Research identifies potential targets for treatment of leishmaniasis

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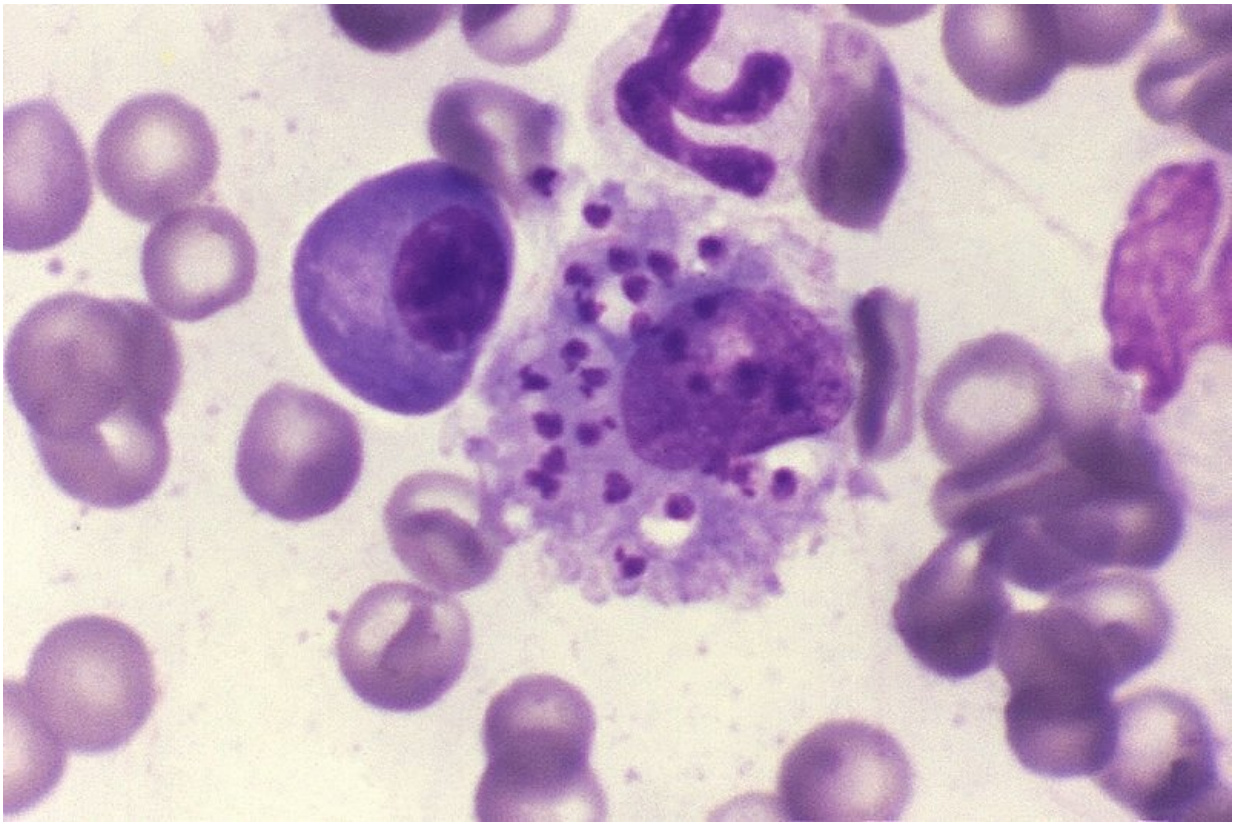

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Brazilian researchers at the University of São Paulo's Bioscience Institute (IB-USP) are starting to unravel the molecular mechanisms by which the parasite that causes cutaneous leishmaniasis circumvents the

host organism's defenses and infects new cells.

Cutaneous leishmaniasis, caused by protozoans of the genus Leishmania, produces skin lesions that are slow to heal. It is usually transmitted to humans and other mammals by bites of blood-feeding insects such as the sand fly (genus Lutzomyia in the Americas).

According to Lucile Maria Floeter-Winter, a professor in IB-USP's Physiology Department and the main investigator of the group, the pathogen's entry into macrophages—defense cells that are the main targets of Leishmania in mammals—is sufficient to alter gene expression in the host. As a result, there is a decrease in the synthesis of nitric oxide, a chemical weapon used by the immune system to ward off invaders.

"Our line of research aims to understand how this interaction between Leishmania and macrophages works so that we can identify molecular targets to interrupt the infection and kill the parasite," said Floeter-Winter.

