

Trypanosoma cruzi: A Molecular Deconstruction of Chagas Disease

Author: Marcelo Serrano Valeriano¹ ;Luis Gómez Peña²
 Research Professor, Universidad Nacional del Oriente¹
 Head of the Medicine Program, Universidad Nacional del Oriente²
Affiliation/Institution: Universidad Nacional del Oriente
Orcid Code: <https://orcid.org/0009-0004-0707-4579>¹
<https://orcid.org/0000-0001-6323-5075>²

Abstract:- The objective of this review article is to compile high-quality information related to the molecular aspects surrounding the parasite *Trypanosoma cruzi*, to obtain a molecular perspective.

The PubMed database was consulted, and this article included documents published within the last five years. Additionally, some older documents with a well-founded and significant basis were considered. Other search filters included the following keywords: molecular aspects of *Trypanosoma cruzi*, *Trypanosoma cruzi* immune. The general search results generated 3,286 documents; 2,761 were excluded due to being older than five years, and another 415 were excluded for not being review articles. This left a total of 110 articles for consideration, of which 25 were consulted to support and develop this article. The strategy for searching and selecting relevant articles involved reading the abstracts. The theoretical analysis-synthesis method was used for the search and processing of empirical, theoretical, and methodological information, aiming to break down the information to extract the essential aspects of the subject of study and progressively contribute to solving the scientific problem.

This article provides information on different aspects and offers a molecular perspective on the mechanism of action of *Trypanosoma cruzi* in the human body and how it fights for its survival at the expense of ours.

Keywords:- *Trypanosoma cruzi*, Molecular Aspects, Biological Cycle, Autoimmunity

I. INTRODUCTION

Chagas disease is caused by the parasite *Trypanosoma cruzi*, which damages the host as its biological cycle progresses within the organism by exploiting the energy of nucleated cells, where it carries out intracellular fission processes. "*Trypanosoma cruzi*, the etiological agent of Chagas disease, is transmitted by triatomine vectors during the invertebrate host's feeding process" (1).

Many factors determine the severity of this disease. The causative agent is a parasite with a still-unknown genetic diversity, creating an unfavorable clinical outlook. "*Trypanosoma cruzi*'s genetic diversity is divided into seven discrete typing units (DTUs): TcI–TcVI and Tcbat" (2). Furthermore, *Trypanosoma cruzi* possesses abilities that allow it to effectively evade the host's defense mechanisms when the parasite is detected. "To overcome host immunity, the trypanosome has an arsenal of evasion strategies linked to the alternation between proliferative intracellular forms and non-proliferative extracellular infectious trypomastigotes. The different morphological forms of the life cycle are associated with adaptive changes in gene expression" (3).

National and international contexts do not inspire confidence in conducting studies that conclude in comprehensive research. Additionally, as previously mentioned, the parasite employs various mechanisms to alter its genomic structure, making it complex to study using conventional clinical trials, requiring specific technologies. However, proteomic studies and assays may pave the way toward favorable conclusions.

➤ *This Leads to the Following Scientific Problem:*

What molecular characteristics of the genome and genetics of *Trypanosoma cruzi* can contribute to controlling Chagas disease?

This scientific question arises because theoretical gaps persist regarding Chagas disease, particularly within the scientific community. These gaps include the uncertainty of controlling an infectious disease. The ability to study the parasite at a molecular level provides highly precise information on its molecular aspects and physiology.

Research Hypothesis: Studies on the molecular aspects of the genome and genetics of *Trypanosoma cruzi* generate relevant information that contributes to controlling Chagas disease.

This hypothesis proposes and asserts that studies and investigations bring us closer to eradicating certain pathologies that severely harm the population. It also creates a circle of interest for the scientific community.

II. METHODOLOGY

A review was conducted to gather data from various virtual documents containing information relevant to the researcher. The PubMed database was consulted, and this article included documents published within the last five years. Additionally, some older documents with a well-founded and significant basis were considered. Other search filters included the following keywords: molecular aspects of *Trypanosoma cruzi*, *Trypanosoma cruzi* immune.

Initially, the general search results generated 3,286 documents. Of these, 2,761 were excluded due to being older than five years, and another 415 were excluded for not being review articles. This left a total of 110 articles for consideration, from which 25 were selected to support and develop this article. The strategy for searching and selecting relevant articles involved reading the abstracts.

