Trypanosoma cruzi I diversity: Towards the need of genetic subdivision?
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**A R T I C L E   I N F O**

Article history:
Received 23 February 2011
Received in revised form 28 March 2011
Accepted 1 April 2011
Available online 9 April 2011

Keywords:
Trypanosoma cruzi I
DTUs
Genetic variability
Mini-exon
Microsatellites

**A B S T R A C T**

Trypanosoma cruzi the aetiological agent of Chagas disease, a complex zoonoses that affects the American continent is a genetically variable parasite subdivided into six Discrete Typing Units (DTUs). T. cruzi I is the most prevalent DTU affecting the northern countries of America with sporadic cases in the southern countries. T. cruzi I has shown great genetic diversity showing plausible subdivisions needed for this group. Recently, Tcl has gained novel importance because of the lately discovered relation with cardiomyopathy manifestations that raises the importance of establishing subdivisions within this DTU.

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1. Introduction

Chagas disease caused by Trypanosoma cruzi is a complex systemic disease that affects over 10 million people in the American continent (WHO, 2007). The nomenclature of T. cruzi has recently been amended to reflect the high phenotypic and genotypic variability of this parasite. Six Discrete Typing Units (DTUs) have been proposed based on various features of T. cruzi (Zingales et al., 2009). Since 1999, when an international consensus on the nomenclature of T. cruzi was reached for the first time (Anon, 1999), two completely different genetic lineages have been discussed. Through isozyme studies, lineage Tcl has been revealed to be a homogeneous genetic group with dimorphisms in the mini-exon gene and in the divergent domain of the rDNA of parasites (Miles et al., 1977; Souto et al., 1996; Tibayrenc and Ayala, 1988; Zingales et al., 1998). After ten years of research aimed at the verification of Tcl homogeneity, several molecular markers have demonstrated the existence of considerable genetic variability within T. cruzi I. Of note, the first classification of T. cruzi I was a lineage linked to the sylvatic transmission cycle of Chagas disease. Since then, a large number of molecular markers have shown high genetic variability in T. cruzi I (Cura et al., 2010; Falla et al., 2009; Herrera et al., 2007, 2009a,b; Llewellyn et al., 2009a, 2011; Mejía-Jaramillo et al., 2009a; Ocaña-Mayorga et al., 2010; Salazar et al., 2006; Spotorno et al., 2008; Triana et al., 2006).

