In search of new spaces: *T. cruzi* (in wine) with vesicles (in yellow) on its body, next to a host cell

Parasite
poised for the attack

Vesicles with proteins help *Trypanosoma cruzi* invade host cells

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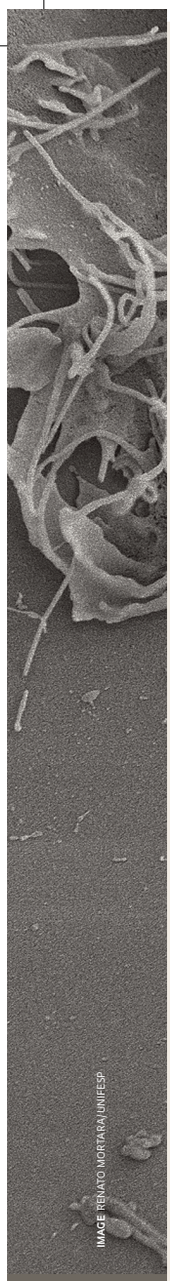


IMAGE RENATO MORTARA/UNIFESP

By examining their own data and that of other groups from the past 40 years, researchers in São Paulo have identified a potential mechanism that may help the protozoa that causes Chagas disease invade host cells. As soon as it settles into a single cell, *Trypanosoma cruzi* differentiates, divides violently enough to rupture the cell and release vesicles with proteins and lipids (fats) that facilitate the establishment of the parasite in other cells.

"It seems to be a wide-ranging phenomenon," says Walter Colli, professor of chemistry at the University of São Paulo (USP) and supervisor of this study, which was carried out by Ana Claúdia Torrecilhas of the Federal University of São Paulo (UNIFESP) in the city of Diadema in collaboration with Robert Schumacher and Maria Júlia Manso Alves of USP. "Other parasite groups and tumor cells also release vesicles that operate in a similar fashion and facilitate the infection of host cells."

Experts in this area, in both Brazil and other countries, are excited about the possibility of using this information to develop new ways to combat or diagnose tropical diseases on a broad global scope. Such is the case with Chagas disease, which affects approximately 10 million people in South America and is becoming a public health problem in the United States.

In an editorial published in May of this year in *PLoS Neglected Diseases*, researchers from the United States and Mexico warned about the advancement of Chagas disease in the United States, mostly among immigrants in the states closest to the Mexican border; an estimated 1 million people are infected. This study refers to Chagas disease as "the new AIDS of the Americas," and although one is caused by a protozoa transmitted by an insect and the other by a virus that is primarily transmitted through sexual contact, both can be passed on through blood transfusions, are more common among the poor and require prolonged treatment. Moreover, Chagas disease has become an important opportunistic infection among people living with HIV / AIDS, and as in the first two decades of the AIDS epidemic, most people with Chagas disease have no access to medical care.

In August, Peter Hotez, a professor at Baylor Medical School, director of the Sabin Vaccine Institute in Houston, Texas, and principal author of the *PLoS* editorial, wrote an article in the *New York Times* arguing that tropical diseases such as Chagas, leishmaniasis, dengue, and cysticercosis were the "new plagues of poverty." He said that 20 million people in the U.S. live in extreme poverty. "Without new interventions," he said, "these diseases are here to stay and will keep people in poverty for decades to come."

