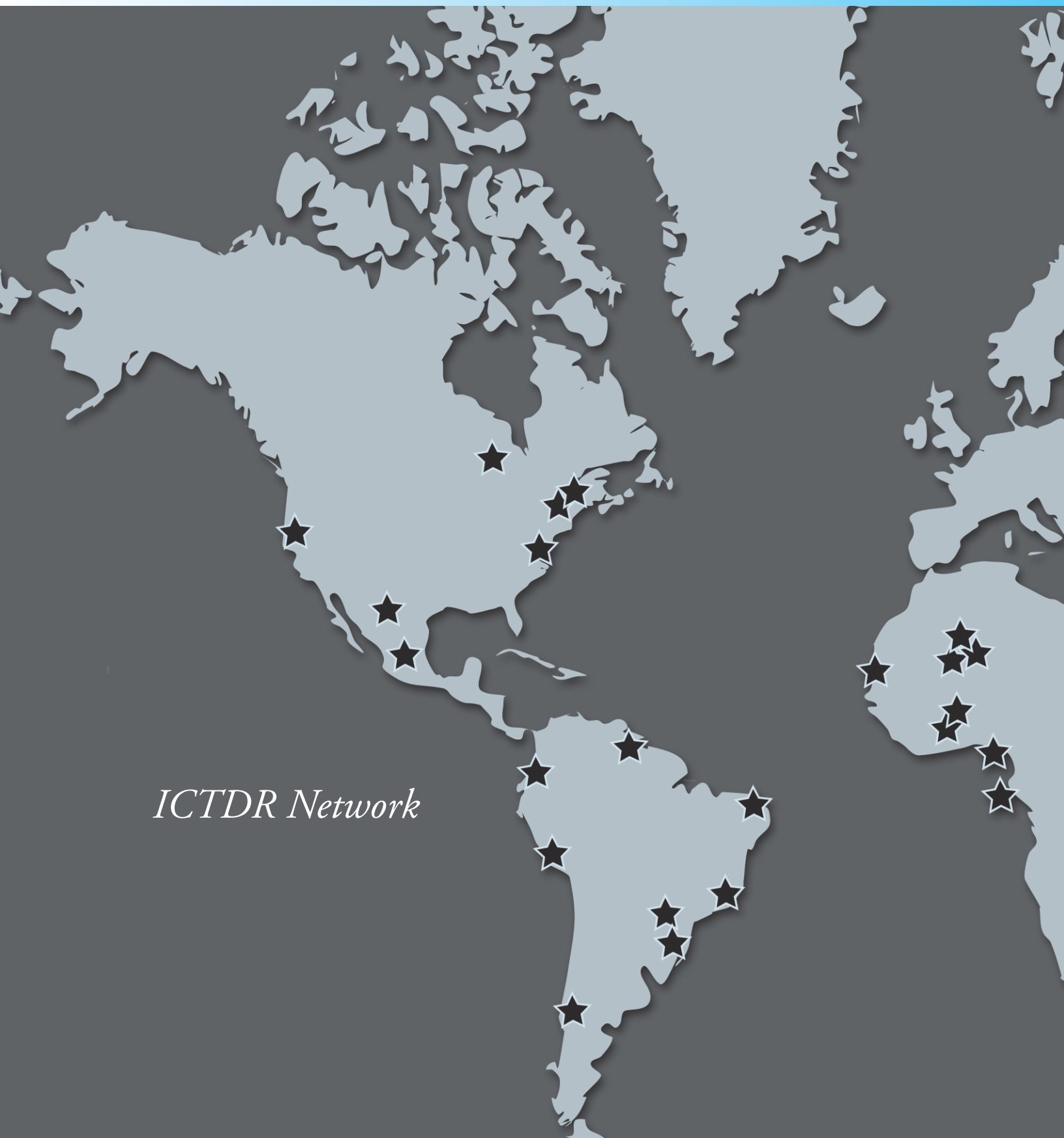
International Centers for Tropical Disease Research Network

10th Anniversary

RESEARCH BEYOND BOUNDARIES

NIAID


ICTDR Network

PREFACE

Introduction

ICTDR: A Decade of Achievements

A decade ago, in 1991, the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) established the International Centers for Tropical Disease Research (ICTDR), recognizing the critical importance of building research capacity and partnerships in countries struggling with the burden of tropical diseases. In doing so, NIAID was paying heed to the changing global dynamics of infectious diseases. The Institute was also hearkening to a tradition dating back more than half a century, well before NIAID was officially established, when its predecessor, the National Microbiological Agency, established its own division for studying tropical diseases.

As one of several steps to commemorate the first full decade of the ICTDR program, NIAID is publishing this 10th anniversary volume. It contains several articles representing some of the tropical diseases research activities that come under this wide-ranging program. Six featured articles provide an in-country perspective of the challenges involved in conducting tropical disease research. In addition, the brochure examines ICTDR accomplishments in the following key areas: developing better diagnostics, new vaccines, innovative approaches to immunotherapy, and new and improved drugs and vector-control measures.

This commemorative volume is not intended to be a comprehensive report on ICTDR research activities. However, we hope it will provide readers with a better appreciation of the valuable contributions this program is making to worldwide health through targeted research on tropical diseases.

This anniversary is also an opportunity to acknowledge our partners around the world who have made this program such a success on so many different levels. Through 10 years of partnerships, the ICTDR has demonstrated that it is possible to conduct research beyond geographic boundaries.

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Preface

Despite significant inroads in dealing with tropical diseases, the microbial pathogens and parasites that are responsible for causing tropical diseases continue to impose a tremendous burden throughout much of the world—claiming huge numbers of lives, inflicting a great deal of morbidity, and very much interfering with the economic livelihoods and productivity of the people that they afflict.

Moreover, the infectious agents responsible for these diseases of the tropics carry no passports and show no respect for national boundaries. Thus, in the face of a variety of forces, ranging from political unrest and guerrilla warfare to burgeoning global trade and adventurous tourism, many of the pathogens responsible for these tropical diseases are also emerging as public health threats in industrialized countries. Programs promoting international research efforts and other tropical disease control measures in the developing world thus can help to protect the health of Americans as well as the health of people living in countries where these diseases have long been endemic.

The ICTDR program brings NIAID-supported intramural and extramural centers of tropical disease research together as part of a focused interactive network. Also, ICTDR is fostering several distinct types of partnerships, including those between domestic and foreign scientists, between NIAID and other private or public international agencies, and between individual investigators and other funding organizations.

The core of the ICTDR program resides in its rich network of partnerships among scientists in the United States and abroad—a set of alliances for conducting cutting-edge research, fostering good will,

and transferring technical knowledge and know-how to research institutes and hospitals in regions of the world where tropical diseases are endemic. The alliances encourage U.S. scientists to work in and obtain valuable research materials from those regions. They also enable investigators from those areas to collaborate on research projects on site and to visit U.S. laboratories and attend scientific conferences and workshops to discuss with global experts the challenges of studying and combating these tropical pathogens.

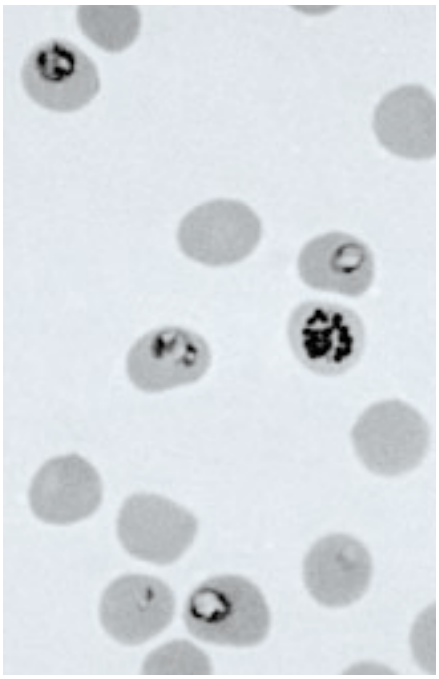
There are four major programs within the ICTDR network:

- The International Collaborations in Infectious Disease Research (ICIDR) program is designed to encourage collaborations between U.S. researchers and their counterparts working in countries where tropical diseases are endemic.
- The domestic Tropical Disease Research Units (TDRU) are awarded to domestic institutions in the United States, whose designated scientists are applying modern biomedical technologies to the discovery of new control measures for parasitic infections.
- The Tropical Medicine Research Centers (TMRC) grants are large awards made to outstanding foreign institutions to provide their staff and appropriate visitors opportunities to study tropical diseases in endemic regions.
- Finally, NIAID also supports several intramural laboratories, particularly the Laboratory of Parasitic Diseases and the Laboratory of Infectious Diseases, that are devoted to research on tropical infectious diseases and thus are an important component of the ICTDR network.

MALARIA

From Laboratory Bench to Field and Back

Gaining Insights on Drug-Resistant Malaria



This micrograph shows red blood cells infected with malaria parasites.

While growing up in a small village in the West African nation of Mali, Abdoulaye Djimde lost two brothers to malaria. "The first one died when I was around 12, and I was very shocked by this," Djimde says. "Since then, I decided to do something when I grow up to ease the pain of malaria."

Djimde is now on staff at the University of Mali Malaria Research and Training Center in Bamako. He is also part of an international team of investigators of the National Institute of Allergy and Infectious Diseases (NIAID) International Centers for Tropical Disease Research (ICTDR) that, through a combination of laboratory and field research in the United States and Mali, is working to understand and overcome the problem of drug-resistant malaria.

Malaria, which is caused by the single-celled *Plasmodium* parasite, strikes an estimated 300 to 500 million people worldwide each year. Roughly 90 percent of all deaths from malaria occur in Africa, where the disease kills about 1 to 2 million people annually—mostly young children (see sidebar [page 16]). In addition to these high rates of disease and deaths from malaria, sharply increasing resistance to the drug chloroquine by the deadliest species of malaria parasite, *Plasmodium falciparum,* is seriously complicating the continuing battle against the disease.

Analyzing Chloroquine Resistance Considered Critical on Several Fronts

What ICTDR investigators in several countries are learning about chloroquine resistance may lead to ways to reverse drug resistance, should help in designing new antimalarial drugs, and could also guide national policies that can prolong drug effectiveness. Recent

insights into the genetic basis of chloroquine resistance are already facilitating surveillance of African populations for resistance patterns that soon will be used to guide government decisions regarding recommended treatments for malaria.

Chloroquine came into use in the late 1940s and soon became the mainstay treatment for malaria because it is cheap, safe, and highly effective. However, the popularity and widespread distribution of the drug contributed to its overuse and misuse, which fostered the emergence of chloroquine-resistant strains of *P. falciparum.* Chloroquine resistance arose in Southeast Asia and South America in the late 1950s, and is now so widespread in these regions that doctors there have largely abandoned its use in favor of drugs that, although effective, are typically more expensive and toxic.

In Africa, chloroquine resistance emerged near the end of the 1970s. Within a decade, however, chloroquine-resistant forms of the malaria parasite had spread across sub-Saharan Africa, leading to a rising death toll from the disease. Nonetheless, chloroquine remains the treatment of choice in many parts of the continent because it is cheap and relatively safe to use. Resistance to this drug in the field often is not a simple either-or issue. For instance, partial immunity to malaria, which is widespread among older children and adults who have had previous bouts with the disease, seems to help chloroquine work even against ostensibly resistant parasites.

Malaria is transmitted to humans through the bite of the female *Anopheles* mosquito. When a mosquito infected with *P. falciparum* feasts on her victim's blood, she injects parasites into the individual's bloodstream along with her saliva. The parasites develop first in liver cells and then infect red blood cells, where they fuel their activities by consuming hemoglobin, the oxygen-carrying

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component of blood. In the red blood cell, the parasites go through another stage of development in their complex life cycle. Once that stage of development is complete, the parasites burst from the red blood cell, ready to infect other such cells. This stage of the parasite's life cycle gives rise to typical, often relapsing, symptoms of malaria, including fever, chills, and sweating accompanied by headaches, nausea, and vomiting. More severe and potentially life-threatening complications of the disease include anemia, kidney failure, and neurological problems.

Search for Drug Resistance Genetic Traits Dates to Mid-1980s

In the mid-1980s, NIAID researcher Thomas Wellems began searching for the *P. falciparum* gene or genes responsible for chloroquine-resistant malaria. Wellems' research group, whose members developed the first methods to genetically manipulate *P. falciparum*, found that a single gene most likely causes chloroquine resistance. "People assumed that the slow spread of resistance indicated involvement of multiple genes," Wellems says. "It turns out that there is genetic complexity—but it doesn't involve mutations in multiple genes, it involves multiple mutations in one gene."

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Although Wellems and his coworkers narrowed their hunt to a stretch of DNA on chromosome 7 of *P. falciparum,* another decade of painstaking analysis was needed before they identified the gene responsible for chloroquine resistance, which they designated *pfcrt*. Chloroquine-resistant strains of the parasite being studied in the lab had eight mutations in the *pfcrt* gene, each of which involves the change of a single letter in the four-letter DNA code. The researchers subsequently learned that the *pfcrt* gene encodes a protein, PfCRT, that spans the membrane of the digestive vacuole of *P. falciparum,* the cell compartment in which hemoglobin from red blood cells is broken down to provide metabolic fuel for the parasite. Chloroquine kills the malaria parasite by preventing detoxification of heme, the iron-containing portion of hemoglobin that is released when the parasite digests hemoglobin. "The mutant form of the PfCRT protein reduces chloroquine access to the vacuole," Wellems says, "apparently enabling the parasite to continue detoxifying heme."

Wellems and his colleagues analyzed the *pfcrt* gene sequence in a series of chloroquine-resistant and chloroquine-sensitive laboratory strains of *P. falciparum* from Southeast Asia, South America, and Africa. Although each resistant strain has multiple mutations in the *pfcrt* gene, one of these mutations is particularly critical for chloroquine resistance. "It looks at this point that for *P. falciparum,* every resistant strain in the world has this mutation, which causes a change in position 76 of the protein encoded by the *pfcrt* gene," Wellems says. He believes that other mutations in the *pfcrt* gene are needed to compensate for the position 76 change in the PfCRT protein, which by itself may alter the protein's structure in a way that is detrimental to the parasite.

Field Studies Bring Insights, Add Puzzles to Drug Resistance Picture

Clinical studies in Mali do much to confirm findings in the Wellems laboratory. The studies in Mali included collaborators from Wellems' group in the NIAID Laboratory of Parasitic Diseases, part of the ICTDR network; from a research group at the University of Maryland School of Medicine led by ICTDR investigator Christopher Plowe; and a group led by Ogobara Dumbo at the Malaria Research and Training Center (MRTC) in Bamako, which is supported in part by one of three Tropical Medicine Research Center grants from the ICTDR program (see sidebar [page 19]). Abdoulaye Djimde, who will return to the University of Mali after completing his doctoral work in Plowe's lab, was one of several leaders of these studies.

"The focus of our lab has been on translating the discovery of mutations in parasite genes that appear to confer resistance in the laboratory into useful molecular [tests] for drug resistance that can be used for surveillance purposes," says Plowe, who spends 30 to 40 percent of his time doing field research on malaria in Africa. "The main objective of our study was to see if this laboratory finding [of a link between *pfcrt* mutations and chloroquine resistance] would hold in the field, in parasites collected from patients," Djimde says.

Based on the extensively studied *pfcrt* mutations, Plowe, Djimde, and their collaborators developed a test to rapidly diagnose patients with chloroquine-resistant malaria and analyze parasites isolated

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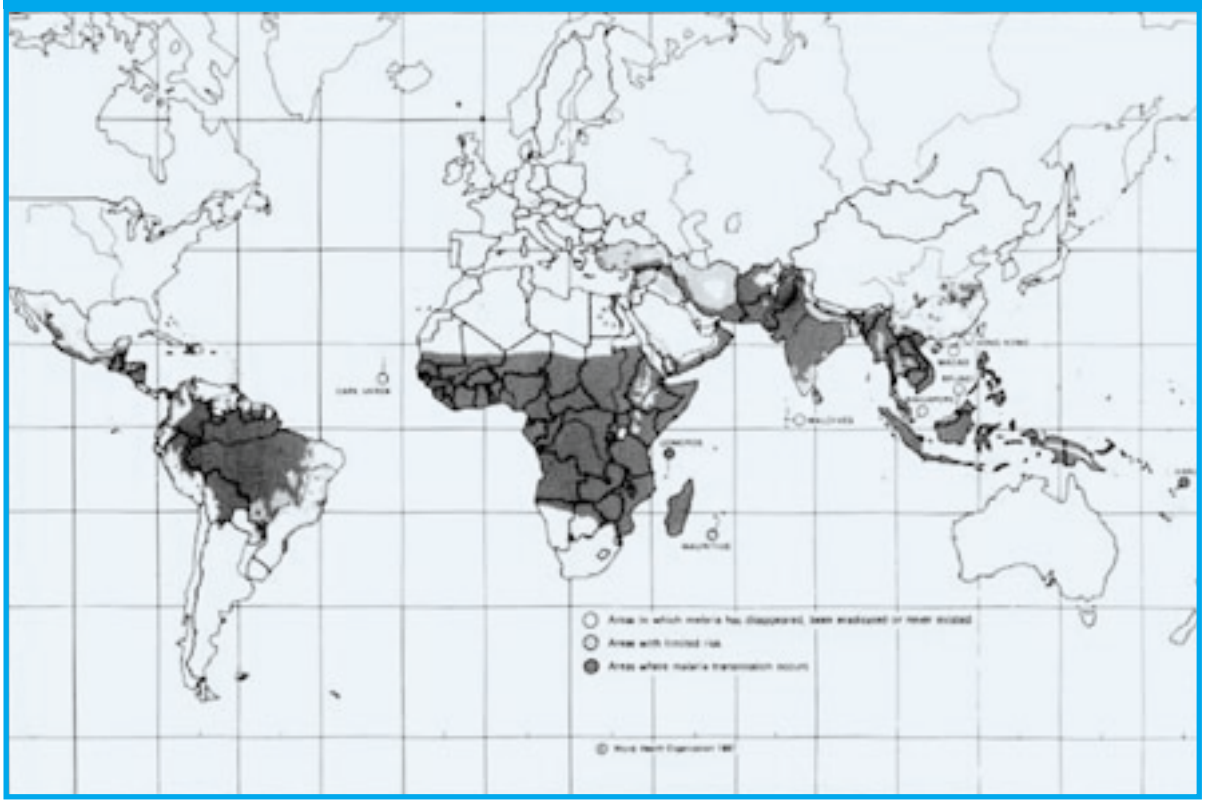
from them. Previously, Plowe says, diagnosing chloroquine-resistant infections took at least 14 days and required multiple blood samples from each patient. The older procedure is so time-consuming, expensive, and labor-intensive that it is impractical for monitoring drug resistance at a population level. “Most countries can do this [older test] only at a small number of sentinel sites, and even so, most don’t do it annually,” Plowe says. However, the new test for detecting resistance takes only a few hours, can detect mutations in the *pfcrt* gene in parasite DNA from a single blood sample, and can be run on hundreds of samples at once.

To evaluate the new procedure, members of the team used blood samples collected before and after chloroquine treatment from patients in two central Mali villages, Mopti and Bamako, where the disease is widespread. The patients, who were at least 2 years old, had participated in a clinical study led by Djimde in 1997 on the effectiveness of chloroquine treatment. In 86 percent of these patients, the drug eliminated infection by *P. falciparum,* indicating that resistance to chloroquine is relatively low in this region.

Epidemiological assessment of the status of malaria



Chloroquine-resistant malaria is defined as an infection that persists after chloroquine treatment or recurs within 2 weeks of treatment.

Ogobara Doumbo reading blood smears for malaria diagnosis

As part of the recent study, Djimde, Plowe, and their colleagues tested *P. falciparum* DNA from the patients' blood samples and detected the position 76 mutation in 100 percent of the post-treatment blood samples from 60 people with chloroquine-resistant malaria. No other mutation was so closely linked with chloroquine-resistant infection. These findings "led us to conclude that, to date, the *pfcrt* gene is the best molecular marker for chloroquine resistance," Djimde says.

In contrast to the post-treatment test results in patients with chloroquine-resistant infections, the position 76 mutation was found in only 41 percent of blood samples obtained from patients who had not received drug treatments. Some patients who carried the mutation nevertheless eliminated the infection after chloroquine treatment, suggesting that other factors besides that mutation play a role in determining responsiveness to the drug. The researchers found that in children less than 10 years old, 69 percent of pre-treatment infections with the position 76 mutation failed chloroquine treatment, compared with 34 percent of such pre-treatment infections in patients over 10 years of age. These findings suggest that partial immunity to malaria, which is more common in older individuals, plays a major role in responsiveness to chloroquine.

Although the test for *pfcrt* mutations cannot be used to predict chloroquine resistance on a case-by-case basis in countries where malaria is widespread, or endemic, the absence of the position 76 mutation could be used to identify those patients who will likely respond to chloroquine. "The way we think these results could be used right now," Djimde says, "is in helping to design a finer map of the distribution of chloroquine resistance in malaria endemic countries." Such information should be extremely useful for government officials trying to establish treatment policies for malaria. "Even in a single country, you can find different areas with very different levels of chloroquine resistance," he adds. "So, in order to define effective treatment policies, we need to follow the evolution of the level of chloroquine resistance across the country."

