Implications of genetic variability of *Trypanosoma cruzi* for the pathogenesis of Chagas disease

Implicações da variabilidade genética do *Trypanosoma cruzi* na patogênese da doença de Chagas

Abstract

*Trypanosoma cruzi*, the etiological agent of Chagas disease, presents a high degree of intra-specific genetic variability, with possible implications for the clinical forms of the disease, like the development of cardiopathy, megaesophagus, and megacolon, alone or in combination. This tissue tropism involved in the pathogenesis of Chagas disease has still not been totally elucidated. Thus, the current review approaches key aspects of *T. cruzi* genetic diversity, the clinical forms of Chagas disease, and the infection of the host cell by the parasite and the immune response. Other aspects discussed here include the release of immunosuppressive factors by the parasite, acting in the host’s immune response pathways; host cell apoptosis inhibition; the pathogenesis of chagasic megaesophagus, which can be related to host-parasite interaction; and finally the association between megaesophagus and increased risk for the development of squamous-cell esophageal carcinoma. However, despite great advances in the understanding of this disease, it is still not possible to establish the true relationship between the parasite’s genetic variability and the clinical form of Chagas disease.

Biography of *Trypanosoma cruzi* and Chagas disease

*Trypanosoma cruzi*, a flagellate protozoan of the Kinetoplastida order, Trypanosomatidae family, is the etiological agent of Chagas disease, a frequent anthroponoosisis in Latin America.

The parasite’s life cycle alternates between vertebrates and insects, with different principal developmental stages in each host, with infective replicative epimastigotes (stage with kinetoplast and flagellar pouch in the anterior position of the nucleus) and metacyclic trypomastigotes (kinetoplast in the extremity posterior to the nucleus) in the hematophagous vector, replicative intracellular amastigotes (rounded form with short inconspicuous flagellum), and bloodstream trypomastigotes in the mammalian host.

Naturally acquired *T. cruzi* infections are initiated in the dermal layers or conjunctival mucosa by infective metacyclic trypomastigote forms that are transmitted by an infected hematophagous triatomine vector and are thereby transformed into amastigotes with the capacity to multiply by simple binary division. Next, they differentiate into trypomastigotes that are released by the host cell into the interstitium and reach the bloodstream and are thus able to invade cells from any tissue to produce a new cycle or be destroyed by host immune mechanisms. For example, the slender forms are lysed during the acute phase of the disease by the comple-
ment system present in the host cell plasma membrane.

The potential vectors for *T. cruzi* cover more than 130 species of triatomine insects from the Reduviidae family, five of which are epidemiologically more significant: *Triatoma infestans*, *Triatoma brasiliensis*, *Triatoma dimidiata*, *Rhodnius prolixus*, and *Panstrongylus megistus*. In addition to triatomines, the parasite can be transmitted in other ways, for example by blood transfusion, which is no longer an important route in the Latin American countries that have established effective routine serological control in blood centers. Transmission from mothers with Chagas disease to their fetuses has also been reported, with the incidence varying from less than 1% to 10%, even outside endemic areas. Outbreaks of acute Chagas disease can also occur due to oral transmission through contaminated foodstuffs like tea, sugar cane juice, or açai juice, described in the Amazon Region and more recently in the Brazilian States of Santa Catarina and Ceará. Although such events are worrisome, they are extremely rare and bear no relationship to the current situation with the control of vector-borne transmission by *Triatoma infestans*, the principal vector species in Brazil. Recently, Cortez et al. observed that the gp82 adhesion molecule expressed by *T. cruzi* is related to oral infection in experimentally infected mice. Thus, the molecule could also be associated with outbreaks due to oral transmission in humans. The factor accounting for the high infectivity of ingested parasites have still not been fully elucidated, but the occurrence of severe cases of infection leading to death in many infected individuals indicates a high pathogenicity of the parasites and their capacity to penetrate the gastric mucosa, despite the action of acid gastric contents. It has been demonstrated that digestion of the gp90 molecule specific to the metacyclic trypomastigote stage by pepsin increases the infectivity of HeLa cells due to the corresponding increase in gp82.

