Ancient Organisms, Modern Scourges

By Lois Baker

Researchers work to disable parasites that cause sleeping sickness and Chagas disease

Trypanosomes, a family of microscopic parasites, cause a mountain of suffering. Among the trypanosomes' most virulent weapons is African sleeping sickness, which is responsible for more deaths than HIV/AIDS in sub-Saharan regions.

PHOTO: WHO/TDR/STAMMERS

In all, the disease threatens more than 60 million people in 36 countries on the African continent, and the World Health Organization estimates that, of this population, only four million are under active surveillance or have access to health centers where reliable diagnosis is available.

Chagas disease, caused by a related trypanosome, affects approximately 18 million people in Central and South America. About 25 percent of the 100 million people living in that part of the world are considered at risk.

Both parasites are wily opponents. These ancient organisms have developed ingenious defenses against attack. The African parasite, transmitted by the bite of the tsetse fly, is enclosed in an infinitely changeable protective protein coat. When the trypanosome comes under attack by the host's immune system or a vaccine, the parasite changes its coat proteins, rendering it impenetrable.

The American parasite, transmitted via the triatomine bug, also known as the assassin bug or kissing bug, has a different type of protein coat and evades the immune system primarily by living in and dividing inside the host cells.

Both defenses make it improbable that a useful vaccine against trypanosomiasis—the general term for these diseases—ever could be created. Unfortunately, the few treatments that do exist are so toxic they can be worse than the symptoms, which are devastating in the extreme.

Making the situation worse for those in areas where trypanosomiasis is endemic is the fact that its variations are difficult to diagnosis, even under the best of circumstances.

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African sleeping sickness exists in two forms, West African and East African, dictated by the geographic range of the species of tsetse fly that spreads the parasite.

Both infections begin with the insect's bite, which initially causes mild symptoms, including fever, weakness, headache, joint pains and itching at the site of the bite. East African sleeping sickness progresses from early to advanced stages within days or weeks, making a diagnosis easier.

West African sleeping sickness, in contrast, may become asymptomatic for months or years before entering its advanced stage.

In both forms, the parasite eventually overwhelms the immune system and invades the body's vital systems, including the brain. If sleeping sickness is not treated early, death is inevitable.

Chagas disease, which may remain asymptomatic for decades, primarily attacks the heart, esophagus and colon. Death results eventually from heart failure.

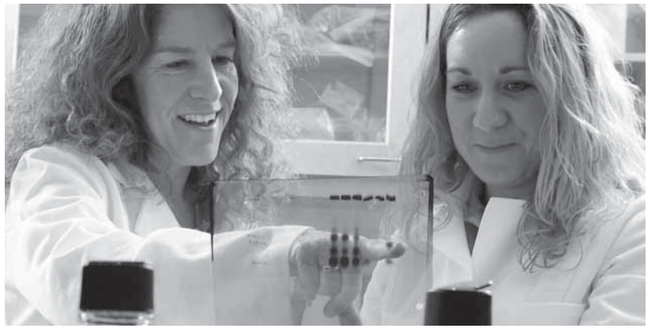
The work of two UB molecular parasitologists, Noreen Williams, PhD, and Laurie Read, PhD, could brighten this dark scenario. Although they work in separate laboratories a few doors apart and are following slightly different routes, both scientists are studying the African sleeping sickness parasite *Trypanosoma brucei* with the goal of finding promising targets for drugs that could treat both of these devastating diseases.

Because the chances of preventing infection by killing the parasite from the "outside" via a vaccine are close to zero, Williams and Read hope to defeat it from the "inside" by creating a chemical Trojan horse. The researchers are looking for ways to disrupt the parasite's most basic mechanisms to prevent it from multiplying in its human host.

Williams, a professor of microbiology and immunology, is investigating regulatory events in *T. brucei*, particularly the role of the enzyme mitochondria ATP synthase in the parasite's life cycle. Her work could identify crucial processes that could become targets for drugs to prevent transmission.

Read, associate professor of microbiology and immunology, studies the basic biological mechanisms of *T. brucei*, concentrating on the processes of RNA editing and RNA turnover. Interrupting any of these events could prevent the parasite from replicating and could identify pathways for drug interventions.

"Our work is very similar, very related," says Read, "and while we don't collaborate in the strictest sense of the word, we interact intellectually on a regular basis, to the benefit of both our labs."



FAR LEFT: Noreen Williams, PhD, and Silvia Brown, a postdoctoral fellow. LEFT: Laurie Read, PhD, and Debbie Pasternack, a PhD student. BELOW: Acute Chagas disease in a young girl from Sao Felipee, Brazil. The eye sign of Romana is present. This is frequently seen in acute cases and is presumed to mark the point of entry of the parasite.

Both Read and Williams are in the top third of UB researchers in terms of active federal grants and together account for more than $7 million in research dollars. Read recently received a $1.5 million grant from the National Institutes of Health to continue studying RNA editing in *T. brucei*. Publications by Williams and Read on trypanosomes total more than 50.

Williams has studied the life cycle of the trypanosome for 19 years, the last 14 in her laboratory in UB's Witebsky Center for Microbial Pathogenesis and Immunology. Introduced to the quirky parasite by fellow researchers early in her career, she found it sufficiently intriguing to abandon her purely chemical interest in the enzyme mitochondrial ATP synthase, which she had been researching for several years, in order to study its function in the organism.

The attraction rested in the trypanosomes' unusual ability to change the process of energy production and metabolism depending on which host they inhabit—insect or mammal. "In mammal hosts, trypanosomes live off of glucose in the blood, but in their insect hosts they survive primarily on amino acids," says Williams. "I wanted to know how mitochondria, the cell's energy producers, change the process of energy production so these organisms can survive in two totally different host environments.