In their latest experiments, the scientists infected macrophages from mice with protozoans of the species Leishmania amazonensis. The cultured cells were divided into two groups: One was infected with the wild-type parasite, and the other with a strain modified in the laboratory to suppress an enzyme called arginase. Results of the study were published in *Scientific Reports*.

The same group of researchers had already demonstrated in 2012 that arginase production is essential to survival of the parasite in the host organism, as reported in an article published in the journal *PLOS ONE*, which described a previous experiment.

The next step was to analyze how the entry of Leishmania into the cell

alters the expression of microRNAs, small RNA molecules that do not code for proteins, but rather perform a regulatory function in several cellular processes.

"MicroRNAs are capable of binding to molecules of messenger RNA [which give rise to proteins], causing their degradation or inhibiting their translation into proteins," Floeter-Winter explained. "So when microRNA expression in the cell increases, it means some cellular process is being inhibited."

The group focused on analyzing a set of 84 microRNAs that are known to be involved in macrophage immune responses, with the aim of seeing which were expressed more when the parasite entered the cell. Expression was measured at four different times: four, 24, 48 and 72 hours after infection.

They found that expression of 78 percent of the 84 microRNAs increased in macrophages infected with wild parasites, compared with 32 percent in macrophages infected with the arginase-knockout mutants. "This shows that the fact that the parasite doesn't produce arginase makes macrophages respond differently to infection," Floeter-Winter said.

Tests with microRNAs' antagonists

Among the microRNAs with upregulated expression in macrophages infected by the wild parasites compared with the group infected with the arginase-knockout mutants, two molecules drew the researchers' attention: miR-294 and miR-721. They used bioinformatics software to look for possible targets of these two microRNAs. The analysis suggested that both were responsible for inhibiting macrophages' production of nitric oxide synthase, an enzyme required by defense cells to secrete nitric oxide and kill the pathogen.

"Penetration of the macrophage by the arginase-knockout Leishmania also increases expression of miR-294 and miR-721, but apparently not enough to inhibit nitric oxide synthase completely and facilitate the parasite's survival," Floeter-Winter said.

Three other experiments were performed to confirm that miR-294 and miR-721 target the messenger RNA region of nitric oxide synthase. The methodology used was described in another article published in the journal *Protocol Exchange*.

In one of the experiments, the researchers placed an antagomir in cultured cells shortly after infection, with the goal of antagomir binding to the microRNAs to prevent them from binding to messenger RNA, their natural target. "We showed that as we increased the dose of the specific antagomir for miR-294 and miR-721, a smaller amount of these microRNAs bound to the messenger RNA for nitric oxide synthase, and so the inhibitory effect didn't occur," Floeter-Winter said.

Next steps

At one point, Floeter-Winter and her group thought that arginase could be an interesting target for the development of drugs against leishmaniasis, but the idea proved impracticable. "In the previous study, we showed that arginase is located in an organelle of the parasite, which in turn is in an organelle of the macrophage. A sufficient amount of a chemical compound to have a biological effect would be unlikely to penetrate all these membranes in order to reach the site where arginase is active," she said.

The two microRNAs, miR-294 and miR-721, appear to be more promising targets, she believes, because they are located in the macrophage cytoplasm.

The next step in this strategy, according to Floeter-Winter, is to repeat the experiment using macrophages from a different strain of mice. "Initially, we used BALB/c mice, which are susceptible to infection by Leishmania. Now, we're going to use macrophages from C57 black mice, which are resistant to the parasite. We want to see what happens in this context to microRNA expression after infection," she said.

The group also plans to repeat the experiment using human macrophages and to perform tests with other species of Leishmania that cause American tegumentary leishmaniasis (ATL), such as L. major and L. braziliensis, and species that cause visceral leishmaniasis, such as L. infantum and L. donovani, among others.

"We began with a broad snapshot of this interaction between parasite and macrophages. Now we need to drill down into the detail of what happens case by case," Floeter-Winter said.

More information: Sandra Marcia Muxel et al. Leishmania (Leishmania) amazonensis induces macrophage miR-294 and miR-721 expression and modulates infection by targeting NOS2 and L-arginine metabolism, *Scientific Reports* (2017). DOI: 10.1038/srep44141

Maria Fernanda Laranjeira da Silva et al. Leishmania amazonensis Arginase Compartmentalization in the Glycosome Is Important for Parasite Infectivity, *PLoS ONE* (2012). DOI: 10.1371/journal.pone.0034022

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