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Effect of vector on infectivity of *Trypanosoma cruzi*

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Summary

A comparative study was carried out on the interaction between *Triatoma infestans* and bloodstream forms (BSF) of *Trypanosoma cruzi* isolates RA and UP, both lethal for mouse, and CA-I nonlethal for this host. Parasite duplication was readily detected in triatomines fed with CA-I, metacyclic (Mtc) differentiation reaching a maximum at an optimum ingestion level of 250 BSF/insect. Progress and differentiation proved much lower for RA, but reached intermediate values for the UP isolate. Assays for infectivity for each isolate after bug passage revealed a drop for the RA and UP, whereas for CA-I an increase was observed indicating that virulence of BSF and Mtc differs. Our results suggest that parasite selection by insect passage modulates infectivity of a given parasite population; however, virulence was independent of the absolute number of Mtc in the insect's feces.

Key words: *Triatoma infestans*; *Trypanosoma cruzi*; parasite infectivity; infectivity modulation.

Introduction

It had been reported that virulence of *Trypanosoma cruzi* bloodstream forms (BSF) decreased after passage through the digestive tract of the kissing bug (Schilling, 1973; Rego and Garnham, 1956; Philips, 1960; Lammel et al., 1981). Carvalheiro and Collares (1965) were unable to confirm these results, although they did find a lowered viscerotropism of the parasite after bug passage. On the other hand, diverse parasite populations are known to evolve differently in the triatome (Brener, 1973; Garcia and Dvorak, 1982). Therefore,

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the individual capacity of each parasite stock to develop and differentiate in the vector's digestive tract may lead to variations in the degree of virulence during its natural cycle. Although up to now bug passage has been uniformly reported only to attenuate the parasite's virulence, this does not exclude the opposite effect. The purpose of the present work was to compare the interaction between *Triatoma infestans* and 3 *T. cruzi* isolates, 2 lethal and 1 nonlethal for mice, and to determine whether passage through the vector can affect the parasite's degree of infectivity.

Materials and Methods

Parasites. BSF of the lethal RA and UP as well the nonlethal CA-I isolates were obtained by bleeding outbred Rockland mice from the orbital sinus at the peak of parasitemia. Dilutions for the desired parasite concentrations for insect feeding were performed in whole normal mouse blood. The RA (González Cappa et al., 1981) and CA-I (González Cappa et al., 1980) parasite stocks had been isolated from an acute and a chronic human case, respectively. Stock UP was isolated from an acute infection in a laboratory worker in 1970. The 3 isolates were maintained by serial mouse transfer.

Insects. Third instar *T. infestans* reared in our laboratory were used throughout the study. As reported elsewhere, a modification of Pipkin and Connors' feeding apparatus was employed to infect the insects with *T. cruzi* BSF (Isola et al., 1980).

Experimental protocol. Batches of 30 insects each were artificially fed with insect mouse blood containing 5×10^1 , 5×10^2 , 5×10^3 or 1×10^6 BSF/ml of either RA or CA-I isolate. Separate lots were fed with 1×10^6 UP-BSF/ml. Insects were weighed in groups of 5 before feeding; after culling those not fully fed, they were weighed again. The number of parasites ingested per insect was then estimated on the basis of the amount of blood ingested per group. Mid and hindguts of the insects were removed 30 days postinfection (pi) and pools from 10 insects each were ground in 1 ml phosphate buffered saline (pH 7.2) in a manual glass tissue grinder unless otherwise stated. Parasites were counted in a Neubauer chamber and percentage of metacyclic forms (Mtc) calculated from either fresh or fixed and stained slide preparation.

Infectivity of the parasites recovered from the insects was evaluated for the batches fed with blood containing 1×10^6 BSF/ml. Groups of ten 21 ± 1 -day-old male Rockland mice were injected subcutaneously with 1×10^3 parasites. Parasitemia and mortality were recorded during 60 days pi. Each experimental group was compared with a control group inoculated with 1×10^3 BSF of the corresponding isolate obtained from infected mice.

Parasites from one of the batches fed with blood containing UP-BSF were recovered and resuspended in fresh normal rabbit serum in order to lyse the epimastigote (Epi) (Yanovsky et al., 1965; Nogueira et al., 1975). The infectivity of the remaining Mtc was evaluated as stated above by inoculating 1×10^3 parasite/mouse. A matched sex and age group infected with 1×10^3 BSF/mouse was used as control.

