Molecular biology technique allows for discovery of novel targets for candidate vaccines against schistosomiasis

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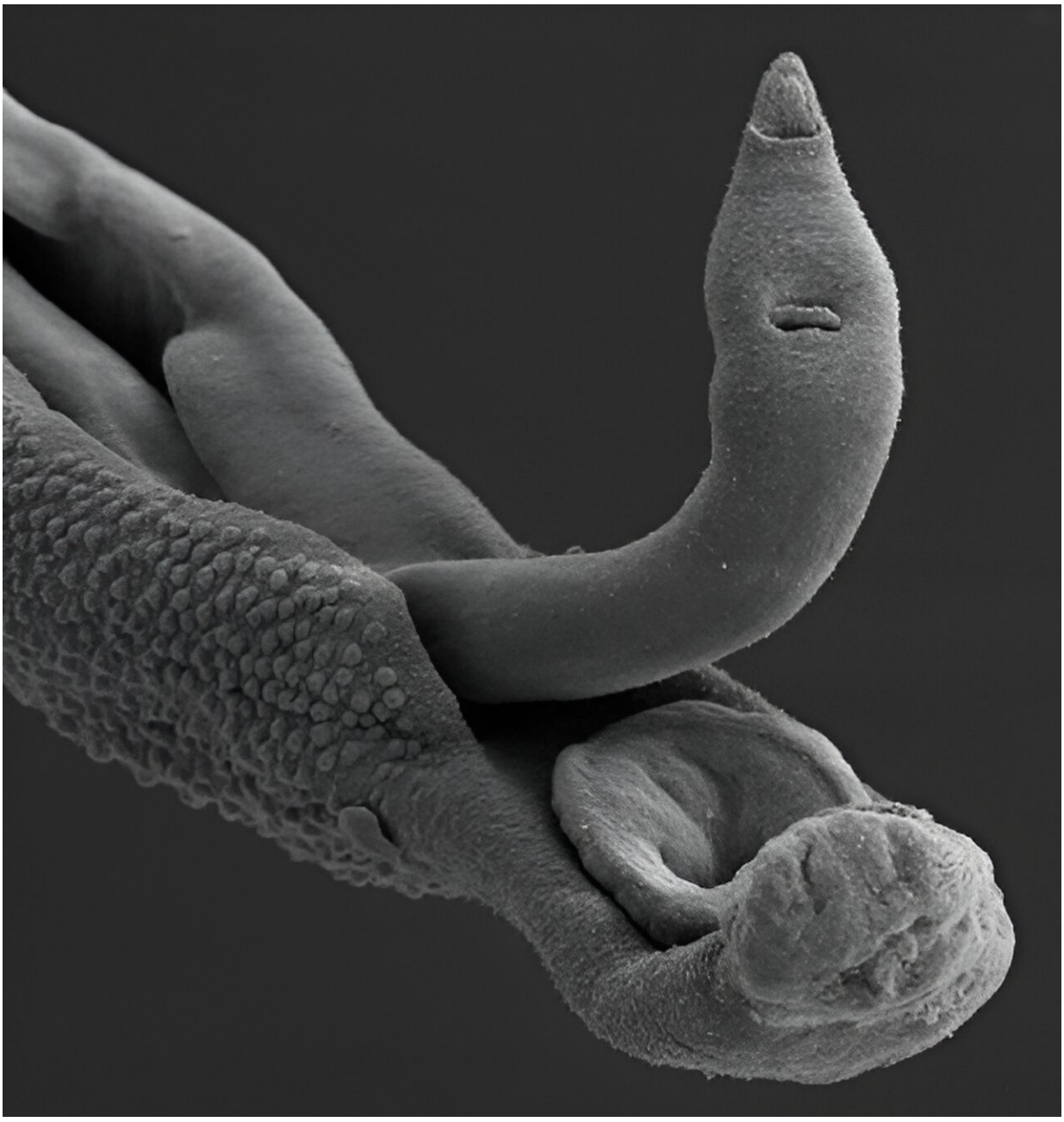

Female inside male of Schistosoma mansoni. Some 200 million people in 74 countries have schistosomiasis due to infection by parasitic worms, including S. mansoni. Credit: Butantan Institute

Researchers in Brazil have used an innovative technique in molecular

biology to identify targets for candidate vaccines against Schistosoma mansoni, the parasite that causes schistosomiasis.

Considered one of the world's 17 neglected tropical diseases (NTDs), schistosomiasis affects some 200 million people in 74 countries, according to the World Health Organization (WHO). Six million are estimated to be infected in Brazil, mainly in the Northeast region and Minas Gerais state.

The scientists used phage display, the study of protein interactions using bacteriophages, viruses that infect bacteria, to screen 99.6% of 119,747 DNA sequences encoding the proteins known to be expressed across all life-cycle stages of the parasite, achieving comprehensive coverage of its proteome.

The results of the study are reported in an article in *npj Vaccines*.

