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Short communication

Differential tissue distribution of *Trypanosoma cruzi* during acute experimental infection: Further evidence using natural isolates

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ABSTRACT

In the present work, we evaluated the effect of mixed *Trypanosoma cruzi* infections, studying the biological distribution of the different parasites in blood, heart and skeletal muscle during the acute phase. Albino Swiss mice were infected with different parasite strain/isolates or with a combination of them. The parasites in the different tissues were typified through specific PCR, population variability was analyzed through RFLP studies and parasitological and histopathological parameters were evaluated. We found a predominance of TcII and TcVI in all tissues samples respect to TcV and different parasite populations were found in circulation and in the tissues from the same host. These results verify the distribution of parasites in host tissues from early stages of infection and show biological interactions among different genotypes and populations of *T. cruzi*.

The variable clinical course in human *Trypanosoma cruzi* infection includes an acute phase, with circulating parasites and symptoms that could persist for one or two months, and a chronic phase, which may be clinically silent and have no evident pathology or evolve into irreversible cardiac and/or digestive lesions. This variable progression, from a clinically silent period to a more severe scenario, is probably the consequence of many factors; it has been suggested, for instance, that genetically different infecting parasite populations or strains may produce different clinical symptoms [1]. This parasite shows high genetic variability, which has been the basis to classify it into six lineages or Discrete Typing Units (DTUs), TcI to TcVI [2]. Other possible factors to explain the variable clinical features include exposure to re-infections [3] and the general health condition and genetic background of the host [4,5]. In recent years there has been considerable progress in understanding the biological diversity of the etiological agent, as well as the population polymorphisms associated with susceptibility to this disease. However, many other aspects such as host-parasite interactions, genetic mechanisms of cell invasion, genetic variability, and tropism are not sufficiently known. Particularly, there is no clear understanding of the effects of genetic diversity in mixed infection (with more than one DTU in the same individual). Within endemic areas there is a considerable risk of both multiple exposures to *T. cruzi*, with a very high prevalence of infection in vectors [6] and of exposure to different genotypes, with vectors commonly found to be harboring mixed *T. cruzi* infections [7], which could lead to mixed infections in humans [8], wild and domestic animals. In these mixed infections, the co-infecting parasites may be interacting among each other within the same host [9] determining the severity of disease symptoms, successful parasite transmission rate and epidemiology of the disease [10]. We have previously described the tissue distribution of two *T. cruzi* natural isolates (both obtained from congenitally infected patients), verifying that they both consisted of a mixture of DTUs; when they were used in experimental infections, the populations present in blood were different to those found in the tissues of the same host [11]. The distribution of these different populations of the parasite in the same individual is a factor that might determine the clinical course of the disease, causing the widespread clinical variability. In the present work, additional parasite strains or natural isolates were used, alone or in combination, to further study their interactions in a murine model of *T. cruzi* infection, providing additional information about the genetic diversity of natural isolates from endemic areas and their combined behavior in the mammalian host. To achieve this, we evaluated the composition and distribution of the different DTUs in blood, heart and skeletal muscle from mice infected with either a single strain/isolate or a combination of them, in the acute phase of the experimental infection.

Adult (8–12 weeks of age) male and female albino N:NIH Swiss mice, weighing 25 ± 3 g, were inoculated, by intraperitoneal injection, with 50 blood trypomastigote forms, from three *T. cruzi* natural isolates (Lucky, Casibla and SGO-Z12) and a strain (Tulahuen). Mice were divided into 10 groups (n = 10 each), and each group was infected with a