Chagas disease currently affects some 16 to 18 million individuals, concentrated in the poorest rural and urban areas of Latin America and representing one of the most serious public health problems in the region, where more than 100 million people are exposed to infection. Brazil (principally the Northeast, Southeast, and South) accounts for a major portion of these millions of infected individuals.

Chagas disease presents a variable clinical course, including an acute or initial phase which can be asymptomatic, oligosymptomatic, or symptomatic, with fever, adenomegaly, unilateral conjunctivitis (Romano’s sign), miocarditis, and meningoencephalitis; the initial phase can be fatal in up to 10% of severe cases, with higher mortality in children under three years of age due to meningoencephalitis. The chronic phase represents a latency period of 10-15 years, referred to as the indeterminate form, after which some 27% of infected individuals develop cardiac symptoms that can lead to sudden death, 6% develop digestive damage, and 3% can present involvement of the peripheral nervous system. Genetic variability of both the parasite and host can influence the clinical forms of Chagas disease.

In Brazil, 50% of 60% of individuals with Chagas disease present the indeterminate chronic phase. Some 2% of 4% these per year progress to the clinically defined severe chronic phase, with cardiac or gastrointestinal involvement, with progressive disease and more frequently involving males beginning in their thirties or forties.

In endemic areas, 15% to 20% of chronic Chagas disease carriers develop digestive tract motility, secretion, and absorption disorders, especially in the esophagus and colon. Such alterations appear first with slow transit and difficulty in emptying, followed by increased caliber of the affected organ and greater difficulty in emptying, characterizing the presence of megasphagus (grades I to IV) or megacolon. Involvement of the enteric nervous system appears to be an essential element and generally precedes the alterations in the motility of these organs.

The mechanisms determining the clinical forms of the disease (cardiac or digestive) have not been totally elucidated, but extensive research points to the importance of genetic diversity in the parasite and host immune response. The current review covers several of these aspects.

**Genetic diversity of Trypanosoma cruzi**

The geographic distribution of triatomines and vertebrate hosts associated with hematophagous insects’ preference for specific blood sources define two *T. cruzi* transmission cycles: a wild cycle involving different triatomines and wild animals and a domiciliary/peridomiciliary cycle in which domestic animals and humans can act as reservoirs.

Parasite clone populations are obtained from triatomine vectors or mammalian hosts. Patients in endemic areas are probably infected by multiple contacts with different triatomines, and the latter in turn can feed on the blood of various infected individuals, thus leading to the formation of multiclonal populations in hosts and vectors. Such populations differ in terms of their
The genetic variability of *T. cruzi* populations has been demonstrated repeatedly, based mainly on enzyme electrophoresis patterns (zymograms) and variation in the kinetoplast DNA (kDNA) 20.

Systematic enzymatic studies were initiated by Miles et al. 21 in *T. cruzi* samples isolated from different hosts in various regions of Brazil. Using electrophoresis methods, the authors described three classes of parasites, distinguished on the basis of specific patterns for a group of enzymes. These classes or “enzymatic lineages” were designated zymodemes (Z1, Z2, and Z3), each including all the parasites with identical electrophoresis patterns for the target enzymes. Epidemiological studies demonstrated that Z1 and Z3 were associated with the wild cycle and Z2 with the domiciliary cycle 22.

Later, Tibayrenc et al. 23 demonstrated the existence of marked polymorphism in *T. cruzi* isolated from different hosts from other regions of South America. By studying 15 enzyme-coding gene loci in *T. cruzi* clones isolated from different geographic areas, they observed much greater diversity and proposed 43 genotypes. This group of researchers also suggested the possibility of a clonal structure for the *T. cruzi* lineages based on the existence of clones without sexual interactions separated by a long evolutionary process. Thus, *T. cruzi* lineages are natural clones, with natural selection favoring only certain types of genetic patterns or combinations, and may result in a limited number of groups of isoenzymes represented by three principal clones 24.

