Trypanosoma cruzi: BEHAVIOR OF METATRYPOMASTIGOTES FROM *Didelphis marsupialis* AND *Panstrongylus geniculatus*

Trypanosoma cruzi: Comportamiento de Metatripomastigotes obtenidos de *Didelphis marsupialis* y *Panstrongylus geniculatus*

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ABSTRACT

Trypanosoma cruzi colonizes the anal scent glands of *Didelphis marsupialis* opossum, differentiating into metatrypomastigotes, which affect mammals through contamination in the same way as do metacyclics formed in the stomach of the triatomine vector. Meta-tryposmastigotes harvested from anal glands of *D. marsupialis* and from the intestine of *Panstrongylus geniculatus*, both of which naturally infected when captured in urban areas of the Caracas valley, Venezuela, were inoculated by the intra-peritonal (i.p.) route in NMRI mice for comparison of infectability, tissue tropism, and virulence. Metacyclic trypomastigotes from both sources produced 100% infectivity and mortality, causing heart, skeletal muscle, pancreas, colon, liver and lung invasion, however glandular metacyclic caused higher tissue parasite proliferation. Mice inoculated with glandular trypomastigotes showed, additionally, invasion of the smooth muscle of testis, epididymus, different conduct and seminal vesicles. Control opposums inoculated i.p. with glandular trypomastigotes, developed parasitemia and colonization of the anal glands. The latter in turn, i.p. inoculated in mice, induced lethal parasistemia. Results are discussed in relation to the extreme variability of *T. cruzi* populations which might correspond to different simultaneous cycles of transmission in particular ecotopes. The epidemiological importance of the presence of *T. cruzi* metacyclic stages in anal opossum glands in rural and urban areas is emphasized.

Key words: *Trypanosoma cruzi*, metatrypomastigotes, *Didelphis marsupialis*, *Panstrongylus geniculatus.*

RESUMEN

Trypanosoma cruzi coloniza las glándulas anales odoríferas de rabipelados o zarigüeyas donde se diferencia a metatripomastigotes que infectan a mamíferos por contaminación, en forma similar a los insectos vectores. Tripomastigotes metacíclicos obtenidos de las glándulas anales de *Didelphis marsupialis* y del intestino de *Panstrongylus geniculatus,* naturalmente infectados y capturados en áreas urbanas del valle de Caracas (Venezuela), fueron inoculados por vía intraperitoneal (i.p.) en ratones NMRI para comparar su infectividad, tropismo tisular y virulencia. Metatripomastigotes de ambas fuentes produjeron 100% de infección y de mortalidad, con histotropismo en corazón, músculo esquelético, páncreas, colon, hígado y pulmón; sin embargo, tripomastigotes metacíclicos glandulares causaron niveles mas elevados de proliferación parasitaria en los tejidos. Ratones inoculados con metacíclicos de glándulas anales de rabipelados mostraron, adicionalmente, tropismo hacia la musculatura lisa de testículo, epidídimo, conducto deferente y vesícula seminal. Rabipelados controles inoculados i.p. con tripomastigotes luminales desarrollaron parásitos sanguíneos y glandulares; estos últimos inoculados i.p. en ratones produjeron parasitemias letales. Los resultados fueron discutidos en relación con la elevada variabilidad de las poblaciones de *T. cruzi*, la cual ha sido señalada como respuesta a diferentes y simultáneos ciclos de transmisión que se producen en ecótopos con particularidades propias. La importancia epidemiológica de la presencia de estadios metacíclicos de *T. cruzi* en las glándulas anales de rabipelados de áreas rurales y urbanas es enfatizada.