The theoretical analysis-synthesis method was employed for the search and processing of empirical, theoretical, and methodological information. This method aims to break down the information search to extract the essential aspects of the study object and progressively contribute to solving the scientific problem.

III. RESULTS AND DISCUSSION

The following section details all the molecular aspects between the causative agent, *Trypanosoma cruzi*, and the human body, focusing on the interaction between the human defense mechanisms and how *Trypanosoma cruzi* fights to evade and degrade these defense levels. This analysis is based on an exhaustive compilation of information.

➤ *General Overview and Genomics*

Trypanosoma cruzi is an intracellular parasite that begins its biological cycle when transmitted indirectly by a vector. "Chagas disease, also known as Chagas-Mazza or American trypanosomiasis, is a systemic, chronic, and potentially life-threatening parasitic disease caused mainly by infection with the protozoan parasite transmitted by triatomines, *Trypanosoma cruzi*" (4). The disease is contracted through inoculation by a vector, commonly known as *Triatoma infestans* or vinchuca, which defecates into the wound caused by its bite, infecting the mammals it feeds on. "The metacyclic trypomastigotes released in the insect's feces during blood feeding enter the mammalian host through skin wounds or mucosal membranes and invade surrounding cells" (5).

Trypanosoma cruzi can be transmitted by different triatomine families, which vary morphologically and regionally. "*Triatoma infestans*, *Rhodnius prolixus*, and *Triatoma dimidiata* are the only competent vectors capable of transmitting *Trypanosoma cruzi* to humans. *Triatoma infestans* is predominantly spread in sub-Amazonian endemic regions, *R. prolixus* is reported in South and Central America, and *T. dimidiata* is reported in Mexico" (6).

The parasite undergoes various phases of metamorphosis to ensure its replication and survival. Epimastigotes differentiate into trypomastigotes and also into amastigotes, each with extracellular and intracellular roles. These stages exhibit distinct characteristics, ranging from motility to binary fission, which is the protozoan's reproduction mechanism. "The transformation of trypomastigotes into amastigotes, a process that takes place within the parasitophorous vacuole, involves a transitional stage resembling the epimastigote form. A similar process occurs at the end of the intracellular cycle when amastigotes transform into trypomastigotes" (7).

The genomic structure and characteristics of *Trypanosoma cruzi* are complex, limiting the extent of studies that can be conducted on it. "More than 50% of the *Trypanosoma cruzi* genome is composed of repetitive sequences, including numerous families of surface proteins (e.g., trans-sialidases, mucins, and mucin-associated surface proteins) with hundreds to thousands of members each" (8).

"*Trypanosoma cruzi* is a highly adaptable microorganism, undergoing various metabolic and structural variations in response to environmental stressors during its reproductive cycle. These variations occur in the different stages of its life cycle and include changes in morphology, kinetoplast location, surface protein expression, evasion mechanisms, and reproductive capacity" (9).

Chagas disease still lacks an effective cure or treatment and remains one of the most prioritized infectious diseases for research. The triatomine vectors responsible for infecting mammalian hosts are widespread throughout the Americas, making their eradication highly challenging. This vector is primarily nocturnal, feeding on unsuspecting mammals. Studying the genomic properties of *Trypanosoma cruzi* is difficult due to its complex structure and its remarkable adaptability to the environment and the biological stage it inhabits.

➤ *Genetic Diversity*

"The distribution of discrete typing units (DTUs) can be loosely defined by several parameters, including ecology, vector and host preference, geography, and association with diseases" (10). Chagas disease is transmitted by different types of triatomines, each specific to a region and associated with a distinct trypomastigote type. While they cause similar symptoms, the pathologies and consequences vary, and the DTUs are identified accordingly. These units are differentiated using alphanumeric codes ranging from TcI to TcVI and TcBat. These DTUs are widely recognized and accepted by the scientific community studying Chagas disease as reliable classification systems. "At a second satellite meeting held in Buzios in 2009, it was recognized that *Trypanosoma cruzi* strains should be classified into six discrete typing units (DTUs), TcI-TcVI" (11).

The DTU TcI is primarily found in North and Central America. This unit is transmitted by triatomine species such as *Triatoma*, *Panstrongylus megistus*, and *Rhodnius prolixus*. "TcI: North America, Central America, and South

America, Rhodnius, Triatoma, Mepraia, Meccus, Panstrongylus, indeterminate, and cardiac" (12). TcII is predominantly located in central and eastern Brazil, with vectors including Panstrongylus megistus and Triatoma infestans. It is associated with cardiomyopathy, megaesophagus, and megacolon. TcIII is uncommon in humans and ranges geographically from Venezuela to Argentina. TcIV is transmitted by Rhodnius, Panstrongylus, and Triatoma. TcV and TcVI are transmitted by Triatoma infestans and cause similar pathologies, including

cardiomyopathies, megaesophagus, and megacolon.