The hypothesis of a correlation between the biological and phylogenetic variability of *T. cruzi* was confirmed by Revollo et al. 25 using various in vitro parameters, like MLEE (multilocus enzyme electrophoresis) and RAPD (randomly amplified polymorphic DNA), based on the genetic distances. Thus, the biological types (biodemes) were correlated with the zymodemes (Z1, Z2, and Z3) described by Miles et al. 22, with type II biodeme corresponding to Z2 and type III to Z1 19. The exception was type I, which presented a peculiar electrophoretic profile, not described previously 26 and was subsequently identified as Z2b.

The identification of nuclear DNA markers with very low evolutionary rates, like the RNAr 24S1 gene and the intergenic region of genes with minieons helped established the existence of two principal phylogenetic lineages within the *T. cruzi* taxon 27. These lineages were name by an expert committee based on biochemical and/or genetic evidence, as *T. cruzi* group I and *T. cruzi* group II. *T. cruzi* group I corresponds to Z1 and *T. cruzi* II to Z2 28. Isolates belonging to Z3 were not included in this classification. Subsequent application of the RAPD, MLEE, and karyotyping techniques indicated that group II was subdivided into discrete typing units (DTUs) displaying distinct geographic and ecological variations, with two groups found principally in the wild environment and three limited to the domiciliary transmission cycle 29,30. Further application of molecular techniques like RAPD and SSR-PCR (simple sequence repeat anchored primer-PCR) led to high homogeneity of *T. cruzi* lineages in chronic patients from different endemic areas of Brazil, thus suggesting that human hosts select specific varieties of the parasite clones from what are probably mixed infective populations 31.

These findings were recently confirmed by the RAPD technique based on *T. cruzi* isolates from patients with the cardiac and indeterminate forms of chronic Chagas disease, with low genetic variability observed between the parasites analyzed, thus clearly indicating the presence of a genetic group that was well correlated with *T. cruzi* populations 32.

Analysis of the parasite population structure based on the discovery of microsatellites in *T. cruzi* has shown that the percentage of multiclinal populations decreases progressively when comparing wild-cycle clones with those isolated from humans, thus reinforcing that human hosts select specific varieties of *T. cruzi* clones from mixed infective populations 33, due to the immune response. Such processes may be related to the reduction in genetic complexity of *T. cruzi* lineages isolated from chronic patients. According to another explanation, at the precise moment the parasite is isolated in the host, one population might predominate over the others, considering that in the parasite’s life cycle, blood trypomastigotes are not released according to a circadian rhythm 32.

The marked variability among chronic patients in the tropism of preferentially infected tissue (cardiac or digestive) and the parasite’s pathogenicity evokes the issue of whether the differential pathogenesis of the disease reflects the distinction between *T. cruzi* lineages 20.

Epidemiological studies have shown evidence suggestive of an association between *T. cruzi* group II and placental mammals and particularly human infection. This fact was observed in distant geographic areas like Brazil and Bolivia, where the majority of Chagas disease carriers were in the chronic phase and with infections limited to *T. cruzi* II 34,35. Additionally, among those with acute Chagas disease, a small propor-
tion was infected with *T. cruzi* I and/or presented *T. cruzi* I/II co-infections 35.

In Brazil, *T. cruzi* I appears to be found preferentially in the wild cycle of Chagas disease transmission, while *T. cruzi* II is heavily associated with the domiciliary cycle 36. This was confirmed recently by the demonstration of *T. cruzi* group II parasites infecting human tissues (esophagus, heart, and colon) in 25 patients with the cardiac or gastrointestinal forms of the disease residing in various cities in the State of Minas Gerais 37. Meanwhile, epidemiological evidence suggests that cases of chronic human infection in the north of South America and in Central America may be due to *T. cruzi* group I 38.

Lauria-Pires et al. 39, using isoenzyme assays and RFLP (restriction fragment length polymorphisms), observed mixed infections in both a patient with the cardiac form of Chagas disease and another with megaeosophagus. The patient with megaeosophagus showed at least three different populations of the parasite, demonstrating that a Chagas disease patient can be infected with genetically diverse populations of *T. cruzi*.

