Bloodsucking worms called schistosomes are among the world’s most worrisome human parasites. A new genome sequence and powerful genetic tools promise to help crack their secrets. By Patrick Skelly

**KEY CONCEPTS**

- Parasitic worms known as schistosomes are a major cause of disability and death in many parts of the world, especially sub-Saharan Africa.
- Although a treatment exists, reinfection is the rule.
- A vaccine would make a world of difference, but none has yet proved effective. Genetic and other tools hold promise for generating new candidates.

—The Editors

Legend has it that vampires create no shadows, cast no reflection and—in more modern versions of the tale—cannot be captured on photographs, film or video. Of course, vampires are only myths. Unfortunately, schistosomes, which behave in some similar ways, are not. These infectious worms dwell in human veins and eat our blood. Among parasitic illnesses, the World Health Organization ranks schistosomiasis, the disease caused by the worms, second only to malaria in terms of the number of people it kills and chronically disables and the drag it imposes on the social and economic development of nations. And, in their own way, schistosomes have achieved invisibility. Cameras can capture these creatures, but our immune system does not.

Investigators have struggled for years against the schistosome’s evasiveness. They have been trying to create vaccines able to rally a defense that would pounce on the parasite quickly, thereby preventing disease, or that would help the body to clear existing infections. Vaccines are a necessary and missing component of a global effort to eradicate this illness. So far the results have been disappointing. But schistosome researchers like myself feel we may be at the start of a great leap forward. Genome projects are laying bare the DNA sequence of the parasite, and scientists are beginning to develop powerful new tools to probe its molecular secrets. These
weapons may help make it possible to enhance immunity and accelerate vaccine efforts.

Preying on Humans
A vaccine would help avoid an enormous amount of suffering. Some 200 million people, mostly in tropical and subtropical countries, have schistosomiasis, meaning they harbor schistosomes in their blood. In children, persistent infection can retard growth and cause cognitive deficits. And in anyone, it can lead to anemia as well as damage to the intestines, bladder, spleen and liver, resulting in symptoms ranging from bloody diarrhea and cramping to life-threatening internal bleeding and kidney failure. Schistosomiasis can drastically reduce someone’s ability to work, crippling both individuals and the economy.

People become infected when they encounter water infested by immature schistosome forms, which, though toothless, easily degrade and penetrate human skin and then enter blood vessels. There the immature parasites develop into adult bloodsucking worms and mate, after which the females begin laying eggs.

Then the eggs make matters worse. As many as half of the hundreds laid daily by each female will lodge in a variety of organs. Unrestrained, they would secrete toxins at a lethal level. The immune system, though usually capable of eliminating the worms, blocks the acute lethality, albeit at the cost of doing damage of its own: it provokes the formation of scar tissue, a major cause of the organ impairment seen in the disease. The immune response to the eggs also apparently helps them to puncture blood vessels, which in the intestinal tract allows them to make their way into feces and thus out of the body to continue development. Eggs that invade the bladder may, alternatively, escape in urine. In water the eggs hatch; then larvae emerge and infect snails. Inside snails the schistosomes replicate asexually before pouring into the water to infect, or reinfect, new human victims. [For more on the worm's complex life cycle, see box on page 97.]

Good sanitation and snail control have limited the disease in many countries. But in poverty-stricken regions, where clean water is still not available, it thrives. A safe antischistosome drug, praziquantel, was developed in the 1970s. It has few side effects and is now relatively cheap; plus, a single treatment can clear the infection. Reinfection, however, occurs frequently, and the worry looms that schistosomes will gain resistance to this drug. Already cases of schistosomiasis have surfaced that require higher than normal levels of the drug to clear—a possible sign of incipient resistance.

It is because of concern over drug resistance and because prevention is always the best medicine that health officials are eager to add a vaccine to the fight against the parasite—if a practical and effective one can be created. Typical vaccines deliver dead or inactive pathogens or distinctive segments of molecules (often proteins) made by those organisms in a way that induces the immune system to behave as if a true infection has occurred. The system produces cells that specifically recognize molecules present in the vaccine; thereafter some of these cells remain on the alert for the pathogen, ambushing it with antibody molecules directed to the recognized targets with other weapons before the menace can cause illness.

Investigators did not initially expect development of a vaccine against schistosomiasis to be as difficult as it has been. The worms’ life cycle suggested the parasites would be a soft target for our mighty immune system. Yet they turn out to be anything but simple to handle.