"In many countries," Plowe says, "chloroquine failure rates are 60 or 70 percent, and that translates into huge numbers of deaths. But without evidence that chloroquine isn't working...it's very difficult for a government to justify switching from a cheap and safe drug to the alternative drugs that are more expensive and tend to be more toxic."

Findings from Other Sites Further Complicate This Drug Resistance Story

Other ICTDR researchers are looking in additional countries for insights into some of these questions. Philip Rosenthal of the University of California, San Francisco (UCSF), and his colleagues from UCSF and Makerere University in Uganda found that, although all the malaria patients they tested in Uganda had the telltale mutation before chloroquine treatment, about half of them nevertheless responded to the drug. Chloroquine resistance is so widespread in East Africa, he says, that "whether or not people respond to chloroquine to a large extent is dependent on other

Terrie Taylor: Fighting Severe Malaria on the Front Lines

For the past 15 years, malaria researcher Terrie Taylor has spent January through June in the East African nation of Malawi. Those 6 months are the rainy season in Malawi, when malaria-transmitting mosquitoes thrive and the number of malaria cases skyrockets. During those stays, Taylor, a professor of internal medicine at the Michigan State University College of Osteopathic Medicine, treats children with malaria while also conducting research on the disease at Queen Elizabeth Central Hospital in Blantyre, Malawi.

"The work in Malawi is seasonal and intense," says Taylor, whose research is funded by the NIAID International Collaborations in Infectious Disease Research program, part of the ICTDR network. "We essentially run a 24/7 pediatric intensive care unit."

Spending time in Malawi is critical for a project like Taylor's, which she and British researcher Malcolm Molyneux started in 1986. "The only way to be involved in something like this is to be there," Taylor emphasizes. "Being on site six months of the year also enhances my credibility in Malawi, and allows me a much more substantive role in beginning to develop research capacity and infrastructure."

About 1 million children in sub-Saharan Africa die each year from severe cases of *Plasmodium falciparum* malaria. Taylor's research focuses on the causes, development, and treatment of cerebral malaria, one of three sometimes overlapping syndromes that occur in severe and complicated cases of the disease. This syndrome, whose precise causes are still poorly understood, is characterized by fever, convulsions,

factors," mainly immunity, which is consistent with the results from Mali.

Plowe says the studies in Uganda and elsewhere indicate that "in general, once you get above 30 or 35 percent resistance, prevalence of the marker is about 100 percent." Therefore, testing for it will likely be useful only in "areas where chloroquine resistance is relatively low." Testing for the position 76 mutation may also be useful for finding out whether or when the level of chloroquine-resistant parasites begins to decline in areas where chloroquine is no longer being used. "In such cases," Plowe says, "you could make an argument for going back in with chloroquine, ideally combined with another drug...so that chloroquine resistance doesn't pop back up again."

The idea of using combination therapy to prevent drug resistance from developing is gaining support among experts in the field of malaria research. Many of them believe that treating malaria patients simultaneously with two drugs could drastically reduce the further development of drug-resistant cases. They say the odds that malaria

(Continued)

and coma. Left untreated, it typically is fatal within 24 to 72 hours.

Taylor and her colleagues have developed regimens for the intramuscular administration of quinine, the current drug of choice for treating cerebral malaria, that allow the drug to be used in remote outpatient settings where intravenous administration is not possible. Their work has also led to a widely accepted definition of cerebral malaria that makes it much easier to compare data gathered at different sites. Their studies of the causes and development of the condition, including its clinical manifestations and—through autopsy studies—how it affects the brain, should lead to more effective treatments.

Terrie Taylor and a young malaria patient

With funding from the ICTDR program, Taylor is helping to establish the first-ever clinical trials network for severe malaria in African children (SMAC). "It is still in its early stages, but we have high hopes," Taylor says of SMAC, a collaboration among research clinicians in Malawi, Kenya, Ghana, Gabon, and The Gambia that should help develop standards of care for children with severe malaria across Africa. "This is a critical collaboration, because as a network of five sites we will be able to enroll enough children in clinical trials of new therapies to conduct mortality-based studies. The mortality rate is the bottom line," Taylor says, "and an intervention that decreases the mortality rate by even 10 percent would make a huge difference."

parasites will develop resistance to two drugs are much lower than the odds of developing resistance to a single drug.

A handful of African nations in which chloroquine resistance is particularly common now use Fansidar, an affordable drug containing two active compounds, sulfadoxine and pyrimethamine, as the first-line malaria treatment. Fansidar is the standard second-line treatment for malaria in several additional African countries. But in a particularly worrisome development, some malaria parasites in Africa have also become resistant to Fansidar. "The big problem is, when Fansidar fails, what will we use next?" Plowe says.

"In Uganda, officially the government still recommends chloroquine as the first-line drug for malaria," Rosenthal says. "They know they should switch, but they are struggling with what to switch to. They know that switching to Fansidar is not a good solution, because resistance to Fansidar develops quite quickly, so they are considering other options," including combination therapies.

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Rosenthal and his colleagues recently completed a study of combination therapy for malaria in Uganda using "two old-fashioned drugs," Fansidar and amodiaquine. Amodiaquine, which is chemically related to chloroquine, "went off the map a while ago because of concerns about toxicity," Rosenthal says. But a combination of amodiaquine and Fansidar proves very effective in treating malaria; moreover, when used in short courses and low doses, amodiaquine does not cause much toxicity. He says this combination of drugs, both of which are inexpensive and available in Africa, may be useful as a "short-term stopgap therapy right now for malaria in Africa, as we are waiting for new drugs."

In Papua New Guinea (PNG), where malaria is an increasing health problem, the government recently announced a change in its guidelines for first-line treatment of uncomplicated (that is, not severe) *P. falciparum* malaria. Instead of chloroquine or a related drug, camoquine, government health authorities now recommend a combination of chloroquine or camoquine plus Fansidar, according to ICTDR researcher James Kazura of Case Western Reserve University, who studies malaria in PNG. The change in recommendations in PNG is "presumably as a response to the fact that there must be increasing chloroquine resistance there," he says, though such increased resistance is not well documented. His

Ogobara Doumbo: Helping People Via World-Class Malaria Research

Cooperation, trust-building, respect for tradition. These are among the tools that Ogobara Doumbo, a physician and epidemiologist at the University of Mali, is using—along with the tools and technologies of modern science—to build a world-class malaria research program with which to battle this disease in rural communities in Mali.

Doumbo, who grew up in a small village in the Dogon region of Mali, says that from an early age he "wanted to be a physician and to help people." He has done that and much more as head of the Department of Epidemiology of Parasitic Diseases and codirector, with medical entomologist Yeya Touré (see feature on malaria vectors), of its Malaria Research and Training Center (MRTC), which is supported by the NIAID ICTDR program.

When Doumbo succeeded his French mentor Philippe Ranque as department head in 1988, he and Touré decided to focus their research efforts on malaria. "By this time," Doumbo says, "we were lucky to meet our NIH colleagues Dr. Lou Miller and Dr. Bob Gwadz," malaria researchers from NIAID's Laboratory of Parasitic Diseases who were seeking collaborators in Africa. Doumbo was struck by Miller's assertion that, if malaria eradication efforts in Africa were to succeed, Africans must build their research capacity and become active and equal partners.

Miller and Gwadz worked with Doumbo and Touré to establish the MRTC, which is supported by the Malian government, NIAID, and several other outside partners. The center's goals are to build the national capacity for research on malaria using new techniques and technologies, to train Malians and other African scientists to conduct malaria research, and to provide technical assistance for Mali's national malaria control program. Doumbo and Touré were among the first Malian scientists trained to do research on parasitic diseases. Now Doumbo is working to build the next generation of scientists in Mali. Since 1992, he has sent 20 young Malians to the United States and Europe for graduate training. Once the trainees complete their Ph.D. work, which must include field studies in Mali, Doumbo insists that they return to Mali to share their newly acquired expertise with others.

"Coming from a village," Doumbo says, "I know that building a strong, trusting relationship is the key issue" for doing successful field research. He routinely spends a year or more building relationships in rural communities before initiating a study. Doumbo insists on securing consent from the chief and elders of a community in addition to the informed consent from individual study participants required by his collaborators from NIH and U.S. universities.

Doumbo, who comes from a family with many traditional healers, recognizes the power these healers have in the community and has enlisted them in his team's research activities. In one ICTDR-funded research project in the village of Bancoumana, Doumbo and his colleagues demonstrated that the number of deaths from cerebral malaria, a severe form of malaria that strikes some children, can be drastically reduced by working closely with mothers and traditional healers to ensure rapid diagnosis and appropriate treatment. "That shows clearly that if you decentralize and build a community-based [health care] system you can fight better against malaria," he says.

This and other key findings by MRTC researchers and their collaborators, including the development and field-testing of molecular techniques to detect chloroquine-resistant parasites in malaria patients, have a clear public health impact, Doumbo says. "I am a scientist, but I always translate my findings into active strategies to help the community."

colleagues, Pete Zimmerman and Rajeev Melhotra, are evaluating chloroquine resistance in PNG by looking for the position 76 mutation in parasites isolated from coastal regions of the country, where malaria is most common. They plan to collect clinical data on chloroquine resistance and correlate the data with their as-yet-unpublished findings on the prevalence of the mutation in PNG.

The combination of laboratory and field research on malaria supported by the ICTDR program should deepen our understanding and lead to new ways of treating the disease. Wellems says that studies on the PfCRT molecule, and the mutations found in resistant parasites, hint that "it may be possible to change the structure of chloroquine and get a renewed lease on life with the drug." Ongoing research by Djimde and leading immunologists on how partial immunity helps eliminate chloroquine-resistant *P. falciparum* infections may help researchers understand the mechanisms that make some people immune to malaria—information that should prove especially useful to investigators trying to develop vaccines to prevent it. ICTDR researchers are also working on new drugs for malaria and studying the basis of *P. falciparum* resistance to drugs other than chloroquine.

The ICTDR program has enabled us to bring resources to colleagues we work with in developing countries.

"There's a lot of bang for the buck in the ICTDR program," says Kazura. It has "enabled us to bring resources to colleagues we work with in developing countries," and also supported training of scientists like Abdoulaye Djimde. After gaining expertise in molecular biology in Plowe's lab at the University of Maryland and Wellems' lab at NIAID, Djimde will return to the MRTC in Bamako where he says he will "join the team...and continue our work in better understanding and better fighting against malaria in the country." And, adds Plowe, "Ultimately, the best work is going to be done by people in endemic areas, because they understand the disease best, and they can best set priorities for tackling the problem."

Accomplishments

Soon after ICTDR was founded, several major developments in biomedical research began to take shape and became part of its scientific programs. Advances in DNA sequencing technology and in bioinformatics, for example, now make it possible to determine and computationally annotate the genomic sequences of the diverse and complex pathogens and vectors that cause and transmit tropical diseases. Tools have also been developed to analyze, on a genome-wide scale, the expression of genes and to identify polymorphisms that are relevant to drug resistance and virulence.

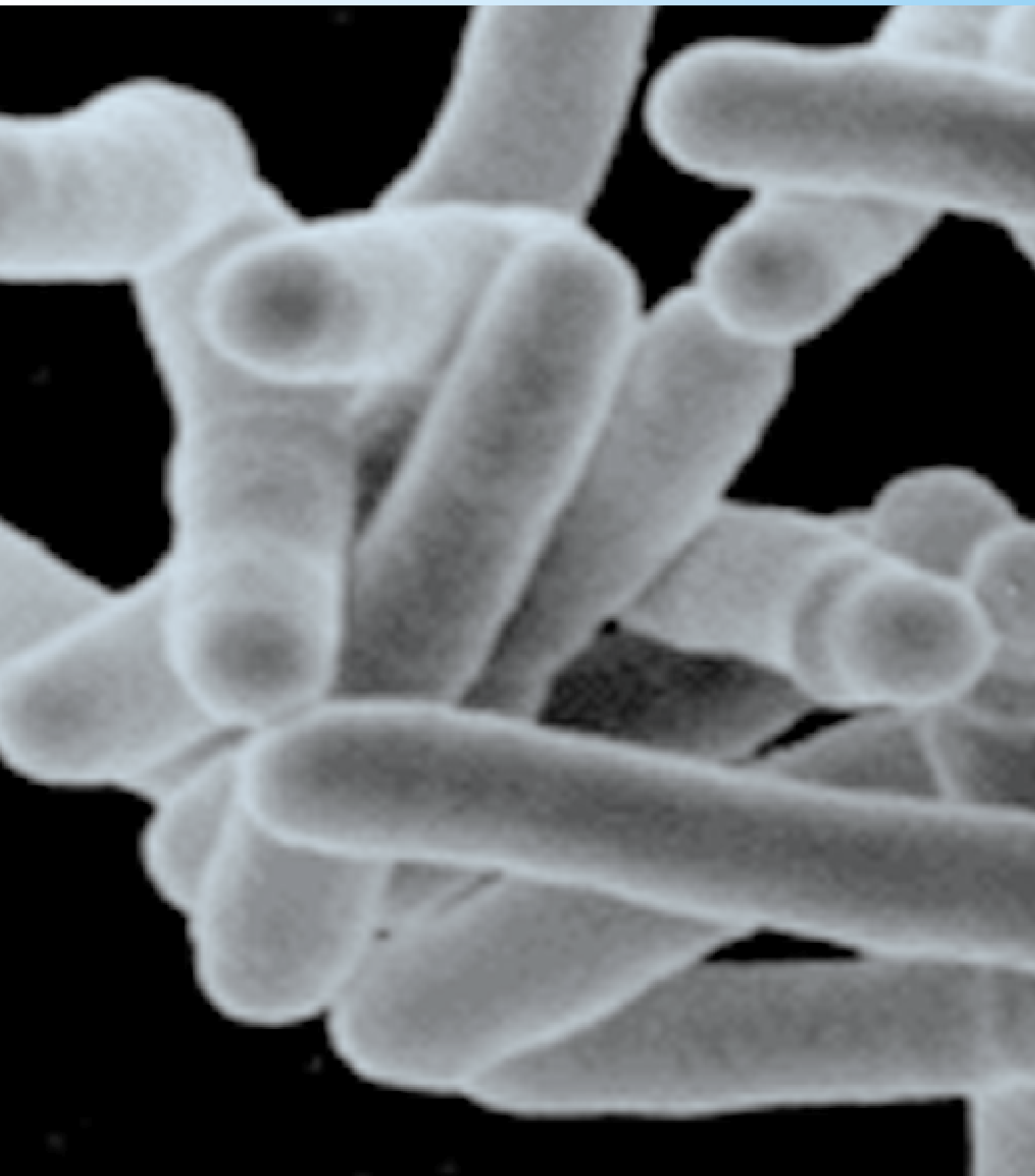
Such information is beginning to reveal a comprehensive picture of the full range of genes that comprise these disease-causing microorganisms, thereby helping investigators to identify critical targets for diagnostic products, new drugs, vaccines, immunotherapies, and vector-control strategies. Relational databases are available to store, access, and query the large amounts of genome sequence and other data that are rapidly being acquired.

With this revolution in genomic analysis has come the development of methods for manipulating the genes of protozoan pathogens, including malaria parasites and trypanosomatids. Investigators are now able to modify gene expression in these organisms in the laboratory and to characterize the functions of individual genes. Moreover, new cell-based analytic methods are enabling scientists to monitor many of these gene products and their trafficking patterns within the cells and organelles of parasites and other tropical disease pathogens.

Another important series of developments has evolved from studies of the human immune system. Immunologists are now developing a more complete understanding of Tlymphocyte subsets and accessory cells that regulate components of the complex host defense system, whose functions often falter during the course of infections with tropical diseases. A deeper understanding of the immune system is crucial for scientists to develop vaccines against, and immunotherapeutic approaches to treat, the full range of tropical diseases.

Throughout the years, ICTDR has enjoyed the participation of many important partners in this endeavor. Representatives from the Fogarty International Center, the National Library of Medicine, the NIH Center for Bioethics, the U.S. Food and Drug Administration, the U.S. Centers for Disease Control and Prevention, the U.S. Army and Navy, the U.S. Agency for International Development, the National Aeronautics and Space Administration, the World Health Organization, the World Bank, the Wellcome Trust, the Burroughs-Wellcome Fund, and the Bill and Melinda Gates Foundation have all been invited to share information on their programs at ICTDR meetings. Two bilateral research programs, the Middle East Regional Cooperation Program and the U.S.-Japan Cooperative Medical Sciences Program, have also been involved in the ICTDR agenda. NIAID has formed alliances for collaborative research with USAID (malaria vaccine development; schistosomiasis vaccine development), NASA (geographic information systems and remote sensing for prediction of infectious disease epidemics), CDC (malaria and schistosomiasis vaccine development), the U.S. Navy and Army (malaria vaccine development), and the Malaria Vaccine Initiative of the Gates Foundation (malaria vaccine development). A 5-year memorandum of understanding for information exchange and cooperation between NIAID and WHO/TDR was signed in 1995. In addition, during the past decade, NIAID has greatly expanded its support and strengthened research capabilities in developing countries where tropical diseases are endemic through partnerships with the Fogarty International Center and the Multilateral Initiative on Malaria.

What follows on pages 33, 45, 59, 73, and 86 in this issue highlights achievements in these targeted research categories during ICTDR's first decade.



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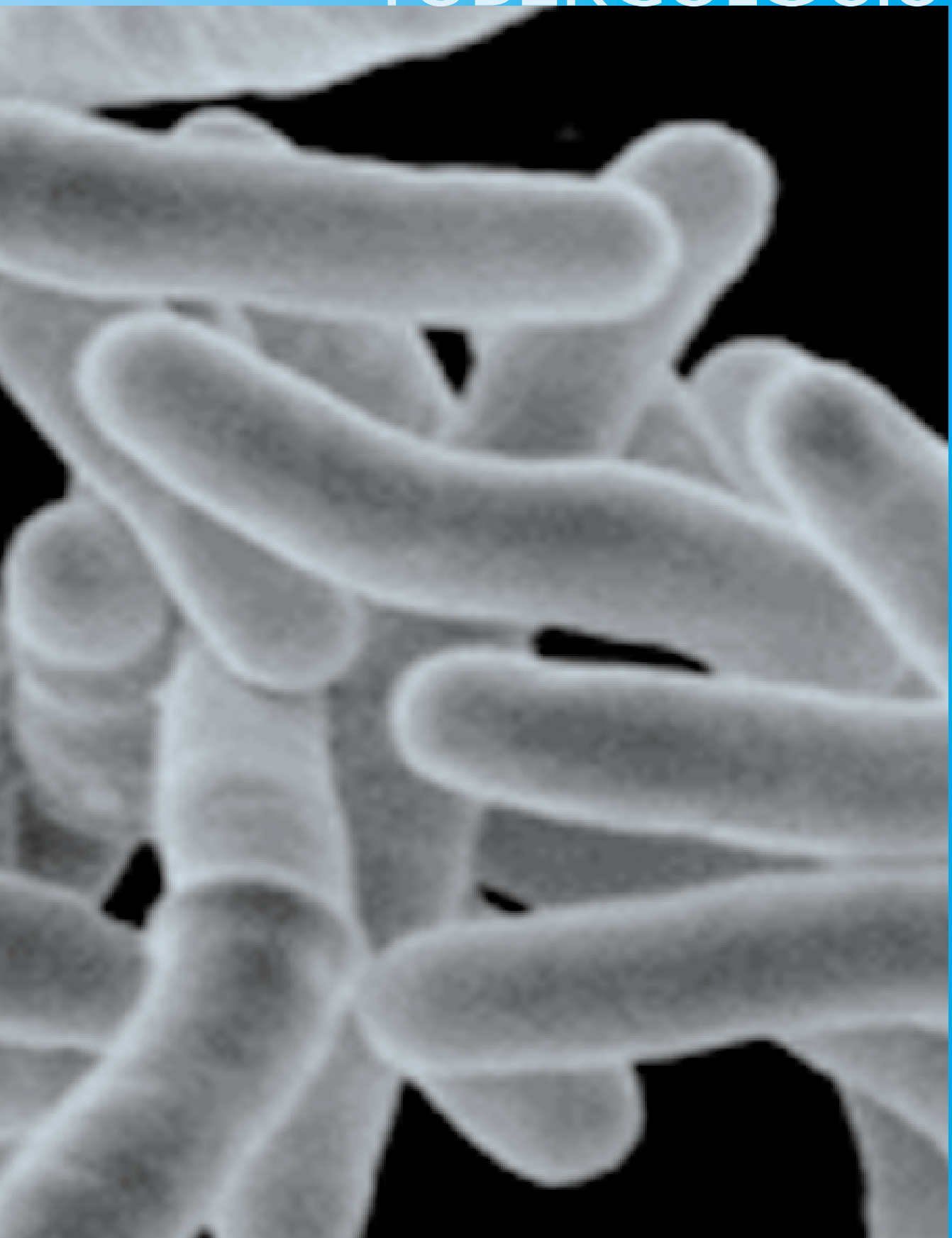


Regional Research Yields Insights to Change TB Control Program in Mexico

"We showed that about a third of the cases in the city were the consequence of recent transmission, which flew in the face of the established dogma," says infectious disease researcher Peter Small.