Although the isoenzymatic variability of *T. cruzi* populations has allowed the identification of three principal groups of zymodemes (Z1, Z2, and Z3), it is important to note that isoenzymes are markers expressed and limited to the control of the parasite’s life stage, requiring 10^9-10^10 parasites for typing, in addition to the fact that the culture conditions allow spurious results, thus limiting the routine use of this technique 16. Therefore, the first major stride with high-resolution techniques for studying *T. cruzi* genetic variability was the discovery of restriction fragment length polymorphisms in kinetoplast DNA 40.

Kinetoplast DNA (kDNA) is a plate-like structure including concatenated circular molecules called maxicircles (analogous to the mitochondrial DNA of other eukaryotes) and minicircles 41, comprising 20-25% of the parasite’s total DNA 1. Maxicircles are large molecules with few copies, containing genes for mitochondrial proteins and RNA. Minicircles are small molecules (1.4kb) with 20-25 thousand copies, associated with RNA editing. They present conserved regions with 20-25 thousand copies, associated with RNAr. Minicircles are small molecules (1.4kb) containing genes for mitochondrial proteins and Maxicircles are large molecules with few copies, comprising 20-25% of the parasite’s total DNA 1. The first studies on *T. cruzi* DNA polymorphisms reported the typing of kDNA minicircles by the RFLP technique, revealing parasite populations with identical or very similar minicircle patterns and referred to as schizodemes (the restriction profile of a lineage) 40. In the minicircles, four variable 330bp portions of kDNA are evaluated which evolved rapidly enough to produce differences between isolated parasites or clones, but not to the point of precluding a stable genetic identity in the lineage. Thus, each lineage and in some cases each clone in the same lineage presents a different schizodeme 40.

Another, more sensitive method to study kDNA polymorphisms is low stringency single specific primer-PCR or LSSP-PCR 44,45, in which the 330bp regions of the minicircles from previously amplified kDNA function as a mold for a second PCR with a single primer specific to it, under very low stringency conditions. The primer specifically hybridizes this complementary region and nonspecifically hybridizes multiple sites within the fragment in a sequence-dependent fashion, producing a highly complex class of reaction products visualized by electrophoresis to generate “genetic signatures”. For the first time, this technique, used with great success in the amplification of this region, produced a profile of the parasites present in the tissues of chronically infected patients 46,47.

Analyses of genetic profiles obtained from the variable kDNA region showed a high degree of intraspecific variability in parasite populations isolated from patients with the same clinical form of the disease, independently of clinical characteristics and disease stages, with unique genetic signatures obtained for each individual patient 48. This intense kDNA gene polymorphism may result from different factors, such as the presence of different classes of minicircle sequences for each parasite, high mutation rates in the hypervariable regions, reversible damage to kDNA sequences, or low identity between them 48.

Despite the indication of a differential distribution of lineages in the wild and domiciliary cycles of *T. cruzi* transmission and Chagas disease pathogenesis, based on the current studies on the parasite’s genetic diversity, it is still not possible to establish a precise association between the parasite’s lineages or clones and the clinical form of the disease.

**Tissue tropism: parasite-host interaction and immune response activation**

In Chagas disease, the existence of different *T. cruzi* clones may help explain the occurrence of areas in which the disease shows a higher incidence of cardiac or digestive involvement than in others 49, but other mechanisms are certainly
specific elements in *T. cruzi* variability and the clinical characteristics of Chagas disease is that the parasite populations represent groups of clones that can present symbiotic relations, while competing heavily for available resources. Experiments in mice have demonstrated that over the course of infection, various *T. cruzi* populations present different forms of blood trypomastigotes (slender, intermediate, and broad). They thus display important behavior differences between populations, with one or another type of morphology predominating.

One explanation for the correlation between specific elements in *T. cruzi* variability and the clinical characteristics of Chagas disease is that the parasite populations represent groups of clones that can present symbiotic relations, while competing heavily for available resources. Experiments in mice have demonstrated that over the course of infection, various *T. cruzi* populations present different forms of blood trypomastigotes (slender, intermediate, and broad). They thus display important behavior differences between populations, with one or another type of morphology predominating.