Results

The average weight gained by feeding was roughly 50 mg per insect. Therefore, the number of parasites ingested was estimated as 2–3, 20–30, $2-3 \times 10^2$ or $4-6 \times 10^4$ per insect, when fed on blood containing approximately 5×10^1 , 5×10^2 , 5×10^3 or 1×10^6 BSF/ml, respectively.

Parasite duplication was evident 30 days pi in triatomids fed with CA-I-

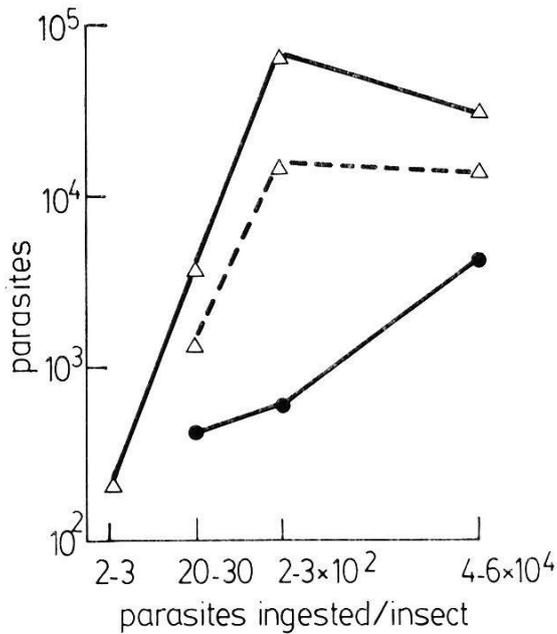


Fig. 1. Parasites recovered after feeding the insects with different number of bloodstream forms of *Trypanosoma cruzi*. Δ — Δ = Total number of parasites recovered per insect after feeding with CA-I isolate. Δ ----- Δ = Number of Mtc recovered per insect after feeding with CA-I isolate. \bullet — \bullet = Total number of parasites recovered per insect after feeding with RA isolate. Mtc were only sporadically seen when insects were fed with RA isolate.

BSF; recovery from feces showed an increase of 2 logarithmic units up to an optimum of $2-3 \times 10^2$ parasites ingested/insect (Fig. 1). When $4-6 \times 10^4$ BSF were ingested at the time of infection, the absolute number recovered 30 days later increased less than 1 logarithmic unit, if at all (Fig. 1 and Table 1). Mtc differentiation increased likewise, peaking at the optimum ingestion amount and without further improvement following higher BSF intake (Fig. 1 and Table 1). In contrast, for the RA stock, no parasites were recovered from insects ingesting 2-3 forms. When BSF intake was 20-30 or higher the number of parasites recovered showed a pattern of a constant increase (Fig. 1). Despite this pattern, the absolute numbers recovered were 10 and 5 times higher for the groups ingesting 20-30 and $2-3 \times 10^2$ BSF, respectively, and lower than the estimated number ingested ($4-6 \times 10^4$) for the group fed with the highest parasite concentration. Mtc were only sporadically seen in any of these groups (Fig. 1 and Table 1). The development of the UP isolate in the triatomids was intermediate between that of RA and CA-I stocks. The percentage of differentiation was similar to that for the RA isolate, but the number of parasites recovered was higher (Table 1). Fixed stained slides exhibited typical Epi and Mtc, as well as a variety of transitional forms. The relative numbers of each form recorded for the 3 isolates recovered after ingestion of $4-6 \times 10^4$ parasites/insect are shown in Table 1.

In infectivity assays on either lethal isolate, parasitemia was lower and occurred later in the animals injected with parasites from the vector than in

Table 1. Forms of the parasites recovered from insects 30 days after artificial feeding with blood containing 1×10^6 BSF/ml

Parasites recovered	Insects fed with		
	RA	UP	CA-I
Epi	87%	82%	49%
Mtc	8%	6%	28%
Trans forms*	5%	12%	23%
Total number/insect	0.5×10^4	1.8×10^4	3.0×10^4

* Any transitional forms between Epi and Mtc

Table 2. Survival of mice injected with either BSF or insect-parasites of RA, UP or CA-I isolates

Isolates	Parasite source	n	ST (days) ^a	S ₆₀ ^b
RA	blood	10	13.8 ± 1.03^c	0%
	insect	10	25.2 ± 3.32^c	20%
UP	blood	10	16.4 ± 1.71^d	0%
	insect	10	31.7 ± 7.63^d	30%
CA-I	blood	10	41.5 ± 3.53^e	80%
	insect	10	39.0 ± 10.00^e	70%