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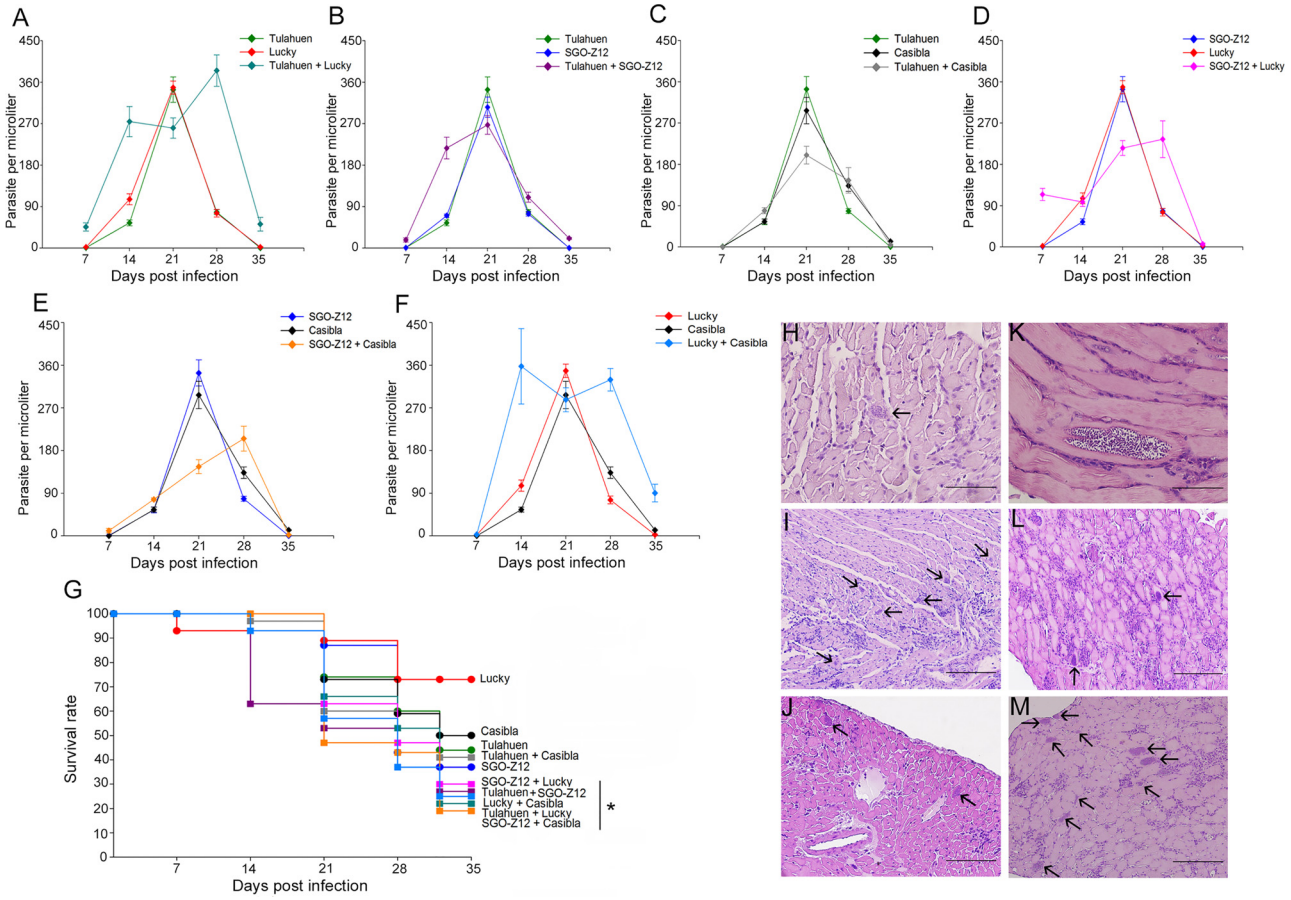

Fig. 1. A–F: Evolution of parasitemia in Albino Swiss mice infected with a single strain/isolate or a combination of them. For all groups: n = 10. G: Survival curve of all the infected groups; this figure was performed using Kaplan Meier Survival test (Y axis shows percentages). The asterisks indicate statistical significance. H–M: Histopathological studies were performed to evaluate structural alterations provoked by the parasite; by day 35 post infection, 3 mice were randomly selected from the ones that survived until this timepoint and cardiac and skeletal muscles were extracted. All mice were sacrificed by decapitation, using Ketamine ClH (Ketalar®, Parke Davis, Warner Lambert Co, USA) anesthesia (10 mg/kg). A total of 9 slices stained with hematoxylin and eosin from each group for each tissue was analyzed and the area of inflammatory infiltrates was determined with a 100X magnification for each slide. The percentage of inflammatory infiltrates per area in each section was quantified using AxioVision Software. H: Cardiac section from SGO-Z12 + Casibla co-infected mice, 600X, bar = 200 µm. I: Cardiac section from Tulahuen + Casibla co-infected mice, 200X, bar = 667 µm. J: Cardiac section from Lucky + SGO-Z12 co-infected mice, 200X, bar = 667 µm. K: Skeletal muscle section from Tulahuen + SGO-Z12 co-infected mice, 600X, bar = 200 µm. L: Skeletal muscle section from Lucky + SGO-Z12 co-infected mice, 200X, bar = 667 µm. M: Skeletal muscle section from SGO-Z12 + Casibla co-infected mice, 200X, bar = 667 µm. Arrows indicate amastigote nests. Cardiac and skeletal muscle parasitism was evaluated by counting the number of parasite nests in the tissue sections. Each tissue section was analyzed for > 10 microscopic fields (200×) and three different tissue sections/mouse were examined.