VARIOUS SIZES

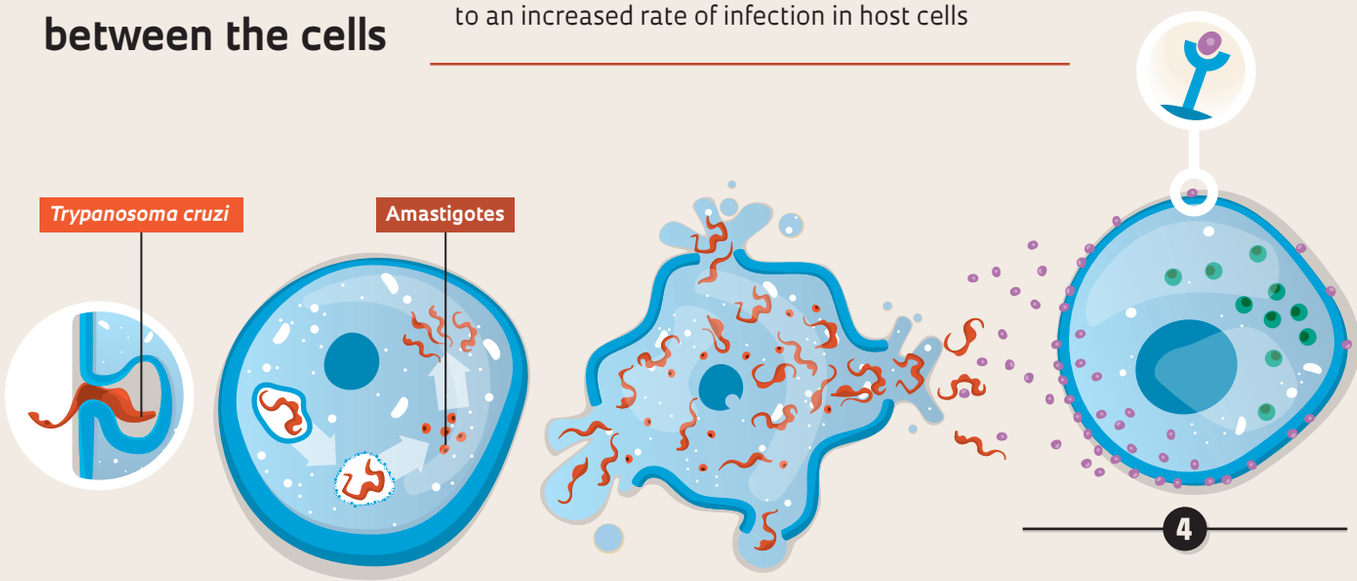
There has been a vast amount of scientific literature about these vesicles – approximately 3,500 articles on these structures have been published over the last five years – but many questions remain unanswered. The first question to be answered: what should these compartments full of protein be called? In an article published in February of this year in *Proteomics and Bioinformatics*, two researchers from La Trobe University, Australia observed that the so-called extracellular microvesicles were given different names based on their size, composition and origin. One type of vesicle, the exosome, which measures 30 to 100 nanometers in diameter, proved significant because of its multiple functions, which include transferring the HIV virus to target cells; the exosome now has its own website, www.exocarta.org, with almost 150 studies and 4,563 identified proteins by early September. There are also other types of vesicles, which for now are less important, such as the ectosome (a large membrane vesicle and apoptotic bubble that may be as large as 5,000 nanometers wide and is released by dying cells).

The *T. cruzi* vesicles are smaller, measuring 20 to 80 nanometers in diameter, and initially attracted little attention. In the late 1980s, upon identifying the vesicles, Marinei Gonçalves, Maria Júlia Manso Alves, Bianca Zingales and other researchers from Colli's team thought, along with others in this area, that the vesicles were just discarded material, although it had already

Battle

between the cells

Trypanosoma cruzi releases vesicles that lead to an increased rate of infection in host cells



