Rev. Inst. Med. trop. São Paulo
38 (1): 23-28, janeiro/fevereiro, 1996.

TRYPANOSOMA CRUZI STRAINS: BEHAVIOR AFTER PASSAGE INTO AUTHOCTONOUS OR FOREIGN SPECIES OF TRIATOMINE (BIOLOGICAL AND BIOCHEMICAL PATTERNS).

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SUMMARY

The behavior of *T. cruzi* strains from S. Felipe - BA (19 SF, 21 SF and 22 SF) classified as Type II Zymodeme 2, was investigated after passage through the authoctonous (*P. megistus*) and foreign vectors (*T. infestans* and *R. prolixus*). For each strain Swiss mice were infected: I - with blood forms (control); II - with metacyclic forms (MF) from *P. megistus*; III - with MF from *T. infestans*; IV - with MF from *R. prolixus*. Inocula: MF from the three species of triatomine, 60 to 120 days after feeding in infected mice, adjusted to 10^4. Biological behavior in mice (parasitemia, morphology, mortality, virulence and pathogenicity) after passage through triatomine was compared with data from the same strain in control mice. Isoenzymic electrophoresis (ASAT, ALAT, PGM, GPI) were also performed after culture into Warren medium. The three strains maintained the isoenzyme profiles (zymodeme 2), in the control groups and after passages through different species of triatomine. Biological characterization disclosed Type II strains patterns for all groups. An increased virulence was observed with the 22 SF strain isolated from *P. megistus* and *T. infestans* and higher levels of parasitemia and predominance of slender forms in mice inoculated with the 19 SF and 21 SF from these same species. Results indicate that the passage through the two species *T. infestans* and *P. megistus* had a positive influence on the virulence of the regional strains of S. Felipe, regardless of being autocthonous (*P. megistus*) or foreign to the area (*T. infestans*).

KEYWORDS: *Trypanosoma cruzi; T. cruzi* strains; Biological characters; Biodemes; Zymodemes; Vector species; Triatomine.

INTRODUCTION

Biological classification of *Trypanosoma cruzi* strains into a limited number of Types or Biodemes [1] has disclosed an ubicuous distribution of the strains [2] with predominance of one Type in one same endemic area. Each biological type has a peculiar isoenzymic profile [3] with correspondence to the zymodemes described by MILES et al. [18]. On the other hand, an adaptation of different zymodemes to the vector species predominant in one area has been demonstrated [10, 19]. Considering vector-parasite relationship several authors have shown higher degree of adaptability of *T. cruzi* strains from one endemic area to the authoctonous species of vectors [5, 6, 12, 22, 23]. Reciprocal vector/parasite influence could be important in the determinism of parasite behavior.

Strains isolated from the endemic area of São Felipe - Bahia have been characterized as Type II [1] and Zymodeme 2 [3]. The present study intends to comparatively evaluate these strains after maintenance in an authoctonus species (*P. megistus*) [16, 20] or in *Triatoma infestans* which is present in other areas of Brasil but not in the Reconcavo Bahiano [20], and *Rhodnius prolixus*, a

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foreign species, occurring in other American countries [17]. The influence of the vector species in the biological and biochemical behavior of these strains was investigated.

MATERIAL AND METHODS

Three strains of *T. cruzi* isolated from patients of the endemic area of São Felipe - BA (Reconcavo Bahiano) and maintained in laboratory by serial passages in mice, has been used in the present study: 19 SF, 21 SF and 22 SF, (Type II and Zymodeme 2). Twenty Swiss mice, weighing 10 to 12g were infected with either of each strain (Inocula 10^5) and used for feeding the triatomine.

Three different species of triatomine (*Panstrongylus megistus, Triatoma infestans* and *Rhodnius prolixus*) reared in the Parasitology Laboratory has been used for *T. cruzi* passage. Five fifth instar laboratory-bred were fed on each infected mice. The inocula were obtained 60 to 120 days after, as metacyclic forms from the insects gut and washed by centrifugation with PBS (3 times 1,000g), and adjusted to 10^4 by counting in Neubauer chamber.

