

BIODEMES AND ZYMODEMES OF *TRYPANOSOMA CRUZI* STRAINS: CORRELATIONS WITH CLINICAL DATA AND EXPERIMENTAL PATHOLOGY

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With the objective of establishing biological and biochemical characteristics of a significant number of Trypanosoma cruzi strains from different geographical areas, 138 strains isolated from naturally infected humans, triatomine or vertebrate hosts were studied; 120 were isolated from different areas of Brazil and 18 from other South and Central American countries. Inocula from triatomine or culture forms were injected into suckling Swiss mice, followed by passages into mice 10 to 12 g. Biological characters and histopathological study permitted the inclusion of the strains into three Types or biodesmes: I, II, III. Isoenzymic analysis confirmed a correspondence between the biodesmes and zymodesmes: Type I and Z2b, Type II and Z2, Type III and Z1. Results showed the ubiquitous distribution of the several types of strains. The predominance of the same Type and zymodeme in one geographical area was confirmed: Type II strains among the human cases from eastern Bahia and east of Goiás; Type III strains from humans of north Brazil and Central America and from silvatic vectors or vertebrates from other geographical areas. The biological types of strains correlate with different histopathological lesions considering cardiac involvement and neuronal lesions. These findings suggest that the biological behavior together with isoenzymes patterns and pathological pictures in the vertebrate host can be an important tool for establishing correlations between strains behavior and clinico-pathological manifestations of Chagas' disease in different geographical areas.

Key-words: Trypanosoma cruzi strains. Geographical distribution. Biodesmes. Zymodesmes. Pathology of Chagas' disease.

Trypanosoma cruzi strains are complex multiclonal populations that differs in their genetic and biological characteristics and in their behavior in the vertebrate host. According to Thompson and Lymbery³⁶ if one considers the extensive genetic heterogeneity within species of protozoan and metazoan parasites, a "strain" is not only genetically differentiated from another populations but also differs in one or more characters of epidemiological significance. The concept of *T. cruzi* strains fits well with this general view. Although genetical studies are important to clarify the intraspecific heterogeneity of the parasite, only the study of the biological behavior and the host-parasite relationships could clarify the importance of different strains, in the determination of clinico-pathological manifestations of Chagas'

disease. *T. cruzi* strains represent subspecies based on intrinsic characteristics such as antigenic composition⁴, morphology¹⁴, susceptibility to chemotherapy^{7 15}, isoenzyme patterns^{12 16} and the genomic profiles of DNA kinetoplast²⁶ as well as in the host-parasite relationship¹. An extensive study of the biological characteristics of the natural strains and the histopathological profile in experimental animals has disclosed the possibility of grouping them into a few well defined Types or biodesmes^{1 2}. Different patterns of behavior classified the strains into the Types: I, II and III that correspond to specific zymodesmes⁹. The distribution of the diverse biodesmes in different endemic areas is important to clarify their influence on the local manifestations of Chagas' disease.

In the present study 138 strains of *T. cruzi* are analysed in an attempt to: 1) correlate biological characteristics with zymodesmes patterns; 2) evaluate pathogenicity and histopathological patterns of lesions in mice; 3) identify the distribution of the Types of strains and zymodesmes in South and Central America.

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MATERIAL AND METHODS

Isolates of *T. cruzi* were received from different areas of Brazil and from other South and Central American countries either in triatomines used for xenodiagnosis or in acellular culture medium. Before inoculation, they were washed three times by centrifugation in PBS, pH 7.2. Suckling Swiss mice were initially inoculated and strains then cultivated in Warren medium. Some strains failed to infect mice, hampering their biological characterization. Seven of them were characterized only by isoenzymic profiles. The present survey refers to 138 strains from different geographical areas, as follows: 120 from different regions of Brazil: north (5), northeast (41), central west (64), central south (3) south (7); 15 strains were from other South American countries: Argentina (6), Bolivia (3), Chile (4), Colombia (1), Perú (1); 3 strains from Central America, Honduras (2) and Guatemala (1). Details about the origin of the strains are shown in Tables 1 and 2.