For example, populations with predominantly slender forms are more infective to human and mouse cells, developing earlier parasitemias, yet more sensitive to circulating antibodies. Meanwhile, populations with predominantly broader and less infective forms take longer to penetrate the cells, developing later parasitemias in mice, yet more resistant to the action of antibodies, thus remaining longer in the bloodstream. Cell tropism also differs between these forms, since slender trypomastigotes preferentially infect cells from the nuclear monophagocytic system in the spleen, liver, and bone marrow, while the broad forms display tropism for smooth, cardiac, and striated muscle cells.

The most probable explanation for this tropism involves molecular interactions on the cell surface between *T. cruzi* invader clones and the host tissues (cardiac muscle, myenteric plexus in the esophagus and colon, etc.). The initial idea of differential tissue tropism with an important role in the pathogenesis of Chagas disease gained new life when it was demonstrated that parasites with different genetic profiles can be found in different tissues (esophagus and heart) in the same patient. Studies with dually infected mice showed a strong correlation between the parasite population profile (JG or Col1.7G2) and type of lesion in cardiac and rectal tissues, as well as the persistence of one type (JG) in the chronic phase of the disease in a tissue previously infected with the other (CL-Brener clone).

During the acute phase of Chagas disease, the parasites are present in different organs, but in the chronic phase they damage specific organs, manifesting genetic heterogeneity among the parasites isolated, which may explain the degree of tropism for different organs. Both factors (host cell and parasite) appear to be involved in the infectivity and may form the basis for histotropism. Invasion of the non-phagocytic host cell by *T. cruzi* depends on parasite surface glycoproteins, and the ability of this infectivity varies among metacyclic trypomastigotes from different parasite populations. Such glycoproteins display differential activity in Ca\(^{2+}\) ion signaling.

The infective trypomastigotes of *T. cruzi* are mobile due to the presence of a flagellum and have the capacity to infect numerous nucleated cells during the acute phase of the disease, establishing residence in the cell cytoplasm and differentiating into replicative amastigotes. Initiating communication between the infective forms of the parasite and mammalian cells requires contact between parasite ligands (soluble or around the membrane) and host cell receptors.

Recognition by the host cell appears to be mediated by various parasite surface glycoproteins coded by multi-gene families, including gp82, gp90, and gp35/50, allowing interaction with different receptors and extracellular matrix molecules. Interaction between these ligands and their purported but unidentified receptors in the host cell appears to directly or indirectly affect the ability of gp82 to engage its receptor and trigger the signaling mediated by Ca\(^{2+}\) ions. The levels of expression of parasite ligands may be an important determinant of tissue tropism. This signaling mediated by Ca\(^{2+}\) ions is triggered by oligopeptidase B (OB) from the trypomastigote form, which activates phospholipase C (PLC) in the host cell and promotes the generation of inositol triphosphate (IP3) and the subsequent mobilization of Ca\(^{2+}\) ions from the intracellular reserves, such as the endoplasmic reticulum. Another entry pathway for the parasite is related to the release of bradykinin (active kinin) from the host cell kininogen, induced by the cruzipain present in the flagellar pouch of the trypomastigote, which binds to B\(_2\)R receptors, stimulating the PLC to generate IP3 and elicit Ca\(^{2+}\) ions from the intracellular reserves.

Recently, a conserved FLY (VTXXNVFLYNR) domain was described that is present in all members of the trans-sialidase/gp85 glycoprotein family located on the surface of trypomastigote forms that increase the efficiency of host cell invasion by binding to cytokeratin 18 from cultured epithelial cells. This process leads to its dephos-
phorylation and reorganization, activating the ERK 1/2 signaling cascade, which culminates in a nine-fold increase in the number of parasites per cell 56.

The increase in the concentration of Ca2+ ions released in the host cell cytoplasm is accompanied by a quinesin-dependent local recruitment of lysosomes towards the cell plasma membrane through microtubules that merge with the membrane to form the lysosome-derived parasitophorous vacuole 57. Exposure of trypomastigotes to the acid environment of the lysosomal vacuole facilitates the activity of the *T. cruzi* pore-forming molecule (Tc-Tox), which facilitates the rupture of the vacuole. Additionally, the low pH acts as an important activator for trypomastigotes to differentiate into amastigotes, which begins inside the vacuole and ends in the host cell cytoplasm 3.