Blood forms obtained from infected mice, washed three times in PBS (3,000g) were used to infect one control group, for each strain, with 10^4 trypomastigotes.

Inoculations were performed intraperitonially in Swiss mice weighing 10 to 12 g. For each *T. cruzi* strain 75 to 100 mice were used, divided into four experimental groups; Table 1 shows the number and distribution of mice according with the experimental group.

Experimental groups: For each *T. cruzi* strain, four experimental groups were done: Group I - mice inoculated with blood forms (controls); Group II - mice infected with metacyclic forms from *P. megistus*; Group III - mice infected with metacyclic from *T. infestans*; Group IV - mice infected with metacyclic from *R. prolixus*.

Biological characterization: Parasitemia was evaluated for each experimental group by daily counting of the number of parasites in peripheral blood in 50 microscopic fields, 400X. Morphology of parasites was evaluated in the 7th, 10th and 14th days of infection in blood smears stained with Giemsa stain, for establishing the percentages of slender and broad forms. Mortality rates represents the number of mice that spontaneously died up to 20 days of infection, excluding those sacrificed during the course of the infection.

Biochemical analysis - Blood forms obtained by heart puncture from mice of the four experimental groups were cultured in acellular Warren medium. Parasites were washed with KRT buffer solution. Enzymic extracts were obtained and maintained in liquid Nitrogen as "pearls" according to MILES et al. [18]. They were submitted to starch gel electrophoresis for the enzymes: Alanine aminotransferase (ALAT) E.C. 2.6.1.2.; Aspartate aminotransferase (ASAT) E.C.2.6.1.1.; Glucose phosphatisomerase (GPI) E.C.5.3.1.9. and Phospho glucomutase (PGM) E.C.2.7.5.1.

Thin-layer starch-gel electrophoresis was performed by application of 30V/cm during 90 minutes for ALAT and 60 minutes for ASAT; 20V/cm during 150 minutes for GPI and 120 minutes for PGM. The enzymes ALAT and ASAT were developed with phosphate buffer solution 0.1M and Beta NAD and examined under ultra-violet light; GPI and PGM were developed with TRIS/HCl buffer solution 0.3M and NADP plus MTT (dimethilthiazole 2-yl 2-5 dipheniltetrazolium bromide) 0.36 mM, agar-gel 0.6% and phenazine metasulphate, 0.03 mM. As control of the enzymes, the enzymic extracts of the prototypes of the three Types of strains (Peruvian, Type I; 21 SF Type II and Colombian, Type III) were submitted to electrophoresis at the same plates.

TABLE 1

Distribution and mortality rates of mice infected with different strains of *T. cruzi* (experimental groups and origin of parasites)

Exper. groups	Origin* of parasites	Strains of *T. cruzi*					
		19 SF		21 SF		22 SF	
		Number	Mortality (%)	Number	Mortality (%)	Number	Mortality (%)
I	Mouse	25	9	27	71.4	25	18
II	*P. megistus*	19	0	25	27	25	27
III	*T. infestans*	6	0	26	8	6	25
IV	*R. prolixus*	24	16.6	24	20	20	16
	Total	74		102		76	

* Inoculum: 10^4 trypomastigotes obtained after passage into mouse (controls) and three species of triatomine

RESULTS

Evolution of parasitemia in the several groups is shown in Figs. 1 (a, b and c). With the exception of the 19 SF strain, the peaks occurred later in the groups infected with metacyclic forms from triatomine as compared with those infected with blood forms. Highest peaks occurred with the 19 SF strain obtained from *P. megistus* and *T. infestans*; with the 21 SF strain and inocula from mice and *T. infestans* and with the strain 22 SF from *P. megistus*.