"You can't kill something if you don't know how it lives," Williams adds. "You have to know how it works, how it responds. When you understand these things, you understand what's essential for the organism's survival."

Researchers in Williams' lab include five graduate students, two postdoctoral associates, a master's student and a medical student. Currently, the group is studying protein complexes—one of which is mitochondrial ATP synthase—that allow the parasite to adapt for survival through transmission into the next host.

"Humans have the same enzyme complex," says Williams, "but we've shown that there are significant differences in the structure and function of the complex in these parasites. This means that we may be able to selectively target the enzyme complex in the parasite with drugs that do not target the enzyme in the human host."

In another project, Williams and colleagues are studying proteins that bind to trypanosome RNA and regulate the expression of proteins encoded by the RNA. "The way the parasites regulate gene expression is quite different from the way their hosts, human or insect, do," she says. "The proteins we study are unique to this family of parasites. Again, these unique features may allow us to target expression of proteins that are essential for the parasites to survive in one host or to make the transition from one host to another. If you can knock out expression of these essential proteins, the parasites can't survive and the disease would be prevented."

Read was studying the biochemistry of malaria parasites until she heard a presentation on RNA editing in trypanosome at a professional meeting and was smitten on the spot. "It's a fascinating organism," she observes.

Read came to UB in 1994 after spending four years as a postdoctoral researcher in molecular parasitology at the Seattle Biomedical Research Institute. She now supervises a master's student, four doctoral students, two postdoctoral fellows and a technician in her laboratory in the Witebsky Center.

"Trypanosomes are important medically because they cause diseases that kill approximately 300,000 people per year," she says. "They are also of basic biological interest for a number of reasons. They are ancient organisms, so what we learn from trypanosomes can help us understand the evolution of fundamental processes in nucleated cells. In addition, they are model organisms for the study of RNA metabolism—gene regulation at the RNA level.

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"Many organisms do a lot of regulation at the level of either making the RNA copy of a gene or not," she continues. "Trypanosomes don't regulate this step; they make all the RNAs all the time. They regulate whether the encoded proteins ever get made by either correctly processing the RNA or not, and by specifically regulating the stability of different RNAs under different conditions."

Trypanosomes provide an especially good model to study RNA metabolism, she points out, because they are specifically geared up to work almost exclusively at this level.

Since the trypanosome makes all its RNA all the time, another approach to finding the parasite's vulnerable targets is to study how it degrades the RNA it doesn't need in order to maintain the necessary balance within the cell," explains Read. "Interrupting this process could prevent the parasite from replicating in its mammalian host."

It's also important to study the trypanosomes themselves, particularly as they differentiate into different life-cycle stages in the mammalian host and the tsetse fly insect vector, says Read, echoing Wiliams' comments. "The parasite expresses a very different repertoire of genes in these two life-cycle stages, so if we can learn how the parasite regulates that gene expression, we might be able to interfere with the process. For example, the type of RNA editing we study occurs only in *T. brucei* and its relatives, such as *T. cruzi*, which causes Chagas disease, and *Leishmania*, which causes leishmaniasis, a disease that is infecting U.S. troops in Iraq and Afghanistan. Any process that takes place in the parasite but not in the host is an excellent point of drug intervention."

WHO/TDR/WELLCOME

Both parasitologists have broadened their research scope in recent years in ways that should enhance such efforts.

In 2000, Williams began collaborating with a laboratory headed by Beatriz Garat, PhD, in Montevideo, Uruguay, where Chagas disease is endemic. The work is focused on regulation of gene expression in *Trypanosome cruzi*, the Chagas disease agent. Several of Garat's students have come to UB to work in Williams' laboratory. One of those students has since established a laboratory in Curitiba, Brazil, and Williams has begun collaboration there as well.

None of these diseases has invaded the U.S., but neither geographical borders nor vast oceans offers protection in the 21st century.

"I think most of us have come to recognize," says Read, "that with immigration to the U.S., travel abroad by so many U.S. citizens, and military deployments to exotic locales, that all 'tropical' diseases have the potential for significant impact on our lives and our health-care system." BP

Mouse Calls

Trypanosomes

The battle against parasites is being waged by researchers from many disciplines, including biology, ecology, epidemiology, public health, toxicology, microbiology, parasitology, and veterinary sciences.

Trypanosomes, a guide on the UB Health Sciences Library web site at http://ublib.buffalo.edu/hsl/resources/guides/trypanosomes.html provides resources on research to control transmission of African Sleeping Sickness, Chagas disease, and Leishmaniosis.

Stanford University Libraries' Highwire Press, which hosts the largest repository of free full-text life science articles in the world, has links to 27 journals that publish on Trypanosomes at http://highwire.stanford.edu/lists/topic_dir/606274/607183/607184/607185/focus.dtl.

A Health Topics page on African Trypanosomiasis at the World Health Organization (WHO) at http://www.who.int/topics/trypanosomiasis_african/en/ tracks activities, reports, news, events and contacts within WHO and collaborating agencies.

The University of Cambridge Department of Pathology Field Lab maintains an informational Web site at http://homepage.mac.com/mfield/lab/programme.html including research activities and publications with the "aim of elucidation of fundamental cell biological processes of trypanosomes."

The Consortium of Conservation Medicine at http://www.conservationmedicine.org/ offers an interdisciplinary arena in which to tackle this and emerging infectious diseases that increase as international travel brings humans and organisms together.

Also included is a selected bibliography of articles published by UB molecular parasitologists Noreen Williams, PhD, and Laurie Read, PhD, and a list of books and journals available in HSL on the topic.

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