

## Biology and Ultra-structure of *Trypanosoma cruzi*: a 90-years Old Challenge for Scientists

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Key words: *Trypanosoma cruzi* - biology - ultra-structure

When Dr Carlos Chagas first observed under the microscope the flagellate that he named *Schizotrypanum cruzi* (today known as *Trypanosoma cruzi*), in honor of his master and friend Oswaldo Cruz, he began to study the biology of this interesting microorganism (Chagas 1909). This unique fact in medicine, a single person's discovery and report of the clinical picture of a new disease, with a new etiological agent, its life cycle, its vectors and wild reservoirs, were later explained by himself (Chagas 1922): a process that he was able to understand after the intense and basic studies on the biology of this parasite, carried out at the Oswaldo Cruz Institute. In Chagas' own words "...quando no sangue periférico de uma criança febril, observamos o flagelado patogênico, de sua biologia já possuíamos noção completa, adquirida em demorados estudos anteriores" "...when we found the pathogenic flagellate in the blood of a children, we had already a complete notion of its biology, acquired under previous and detailed studies" (Chagas 1922).

Since then, the intriguing biology of this special parasite has been studied. Its morphology, depicted under the optical microscopy of Carlos Chagas and Garpar Vianna, was first investigated under an electron microscope by Dr Hertha Meyer (Meyer & Porter 1954). Dr Carlos Chagas Filho, Chagas' son who founded in Rio de Janeiro the famous Institute of Biophysics that later took his name, achieved to establish Dr Hertha Meyer in this Institute during the II World War. She started a whole school of cell biology of *T. cruzi* (as well as other tropical disease parasites), introducing *in vitro* cell culture of muscle and nerve cells, and the ultrastructure study of all the parasite developmental stages (reviewed in De Souza 1984). Since Dr Chagas' time, some basic questions still chal-

lenge the intelligence and curiosity of scientists all over the world. Why and how does the parasite evolve among three different morphological and functional states of differentiation over its life cycle (ama-, epi- and trypomastigotes)? Does the *T. cruzi* slender and stout forms found in blood stream of different host represent sexual dimorphism or anything else? How does *T. cruzi* enter so many different cells? How does at least part of the parasite population that infects a person or animal escape the vigorous specific immune response elicited in the host by the infection, leading to the finding of viable forms during the decades-lasting chronic development of the disease?

During the 90 years that followed since Chagas' classic report of a new disease, much information was obtained on the biology and ultrastructure of its causative agent. Recent reviews from Dr Wanderley De Souza (1984, 1995) summarize the knowledge in this area. A large knowledge was obtained on the understanding of its clonal composition (Heckert et al. 1994, Tibayrenc 1995), as well as in its population distribution and associated biological and molecular markers (Zingales et al. 1998). Another important field on parasite biology investigation concerns its metabolic pathways (reviewed in Cazzulo 1992), that engages the design of new drugs based on differences encountered. Key organelles from which the parasite gets energy such as acid calcisomes (DoCampo et al. 1995) and reservosomes (Figueiredo et al. 1994) are under intense study. The same happens with some pivotal enzymes for the parasite that are involved in energy production (such as glycosomal enzymes, purine and sterol biosynthetic pathways), detoxification (such as trypanothione reductase, ornithine decarboxylase and S-adenosylmethionine decarboxylase), or virulence (such as cysteine proteases) mechanisms. Second generation anti-trypanocidal drugs will certainly emerge from this field of research (Krauth-Siegel & Schoneck 1995, Cazzulo et al. 1997, Croft 1999, Urbina 1999) to substitute for the same two old and toxic drugs that are currently available for clinical use since the sixties (benznidazole and nifurtimox).