Morphological evaluation of blood forms of the parasites from mice infected with the 19 SF and 21 SF strains is shown in Figs. 2 and 3. For the 19 SF strain the controls infected with blood forms showed predominance of slender forms (60%) by the 10th day and of broad forms (70%), by the 14th day of infection; metacyclic forms from *P. megistus* and *R. prolixus* determined a predominance of slender forms from the 10th to the 14th day; in the infection with trypomastigotes

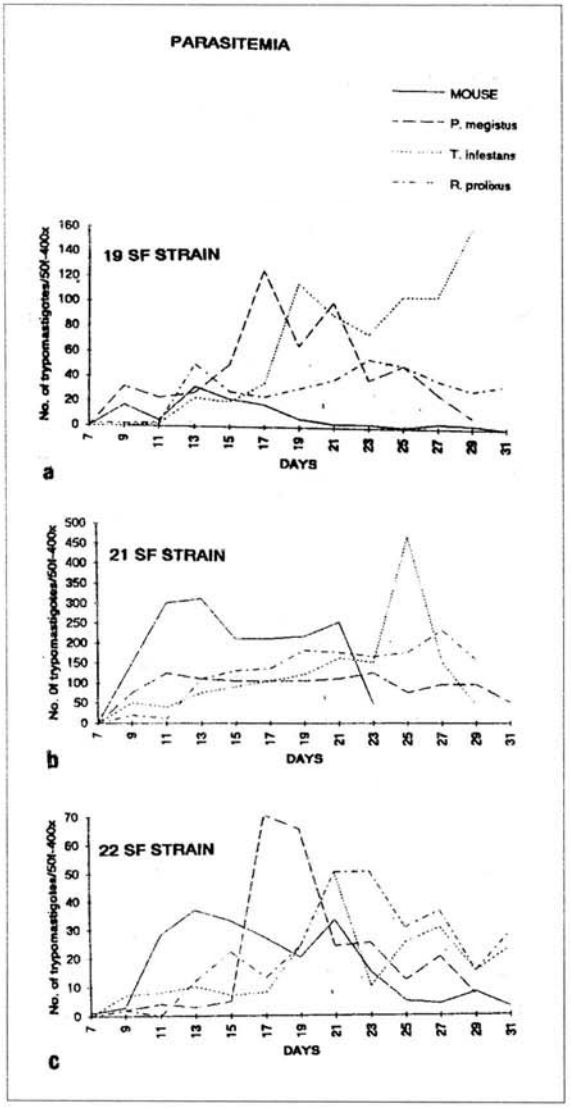

Fig. 1 - Evolution of parasitemia in mice infected with inocula from mice (controls) or from triatomine (three different species) - **a) 19 SF** strain of *T. cruzi:* highest levels were observed in those infected with inocula from *P. megistus* and *T. infestans*; **b) 21 SF** strain: the inocula obtained from mice (blood forms) determined early peaks but the highest levels were observed with inocula from *T. infestans*, in a late stage. **c) 22 SF** strain: the highest levels of parasitemia were observed in those infected with metacyclic from *P. megistus*.