Palabras clave: *Trypanosoma cruzi*, metatripomastigotes, *Didelphis marsupialis, Panstrongylus geniculatus.*

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INTRODUCTION

Chagas´ disease is normally transmitted by contamination of the skin and mucous membranes of the mammal host by feces and urine of hematophagous insects (Hemiptera: Reduviidae: Triatominae) containing infecting metacyclic trypomastigote stages of *Trypanosoma cruzi* [14, 27]. Deane *et al.* [9] found these *T. cruzi* metacyclic stages in the fluid of the anal scent glands of opossums, where the parasite developed in a manner analogous to the normal course in the intestine of the insect vector.

Therefore, the present study was undertaken to compare infectivity, tissue tropism, and virulence produced by metacyclic trypomastigotes harvested from the anal scent glands of the opossum *Didelphis marsupialis* (Marsupiala, Didelphidae) from the Caracas´valley, Venezuela, and from the intestine of *Panstrongylus geniculatus*, the only insect vector in this area [23] in an attempt to clarify the behavior of a parasite which could develop its life cycle under such different conditions as the gut of an insect and the anal scent glands of a mammal.

MATERIALS AND METHODS

Parasites

T. cruzi metatrypomastigotes were obtained from opossums, naturally infected, captured in the urbanized areas of Colinas de Bello Monte (strain IN16) and Parque del Este (strain CO79), Caracas and from naturally infected adult *P. geniculatus* captured in Colinas de Bello Monte (strains VP24, VP25), La Vega, and Petare, as well as urban areas [23] (strains VP27, VP28, respectively). These strains were characterized by us [12], through Randomly Amplified Polymorphic DNA (RAPD) analysis according to Carrasco *et al.* [5] as belonging to the Z1 zymodeme [20].

Experimental Infections

Samples from anal glands of the opossums and from the guts of the insects were diluted 1:1 in sterile 0.85% saline for counting the metacyclic stages according to Brener [3]. Metatrypomastigotes from the six strains were inoculated i.p., at a dose of 130 trypomastigotes/g body weight into six groups of five 15g NMRI outbred mice (n=30). Three days post-inoculation and thrice weekly thereafter, parasitemia was measured [3] until it disappeared or the animal died. One mouse with high parasitemia from each lot was sacrificed by cervical dislocation and tissue samples were taken from heart, skeletal muscle, pancreas, liver, spleen, colon, duodenum, kidney, lung, and skin for immediate fixation in 10% formalin. After imbedding in paraffin, 5μ sections were cut and stained with hematoxylin-eosin. For study of the histotropism, intracellular parasites were detected by 1,000 X microscopy, checked by two independent observers. The tissular parasitism was quantified with respect to the number of pseudocysts/ 50 fields at 400 X (0, 1-10, 11-20, 21-30 and >30, respectively); thus, the degree of parasitism was graded as: -, +, ++, +++, ++++, corresponding to absent, scarce, moderate, abundant and intense infection, respectively.

The genital organs are regularly parasitized, in experimental infections, by *T. cruzi* strains isolated from vectors and reservoirs captured in the Caracas´valley [12, 13, 25]; thus, to study the tropism of *T. cruzi* metatrypomastigotes from the anal glands of opossums to such organs, five 15g mice were inoculated as explained above with strain CO79 (from opossum). Sections were made from samples of testicle, epididymus, *vas deferens*, and seminal vesicle of a mouse with high parasitemia. Mortality was recorded daily.

Two groups of three juvenile opossums (average wt 350 g), taken as sucklings in the marsupium of trapped wild females, negative for *T. cruzi* by blood examination and xenodiagnosis with 12 III-stage laboratory-bred nymphs of *Rhodnius prolixus*, were inoculated i.p. as controls of the infection with 200 metatrypomastigotes/g body wt [15] harvested from the anal glands of the two donor opossums. The anal glands of the experimentally infected opossums were examined weekly. Samples of the gland contents showing parasites were inoculated i.p. into mice, which were examined as above.

RESULTS

T. cruzi metacyclics harvested from both glandular fluids of opposums and guts of the triatomines infected all rodents (FIG. 1). Mice inoculated with anal gland metacyclic stages from opossum (strain IN16) developed a patent parasitemia reaching an average peak of 1.2 x 10^6 trypomastigotes/ ml blood, when the last animals died, showing erection of hair, copious urination, and minimal movement, typical of a severe *T. cruzi* infection.