The scientists leading the tuberculosis (TB) research project in southeastern Mexico funded through the International Centers for Tropical Disease Research (ICTDR) program are purposeful iconoclasts. Confronted with stubbornly high TB rates, they generated findings about transmission of this disease and drug resistance patterns that seem to oppose conventional wisdom and even challenge a control strategy that experts believe can tame worldwide TB. However, in challenging some of these prevailing ideas, they are helping to change TB control policies in Mexico, while making their district a model for controlling this disease throughout the country and possibly elsewhere in the developing world.

The foundations for the TB projects in Mexico were laid in the early 1990s when researchers developed a new approach for tracking TB in San Francisco. Infectious disease researcher Peter Small and his colleagues from the University of California, San Francisco, began using DNA fingerprinting techniques to help monitor the spread of TB throughout that city, collecting data on specific mycobacterial strains in patient sputum samples. By comparing strains, the researchers could pinpoint how TB spreads among individuals in a community. With this information they made a startling discovery. "We showed that about a third of the cases in the city were the consequence of recent transmission, which flew in the face of the established dogma," says Small, now head of the Stanford University Center for Tuberculosis Research. The surprisingly large number of new cases explained why the disease rate kept increasing in the city despite a 97 percent therapy completion rate among identified cases.

Molecular Epidemiology Provides a New Approach to TB Control

Convinced that DNA fingerprinting provides a powerful mechanism to understand the dynamics of TB transmission within a community, Small sought an opportunity to test the technology outside the United States.

Mexico stood out as an obvious choice for two reasons: first, Stanford researchers have a 30-year history of research collaborations with their counterparts across the border; second, the Orizaba health jurisdiction in the southeastern Mexican state of Veracruz offered an ideal testing ground for the new molecular epidemiology techniques. This 216-square-kilometer region is home to a population of about 282,000 with a high rate of TB. "One of the reasons that we are working in this area is because it's pretty prototypical of TB in high-burden areas," says Maria de Lourdes Garcia, the Mexican principal investigator based at Mexico's National Institute of Public Health (INSP) in Cuernavaca and an International Research Scholar with the Howard Hughes Medical Institute. "It's our hope that we'll demonstrate not just what's going on in this small area, but also basic principles that can be generalized to any similar situation globally."

Most of the rural area of the Orizaba jurisdiction is devoted to agriculture, but industry—paper, textiles, oil, sugar, and production of other foods—serves as the region's main economic engine. Although most residents enjoy comfortable living standards, what made the area particularly suitable for the research project is a persistently high rate of TB—42.6 per 100,000 inhabitants—despite the efforts of a well-established TB control

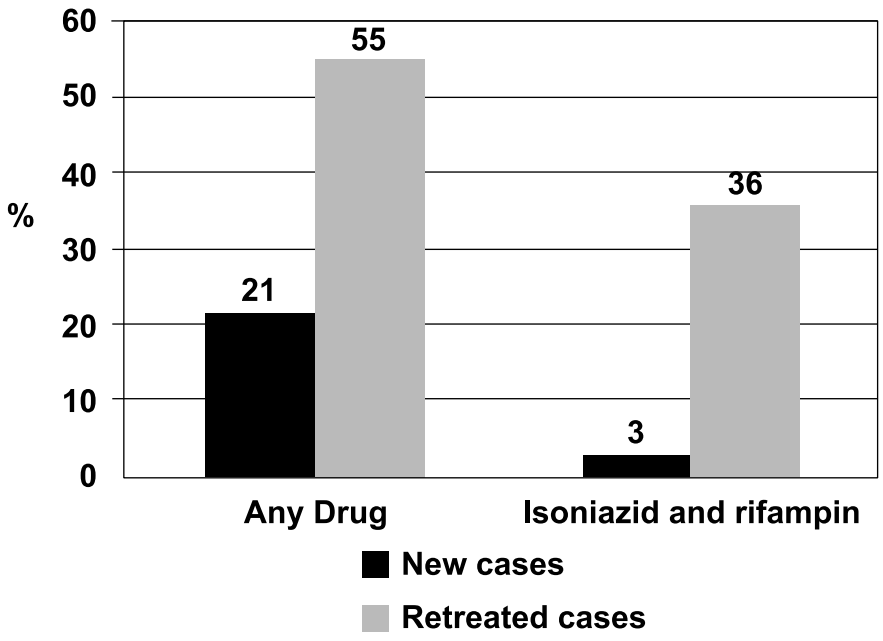
In challenging some of these prevailing ideas, they are helping to change TB control policies in Mexico, while making their district a model for controlling this disease throughout the country.

program. In 1996, local health authorities began implementing DOTS (directly observed treatment short course), the TB control strategy promoted by the World Health Organization (WHO) as the surest means of eradicating TB worldwide. Despite an overall cure rate of 77 percent, the disease maintains a stubborn grip on the region.

A Nation's TB Control Program Revised

With support from their first ICIDR grant in 1994, the collaborators undertook a retrospective analysis of TB treatment outcomes over the previous 5-year period in the Orizaba health jurisdiction. The results confirmed that the incidence of disease had not decreased as a result of DOTS intervention. To better understand this obstinacy, the team used DNA fingerprinting to compare strains of the bacterium from different patient samples. The molecular examination identified one group of seven patients who shared the same strain. The team's field workers, after talking with these patients, eventually deduced that the individuals

Mycobacterium turberculosis resistance to drugs in 232 patients in Orizaba, Mexico



frequented the same unlicensed bars. This social network accounted for a quarter of TB transmissions that progressed rapidly to full-blown disease.

Despite well-organized community-level TB control measures, this pocket of TB transmissions was continuing essentially unchecked. "Alcoholics are notoriously difficult to get to complete therapy," Small says. Even though the goal of DOTS is to ensure that a high proportion of TB patients in an area complete therapy, by-the-book application of this strategy inadvertently missed some of the most difficult-to-treat patients. "So early on, we were coming to this realization that the DOTS program, though an essential first step, was not sufficient to decrease TB transmission," he says. Because the research team worked closely from the outset with officials from the local TB control program, their discoveries were quickly translated into useful changes in their practices. "It resulted in a shift in their focus and a more intense scrutiny of this population," Small says. "We're starting to see tuberculosis rates go down in this community in part because of the changes in practices triggered by the insights learned from the study."

Because the research team worked closely from the outset with officials from the local TB control program, their discoveries were quickly translated into useful changes in their practices.

The research team efforts also are having an impact on the nationwide DOTS policy. Mexican officials faced a difficult decision. Although many countries were implementing DOTS regimens by treating patients with four drugs, Mexican officials were deploying only three such drugs while still attempting to extend DOTS to everyone in the country with TB, including those living in hard-to-reach areas. Because of costs, officials planned to take one of these steps while delaying the other. "The advantage of just expanding the program was that you could do it faster if you didn't take the time up-front to change from three to four drugs," Small explains. "The advantage of going to four drugs was that it would help to decrease the problem of drug-resistant TB if there were already high rates of resistance."

Mexican officials, however, did not have the data on drug resistance that might have helped them in making this decision, Small says. In filling this gap, the research team found that drug resistance among infected residents in Orizaba was disturbingly common. Bacteria from more than 25 percent of patients were resistant to one of the TB drugs, and bacteria from 11 percent were resistant to two or

more drugs. Moreover, treatment failed for 56 percent of the patients with multi-drug-resistant strains, and the disease proved fatal for 28 percent of patients in this group. "Giving three drugs to those kinds of patients had an inadequate result," Small says.

These, along with results generated in a broader nine-state survey of drug resistance by investigators from the U.S. Centers for Disease Control and Prevention, prompted Mexican officials at the end of 2000 to upgrade their DOTS regimen to include four drugs. "I feel as though the data that this project provided was helpful to them in making that decision, and in my mind, it's really one of the major accomplishments of this project," Small says. The Mexican ministry of health agreed, and honored the collaborators with two awards recognizing the importance of their research.

These changes in Mexico's DOTS policy may well encourage other countries to follow suit.

These changes in Mexico's DOTS policy may well encourage other countries to follow suit, says team member Jose Sifuentes, manager of diagnostic needs for the projects and the chief of the clinical microbiology lab at the National Institute of Medical Sciences and Nutrition (INCMN) in Mexico City. "I am optimistic about this, since Mexico has a strong influence among Latin American countries, so I see good prospects for our findings."

Small credits the team's Mexican scientists for insisting that drug resistance studies be made part of the ICIDR projects in Orizaba. Drug-resistant TB had not been an issue in the earlier San Francisco studies, he says. "Were I doing these projects independently, I never would've taken the question on. But as soon as I started talking with people in Mexico, they said, 'listen, we're really concerned about drug resistance, and we need to incorporate this aspect into the protocols.' So it became part of our agenda early on and, I think, rightly so, in that it's one of the most important findings of the work we've done."

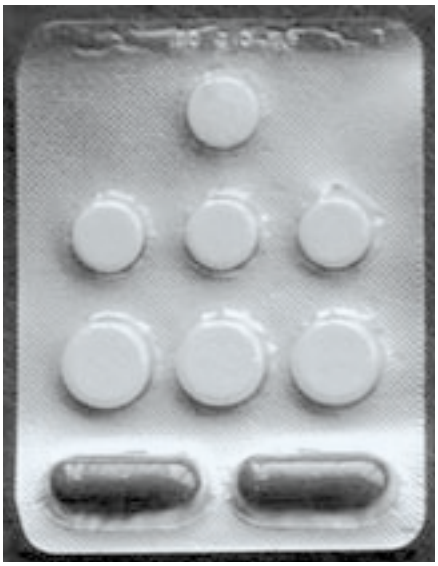
The Right Combination of Talents

These findings that are redefining TB control stem from the early visions of the principal investigators in charge of the 1994 ICIDR projects. "In the beginning, it was literally just Lourdes and I—and reams of paper on which we'd drafted a variety of different scenarios," Small says, referring to Maria de Lourdes Garcia. The two joined forces to develop the ICIDR TB grant projects in

response to recommendations from their respective mentors. Since then, their projects have expanded to include some 40 people working on TB in Mexico.

Two other key players who later joined the ICIDR projects are Sifuentes from INCMN and Alfredo Ponce de Leon, a medical internist at INCMN who learned DNA fingerprinting and other molecular techniques in Small's Stanford lab. Sifuentes manages the laboratory that provides diagnostic support for the projects, which includes culturing bacteria from patient sputum samples to confirm microscopic diagnoses. Ponce de Leon's lab handles all the genetic typing of cultured bacteria and coordinates the conventional mycobacteriology done at the field site in Orizaba. Garcia serves as the chief epidemiologist for the projects and keeps all the field activities humming. She supervises patient recruitment and the running of the clinical offices at the field site. In addition, she coordinates collaborations with local health authorities and community institutions, while overseeing patient data analysis at the INSP.

Health care worker in Orizaba providing DOTS



Prepackaged DOTS medication

"There were times when it seemed like we were never going to get these projects off the ground," Small recalls. One early difficulty was convincing community members that individuals with chronic coughs should be treated right away. Then the research team needed to persuade local health care providers to obtain sputum samples from such patients for routine testing. Provisions were made to examine samples locally and then to send positive samples to Mexico City where confirmatory testing is conducted. Back in the community, TB patients are revisited and their close contacts tested. The team set up a system for recording and analyzing patient data. "It was really an incredibly daunting task," Small says, "but we knew that…if we could pull it off, there would be unique insights that would have practical implications for tuberculosis control."

From the beginning, Garcia and Small recognized the importance of working closely with local health authorities, particularly those running the local program for TB prevention and control. The Orizaba health jurisdiction includes 3 general hospitals, 13 primary health care centers, and 47 community health committees, along with several private clinics. "We wanted them to feel our presence not as an intrusion, but as collaborators whose ultimate purpose is the welfare of patients," Garcia says.

To that end, the research team consults with local authorities and apprises them of its plans and results, inviting them to participate in presentations and seminars. In turn, the local jurisdiction provides the team administrative assistance, lab space, and use of vehicles, and serves as a facilitator of cooperation with local political and educational leaders. The large field team "has been very well received," Garcia says. "As our group is involved in initial diagnosis of patients and represents the first contact they have with the health system, many times the local health program asks their support to bring in patients who default on treatment."

Likewise, the community is very receptive to the team. "For example, we have been able to work with different private and public associations such as those dealing with alcoholics, homeless shelters, schools, factories, and prisons," Garcia says. Field team members are recruited from the nursing, medical, and biological science schools in Orizaba. They are members of the community in

which they are recruiting patients, "so they understand the subtleties of the social situation," Small notes.

The TB Projects Are Having Broader Impacts Than Ever

The collaborators were awarded a second ICIDR grant in 1999, and they are now focusing on the impact of TB diagnostic practices. Typically, health care workers who are implementing the DOTS strategy base diagnoses on simple microscopic examinations of sputum samples. Although that approach is pragmatic, even WHO officials acknowledge that they can miss perhaps 50 percent of all TB cases, according to Small. To determine whether capturing the missed cases could dramatically decrease overall disease rates, the team plans to conduct culture-based diagnostic tests on all presumptive TB patients, including those who test negative on the basis of standard microscopy, to determine what difference this more

Member of Mexican ICID team working in a local laboratory

Health care worker examining TB patient

aggressive diagnostic approach can make in stamping out the disease in the Orizaba district.

The TB ICIDR projects are affecting communities throughout Mexico in other important ways. For instance, the Orizaba health jurisdiction's TB control program now serves as a model for the training of health care workers administering DOTS in other states, Garcia says. "We have been able to develop a small, but strong core group of tuberculosis research in Mexico," Sifuentes adds. Moreover, the efforts of Sifuentes and Ponce de Leon have helped hospital authorities to improve their capabilities for diagnosing other kinds of bacterial infections.

Individual members of the research team have gained personally from these efforts, too. Ponce de Leon, for example, says that seeing the changes in the national TB control policy and knowing that they will have a direct impact on his fellow Mexicans "made it for me!" From his perspective, Small says, "to have a project like this keeps me in touch with the importance of the work that I'm doing. It's important when you live in the middle of Silicon Valley like me and hear people whining about scratches on their BMWs to be reminded what the real world is all about. I don't think when I got started that I recognized the magnitude or the complexity of doing this in a totally different environment."

Ponce de Leon agrees. "International collaborations are difficult, extremely difficult," he says. "At times we have felt the patronizing attitude of Americans, and they have felt the agonizing experience of dealing with the Latin temperament." He adds emphatically, however, that these prove to be minor distractions, and the overall project "is worth it. You make a lot of friends, you spend money on good things, and you do great science."

Accomplishments

CHEMOTHERAPY

ICTDR investigators have pursued several related strategies aimed ultimately at developing new drugs or enhancing those already available for combating tropical diseases. These strategies include investigating essential parasite metabolic pathways or other unique targets for potential chemotherapeutic agents; studying parasite and host enzymes and receptors with similar functions to develop highly selective inhibitors of parasite activity; understanding the molecular mechanisms of drug resistance and identifying methods to reverse resistance and extend the usefulness of available drugs; evaluating agents identified through conventional means or from alternative sources for their efficacy in treating tropical diseases. Discoveries made within the past decade that may lead to potential new drug targets and inhibitors and a better understanding of drug resistance include the following:

- Malaria parasite proteases that play a key role in digesting hemoglobin have been identified as potential new targets for drug intervention.
- Malaria parasites within human red blood cells detoxify heme molecules from degraded hemoglobin by forming them into nontoxic hemozoin polymers, a process that provides several potential targets for drug development.
- Inhibitors of cysteine proteases appear to be essential for growth of infectious-stage intracellular *Trypanosoma cruzi* amastigotes. Inhibitor-based candidate drugs block acute or chronic *T. cruzi* infections with minimum toxicity to host animals. Collaborators in industry are screening potential inhibitors of cysteine proteases from *T. cruzi.* One promising inhibitor of the *T. cruzi* protease has been through extensive follow-up evaluations. Similar cysteine proteases have also been identified in *Trypanosoma brucei, Leishmania major, Schistosoma mansoni, Entamoeba histolytica, and Toxoplasma gondii.*
- Some enzymatic pathways in parasites, such as *Plasmodium, Cryptosporidium,* and *Toxoplasma,* that function in plants but not in mammals are potential new targets for drug discovery and development. For example, an enzyme within the shikimate pathway, which is responsible for synthesizing compounds such as folate, ubiquinone, and certain amino acids essential to these parasites, is inhibited by the plant herbicide, glyphosate.
- An enzyme that functions as a vacuolar-type proton translocating pyrophosphatase in the plasma membrane and acidocalcisomes of *T. cruzi* is not found in mammalian cells and, therefore, represents a potential chemotherapeutic target.
- The variant surface glycoprotein of bloodstream-stage African trypanosomes contains large quantities of the fatty acid, myristate. Since the fatty acid is not found in mammalian cells, this parasite synthetic pathway represents a potential chemotherapeutic target.
- The glycosome, a membrane-bound organelle that houses important metabolic pathways in *Leishmania donovani* and other trypanosomatid protozoa, contains a protein associated with import of molecules into the glycosome. This glycosomal protein appears to be essential for parasite survival, suggesting that this pathway may be a useful drug target.
- Leishmania and trypanosomatid parasites contain kinetoplasts, massive networks of DNA circles containing topoisomerase enzymes that are crucial for cell viability and could be potential drug targets.
- Lipophosphoglycan, the major surface glycoconjugate of *Leishmania,* appears to be an important virulence factor that contributes to parasite survival in its sand fly vector and in the mammalian host; enzymes involved in synthesizing the glycoconjugate offer a potential target for chemotherapy.
- An adenosine analog specifically inhibits the enzyme glyceraldehyde-3-phosphate dehydrogenase—but not the human enzyme—of the glycolytic pathway in *T. brucei and T. cruzi.*
- Molecular markers, correlating with *P. falciparum* resistance to mefloquine pyrimethamine-sulfa, and chloroquine, have been identified.
- Verapamil, a calcium channel-blocking agent used to treat cardiac arrhythmias, reverses chloroquine resistance in malaria trophozoites.
- Certain drug-resistant *P. falciparum* variants rapidly develop resistance to multiple drugs in a process that appears to be due to a hypermutability phenotype.
- Several aminoquinoline analogs of chloroquine appear effective against resistant *P. falciparum,* and intensive structure-activity analysis is under way. The propyl analog known as AQ-13 is in phase I clinical testing.
- Single dose treatments of infected pigs with oxfendazole proved effective in Peruvian studies for preventing the transmission of cysticercosis to humans, caused by consuming *T. solium* cysts in undercooked pork.
- New stable glutamine derivatives are being tested as alternative agents for oral rehydration and nutrition therapy.
- Artemisinin (qinghaosu), a compound derived from a traditional Chinese herbal remedy, is being used to treat malaria, including cases that are resistant to alkaloid drugs such as quinine. Whereas the chemical synthesis of artemisinin is very complicated, several simpler and easier to produce artemisinin-like synthetic tricyclic 1,2,4-trioxanes have been developed that show antimalarial effectiveness in mice and monkeys.

DIAGNOSING PARASITIC DISEASES

Diagnosing Parasitic Diseases

Offering Hope for Prevention and Control

Filarial parasite

Accurate diagnoses of parasitic diseases are important for several reasons—the most obvious being the ability to target treatments to particular pathogens. However, diagnosis of these diseases also has important implications for public health and research efforts. For example, without accurate diagnostic methods, researchers and public health officials cannot determine the true prevalence and burden of a disease. Such information can enable them to more accurately target population-based interventions and allocate often-scarce resources to combat one or another of these diseases. It can also encourage companies to work on additional diagnostics as well as new treatments or vaccines, while providing valuable clues to academic or other investigators about how humans acquire immunity to a particular disease and, thus, indicate avenues worth following in developing vaccines to protect against it.

Despite the many valuable roles that good diagnostic tests can fill, a variety of challenges has continued to thwart efforts to make them available for many parasitic diseases. Until recently, many tests lacked sufficient specificity and sensitivity to pinpoint a diagnosis, and they were usually too expensive and laboratory-intensive to be practical for wide use in developing countries. However, through the National Institute of Allergy and Infectious Diseases (NIAID) International Centers for Tropical Disease Research (ICTDR), scientists from key sites, including in the tropics, have been developing and field-testing several new diagnostic tools whose collective impact goes far beyond deciding appropriate treatments for individual patients. By funding collaborative research efforts to develop and field test new diagnostics for a series of specific tropical diseases, ICTDR is fostering unique partnerships that have the potential to contribute substantially to the eventual prevention, control, and even elimination of several parasitic diseases of major public health significance.

Amebiasis: From Diagnosis to Preventive Vaccines

A prime example of ICTDR-sponsored research producing new diagnostic tools with broad implications for public health is its international amebiasis project. Collaborations among NIAID-supported scientists in academic laboratories, industry, and in the field are helping to make a highly specific diagnostic test for amebic dysentery available in sophisticated clinics as well as in villages where this parasite is apt to strike. Scientists are now applying knowledge gained from this test to the development of a vaccine to prevent amebiasis.

