Study describes enzyme's key role in immune response to Chagas disease parasite

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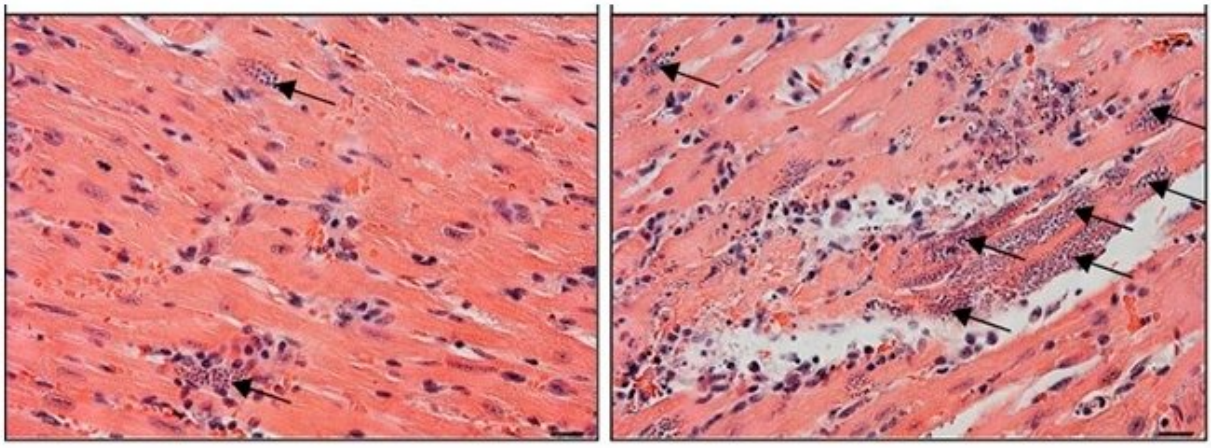

A study published in *Nature Communications* shows that the expression of PI3Kγ protein increases during infection by T. cruzi, an essential response in avoiding excessive inflammation and controlling parasitemia . Credit: heart tissue in PI3Kγ-deficient mice [R] contains significantly higher levels of parasites than that in control mice [L] 18 days after infection/ *Nature Communications*

In an article published recently in the journal *Nature Communications*, researchers affiliated with the University of São Paulo (USP) in Brazil describe the central role played by an enzyme called phosphatidylinositol 3-kinase gamma (PI3Kγ) in regulating the immune response against Trypanosoma cruzi, the protozoan parasite that causes Chagas disease.

"Our results show that the expression of PI3Kγ increases during infection by T. cruzi in both mice and humans. This appears to be essential to avoid excessive inflammation that might injure the organism and also to control heart parasitemia," said Maria Claudia da Silva, lead author of the study and a researcher at the University of São Paulo's Ribeirão Preto Medical School (FMRP-USP).

According to the authors, molecules capable of modulating the cellular signaling pathway mediated by this enzyme may in future be tested as a treatment for Chagas disease, which affects some 7 million people in Latin America—2 million to 3 million in Brazil alone.

The study was conducted at the Center for Research on Inflammatory Diseases (CRID). It was part of the master's and Ph.D. research of Maria Claudia da Silva, supervised by João Santana da Silva and Thiago Mattar Cunha, professors at FMRP-USP.

Transmitted by insects known as triatomine or kissing bugs (mainly Triatoma infestans in South America), as well as by transfusions of blood from donors with Chagas disease and by the ingestion of contaminated food, T. cruzi stays with the patient for life. The initial acute phase of infection may be asymptomatic or may cause fever, nausea, headache, inflamed and painful lymph glands, skin rash, swollen eyelids and enlarged liver and spleen.

Without treatment, complications can arise years later in the chronic phase. The most frequent are heart ventricle enlargement, which affects approximately 30 percent of patients and typically leads to heart failure, and esophagus or colon enlargement, which affects up to 10 percent of patients and can lead to loss of peristaltic movement and sphincter dysfunction. Most infected people remain asymptomatic even with large numbers of parasites circulating in the organism.

"In our experimental model, we used a strain of the parasite that prefers heart tissue," João Santana da Silva said. "The initial tests showed that PI3Kγ-deficient mice developed severe cardiomyopathy in the acute phase and died after a short time, but we had no idea why this happened."