Metatrypomastigotes from *P. geniculatus* (VP24, VP25, VP27 and VP28 strains) and from the other opossum (strain CO79) killed all mice, although the average peak parasitemias were lower.

Glandular metatrypomastigotes (strains IN16, CO79) infected all opossums, producing average peaks of 1.7 x 10^5 and 1.5 x 10^4 trypomastigotes/ml blood respectively as well as glandular colonization. No opossums died, while those mice inoculated with glandular material from experimentally infected opossums died showing parasitemias that reached 10^5 parasites/ml blood.

The histotropism observed in experimental mice is summarized in TABLE I. Metacyclic forms from both origins, opossum scent anal glands and insect feces, produced marked myotropism with intense invasion of heart and skeletal muscle by the strains from anal glands; abundant to scarce invasion in skeletal and smooth muscles was produced by the vector strains; limited macrophagotropism and scarce invasion of pancreas were produced by both types of strains. In addition, male genital organs were infected by strain CO79 of glandular origin.

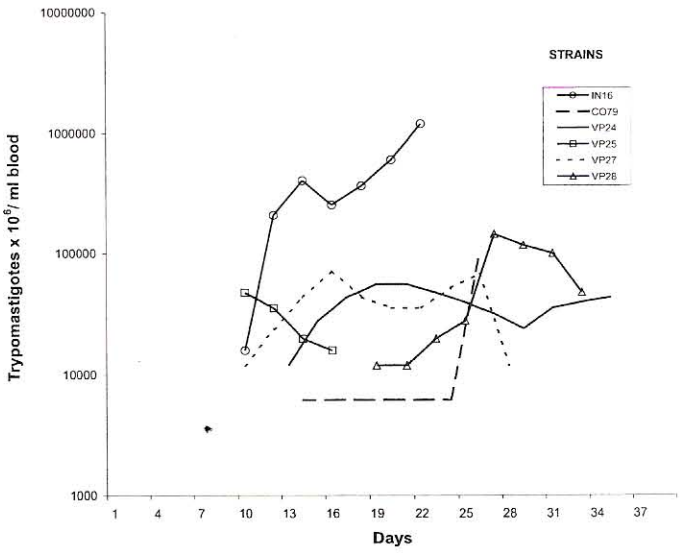

FIGURE 1. PARASITEMIAS OBSERVED IN NMRI MICE INOCULATED WITH METACYCLICS OF *TRYPANOSOMA CRUZI* ISOLATED FROM ANAL GLANDS OF *DIDELPHIS MARSUPIALIS* (STRAINS IN16 AND CO79) AND *PANSTRONGYLUS GENICULATUS* (STRAINS VP24, VP25, VP27, VP28).

DISCUSSION

Differentiation of *T. cruzi* in the insect vector, where morphogenesis through the developmental stages in the gut results in the infective metatrypomastigote, reflects the adaptation of the parasite to this microenvironment. The kinetics of metacyclogenesis and certain controlling factors in the natural condition for development of *T. cruzi* in the vector have been studied *in vitro* [8, 10, 17] and *in vivo* [11, 26]. The biochemical characteristics of the strains of *T. cruzi* have been emphasized [11], whereas other workers consider the process to be vector-dependent [21]. Mello *et al.* [19] illustrated the complexity of this interaction, indicating that important determinants come from both the parasites and the triatomine hosts.

The biological properties of the metacyclic stage are altered by cultivation *in vitro* and in serial passage through vertebrate hosts and insect vectors. The changes in the behavior of the strains of *T. cruzi* are the result of selection among the subpopulations of the parasite, with the resulting morphogenetic plasticity that permits adaptation to different microenvironments [8]. On the other hand, Alves *et al.* [1] suggest that the genome of *T. cruzi* may be altered by environmental conditions and subcloning.