Concerning the mechanisms of invasion, despite a search in the MedLine database find 155 indexed references on this matter among the 5206 references found with the key-word *T. cruzi* in the last 45 years (about 3%), much efforts are yet to be done before a clear and consensual comprehension emerges. Since the classical studies of Dias (1934), Kofoid et al. (1935) and Hertha Meyer (Meyer & Xavier-de-Oliveira 1948), we know that, depending on the parasite strain, it takes less than 20 min to *T. cruzi* to enter a cell. It proliferates through binary division along 2 to 9 cycles and fully fills the host cells' cytoplasm in 4-5 days, leading to cell rupture and release of new trypomastigotes. After invasion, it takes 1-2 hr for the parasite to disrupt the vacuolar membrane (Burleigh & Andrews 1995), 2-3 hr to transform into amastigotes and enter the G1/G2 phases of the cell cycle, 24-44 hr to synthesize DNA. Amastigotes' generation time takes 8-15 hr, depending on the parasite strain, but cytokinesis is fast, taking 20-30 min (Hyde & Dvorak 1973). In all cell types studied up to now the same negative binomial distribution of infected-cells was found (Hyde & Dvorak 1973, Pécora et al. 1980), indicating that during the first 24 hr of parasite-cell contact, cells that are already infected are more susceptible to a second invasion, probably because cell surface charge changes after infection (Soeiro et al. 1994). *T. cruzi* can enter experimentally enucleated cells but these cells do not support the complete development of the parasite (Osuna et al. 1983). In primary cultures of macrophages and muscle cells (not in some established cell lineages) the parasite can only invade living cells (Barbosa & Meirelles 1995), indicating an active role of the host cell itself. A complex panel of multiple ligands and receptors involved in host cell recognition, adhesion and penetration emerge from many studies and were reviewed in recent work (Araújo-Jorge et al. 1992, Vermelho & Meirelles 1994, Burleigh & Andrews 1995, 1998, Coutinho et al. 1998). This multiplicity of molecules involved in *T. cruzi* invasion, delayed the theoretic development of parasite entry blockade through rationally designed drugs or vaccines. However, successful approaches for DNA based-vaccines were recently reported, (a) for a transialidase gene (Costa et al. 1999), and (b) for a trypomastigote surface antigen that is a target of anti-*T. cruzi* antibody and major histocompatibility complex class I-restricted CD8+ cytotoxic T-lymphocyte responses (Wizel et al. 1998).

Different strategies for invasion were found to be used when the parasite faces different host cell types: active penetration, active induction of receptor-mediated phagocytosis, and opsonin-medi-

ated phagocytosis. Especially important contributions came from *in vitro* studies using primary cardiomyocyte cell cultures, the most relevant host cell for the parasite *in vivo*. The main process of invasion occurs by active induction of phagocytosis, which is 70% sensitive to cytochalasin blockage (Barbosa & Meirelles 1995). Parasitophorous vacuole formation and phago-lysosome fusion occurred (Meirelles et al. 1986), but in muscle cells vacuole membrane formation is not strictly dependent on lysosome recruitment, as described for cell lineages (Tardieux et al. 1992). Activation of cardiomyocyte trypanocidal mechanisms under cytokine effect was very recently found, through NO synthase induction and NO secretion (Chandrasekar et al. 1998, Machado 1999).

Concerning the mechanisms that the parasite evolved to ensure safe survival for at least part of its population, under the vigorous pressure of a specific immune response elicited in the host by the infection, there is also much effort to be addressed. How the parasite renders almost invisible an infected cell in an immune-competent chronic infected person or animal? Parasite molecules that depress directly or indirectly the immune response or interfere with it (Frank 1992), modulation of the infected host cell adhesion molecules, such as MHC (Meckert et al. 1991, Stryker & Nickell 1995, Zhang & Tarleton 1996, La Flamme et al. 1997), integrin (Savino & Barbosa 1996) and lectin receptors (Kahn et al. 1995, Soeiro et al. 1999), alterations of the extracellular matrix (Andrade et al. 1989, Morris et al. 1990), or even parasite DNA incorporation in the cell genome (Teixeira et al. 1994) were already reported, but have not completed the puzzle. Recent work also re-analyzed important changes induced inside the infected cell, such as cell uncoupling by affecting gap junctions (Carvalho et al. 1994), cytoskeletal alterations (Pereira et al. 1993) and organelle rearrangement.

The round table that Dr Brener and I have the pleasure to introduce in this meeting will discuss some of the fascinating points that are still unknown in these fields. As throughout animal kingdom, life for a trypanosome is essentially a question of eat, survive and reproduce under different nutritional conditions and sources. *T. cruzi* has evolved an interesting biology, adapted to different environments in vertebrate and invertebrate hosts, and to store enough energy source to assure its reproduction and survival under so different situations. I am sure that answers and new questions will clearly appear during the presentations of the round-table components.

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