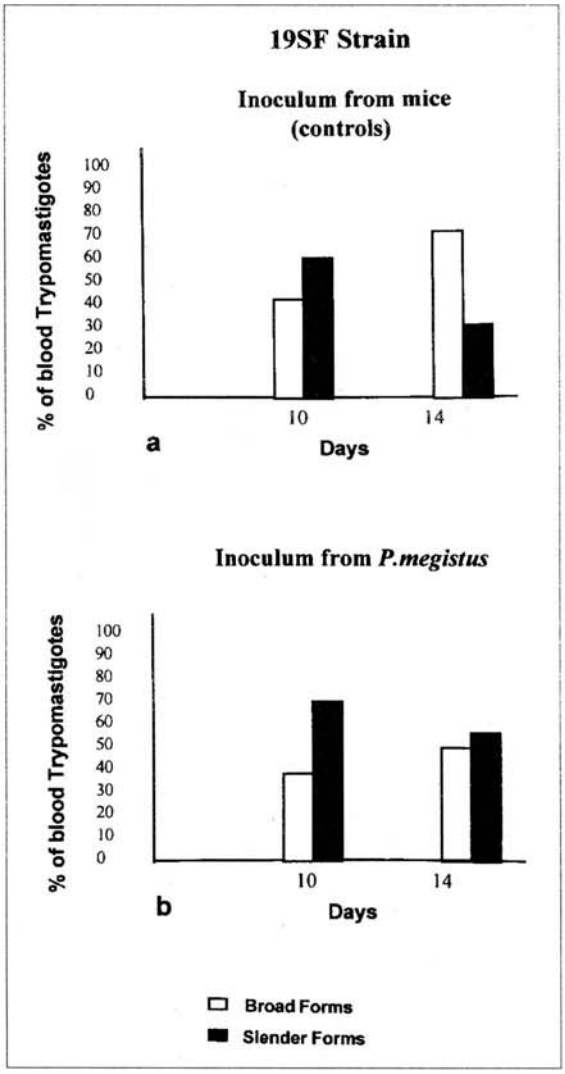

Fig. 2 - Morphological aspects of the 19 SF strain in peripheral blood of infected mice: **a)** predominance of slender forms in the 10th day and of broad forms in the 14th day in the infection with blood forms; **b)** slender forms predominate in mice infected with metacyclic from **P. megistus** in the 10th and 14th day of infection.

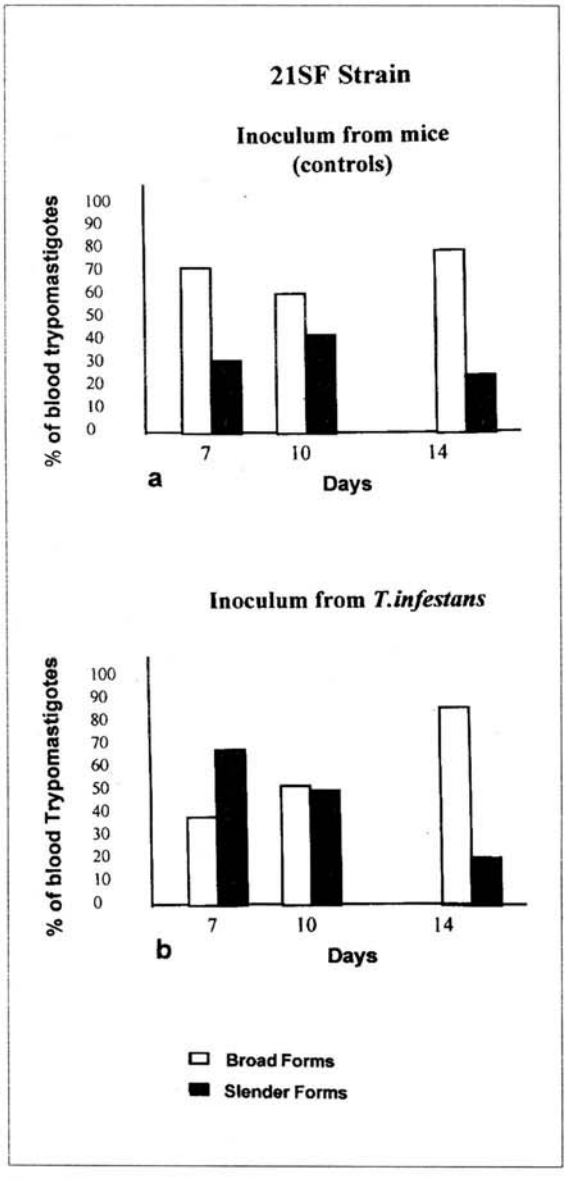

Fig. 3 - Morphology of parasites in peripheral blood of mice infected with the **21 SF** strain: **a)** controls infected with blood forms: there are predominance of broad forms from the 7th through the 14th day; **b)** predominance of slender forms in mice infected with metacyclic from *T. infestans* (7th day) and of broad forms by the 14th day.

from *T. infestans* there were predominance of broad forms by the 14th day. The 21 SF strain in mice inoculated with blood forms determined a predominance of broad forms from the 7th through the 14th day. In mice inoculated with the trypomastigotes from triatomine an increased number of slender forms for all the species was seen, as compared with the control group. However broad forms predominate by the 14th day in those inoculated with forms from *P. megistus* and *T. infestans*.