single strain/isolate or a combination of them. Lucky and Casibla were originally obtained from congenitally infected newborn children from an endemic area in Argentina; SGO-Z12 was also obtained from an endemic area of Argentina (Santiago del Estero) through xenodiagnosis. The Tulahuen strain was originally obtained from an infected vector insect from Chile; the parasites from this strain used in the present work were obtained several years ago (1979) from the Instituto Nacional de Parasitología "Dr. Mario Fatala Chabén" (Argentina) and has been kept in mice ever since through successive infections of new uninfected animals every 15 days. We have previously typified these parasites as TcV [11]. Mice were kept in controlled housing conditions (12 h' light period, 23 ± 3 °C, with food and water *ad libitum*) and the experimental procedures had been approved by the Institutional Committee for the Care and Use of Laboratory Animals from the Faculty of Medicine, National University of Córdoba, Argentina.

Parasitemia levels (Fig. 1A–F) were measured in a Neubauer hemocytometer using blood samples obtained from the tail of the mice until day 35 post infection (p.i.), which corresponds to the acute stage [11]. The survival was monitored daily (Fig. 1G). The groups infected with Tulahuen + Lucky, Tulahuen + SGO-Z12 and Lucky + Casibla presented a peak in the parasitemia earlier in time (14 days p.i.) than the corresponding groups infected with a single strain/isolate; additionally, in these groups, circulating parasites were observed for a longer period. This was expected since these groups were initially inoculated with twice the number of parasites than the groups infected with a single strain/isolate (100 *vs*. 50 trypomastigotes, respectively). However, the co-infected groups Tulahuen + SGO-Z12, Tulahuen + Casibla and SGO-Z12 + Casibla presented a total amount of circulating parasites lower than the groups infected with the single strain or isolate. In accordance with these results, studies involving *T. cruzi* mixed infections have shown that peripheral blood parasite load could either be increased or decreased in the presence of a co-infecting strain [9,12]. The survival rate in the co-infected groups was significantly lower than in the groups with the corresponding single-infections, being the higher initial amount of parasites the probable cause of these findings.

Histopathological studies showed higher percentage of inflammatory infiltrates in skeletal muscle (two to three times higher) than in cardiac muscle ($P < 0.05$ Chi square test), except in SGO-Z12, Tulahuen + Casibla and Tulahuen + SGO-Z12 in which the percentage of inflammatory infiltrates was similar in both muscles. The percentages of inflammatory infiltrates in cardiac muscle varied from 3.56% in

mice infected with Lucky to 0.55% in mice infected with SGO-Z12. Mice infected with Tulahuen, Casibla or the mixtures of parasites showed intermediate values among those previously mentioned. In most cases, when the area covered by inflammatory infiltrates in the co-infecting groups was compared with the respective single infections, similar or intermediate values were found.

Cardiac and skeletal muscle parasitism was evaluated by calculating the average number of amastigote nests in each tissue section from each group. We found between 1 and 2 amastigote nests per section of skeletal muscle in the groups of mice infected with a single strain or isolate; no amastigote nests were found in the cardiac muscle sections. The co-infected mice on the other hand, showed between 3 (Tulahuen + SGO-Z12) and 18 (Tulahuen + Casibla) amastigote nests per section of skeletal muscle and up to 4 per section of cardiac muscle (Tulahuen + Casibla); Tulahuen + SGO-Z12 however, did not show amastigote nests in this latter organ (Fig. 1H–M). The persistence of the parasite in cardiac, skeletal and smooth muscle cells is an important aspect for the development of the chronic phase of the infection; the ability of the host to establish an immune response to control the infection is therefore crucial for the disease outcome [13].

Studies involving *T. cruzi* mixed infections showed that co-infected animals presented levels of parasitemia, inflammatory reaction and parasitism in the heart at an intermediate level compared to those single-infected [9,14]. In the present work, we found similar inflammatory response in co-infected mice compared to single strain/isolate infected mice and an increased cardiac and skeletal muscle parasitism and mortality. The increased tissue parasitism in co-infected mice could be related to the parasites remaining in circulation for longer periods of time (Fig. 1A–F), which, added to a similar inflammatory response, resulted in less control of the infection in the organs. The higher mortality of the co-infected groups may have been due to this cumulative damage in these and other tissues by isolates that differed in their tropism and pathogenicity [15]. In the present work we only studied cardiac and skeletal muscles, but many other studies had shown that *T. cruzi* infects and damage other tissues during acute infections [11,15]. In this later work however [15], no association was found between mixed infections and an increased host morbidity or mortality; nevertheless, a more disseminated infection, with multiple infected hosts displaying a greater number of organs containing *T. cruzi* than their singly infected counterparts, was observed.