TABLE I

TISSUE TROPISM PRODUCED IN NMRI MICE BY ISOLATES OF *TRYPANOSOMA CRUZI* FROM *PANSTRONGYLUS GENICULATUS* (STRAINS VP 24, VP 25, VP 27, VP 28) AND FROM ANAL GLANDS OF *DIDELPHIS MARSUPIALIS* (STRAINS IN16, CO79) CAPTURED IN URBANIZED AREAS OF CARACAS, VENEZUELA

Organ	Strain					
	VP24	VP25	VP27	VP28	IN16	CO79
Heart	+	+	+	+	++++	++++
Skeletal Muscle	+	+	+++	+++	+++	++++
Liver	-	-	-	+	+	-
Spleen	-	-	-	-	-	-
Duodenum	-	-	-	-	$+^{a}$	$+^{a}$
Colon	-	-	$++^{a}$	-	$+^{a}$	$+^{a}$
Lung	-	-	$+^{a,b}$	$+^{a}$	$+^{a,b}$	-
Kidney	-	-	$+^{c}$	$+^{c}$	-	-
Skin	-	$+^{d}$	$+^{d}$	$+^{d}$	-	-
Pancreas	$+^{e}$	$+^{e}$	$+^{e}$	$+^{e}$	$+^{e}$	$+^{e}$

Mice inoculated with strain CO79 showed, in addition, invasion of the smooth muscle of the male genital organs.

The superscript letters indicate the presence of parasites in the following tissues: a-smooth muscle; b-alveoli; c-capsule connective tissue; d-dermis connective tissue; e-acini.

Although some parameters that characterize the strains of *T. cruzi* isolated from *D. marsupialis* and *P. geniculatus* have been studied [2, 6, 13, 16, 24, 25], the comparative behavior of metacyclics from naturally infected anal glands of marsupials and insect vectors from similar geographical areas has not yet been investigated.

It should be emphasized that the strains of the present work characterized as belonging to the Z1 zymodeme, produced similar infectivity and mortality but parasitemia and histotropism were different; virulence (grade of tissular parasite proliferation) was higher in animals infected by glandular trypomastigotes. Strain IN16 from the opossum anal glands induced high level of blood parasites, mortality and virulence; this may be a particular trait of this subpopulation of *T. cruzi* [25]. The possible alteration of these biological properties as *T. cruzi* penetrates the microhabitat of those glands should be investigated. This parasite can adapt to this unique habitat with morphological and physiological changes that differ from that of the vector [7]. The few observations of possible regulatory factors in the anal glands of opossums have not shown interference by the host tissue in the process of metacyclogenesis [18], which in the triatomines requires the adhesion of the epimastigotes to the rectal cuticle of the insect through hemidesmosomes. This parasite behavior does not seem to occur in the scent glands of marsupials, in which structures for this adhesion have not been observed [7], even though differentiation to infective metatrypomastigotes occurs.

The high levels of mortality and tissular parasite proliferation shown by the strains of this study is in contrast to other reports [2, 6, 13, 16, 22, 25] and demonstrates the extreme variability of *T. cruzi* [4, 16]. Our results support the suggestion of Jansen *et al.* [16] that wild transmission of *T. cruzi* can occur through different and simultaneous cycles, wherein each ecotope would be considered a distinct system.

The ejection of the glandular material of the opossums in territorial marking or in response to threat [9], the experimental infection of opossums and mice with this material through oral and ocular instillation [29], the close anatomical relationship between the anal glands, the rectum, and the urogenital organs as well as the presence of metyacyclic stages in the urine and feces of opossums [18, 28] could explain the cases of Chagas' disease from non-endemic areas where triatomines have not been found [9]. Our studies have been made in areas of intensive urbanization of the valley of Caracas, where infected synanthropic opossums cohabitate with a large human population and domestic animals [13]; the epidemiological importance of these facts becomes obvious.

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