The battle against a worm

Genes identified by a method created in Brazil are promising targets for new drugs against schistosomiasis

Salvador Nogueira

As a result of genomic research, begun in Brazil about 15 years ago, Brazilian researchers have found promising targets for the development of a vaccine against schistosomiasis, a disease that affects more than 200 million people worldwide. A team from the University of São Paulo (USP) and the Butantan Institute has identified a set of nine genes that demonstrate an ability to reduce by up to 28% the number of worms in the bodies of 22% of inoculated mice, compared to a group of untreated mice.

The causative agents of schistosomiasis are three worms of the genus *Schistosoma*: *S. haemotobium*, *S. japonicum* and *S. mansoni*. This third worm of the genus is the parasite found in Brazil, which was brought from Africa with the slaves during Portuguese colonization. Up to now, palliative strategies that were not completely effective have been the only way to fight it. Drugs cut the risk of death from the parasite

No sewerage system or running water: favorable conditions for infection

RICARDO AZOURY

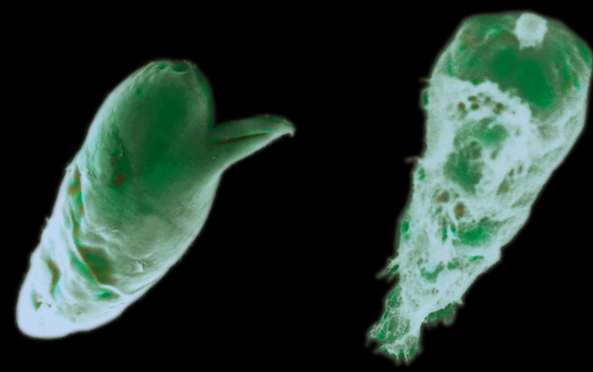
in half, but they do very little to contain the spread of the disease, which, in the view of researchers, justifies the development of other strategies. Vaccination is, in theory, one of the most promising.

One of the challenges in identifying ways to fight the worm is its complex life cycle. The microscopic eggs come from the feces of an infected human host and, in fresh water, hatch and produce a larval form called miracidium that infects snails and develops into another form of the worm, the cercaria. Each miracidium can produce 10,000 cercariae, which return to water and seek vertebrate hosts – people.

Provided with a bifurcated tail, the cercaria penetrates the skin and enters the bloodstream within less than 15 minutes. Upon entering the body, it loses its tail, is now called a schistosomula and lodges itself in the veins of the intestine, where it develops into a worm and lays eggs. Some cercariae may settle in the liver while others penetrate the intestinal wall and are excreted with feces, thus restarting the transmission cycle.

One of the genes increased by 28% the response to infection caused by the worm

Propagation and contagion



Eliminated with human feces, the eggs of *Schistosoma mansoni* get into rivers and lakes

The eggs release miracidia, larvae that become lodged in snails of lakes and streams

With so many changes along the way, the route chosen by the group led by Sergio Verjovski-Almeida, a researcher at the USP Chemistry Institute, to investigate the genes of *Schistosoma mansoni* was the so-called transcriptome. The term is derived from transcription because only the genes that are being replicated (transcribed) in the form of RNA to take an active role in metabolism are to be investigated. Thus, not only is it possible to identify a significant portion of the worm's genes, but it is also possible to correlate which genes are activated and in which phase of the worm's complex life cycle they are activated.

In 2003, the group made a significant advance when it published the results of two years of work deciphering the transcriptome of *Schistosoma mansoni*, determining, in whole or in part, the sequences of 92% of the approximately 14,000 genes of the parasite. The work, published in the journal *Nature Genetics*, was included on a recent list of *hot articles* in Brazilian science prepared by Marco Antonio Zago, dean of research at USP.

FROM GENOMICS TO APPLICATION

The transcriptome produced in 2003 was made possible by a gene identification method known by the acronym Orestes (Open Reading frame ESTs), developed by Emmanuel Dias Neto and Andrew Simpson, then working at the Brazilian branch of the Ludwig Institute for Cancer Research. Using that method, the team led by Verjovski-Almeida and his colleagues have mined the data in search of information to help us understand, in molecular terms, how the parasite acts to circumvent the body's defense systems.