Table 1 - Strains of *T. cruzi* from different areas of Brazil.

Area	State	Biodeme	Zymodeme	Nr	Origin
North	Pará	III	Z1	5	human
Northeast	Ceará	II	Z2	1	human
		III	Z1	6	animals
	Paraíba	III	Z1	3	human
	Bahia: east ¹	II	Z2	23	human
	southeast	II	Z2	1	triat.
	north	III	Z1	8	opos
Center W	Goiás ²	II	Z2	28	human
		III	Z1	15	human
	MG and BA ³	II	Z2	16	human
		III	Z1	15	human
Center S	MG	II	Z2	3	human
South	SP, SC, RS	I	Z2b	2	human opos.
		II	Z2	1	human
		II	Z1	4	triat. opos.

1 Recôncavo bahiano; 2 Mambai-GO; 3 Montalvania-MG and neighboring localities of Bahia. BA = Bahia; MG = Minas Gerais; SP = São Paulo; SC = Santa Catarina; RS = Rio Grande do Sul.

Table 2 - Strains of *T. cruzi* from other countries of South and Central America.

Country	Area	Biodeme	Zymodeme	Nr	Origin
Argentina	San Luis (AWP)	I	Z2b	1	human
	San Luis (CA-I)	III	Z1	1	human
	San Luis	-	Z2b	1	human
	Apipe Gde.	-	Z2b	1	human
	Corrientes	-	Z2b	1	human
	La Pampa (RA)	II	Z2	1	human
Bolivia	Santa Cruz	III	Z1	1	triat.
	Chiwisivi (C-8)	III	Z1	1	triat.
	Chiwisivi (C-50)	-	Z2b	1	triat.
Colombia*		III	Z1	1	triat.
Peru**		I	Z2b	1	human
Chile	Vale Elqui	II	Z2	3	human
	Antofagasta	III	Z1	1	triat.
Honduras		III	Z1	2	triat. human
Guatemala		III	Z1	1	human

* prototype of Type III; ** prototype of Type I.

Biological characterization. Inocula (10^5 blood forms) were injected intraperitoneally into mice weighing 10 to 12g. Parasitemia were evaluated daily by microscopic examination of peripheral blood. Morphology: evaluation of the percentage of broad and slender forms were performed on the 7th, 10th, 14th day post infection. Histopathological study during the acute phase was performed on the 7th, 10th, 14th, 20th and 30th day after inoculation (three mice on each day) and in the chronic phase, with 150 and 180 days of infection. Paraffin sections (5m thick) of heart, skeletal muscle, liver and spleen, stained with hematoxylin and eosin were examined by optical microscopy. For each strain this methodology was repeated in three different passages into mice. The strains were classified into three biological Types or biodemes (I, II, III) as previously described¹.

Isoenzymic characterization. The parasites were cultured in Warren medium, for obtention of enzymic extracts, according to Miles et al²³. The following enzymes were investigated: aspartate aminotransferase (E.C.2.6.1.1. ASAT); alanine aminotransferase (E.C. 2.6.1.2. ALAT); phosphoglucomutase (E.C.2.7.5.1. PGM); and glucosephosphate isomerase (E.C.5.3.1.9. GPI). Thin-layer starch-gel electrophoresis was performed by application of 30V/cm, during 90 minutes for ALAT and 60m for ASAT and of 20V/cm during 150m for GPI and 120m for PGM. The enzymes ALAT and ASAT were developed with phosphate buffer solution 0.1M and BNAD, examined by ultra-violet light; for the enzymes GPI and PGM, TRIS/HCl buffer solution 0.3M and NADP were used besides the MTT (dimethylthiazole 2-yl 2-5 diphenil tetrazolium bromide) 0.36mM, agar-gel 0.06% and phenazine metasulfate, 0.03mM. As control of the isoenzyme characterization the prototypes of each of the three morphobiological patterns were included on each electrophoretic run: Peruvian (Type I), 12 SF (Type II) and Colombian (Type III). The nomenclature here used for the zymodeme patterns is based on that established by Miles et al²³ and the genetic variant Z2b described for the Chilean strains by Miles et al²⁴ with a three banded GPI pattern that corresponds to Bolivian Z2 described by Tibayrenc et al³³.