After the parasitophorous vacuole ruptures, trypomastigotes are released into the cytoplasm and conclude the differentiation into amastigote forms, which begin to divide approximately 24 hours after infection 3. These forms multiply by simple binary division every 12 hours for a total of nine generations, differentiate into trypomastigotes by the elongation process, and are released into the intercellular space due to the rupture of the host cell 2, infecting neighboring cells or reaching the bloodstream, where they can infect cells from other tissues or be ingested by the insect vector and complete the life cycle. Only the trypomastigote forms are capable of rupturing the vacuole membrane, while the epimastigote forms are destroyed inside it. It has been demonstrated that the membrane surrounding the parasitophorous vacuole differs according to the parasite’s developmental phase 57.

Southern blot DNA analysis of macrophage lineages transfected with *T. cruzi* trypomastigote forms, once inside the host cell, showed band patterns formed by hybridization with the minicircle kDNA probe, thus demonstrating horizontal transfer of sequences of this molecule that are integrated into the host cell DNA by natural infection. Immunofluorescence assays suggested that the integration of kDNA into the genome of macrophage clone lineages leads to alteration of the host cell expression. Thus, kDNA integration sites associated with modifications in the host genome may represent a critical biological element in the host-pathogen interactions leading to the chronic clinical manifestations of Chagas disease 58.

According to Teixeira et al. 59, persistent, lifelong chronic infection in the Chagas disease patient is sufficient to allow the occurrence of new kDNA integration events in multiple genome sites. In some patients, accumulated mutations caused by insertion in the host genome may trigger the immune system’s rejection mechanism in the heart and other organs, leading to a gradual increase in the number of host protein alterations.

The nature of the variability of Chagas disease in humans probably results from the variation in the immune response efficiency in different individuals; for example, efficient immune responses control the parasite level, limiting the tissue damage, while inefficient responses fail to adequately control the parasite burden, thus leading to persistent inflammatory reactions and a more severe clinical condition 60.

The cellular immune response is an important factor in the control of *T. cruzi* in all stages of the disease, and an immune imbalance can result in increased blood and tissue parasitism 61. The correlation between the frequency and intensity of the tissue inflammatory process in the presence of the parasite can be observed especially in cases of advanced megasophasagus. This was observed by Lages-Silva et al. 62 in 80.8% of the tissues examined in megasophasagus, where they found an association between the presence of *T. cruzi*, its antigens, or genomic fragments detected by immunohistochemistry and PCR. However, these same authors also observed the parasite’s presence in tissues without inflammation, which in such cases may be due to recent invasion in which there was still no focal inflammatory response or no degree of host immunosuppression 63.

According to one hypothesis, immunosuppressive *T. cruzi* excreted-secreted antigens (ESA) are involved, serving to maintain chronic infections, acting as regulatory (activation/inhibition) factors for the host immune cells (B and/or T-cells, macrophages, and dendritic cells). Such antigens are molecules belonging to the glutathione S-transferase (GST) super-family, or so-called Tc52, which is expressed by intracellular amastigotes. Elevated levels of this protein in the bloodstream of mice experimentally infected with *T. cruzi* occur during the acute phase of the disease and are associated with the decreased T-cell response to the mitogen 64.

Under experimental conditions, Tc52 appears to be relatively silent, immunologically, during the early acute phase. This protein’s molecule presents discrete domains that can minimize its antigenicity, whereby it fails to stimulate significant antibody levels and lymphocyte proliferation. A 28kDa fragment (Tc28k) located in the carboxy-terminal portion of the Tc52 protein was identified with the capacity to inhibit T-cell activation 64. This peptide is related to low secretion of IL-2 and IFN-γ and may allow the parasites to
escape the immune surveillance and grow unimpeded due to the blockade of normal immune responses. In such cases, the host can become susceptible to opportunistic infections 64.