"It is important to understand both the full extent of the parasite's genetic diversity and the geographic range of the various lineages, as strain diversity may be associated with the variety of clinical manifestations and specific transmission cycles of the parasite" (13).

Figure 1 illustrates the geographic locations where each discrete typing unit is found across the Americas.

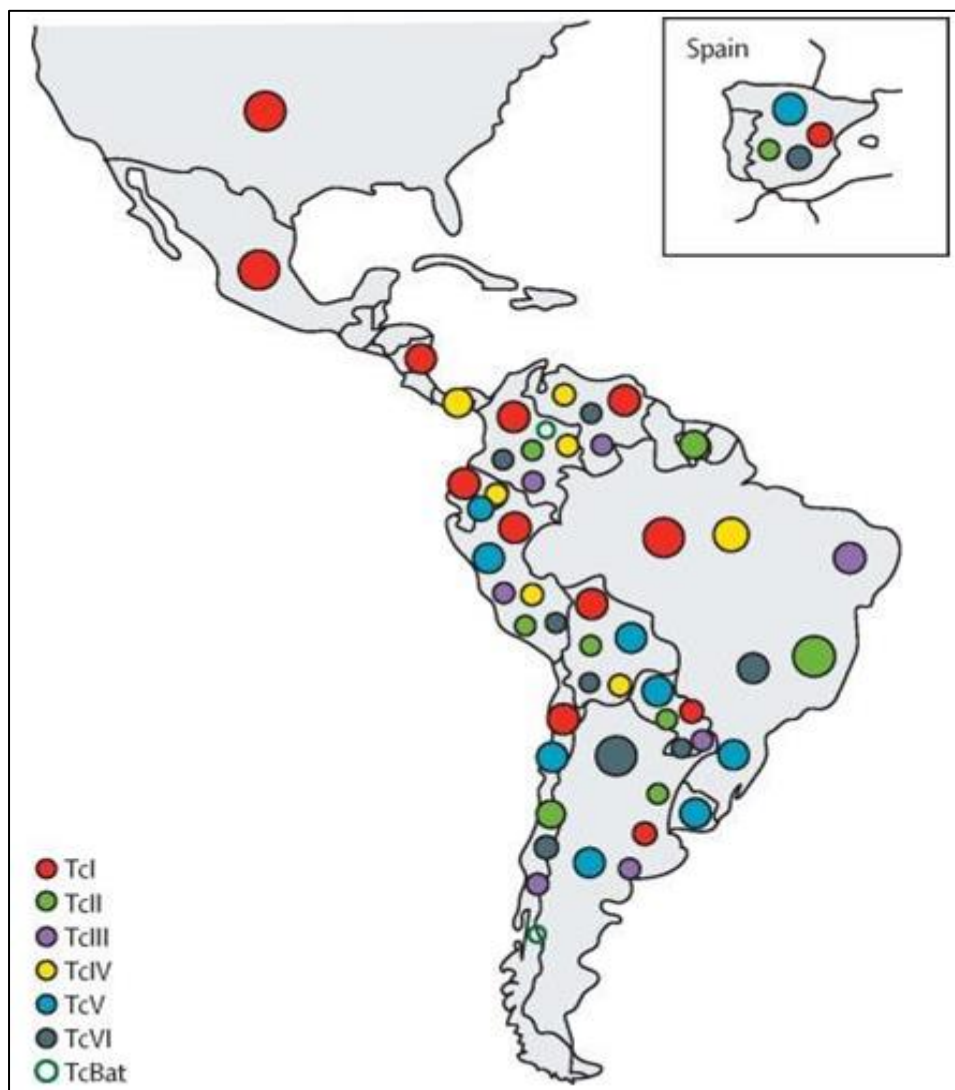


Fig 1. Geographic Distribution of Recognized DTUs

Source: **Adapted from** Luísa M D Magalhães PhD, Kenneth J Gollob PhD, Bianca Zingales PhD, Walderez O Dutra. Pathogen diversity, immunity, and the fate of infections: lessons learned from *Trypanosoma cruzi* human–host interactions

The genetic diversity of *Trypanosoma cruzi* creates a complex scenario when studying its molecular aspects, as each variant has a different mechanism of action in the host organism. Identification systems, such as DTUs, are useful and provide reliability for any study. It is important to understand that each variant will lead to a different clinical state.