RESULTS

In the present study the basic characteristics of the three biodemes were taken to classify all

the studied strains, as previously published². Briefly: Type I - macrophagotropism in the initial phase of infection, high virulence with 100% of mortality within 12 days, maximum parasitemia on the 7th to 12th days and predominance of slender blood forms in the initial phase of infection; Type II - myotropic, especially involving the heart, predominance of broad forms but with a percentage of slender forms in peripheral blood, parasitemic peaks from 12 to 20 days when mortality reaches a maximum; this type of strain can presents low, medium or high virulence; Type III, myotropic strains, mainly parasitism of skeletal muscle, predominance of broad forms, parasitemic peaks from 25 to 30 days or later, low mortality within 30 days.

Zymodeme patterns for all strains were identified as Z1, Z2 and Z2b based on the profiles of GPI, PGM, ASAT and ALAT (Figures 1 a, b and 2 a, b for the prototypes). A concordance was detected between the biological type and the zymodeme: Type I corresponded to zymodeme Z2b, a variant of Z2. Some strains with this zymodeme (Z2b) did not show the high virulence characteristic of the Type I

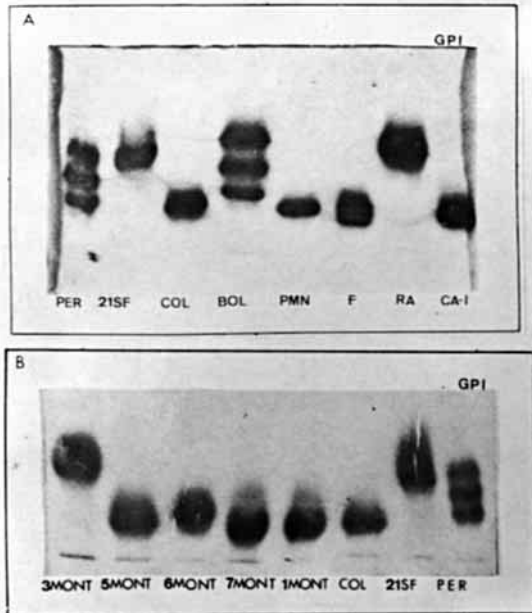


Figure 1 - Electrophoretic patterns for the enzyme GPI of *T. cruzi* strains: a) the prototypes of Type I (Per) Z2b; Type II (21 SF) Z2; Type III (Col) Z1 and strains from Bolivia (Bol); northeast Brazil (PMN-Ceará); Argentina (RA and CA-I). b) the same prototypes as above and five strains from Montalvania, MG one of which showing the zymodeme Z2 (3 Mont) and four Z1 (1 Mont, 5 Mont, 6 Mont and 7 Mont).

