugs of choice. Chloroquine destroys merozoites in the blood, while primaquine destroys schizonts hidden in the liver. Together, the two drugs are very effective against the susceptible malaria strains that still predominate in Africa, India, and Central America.

In late 1959, however, two American engineers working in Colombia at the headwaters of the Amazon River developed a malaria that was resistant to chloroquine. About a year later, a second set of cases developed in northern Brazil. Scientists initially wrote off these episodes as isolated incidents, but in 1962, chloroquine-resistant malaria began to be observed frequently in Malaya, Thailand, Vietnam, and Cambodia. The chloroquine-resistant strains soon predominated in Southeast Asia and along the Amazon River.

In 1964, the Walter Reed Army Institute of Research was assigned the responsibility to search for new antimalarial drugs. Since then, Walter Reed and its contractors have screened nearly 300,000 compounds for antimalarial activity. Almost 3 percent of these were found to have some activity against *Plasmodium* species that infect mice. These were subjected to various other tests, including a test for activity against *P. cynomolgi* in rhesus monkeys. And finally, about 200 of the best compounds to emerge from these studies were tested against *P. falciparum* and *P. vivax* in the South American owl monkey (*Aotus trivirgatus*).

Adapting human malaria strains to grow in the owl monkey was one of the major accomplishments of the drug research program, since there are no other animals in which the human parasites flourish. The first step was taken in 1966 by Martin Young of the Gorgas Memorial Hospital and Research Institute in Panama City. Young succeeded in infecting owl monkeys with *P. vivax*. Spurred by this success, Quentin Geiman of Stanford University was able to infect the monkeys with several different strains of *P. falciparum*, including strains that are resistant to chloroquine and other drugs. A large breeding colony of owl monkeys is now maintained at the National Center for Primate Biology in Davis, California, but their total numbers are still small and their use is carefully rationed.

From the animal program have emerged 29 drugs that were submitted to clinical testing in humans. These studies were conducted at several different prisons until mid-1975, when they were terminated because of public concern about prisoners' ability to give whole and voluntary concern and because of the loss of key personnel. Preliminary clinical trials for drug safety are now conducted with paid civilian volunteers at the Washington Hospital Center in the District of Columbia. Field trials are conducted at the SEATO Medical Research Laboratory in Bangkok, Thailand. Many of the drugs that were effective in monkeys proved to be ineffective or not well tolerated in man, but seven drugs and two combinations of drugs have so far been shown to be effective in humans. Several of these have already been used to cure U.S. servicemen who contracted chloroquine-resistant malaria, but all the drugs still have an investigational status and studies are continuing.

One of the best of the new drugs, says Craig J. Canfield, the director of the Walter Reed program, is mefloquine. Mefloquine is a structural analog of drugs that had been discovered to be effective against malaria, but that produce toxic side effects. Mefloquine has been shown to cure chloroquine-resistant falciparum malaria with only one dose and is almost completely free of side effects. One dose provides protection against malaria infections for as long as 30 days. Mefloquine is also effective in terminating acute attacks of vivax malaria, but must be accompanied by a schizonticidal drug such as primaquine for complete eradication of the disease. Several other drugs that are nearly as effective as mefloquine are also being tested for use against the chloroquine-resistant species.

Unfortunately, none of the new drugs are being used to any significant extent in the areas where chloroquine-resistant malaria is endemic. All the new drugs are complex chemicals that are relatively expensive to synthesize, and the people who are most in need of the drugs are not able to pay for them. Consequently, few drug companies have shown any interest in the drugs developed at Walter Reed. Only Hoffmann-LaRoche, Inc., of Basle, Switzerland, has expressed interest so far, Canfield says. That company has produced some mefloquine and made it available to the World Health Organization (WHO) for further studies in Africa and South America. What is particularly distressing, Canfield says, is that none of the national drug companies in countries with endemic malaria have expressed any interest. These government-backed companies could subsidize the drugs and sell them at a low cost.

One bright hope appears to lie in WHO's Special Program for Research and Training in Tropical Diseases. One facet of this program is the application of drugs that have already shown some promise against malaria. Presumably, most of the drugs studied in this program will be those developed at Walter Reed. The program will try to develop new ways to use the drugs for both prevention and cure of malaria in the participating countries. If this program proves successful, then perhaps some of the countries will themselves take an interest in the drugs and the products of 13 years of research will finally be used as they were intended. Even then, it must be remembered that the parasite has a demonstrated ability to develop resistance to new drugs after they have been used for a short time. Thus the search for newer and better drugs must continue.—T.H.M.

tional Institute for Medical Research in London vaccinated monkeys with erythrocytes parasitized by attenuated *P. knowlesi*. They found that immunity developed toward the specific strain that was used for the vaccination; but when the monkeys were inoculated with the nonattenuated form of the same strain, a slightly different strain that was no longer recognized by the monkey's immune system emerged in their blood.