A Deadly Parasitic Disease

Amebiasis, an infection caused by the parasite *Entamoeba histolytica*, is responsible for approximately 50 million illnesses and 100,000 deaths each year, making it the third leading cause of deaths worldwide due to parasitic diseases in humans. Although amebiasis can occur almost anywhere, it commonly strikes residents and visitors in underdeveloped areas, especially Central and South America, Africa, and Asia. In the United States and other developed countries, cases of amebiasis are most likely to occur among immigrants from and travelers who visit endemic regions.

Individuals typically become infected with *E. histolytica* when they unsuspectingly ingest cysts in fecally contaminated food or water. When those cysts reach the intestine, they swell and release the motile, symptom-inducing form of *E. histolytica*, called the trophozoite, that can remain there and even form new cysts without causing disease symptoms. However, trophozoites can also invade the

Without accurate diagnostic methods, researchers and public health officials cannot determine the true prevalence and burden of a disease. Such information can enable them to more accurately target population-based interventions and allocate often-scarce resources.

lining of the colon, killing host cells and causing amebic colitis, acute dysentery, or chronic diarrhea. In addition, trophozoites can be carried through the blood to other organs, most commonly the liver, where they may form life-threatening abscesses.

Barriers to Accurate Diagnosis

Historically, diagnosis of amebiasis was complicated and often unreliable for various reasons. For instance, several areas of the body can be affected, symptoms may be similar to other conditions such as inflammatory bowel disease, and early tests were not highly specific. Before antigen-based tests were developed, diagnosing amebiasis routinely entailed examining stool samples by microscope in search of cysts. However, this method is laborious, often requiring scans through more than one specimen because the number and distribution of cysts in stool specimens vary greatly. In addition, the microscopic examination of stool specimens is not only tedious, but notoriously limited in its sensitivity and specificity. For one thing, macrophage cells from the host look a lot like the cysts. For another, cysts of *E. histolytica*, which causes amebiasis, and those of *E. dispar*, which does not, are indistinguishable under a microscope.

By funding collaborative research efforts to develop and field test new diagnostics for a series of specific tropical diseases, ICTDR is fostering partnerships that will contribute to the eventual prevention, control, and even elimination of several parasitic diseases…

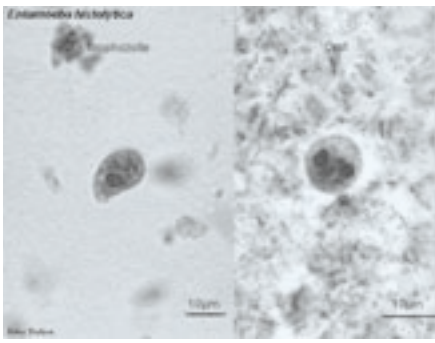
Amebiasis outside the intestine has been even more difficult to diagnose. Clinical manifestations vary widely, and fewer than 10 percent of patients with amebic liver abscesses have identifiable *E. histolytica* in their stools. Noninvasive diagnostic procedures such as ultrasound, computer tomographic (CT) scan, and magnetic resonance imaging (MRI) can detect liver abscesses but cannot distinguish between abscesses caused by ameba and those caused by bacteria, thus hampering proper treatment of the condition. Until recently, the most accurate diagnostic test involved examining a sample of the abscess tissue obtained by needle aspiration, a procedure that is painful, potentially dangerous, and relatively insensitive, identifying amebic trophozoites only 20 percent of the time.

Development of Highly Specific Tests

To address the need for a more specific diagnostic test for amebiasis, a team of laboratory scientists conducted studies on the biology of *E. histolytica*, particularly the mechanism by which amebic

trophozoites adhere to and kill human host cells. Led by William A. Petri, Jr., at the University of Virginia, the team identified a characteristic carbohydrate-containing protein, designated Gal/GalNAc lectin, on the surface of the ameba that is required for trophozoites to adhere to human host cells. This lectin protein also is a major antigen that is recognized by the human immune system, which produces anti-lectin antibodies that are specific for *E. histolytica* but not for *E. dispar*.

When they found that this *E. histolytica* antigen elicits specific antibodies, the ICTDR research group recognized the makings of a new diagnostic test for disease-causing ameba. Petri and his immediate collaborators quickly teamed up with members of a small technology company (TechLab), who were under the direction of David Lyerly and Tracy Wilkins. With additional ICTDR support, they developed a diagnostic test for use on stool specimens that accurately discriminates between *E. histolytica* and *E. dispar*.



E. histolytica *trophozoites and cysts*

From the early stages of these diagnostic test development efforts, Rashidul Haque, another member of this ICTDR consortium, and his collaborators at the International Center for Diarrheal Disease Research in Bangladesh cooperated by field-testing several versions of the new diagnostic procedure. Those field studies established that the sensitivity and specificity of an early version of the test exceeded 90 percent, and subsequent testing indicated that an improved version of the test attains an even higher 95 percent sensitivity, an achievement that helped to gain regulatory approval for wide use in clinical settings of this diagnostic procedure.

The antigen-based *E. histolytica* diagnostic test is simple to conduct, and it provides results rapidly and cheaply. More important, this test can detect amebic infections before serious symptoms appear. Thus, infected individuals can begin treatments very quickly, thereby preventing invasive amebiasis and minimizing further spread of infections to other individuals. Moreover, quick and easy follow-up testing with this simple procedure can be done to determine whether the symptomless intestinal infection is actually gone. ICTDR is supporting follow-up efforts to make the test even more user friendly and less expensive for developing countries to use. This research will move the test from a format that needs to be followed in laboratories

to a simpler dipstick test that can be done readily in clinics, other such settings, or the home.

The discovery by Jonathan Ravidin of the University of Minnesota that the same amebic protein, Gal/GalNAc lectin, circulates in the blood of infected patients forms the basis for another application of the diagnostic test for detecting and helping to evaluate treatments for amebic liver abscesses. Field studies of the noninvasive antigen test show that it has 96 percent sensitivity when used to detect Gal/GalNAc lectin in the blood of patients with amebic liver abscesses before they began treatment with the drug metronidazole. This version of the test, although not yet approved by regulatory authorities, could be used on blood samples to diagnose amebic liver abscesses as well as to assess the effectiveness of such treatments.

Use of the Diagnostic Test in Research

The *E. histolytica* antigen detection test provides a useful tool to researchers exploring strategies to control amebiasis, offering them a ready means to determine what percentages of adults and children are sick because of amebiasis. Accurate prevalence statistics can demonstrate to local public health authorities the relative importance of this disease, providing guidance to them about allocating

Mothers and children in Dhaka hospital

resources and providing tangible incentives to companies considering investment in developing treatments and vaccines for this (or other) tropical diseases.

In addition, researchers can use the diagnostic test to address questions about whether local populations develop immunity to this protozoan parasite. For instance, ICTDR is supporting a study in Dhaka, Bangladesh, where diarrhea is the leading cause of death in children younger than 6 years old. A controlled prospective study is following a cohort of preschool children in refugee camps to determine whether those who are infected once with *E. histolytica* develop an antibody response to the parasite. Preliminary results suggest that infected children who develop an IgA antibody response against the lectin are half as likely to develop amebiasis again, as are children without previous infection. These results indicate that humans acquire at least limited immunity to the parasite, and offer hope that scientists can develop a vaccine that mimics this natural protective response. Researchers in Bangladesh are interested in someday following these efforts with a more ambitious program to test the safety and efficacy of candidate vaccines to prevent amebiasis.

Petri's team already is working to develop such a candidate vaccine based on use of amebic lectin that is used in the diagnostic test.

With support from the ICTDR Tropical Disease Research Units, Petri's team already is working to develop such a candidate vaccine based on use of the Gal/GalNAc lectin, the same amebic protein that is used in the diagnostic test and is required for trophozoite adherence to and killing of human cells. Eric Houpt at the University of Virginia is leading a team that includes Lyerly and Randy Vines to develop the first mouse model of intestinal amebiasis to study intestinal infection with *E. histolytica* and the resulting immune response. Researchers used this model to test a vaccine that elicits an antibody response against Gal/GalNAc lectin and blocks adherence of the ameba to mouse cells.

Building on this vaccine, which protects mice from amebiasis, researchers are now trying to learn which regions of the amebic protein produce critical responses and thus offer the best protection when used in a vaccine. Meanwhile, parallel research projects are studying the use of plants as an alternative way of producing and delivering edible versions of this or other vaccines. Carole Cramer and Fabricio Medina-Bolivar at Virginia Tech are developing plant-based

mucosal adjuvants for use in edible vaccines, while Barbara Mann and Michael Timko at the University of Virginia and other collaborators are attempting to express the Gal/GalNAc lectin in tomatoes and carrots. Producing vaccines in plants that people commonly consume as food offers a promising approach that could make such products less expensive, easy to administer, and in some cases capable of being transported and stored without refrigeration.

Filariasis and Cysticercosis: From Diagnosis to Disease Elimination

ICTDR-sponsored research is yielding other diagnostic tools whose impact reaches beyond identifying individual patients with a particular tropical disease. These new tests are part of a broader strategy for controlling and possibly eradicating two additional important diseases of the tropics, filariasis and cysticercosis.

Filariasis

New antigen, antibody, and DNA tests for lymphatic filariasis are poised to contribute to efforts by the World Health Organization (WHO) to eliminate this disabling and disfiguring parasitic disease. More than one billion people, about 20 percent of the world population, live in tropical and subtropical areas where they are at risk for infection with *Wuchereria bancrofti*, the mosquito-transmitted worm that causes filariasis. Approximately 100 million people already are infected with the parasite, and more than 40 million of these individuals are seriously incapacitated and disfigured by resulting chronic conditions such as grotesque enlargement, or elephantiasis, of the extremities and genitals. Lymphatic filariasis is a significant cause of social stigmatization and poverty, and consumes considerable health resources in endemic areas.

Until recently, diagnosis of lymphatic filarial infection involved examining blood samples by microscope to detect microfilariae—minute larvae—that the adult worm produces in infected individuals. The test is insensitive, and it also is inconvenient because the method depends on collecting blood samples from individuals at night when microfilariae tend to be active. Not easy to do under the best circumstances, microscope-based tests can prove exceedingly difficult as well as inconsistent when attempted under field conditions.

Having simple and effective diagnostic procedures for lymphatic filariasis is especially important for success of the WHO filariasis eradication strategy, which depends on diagnosing cases at the community level and repeatedly treating infected community members to interrupt transmission of infections by mosquitoes. ICTDR funding has been crucial for moving scientific advances from the laboratory to the field, where new diagnostic tools are helping researchers and public health officials to plan and monitor progress in Egypt's lymphatic filariasis elimination program, according to Gary J. Weil at Barnes-Jewish Hospital in St. Louis, Missouri.

(Continued)

ICTDR-supported filariasis research in Egypt has led to the development of important new tools for studying the epidemiology of this parasite. The current project aims to show how these tools can be used to monitor progress toward eliminating filariasis. Moreover, lessons learned in Egypt should be applicable to other countries planning their own such programs. In field studies, a new filarial test proved highly sensitive for detecting products released by *W. bancrofti* in blood samples from filariasis patients. Equally important, the test can be performed simply and rapidly using very small samples of blood collected from a finger prick during the day or night. In Egypt, this commercialized rapid-format card test is being used to map communities infected with *W. bancrofti* for inclusion in the national filariasis elimination program.

Another new test detects human antibodies to a recombinant filarial protein cloned in Weil's laboratory with the help of Reda Ramzy at Ain Shams University in Cairo, Egypt. This test can tell when a person has been infected with or heavily exposed to *W. bancrofti*. It is being used in Egypt to test young children as a means of documenting whether filariasis is still being transmitted in particular communities. Children are a sentinel population because first exposure to *W. bancrofti* typically occurs during childhood. By testing children every year before and after communitywide mass treatments, researchers expect to determine whether transmission of the parasite is being interrupted, as indicated by declines in antibody rates among children. A new DNA test for filariasis, developed by Ramzy and colleagues, detects parasites in human blood or mosquitoes using the polymerase chain reaction (PCR). This diagnostic tool will be used to determine whether treatment produces a decline in the percentage of houses in a community that have mosquitoes infected with the parasite. Such a decline would be an early indicator of the success of the Egyptian filariasis elimination program and would also provide crucial incentives for the sustained involvement of industry partners throughout the 20 years that WHO estimates will be needed to eliminate lymphatic filariasis worldwide. The Global Program for Elimination of Lymphatic Filariasis calls for coordinated efforts by governments, nongovernment organizations, and industry partners over the next two decades. Weil believes that the ability to measure and monitor progress toward eliminating filariasis will be critically important until this goal is met.

Cysticercosis

ICTDR also helps support the Cysticercosis Working Group in Peru, an international consortium of scientists that includes Hugo Garcia, Robert Gilman, Armando Gonzalez, and Victor Tsang. The working group brings skills in tropical medicine, neurology, epidemiology, veterinary medicine, and medical technology to the task of controlling and perhaps eliminating cysticercosis. This parasitic disease affects millions of people annually in Latin America, Asia, and Africa and is found increasingly in North America, where immigrants from endemic areas inadvertently bring the infection with them. Although WHO officials consider cysticercosis an eradicable disease, the absence of an effective and sustainable control strategy so far keeps this goal from being realized.

Humans acquire cysticercosis when they ingest food or water that is contaminated with the eggs of the tapeworm, *Taenia solium*, that infected pigs shed in feces. These microscopic eggs contain larval embryos that travel through the human bloodstream to various tissues in the body where they can form cysts. Cysts in the brain can cause life-threatening neurologic problems such as seizures, making neurocysticercosis the major cause of late-acquired

epilepsy in developing countries. Because pigs are the intermediate host to *T. solium,* farmers in developing countries suffer significant economic losses when they try to market infected stocks.

Until recently, human cysticercosis was diagnosed using CT scans and MRI, neither of which can reliably distinguish neurocysticercosis from several other brain diseases. Moreover, these costly technologies are not feasible for use in large-scale epidemiologic surveys in the field. Determined to develop a simpler and more reliable alternative, a team led by Tsang developed an enzyme-linked immunoblot (EITB) test for cysticercosis. The highly specific and sensitive test identifies individuals who produce specific antibodies that react with antigens produced by *T. solium* larval cysts. The commercially available test enables clinicians to establish a specific diagnosis for infected patients and begin treatment, while also helping to break the chain of infections within the wider community.

The EITB test simplifies the task of individual diagnoses and, compared with the arduous, impractical CT or MRI, makes dramatically easier a wide-ranging epidemiologic study. In short, from a public health perspective, this new highly specific and sensitive blood test is revolutionary. The test is enabling epidemiologists to calculate the magnitude of the human cysticercosis disease burden, which they find is much greater than previously recognized. Led by Garcia and Gilman, the Cysticercosis Working Group in Peru now estimates that 75 million people in Latin America live in areas where they are at risk for infection, and approximately 400,000 Latin Americans have symptomatic disease.

This same research consortium continues to build the scientific foundations for eventually controlling and possibly eradicating cysticercosis. For instance, the blood test that the group developed for detecting *T. solium* in pigs allows other researchers to monitor this parasite throughout specific communities by tracking rates of new infections among pigs. When introduced into an area, "clean" pigs quickly become infected wherever tapeworm carriers are found, thus helping to detect the source of cysticercosis infections. Another diagnostic test developed by the team identifies human carriers of the adult tapeworms, which are the key sources of transmission.

Scientists led by Gonzalez evaluated oxfendazol, a previously overlooked veterinary drug, for treating cysticercosis in pigs. An inexpensive, single-dose treatment with oxfendazol kills all *T. solium* in pigs, suggesting this drug could provide a practical means for interrupting a vital link in parasite transmission and thus for preventing human infections. Gonzalez, Gilman, and their collaborators also are using pigs infected with *T. solium* to evaluate other therapeutic drugs or potential vaccines for cysticercosis. Garcia and his colleagues also are attempting systematically to define better procedures for managing and treating patients with cysticercosis.

The Cysticercosis Working Group in Peru is conducting research aimed at developing a systematic and integrated approach to the control of cysticercosis that builds on the advances of new diagnostic tools, drug treatments, and animal models. Researchers are developing a diagnostic test in dipstick format that will be more practical for use in the field than the current laboratory-run EITB tests. Additional efforts are focused on developing a vaccine to prevent cysticercosis that will be tested in the new pig model. The working group is exploring control measures in both human and pig populations in endemic areas of Peru. Strategies include using the new diagnostic tools to identify and monitor areas of *T. solium* infection as well as a combination of prevention and control measures such as vaccination of pigs along with drug therapy for pigs and humans.

Accomplishments

DIAGNOSTICS

ICTDR investigators have developed many new or improved diagnostic techniques, several of which appear to be cost-effective and are either already commercially available or are being evaluated for possible licensure and use in clinical settings and as part of tropical disease control programs. Examples include the development of

- An ELISA-microwell assay to distinguish pathogenic *Entamoeba histolytica* from morphologically indistinguishable, nonpathogenic *Entamoeba dispar* and intestinal and hepatic amebiasis.
- A qualitative enzyme immunoassay for detecting *Giardia lamblia, E. histolytica/dispar,* and *Cryptosporidium parvum* in fecal specimens.
- An ELISA-based assay for detecting antibodies to *Trypanosoma cruzi* (Chagas' disease).
- An antibody-based ELISA assay for detecting active visceral leishmaniasis.
- Reagents needed to develop, improve, and evaluate a filariasis test.
- An immunoblot assay, using antigens collected from cultured tapeworms for identifying adult *Taenia solium* carriers.
- Lactoferrin testing for inflammatory diarrheal illnesses that are culturable and potentially treatable. This simple, inexpensive test is now being adapted to a dipstick format with the potential for use in rapid field diagnosis.
- A resin-dye test for the noninvasive diagnosis of hypochlorhydria that could be used for determining what fraction of individuals infected with *Helicobacter pylori* has a high risk for developing gastric carcinoma.
- An adaptation of an ELISA technique for diagnosis of *Cryptosporidium*.
- Specific anti-*Giardia* and anti-*Cryptosporidium* assays that can be used to detect antibodies in infants before the appearance of clinical signs of disease.
- A high-sensitivity, 6-hour PCR assay for detecting dengue viremia.
- DNA sequence-based diagnostic tools for paragonimiasis to clarify the main species infecting humans and responsible for pulmonary disease.
- The Malaria Research and Reference Reagent Resource Center, which provides scientists with reagents needed for detecting malaria drug resistance markers by PCR.

MALARIA VECTORS

Breaking the Chain

Mosquitoes May Be Vulnerable Link in Malaria Cycle

Like other parasites, *Plasmodium falciparum*, the organism responsible for causing the deadliest form of malaria to humans, cannot survive on its own. Indeed, it depends alternately on humans and mosquitoes for food and shelter, shuttling between these two host species during different phases of its complex life cycle. Seeking a new way to break this vicious cycle, researchers are studying the biology, ecology, and behavior of mosquito vectors, or carriers, of *P. falciparum* and their interactions with both people and parasites. These studies are an important component of a multipronged approach to reduce the impact of malaria in Africa, where the disease kills about 1 million people each year—mostly young children.

Two investigators supported by the National Institute of Allergy and Infectious Diseases (NIAID) International Centers for Infectious Disease Research (ICTDR) program, Yeya Touré of the Faculty of Medicine, University of Mali, and John Beier of the School of Public Health and Tropical Medicine at Tulane University, are leading research on malaria vectors in Africa. Their overall goal is to develop novel strategies to reduce disease transmission. These strategies include designing new methods to kill mosquitoes, blocking transmission of malaria parasites from humans to mosquitoes (or vice versa), and interfering specifically with the parasite's life cycle within mosquitoes.

When a female *Anopheles* mosquito carrying malaria parasites feeds on human blood, she injects the infectious sporozoite form of the parasite into the victim's bloodstream along with her saliva. The parasites undergo several stages of development in humans, first in the liver and then in red blood cells. Some of the parasites that infect red blood cells develop into male and female sexual forms, called

gametocytes, which can be transmitted to another female mosquito when she consumes that person's blood. In the mosquito, the parasites complete their life cycle by reproducing sexually to form oocysts that mature in the insect's gut. Each mature oocyst releases a thousand or more sporozoites that migrate to the mosquito's salivary glands, ready to renew the cycle of transmission the next time that mosquito bites a person.

Anopheles gambiae *mosquito*

A Need to Go Beyond Existing Methods of Vector Control

One way to break this cycle is to eliminate the mosquito vector. The insecticide DDT was a mainstay of malaria eradication programs in the 1950s and 1960s and is still used for indoor spraying of houses. But many mosquitoes are now resistant to DDT, and other insecticides used for such indoor spraying are more costly. Mosquito nets impregnated with biodegradable insecticides and placed around beds are being used extensively in Africa and other regions where malaria is endemic. Although use of these nets has significantly reduced childhood mortality, mosquitoes in some areas are developing resistance to the insecticides used to treat the nets.