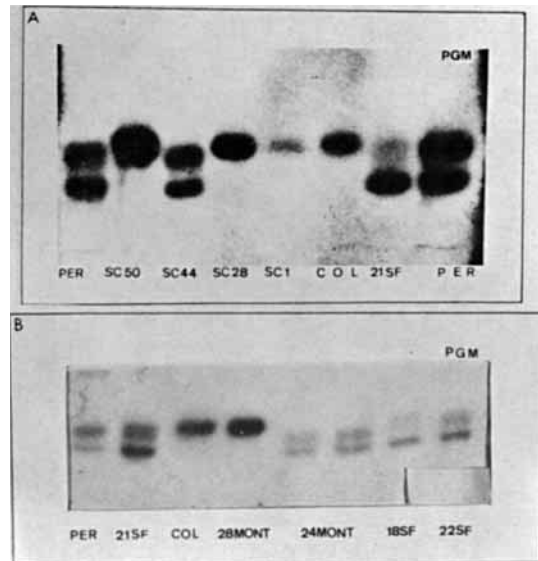


Figure 2 - Isoenzymic patterns for the enzyme PGM of *T. cruzi* strains; a) the prototypes of Type I (Per) Z2b; Type II (21 SF) Z2 and Type III (Col) Z1; four strains from Santa Catarina state -South Brazil: SC-1, SC-28, SC-50 - Z1; SC-44 - Z2b; b) the same prototypes as above and two strains from Montalvania, MG : 28 Mont (Z1) and 24 Mont (Z2); two strains from São Felipe, Bahia: 18 SF and 22 SF, both with the profil of Z2.

strains (SC-44 from Santa Catarina and another from Argentina). Type II strains corresponded to Z2. Type III strains to Z1. For each biodeme a characteristic histopathological picture in acutely infected mice was disclosed as previously described¹. Macrophagotropism was evident for the Type I strains (Figure 3),

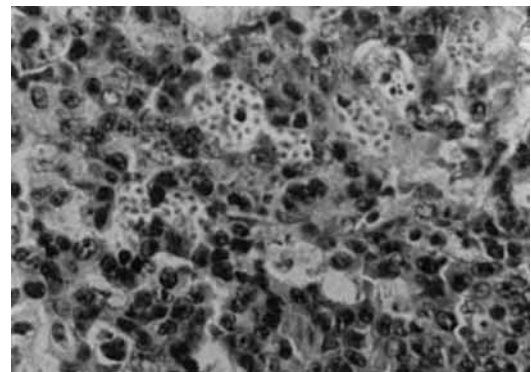


Figure 3 - Section of mouse spleen in the acute phase of infection: Type I strain - macrophages are loaded with intracellular amastigotes of *T. cruzi* (macrophagotropism), 400X.

together with intense cardiac lesions. The Type II strains determined intense cardiac parasitism and myocarditis. Type III strains determined predominant skeletal muscle lesions and also cardiac parasitism and myocarditis. In chronically infected mice, with the three types of strains a variable degree of inflammatory lesions in the myocardium were present, from mild to intense. Cardiac lesions were more frequent and more intense in those infected with Type III strains (Figures 4 a, b). Involvement of the myoenteric plexus was more evident in mice

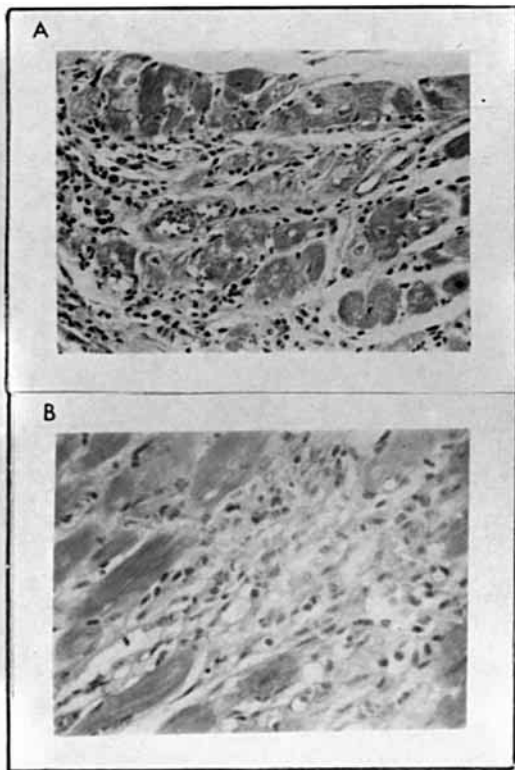


Figure 4 - Heart lesions in mice chronically infected with Type III strain of *T. cruzi*: a) myocardium showing chronic diffuse myocarditis with mononuclear cell infiltration, fibroblast proliferation and interstitial fibrosis, 400X; b) focal area of cardiac cell destruction, fibroblast proliferation, matrix deposits and mononuclear cell infiltration, 400X.

infected with the Type I and II strains (Z2b, Z2), with the presence of amastigotes, inflammatory infiltration and neuronal cells destruction (Figures 5 a, b).