2. T. cruzi I and first subdivisions

Pioneering work by Brisse et al. (2001) presented trees constructed using Random Amplified Polymorphic DNA (RAPD) and Multi Locus Enzyme Electrophoresis (MLEE) analyses, and these demonstrated a significant amount of polytomies in the Tcl group. Barnabé et al., 2000 analysed a significant set of T. cruzi stocks from a wide ecogeographical range demonstrating the high genetic variability in T. cruzi based on 22 loci using MLEE, in this tree the branches within Tcl shows a divergent topology suggesting the first attempts in the path to elucidate the genetic diversity within this formerly neglected DTU. Other reports from Colombia and Venezuela uncovered a large number of isolated groups of Tcl using techniques such as MLEE (Montilla et al., 2010; Saravia...
et al., 1987; Widmer et al., 1984). Later, the analysis of data from LSSP-PCR and karyotyping of the variable region of kinetoplast DNA (kDNA) demonstrated an association between genetic variation and the geographical origin of isolates (Salazar et al., 2006; Triana et al., 2006). These two studies revealed that Tcl is not homogenous; however, because of the high number of copies of kDNA and genetic divergence of each copy, this assessment was not robust enough. According to the results published by Fernandes et al. (1998, 2001) and O’Connor et al. (2007), the intergenic region of the mini-exon gene was determined to be a suitable marker for the assessment of Tcl genetic variability. In 2007, four genotypes formerly named haplotypes present in Colombian isolates of Tcl were reported, along with their relationship with the transmission cycles of Chagas disease (Herrera et al., 2007, 2009b). Genotype Ia, which is associated with human infection and domestic vectors, is present in a specific pattern at positions 28 with an Adenine and a motif TGTGTG at positions 35–40; genotype Ib, which is associated with human infection and peridomestic vectors, contains a T-C substitution at position 44; genotype Ic, which is not very robust due to the low number of isolates correlated with domiciliary vectors, is characterised by a TATATA sequence at position 35–40 and genotype Id, which is associated with the sylvatic cycle is characterised by a deletion of nine nucleotides at positions 15–23 of the microsatellite region of the mini-exon gene. According to the characteristics and the insertions, deletions and SNPs identified for each genotype, specific primers were developed to allow for the differential identification of three of the four genotypes (Ia, Ib and Id) (Falla et al., 2009). This work pioneered the study of genetic diversity of the Tcl group and prompted the scientific community to continue the search for other molecular markers to corroborate the genotypes of Tcl identified using the mini-exon gene. Subsequent work using the cytochrome b gene revealed the presence of two phylogenetically robust subgroups. The first subgroup is associated with human infection and vectors from Chile, Brazil and Colombia, whereas the second subgroup is mainly associated with caviomorph reservoirs, demonstrating the importance of these mammals in the evolution of trypanosomes (Spotorno et al., 2008). In 2009, Llewellyn et al. reported 135 Tcl samples from various geographical regions endemic for Chagas disease in Latin America. The samples were subjected to microsatellite analysis and also demonstrated that the Tcl group was highly diverse besides the work developed in Ecuador using a significant set of Tcl isolates unravelling the genetic structure of Tcl featuring two genotypes associated to the domestic/peridomestic cycle and the sylvatic cycle of transmission (Ocaña-Mayorga et al., 2010). In Brazil, a new Tcl genotype has been described as TclBat due to its relatedness with bats from the genus Myotis and Noctilio based on Histone 2B, SSU rDNA and Cytb (Marcili et al., 2009a). This genotype is very related to Tcl and also to the hybrid DTUs TclIII and TclIV when the concatenated analysis was developed. This suggest and corroborates the high genetic diversity displayed by Tcl taxon and could show clues about the evolutionary history of T. cruzi I with the host-relationships features.

Both sequence variation and microsatellite markers demonstrate that genotypes involved in human infections in Venezuela and Colombia are distinct from sympatric sylvatic variants. This is intriguing as it was recently demonstrated that the main vector in the region, Rhodnius prolixus, demonstrates panmixia between domestic and sylvatic environments across the western endemic region in Venezuela (Fitzpatrick et al., 2008). In 2009, when the nomenclature of T. cruzi was revised for the second time (Zingales et al., 2009), no divisions in Tcl were created, based on the argument that studies with other markers should be completed. Recently, Cura et al. (2010) analysed a set of 105 Tcl samples from most of the endemic regions of Latin America and North America and confirmed the previously reported Ia, Ib, Ic and Id genotypes. Cura et al. also reported the existence of genotype Ie, which can be found in Bolivia, Argentina and Chile and is characterised by a 44-bp motif in the intergenic region of the mini-exon gene detected using specific primers designed for this purpose. In addition, it was determined that this variant is closely related to domestic cycles in Argentina, Bolivia and the sylvatic cycles of Chile, where Mepraia gajardoi and Mepraia spinolai play an important role in the transmission cycle of Chagas disease (Fig. 1). All these studies were pioneers in elucidating the genetic variability within Tcl (Table 1).

**Table 1** Trypanosoma cruzi I subdivisions reported in the literature based on different molecular markers.

<table>
<thead>
<tr>
<th>Refs.</th>
<th>Molecular marker</th>
<th>Number of subdivisions</th>
<th>Association of subdivision</th>
<th>Geographical setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salazar et al. (2006)</td>
<td>LSSP-PCR (kDNA)</td>
<td>2</td>
<td>Geographical clustering</td>
<td>Colombia</td>
</tr>
<tr>
<td>Herrera et al. (2007)</td>
<td>Mini-exon gene</td>
<td>4</td>
<td>Transmission cycles</td>
<td>Colombia</td>
</tr>
<tr>
<td>Herrera et al. (2008)</td>
<td>Cytochrome b gene</td>
<td>2</td>
<td>Transmission cycles</td>
<td>Chile</td>
</tr>
<tr>
<td>Herrera et al. (2009b)</td>
<td>Mini-exon gene</td>
<td>5</td>
<td>Transmission cycles</td>
<td>America</td>
</tr>
<tr>
<td>Llewellyn et al. (2009a)</td>
<td>Microsatellites</td>
<td>2</td>
<td>Transmission cycles</td>
<td>America</td>
</tr>
<tr>
<td>Marcili et al. (2009b)</td>
<td>Histone 2B, SSU rDNA and Cytb</td>
<td>2</td>
<td>Hosts (Tcl and TclBat)</td>
<td>Brazil</td>
</tr>
<tr>
<td>Cura et al. (2010)</td>
<td>SL-IR Mini-exon gene</td>
<td>5</td>
<td>Transmission cycles</td>
<td>America</td>
</tr>
</tbody>
</table>
3. Ecobiological features and clinical trends of \textit{T. cruzi} I