Another proposed mechanism for the parasite’s action on the host immune system is apoptosis, by means of the release of factors that kill the immune system’s cells by activating the programmed cell death machinery. A principal surface glycoconjugate from T. cruzi, GIPL, has been shown to induce macrophage apoptosis through its lipid ceramide domain 65. Paradoxically, there is evidence that the parasites can inhibit host cell apoptosis, such that parasite-derived molecules can interfere in cell growth. For example, T. cruzi trans-sialidase can impede nerve cell apoptosis, since it can activate expression of the bcl-2 gene, leading to the protection of rat pheochromocytoma PC12 cells (a lineage that displays various neuronal characteristics) against apoptosis induced by growth factor deprivation 65. It is thus reasonable to suggest that among the molecules released by the parasite, those conferring a selective advantage to it represent potential targets for the development of treatment strategies to moderate the host immune system dysfunction 65.

Although the abundant heterogeneous glycopolymers on the surface of T. cruzi trypomastigotes possess affinities with extracellular matrix proteins from mammalian cells and thus guarantee access to the host cell during the acute phases of infection, it is still not clear which signaling pathways induced by the parasite are integrated to coordinate the invasion, support intracellular replication, or influence tissue tropism 3.

**Chagas disease megaesophagus and its association with esophageal carcinoma**

Megaesophagus is one of the clinical manifestations of the digestive form of Chagas disease, occurring in some 2% to 8.8% of patients in the chronic phase in Central Brazil 66, developing in patients infected with T. cruzi clones that present preferential tropism for esophageal tissue muscle cells. One of the serious late consequences of megaesophagus is the increased risk of these patients developing esophageal carcinoma 49. Our research group has studied these diseases with a specific focus on potential molecular markers for early diagnosis of carcinoma of the esophagus in patients with chagasic megaesophagus, as well as a possible association between T. cruzi genetic diversity and megaesophagus.

Megaesophagus occurs as a consequence of achalasia, characterized by the destruction or absence of intramural nerve plexi in the esophagus due to T. cruzi infection and leading to loss of peristalsis in the esophageal body and the lack of opening of the lower esophageal sphincter in response to swallowing, thereby causing esophageal stasis (stagnated food passage), accentuated initial discoordination, and progressive dilatation and decreased contractile capacity of the organ. Chagas disease is the only proven etiological factor for megaesophagus. However, the cause of megaesophagus is unknown in the rest of the world and has been associated with a viral etiology, genetic influence (association with HLA), and autoimmune processes 67,68,69. Dysphagia (difficulty in swallowing) is the most prominent clinical symptom of megaesophagus and generally shows a long evolution, installing progressively, initially for solid foods, later for semi-solids, and later also for liquids 49.

Patients with megaesophagus display mucosal inflammation due to esophageal stasis and the action of bacteria present in stagnated foods, growing in suspension in the lumen. Such factors can favor the development of epithelial dysplasia, culminating in chronic esophagitis affecting the entire esophageal mucosa. This may be the first step in the development of squamous-cell esophageal carcinoma, which is 33 times more frequent in these patients than in the general population 70 and whose incidence varies from 2% to 8.6% 49,71.

Fein et al. 72 suggested the action of the microbiota on the esophagus based on an experimental study of esophageal carcinogenesis. Later, patients with advanced megaesophagus were shown to have a high concentration of bacteria in the stasis liquid with the capacity to reduce dietary nitrates into nitrites and N-nitrous compounds with DNA mutagenic properties. Bacterial concentration was correlated with the degree of esophageal dilatation 73,74. Chronic stasis thus favored the prolonged persistence of mutagenic compounds in contact with the mucosa, thus explaining the increased risk of esophageal cancer in patients with megaesophagus 74.