Results of the biological and isozymic characterization of the 138 strains are summarized in Tables 1 and 2. Table 1 shows the exclusive presence of Type III, Z1 strains in the north

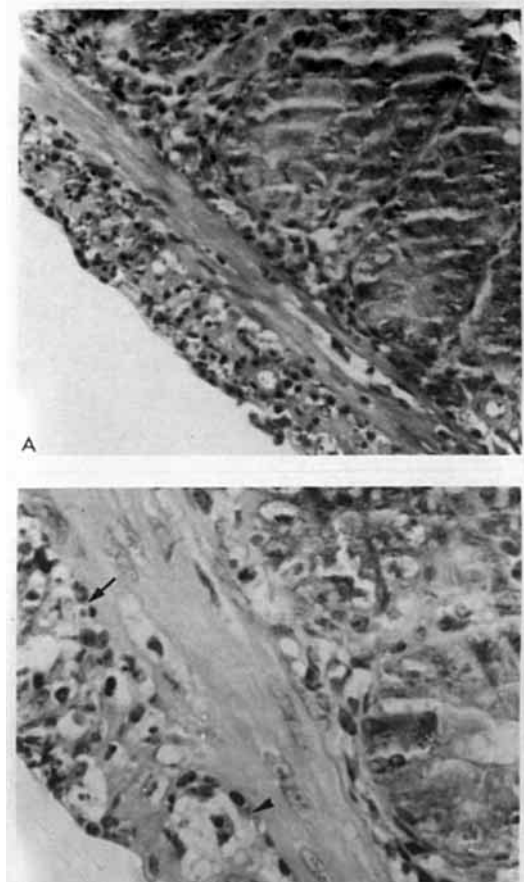


Figure 5 - Myoenteric plexus in mice infected with Type I strain (acute phase): a) inflammatory infiltration, neuronal cell destruction of the Auerbach plexus and parasite debris, 250X b) Auerbach plexus - intracellular amastigote forms (arrow), mononuclear infiltration and neuronal cell vacuolization (arrow head), 400X.

and the predominance of Type II, Z2 in the east of Bahia State for the strains from patients of São Felipe (Table 3). In the central west region of Brasil (Table 1) two areas deserve consideration: east of Goiás State (Mambai) where 28 strains from human cases (Table 4) disclosed Type II, Z2 and north of Minas Gerais State (Montalvania) and west of Bahia from where 32 strains isolated from human cases (Table 5) were either Type II, Z2 (17 strains) or Type III, Z1 (15 strains). From the south of Brazil (Table 1) of the four strains from Santa Catarina State, three were classified as Type III, Z1 - *P. megistus* (2) and opossum (1) and one strain from an opossum was classified as Type I, Z2b, although disclosing a

Table 3 - Identification of the strains from São Felipe, Bahia.

Strain	Patient	Age	Sex	Clin. diag.	Type	Zym
1 SF	EMR	12	F	chr. assym.	II	Z2
7 SF	MES	32	F	chr. assym.	II	Z2
9 SF	MRS	30	F	chr. assym.	II	Z2
10 SF	CSF	18	M	indet.	II	Z2
11 SF	PIA	55	M	chr. card.	II	Z2
12 SF	ECP	17	M	acute	II	Z2
15 SF	NCS	5	M	acute	II	Z2
16 SF	RCH	4	F	acute	II	Z2
17 SF	DPS	13	F	acute	II	Z2
18 SF	RC		M	acute	II	Z2
19 SF	DPS	4	M	acute	II	Z2
20 SF	MRB	4	F	acute	II	Z2
21 SF	MPS		M	acute	II	Z2
22 SF	ABS	4	M	acute	II	Z2
23 SF	MPRS	16	F	acute	II	Z2
24 SF	JCF	10	M	acute	II	Z2
25 SF	MLS		M	acute	II	Z2

low virulence for mice. Table 2 shows the distribution of the three types of strains for various countries of South America. Of the six strains from Argentina, the CA-I and RA strains described by Gonzalez Cappa^{17 18} were classified as Type III (Z1) and Type II (Z2) respectively; other strains from Corrientes did not infect mice and showed the zymodeme

Table 4 - Identification of the strains from Mambai, Goiás.