The distribution of \textit{T. cruzi} genotypes and reservoirs has important implications for the divisions of Tcl. In the southern part of the continent, infection of \textit{Canis familiaris} has been associated with Tcl, V and VI, whereas infections in the north are associated with genotypes la and lb (Falla et al., 2009; Herrera et al., 2009b). Furthermore, a significant number of \textit{Diplodiplas marsupialis} in Colombia are infected with Tcld genotype, which suggests an association with the sylvatic transmission cycle. Similar studies in pinnipeds have demonstrated that Tcl predominantly infects arboreal reservoirs (Cura et al., 2010; Falla et al., 2009). There are several hypotheses regarding the distribution of the different genetic groups of \textit{T. cruzi}, suggesting that these reservoirs belonging to the arboreal ecotopes are preferentially infected with Tcl and that terrestrial ecotopes are infected with Tcl-TcVI (Yeo et al., 2005). This hypothesis is controversial in light of recent reports demonstrating that the arboreal ecotope reservoirs \textit{Monodelphis breviceudata}, \textit{Philander frenata} and \textit{Didelphis aurita} are infected with Tcl, TcIV and Tcl, respectively (Marcili et al., 2009b; Llewellyn et al., 2009b).

However, the associations are not absolute, and in the case of Tcl, there was no apparent clustering of particular Tcl genotypes with \textit{Diplodiplas} in comparison to isolates from other arboreal mammals (Llewellyn et al., 2009b). Also for phylogeographical analyses of Tcl, the results indicate that isolates cluster according to geography rather than host association (Marcili et al., 2009b; Llewellyn et al., 2009b). This could also be supported for the recent analysis developed in mammals naturally infected with Tcl using microsatellites markers revealing the role of the mammals reservoirs in the diversifying selection of \textit{T. cruzi} (Llewellyn et al., 2011). Two interesting studies of host response to different strains have confirmed, by comparative artificial infection, that, in the southern USA two species of opossum (\textit{Monodelphis domestica} and \textit{Diplodiplas virginiana}) seem to be resistant to Tclv (Roellig et al., 2009a; Roellig et al., 2009b). This highlights a mechanism for the association of a vertebrate host with one strain over others. The strong association between Tcl and \textit{Rhodnius} species can also be explained by a similar mechanism: comparative artificial infection studies of \textit{R. prolixus} with various strains revealed a tendency for it to be resistant to infection by Tcl (Mello et al., 1996). For triatomines, susceptibility or resistance to trypanosome infections seems to be modulated by the intestinal symbionts, which are vital for development (Azamujba et al., 2005). \textit{T. cruzi} is considered to be subpathogenic to triatomines, whereas \textit{Trypanosoma rangeli} is another species that commonly infects \textit{Rhodnius} species and causes pathogenicity based on a reduction of the number of symbionts (Vallejo et al., 2009). Some studies using different species of triatomines such as \textit{Rhodnius pallescens}, \textit{Triatoma dimidiata}, \textit{Rhodnius colombiensis} and \textit{Panstrongylus geniculatus} have shown the affinity of Tcl to infect these species in comparison with TcV (Mejia-Jaramillo et al., 2009b). A study of four \textit{Rhodnius} species artificially infected with different strains of \textit{T. rangeli} showed a measurable difference in response and indicated adaptation of trypanosome strains to the local vector species (Machado et al., 2001). At least half of all species of triatomine bugs have been found naturally infected with \textit{T. cruzi} (Lent and Wygodzinsky, 1979; Schofield, 1994). Unfortunately, the vast majority of these records do not include specific strain associations. Clearly this is an area of potential research. In the context of dispersal triggered by starvation, there is evidence that starvation decreases \textit{T. cruzi} infection in triatomines (Kollien and Schaub, 1998) and in some species starvation may clear infection altogether (Phillips and Bertram, 1967; Vargas and Zeledon, 1985). This factor could go towards explaining paradigms such as in Venezuela where sylvatic and domestic bugs seem to be in panmixia but Tcl shows discrete general clustering of sylvatic and domestic cycles (Fitzpatrick et al., 2008; Llewellyn et al., 2009b).