In further tests, the CRID researchers found that the levels of the parasite in the blood of mice genetically modified not to express PI3Kγ were the same as in wild mice, which could express the enzyme and survive infection.

According to Santana da Silva, the mice that died from infection by T. cruzi were expected to have more parasites in their bloodstream than those that survived.

"However, when we looked at their hearts, we found that PI3Kγ-deficient mice had far higher parasitemia levels and much more severe inflammation [myocarditis]," he said. "The immune system was producing proinflammatory molecules in an uncontrolled manner, injuring the heart tissue, and even so, it was unable to kill the parasites efficiently."

Defective macrophages

During Maria Claudia da Silva's Ph.D. research, the group investigated how the immune response to the parasite is modified by the absence of PI3Kγ. According to Cunha, studies in the literature show that the enzyme participates in a signaling pathway that plays a key role in the migration of defense cells to inflammation sites in the organism.

In the case of infection by T. cruzi, under normal conditions, signaling proteins called chemokines are produced by the parasite when it infects host cells. The chemokines activate macrophages and dendritic cells, the

front line of the immune system, which migrate to the site and kill the invaders.

Although the defense mechanism is not 100 percent efficient, the researchers explained that it manages to keep parasitemia levels low, and most infected individuals have no symptoms during the acute phase.

"Our results show that when the signaling pathway mediated by PI3Kγ is not active in macrophages, these defense cells lose their capacity to kill the parasites and control the inflammation," Cunha said. "To prove that the problem resided specifically in macrophages, we used an animal model called conditional knockout, in which PI3Kγ is missing only in macrophages."

The CRID group did not fully elucidate the mechanism but found that without the enzyme, the macrophages produce less nitric oxide, which is required to kill the parasites and acts in conjunction with a pro-inflammatory cytokine called interferon gamma (IFNγ).

"If the macrophages don't express PI3Kγ, they can't kill the parasites even in the presence of IFNγ," Santana da Silva said.

Evidence in humans

In partnership with Edecio Cunha Neto, a researcher at USP's Medical School in São Paulo (FM-USP), the CRID team studied tissue from patients who developed cardiopathy in the chronic phase of Chagas disease and who underwent biopsy or heart transplant. They also analyzed a database containing information on all molecules expressed in the heart tissue.

The study showed that individuals with higher levels of PI3Kγ had lower levels of heart parasitemia than those who expressed less PI3Kγ,

although both groups presented with myocarditis. In addition, all had higher levels of PI3Kγ and of all molecules in the pathway mediated by this enzyme than non-Chagas patients with chronic congestive heart failure.

"These findings suggest that this enzyme is also involved in control of the parasite in humans," Santana da Silva said. "In our in vitro experiments, we observed that human macrophages failed to kill the intracellular pathogen when infected with T. cruzi after treatment with the PI3Kγ inhibitor. How this happens is something we have yet to understand."

Preliminary results also show that in patients who develop cardiopathy in the chronic phase of infection by T. cruzi, there is a higher incidence of a polymorphism (a variation in the gene that encodes the enzyme) that may be associated with lower activity of PI3Kγ than in patients who develop chronic disease in other organs, such as the spleen or intestine.

"We're currently writing another paper in which we raise the hypothesis that people with a certain polymorphism in the gene that encodes PI3Kγ run a greater risk of developing cardiopathy in the chronic phase," Cunha said.

Another possible line of investigation, he added, would be to determine whether the rare cases of patients who die of sudden myocarditis during the acute phase of Chagas disease are associated with lower levels of PI3Kγ, as the researchers observed in mice.

Santana da Silva is also interested in investigating the signaling pathways that modulate PI3Kγ production in the human organism.

"Drugs are available to inhibit PI3Kγ production but not to stimulate it," he said. "We now need to investigate the regulatory pathways mediated

by PI3Kγ in search of molecules that induce the release of these substances. We've already performed lab tests on some molecules that display this type of action. It's basic science, but there are potential applications for the control of parasitemia in both the acute and chronic phases."

According to Cunha, activating the enzyme "is no easy task" and could have implications for other pathological conditions. "Inhibitors of the PI3Kγ pathway are being tested for the treatment of cancer and inflammatory diseases," he said.

More information: Maria C. Silva et al, Canonical PI3Kγ signaling in myeloid cells restricts Trypanosoma cruzi infection and dampens chagasic myocarditis, *Nature Communications* (2018). DOI: 10.1038/s41467-018-03986-3

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