Strain	Patient	Age	Sex	Clin. diag.	Type	Zym
1 MAM	ASG	8	F	indet.	II	Z2
2 MAM	JA	35	M	indet.	II	Z2
3 MAM	VM	6	M	indet.	II	Z2
4 MAM	SBA	26	F	indet.	II	Z2
5 MAM	JV	12	M	indet.	II	Z2
6 MAM	FRV	6	M	indet.	II	Z2
7 MAM	PMJ	18	F	acute	II	Z2
8 MAM	CMC	16	F	acute	II	Z2
10 MAM	NMM	9	F	indet.	II	Z2
11 MAM	ARPS		F	indet.	II	Z2
13 MAM	MCM	34	F	indet.	II	Z2
14 MAM	EM		F	indet.	II	Z2
17 MAM	JPM	45	F	indet.	II	Z2
18 MAM	MSS	12	M	indet.	II	Z2
19 MAM	JSC	10	M	indet.	II	Z2
20 MAM	MJV	38	M	indet.	II	Z2
21 MAM	CRV	57	F	chr. card.	II	Z2
22 MAM	VMM	34	M	indet.	II	Z2
23 MAM	JML	9	M	indet.	II	Z2
24 MAM	LJS	12	F	indet.	II	Z2
25 MAM	JGA	44	M	megaesoph	II	Z2
26 MAM	MJJ	25	F	indet.	II	Z2
27 MAM	MRS	61	M	indet.	II	Z2
28 MAM	MMM	62	F	megaesoph	II	Z2
29 MAM	DPS	45	M	indet.	II	Z2
30 MAM	Miltina	48	F	indet.	II	Z2
31 MAM	MCS	45	F	chr. card.	II	Z2
32 MAM	JPM	66	F	megaesoph	II	Z2
33 MAM	EMA	34	M	chr. card.	II	Z2

Z2b. Strains from Bolivia, Chile, Colombia, Peru, were classified into the three types (I, II, III) and zymodemes (Z2b, Z2, Z1). From Central

America (Table 2) the 3 strains were included into Type III, Z1.

Table 5 - Identification of the strains from Montalvania.

Strain	Patient	Age	Sex	Clin. diag.	Type	Zym
1 MONT	TFO	13	F	acute	III	Z1
2 MONT	FFM	35	M	sub-acute	III	Z1
3 MONT	CCL	17	F	indet.	II	Z2
4 MONT	MA	49	M	card+dig.	II	Z2
5 MONT	LFC	2	M	acute	III	Z1
6 MONT	ZNC	25	F	acute	III	Z1
7 MONT	ERM	16	M	acute	III	Z1
8 MONT	JFP	40	M	acute	III	Z1
10 MONT	EMS	11	F	acute	II	Z2
11 MONT	MMJ	7	F	acute	III	Z1
12 MONT	MRSG	5	F	acute	II	Z2
13 MONT	AAS	10	M	acute	III	Z1
14 MONT	FHS	27	M	acute	III	Z1
15 MONT	MLSG	13	M	acute	II	Z2
16 MONT	SLJ	61	F	acute	II	Z2
17 MONT	JPS	22	M	acute	III	Z1
18 MONT	CPO	54	F	acute	II	Z2
19 MONT	TCF	10	F	acute	III	Z1
20 MONT	HVNM	12	F	acute	II	Z2
22 MONT	JLR	32	M	acute	II	Z2
23 MONT	AGS	15	M	acute	II	Z2
24 MONT	EPC	6	F	chr. card.	II	Z2
25 MONT	ICRS	11	F	acute	III	Z1
28 MONT	MMS	35	F	acute	III	Z1
29 MONT	CGM		F	acute	II	Z2