Squamous-cell carcinoma of the esophagus has a multifactor etiology, including environmental exposure, dietary habits, chronic esophagitis, cultural influences, and possibly genetic predisposition 75. Esophageal carcinoma generally affects patients with a long history of dysphagia, with late diagnosis, when the symptoms have remained disguised. This delay allows much greater tumor growth, to the point of obstructing the dilated esophagus, leading to a poor prognosis. Early diagnosis of the cancer can be aided by routine endoscopic examination of patients with megaesophagus 49.
Although research on genetic alterations in chagasic megaesophagus is still limited, one study reported a mutation in the TP53 tumor suppressor gene and increased expression of its protein in the mucosa of patients with advanced chagasic megaesophagus and in tumor mucosa associated with advanced megaesophagus, indicating the presence of mutant p53 protein and propensity to malignant transformation. Chino et al. also observed increased expression of p53 protein both in carcinoma associated with idiopathic achalasia and in dysplasia, suggesting that dysplastic alterations in patients with advanced achalasia may be related to increased risk of tumor development. These authors also detected loss of p16 protein expression, which together with p53 participates in cell cycle regulation in the invasive parts of the carcinoma and in situ carcinoma in relation to the areas with dysplasia, hyperplasia, and normal epithelium.

Our research group recently described the occurrence of aneuploidies (principally trisomies) in chromosomes 7, 11, and 17 and deletion of the TP53 gene in the esophageal mucosa in individuals with chagasic megaesophagus with no history of cancer, and frequent increased involvement of these alterations in patients with esophageal carcinoma. Although limited, these data suggest that such alterations may be involved in the initial phases of malignant transformation, increasing the risk of developing squamous-cell carcinoma of the esophagus in this type of disease.

Final remarks

Although Chagas disease was discovered approximately nine decades ago, its pathogenesis and the mechanisms in the parasite-host relationship are still not well understood. It is known that the pathogenesis of megaesophagus may be related to host characteristics as well as those of different T. cruzi clones.

Throughout these nine decades, much has been discovered on the biological characteristics involving Chagas disease, such as: T. cruzi genetic diversity and the mechanisms of the parasite’s entry into the host cell as regards the signaling pathways involving surface molecules on both the parasite and host cell, allowing the parasite to reach the cytoplasm and to replicate; factors released by the parasite that elicit different inflammatory responses, that may or may not lead to host cell apoptosis; mechanisms in the integration of T. cruzi kDNA fragments into the host cell genome, functioning as mutations that lead to changes in the host cell phenotype, evoking the immune system’s rejection of cells containing the mixture of the expression of its proteins with those of the inserted kDNA; and evidence that different clones present differential tissue tropism, depending on the biological form of the parasite during infection.

Due to the intraspecific diversity of T. cruzi populations isolated from patients presenting the same clinical form of Chagas disease, at present it is still not possible to establish an association between the parasite’s genotype and the manifestation of the disease. It will be necessary to search for other genetic markers in the nuclear genome or kDNA in order to better characterize the role of T. cruzi in the pathogenesis of Chagas disease.
Resumo

O Trypanosoma cruzi, agente etiológico da doença de Chagas, apresenta elevado grau de variabilidade genética intra-específica, com possíveis implicações na forma clínica da doença, como o desenvolvimento de cardiopatia, do megaesôfago e do megacôlon de forma isolada ou em associação. Este tropismo tecidual envolvido na patogênese da doença não está totalmente esclarecido. Assim, nesta revisão são abordados alguns aspectos referentes à diversidade genética dos parasitas isolados, às formas clínicas da doença de Chagas, ao processo de infecção do parasita na célula hospedeira e resposta imune. Outros aspectos também são enfocados, como os fatores imunossupressivos liberados pelo parasita que atuam na regulação das respostas imunes, a inibição da apoptose da célula hospedeira, assim como da patogênese do megaesôfago chagásico que pode estar relacionada à interação hospedeiro-parasita e sua associação com risco aumentado para o desenvolvimento do carcinoma epidermóide do esôfago. Porém, apesar dos avanços no entendimento desta doença, ainda não é possível estabelecer o verdadeiro perfil da variabilidade genética do parasita com a forma clínica da doença de Chagas.

Acalasia Esofágica; Trypanosoma cruzi; Variação (Genética); Doença de Chagas

Contributors

F. S. Manoel-Caetano participated in the literature search and drafting of the manuscript. A. E. Silva collaborated in the reading and review of the manuscript, inserting modifications in the paper’s structure and content, based on the bibliographic references utilized.

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