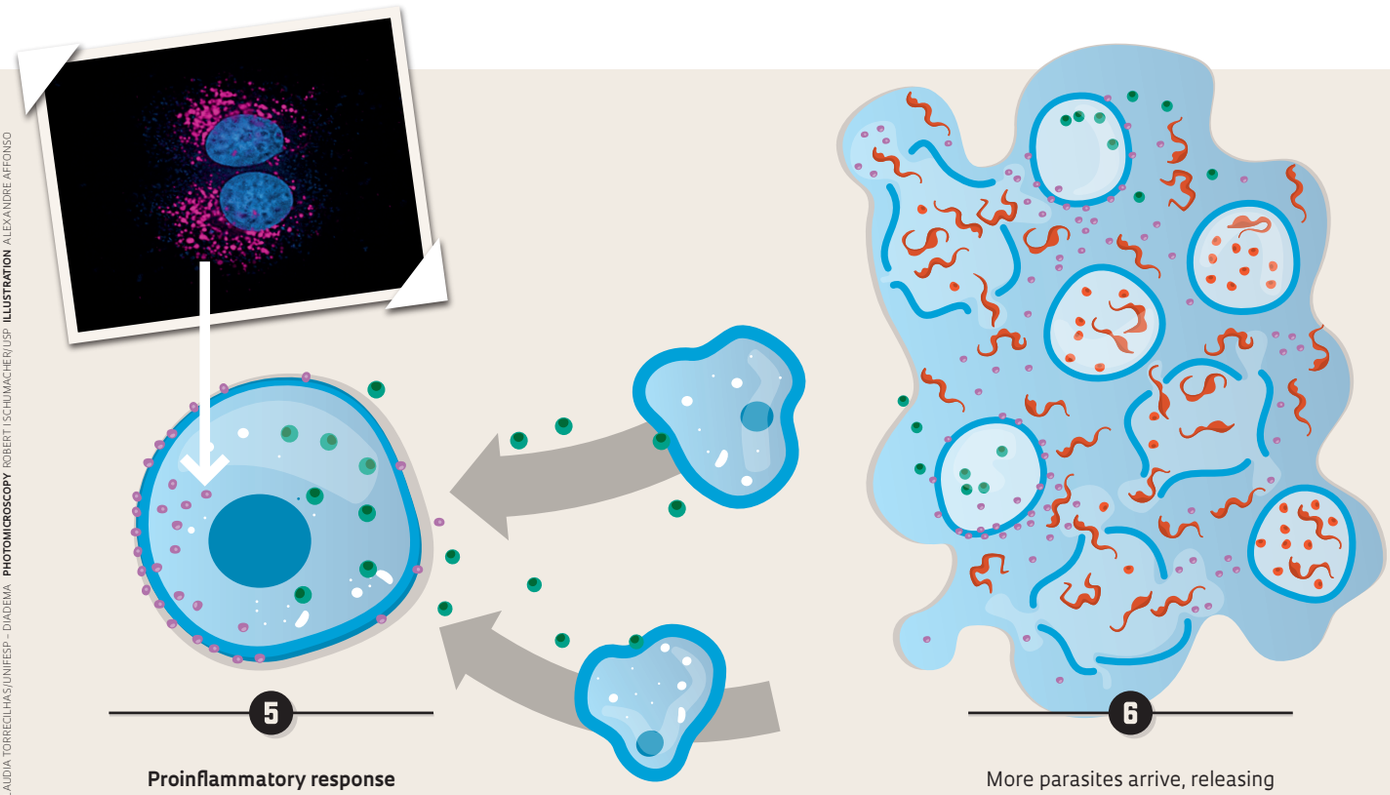
been observed that the more virulent varieties of *T. cruzi* released more vesicles that the less virulent ones.

In subsequent years, other studies and more sensitive equipment showed that vesicle proteins and lipids could facilitate host cell infection by the parasite. In her doctoral and post-doctoral work, performed under the direct supervision of Alves, Ana Claudia Torrecilhas found that vesicle proteins increased the amount and the activity of the parasites in the tissues and induced an inflammatory response in the host. In a study using mice, she observed that the vesicles facilitated the entry of the parasite into the heart cells and hastened the death of the animals.

Now, in a study to be published in October in *Microbes and Infection*, researchers from USP and UNIFESP note that nearly half of the vesicle content is made up of glycoproteins (proteins with sugars attached). One of these is trans-sialidase, an enzyme specific to this parasite, as well as others encoded by a super-family of genes called gp85 that contains approximately 700 active genes and 700 pseudogenes, also discovered in Colli's laboratory.