Swimming with the Enemy
One reason schistosomes initially seemed like they should be an easy target is that they are relatively large and make no effort to find hiding places in the body. The first sight of an adult worm always surprises my graduate students. These biologists are familiar with the microscopic bacteria and viruses that can live in our bodies and that often evade immune attack by hiding inside cells or by outcompeting immune cells through high-speed reproduction: one virus or bacterium can beget millions, indeed billions, of others during the course of an infection.

Schistosomes, on the other hand, are big enough to be viewed by the naked eye. An adult is a centimeter long. Furthermore, the worms that start an infection on day one are the same ones present days, years or even decades later; inside the human body their numbers do not grow, except, of course, by new infections.

And evolution has chosen a hostile home for schistosomes. Lying exposed in the bloodstream would not appear to be an ideal habitat for a parasite. Blood, though nutritious, is a major conduit for all the forces of immunity, which, somehow, the worms avoid.

Beyond being big and brazen, schistosomes possess other features that suggest the immune system could be induced to recognize them if conditions were right. The body’s strong reac-
FAST FACTS

Schistosomes probably originated in Asia and then dispersed to India and Africa. They jumped to the Americas in the blood of African slaves.

The worms lack an anus, so they vomit wastes out the mouth, for the host’s bloodstream to whisk away.

Females do not mature unless they have contact with males; removed from a male’s slit, a female will physically regress.

Schistosomes that sicken humans replicate in aquatic snails. Governments could help limit the worms’ spread by eradicating snails from freshwater and by preventing them from colonizing new bodies of water, such as lakes formed when dams are built. Many fear, for example, that construction of the Three Gorges Dam in China will foster new schistosome infections.

In snails, schistosome larvae often compete with other parasites, some of which like a rash known as swimmer’s itch in the U.S. and elsewhere. Parasitologists William W. Cort discovered the worm link in 1927, by placing larvae from contaminated water on his own skin and observing the symptoms.

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Wormy Tactics

Certainly, knowing how schistosomes typically escape immune detection is important if we are to develop vaccines that can overcome that propensity. The parasites have several tricks at their disposal that may explain their seeming invisibility to our defenses. One is that they come armed with a variety of molecules that may allow them to disable or “blind” the immune system. Kalyanadasaram Ramaswamy and his colleagues at the University of Illinois have shown, for instance, that some schistosome molecules can, at least in a test tube, inhibit proliferation of immune cells or induce the cells’ death.

In addition, some newly identified schistosome genes look like human ones that are switched on in immune cells. Other genes encode receptors, or docking sites, that are closely related to human receptors that bind small molecules called cytokines (which control the activity of immune cells) or hormones (which convey messages between cells over longer distances). It stands to reason that the parasites would benefit...
from intercepting signaling molecules that help our bodies to react to infection. The worms presumably use their receptors to essentially spy on intercellular chatter, to gain information about the state of their environment and to prepare counteractive measures before immune cells have a chance to strike.

Schistosomes also possess what seems to be a cloak of invisibility: an unusual covering known as the tegument. Most parasites are covered by a single oily membrane. In addition to that membrane, the outer part of the tegument sports a second, external one that contributes to the parasite’s ability to hide. The tegument provides ample protection to the worm as it migrates through our blood, but in the hands of scientists, it is extraordinarily fragile and nebulous. This fragility has made it difficult to answer even basic questions about the tegument’s biology, such as which proteins reside in it and whether any protrude from its surface. This last question is of keen interest to vaccine designers, because the targets of most successful vaccines are proteins or other molecules that appear on the outside of a pathogen.

We do know, though, that this outer coat can actually acquire human molecules from the blood. It is possible to detect, for instance, our own blood-group molecules (which establish the familiar blood types A, B, and so on) attached to the worm’s surface. One controversial idea is that these stolen human molecules could act as a disguise, covering the parasite’s own molecules and making them invisible to immune surveillance.

**Tricks of Our Own**

For decades, researchers have tried to pierce this impressive armor of disappearing tricks using the classic tools of molecular biology: isolating schistosome proteins and their genes one by one, then trying to discern the proteins’ functions and turn those molecules into effective vaccines. Now this slow and meticulous process may be thrown into higher gear by new technologies and the approaches they make possible.