After this general description of the evolution of each infection, we evaluated the presence and distribution of the different *T. cruzi* DTUs in blood and cardiac and skeletal muscles from these animals, to determine if the parasite populations in blood were different from those found in the tissues of the same host. To achieve this, sample preparation, parasite detection and DTU identification were performed as previously described [11]. The parasite DTU in each tissue was determined using two PCR algorithms [16,17] that together allowed a better identification of the DTUs present in each case [11]. To verify if there was intra-lineage diversity, restriction fragment length polymorphism (RFLP) studies were performed using the mini-exon region [11].

Table 1 shows the results obtained after the identification of *T. cruzi* DTUs and the number of samples of each organ from the same individual with different RFLP pattern than the one found in blood (patterns shown in supplementary table). The mini-exon region has previously demonstrated to differentiate parasite populations [18]. However, while in some cases the RFLP patterns found in the co-infected groups were, as expected, a combination of the patterns found in the corresponding single-infected groups, in most cases the RFLP patterns were similar to only one of those groups, probably revealing different proportions of the different parasite populations. In a few cases (particularly Tulahuen + Lucky), although the RFLP patterns were consistent among the samples, they were not the expected fragments for the combination. Further studies are therefore needed to properly assess the usefulness of this gene for parasite discrimination at intra-DTU level. In TcI parasites for instance, the mini-exon fragment has shown limited efficacy to establish subpopulations [19]. However, in present results, emphasis is placed on what happens within each individual (blood *vs.* organs), with no intention of generalizing the RFLP patterns to all the animals from any given group. Although the extent to which our findings could be generalized to human infections is still unclear, the different parasite populations found in each individual reflect the high genetic variability of *T. cruzi* and their differential tissue distribution [9,15,20,21]: while some parasites remain in circulation, others successfully infect the heart and others lodge in the skeletal muscle. This differential distribution, among other factors, could influence the clinical outcome of the infection [21].

Table 1
DTUs detected by PCR from blood, heart and skeletal muscle samples and different RFLP patterns in tissues (heart or skeletal muscle) to those found in blood of the same host, after the digestion of the mini-exon fragment with Bce AI.

Stain / isolates	DTUs			Samples with different RFLP patterns in tissues to those found in blood	
	Blood	Heart	Skeletal Muscle	Heart	Skeletal Muscle
Tulahuen	V	V	V	**1/3**	0/3
SGO Z12	II - VI	II - VI	VI	0/3	**3/3**
Lucky	II - VI	II - VI	II - VI	0/3	**2/3**
Casibla	II - VI	II - VI (2/3) VI (1/3)	II - VI (2/3) VI (1/3)	**1/3**	**3/3**
Tulahuen + SGO Z12	VI	VI	VI	0/3	**3/3**
Tulahuen + Lucky	II - VI	II - VI	II - VI	0/3	0/3
Tulahuen + Casibla	II - VI	II - VI	II - VI	**1/3**	**2/3**
SGO Z12 + Lucky	II - VI	II - VI	II - VI	0/3	0/3
SGO Z12 + Casibla	II - VI	II - VI	II - VI (2/3) VI (1/3)	0/3	0/3
Lucky + Casibla	II - VI	II - VI	II - VI	**1/3**	**3/3**

T. cruzi DTU classification was performed following Ramirez and collaborators [16] algorithm, modified for three molecular markers and Cosentino and Agüero typing assay [17]. PCR products were separated by electrophoresis in a 2.5% agarose gel stained with ethidium bromide and examined under UV light. Each reaction was performed in triplicate. Sample obtaining: by day 35 post infection, blood and cardiac and skeletal muscles were extracted from 3 mice randomly selected from the ones that survived until this timepoint. All mice were sacrificed by decapitation, using Ketamine ClH (Ketalar®, Parke Davis, Warner Lambert Co, USA) anesthesia (10 mg/kg). The RFLP studies were performed using the amplified mini-exon region (300 pb) and the enzyme Bce AI (New England Biolabs, Inc.) [11]; digestion conditions suggested by the enzyme's manufacturer were followed. The obtained fragments were separated by electrophoresis in 2.5% agarose gels stained with ethidium bromide and examined under UV light.