^a mean survival time \pm standard deviation

^b percentage of mice surviving after 60 days of parasite inoculations

^c $P < 0.001$

^d $P < 0.001$

^e $0.8 < P < 0.9$

P was calculated by Student's "t" test

controls (Fig. 2, a and b). Deaths were also delayed and 60 days pi few animals inoculated with either RA or UP were still alive (Table 2). In the group inoculated with CA-I mortality was relatively low, reaching 30% and 20% for the experimental and control groups, respectively. Parasitemia developed earlier and was higher than in the group injected with BSF on days 21 and 31 pi (Fig. 2, c and Table 2). The differences between experimental and control groups were statistically significant at the 5% level, but the tendency to develop higher parasitemia among the mice infected with parasites obtained from the vector's feces was similar in the 3 experiments performed with the CA-I stock.

In order to determine whether attenuation of lethal isolates was independent of the absolute number of Mtc injected per mouse when vector-derived parasites were used, a group of animals was inoculated only with UP Mtc, recovered following Epi lysis with normal rabbit serum. All controls infected

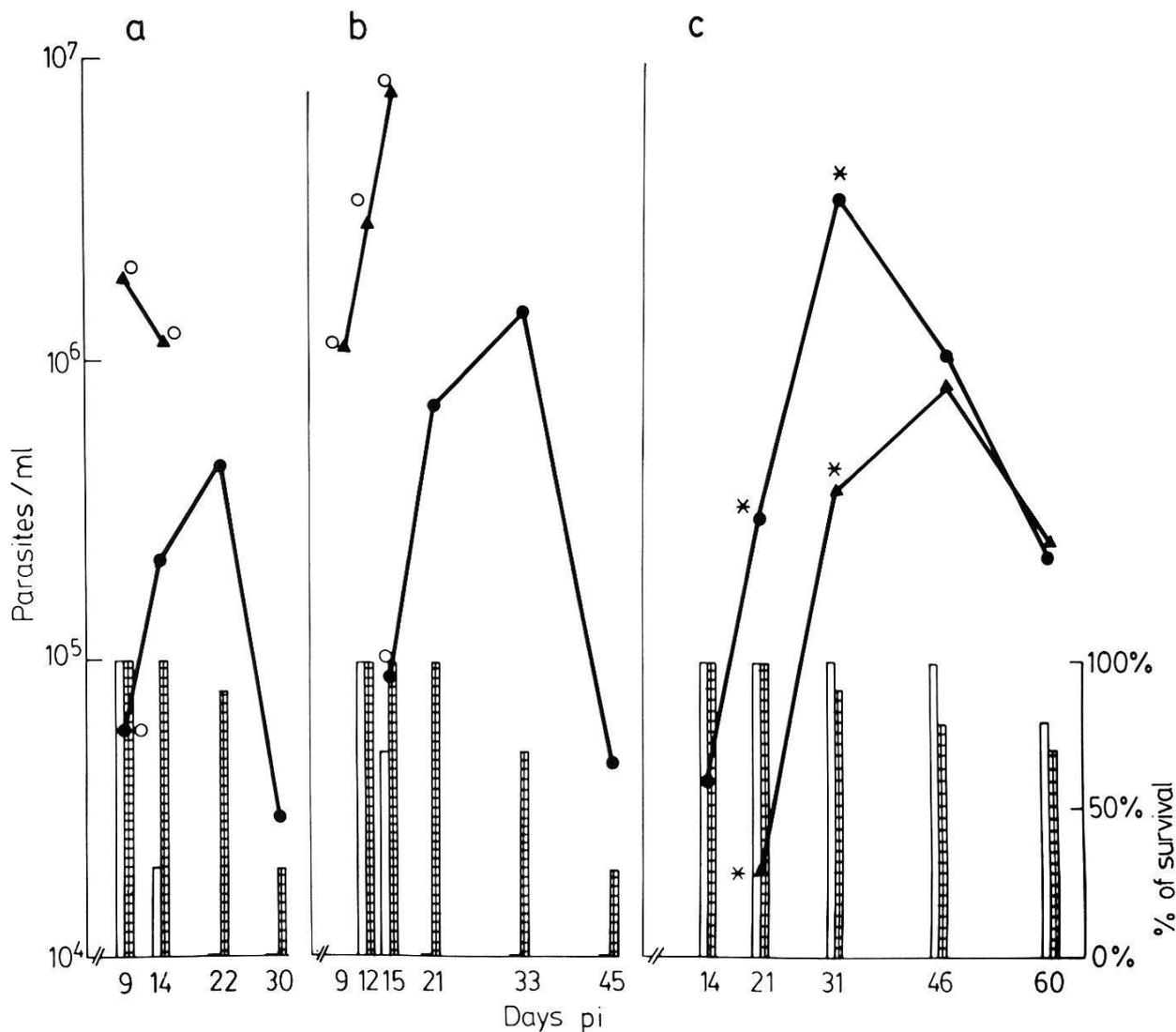


Fig. 2. Parasitemia patterns and survival of mice inoculated with the insects' infected feces or with bloodstream trypomastigotes. a = Mice inoculated with RA isolate. b = Mice inoculated with UP isolate. c = Mice inoculated with CA-I isolate. ▲ = Parasitemia patterns of mice inoculated with bloodstream trypomastigotes. ● = Parasitemia patterns of mice inoculated with insects' infected feces. □ = Percentage of survival of mice inoculated with bloodstream trypomastigotes. ▣ = Percentage of survival of mice inoculated with insects' infected feces. ○ $P < 0.001$, * $0.05 < P < 0.1$ (P was calculated by Student's "t" test).

with BSF died within 18.5 ± 0.71 days pi while 70% of those injected with a similar number of Mtc died within 25.0 ± 3.79 days. The remaining 30% survived the 60-day experimental period.

Discussion

It is generally accepted that the virulence of many *T. cruzi* isolates increased progressively following successive passages in mice until they become lethal. Schilling (1973), Rego and Garnham (1956), Philips (1960) and Lammel et al. (1981) had previously reported that increased BSF virulence can decline

after a single passage of the parasite population through the vector's digestive tract, as happened with RA and UP isolates in the present study. As virulence is attributed to Mtc, the resultant attenuation might simply be due to the low percentage of Mtc in the mixed population serving as inoculum, rather than to a true impairment of their infective capacity. In this work, a true loss of infectivity for the UP lethal parasite isolate, which proves that virulence of BSF and Mtc differs, was demonstrated when, following Epi lysis, a similar number of Mtc and BSF were used to infect mice.