Mortality rates are expressed in Table 1, showing a high variability according with the strains. For the 21 SF strain the highest mortality index was seen in mice infected with blood forms (71.4%) and with forms from *P. megistus* (27%). For 22 SF strain, maximum mortality occurred with inocula from *P. megistus* and *T. infestans* (27 and 25% respectively). The 19 SF strain, obtained either from blood forms or metacyclic from triatomine, determined low mortality (0 to 16.6%).

Isoenzymic analysis showed the same profile for the strains after passage in triatomine as compared to controls, corresponding to Type II and zymodeme 2 (Figs. 4 and 5).

DISCUSSION

The stability of biological, biochemical and genetic characteristics of *T. cruzi* strains under different conditions has been shown by several authors [4, 9, 13, 15]. Virulence of these strains is however a variable character depending on several factors, including *T. cruzi*/vectors relationship [11]. In a previous paper [14] increasing of virulence was observed with the 12 SF strain, the

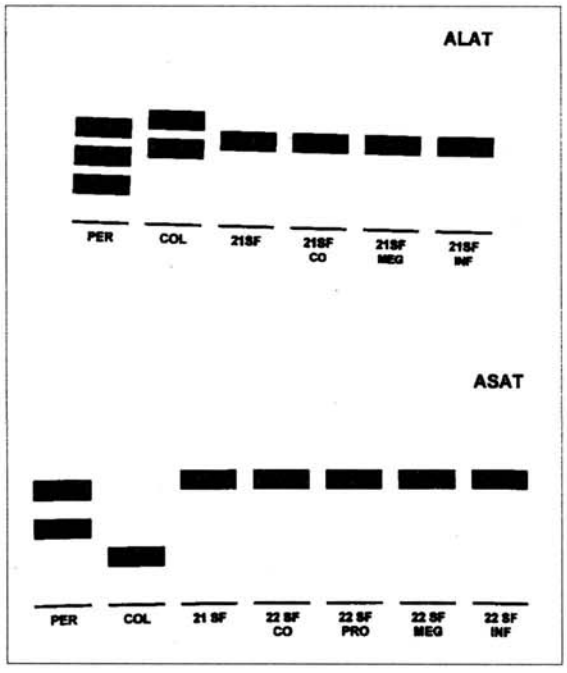

Fig. 4 - Enzymic profile of **ALAT** and **ASAT** of the strain **21 SF** showing the patterns of the Type II strains (Zymodeme 2) after passage in mice, and in three different species of triatomine.

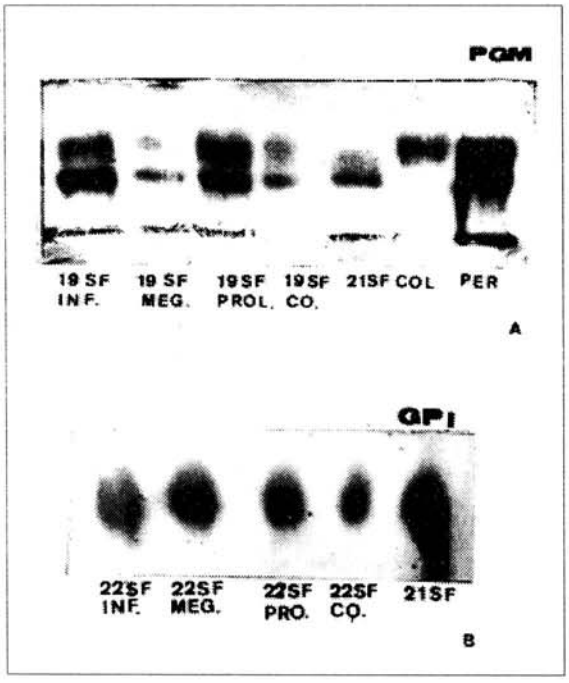

Fig. 5 - Isoenzymes patterns of the **PGM (a-19 SF)** and **GPI** enzymes (**b-22 SF**) after passages in mice and three different species of triatomine. The patterns of Type II, (Zymodeme 2), are maintained.