The SGO-Z12 isolate consisted of TcII and TcVI: both DTUs were present in blood and cardiac muscle samples; however, only TcVI was present in the skeletal muscles from the three mice analyzed. All these skeletal muscle samples showed different RFLP patterns to those found in circulation and the cardiac muscle of the same host, confirming the existence of a different type of parasite in this tissue. The RFLP patterns in the heart samples were similar to those found in blood. Since both DTUs were present here (blood and heart), there seems to be a different parasite population within TcVI with a tropism for the skeletal muscle. Some abnormalities in the function and structure of skeletal muscle fibers had been found with increasing frequency associated with Chagas disease and other pathologies [22]. Parasites targeting this tissue could be the cause of these abnormalities.

Lucky and Casibla were previously genotyped in our laboratory [11], these isolates also consisted of a mixture of TcII and TcVI. The presence of TcII and TcVI in the three isolates used in the present study

reflects the fact that these are two of the lineages of *T. cruzi* (together with TcV) more frequently associated with the domestic cycle of infection in Central and South America [2,8]. All the mice infected with Lucky presented both DTUs in all the samples analyzed and different RFLP patterns were only found in one skeletal muscle sample and one cardiac muscle. Previously, this isolate showed higher variability in organs not studied in the present work (liver and spleen) [11].

Most of the samples from the mice infected with Casibla presented both DTUs, with the exception of one cardiac muscle sample and one skeletal muscle sample (not from the same animal) which showed the presence of only TcVI; all the skeletal muscle samples and the cardiac sample infected with TcVI showed different RFLP patterns to those found in circulation. As found here, this isolate previously showed high variability within a given host [11]: all the samples analyzed (cardiac and skeletal muscles, liver and spleen) presented different RFLP patterns to those found in circulation. Other studies have also demonstrated differences between prevalent genotypes in bloodstream and cardiac tissue [13,16,23]. This can complicate treatment related to heart transplantation for example, where chemotherapy is used to counter reactivation of the parasite following post-surgical immunosuppressive therapy, since in mixed infections parasite reactivation can occur at different timepoints [23]. Taking all these results in consideration, Casibla seems to have the highest variability when compared with the rest of the isolates analyzed.

Only TcVI was found in blood, heart and skeletal muscle from the mice co-infected with Tulahuen + SGO-Z12; however, different RFLP patterns were found in all the skeletal muscle samples analyzed and they were all similar to those found previously in the SGO-Z12 group, suggesting that these populations were present in this isolate. Intra-lineage diversity has been previously demonstrated using different gene fragments, showing that the genotypes identified at the intra-DTU level could vary widely, depending of the locus studied [24]. TcV and TcVI have previously been shown to have homogenous population structures with low intra-lineage diversity [25]. In our case, TcV showed nearly no variability while TcVI showed at least three different populations: two present in SGO-Z12, as stated earlier, one of which is shared with Casibla, and a different one present in Lucky. The mini-exon region therefore could partially differentiate parasite populations within TcVI, as has been demonstrated with other genes [24]. This fragment, however, might have some limitations, as stated earlier, and further studies are needed to properly assess its usefulness to differentiate parasite populations.

It is important to take into consideration that the initial inoculum for Tulahuen + SGO-Z12 group, as well as for all the co-infected groups, was higher than for the single-infected ones. Even though the variation in the DTUs present in the different tissues from the mice from the co-infected groups could be related to this different inoculum size rather than due to a differential distribution, the groups infected with only one isolate (SGO-Z12, Lucky or Casibla) also presented some kind of variation and they were all infected with a comparable parasite inoculum (50 parasites per animal). Regarding the infecting parasite inoculum, it is important to highlight that other authors report no observable differences in the pathology, host outcome or disease progression for individuals that received higher amount of parasites due to multiple exposures or mixed infections [15].

This initial inoculum for Tulahuen + SGO-Z12 group consisted of a mixture of three DTUs (TcII, TcV and TcVI), but the only DTU found in these samples was TcVI. The predominance of TcVI over other DTUs was also observed by Ragone and collaborators that performed experiments with different mixed infections involving combinations of two isolates (TcIII + TcVI and TcV + TcVI) in C57BL/6J mice and detected only the presence of TcVI in the acute phase (also in blood, heart and skeletal muscle) [9]. This result could be due to several factors: the survival of only one isolate in a given organ/tissue could be related to different mechanisms associated with its ability to escape from the host immune system [12], or due to a selective process within the host cells in favor of a given DTU [26], which could result in the dissimilar proportions of the different parasite populations discussed earlier. This behavior is epidemiologically relevant since it would imply less parasite availability of a given DTU to vectors circulating in the endemic area [27].