They follow on from those of a previous study that revealed the mechanism whereby the Rhesus macaque Macaca mulatta naturally develops a lasting immune response against schistosomiasis by inhibiting certain of the parasite's genes so that it cannot multiply in the host organism. This immune response leads to self-cure after first contact with S. mansoni and enables the animal to react faster to a second infection.

"Phage display had never been deployed for this purpose in research on parasitic diseases, which normally involves preselection of a few targets for testing of candidate vaccines. In this study, we screened 12,000 proteins of S. mansoni at the same time to identify which ones were targeted by the macaque's antibodies, both after initial infection and reinfection and after reinfection and self-cure, a key innovation. Both the technique and the model for the study were innovative," said Murilo Sena Amaral, a researcher at Butantan Institute's Laboratory of Cell

Cycle.

Amaral is the penultimate author of the article. The last author, as principal investigator for the study, is Sergio Verjovski-Almeida, also a researcher at Butantan Institute and a professor at the University of São Paulo's Institute of Chemistry (IQ-USP).

Methodology

The researchers investigated the immune response of ten macaques infected by S. mansoni during the stages of self-cure and resistance to reinfection using a recently developed technique called peptide library-based phage immunoprecipitation sequencing (PhIP-Seq).

They constructed a phage display library that comprised 119,747 DNA sequences encoding 11,641 known proteins from S. mansoni in all stages of its life cycle. The library was incubated with antibodies collected from rhesus macaques in a previous study at different points during the process of self-cure and resistance to reinfection. The aim was to isolate and identify specific targets of the animal's immune response to the parasite.

Library screening with antibodies from the early phase of parasite infection identified significantly enriched epitopes of parasite extracellular proteins known to be expressed in the host's digestive tract, shifting toward intracellular proteins during the late phase of parasite clearance (released owing to its death). Epitope refers to the specific target against which an individual antibody binds. When an antibody binds to a protein, it bonds not to the entire protein but to a segment known as an epitope.

The enriched peptides were analyzed with bioinformatics tools to identify potential candidates for vaccines. The most promising

candidates were tested in a pilot vaccination assay, in which mice were immunized with a selected pool of PhIP-Seq-enriched phage-displayed peptides. The result was a significant reduction of worm burden in the immunized mice.

"You often hear the argument that a schistosomiasis vaccine isn't feasible, but our discoveries have revealed a great deal of the immune response and opened up promising prospects for the development of an effective vaccine. We worked with the 12,000 proteins key to all stages of the parasite's life cycle and succeeded in identifying the most reactive targets," Verjovski-Almeida told Agência FAPESP. The technique can be used for other types of parasite, he added.

In an article published in May 2023, the group described their discovery of a way to "separate" male and female parasites so as to prevent reproduction and egg release. Male-female pairing, with the female living inside the male, is essential to their survival. Without it, they die. In the study, the researchers showed that male-female separation could be obtained by silencing specific long noncoding RNAs (lncRNAs), which are therefore a promising target for treatment of the disease.

How the worm works

Schistosomiasis is a parasitic disease associated with poor hygiene and a lack of basic sanitation. It is transmitted when an infected person excretes feces containing schistosome eggs into the environment. The eggs hatch in freshwater, releasing larvae that infect snails. The snails are intermediate hosts, while humans are definitive hosts.

After four weeks, the larvae leave the snail as cercariae, the free-swimming larval stage. When humans come into contact with contaminated water, they acquire the disease via active skin penetration by cercariae.

In the human bloodstream, the cercariae progress to the schistosomule stage, eventually becoming adult worms that lodge in the veins of the intestines. The first symptoms of the disease appear two to six weeks after infection.

The disease is diagnosed by laboratory analysis of feces. Simple cases can be treated by a single dose of praziquantel, a drug discovered in the 1970s and distributed in Brazil by the national health system (Sistema Único de Saúde, SUS). However, it does not assure continuous protection. Patients taking it can be reinfected, and there are reports of parasite drug resistance.

"The next step is to develop a suitable vaccine formulation containing adjuvants and a novel mechanism for delivery of these antigens so that they produce better protection in the host. We have some targets with higher response levels," Verjovski-Amaral explained. Butantan Institute has applied for a patent on the group's discoveries linked to possible vaccine targets.

Oswaldo Cruz Foundation (FIOCRUZ), an arm of the Brazilian Health Ministry, has been working for years on what could be the world's first schistosomiasis vaccine. Called Schistovac, it is in the testing stage and contains a modified version of the Sm14 protein found in S. mansoni. The protein normally plays a key role in trafficking fatty acids, which are essential to the parasite's cellular functions. The modified version is designed to prevent proliferation.

More information: Daisy Woellner-Santos et al, Schistosoma mansoni vaccine candidates identified by unbiased phage display screening in self-cured rhesus macaques, *npj Vaccines* (2024). DOI: 10.1038/s41541-023-00803-x

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