➤ *Resistance to Cellular Oxidative Stress*

A fascinating characteristic of the *Trypanosoma cruzi* parasite is its ability to evade the phagocytic action mechanism of lymphoid cells. "*Trypanosoma cruzi* has evolved to such an extent that it utilizes a line of peroxidases to degrade reactive oxygen and nitrogen species released by the host's defensive cells" (14). Cellular oxidative stress is a critical process in the context of Chagas disease, playing a key role in both the infection's pathophysiology and the associated tissue damage. This disease, caused by the

protozoan *Trypanosoma cruzi*, generates an inflammatory response and immune dysfunction that promotes a pro-oxidant environment in the host's tissues. During infection, the host's immune system produces reactive oxygen species (ROS) and reactive nitrogen species (RNS) as part of the response to eliminate *Trypanosoma cruzi*.

Activated macrophages generate ROS through NADPH oxidase and RNS through inducible nitric oxide synthase (iNOS). Although these molecules have antimicrobial properties, their overproduction contributes to oxidative damage in the host's tissues. "When a host is infected by *Trypanosoma cruzi*, there is an attempt to control the infection by increasing ROS and NO levels, which leads to oxidative stress in both the acute and chronic phases of Chagas disease" (15).

It has been demonstrated that components produced by our own body cause damage to tissues and cells, as the excessive production of ROS results in oxidative damage to lipids and proteins. However, *Trypanosoma cruzi* can degrade these compounds; for example, the peroxidase TcGPXI is found in the cytosol and can degrade exogenous hydroperoxides. TcGPXII, located in the endoplasmic reticulum, inactivates lipid- hydroperoxidase, and the hemoperoxidase TcAPX disables the binding of hydroxyl anions to oxygen, in conjunction with triparedoxin peroxidases TcPX and TcMPX. "To survive and establish a productive infection in macrophages, intracellular parasites confined to phagolysosomes must overcome the intense oxidative burst induced by the activation of NADPH oxidase associated with the macrophage membrane and SLAMF1, which together are responsible for the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS)" (16).

Trypanosoma cruzi possesses many mechanisms to evade the body's natural defenses, increasing human and scientific interest in uncovering additional, undiscovered mechanisms. More in vivo clinical trials are needed to further understand these evasion mechanisms. Manipulating certain proteins in the structure of *Trypanosoma cruzi* through modified antigens could yield promising results, potentially compromising important functions such as motility and replication, among others.

➤ *Molecular Effects in the Host*

"The innate immunity, composed of phagocytes, especially macrophages, neutrophils, and dendritic cells, constitutes the first line of defense against *Trypanosoma cruzi* when it invades a vertebrate host" (6). When the parasite divides through binary fission, it is released into the extracellular space, causing the lysis of the cell that was undergoing this process. It transitions from amastigotes to trypomastigotes, at which point the innate immune response is activated with the release of lymphoid lineage cells. "Toll-

like receptors (TLRs) are the main mediators of the response; these transmembrane proteins are part of a group of molecules known as pattern recognition receptors (PRRs), associated with pathogen-associated molecular patterns (PAMPs)" (17).

Regarding cellular activity, the innate immune response is characterized by the release of a specific set of lymphoid lineage cells that generate the production of certain interleukins such as IL-6, IL-12, as well as some cytotoxic types like interferon (IFN), and tumor necrosis factor alpha (TNF- α), all aimed at counteracting *Trypanosoma cruzi*. "The antigens of *Trypanosoma cruzi* act as recognition signals and regulate the expression of pro-inflammatory cytokines in macrophages such as IL-1, IL-12, TNF- α , and IL-10" (6).

Both phenotypes 1 and 2 of macrophages play an important role in the host's innate immune response, acting as mediators of processes such as pro-inflammatory responses. "The activation of macrophages towards microbicidal M1 responses depends on macrophage receptors for pathogen-associated molecular patterns (PAMPs) and cytokines derived from T cells, such as IFN- γ and TNF- α , which induce the expression of iNOS and help control intracellular infection" (18).