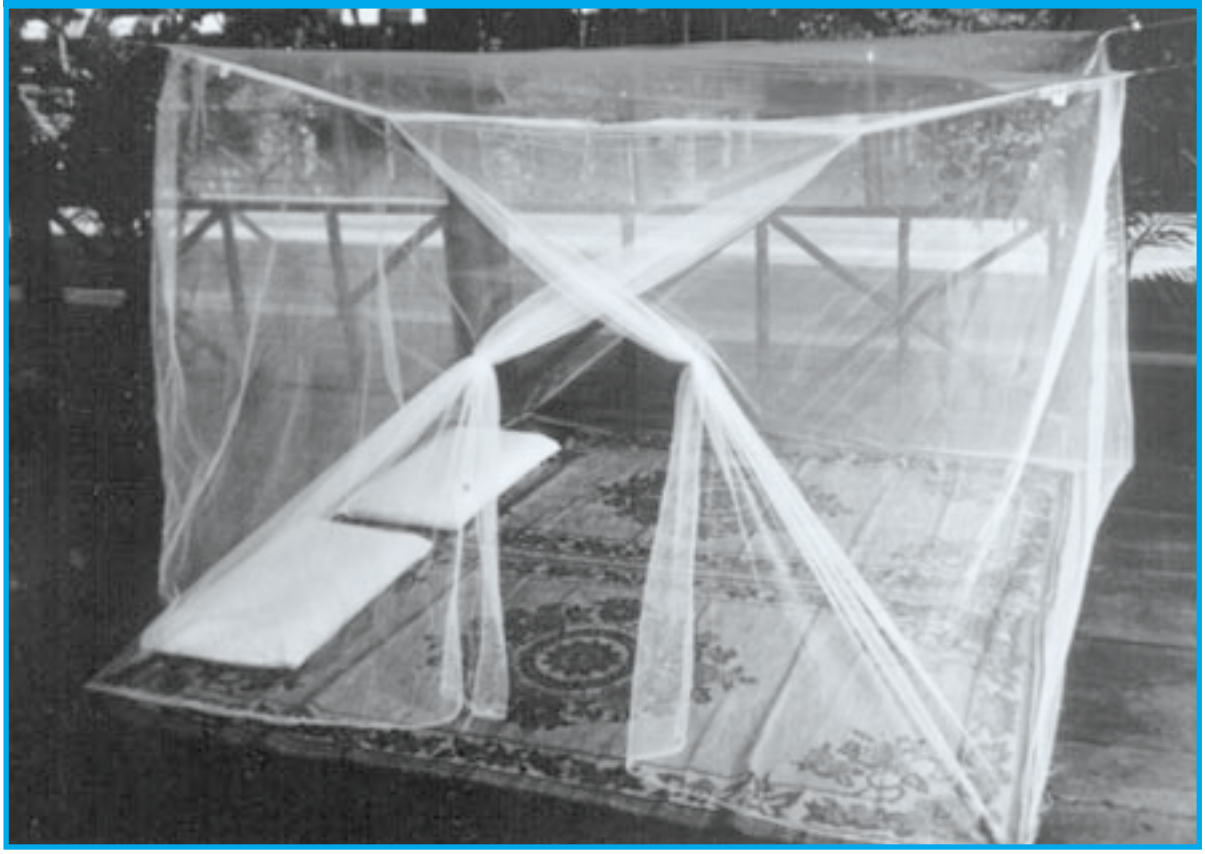
"If bed nets can come in and knock down the vectors by 80 percent, where our program comes in is what to do about the other 20 percent," says Beier, who in 1999 received an International Collaborations in Disease Research (ICIDR) grant from the ICTDR program to continue his work on malaria vectors in Kenya. Beier is working with researchers from the International Centre of Insect Physiology and Ecology (ICIPE)—the largest group of scientists studying insects in Africa—and from the Kenya Medical Research Institute (KEMRI). "The nice thing about Kenya," Beier says, "is

that we can do our field work almost anywhere in the country through a network of field stations" run by ICIPE and KEMRI. In 1996 Beier helped launch ICIPE's malaria vector program, which he directs with ICIPE/KEMRI scientist John Githure.

In Kenya, Researchers Learn More About Malaria-Bearing Mosquitoes

Beier's research in Kenya has three components focusing on the larval ecology of mosquitoes, adult behavior of mosquitoes, and vector competence, which is the ability of mosquitoes to support malaria parasite transmission and survival. *Anopheles* and other mosquitoes normally lay their eggs in water, where they hatch to produce larvae, the earliest life stage of the mosquito. The wingless larvae live in water and develop first into pupae and then adult mosquitoes. Beier's project on larval ecology, a subject he says has not been studied much in Africa, is aimed at learning more about the habitats and habitat preferences of mosquito larvae in different

Insecticide-impregnated bed nets



ecological zones throughout Kenya. "This is important, because we have to be able to identify the larval habitats to know how to control the larvae," he says.

Beier and his colleagues discovered that *Anopheles gambiae* eggs can survive in dry soil in a dormant state for 2 to 3 weeks, indicating that mosquitoes need not always lay their eggs in water. This capacity of eggs to survive in dry soil means that when the rains come, the widely distributed eggs are ready to produce larvae. The researchers are now trying to understand how *A. gambiae* eggs can live without water.

The Kenya team has also begun studying aquatic species that eat mosquito larvae in nature. Because such predators tend to be prevalent in large, open permanent water bodies such as swamps or ponds, mosquito production in such habitats is relatively low. By contrast, mosquito production in small temporary pools can be very high, because the eggs hatch before potential predators can get there. "This is important, because if we just spray the permanent water bodies with insecticides, all we are going to do is kill off the predators, and the mosquitoes are going to colonize these habitats before the predators come back in, after the insecticides wear off," Beier explains.

The researchers are now trying to understand how *A. gambiae* eggs can live without water.

The larval ecology project will eventually include community demonstration projects of larval control. In the coastal city of Malindi, for instance, Beier and his colleagues are working with one community group that is interested in helping with malaria control. "If the communities and local governments are going to get involved, we have to work with them on some basic elements, including the basic biology of malaria vectors, identifying habitats, and then see if some of these habitats can be eliminated," Beier says. Killing mosquitoes in the larval stage is more likely to be an effective vector control method in cities than in rural communities in Kenya and elsewhere because many areas of standing water in which mosquitoes like to breed are also used by families for growing rice. Spraying these areas with insecticide would not be feasible in communities in which people rely on such small plantings for most of their food.

In other studies aimed at understanding key adult mosquito behaviors, Beier and his colleagues are seeking natural chemicals that stimulate egg-laying (oviposition) behavior or attract mosquitoes to certain people. Initial studies of oviposition behavior suggest that water from breeding sites contain bacteria producing odors that attract mosquitoes, according to Beier. In collaboration with the chemical ecology division at ICIPE in Nairobi, the researchers are also conducting experiments to identify chemical components of human odors that attract mosquitoes. The goal in both cases is to identify natural chemicals that attract mosquitoes, find ways to produce these chemicals in the laboratory, and use them to bait mosquito traps that would be placed throughout a community.

"The concept we're trying to promote is one of integrated vector management," in which a range of techniques could be used to control mosquitoes in areas of varying transmission intensity, Beier says. For example, areas of low transmission might require only bed nets for sufficient vector control, whereas high-transmission areas might require bed nets and larval control and odor-baited traps.

…Areas of low transmission might require only bed nets for sufficient vector control, whereas high-transmission areas might require bed nets and larval control and odor-baited traps.

Several Approaches to Studying Human-to-Mosquito Transmission

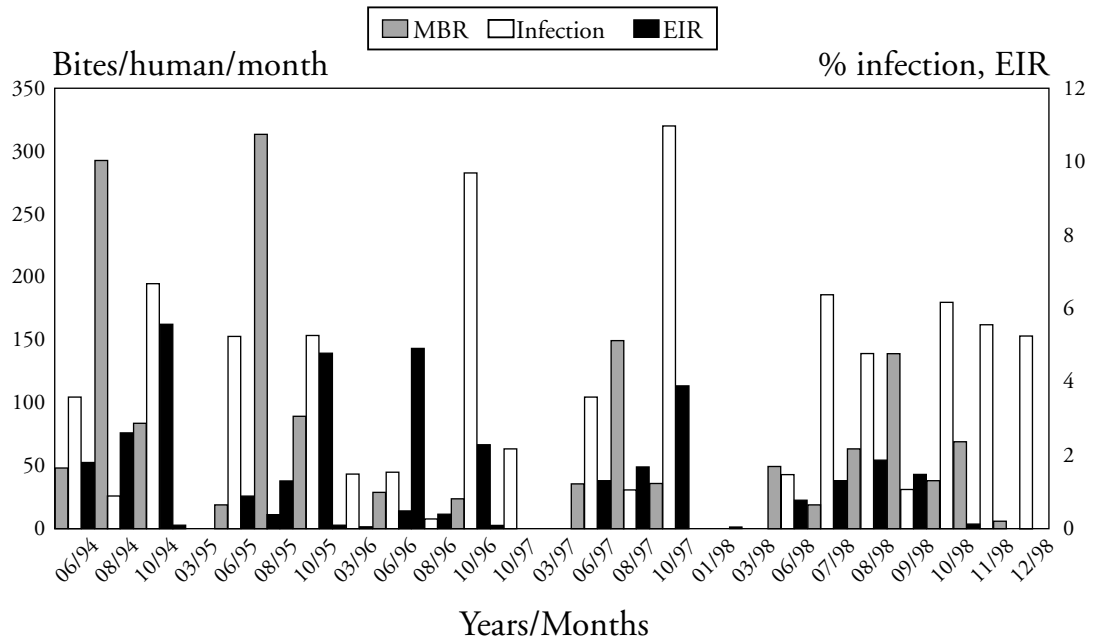
Although researchers around the world, including Yeya Touré's group in Mali, are working on vaccines to block transmission of malaria gametocytes from people to mosquitoes, "we wanted to take a little bit different approach" to studying transmission, Beier says. Thus, studies on vector competence in Kenya focus on how environmental and genetic factors affect a mosquito's susceptibility to infection and how these factors affect parasite development in the mosquito once she becomes infected. As part of this work, the researchers are starting to examine how temperature variations affect vector competence.

An exciting although technically challenging part of Beier's research on vector competence entails looking for genetic factors that may explain why some mosquitoes are refractory, or resistant, to infection by *P. falciparum* while others remain susceptible. Some mosquitoes do not become infected with malaria when they bite a person carrying gametocytes, and Beier plans to select for such refractory mosquitoes by breeding them in the laboratory and then

using them to search for genes involved in refractoriness. Knowledge about such genes and how they function could lead to ways to manipulate refractoriness in wild mosquitoes. Before these studies can begin, however, the researchers are faced with the challenge of developing a successful laboratory system for infecting mosquitoes with malaria gametocytes, which Beier describes as "more of an art form than a science."

Beier is also assisting with research on malaria vectors in Mali, where he collaborates with medical entomologist Yeya Touré as part of an ICTDR Tropical Medicine Research Centers (TMRC) grant awarded jointly to the University of Mali, located in the capital city of Bamako, and Tulane University. Touré, head of the University of Mali's Malaria Research and Training Center (MRTC), is leading a series of studies on malaria vectors in Bancoumana, a village of around 8,000 inhabitants located about an hour's drive from Bamako. The village is in the southern part of Mali, where malaria is hyperendemic—that is, more than 75 percent of children between the ages of 2 and 9 carry the malaria parasite.

Monthly variations of the EIR of *A. gambiae*

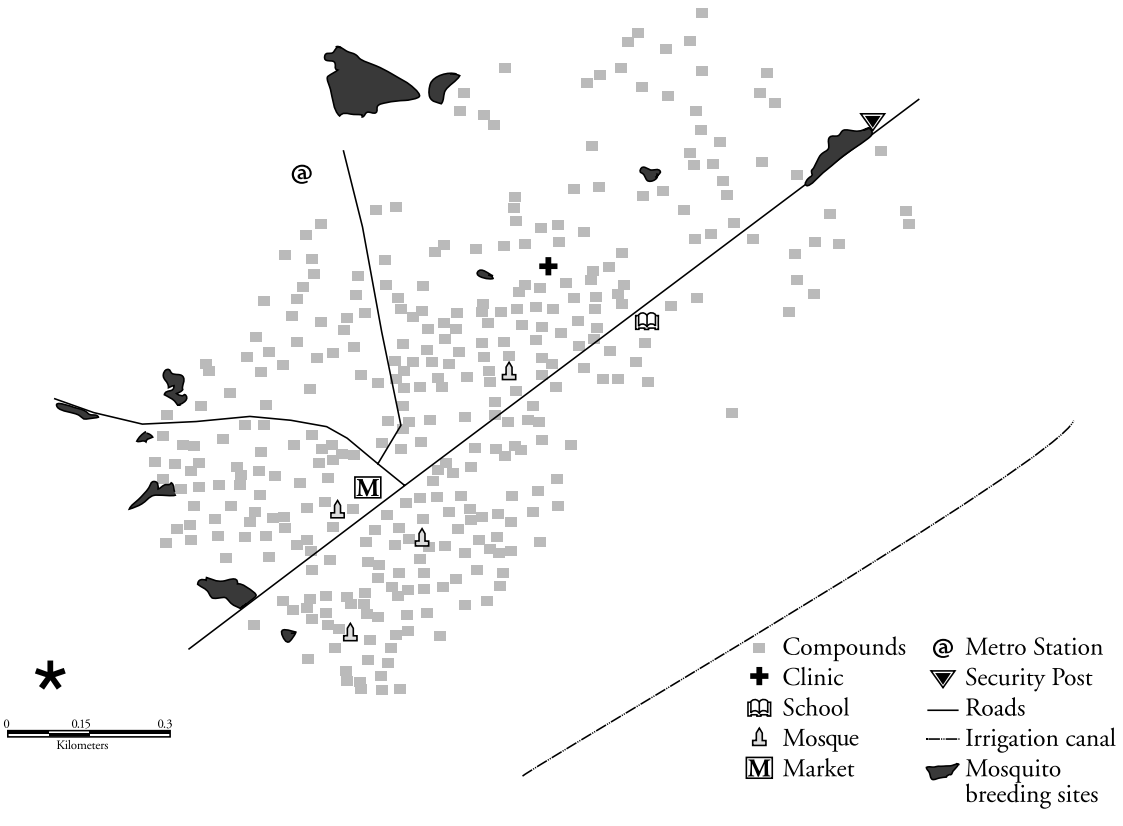


Bancoumana, June 1994 to December 1998

Unlike in western Kenya, where malaria transmission occurs year-round, transmission of the disease in Bancoumana occurs mainly during the rainy season, from June to October. Even during the dry season in Kenya, there is enough water to support mosquito breeding, whereas "in the dry season in Mali, everything shrivels up and dies," says Beier. In his visits to Bancoumana, he has been impressed by the good relations between the MRTC researchers and the people of the village. Touré and his colleagues chose Bancoumana as their study site because it is reasonably accessible from Bamako and because the MRTC researchers had already established a highly effective collaboration with the villagers (see sidebar on Ogobara Doumbo, page 19).

Under the TMRC grant, Touré and his colleagues have been studying the dynamics of parasite transmission from people to mosquitoes. They began by finding out who among the population was carrying the gametocyte stage of the parasite—the stage that is infectious to mosquitoes. About 12 percent of all people in

Village of Bancoumana GPS-based map



Bancoumana have *P. falciparum* gametocytes in their blood, with young people between the ages of 2 to 18 carrying relatively high levels of gametocytes that prove more infectious to mosquitoes than do those carried by people over 18. "This is quite an important finding because it has implications when it comes to the testing of a transmission-blocking vaccine," Touré says. For example, he explains, it will enable investigators to understand exactly what population to use and what sample size to work with in vaccine studies.

Touré's team found significant seasonal variations in the ability of people to infect mosquitoes with the malaria parasite. "We observed quite a high infectivity rate during the rainy season, with a peak in October," he says. To their surprise, the researchers also observed a second, smaller peak in February, during the dry season. Their findings indicate that even though there is almost no detectable transmission occurring during the dry season, people are still producing gametocytes and these gametocytes are infectious for mosquitoes that feed on their blood. These findings "show a high potential for continuation of transmission," Touré says. "Once the first rains come and the mosquito density increases, immediately you have mosquitoes that will be infected because the gametocytes are already there."

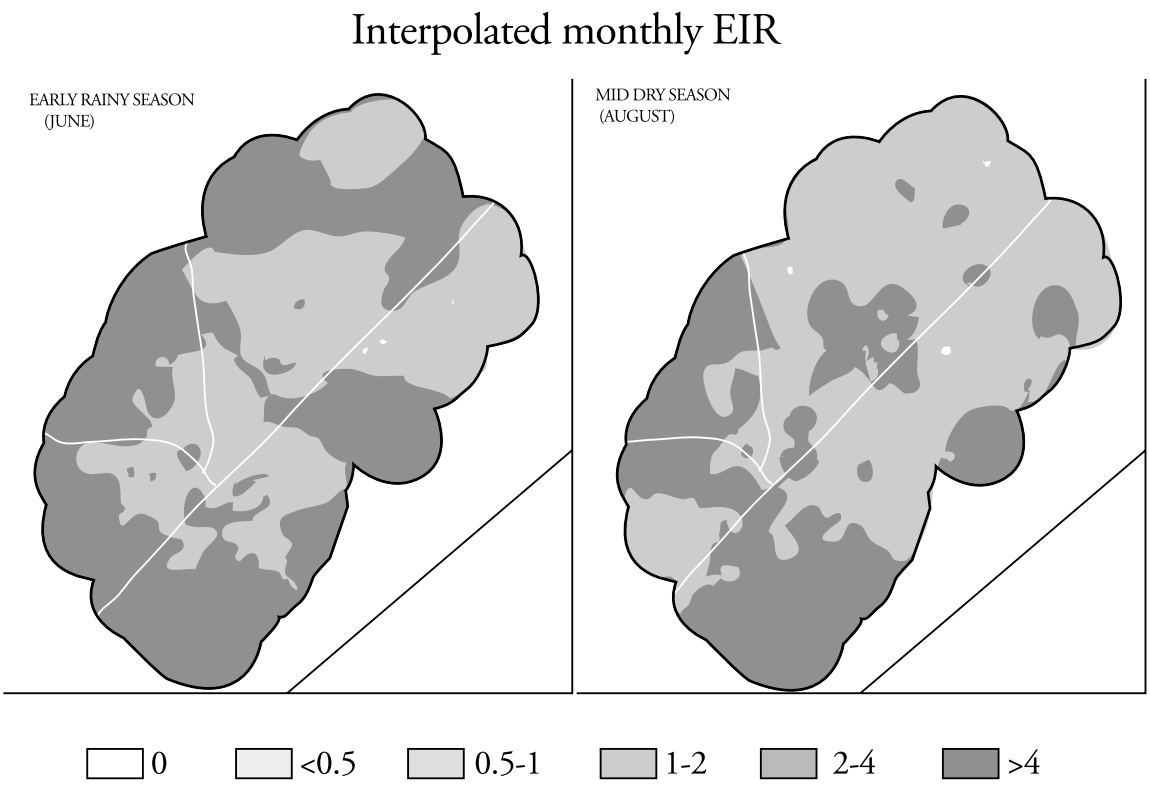
About 12 percent of all people in Bancoumana have *P. falciparum* gametocytes in their blood, with young people between the ages of 2 and 18 carrying relatively high levels of gametocytes that prove more infectious to mosquitoes than do those carried by people over 18.

Progress in the Quest for Transmission-Blocking Vaccines

Knowing more about the infectivity of humans for mosquitoes will help the Malian researchers who are trying to develop a vaccine that breaks the cycle of malaria transmission between human and mosquito. Touré and his colleagues are looking for people who might carry natural transmission-blocking components in their blood serum. By comparing blood samples from gametocyte carriers for their relative ability to infect mosquitoes in the laboratory, the researchers found that about 18 percent of carriers have something in serum that blocks gametocyte transmission. Researchers are analyzing serum from people who have this transmission-blocking capacity, hoping to identify specific antibodies that block transmission, according to Touré. This information could be used to design a transmission-blocking vaccine that works by stimulating production of these antibodies.

Touré and his colleagues are also studying the other side of the malaria transmission cycle, looking at patterns of transmission of *P. falciparum* from mosquitoes to humans. According to a 4-year study of malaria transmission in Bancoumana, the highest number of mosquito bites per person per month usually occurs in August. However, the peak in the number of *infective* bites (known as the entomologic inoculation rate or EIR)—that is, bites by mosquitoes carrying the sporozoite form of the parasite, which infects humans and causes disease—occurs in October, at the end of the rainy season. Moreover, the genetic diversity of *P. falciparum* parasites in mosquitoes varies throughout the year and peaks in October, coinciding with this peak of transmission. Although the researchers do not understand the connection between parasite diversity and transmission intensity, Touré says that "the two together are probably driving the highest level of infection and of clinical cases" of malaria that occurs at the end of the rainy season.

Spatial analysis of the entomological characteristics of malaria transmission in Bancoumana, Mali



What are the implications of these results in terms of vector control? Knowing that the bulk of parasite transmission and clinical cases of malaria occur in October will enable the design of "a selective, targeted control method" to reduce malaria transmission, Touré says. Such a targeted approach will allow the most efficient and economical use of limited resources. For example, he says, by spraying houses in July or August with an insecticide that lasts for 3 months, "you might be able to affect something like 90 percent of the transmission. You will interrupt the peak of transmission and the peak of clinical cases."