prototype of type II, after passage through *T. infestans* and *P. megistus* and of the Colombian strain, prototype of Type III after passage through *P. megistus* and *R. prolixus*. The present investigation have shown that the biological and biochemical characters of the three strains were unaltered after the passage through three different species of triatomine. Considering the virulence, evaluated by the capacity of multiplication in the vertebrate host and mortality rates, there were a positive influence from passage through *T. infestans* and *P. megistus* in reference to the 22 SF strain, although for the 21 SF strain the inoculation with blood forms determined the highest mortality and parasitemic peaks. The 19 SF strain maintained low virulence either for the inoculum from mice or from the three species of triatomine although with higher parasitemic levels in this latter. The maintenance of the biochemical characters, revealed by the isoenzyme profiles confirms the hypothesis that virulence can be a variable parameter but the genetic phenotypic patterns is stable, as have been shown for several strains after they were submitted to chemotherapeutic treatment [15]. It seems interesting to investigate whether the behavior of one determined strain is influenced by the vector that predominates in the endemic area. Geographical distribution and importance of the triatomine species in Brasil and South America have been well established [7, 8, 17, 19, 21]. According to MILES et al. [19] the distribution of different triatomine species could be correlated with different zymodemes and different geographical manifestations of Chagas' disease. For these authors, the wide distribution of *P. megistus* and *T. infestans* could influence the adaptability of the strains of *T. cruzi* to these two species of vectors. However, other authors [5, 6, 12, 22, 23] have shown that local strains are more adapted to authoctonous vectors. In the present study the three strains from São Felipe have shown either higher virulence or increased parasitemia levels and predominance of slender forms after passage into *P. megistus*, an authoctonous species or *T. infestans*, not found in that area [8, 20]. This seems to indicate an influence of these two vector species on the virulence of *T. cruzi* strains and, consequently, on its infectivity for the vertebrate host, in comparison with *R. prolixus*. The peculiar factors of the vectors *P. megistus* and *T. infestans* which are responsible for strain behavior, regardless of being authoctonous or foreign to the specific endemic area, deserve further investigations.

RESUMO

Cepas do *Trypanosoma cruzi:* comportamento após passagem em triatomíneos autóctones ou não autóctones (padrões Biológicos e Bioquímicos).

Foi estudada a influência da passagem de cepas do *T. cruzi* de São Felipe - BA (19 SF, 21 SF e 22 SF), Tipo II, Zimodema 2, no vetor autóctone (*P. megistus*) e em vetores não autóctones (*T. infestans* e *R. prolixus*). Para cada cepa, camundongos suiços de 10 a 12 g foram inoculados com I - formas sanguícolas (controles); II - metacíclicos do *P. megistus*; III - metacíclicos de *T. infestans*; IV - metacíclicos do *R. prolixus*. O inóculo para cada grupo foi de 10^4 tripomastigotas. A obtenção dos metacíclicos foi feita 60 a 120 dias após infecção dos triatomíneos. O estudo morfobiológico em camundongos (parasitemia, morfologia, mortalidade, virulência e patogenicidade) foi realizado após as passagens cíclicas e comparado ao dos controles. Foi feita a análise isoenzimática de todos os grupos experimentais para as enzimas ALAT, ASAT, PGM e GPI, após isolamento dos parasitos do sangue periférico dos camundongos e cultivo em meio de Warren. As três cepas mantiveram o padrão do tipo II; entretanto, a passagem das cepas por *P. megistus* e *T. infestans*

determinou um aumento de virulência da cepa 22 SF traduzida por índices mais elevados de mortalidade e de parasitemia; houve maior proliferação parasitária com predominância de formas delgadas no sangue periférico nos animais infectados com as cepas 19 SF e 21 SF provenientes das duas espécies de triatomíneos. Os resultados indicam que as passagens por *P. megistus* e *T. infestans* tiveram uma influência positiva na virulência das cepas de *T. cruzi* de São Felipe - BA, independente de ser o vetor autóctone (*P. megistus*) ou não autóctone (*T. infestans*).

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Recebido para publicação em 31/08/1995.
Aceito para publicação em 08/12/1995.