It appears that the parasite is able to change some of the antigens on its surface. This is analogous to the change in antigenic determinants exhibited by the influenza virus. But, whereas the human influenza virus undergoes antigenic change by recombining with other influenza viruses in an animal host and exchanging genetic information, it appears that *Plasmodium* merozoites can change their antigenic determinants in response to various environmental stimuli. This ability of the parasites made scientists justifiably pessimistic about the possibility of preparing a vaccine against the asexual form.

In 1975, however, Sidney Cohen and his associates at Guy's Hospital in London demonstrated that immunization could be achieved by inoculating purified merozoites in Freund's complete adjuvant. Protection is developed, they find, against all antigenic variants produced by the strain used for vaccination, and even against some other strains. The immunity lasts for at least a year. This approach is particularly promising because even partial immunization would reduce the severity of malaria when it is contracted, and because merozoites have been obtained in small numbers from the culture systems.

Complete Adjuvant Necessary

The only sour note in Cohen's work is that immunity could not be achieved if the merozoites were combined with Freund's incomplete adjuvant or with any of the other substances that are generally agreed to be less toxic than Freund's complete adjuvant. Merozoite vaccination will thus have no application to humans until a safe, suitable replacement for Freund's complete adjuvant is found or until some other way is found to render the merozoites more immunogenic.

The third approach is somewhat more unusual in that it involves, in effect, immunizing mosquitoes against the sexual stage of *Plasmodium*. Robert Gwadz, Richard Carter, and David Chen of NIAID inoculated chickens with *Plasmodium* gametes attenuated by x-irradiation. The chickens produced antibodies against the gametes, but this does not affect the chickens' susceptibility to malaria because the gametes are rarely present in the host's blood during an infection. When mosquitoes feed on an infected, immunized chicken, however, they ingest the antibodies along with gamete-infested erythrocytes.

When the gametes are freed from the erythrocytes in the mosquitoes' stomach, they are immobilized by the antibodies and fertilization does not occur. This type of vaccination does not really protect the individual who is vaccinated, but it does halt the spread of the disease. It is thus analogous to mosquito eradication programs in which insecticides are used to spray surfaces on which mosquitoes rest after a feeding. If this technique were used to prevent spread among humans, then it could be possible to eradicate malaria where it is used since there are no animal reservoirs for the disease.

The vaccination may provide some protection, however. Gwadz and Ira Green of NIAID have extended the studies to monkeys and have some evidence that gamete immunization may be equally effective in primates. They have found, furthermore, that vaccination reduces the severity of infections in the monkey. This discovery seems promising. Unfortunately, however, vaccination of monkeys requires Freund's complete adjuvant, whereas vaccination of the chickens does not.

Aside from the adjuvant problem, the main drawback to Gwadz and Green's work is that there is at present no way to obtain large quantities of the gametes for the production of vaccines. The culture systems developed by Trager, Jensen, and Haynes, however, contain small numbers of gametocytes. It should thus be possible, Gwadz says, to find a way to increase the number of gametocytes in the culture, and perhaps even to adapt the culture medium to the growth of gametocytes.

Novel approaches to vaccination might also come out of studies of the way in which merozoites invade erythrocytes. Miller and James Dvorak of NIAID have shown that this does not occur unless there is a specific receptor on the surface of the cell. They have found, for example, that the receptor for *P. knowlesi* and *P. vivax* is closely associated with the blood group determinant known as the Duffy group. Individuals who do not have this determinant, and are thus known as Duffy negative, are not susceptible to infection by either of the two species. More than 90 percent of the West African black population and 65 percent of the American black population are Duffy negative, and thus naturally immune to vivax and knowlesi malaria.

Miller and his associates have recently shown that the receptor for *P. falciparum* is part of a protein or glycoprotein different from the receptor for *P. knowlesi*. Neither the structures of the receptors nor the structure of the Duffy determinants is known yet, but the development of the erythrocyte culture provides a feasible method for determining them. Identification and characterization of the receptors should yield new information about the physiology of *Plasmodium*. In addition, characterization may make it possible to block the receptors chemically or immunologically and thereby prevent infection.

Much Work Necessary

Extensive work is still necessary before there will be usable biological methods to halt malaria infections. One of the great ironies is that most of this work is being done in the United States and Great Britain, countries where malaria has already been eradicated. This situation arises, at least in part, from the optimism that surrounded the introduction of mosquito eradication programs nearly 30 years ago. Scientists and public health officials were so confident of the eventual success of the eradication program that countries where malaria is endemic saw no need to establish research programs on malaria, and young scientists chose not to embark on careers studying a disease that they assumed would soon no longer exist. When the eradication programs met setbacks, those countries were left without scientific resources to consider other approaches.

This situation may soon change, however. WHO has established a Special Program on Research and Training in Tropical Diseases that is designed to stimulate studies of malaria and five other tropical diseases (schistosomiasis, trypanosomiasis, leishmaniasis, filariasis, and leprosy) in the countries where those diseases are endemic. The program emphasizes the expansion of research facilities in those countries and the training of young scientists to enter those fields. The plans for the program include about $45 million to be spent on malaria research by 1981 and on facilities for testing new antimalarial drugs and vaccines as they are developed. The accompanying increase in the number of people studying the problem intensively should finally make it possible to halt the spread of malaria.

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