An experiment demonstrating the critical role of interleukins and cytokines in counteracting parasitic load involves tests on mice to check if mice lacking or deficient in these compounds are more susceptible to mortality. "The histological analysis of the heart showed high parasitism and a diffuse, moderate to intense mononuclear inflammatory process. Ultrastructural studies revealed a large number of macrophages, as well as lymphocytes and undifferentiated cells" (19).

Another mechanism by which *Trypanosoma cruzi* evades the immune system is through its ability to circumvent the complement system processes using specific molecules. The complement system is part of the innate immune response, consisting of a set of proteins that interact through the activation of cell surface enzymes. "The complement system is one of the main components of the immune response and is made up of proteins that interact via activation of surface enzymes" (20).

This system has three activation pathways: the classical pathway, lectin pathway, and alternative pathway, each dependent on a specific activator. The activation of this system results in the production of enzymatic complexes like C3 and C5, leading to phagocytosis and pro-inflammatory anaphylatoxins like C3a, C4a, and C5a, which attract other immune cells. A more detailed explanation of this is presented in Figure 2.

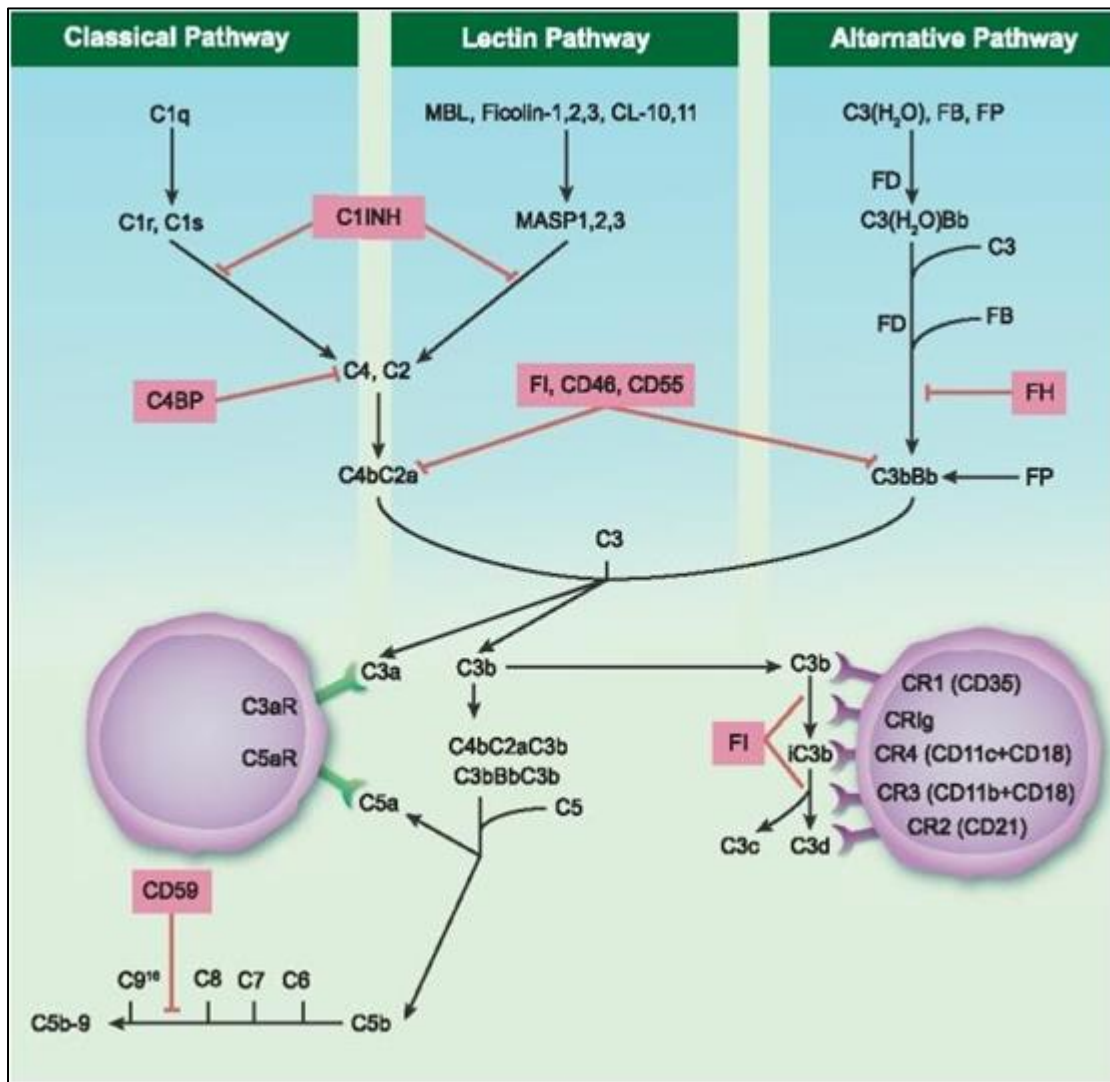


Fig 2. Mechanism of Action of the Complement System

Source: taken from R Lubbers, M F van Essen, C van Kooten, L A Trouw. Production of complement components by cells of the immune system

The different mechanisms involved in inhibiting the action of the complement system include "the surface expression of molecules such as glycoprotein 58/68 (gp 58/68), the complement regulatory protein of *Trypanosoma cruzi* (TcCRP), the tripomastigote decay accelerating factor of *Trypanosoma cruzi* (T-DAF), the calreticulin of *Trypanosoma cruzi* (TcCalr), the C2 transmembrane receptor inhibitor (CRIT), the secretion or acquisition of molecules from the host bloodstream, such as factor H (FH), and the extracellular vesicles of the host induced by *Trypanosoma cruzi* (EV)" (21).

When the parasite interferes with the normal state of the organism, many protective functions are activated. Interleukins are pro-inflammatory products generated in order to eradicate or reduce parasitemia, causing damage over the years to the host. Additionally, the complement system is activated, generating convertases to induce inflammation, lysis, and destruction of the causal agent. However, *Trypanosoma cruzi*, in its evolutionary adaptation to the organism, possesses various mechanisms that are triggered through certain proteins which degrade the

functions of the complement system, both in its initial phase and in the action of the convertases.

➤ *Bim - Sensitization and Trans-Sialidase*