Overcoming the known and yet undiscovered schistosome evasions would be vastly accelerated by having a catalogue of all the worm’s proteins. For that reason, schistosome researchers have been eager to decipher the organism’s genome, the complete sequence of DNA codes it uses as a blueprint for constructing every protein it contains.

But like so much else about these creatures, this goal initially proved elusive. For one thing, the schistosome genome—with more than 300 million nucleotide base pairs (the units of DNA)—is the largest parasitic genome that biologists

**INFECTED AGAIN**

Reinfection by schistosomes is common even after successful treatment because few individuals develop protective immunity and because in many areas, such as Morogoro, Tanzania, people have little choice but to wash clothes, bathe or cool off in infested water. The high rate of reinfection underscores the urgent need for a preventive vaccine.

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**A COMPLEX LIFE CYCLE**

The intricate life cycle of the schistosome includes multiplying prodigiously in snails and laying eggs in a person’s blood (diagram). Those eggs account, by and large, for the long-term effects of infection (panel at far right).

1. **Schistosome eggs**
   - produced in infected individuals enter freshwater in urine or feces

2. **Snail-invading larvae**
   - called miricadia hatch from the eggs

3. **Larvae in snails**
   - reproduce and morph repeatedly, ultimately into a human-infecting form

4. **Released larvae**
   - cercariae—swim to a new victim, usually emerging in midday to maximize the chance of finding a host

5. **Cercariae**
   - bore through the skin (despite being toothless), transform into schistosomula and enter veins

6. **Schistosomula**
   - float to the liver circulation, where they pair up and mature into adults

7. **Worm pairs**
   - migrate (against the flow of blood) to distant sites to lay eggs

8. **Eggs**
   - lodge in the intestines or bladder and enter feces or urine, starting the cycle anew

9. **SCHISTOSOME EGG**

**How Worm Eggs Cause Chronic Disease**

Schistosome eggs do harm by working their way into tissues and eliciting destructive immune reactions.

Responses to *S. mansoni* and *S. japonicum* eggs often compromise the liver and intestines and can also lead to bloody diarrhea, lethal internal bleeding and, possibly, colon cancer.

Responses to *S. haematobium* eggs can damage the urinary tract and kidneys and may induce bladder cancer.
have yet attempted to sequence. (For comparison, the genome sequence of the malarial parasite *Plasmodium* is more than 10 times as small.) Just as daunting was the discovery that almost half the genome is composed of repeated DNA sequences that perform no known function. For researchers, such “junk” DNA makes deriving a completed sequence much more difficult.

Nevertheless, in an international effort spearheaded by Philip T. LoVerde, now at the Southwest Foundation for Biomedical Research, the genome of *S. mansoni* has recently been sequenced, and the sequence is available online for all to analyze. And the Chinese National Human Genome Center in Shanghai is closing in on a listing of all of *S. japonicum*’s active genes.

One great advantage of revealing the full schistosome genome is that every gene can now be seen in context of this organism’s entire genetic background. We have learned, for instance, that the parasite has more than one version of some proteins that vaccines could potentially target; this variety might allow schistosomes to function in spite of vaccine-induced immune activity—by using the nontargeted version. Genomic analysis can now identify common structural features shared by such proteins so that those features might be incorporated in a vaccine and thus prevent the worms from escaping immune attack.

Alex Loukas and his colleagues at the Queensland Institute of Medical Research in Australia have taken advantage of the full genome sequence in another way. They screened it for genes whose features suggested the encoded proteins probably protruded from the tegument. The so-called tetraspanin molecules that emerged from the screen have long domains made of greasy amino acids that would be expected to span the oily surface of the outer membrane, leaving two protein loops exposed on the surface. Recently Loukas’s team reported that two of these newly identified proteins, TSP-1 and TSP-2, when used to vaccinate mice, resulted in a substantial reduction in the number of adult worms and eggs in animals; in the case of TSP-2, the reduction was by more than half. The group then showed that in rare cases, people who are putatively resistant to schistosomes—who have avoided infection with the parasite despite years of known exposure—have antibodies against TSP-2 in their blood. In contrast, those who are chronically infected have no detectable level of these antibodies. This finding suggests that recognition of TSP-2 is a component of rare, natural immunity to schistosomes and that the protein might be useful for eliciting protective immunity in a vaccine as well.