By 2003, the USP group had already managed to identify functions for 45% of the sequenced genes of the worm. The process of identifying them was basically done by comparing the genes of *S. mansoni* to other organisms whose genes had already been characterized in public databases. For half of the genes there was no known equivalent in other organisms.

Working with these unique sections of the parasite – and it is assumed they are because they are important from an evolutionary standpoint – the team led by Verjovski-Almeida, collaborating with the group led by Luciana Leite of the Butantan Institute, found promising targets for the development of vaccines. The work, published last year in the scientific journal *Parasitology Research*, is still not a perfect solution to the disease, but a path that is worth exploring.

AT A CERTAIN STAGE

We know that in principle a vaccine against Schistosoma is possible because of the simple fact that there are certain individuals who demonstrate a natural resistance to the infection – a sign that their immune systems can deal with the invaders and defeat them. Based on studies that attempt to discover how resis-

The six major stages of the life cycle of the parasite

The miracidia produce thousands of germ cells, another form of the worm

The larvae are a young form of the parasite, called cercariae, which infect humans

As they penetrate the skin, the cercariae lose their tails and migrate to the liver where they become adults

Lodged in the intestine, the worms lay eggs that are released with feces

PHOTOS DEPARTMENT OF BIOCHEMISTRY AND IMMUNOLOGY / FMRP-USP

tant organisms fight off an attack, the group decided to search for genes that are especially active in one stage of the parasite's life cycle, the schistosomula. Once this form of the parasite has invaded the body, the pathogen begins to develop within the host.

Mining the transcriptome data in search of genetic targets that were unique to *Schistosoma* and were being expressed more at this stage, the group came up with a set of nine genes. The next step was to test their protective potential – to see if, when injected into the body, they could tell the immune system how to recognize and destroy the *S. mansoni*. For this, the Butantan group used the protocol known as DNA vaccine, in which genes are injected into the body and then replicated by the cellular machinery, increasing the production of proteins, which are then recognized by the body's defenses.

In tests on mice, researchers found two genes that can indeed have a protective effect. The gene designated Dif 5 demonstrated an ability to reduce the number of worms in the bodies of 22% of inoculated mice, compared with a group of untreated mice. But the most important finding was the Dif gene 4, which reduced the level of infection by 25%. And the number for that specific gene improved to 28% when they used a second protocol, encapsulating the material in microspheres. Still not enough to say that we have a vaccine, but it is a promising sign, by showing that there was an enhanced immune response.

To Sergio Verjovski-Almeida and his colleagues, the prospects are good despite the relatively modest numbers. "In the search for vaccines against *Schistosoma*, many studies using DNA vaccines begin with low protection values, which are then augmented by the use of adjuvants or other reinforcement strategies." And an interesting aspect of the work is that it could eventually be possible at a lower cost, since its primary database of the transcriptome comes from 2003, analyzed by bioinformatic techniques. ■

PROJECTS

1. *Genome / transcriptome of the Schistosoma* – No. 2001/04248-0 (2001-2004)
2. *Schistosoma mansoni: molecular characterization of the interaction between parasites and between them and their human host* – No. 2010/51687-8 (2010-2012)

GRANT MECHANISM
Regular line to assist the research project – Genome Program

COORDINATOR
Sergio Verjovski-Almeida – USP

INVESTMENT
1. R$564,829.31
2. R$429,444.90

SCIENTIFIC ARTICLES

1. VERJOVSKI-ALMEIDA, S. et al. Transcriptome analysis of the acoelomate human parasite *Schistosoma mansoni*. **Nature Genetics**. v. 35, n. 2, p. 148-57, 2003.

2. FARIAS, L. P. et al. Screening the *Schistosoma mansoni* transcriptome for genes differentially expressed in the schistosomulum stage in search for vaccine candidates. **Parasitology Research**. v. 108, p. 123-35, 2011.

FROM OUR ARCHIVES

Inside the parasite Issue No. 92 – October 2003