Triatomine bugs directly determine the etiology of the strains of \textit{T. cruzi} involved in human transmission cycles. This is clear because despite Tcl and \textit{Diplodiplas} being widespread, it is the northern distribution of \textit{Rhodnius} that corresponds with its occurrence in human cycles. Overall, the aspects of epidemiological relevance are that associations between terrestrial ecological, \textit{Triatoma} infestations, terrestrial mammals, and \textit{T. cruzi} strains Tcl/TcV have lead to the prominence of Tcl, TcV, and Tclv in human infections in the southern region of South America. In the northern regions human American Trypanosomiasis infections seem to stem from Tcl associated with arboreal \textit{Rhodnius} and arboreal mammals.

Molecular epidemiology studies of \textit{T. cruzi} have attempted to establish the effects of different DTUs in the clinical progression of Chagas disease. Several studies have shown the effect of genetic variability on the host immune response (dos Santos et al., 2009; Melquiades-Rodriguez et al., 2010; Ramirez et al., 2009). It was previously known that cardiopathies in southern cone countries were caused by Tcl, TcV and TclI, but it has recently been demonstrated that Tcl can play an important role specifically in severe cardiopathies related to Chagas disease. Studies of cardiac biopsies from Argentinean patients revealed that patients with severe myocarditis were infected with Tcl, whereas those with moderate or absent myocarditis were infected with Tclv or TcV (Burgos et al., 2010). At the same time that the Tcl genotype was found in the severe myocarditis patients, it was demonstrated that in patients with chronic chagasic cardiopathy, the Tcla genotype was most commonly found in the bloodstream, whereas Tcld was most commonly found in cardiac biopsies. These results are consistent with reports from patients in Colombia, where the least and most prevalent Tcl genotypes in adult patients with chronic chagasic cardiopathy were Tcld and Tcla, respectively (Ramirez et al., 2010). This suggests a possible type of histotropy by Tcl genotypes and the epidemiological importance of this DTU in the southern countries, where cardiopathies were previously thought to be caused primarily by Tcll, TcV and Tclv.

4. Concluding remarks

Recent studies have confirmed the presence of the Tcl genotypes in Argentina, Brazil, Bolivia, Chile, Colombia, Mexico, Panama, Paraguay, French Guiana, Venezuela and the USA (Cura et al., 2010; Tomazini et al., 2010). Furthermore, intergenic region of mini-exon gene sequence studies have largely confirmed these genotypes in Bolivia, Mexico, Brazil and Argentina (Herrera et al., 2009a), which suggest that the Tcl genotype reported by Cura et al. (2010) may correspond to the Bolivian group reported by Herrera in 2009. Based on the intergenic region of the gene mini-exon, there is a wide distribution of genotypes of Tcl in the Americas, a premise that has been confirmed by Llewellyn et al. (2009a) using microsatellite markers in which there are specific genotypes associated with human infection in Bolivia, Brazil, Venezuela and Ocaña-Mayorga et al. (2010) in Ecuador. The wide genetic diversity of Tcl in the Americas is shown in Fig. 1 where has been established the geographical distribution of Tcl genotypes along the Americas.

The implications of these studies in the clinical manifestation of Chagas disease are important and suggest that the need to pursue additional phylogeographic studies using other molecular markers to develop and implement strategies aimed at the mitigation of Chagas disease across the continent. After all these published works the question remains and arises, is it necessary a subdivision within Tcl?, and should the nomenclature be revised?, maybe that is a question that probably will be answered in nine years where the third revision of the \textit{T. cruzi} nomenclature will be held when we celebrate 110 years of the discovery of Chagas disease and maybe new molecular markers and phylogeographical data will help to solve this enigmatic question.
References