DISCUSSION

As postulated by Thompson and Lymbery³⁶ parasite strains must be described by a combination of genetical and biological characteristics since reliance on the genotype alone may confer significance on a feature of little biological relevance. Concerning *T. cruzi* strains, genetical studies based on isoenzyme patterns have demonstrated the multiclonal structure of natural parasite strains^{33 35}. Intra-zymodeme variability has been demonstrated by the genetic distances or expressed by different alleles in the same zymodeme^{12 23 34}. However, we admit that stable populations may represent the equilibrium of multiple clones with predominance of a characteristic biological behavior. Experimental evidences of the stability of strain behavior has been obtained by biochemical and biological characterization after parasites were passed through different conditions of maintenance and cultivation²¹; strains obtained from mice submitted to treatment with different drugs and not cured, which supposedly submitted them to pressure by clonal selection, also maintained their biological behavior and isoenzyme profiles²².

Studies with cloned populations of natural strains have demonstrated either homogeneity or heterogeneity of several clones¹³ including

differences in virulence and pathogenicity²⁷. However, in an epidemiological study, cloned populations can not be taken as representing the strains isolated from different geographical areas. Only "natural" strains can be taken as representatives of the epidemiological profiles of those areas. The present study confirms previous observations indicating the predominance of one type of strain in the same geographical area¹, showing a large distribution of Type II, Z2 strains in Brazil. The zymodeme corresponding to Type I strains has been first described by Andrade et al⁹ in 1983 and after that in Bolivia³⁴ and Chile²⁴ being designated as a variant of the Z2 (Z2b); it is rarely found in Brazil being represented by the Y strain and the strain SC-44 isolated from an opossum from Santa Catarina; this same zymodeme has been identified in one case of congenital transmission of Chagas' disease in Bahia¹¹. The type III strains (Z1), associated with the silvatic cycle²³, has also occurred in human patients in north and northeast states of Brazil. In Montalvania, MG and neighboring localities of west central Brazil²⁰ an overlap between the silvatic and domiciliary transmission cycles determined the concomitance of Types II and III in the same geographical area. Schlemper Jr.³⁰ studying 23 strains from Minas Gerais (south central region of Brazil), detected in all of them the same zymodeme A as described by Romanha²⁸, corresponding to Z2²³. In southern Brazil especially in Santa Catarina, where the parasite was isolated from silvatic vectors or vertebrate hosts with no human cases being registered, Type I (Z2b) and III (Z1) strains were identified, confirming data of Steindel et al³². The small number of strains from other countries of South and Central America was not sufficient to evaluate the distribution of the strain Types and zymodemes, but confirmed findings from other authors, such as Apt et al¹⁰ in Chile and Tibayrenc et al³⁴ in Bolivia, who registered the presence of Z2 and genetic variants in the domestic cycle and Z1 in the silvatic reservoirs; in the present study the three biodemes (I, II, III) have been detected in the several countries. In Central America as well as in northern Brazil, Type III, Z1 strains were predominant.

The biological and biochemical characteristics of strains are correlated with different tissue lesions, as first observed in acute infection of mice with strains of different Types^{1,2}. In the chronic phase, a clearcut influence of the