On the other hand, certain isolates, such as the CA-I, have been shown to remain nonlethal for this host (Andrade and Andrade, 1976; González Cappa et al., 1980). When this isolate was passaged through the bug, recovered parasites induced higher levels of parasitemia when compared to the original stock, suggesting virulence enhancement. These results also indicate differences in the degree of virulence between BSF and Mtc. As far as we know, no increase in virulence of BSF after passage through the bug's digestive tract has been reported. This may be due merely to the almost exclusive use of highly virulent isolates in BSF-insect interaction studies. Reports on parasite-insect interaction with extremely attenuated population only deal with Epi cultures (Santos, 1971; Chiari et al., 1973; Lammel et al., 1981).

Regarding the vector's influence, Garcia and Dvorak's (1982) work using cloned parasite stocks and based only on morphological features, supports a selection mechanism for Mtc differentiation, rather than changes in the parasite itself. When uncloned isolates are used, the behaviour of the parasite stock will reflect the average progress capacity of each individual trypanosome. Brener (1973) was the first to draw attention to parasite selection by insect passage, reporting a variable degree of success of BSF of different stocks to develop in the triatome's digestive tract. Our results agree with his findings: the predominantly slender RA isolate develops less readily within the triatome and the CA-I isolate, predominantly broad, achieved the best development in the vector's digestive tract, as shown by the absolute number of parasites recovered and by the percentage of Mtc differentiation. These results indicate a degree of success indirectly proportional to the virulence of the stock; if this behaviour is a general condition, the parasite selection to progress in the insect digestive tract, might promote chronicity in most infected mammals in the natural cycle and thus increase the survival of parasite stocks.

The three stocks considered here evolved different in the *T. infestans* resulting in diverse degree of success in reproduction and differentiation. Nevertheless, as further confirmation of the lack of correlation between the absolute number of Mtc and the lethal capacity of a given parasite stock (Bice and Zeldon, 1970; Lammel et al., 1981) the RA and UP isolates, containing 8% and 6% Mtc respectively, were found to kill over 70% of mice whereas CA-I, with 28% Mtc, killed only 30% of the animals.

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