The Australian group’s work is encouraging for another reason. One might reasonably wonder whether molecules that fail to evoke an immune response in the human body during an infection would be able to do so when delivered as a vaccine. Loukas’s team and others, however, have demonstrated in mice that if these molecules are presented to the immune system in the right way, they can indeed, at times, elicit a strong protective response.

In parallel with examining the schistosome genome, researchers are working to understand the functions of the proteins made by the para-

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**A BRIGHT SIDE?**

In experimental animals, schistosomes can prevent or ameliorate a range of debilitating autoimmune disorders, such as Crohn’s disease, which causes chronic intestinal inflammation (colitis) in humans. Studies conducted by Joel Weinstock, now at the Tufts University School of Medicine, and his colleagues showed that after mice with colitis were injected with schistosome eggs, they suffered less intestinal swelling and were better protected from lethal inflammation than other mice were.

It turns out that the eggs and Crohn’s disease invoke diametrically opposite immune responses. In this immunological tug-of-war, the response elicited by the eggs has the upper hand. Investigators are now hunting for the molecules that elicit these responses, because some might be valuable as therapies for autoimmune diseases. —P.S.
sites. Such information can help pinpoint which proteins might be the most reasonable to pursue as vaccine candidates. For instance, molecules that the worm always requires to survive or to make eggs in the human body could be useful, because an immune response targeted to them should in principle be deadly to the parasite or limit the destructive egg production.

**Playing the Function Card**

Several years ago knowledge of protein function led Charles Shoemaker of the Tufts Cummings School of Veterinary Medicine and me to proteins that look promising as vaccine components. These proteins are involved in importing nutrients, such as sugars and amino acids. Schistosomes, as they bathe in blood, not only gobble food through their mouth but also take in many nutrients directly through their tegument, and they require nutrient-importing proteins for this purpose. We also know that to work properly, these proteins must be in direct contact with the host’s blood. These molecules are potentially very attractive as vaccine targets because prompting immunity against them could both direct a damaging attack against the parasite (because these proteins are on its surface) and impede its ability to absorb food from the blood.

A focus on function has also raised the possibility of making a vaccine from proteins that the parasites secrete. At first blush, that idea might seem silly: an immune response directed to such molecules would literally miss the target, because these molecules float away from the worm body. But if immune system components bind to these factors and thereby keep the secretions from doing jobs important to the parasite, the vaccine might reduce the worm’s survival or its ability to cause disease. An obvious next step would be to shut off secreted genes one at a time, to see which ones are needed the most and would therefore be the best candidate for this approach.

Until recently, standard tools for shutting off genes did not work in schistosomes. But my laboratory and that of Tim Yoshino of the University of Wisconsin–Madison have taken a leaf from the book of 2006 Nobel Prize winners Andrew Z. Fire of Stanford University and Craig C. Mello of the University of Massachusetts Medical School and developed methods for silencing specific schistosome genes using a technique called RNA interference [see “Censors of the Genome,” by Nelson C. Lau and David P. Bartel; *Scientific American*, August 2003]. So it is now possible to silence the genes of secreted proteins and other schistosome proteins to probe their function.

Going forward, vaccine researchers will have other new tools for uncovering the function of schistosome proteins, where they reside and when in the parasite’s life cycle they are made. Notably, Paul Brindley of George Washington University, Christoph Greweling of the University of Düsseldorf in Germany and Edward Pearce of the University of Pennsylvania are developing methods for genetically engineering worms, making it possible to add distinctive tags to a selected parasite protein; such tags will allow scientists to easily track the protein’s production and location. Among other advantages, this technique could put to rest the question of which proteins normally reside in the tegument and protrude from its surface. Taking another tack, various groups, including that led by Karl Hoffman of the University of Wales, have created devices called DNA microarrays (commonly called gene chips) that can reveal which mixtures of schistosome genes are switched on at each stage of development.

The many fresh approaches to studying the parasite may yield benefits beyond ideas for vaccines. Knowing this organism’s complete genetic makeup, for example, should help pinpoint proteins that are unique to schistosomes and crucial for their survival; novel drugs might then be found that act on those proteins to defeat the worm. Of course, the path from all this new knowledge and how-to an effective vaccine or treatment is not straightforward or certain. Success will depend on researchers’ intellect, intuition, dumb luck, and the level of funding governments and foundations provide. But it is exciting to know that schistosome researchers are moving in directions that were not on the map even a few years ago.