Global Systems Provide Precise Mappings of Malaria

Another part of Touré's TMRC project uses global positioning system (GPS) and geographic information system (GIS) technologies to map malaria transmission patterns in different parts of Bancoumana, tracking how they change over time. The researchers began by creating a GPS-based map of the village. The map shows the location of the different compounds in the village (clusters of 2 to 10 houses belonging to members of an extended family) and of various landmarks and geographical features such as the nearby Niger River and a seasonally flooded area between the river and the village that is used to cultivate rice and is a breeding ground for mosquitoes.

Knowing that the bulk of parasite transmission and of clinical cases of malaria occurs in October will enable the design of "a selective, targeted control method" to reduce malaria transmission.

When Touré and his colleagues plotted malaria transmission rates on this map at different times of the year, they found that in the beginning of the transmission season in June, the level of transmission is relatively low and occurs only in the peripheral areas of the village, which are closest to mosquito-breeding grounds. In October, however, transmission occurs throughout the village but is most intense in the periphery. In the dry season of January through March, a very low level of transmission occurs only on the outskirts of the village.

These observations provide valuable data for targeted malaria control strategies, Touré says. "You can undertake such selective and well-targeted vector control only when you have enough epidemiological data, vector biology, and ecological data," he says. "You must be able to know what the problem is, where it is, and

how it is at different periods." If such information were available for regions throughout the country, it "would allow us to set priorities, focus resources, and improve implementation of control measures."

With the availability of new technologies like GPS and GIS, gathering such information countrywide is becoming more and more possible, Touré says. "It is just a matter of developing human capacity to conduct such surveys, having the researchers and the government bodies work together, and step by step we will be able to solve the problems in the country. It will surely take time, but it is a matter of starting."

Accomplishments

VECTOR CONTROL

ICTDR and other NIAID-supported investigators are targeting insects and other invertebrate organisms that transmit pathogens to humans as another strategy for controlling tropical diseases. These efforts involve identifying new ways either to control the vector populations themselves or inhibit their ability to transmit pathogens. Highlights from research to understand the mechanisms of pathogen development within invertebrate vectors include the following:

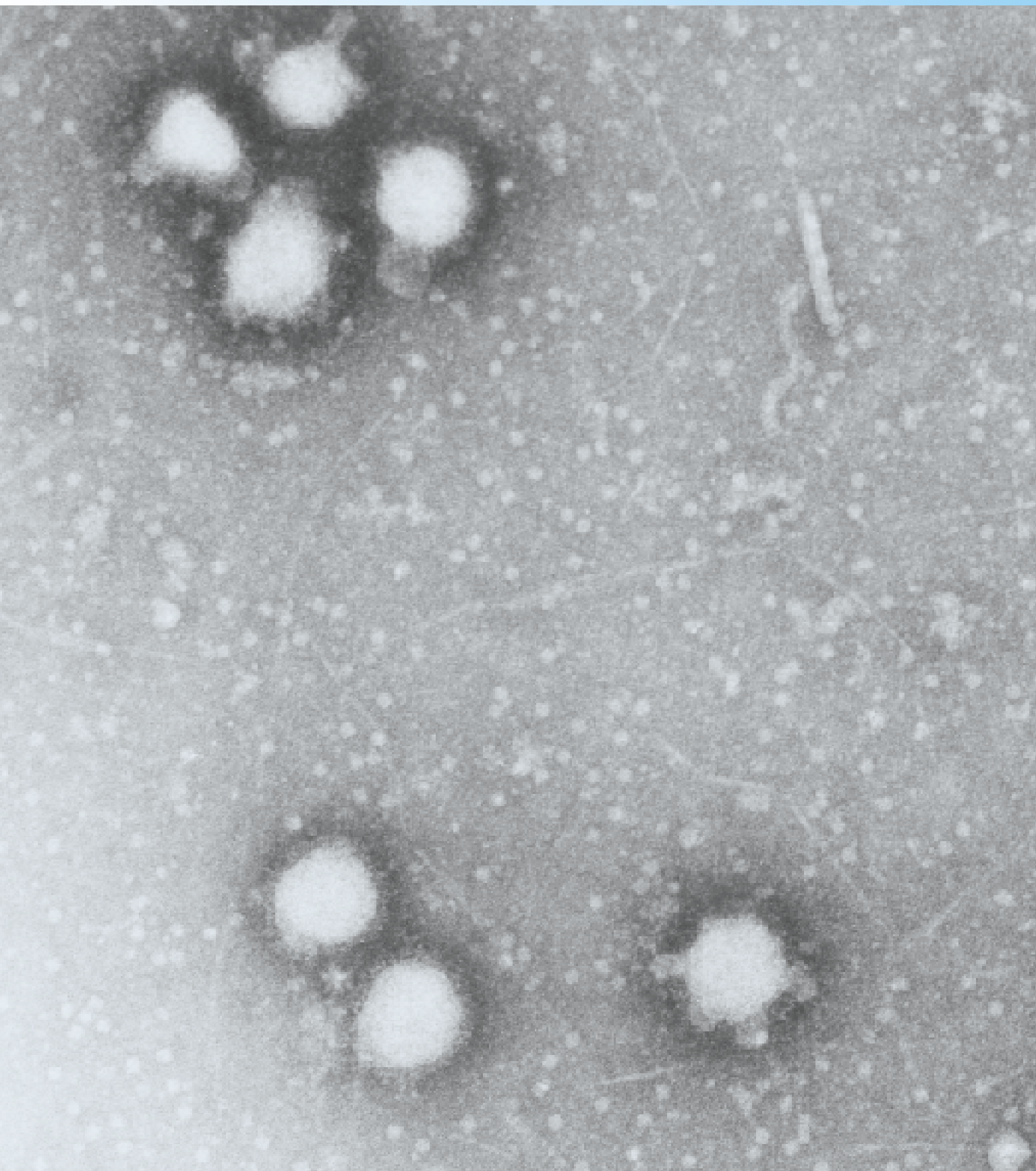
- Invertebrates, like other organisms, have innate immunity to various pathogens. Immunoresponsive genes from *Anopheles gambiae* mosquitoes are being analyzed in search of immune system components that can inhibit pathogen development in the vector. Some genes were identified that discriminate between bacterial pathogens and malaria parasites; among such components is a protein similar to the protease inhibitor alpha-2-macroglobulin.
- Genes have been cloned that encode secreted factors and receptors from the salivary glands of *A. gambiae* mosquitoes that are likely involved in blood-feeding.
- Numerous pharmacologic mediators that facilitate pathogen transmission by inhibiting blood clotting and vasoconstriction have been identified in the saliva of blood-sucking insects.

The following breakthroughs have been made in finding ways to modify insect vectors so as to make them less capable of transmitting pathogens:

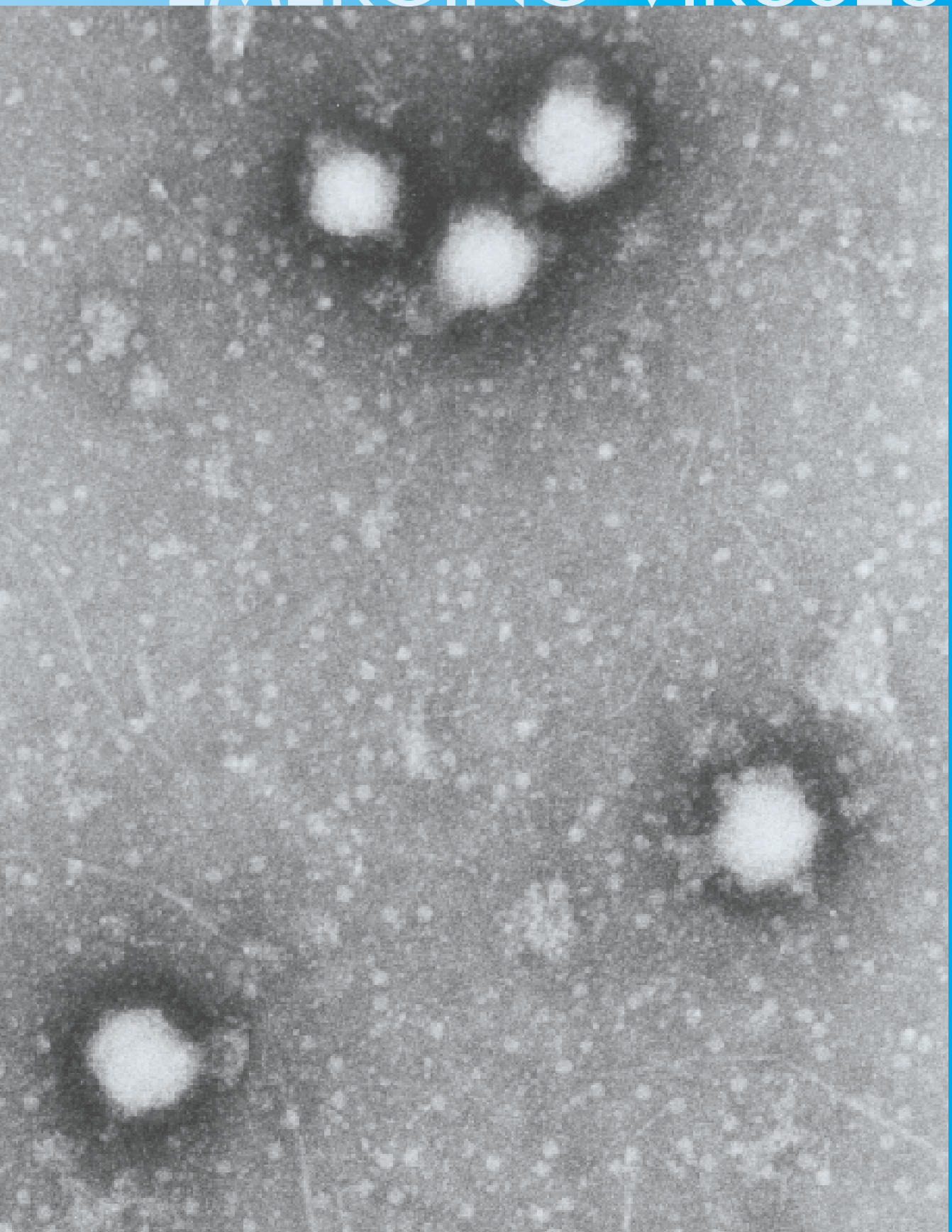
- The regulatory region of the vitellogenin (Vg) gene of *Aedes aegypti* was used to drive expression of the defensin A coding region, one of the major insect immune factors.
- The mosquito-borne alphavirus, sindbis (Togaviridae), is being modified to provide a means for inhibiting targeted mosquito genes. An engineered sindbis virus can infect *A. aegypti* mosquitoes and interfere with their ordinary capacity to transmit dengue virus, providing proof that molecular approaches can make vectors incompetent for transmission of pathogens.
- The *Aedes* densonucleosis parvovirus can express genes in mosquitoes and is being studied as a potential delivery system for mosquito-specific toxins; this virus is target specific, does not infect vertebrates, and can remain infectious in breeding sites for extended periods.
- Transposable elements are being used to genetically modify important vector mosquitoes, including *A. aegypti*, which carries yellow fever and dengue viruses.
- The endosymbiont of the Chagas' disease vector, *Rhodnius prolixus*, has been transformed to express cecropin A, a peptide lethal to the *Trypanosoma cruzi* parasite, providing a useful model for constructing insects carrying transformed symbionts with compromised ability to transmit pathogens.

Ecological studies of vectors will help to identify stages at which environmentally sound control strategies may be targeted. Observations that may contribute to this goal include:

- When marked *A. aegypti* mosquitoes were released in a residential community, follow up analysis indicated that oviposition site availability inversely correlates with the potential for female *A. aegypti* to disperse, suggesting that campaigns to reduce larval sites during dengue outbreaks could actually help to disperse infected adult female mosquitoes.
- Sampling of the larval habitats of anopheline mosquitoes in the Suba District of western Kenya indicates that, in overall terms, *A. arabiensis* outnumbers *A. gambiae*. Distance to the nearest house and substrate type were the factors most closely associated with the relative abundance of *A. gambiae*.
- Gene sequence data led to discovery of new paragonimiasis-carrying snail hosts and demonstrated the genetic diversity of schistosomiasis-carrying snails in China.
- Several tools have been developed to help monitor the distribution and population genetics of vectors responsible for pathogen transmission in endemic areas. These include identification of microsatellite markers on the polytene chromosomes of *A. gambiae;* isozyme, random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR), and internal transcribed spacer 2 (ITS2) markers; and a method to determine the age of mosquitoes by changes in hydrocarbon composition of the cuticle from their legs.
- It is important to detect and monitor not only the arthropod vectors, but the pathogens they carry. Sequences specific to the small subunit ribosomal RNA of the sporogonic stages of *P. falciparum* are being used as part of an assay that can detect 0.1 sporozoites in total RNA purified from potentially infected mosquitoes. This assay can accurately measure sporozoite numbers over at least three orders of magnitude.
- Pathogens migrate to new regions and countries with increasing speed, causing severe disease burdens when the public health system is caught unawares. A new method of monitoring subtle genetic changes, called single strand conformational polymorphism analysis, is being used to monitor the emergence of new dengue virus genotypes, such as the dengue 2 genotype that was detected recently in the Yucatan.

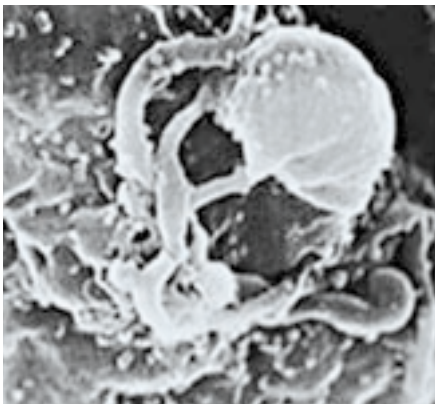


EMERGING VIRUSES



Multidisciplinary Approach to Studying—and Combating—Hantavirus and Dengue

Tracking Dangerous Viruses in the New World



Hantavirus

In the spring of 1997, a 30-year old Chilean man suffering from hemorrhagic fever appeared unannounced at the hospital in Coyhaique, a rural town in the Patagonia region of southern Chile. The case proved to be only the first in an outbreak involving 144 cases of hantavirus cardiopulmonary syndrome (HCPS), which kills its victims by filling their lungs with fluids and weakening the pumping action of the heart. While the disease struck mainly in sparsely populated areas within the Andes, its lurid manifestations and high mortality rate raised public alarm throughout Chile and cast a cloud over tourism in the region.

Except for rare cases of rabies, the HCPS epidemic is the first major wildlife-borne infectious disease to strike Chile. Though the country boasts its share of capable medical research institutions, none was fully prepared to tackle such an unfamiliar infectious scourge. Thus, Chilean scientists began collaborating with hantavirus experts at the University of New Mexico in Albuquerque (UNM) whose expertise was honed only a few years earlier when they dealt with the first recognized HCPS outbreak in the Western Hemisphere. Wider concerns about these rapidly emerging viruses also prompted a response from the National Institute of Allergy and Infectious Diseases (NIAID) International Centers for Tropical Disease Research (ICTDR) program. Struck by the value of this UNM-Chilean collaboration, the program began to support these ongoing research efforts in 1999 through an International Collaborations in Infectious Diseases Research (ICIDR) grant.

The hantavirus ICIDR grant already has had a great impact on Chile and the hantavirus research community, fortifying the country's clinical and field research infrastructure and assembling valuable new knowledge about HCPS and the viral agents that cause it. The ICIDR project also provided a foundation to deal with another hantavirus outbreak that unexpectedly hit Panama in early 2000. In response, members of the UNM-Chilean team quickly mustered a contingent to study this latest New World hantavirus outbreak with support from a special ICTDR fund.

Soon after the American and Chilean scientists began plotting their approach to the hantavirus outbreak in the South American nation, they realized the country's infrastructure for clinical and field research needed bolstering with scientists from several disciplines.

A New Scourge for Humans in the Western Hemisphere

"Through our studies we hope to better understand the natural history, the pathophysiology of the disease," says Greg Mertz, chief of the division of infectious diseases at UNM and the American principal investigator for the grant projects. "It's only through that understanding that we can come up with better ideas either in terms of prevention or treatment." Hantaviruses cause no apparent harm to their natural hosts—rats, mice, and voles—but they exact death tolls of 50 percent or higher when they infect humans. The hallmarks of HCPS are pulmonary edema and cardiogenic shock. Leaking capillaries in the lungs drown victims in their own fluids, and an unknown myocardial depressant causes the heart to falter.

While hantavirus infections undoubtedly have caused sporadic infections in the Americas for many years, the destructive potential

of these viruses first surfaced in the Western Hemisphere in 1993 during the HCPS outbreak in the U.S. Southwest. The epidemic quickly killed half the infected patients, most of whom were young and healthy. The Sin Nombre virus responsible for the epidemic was the first such hantavirus to be isolated outside Asia and Europe.

Sin Nombre is a close cousin of the Andes virus that is the culprit behind HCPS in rural Argentina and Chile. The spate of recent HCPS epidemics in several parts of the Western Hemisphere is one reason that the hantavirus research projects merited ICTDR support, says David Morens, the NIAID epidemiologist overseeing the hantavirus grant. Until the last decade, hantaviruses were thought to be a public health issue only in Asia and Europe. "In just six years, sizeable [HCPS] outbreaks have been recognized in North, South, and most recently Central America," he says.

Biosafety tent for research under field conditions

Soon after the American and Chilean scientists began plotting their approach to the hantavirus outbreak in the South American nation, they realized the country's infrastructure for clinical and field research needed bolstering and that scientists from several disciplines would need to study not only viral pathology but also how the hantavirus is transmitted. The ICIDR grant has provided help in doing both, says Chilean principal investigator Pablo Vial, head of the infectious diseases program at the Catholic University School of Medicine in Santiago. "The projects and the financial resources have allowed us to develop professional teams and build research and clinical capacity with great impact, both in isolated regions of our country and in our reference laboratory and clinical facilities as well."

Chile's bustling capital city, Santiago, boasts a number of major research institutions, including Catholic University and the Chilean Institute of Public Health (ISP). When the first several cases of HCPS struck, the Chilean scientific community based in Santiago responded rapidly, convening a hantavirus working group made up of public health physicians. The government allocated funds to convert a laboratory at the ISP into a biosafety level 3 (BL3) lab capable of handling live hantavirus. However, in the rural areas where the Andes virus has been taking its toll, local clinics such as the one in Coyhaique are often the first facilities to deal with the outbreak. Victims typically do not seek medical attention until they

have reached the acute stage of disease at which time pulmonary edema and cardiogenic shock progress rapidly, outstripping the capabilities of these small facilities.

A Three-Pronged, Multidisciplinary Approach to Combating Hantavirus

When the hantavirus ICIDR team members gathered in 1999 in New Mexico, they developed a three-pronged strategy for combating the outbreak in Chile—the first, focusing on the virus in its rodent hosts and the development of models with which to predict outbreaks, the second on laboratory- and population-based studies to determine the natural history of infection, and the third to explore various options for treating Andes virus-induced HCPS.

To study the natural history of the Andes virus, biologists on the team are trapping rodents, using sampling procedures similar to those developed to characterize the ecology of the Sin Nombre virus in the American Southwest. Every 2 months, trappers revisit three designated sites in Chile to capture mice and take blood samples. Information on infected rodents, rainfall, vegetation growth, and weather patterns is compiled into a database as the basis for developing a model for predicting patterns of disease.

These studies should help to reveal the ecology of Andes virus-induced HCPS, perhaps explaining why it is creeping northward from Coyhaique. Recently, cases turned up within Santiago, a thousand miles north of that rural community, touching off a new level of public alarm. This migration "probably represents some kind of cycle which depends on conditions that we have not yet characterized," Catholic University's Vial says. "The work that the research team has done in assessing the reservoir of the virus in nature has changed the view of the disease in Chile. People have come to realize that this is a highly dynamic infection. As a result, there has been a growing interest among many researchers and health services to dedicating resources and effort to create predictive models of the disease."

Meanwhile, other efforts focusing on how this virus operates on the molecular and cellular levels are capitalizing on the new BL3 lab at the ISP currently dedicated to hantavirus research. Scientists who use the new lab now are conducting world-class virology research,

Extensive familial clustering of cases in Chile… suggests that person-to-person transmission may be occurring at some low level whenever there's an Andes virus outbreak, Mertz says.

maintaining their own stocks of live virus and other reagents while serving as a reference lab for the region. One major question—what factors determine whether a patient survives a mild infection or dies from severe disease—has led the investigators to realize that the familial clustering of cases distinguishes the Andes virus outbreak from those associated with other hantaviruses, suggesting an unusual mode of transmission.

The hantavirus grant projects are supported by two core virology laboratories, the BL3 lab and another facility at Catholic University. Although these labs were stocked with state-of-the-art equipment managed by well-trained scientists, they still needed technologies such as diagnostic assays and viral isolation techniques specific to hantavirus research. Personnel from both labs traveled to New Mexico for a 2-week immersion in procedures for growing hantavirus, running diagnostic assays, and measuring antibody levels in patient serum samples in the UNM lab of pathologist Brian Hjelle.