These authors also reported that TcIII and TcVI induce a more variable serological response than TcV [27]; in consequence, in co-infections between TcIII or TcVI and TcV, this latter DTU could be susceptible to the immunological response induced by the other two and its availability in the host would be reduced (or it could even be eliminated from some of the tissues/organs). In our case, TcV could not be found in the samples from the groups co-infected with Tulahuen through the PCR algorithms; its presence however could be inferred by the RFLP patterns in some of the samples. The amount of TcV parasites were probably reduced by the immunological response triggered by the other parasites present in the co-infections to levels that could not be detected by the methods applied here. All co-infected groups presented parasitemias that lasted for longer periods and sometimes higher tissue parasitism (particularly in skeletal muscle) than the single-infected groups; this could generate a stronger immunological response that would affect the more susceptible populations of parasites.

Something similar could be happening with TcII in the samples from Tulahuen + SGO-Z12 co-infected mice: the host immune system could be eliminating TcII and TcV parasites at levels that could not be detected here and only TcVI would be found in these samples (both TcII and TcV can be detected with the proposed methodology and would not be covered by the presence of other DTU, if present). Three DTUs were present in this group, each of which could trigger a different immunological response; TcII parasites present in SGO-Z12 would be susceptible to this response and would not be detected in the samples analyzed. However, TcII and TcV parasites could be present in other tissues that were not studied in the present work. As stated earlier, previous results from our laboratory have shown that liver and spleen are highly parasitized organs, and the parasites present there may be different (even from a different DTU) to those present in circulation [11]. Other organs such as brain, lung and kidney may represent reservoirs of parasites with probable different subpopulations [28].

In the mice co-infected with Tulahuen + Lucky and Tulahuen + Casibla, TcII and TcVI were present in all the samples analyzed. These groups were also originally infected with three DTUs (TcII, TcV and TcVI), but TcV was not detected in any of the samples analyzed except for two samples from Tulahuen + Casibla. In contrast to what was observed in the group co-infected with Tulahuen + SGO-Z12, in Tulahuen + Lucky and Tulahuen + Casibla, TcII was found in all the samples analyzed. Therefore, the TcII parasites present in the Lucky and Casibla would be less susceptible to the immune response than the TcII parasites present in SGO Z12. Moreover, whenever Lucky or Casibla isolates were part of the infection (either in single-infection or in co-infection with another strain/isolate), TcII and TcVI were found in most of the samples. It is important to note that TcII was always found in association with TcVI (ie, there were no samples with only TcII), whereas samples (mostly cardiac or skeletal muscles) with only TcVI were found. Regardless this differential distribution, no differences were observed in the amount of inflammatory infiltrates, either in heart or skeletal muscle, with respect to the DTU detected in each tissue. Other authors [20] found association between the parasite strain and the inflammatory process: in mice infected with two strains, only one of these strains predominated in certain organs (similarly to what was found here through the RFLP patterns, as discussed earlier), and their histopathological aspects were similar to those found in the animals infected with only this predominant strain. In our results, the use of natural isolates, composed of a mixture of DTUs, as opposed to pure parasite strains, could originate a more complex immune response in which no direct association could be observed.

Taken all together, these results reaffirm that the distribution of the different types of parasites in host tissues could be influenced by the

presence of other types of parasites (ie. from different DTU), as has been previously shown [20,21,29]; parasite/parasite interactions can therefore be acting to determine the type of parasite/DTU present in each tissue or organ at a given moment of the infection. For example, competitive exclusion interactions have been described for other parasites, in which similar strains/species can infect the same host and the different kind of parasites would be in different tissues within the host (this has been described for filariasis) [30] or compete until one of the populations is eliminated (this has been described for malaria) [31]. Such interactions could be taking place in our co-infected groups since only one or two DTUs were found in the infected animals that were infected with three of them (when the presence of the three DTUs would be expected). As discussed above, the "absent" DTU could be present in other tissues not analyzed in the present work or have been eliminated by parasite/parasite or parasite/host interactions. On the other hand, the presence of a given *T. cruzi* population in certain organs has been associated with the clinical manifestations of the disease [21]. Our results are in concordance with the notion that natural isolates of *T. cruzi* may be composed of swarms of clones that upon infection will compete for available host resources, including tissue targets [20].