biological type of strain on the histopathological lesions has also been detected. In a previous study of 200 chronically infected mice³, it was demonstrated that the Type III strains (Z1), were the most pathogenic, determining intense cardiac and skeletal muscle lesions with patent parasitism of tissues even in a late stage of infection; electrocardiographic alterations were more frequent and intense in mice chronically infected with Type III strains²⁹. Cardiac lesions in the chronic phase occurred also in mice infected with Type I and II strains. Besides cardiac lesions, these two types of strains determined significant involvement of neuronal cells of the myoenteric plexus as previously described for the strains of S. Felipe - Bahia, Type II¹ and for the Y strain Type I⁵. A quantitative study of neuronal cells in myoenteric plexus during the chronic phase of infection with the Y strain has shown a significant decrease, as compared with the Colombian strain (Type III)⁶. Recently³¹, segmentar inflammatory alterations in the ganglionic cells of the autonomic nervous system has been detected with the three types of strains showing that the inflammatory lesions caused by the Y strain (Type I) are more destructive to the neuronal tissue than that caused by the other two strain types. Taking into account that the zymodeme Z2b (Type I) is genetically closely related to Brazilian Z2 (Type II), probably the two Types are related to identical pathologies in chronic disease, particularly considering the neuronal destructions in myoenteric plexus. The Zymodeme 2 has been identified by Lauria-Pires¹⁹ in one stock and several clones, from a patient with the digestive form of Chagas' disease, a direct demonstration of the participation of this zymodeme, corresponding to Type II strain in the pathogenesis of megasyndromes in Chagas' disease.

The ubiquitary distribution of *T. cruzi* strains cause difficulties to interpret the different manifestations of Chagas' disease and their correlation with strains variability. It is conceivable that the predominance of the same biodeme and zymodeme in well defined endemic areas can be related with the main manifestations of the disease in these areas. The histopathological evidence that cardiac lesions occurred with the three types of strains, correlates well with the occurrence of cardiopathy as the main manifestation of human Chagas' disease anywhere this parasitic disease is endemic. A similar correlation could

also be made with the megasyndromes in areas in which the types I and II strains (Z2b and Z2 zymodemes) are predominant, and their absence in areas without these biodemes. The transfer of experimental data to the interpretation of human disease is always difficult. However, a direct correlation between the strains behavior in humans and in experimental mice was observed in the response to chemotherapy in strains isolated from patients from Montalvania, classified into Types II and III, with a concordance in 81 per cent of the cases⁸. This is an evidence that experimental data can contribute to the understanding of the human disease.

Therefore, experimental data correlating the biological behavior, histopathological pictures and zymodeme patterns confirms epidemiological evidences indicating an influence of parasite strains on the histopathological lesions² and clinical presentations²⁵ of Chagas' disease in different geographical areas.

RESUMO

Foram estudados os caracteres biológicos e isoenzimáticos de 138 cepas do Trypanosoma cruzi de diferentes áreas geográficas, sendo 120 do Brasil e 18 de outros países da América do Sul e Central. Camundongos recém-nascidos foram inoculados com formas metacíclicas de triatomíneos ou de culturas axenicas, seguindo-se passagem em camundongo de 10 a 12g. Os caracteres biológicos e o estudo histopatológico permitiram incluir todas as cepas em três Tipos ou biodemas: I, II e III. A análise isoenzimática para PGM, GPI, ASAT e ALAT confirmou a correspondência entre os biodemas e os zimodemas: Tipo I e Z2b, Tipo II e Z2, Tipo III e Z1. Os resultados mostraram a distribuição ubíqua dos diversos Tipos de cepas, observando-se a predominância do mesmo biodema em uma mesma área geográfica: cepas de Tipo II de casos humanos do Leste da Bahia e Leste de Goiás; cepas de Tipo III do Norte do Brasil e da América Central e de vetores ou vertebrados silvestres em várias áreas geográficas. Os biodemas correlacionam com diferentes lesões histopatológicas na fase aguda e crônica da infecção, considerando-se o envolvimento cardíaco e as lesões neuronais. Estes achados sugerem que o comportamento biológico, os padrões isoenzimáticos e o quadro patológico podem se constituir em importantes elementos para o estabelecimento de correlações entre as cepas do parasito e as manifestações clínico-patológicas da doença de Chagas em diferentes áreas geográficas.

Palavras-chaves: Cepas do Trypanosoma cruzi. Distribuição geográfica. Biodemas. Zimodemas. Patologia da doença de Chagas.

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