Hanta in the New World

After returning to the ISP lab in Chile, research scientist Hector Galeno put these skills to work in isolating Andes virus from the serum of a 10-year-old patient, the first time a New World hantavirus had been isolated from a human source. The achievement helped cement the ISP's status as a major facility for hantavirus research and gave it an extra measure of autonomy. "When ISP obtained the virus, they no longer had to wonder if another lab would smile on them and send the virus," Hjelle says.

Some of the team's studies indicate that the Andes virus-induced disease causes more hemorrhaging and greater renal impairment than does the Sin Nombre virus, a trait more typical of Old World hantaviruses. The team's research also reveals a tendency to familial case clustering in the Andes outbreak, a rarity compared to other hantavirus outbreaks. Multiple infections in the same family had been noted since the start of the Chilean outbreak as well as during an earlier 1996 HCPS outbreak in neighboring Argentina. Realizing that they should document the full extent of this phenomenon, team members pored over dozens of case reports and determined that roughly a third of the patients were from the same families, much more than they expected. "When we saw that, we were shocked," Mertz says. "We realized that we were dealing with something very different about the epidemic in Chile that we hadn't fully appreciated."

The Panamanian outbreak is characterized by an often milder form of disease—and very high seroprevalence rates that surpass those seen with hantaviruses in almost every other area in the Americas studied so far.

This extensive familial clustering raises the possibility of spread through person-to-person contacts, a transmission mode not seen before with hantaviruses. Scientists had long since determined that the viruses spread through dried and aerosolized urine, feces, and saliva deposited by infected rodents. The regularity of familial clustering in Chile gives Mertz pause. "What makes these Chilean clusters a concern to me is that they suggest that person-to-person transmission may be occurring at some low level whenever there's an Andes virus outbreak," he says.

"Transmission from person to person could be occurring before the patients get sick," Vial says. "This is of particular interest and will be explored by a project of the ICIDR grant." However, he adds, the family clusters could also be the result of common exposure to the same infectious source in a household. "Among the cases seen at El Bolson, Argentina, one cluster provided strong evidence for

person-to-person transmission. Other evidence is nonexistent, or at least very rare," he says. Further careful studies are needed before any conclusions can be drawn.

During the 1993 Sin Nombre outbreak, Hjelle's lab discovered that high serum levels of a special neutralizing antibody (NAb) correlate with patients having suffered only mild disease. The finding directly

Studying dengue outbreak patterns in the Yucatan

As recently as 1981, the mosquito-borne viral disease dengue hemorrhagic fever (DHF) was unknown in the Americas. Following a major DHF outbreak in Cuba that year, however, the disease has become endemic throughout several countries in the American tropics. Worldwide, dengue disease threatens some 2.5 billion people and causes tens of thousands of cases of hemorrhagic fever annually.

Although research indicates that four main virus types can cause dengue, relatively little is known about other factors that contribute to outbreaks or the risk of severe disease, says NIAID epidemiologist David Morens. To address such questions, American vector-borne disease specialists Barry Beaty, Bill Black, and Ken Olson from Colorado State University (CSU) and Mexican scientists Ildefonso Fernandez-Salas from the University of Nuevo Leon in Monterrey, Lourdes Munoz from Instituto Politecnica in Mexico City, and Jose Farfan from Autonomous University of Yucatan (AUY) in Merida are studying dengue and DHF in Mexico with support through an International Collaborations in Infectious Disease Research grant.

"We put together these projects with our collaborators to look at what could be conditioning this emergence of dengue hemorrhagic fever," says Beaty, director of the CSU Arthropod-borne and Infectious Diseases Laboratory. "Our basic approach is to apply population genetics and molecular virology techniques to look at both the mosquito vectors and the viruses to see if there are juxtapositions of these two factors of the disease." By delving into the inner workings of the viruses and their carriers, the researchers hope to spot the convergence of virulent viruses and particularly susceptible vectors and, with such information, develop more targeted control measures in high-burden areas.

In 1995, the severe hemorrhagic form of dengue fever abruptly began increasing in Mexico, particularly throughout the Yucatan. Beaty and other members of the research team have access to a large pool of samples collected from dengue patients and maintained at the AUY in Merida, where their collaborator Jose Farfan directs a special dengue laboratory at the university. In addition, Fernandez-Salas, head of the major medical entomology program in Mexico at the University of Nuevo Leon, Monterrey, has trained almost every head of the mosquito control districts throughout Mexico.

With these resources, the researchers have determined the genetic characteristics of a large number of dengue viruses obtained from patients throughout the Yucatan and the surrounding territory, creating a viral genetic database for the region. Using a genetic analysis technique called single-strand conformation polymorphism analysis, they determined which subtypes of the four main

contradicted the prevailing wisdom of the time, which said that since patients' blood teems with antibodies both early and late in the course of infection, antibodies simply did not provide any indication of disease severity. However, no one had looked specifically at NAb. "All this work that Brian's lab did was with Sin Nombre," Mertz notes. "So we realized we needed to do the work in Chile with Andes virus to see if the same correlation was true there." As of

(Continued)

dengue virus types have been circulating in the region and which are linked to appearances of hemorrhagic fever. Surprisingly, within this collection, the team found a virulent subtype from Sri Lanka thought never to have appeared outside southeastern Asia. So far, however, it seems not to have caused an epidemic in Mexico.

The researchers also are studying the regional population genetics of *Aedes aegypti*, the principal vector for dengue in the Americas. Fernandez-Salas has trained personnel who work throughout Mexico in mosquito control districts, a factor that "greatly facilitates" these efforts, Beaty notes. "We can tap right into that ongoing informational pipeline and the human resources associated with it."

The results from these mosquito population studies point to a surprising degree of genetic exchange among the insects. "We've found to our amazement that there are three main populations of *A. aegypti* in Mexico that seem to be pretty much free in terms of gene flow," Beaty says. While there are pockets of nearly isolated vector populations within these areas, "you would think that there would be more genetic isolation. This is a mosquito that doesn't fly far. But apparently what's happening is there's lots of traffic in breeding sites." Increased commerce, travel, and resident mobility are contributing to the spread and interbreeding of these disease vectors throughout this region and, possibly, the world.

With a handle not only on the genetic diversity of viral subtypes, but also on how far the mosquito vectors travel, the investigators are now trying to bring these knowledge sets together through population genomics, the marriage of population genetics and genomics studies. The strategy uses molecular approaches to explore whole genomes and identify particular genes or locations on chromosomes that may enable mosquito hosts to more readily harbor or transmit a virus, Beaty explains.

Using maps of mosquito genes, the collaborators recently learned that specific genes that prevent viruses from infecting the mosquito midgut differ greatly among populations of *A. aegypti*. One of these genes codes for "early" trypsin; a protein that stimulates digestion of blood meals and may help process dengue virions, enhancing midgut infections. Insects with two susceptibility alleles of the early trypsin gene are readily infected by dengue while heterozygous mosquitoes are only moderately susceptible. The researchers are now examining whether the trypsin gene correlates with dengue seroprevalence rates. Such a correlation could provide a biomarker to help determine the risk of disease transmission in a given area. "This is some of the hottest stuff in our shop," Beaty says, cautioning that these population genomics studies are still in their infancy.

February 2001, eight samples had been tested and early indications suggest that the correlation between NAb levels and survival will be the same.

These findings alone "don't prove that you could give this antibody to someone and alter the course of disease," Mertz says. "But it certainly suggests that you would want to consider that kind of research." Even though significant safety issues and logistical hurdles would have to be addressed, it would be inappropriate not to try to pursue a promising therapeutic strategy in the ICIDR project, he says. "If the NAb story holds up with Andes virus, we are going to do everything we can to see if we can develop an experimental treatment protocol."

The sheer logistics of working in a long, narrow country presented unique difficulties, as study centers in some cases are separated by hundreds of miles.

Creating a Clinical Research Network

The third major grant project involves initiating an experimental treatment protocol in Chile based on a similar protocol in the United States. This protocol is currently under review by the National Institutes of Health. In the meantime, the team has focused on establishing a clinical research infrastructure to support the project's clinical work. Currently, the team has enrolled 10 study sites throughout the affected region, each with a designated clinical investigator and research nurse.

Getting this network organized has posed some interesting challenges for the team. The sheer logistics of working in a long, narrow country presented unique difficulties, as study centers in some cases are separated by hundreds of miles. The effort also is at the mercy of when and where the virus happens to appear. "Cases keep presenting in new locations, and we have literally had to follow the disease, activating centers in new regions as they are struck by infection," Vial says. This means that some clinics have seen no additional cases since being brought into the project.

Some of the challenges facing the team are of human origin, however. One example is the differing standards for research held by the different countries. "The guidelines and norms for scientific research set by the NIH are not always easy to meet, especially in clinical centers that are not used to doing clinical research," Vial says. To streamline the approval process, the team worked with the Chilean ministry of health to create a centralized institutional review

board to which study sites now delegate oversight of the project protocols. "We have enjoyed seeing a real research network develop under sometimes adverse circumstances," Vial says.

Leveraging Beyond the Andes: The Panama Project

In January 2000, the first case of a new hantavirus outbreak turned up at a clinic in the small, middleclass, farming community of San Jose located on the Azuero Peninsula of Panama. Before then hantavirus infection had not been diagnosed in Central America, but by February 2001 some 26 cases have been detected—and attributed to a newly identified hantavirus, designated Choclo.

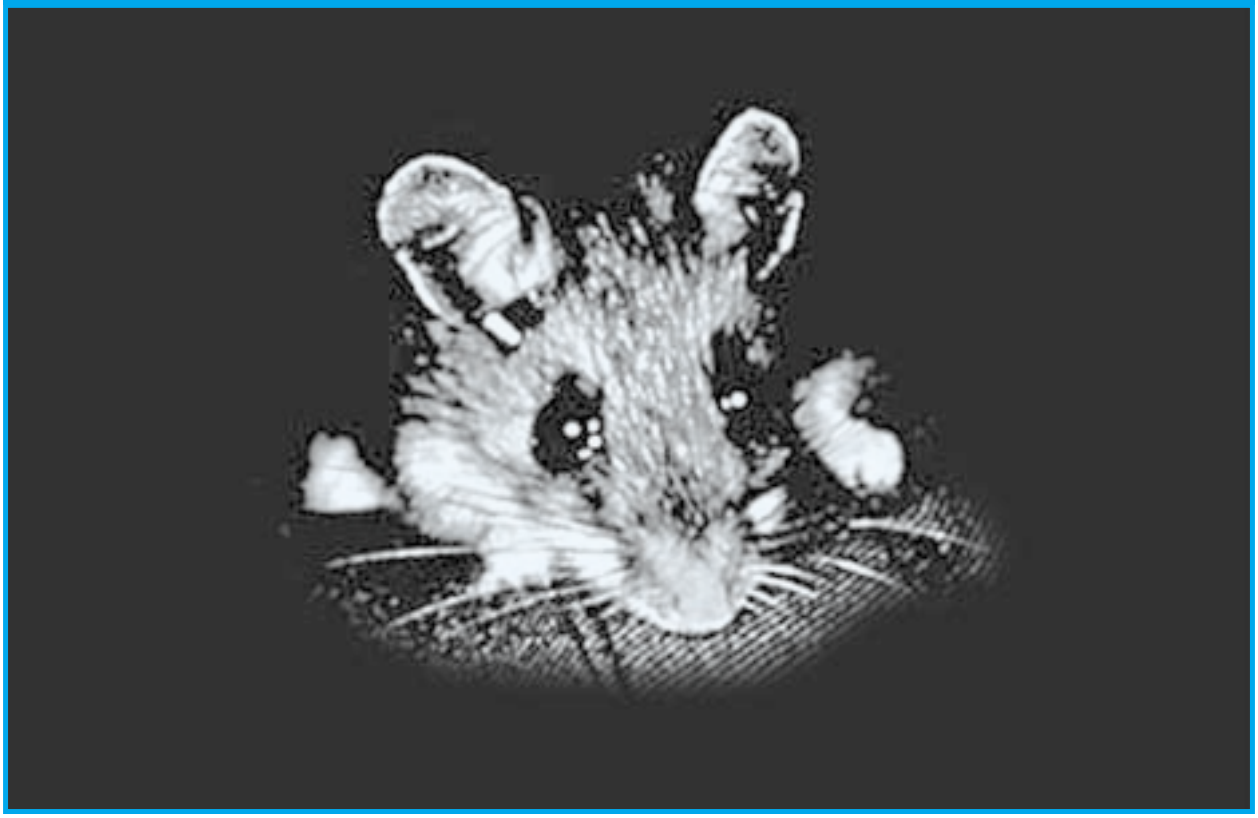
Recognizing an opportunity to further their understanding of New World hantaviruses, members of the Chilean hantavirus ICIDR project proposed to leverage their knowledge, tools, and resources in Panama through the ICTDR Resource Opportunity Pool. This special fund supports research into emergent problems related to ICIDR grants in progress. By adding $100,000 to hantavirus research being supported by the Panamanian ministry of health, the ICTDR resource fund enabled Fred Koster, an infectious disease specialist at UNM, and Fernando Gracia, the director of the Gorgas Memorial Institute in Panama City, to begin a 1-year project in mid-2000.

"The work attempts to localize by serosurveys the geographic areas of involvement and to address the reasons for two interesting observations," Koster says. One of those observations is that the Panamanian outbreak is characterized by an often milder form of disease lacking the cardiogenic shock that makes Sin Nombre and Andes virus infections so traumatic. The second notable factor about the Panamanian outbreak is high seroprevalence rates for the virus that surpass those seen with hantaviruses in almost every other area in the Americas studied so far, he says. Testing throughout the Azuero region reveals that about 12 percent of inhabitants harbor antibodies to the virus, with the rate in two towns exceeding 30 percent. These high rates in Panama "will permit a future prospective study of febrile hantavirus infection and identify the milder infections that don't require hospitalization," he says, noting that more than one virus may be circulating in Panama.

As we have had the benefit of technology transferred to us, we have also tried to transfer the same benefits to other regions of the country… We are just starting to know this disease.

The hantavirus ICIDR grant has already had a large impact on the hantavirus research community throughout the Western Hemisphere by laying the foundations of a store of new knowledge about HCPS and its viral agents. These efforts are necessary steps toward developing better treatments and prevention measures. "We are frequently asked what local people or institutions will gain from being part of the project," Vial says. "Many times we have to recognize that there may not be many gains in the short term and explain that the grant will not immediately solve many local needs. However, as we have had the benefit of technology transferred to us, we have also tried to transfer the same benefits to other regions of the country." The more capable investigators throughout the Western Hemisphere are to discover the secrets of hantaviruses, the better. After all, Vial notes, "we are just starting to know this disease."

Mice are one of several natural hosts for hantavirus.



Accomplishments

VACCINE DEVELOPMENT

ICTDR investigators are identifying antigen targets for vaccines that could be used to prevent tropical diseases, and they are exploring other means to provide individuals with protective immunity against tropical diseases, a long-term goal that often has proved elusive when dealing with the complex and multifaceted pathogens that are responsible for these diseases. Some of these additional efforts entail identifying novel adjuvants that can enhance host immune responses to vaccines. Highlights over the past decade include the following:

- The *Schistosoma mansoni* oligosaccharide, lacto-N-fucopentaose III induces interleukin-10 (IL-10) and prostaglandin E2, two mediators that downregulate Th-1 responses and possibly account for why the host immune system fails to detect this parasite or protect individuals against it. Because similar sugars are detected on other pathogens as well as some metastatic tumors, this may be a general phenomenon. Lacto-N-fucopentaose is a potential adjuvant for driving Th-2-like immune responses.
- The *S. mansoni* surface enzyme triose phosphate isomerase (TPI) illustrates a common problem with parasite vaccine candidates, in that it is very similar to the human enzyme. A multiple antigen peptide construct of TPI, containing T- and B-cell epitopes that are not homologous to the mammalian enzyme, is undergoing preclinical testing for protective activity. Several recombinant protein antigens are also being evaluated preclinically for vaccine potential against schistosomiasis.
- The cytokine IL-12 serves as an effective adjuvant for inducing resistance to *Leishmania major* in mice. Moreover, a leishmania protein, LeIF, that induces IL-12 and mediates Th-1-type responsiveness is being tested as a vaccine adjuvant.
- *L. major* mutants that cannot complete important biosynthetic pathways infect both susceptible and immunodeficient mice but are incapable of causing disease and protect mice against subsequent challenge with virulent organisms. These genetically modified parasites may be further engineered as delivery systems for other vaccine immunogens.
- An immunodominant leishmania antigen, LACK, appears capable of transferring resistance to *L. major* to a susceptible mouse strain, particularly when administered with IL-12.
- NIAID support has contributed to identification of recombinant antigens from *Trypanosoma cruzi*, *Entamoeba histolytica*, and hookworms, as well as additional leishmania antigens, which are being evaluated in animal models for their vaccine potential.
- Asexual erythrocytic stages as well as sexual stages (gametocytes) of *Plasmodium falciparum* can be maintained in mice with immune defects as a model for studying vaccines. One protein, designated Pfg27, is abundantly expressed at the onset of gametocytogenesis and has been shown by genetic disruption studies to be essential for maintaining the sexual phenotype of this parasite.
- Molecular studies using a malaria parasite that infects rodents revealed that the gene encoding the sporozoite surface protein, called TRAP (thrombospondin-related anonymous protein), is critical for parasite invasion of mosquito salivary glands as well as mammalian liver cells and also for parasite gliding motility. Additionally, the surface molecule circumsporozoite protein is a crucial element in sporozoite morphogenesis and attachment to vertebrate host hepatocytes.
- Several *P. falciparum* genes encode variant parasite antigens expressed on the surface of infected erythrocytes that enable parasitized cells to attach to the vascular endothelium. The variant antigens are related to the Duffy antigen-binding protein of *P. vivax* and to the erythrocyte-binding protein of *P. falciparum*, both of which are involved in invading host red cells.
- NIAID has supported Phase I clinical trials of two candidate malaria vaccines, a multiple antigen peptide vaccine directed against pre-erythrocytic stages of *P. falciparum* and also a recombinant protein vaccine produced in *Saccharomyces cerevisiae* that is directed against the *P. falciparum* MSP-1 protein from the surface of asexual erythrocytic stages of this parasite.
- NIAID is participating in a variety of partnerships for malaria vaccine development including contracts, cooperative research and development agreements, and small business grants in the private sector, as well as agreements with several U.S. government agencies, the World Health Organization, and other international agencies. NIAID is also supporting Malaria Clinical Research and Trial Preparation sites in Mali and Ghana and working with several other organizations in Africa to provide infrastructure, training, and testing of various vaccines to protect against tropical diseases.

IMMUNOPATHOGENESIS

Studies in Brazil Provide Insights into Immune Responses to Parasitic Diseases

Edgar Carvalho with patient

When Edgar Carvalho completed his medical training more than two decades ago, he hungered for an opportunity to learn more about the amazing defenses the body uses to protect itself from disease. His training in internal medicine and infectious diseases had provided just a taste of immunology, but no courses or research projects that focused on immunology were then available at his alma mater, the Federal University of Bahia in Brazil. Now, more than 20 years later, a banquet of immunology and immunogenetics research is being done in Bahia thanks to Carvalho, his equally enterprising colleagues, and steadfast support from several sources, including the National Institute of Allergy and Infectious Diseases (NIAID) International Centers for Tropical Disease Research (ICTDR) program.

Although collaborations between Cornell University and the University of Bahia stretch back at least to the mid-1960s, a joint 1979 grant to study leishmaniasis and Chagas' disease enabled tropical disease research in Bahia to expand dramatically. Now its programs encompass four Brazilian research institutions, several American university partners, and some 40 people who are now actively engaged in such research at universities and field sites throughout Brazil. Recognizing the growing importance of these programs a decade ago, NIAID conferred Tropical Medicine Research Center (TMRC) status on the University of Bahia in 1991. And, over more than two decades, these wide-ranging research efforts have yielded critical insights into the pathogenesis and immunology of several parasitic diseases, including schistosomiasis

and leishmaniasis, and, more recently, diarrheal infections that long have plagued the people of Brazil (see sidebar page 82).

The evolution of the research enterprise in northeastern Brazil is a perfect example of how the ICTDR program is boosting research capabilities that benefit the global research community. The change in Brazil has been striking says infectious disease researcher Warren Johnson of Cornell University, who has worked closely with the Brazilian team for more than three decades. The University of Bahia "has evolved into one of the major research institutions in the field of infectious disease and tropical medicine," he says. "I first went to Brazil in 1971 for 6 months to do research on typhoid fever, and there was only one person at the medical school there who was doing any type of serious research. They're now among the top [tropical disease research centers] in the whole world."

The evolution of the research enterprise in northeastern Brazil—now among the top tropical disease research centers in the whole world—is a perfect example of how the ICTDR program is boosting research capabilities that benefit the global research community.

Start of the TMRC in Brazil

The TMRC in Brazil was officially launched in 1991, expanding collaborations between the University of Bahia and Cornell University to focus on visceral leishmaniasis, cutaneous leishmaniasis, and schistosomiasis, and on exploring immunotherapies for these diseases. "We had in mind that we should improve our ability in terms of pathogenesis—understanding more how parasites cause disease in humans—and also that you could improve the therapy or control of these diseases," Carvalho sums up.