A greater diversity of parasites would trigger a "more varied" immune response that would restrict those more susceptible parasites to certain organs or even remove them from the host. The results obtained in the present work suggest that any particular population of *T. cruzi* could infect different tissues within the host, but their presence in a particular organ would be determined by the other parasites present in the co-infection (in the case of infections with different DTUs) and by host/parasite interactions. Novel studies are needed to clarify these interactions and the importance of tissue tropism in the development of infection.

These results, highlight the necessity to conduct studies based on the use of tissue and bloodstream samples from infected patients to compare the genotypes present in the same individual [16,32], since they may be quite different as shown here, which could influence the clinical course of the disease and the possible treatment outcome.

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References

[1] A. Hidron, N. Vogenthaler, J.I. Santos-Preciado, et al., Cardiac involvement with parasitic infections, Clin. Microbiol. Rev. 23 (2010) 324–349.
[2] B. Zingales, M.A. Miles, D.A. Campbell, M. Tibayrenc, et al., The revised *Trypanosoma cruzi* subspecific nomenclature: rationale, epidemiological relevance and research applications, Infect. Genet. Evol. 12 (2012) 240–253.
[3] J.M. Bustamante, M. Novarese, H.W. Rivarola, et al., Reinfections and *Trypanosoma cruzi* strains can determine the variability and the prognosis of the chronic Chagas disease, Parasitol. Res. 100 (2007) 1407–1410.
[4] W.O. Dutra, C.A. Silva Menezes, F.N. Amaral Villani, G. Carneiro da Costa, et al., Cellular and genetic mechanisms involved in the generation of protective and pathogenic immune responses in human Chagas disease, Mem. Inst. Oswaldo Cruz 104 (2009) 208–218.
[5] R.H. Vasconcelos, S.M. Montenegro, E.A. Azevedo, et al., Genetic susceptibility to chronic Chagas disease: an overview of single nucleotide polymorphisms of cytokine genes, Cytokine 59 (2012) 203–208.
[6] S.A. Kjos, K.F. Snowden, J.K. Olson, Biogeography and *Trypanosoma cruzi* infection prevalence of Chagas disease vectors in Texas, USA, Vector Borne Zoonotic Dis. 9 (2009) 41–50.
[7] G. Ribeiro Jr., R. Gurgel-Gonçalves, E.B. Reis, et al., Frequent house invasion of *Trypanosoma cruzi*-infected triatomines in a suburban area of Brazil, PLoS Negl. Trop. Dis. 9 (2015) e0003678 9.
[8] A. Abras, M. Gállego, C. Muñoz, N.A. Juiz, et al., Identification of *Trypanosoma cruzi* Discrete Typing Units (DTUs) in Latin-American migrants in Barcelona (Spain), Parasitol. Int. 66 (2017) 83–88.
[9] P.G. Ragone, C. Pérez Brandán, M. Monje Rumi, et al., Experimental evidence of biological interactions among different isolates of *Trypanosoma cruzi* from the Chaco Region, PLoS One 10 (3) (2015) e0119866.
[10] A.B. Pedersen, A. Fenton, Emphasizing the ecology in parasite community ecology, Trends Ecol. Evol. 22 (2007) 133–139.
[11] M.S. Lo Presti, B.H. Esteves, D. Moya, et al., Circulating *Trypanosoma cruzi* populations differ from those found in the tissues of the same host during acute experimental infection, Acta Trop. 133 (2014) 98–109.
[12] H. Sales-Campos, H.B. Kappel, C.P. Andrade, et al., A DTU-dependent blood parasitism and a DTU-independent tissue parasitism during mixed infection of *Trypanosoma cruzi* in immunosuppressed mice, Parasitol. Res. 113 (2014) 375–385.
[13] A.M. Macedo, C.R. Machado, R.P. Oliveira, et al., *Trypanosoma cruzi*: genetic structure of populations and relevance of genetic variability to the pathogenesis of chagas disease, Mem. Inst. Oswaldo. Cruz. 99 (2004) 1–12.
[14] C.M. Rodrigues, H.S. Valadares, A.F. Francisco, et al., Coinfection with different *Trypanosoma cruzi* strains interferes with the host immune response to infection, PLoS Negl. Trop. Dis. 4 (10) (2010) e846.