The parasites that cause sleeping sickness and leishmaniasis also release vesicles

These molecules are able to activate receptors from the outer membrane of immune cells such as macrophages, dendritic cells and lymphocytes. In turn, these receptors stimulate the production of nitric oxide and other molecules such as interferon-gamma, tumor necrosis factor and interleukin-12. These molecules increase the inflammatory response of the host cells and attract more cells, which destroy the parasites but also damage the organism's cells, thus facilitating invasion by the parasites that survived the battle or that quickly follow. After *T. cruzi* invades a cell, one thing it will never lack is a parasite. According to Colli, a single *T. cruzi* parasite divides rapidly. Within hours it can multiply 500-fold, causing the host cell to explode and releasing the parasites into the extracellular environment and the bloodstream, allowing them to reach other cells.



SOURCES WALTER COLLI/USP; ANA CLAUDIA TORRECILHAS/UNIFESP – DIADEMA **PHOTOMICROSCOPY** ROBERT I SCHUMACHER/USP **ILLUSTRATION** ALEXANDRE AFFONSO

OTHER PARASITES

"The *T. cruzi* vesicles can fuse with macrophages within 15 minutes," says Torrecilhas. Other researchers have found that two other groups of protozoa also release vesicles with similar functionality, although the content is most likely distinct. The first group contains the protozoa of the genus *Leishmania*, which causes leishmaniasis, a disease that has spread to 98 countries and registers 2 million new cases per year. The second group is *Trypanosoma brucei*, with subspecies (*T. b. gambiense* and *T. b. rhodesiense*) that cause sleeping sickness, a disease that affects approximately 70 million people in sub-Saharan Africa.

Two other parasites, *Plasmodium falciparum*, which causes malaria and is responsible for approximately 1 million deaths per year in Africa, and *Toxoplasma gondii*, which causes toxoplasmosis, act in a different manner: they invade host cells and quickly produce vesicles with proteins of the invading microorganisms, which are then released to alert other immune cells.

The researchers are attempting to learn as quickly as possible which protein vesicles from *T. cruzi* and other protozoa activate inflammatory responses in the host cells and how the organism's response is altered to benefit the parasites. It is now clear, however, that the vesicles function as a means of signaling or communicating the distance between the parasites and the host cells.

In recent years, several cellular structures have been recognized as being able to destroy or, conversely, benefit other cells. Other studies have shown that cells can produce structures called tunneling nanotubes, which measure from 50 to 200 nanometers in diameter and can have a length equivalent to the diameter of several cells. Through these tubes, a lymphocyte can bind to another lymphocyte and send nutrients or cellular components that, in the case of immune cells, help prolong – and usually win – the battle against parasites and tumor cells. ■

Projects

1. Interaction between *Trypanosoma cruzi* and host: ligands, receptors and determinants of intracellular development (nº 4/03303-5); Thematic project; **Coord.** Maria Júlia Manso Alves/IQ-USP; **Investment** 1,248,031.59
2. Vesicles released by T. cruzi: role of their components in infection (nº 4/08487-7); PhD scholarship; **Grant recipient** Ana Claudia Torrecilhas/IQ-USP; **Investment** R$ 204,190.40 (FAPESP)

Scientific articles

GONÇALVES, M.F. *et al. Trypanosoma cruzi*: Shedding of surface antigens as membrane vesicles. **Experimental Parasitology**. v. 72, n. 1, p. 43-53. 1999.
TORRECILHAS A.C. *et al.* Vesicles as carriers of virulence factors in parasitic protozoan diseases. **Microbes and Infection** (*in press*). 2012.
TORRECILHAS A.C. *et al. Trypanosoma cruzi*: parasite shed vesicles increase heart parasitism and generate an intense inflammatory response. **Microbes and Infection.** v. 11, p. 29-39. 2009.
SIMPSON, R. J. and MATHIVANAN, S. Extracellular microvesicles: the need for internationally recognized nomenclature and stringent purification Criteria. **Proteomics & Bioinformatics.** v. 5, n. 2, p. ii. 2012.
HOTEZ P.J. *et al.* Chagas Disease: the new HIV/AIDS of the Americas. **PLoS Neglected Tropical Diseases.** v. 6, n. 5, p. e1498. 2012.