Many of the synergistic mechanisms between a causal agent and the host are due to the interaction of mechanisms and molecules generated both by the host and by the foreign agent. This interaction often results in a benefit for the foreign agent. In this case, where *Trypanosoma cruzi* is capable of modifying some molecular components of the organism, it is of vital importance to understand the processes or mechanisms performed by the human body. "Among the strategies of evasion by *Trypanosoma cruzi*, the manipulation of the sialic acid (SA) signature of the infection stands out" (22).

"The Trans-sialidase (TS) genes are part of the largest multigene superfamily in *Trypanosoma cruzi*, composed of 1430 genes and 639 pseudogenes in the genome of the CL Brener strain" (23). "TS, transmitted by parasites (or injection of recombinant enzyme), induces several histological abnormalities such as apoptosis in central and

peripheral immune organs" (24).

Bim-sensitization is an important mediator of the immune response in the organism. "Bim is a potent pro-apoptotic member of the Bcl-2 family involved in various aspects of immune regulation, such as the negative selection of autoreactive thymocytes and the elimination of specific T cells" (25).

Unfortunately, there is limited information about the mechanism of action of Bim-sensitization, beyond the results obtained in an article that states, "Our data suggest that Bim is important for controlling parasitemia, and its absence impairs survival during the acute phase of *Trypanosoma cruzi* infection" (25).

The qualities of *Trypanosoma cruzi* and its relationship with the human organism will continue to generate discussion among the scientific community, as there is still much to study. However, each time a new study, trial, or clinical test is conducted, it provides important results for the scientific record. The molecular characteristics of such a well-adapted parasite present a challenge for any scientist studying it. Its changing morphology, biological cycle stages, genomic structure, genetic diversity, different evasion mechanisms against the body's natural defenses, and the inactivation enzymes and proteins in *Trypanosoma cruzi*'s arsenal create a sometimes discouraging scenario, but also provide another opportunity for further study.

IV. CONCLUSIONS

- The genetic diversity of the parasite *Trypanosoma cruzi* makes the study of each DTU different and requires a variable procedure.
- In the biological cycle of the parasite, the differentiation of one morphology to another occurs, which expresses its genomic structure in different ways.
- The parasite's evasion mechanisms against the body's protective mechanisms, such as innate immunity and oxidative stress, allow it to persist in the host organism until it ultimately overcomes it.
- *Trypanosoma cruzi* possesses different proteins and enzymes as inactivation mechanisms of the complement system, attacking and degrading functions both in the initial stage and in the final product, which are the convertases.

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