[15] C.J. Perez, R.C.A. Thompson, S.K. Keatley, et al., The effect of reinfection and mixed *Trypanosoma cruzi* infections on disease progression in mice, Acta Trop. 178 (2018) 107–114.
[16] J.D. Ramírez, F. Guhl, L.M. Rendón, et al., Chagas cardiomyopathy manifestations and *Trypanosoma cruzi* genotypes circulating in chronic chagasic patients, PLoS. Negl. Trop. Dis. 4 (2010) e899.
[17] R.O. Cosentino, F. Agüero, A simple strain typing assay for *Trypanosoma cruzi*: discrimination of major evolutionary lineages from a single amplification product, PLoS Negl. Trop. Dis. 6 (7) (2012) e1777.
[18] M.G. Rivera, L. Herrera, A. Morocoima, et al., Genetic variability of *Trypanosoma cruzi* TcI isolates from rural and urban areas of Venezuela, J. Vector Borne Dis. 52 (2015) 23–29.
[19] C.P. Herrera, C. Barnabé, S.F. Brenière, Complex evolutionary pathways of the intergenic region of the mini-exon gene in *Trypanosoma cruzi* TcI: a possible ancient origin in the Gran Chaco and lack of strict genetic structuration, Infect. Genet. Evol. 16 (2013) 27–37.
[20] L.O. Andrade, C.R.S. Machado, E. Chiari, et al., Differential tissue distribution of diverse clones of *Trypanosoma cruzi* in infected mice, Mol. Biochem. Parasitol. 100 (1999) 163–172.
[21] A.R. Vago, L.O. Andrade, A.A. Leite, et al., Genetic characterization of Trypanosoma cruzi directly from tissues of patients with chronic Chagas disease: differential distribution of genetic types into diverse organs, Am. J. Pathol. 156 (2000) 1805–1809.
[22] S. Iqbal, D.A. Hood, The role of mitochondrial fusion and fission in skeletal muscle function and dysfunction, Front. Biosci. (Landmark Ed.) 20 (2015) 157–172.
[23] J.M. Burgos, M. Diez, C. Vigliano, et al., Molecular identification of *Trypanosoma cruzi* discrete typing units in end-stage chronic Chagas heart disease and reactivation after heart transplantation, Clin. Infect. Dis. 51 (2010) 485–495.
[24] M. Yeo, I.L. Mauricio, L.A. Messenger, et al., Multilocus Sequence Typing (MLST) for lineage assignment and high resolution diversity studies in Trypanosoma cruzi, PLoS Negl. Trop. Dis. 5 (2011) e1049.
[25] M.D. Lewis, M.S. Llewellyn, M.W. Gaunt, et al., Flow cytometric analysis and microsatellite genotyping reveal extensive DNA content variation in *Trypanosoma cruzi* populations and expose contrasts between natural and experimental hybrids, Int. J. Parasitol. 39 (2009) 1305–1317.
[26] D.A. Pena, I. Eger, L. Nogueira, et al., Selection of TcII *Trypanosoma cruzi* population following macrophage infection, J. Infect. Dis. 204 (2011) 478–486.
[27] P.G. Ragone, C. Perez Brandan, A.M. Padilla, et al., Biological behavior of different *Trypanosoma cruzi* isolates circulating in an endemic area for Chagas disease in the Gran Chaco region of Argentina, Acta Trop. 123 (2012) 196–201.
[28] D. del Valle Pugliese Uliarte, L.A. Trombetta Durante, D. Moreno Rivas, et al., Miocarditis aguda y meningoencefalitis por *Trypanosoma cruzi* en un paciente seropositivo al VIH, Rev. Cubana Med. Trop. 66 (2014) 458–464.
[29] L.M. De Pablos, A. Osuna, Multigene families in *Trypanosoma cruzi* and their role in infectivity, Infect. Immun. 80 (2012) 2258–2264.
[30] D.H. Molyneux, E. Mitre, M.J. Bockarie, et al., Filaria zoogeography in Africa: ecology, competitive exclusion, and public health relevance, Trends Parasitol. 30 (2014) 163–169.
[31] H.M. Abkallo, J.A. Tangena, J. Tang, et al., Within-host competition does not select for virulence in malaria parasites. Studies with Plasmodium yoelii, PLoS Pathog. 11 (2) (2015) e1004628.
[32] C. Cura, A.G. Schijman, Relación entre los genotipos de *T. cruzi* y la presentación clínica de la enfermedad de Chagas, Rev. Esp. Salud Pública 86 (2013) 9–16.