Initially, the TMRC was centered in the immunology service laboratory headed by Carvalho at the University of Bahia. However, since 1995 the TMRC expanded to include two other Brazilian universities that had come forward as emerging centers of research excellence: the Federal University of Ceará (UFC) in Forteleza and the Federal University of the Rio Grande (UFRG) in Natal. The Oswaldo Cruz Foundation also became a TMRC partner when one of the original TMRC principal investigators moved there from the University of Bahia 2 years ago. In addition, the scope of the projects was widened to include diarrheal diseases that are a scourge among children in northeastern Brazil. Amid these changes, Carvalho's lab continues to be the nucleus for the TMRC.

"These aren't programs between universities or institutions; they're programs between people," Johnson says. "It's that personal bond; it transcends changes in governments, changes in funding, misinterpretations."

As the scope of the projects and the number of Brazilian institutions participating in TMRC activities have expanded, so too has the number of American partner institutions. Now in addition to Cornell, researchers from the University of Virginia, the University of Iowa, and Harvard University, among others, are participating in these efforts. "Edgar has been able to productively involve exceptional people to the enrichment of the program," Johnson says. "He had a focus on building a program in his university, not as a self-aggrandizement type of thing, but a program that would really enrich the students in Bahia," who could then join in broader efforts to control parasitic diseases that have caused so much misery in Brazil and its neighboring South American countries. "These types of research centers can sometimes become very personal and very narrow," Johnson adds. "This program under Edgar's direction has been broad and welcoming."

Confluence of People and Places

Ask any of the researchers involved in the TMRC projects about the secret of their success, and they will tell you it all comes down to building strong relationships among collaborators. "These aren't programs between universities or institutions; they're programs between people," Johnson says. "It's that personal bond, it transcends changes in governments, changes in funding, misinterpretations."

Johnson and Carvalho, once mentor and student, now not only are peers but also are steadfast friends. Johnson first met Carvalho in

1979. "Edgar was identified by one of his former professors as one of the most outstanding investigators that the institution had produced in a long time," Johnson recalls. "So Tom [Jones, another Cornell collaborator] and I offered Edgar a position in the research program. It has been a sustained relationship from that time to the present." The two visit each other's homes and even share vacations with their families.

Researchers from other participating institutions are caught up in these amicable collaborations. "The University of Virginia (UVA) was the place where I started, and it really had an important role in putting these things together," Carvalho says, recalling his 1977-1979 immunology fellowship at the UVA School of Medicine. Among his other duties there, he was asked to support research being conducted by Selma Jeronimo of the University of Rio Grande in Brazil. Soon, he was talking with her and her colleagues in Natal about areas of mutual research interest. At UVA, Carvalho also became close with Aldo Lima, a diarrheal disease specialist at the University of Ceará who was studying in Virginia. Years later, they renewed that friendship while collaborating on diarrhea research, now the third pillar of the TMRC efforts.

Indeed, Lima is the TMRC principal investigator for diarrhea research, working closely with Richard Guerrant from UVA (see sidebar on page 82). Jeronimo now heads the visceral leishmaniasis studies, collaborating with Johnson, Richard Pearson at UVA, and Mary Wilson at the University of Iowa. Manoel Barral-Netto, a fellow medical student with Carvalho at Bahia, and now a scientist with the Oswaldo Cruz Foundation, directs the cutaneous leishmaniasis studies, working with several of the same American collaborators. Carvalho and Mittermayer Reis of the Oswaldo Cruz Foundation oversee schistosomiasis studies with Donald Harn of Harvard and Edward Pearce of Cornell.

Another important factor for ongoing efforts at the TMRC in Brazil is its capacity to combine fully modern laboratory-based experiments with field studies centered in endemic regions. It is necessary to have constant contact with the endemic area, Carvalho says, noting that he and his collaborators spend at least several days every month visiting parts of Brazil where leishmaniasis, schistosomiasis, or other tropical diseases tend to be prevalent. The constant contact with the

Constant contact with the endemic area provides a source of ideas for new studies, and, in the field, collaborations are very important, according to Carvalho.

Brazilian children in shantytown setting

diseases is a constant source of ideas for new studies, he says. Although much of the work may be done by individuals in the lab, he adds, in the field, collaborations are very important. Moreover, when U.S. collaborators come to Brazil, they often visit disease-endemic regions to gain a fuller sense of the research and public health challenges that all of the TMRC team members are facing, he points out.

Modulating Host Immune Responses to Control Leishmaniasis

Researchers associated with the Brazilian TMRC emphasize the parasitic diseases that ravage Brazil and other tropical regions of the world. "Protozoan and helminth diseases are highly endemic in tropical areas," Carvalho explains. "Bahia has the highest prevalence of both leishmaniasis and schistosomiasis in Brazil." Leishmaniasis, a major focus for many years, remains a central research interest for good reason. More than 100,000 cases of cutaneous leishmaniasis occur in Brazil every year, and some 10,000 cases of the rarer but more serious visceral form of the disease turn up annually, he estimates.

Parasitic diseases ravage Brazil, with more than 100,000 cases of cutaneous leishmaniasis every year and some 10,000 cases of the rarer but more serious visceral form of the disease, Carvalho says.

Biting, blood-sucking sand flies spread members of the protozoan genus *Leishmania* that cause leishmaniasis. Some species do not spread in humans beyond the site of infection, where they may trigger development of festering ulcers that can take several months to heal. However, in a small percentage of cutaneous infections, the parasites travel to the mucous membranes of the mouth and nose, leading to severe disfiguring of the facial tissues if left unchecked. Other more invasive species travel to the liver and spleen, causing a more serious visceral form of the disease that can prove fatal.

Both forms of the disease tend to attack people living in rural areas, often helped along by crowded conditions and the customary practice of keeping dogs, chickens, and other domestic animals in backyards or even under the same roof. However, the 1990s saw several flare-ups of visceral leishmaniasis in the cities of Belo Horizonte, Teresina, and Natal, abetted by droughts and the migration of families to urban centers, creating densely populated settlements in the peripheral suburbs of these midsized Brazilian cities.

The drug pentavalent antimony is available for treating leishmaniasis. However, the drug is administered intravenously, requiring hospitalization, and this treatment is associated with a high failure rate. Unsatisfied with this situation, the TMRC researchers designated the village of Corte Pedra in Bahia as a field site for leishmaniasis population studies, aiming to better understand the immunological response of infected individuals to the parasites and, on that basis, to explore more effective therapies for treating both forms of the disease.

TMRC researchers find that deficiencies in cytokine production help lead to the visceral form of the disease. Only a small fraction of people infected by the parasite that causes visceral disease ever develops symptoms. Those who do "are not able to produce IFN-γ [interferon gamma], the cytokine that is the most important in terms of activating macrophages that kill *Leishmania*," Carvalho says. The team traced this deficiency to insufficient production of yet another cytokine, interleukin-12 (IL-12), which stimulates T cells to make IFN-γ.

Sand flies are a vector for Leishmania.

Whereas IFN-γ and other pro-inflammatory cytokines appear to be involved in control of parasites in the early stages of infection, the immune response may become unbalanced in later stages. Observing that patients who exhibit a strong immune response to cutaneous leishmaniasis tend to heal slowly and often require more than one series of antimony treatment, the TMRC scientists began probing which components of the immune defenses come into play during these infections. They soon learned that the parasite does not directly cause later skin lesions as many investigators believed. Rather, such lesions are collateral damage caused by the "friendly fire" of cytokines such as IFN-γ. "It's an exacerbated immune response that, on one hand, is not able to control the infection and, on the other hand, leads to tissue damage," Carvalho says.

The TMRC group has spent years studying how combination therapy with drugs and/or cytokines can enhance the effectiveness of leishmania treatment by appropriately balancing the immune response. Several years ago, Carvalho and his colleagues learned that supplementing antimony therapy with granulocyte macrophage colony stimulating factor, or GMCSF, can speed the resolution of visceral leishmaniasis, reducing the number of secondary infections

as compared to patients receiving antimony alone. Meanwhile, the researchers also are testing the effects of a drug, pentoxiphylline, that inhibits the pro-inflammatory cytokine called tumor necrosis factor alpha (TNF-α). In an initial study, pentoxiphylline boosted the effectiveness of antimony in patients with the face-disfiguring

ICIDR studies unveil impact of chronic diarrhea among poor Brazilian children

More than 20 years ago, public health officials hailed oral rehydration therapy (ORT) as a safe and cost-effective life-saving weapon for treating cases of acute diarrhea, points out Richard Guerrant of the University of Virginia in Charlottesville. However, although ORT continues to save millions of lives each year, it leaves untouched other more subtle, longer-term health effects that arise from overlooked or barely recognized cases of chronic diarrhea, particularly those occurring among young children during the first 2 years of life. "This is a hugely important message," he says. "Such children may do poorly in terms of overall growth, fitness, cognitive functions, and school performance. The cost of these early childhood illnesses is far greater than anyone realizes."

Guerrant and Aldo Lima of the Federal University of Ceará, Brazil, and their respective colleagues, working together as part of a NIAID International Collaborations in Infectious Diseases Research (ICIDR), began more than a decade ago to monitor such cases among a cohort of children from poor families who live in a shantytown in northeast Brazil. Some of the first hints about these problems came from rather unusual population field studies, according to Guerrant. "A decade ago, we did 'verbal autopsy' studies, with one of our colleagues visiting grave diggers and then hiking into the hills to find out what happened to the children who had died," he recalls. "We found that about 70 percent of the deaths were attributable to diarrhea, and about half of those were cases of persistent diarrhea.

"Of course, more than 20 years ago, more would have died from acute dehydration because there was no ORT," he continues. "But often, we saw that little Marcello and some of his friends, who were not acutely ill with diarrhea, never did all that well" in terms of their overall health in the years that followed and long after the children were thought to have fully recovered from those bouts with acute diarrhea.

Skeptical about the fullness of those recoveries, Guerrant and Lima decided to examine the long-term health and other health-related impacts on such children—focusing on chronic diarrhea as well as the effects of certain other enteric infections, including those caused by bacteria such as *Escherichia coli* and parasites such as *Cryptosporidium parvum*. Although such microorganisms may sometimes cause severe attacks on the gastrointestinal (GI) system, certain "enteroaggregative" types of *E. coli* as well as *C. parvum* also can produce rather mild symptoms or may give rise to long-term GI infections without obvious symptoms. But even while these microbial agents are symptomatically invisible, they may be lurking in the GI tracts of children where they can "cause smoldering malabsorption" because of a condition that is known colloquially as "Peace Corps gut," Guerrant says.

Much of the surface of the GI tract is decorated with protruding structures called villi that add immensely to its surface area and thus play a key role in its central nutrient-absorbing activities. "The villi of a

mucosal form of the disease, even after these patients had failed previous treatment with antimony alone. The research teams are currently studying the clinical efficacy of antimony plus pentoxiphylline for treating mucosal leishmaniasis patients and of topical GMCSF for treating cutaneous leishmaniasis.

(Continued)

healthy adult are comparable in area to a tennis court," Guerrant says. However, a great deal of that functional surface area is "wiped out in these kids. The ordinarily luxurious villi are flattened and blunted." The long-term health effects can be devastating—"probably more important than those attributable to cholera and other acute cases of diarrhea, and the consequences may well be much more long lasting," he says.

To be sure, ORT is saving lives during cholera or similar epidemics caused by infectious agents of diarrheal diseases in Brazilian shantytowns and throughout the globe. But in the poorest areas there and in other similar settings in Peru, elsewhere in South America, and in Africa, longer term effects from more subtle enteric infections and milder forms of persistent diarrhea are having a broad impact on children's health and may well be undermining their futures, Guerrant points out. The story very much parallels what investigators find for children who are parasitized by worms, he says. "We used to be taught that worms were no big deal…until about 8 years ago when researchers in Kenya and Jamaica found that children treated with a single dose of an effective antihelminthic drug subsequently show better growth, fitness, and cognition."

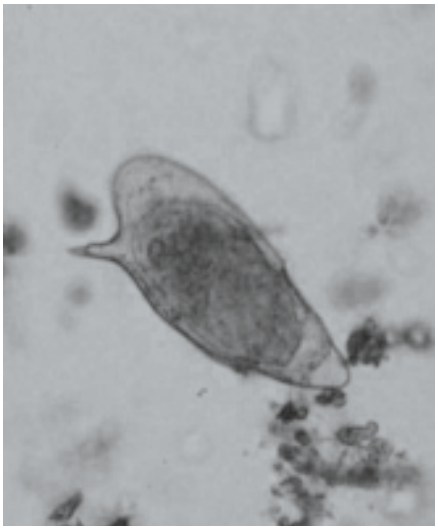
With no comparable "magic bullet" for clearing up long-term *E. coli*, *C. parvum*, and other such enteric infections, Guerrant admits to at first feeling "despondent" about the prospects facing Brazilian and other children plagued by chronic diarrhea. However, recognizing that the amino acid glutamine plays an important role in supplying energy and repairing damaged GI surfaces, Guerrant, Lima, and their collaborators are reformulating conventional ORT mixtures to include stabilized forms of this amino acid as part of a new glutamine-based oral rehydration and nutrition therapy (ORNT). Tests indicate that these and other mixtures that are further supplemented with vitamin A and zinc can speed the repair of diarrhea-damaged intestinal barrier function among some of these children and provide other short-term health benefits. With such information in hand, the team is beginning to evaluate potential long-term health benefits of the ORNT approach.

"The ICTDR program allowed us to develop prospective data, forge long-term relationships with the children and their families in this shantytown, and to develop an understanding of what causes these deranged physiologies," Guerrant says. The first rounds of ORNT testing indicate that glutamine and other supplements may provide "an approach to ameliorating intestinal injury."

The ICTDR enabling of collaborative partnerships was critical for fostering these breakthrough efforts. According to Guerrant, "Lima trained with us 15 years ago, went back to Brazil, and was willing to take on the responsibilities of directing the community team there, do prospective studies, and meet with community members every week," he says. "This is an incredible collaboration, and his contributions are remarkable. We're fortunate in that he's multidimensional—a superb physiologist and also coordinating these field studies."

Immune Responses Also Key to More Severe Forms of Schistosomiasis

Schistosomiasis, a disease caused by parasitic blood flukes, is thankfully on the decline in Brazil. Improved socioeconomic conditions are lowering rates of infection in endemic areas, and drug treatments are proving helpful in controlling the disease among those who still become infected with the parasites. Nonetheless, even though few cases of the serious South American form of the disease marked by distended liver and spleen occur anymore, treatment for the remaining infections still costs the Brazilian government a significant sum each year. And acute schistosomiasis can present a serious problem among tourists.



Egg from S. mansoni

Individuals typically encounter schistosomes through contact with contaminated water. Missile-shaped schistosome larvae burrow into the skin and travel through the body, seeking and finding a suitable mate, and lodging in a vein draining the intestines. Because the adult form of schistosome is adept at evading host immune system responses, they tend to cause relatively little damage. But a female fluke can release hundreds of eggs per day, many of which drill through the small intestine and escape the body through the feces, but some of which become lodged in the intestinal wall or are swept up in the bloodstream and deposited in the liver. The immune system responds by encasing those eggs in fibrous shrouds called granulomas. The hemorrhaging and scarring caused by the eggs and the immune system's response to them result in a chronic, disabling disease that can prove fatal.

TMRC researchers are particularly interested in schistosome-induced liver fibrosis. In the endemic study site of Caatinga do Moura, a village of about 4,000 in Bahia, the scientists noticed that a fraction of the population remains free of this disease despite repeated exposure to contaminated waters. Comparing these resistant individuals to those who develop schistosomiasis, the scientists learned that both T-cell and antibody responses play roles in determining who resists such infections. Importantly, those who succumb mount only a poor Th-1 immune response, according to Carvalho. "These patients do not produce much IFN-γ or TNF-α," he says. Studies of the reaction of cells from schistosomiasis patients to parasite antigens suggest that

production of the modulatory cytokine interleukin-10 plays an important role in how people repond to this infection.

About a year ago, a large outbreak of acute schistosomiasis among Carnaval revelers who swam in a lagoon near the northern border of Bahia gave the TMRC team an opportunity to correlate clinical manifestations of the infection with immunological responses. Many of those schistosomiasis patients produced very high levels of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α. "This was important because before these studies, the idea was that the pathology of acute schistosomiasis was associated with allergic reaction since patients with acute schistosomiasis have eosinophilia and pulmonary manifestations," Carvalho says. These studies suggest that it was really severe inflammation. People that are born in endemic areas do not have acute schistosomiasis because in these cases long-term exposure to the parasite establishes a different immunologic balance.

While TMRC researchers are learning about specific immune-system responses to schistosomiasis and leishmaniasis, their discoveries also point to exciting new possibilities for treating other ailments. "We are moving in a completely different direction in terms of schistosomiasis," Carvalho says. "For instance, understanding how schistosomal helminths disable the host immune response may be useful for treating allergies," he notes. Indeed, residents of Caartinga do Moura with chronic schistosomiasis tend not to have allergy problems. "In more than 20 years working in this endemic area, I never saw a patient that had severe asthma," Carvalho says. "We looked at whether they are exposed to allergens, and they are." Remarkably, the people with schistosomiasis show much weaker reaction to common allergens than those who do not have the disease.

The TMRC researchers are trying to identify the schistosomiasis-associated agents that modulate immune response in hopes that they may be clinically useful. Recently, for example, Harvard collaborator Donald Harn found that certain carbohydrates made by schistosome eggs induce a strong anti-inflammatory response in mice. "It is possible that these sugars are also responsible for some of the differences in response to allergens and other pathogens that we are observing in patients with chronic schistosomiasis," Carvalho says with cautious enthusiasm.

The TMRC researchers are trying to identify the schistosomiasis-associated agents that could be responsible for this protective effect in hopes that they lead to clinically useful treatments for asthma or other allergy-linked symptoms.

Accomplishments

IMMUNOTHERAPY

Many tropical diseases are exacerbated, or even caused, by a misdirected or overzealous host immune reaction to the pathogen. ICTDR investigators, recognizing the critical role of host immune responses in tropical diseases, are testing inhibitors of deleterious host immune responses and agents that augment beneficial host immune responses to determine if they can help in treating such infections. Promising leads, important observations, and other highlights of these efforts include the following:

- In visceral leishmaniasis *(L. chagasi)*, TGF-ß in the liver plays a role in inhibiting protective gamma interferon responses in infected mice, facilitating parasite replication.
- Infections with the protozoan pathogen *Leishmania donovani*, a causative agent of visceral leishmaniasis in humans, are characterized by an absence of delayed type hypersensitivity immunological responses to parasite antigens; parasite antigens fail to stimulate IFN-γ and IL-2 production or lymphocyte proliferation *in vitro.* Treating lymphocytes from such patients with an antibody that blocks cytokine IL-10 increases IFN-γ production and lymphocytes proliferate, indicating that blocking IL-10 holds promise as an intervention strategy.
- Studies investigating mechanisms of susceptibility to the South American species of *Leishmania* indicate that while nonhealing infections with *L. major* are associated with a Th-2 response, non-healing infections with several South American species of *Leishmania (L. amazonensis; L. mexicana)* fail to heal due to an inability to develop an immune response.
- Combination therapy with IL-12 and pentostam can cure BALB/c mice that already are infected with *L. major* by switching an established Th-2 response to a Th-1 response.
- Mice sensitized with *S. mansoni* eggs plus IL-12 develop liver granulomas on subsequent infection that are smaller and less fibrotic than those in nonsensitized mice. The protective response is accompanied by a shift from a Th-2-like cytokine profile to a more Th-1-dominant profile.
- When IFN-γ is administered along with the antileishmanial drug antimony to patients with visceral leishmaniasis, the cytokine immunotherapy appears to augment their responses to conventional chemotherapy.
- T cells of patients infected with *Mycobacterium leprae*, which causes leprosy, fail to respond to antigens from this pathogen, and this failure correlates with the ability of $CD8^+$ T cells to suppress the response of $CD4^+$ T cells through IL-4 production.
- Plasma levels of IFN-γ, TNF-α, soluble tumor necrosis factor receptors, soluble interleukin-2 receptors, and soluble CD8 are higher in children with dengue hemorrhagic fever than in children with less severe dengue fever.
- Mice with neurocysticercosis, a model in which the cestode parasite, *Mesocestoides corti*, is injected into the cranium of such animals, develop extensive infiltration of γδ T cells into the brain, accompanied by a predominant Th-1-like cell-mediated response. Moreover, among humans with neurocysticercosis due to natural exposure to *Taenia solium*, there is a comparable predominance of Th-1-related cytokines, indicating a different response to helminth antigens in the brain than occurs in other tissues.
- Studies of patients with a milder form of schistosomiasis versus the more severe hepatosplenic form suggest that immunologic mechanisms involving IL-10, IL-4 and IL-5 (Th-2-associated cytokines that modulate Th-1 response) and idiotypic/anti-idiotypic networks work to modulate disease severity.
- The Th-2 response driven by schistosome infections alters subsequent patterns of cytokine responsiveness to tetanus toxoid and BCG vaccines, suggesting that concomitant helminth infection may affect vaccination effects.

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