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TRYPANOSOMA CRUZI: **CELL CHARGE DISTRIBUTION**

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SUMMARY

Differences in cell charge between epimastigote and trypomastigote populations were compared in Y, Cl and Colombiana strains of **T. cruzi.** Trypomastigote populations were more homogenous in relation to cell charge than epimastigotes. This homogeneity of cell charge was not the result of the selection of trypomastigote sub-populations by the host immunosystem, but may be the result of a surface coat formed by host blood components.

INTRODUCTION

Little is known about the ability of **Trypanosoma cruzi** to escape humoral immune response. In African Trypanosomes, variation in surface changes with consequent antigenic variation seems to constitute the escape mechanism against antibodies (CROSS [6]; VICKERMAN [21]; TURNER [20]; BORST & CROSS [5]). There is no evidence of antigenic variation in **T. cruzi,** but there are some differences in cell surface properties between epimastigotes and trypomastigotes including lectin-induced agglutination surface structures involved in macrophage attachment cell charge, and coat thickness (ALVES & COLLI [2]; PEREIRA et al. [17]; ZENIAN & KIERZENBAUM [24]; DE SOUZA et al. [8]; Membrane components responsible for eliciting antibody response able to eliminate the entire **T. cruzi** population in the blood of infected animals have not been unequivocally demonstrated. Whether a population diversity with a sub-population showing distinct characteristics is important or not in the escape mechanism is not clear. The purpose of this work is to compare the cell surface charge variation within populations of **T. cruzi** epimastigote and trypomastigote forms, and discuss this surface charge variation in relation to the antibody escape mechanism.

MATERIALS AND METHODS

Cultivation of T. cruzi **epimastigotes** — Epimastigote forms of the Y (SILVA & NUSSENZWEIG [18]) Colombiana (FEDERICI et al. [9]) and CL (BRENNER & CHIARI [4]) strains were grown in Warrens medium at 28°C (1960). Cultures of Y and CL strains were incubated for 7 days and the Colombiana strain for 2 days. Cell was collected by centrifugation at 4,000g and a suspension of 10^7 cells/ml was prepared in phosphate-saline-glucose buffer (PSG) at pH 7.8 with the ionic strength similar to the one in the first chromatography elution buffer.

Cultivation of T. cruzi **trypomastigotes** — Blood trypomastigotes of the Y, CL, and Colombiana strains were obtained from albino mice during the acute phase of infection. The blood was collected from 3 to 5 mice using 50 I.U. of heparin. Blood samples from these mice were pooled and counted as previously described (BRENNER [3]). Experiments were also performed using Y strain bloodstream try

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pomastigotes obtained from mice previously irradiated with 600 rads from a cobalt source.

DEAE-cellulose chromatography of whole cells — The procedure used was a modification of the method of LANHAM & GODFREY [15], and SOUSA [19]. DEAE-cellulose (Whatman 52) was equilibrated with PSG buffer pH 7.8, ionic strength 0.234. Fourteen to 15 ml of this slurry were packed in a 20 ml syringe on glasswool and filter paper (Whatman 5). The sample containing about 10^7 parasites was counted previously and then added to the column; elution started with I = 0.234. The ionic strength was then increased to I = 0.311 PSG (pH 7.8) and I = 0.389. In other experiments, DEAE-cellulose was eluted with buffers of ionic strength 0.181, 0.217 and 0.253. The effluents of each elution were collected in 10 ml fractions. In epimastigote experiments, after elution, cell mobility remains high. Formalin was added to final concentration of 1% to prevent lysis because cell counting sometimes was made hours later. Differently, trypomastigotes were transfered to a 0.5% bovine serum albumin solution to maintain cells alive. This makes the counting easier since some fractions were contaminated with blood cells, and, under these conditions, fixed trypomastigotes are difficult to be recognized. Cells retained in the column were measured by cell counting in diluted resin after chromatography run. Lysis was calculated as the difference between the number of cells added to the column, and the cells recovered in eluted fractions plus those retained in the column. All these experiments were conducted at least in duplicate.

Control of the elution of epimastigotes — Epimastigotes from a culture in Warren's medium were separated by centrifugation, ressuspended, and eluted from a DEAE-cellulose column in PSG buffer. To check if the presence of blood could influence elution of the parasites from DEAE-cellulose, non-infected mouse blood was chromatographed before adding Y strain epimastigote to the column.

RESULTS

Influence of the culture medium on the elution of T. cruzi cells — Control experiments were performed with epimastigotes in conditions similar to that where trypomasigotes are eluted. Data on the influence of the culture medium on the elution of **T. cruzi** are presented in Table I. Additional control experiments (unpublished) where performed by chromatography of epiamstigotes suspended in serum previously inactivated at 56°C. Similar distribution patterns could be observed.

TABLE I

Elution of epimastigotes from a DEAE-cellulose chromatography using buffers of increasing ionic strength expressed in cell recovery percentage

Parasites suspensions	I = 0.234	I = 0.311	I = 0.389	Total recovery	Resin retained	Lysis
Epimastigotes eluted from a column previously treated with mouse blood	17	6	10	33	20	47
Epimastigotes	16	6	8	30	8	62

Distribution profile of PSG with constant ionic strength — The average of 4 experiments with epimastigotes eluted at constant ionic strength 0.234 PSG, showed 68% recovery in the first 7 fractions, 27% in the next 7 fractions, and 5% in the last 7 fractions. With trypomastigotes, 70-80% of the eluted cells was collected in the first 7 fractions using PSG I = 0.311 (Table II). The small total recovery of epimastigotes eluted with constant ionic strength PSG was the reason why in the following experiments buffers were used in a discontinuous ionic strength gradient to allow cells to be eluted under different conditions.

Separation of Y — strain epimastigotes and trypomastigotes — A comparison of the distribution of Y-strain epimastigotes and trypomastigotes is found in Table II. Compared with epimastigotes, there was a high recovery of trypomastigotes using PSG of ionic strength 0.311. Epimastigote forms showed a broader distribution of cells with different surface charge desinties in all experiments (Fig. 1). Trypomastigotes were completely eluted in the first 20

TABLE II

Comparative elution of epimastigotes and trypomastigotes of Y strain in DEAE-cellulose chromatography expressed in cell recovery percentage

	I=0.181	I=0.217	I=0.234	I=0.253	I=0.311	I=0.389	Total recovery	Resin retained	Lysis
Epimastigote	3	4		23			30	33	37
Trypomastigote	2	5		5			12	3	85
Epimastigote			15(12 tubes)	9(12 tubes)			24	6	70
Trypomastigote			5(12 tubes)	27(12 tubes)			32	0	68
Epimastigote			16		6	8	30	8	62
Trypomastigote			15		83	0	98	0	2
Trypomastigote from irradiated mice			1		84	1	86	5	9

fractions, but epimastigotes continued to be eluted after the 50th fractions. Lower ionic strength buffers the recovery of trypomastigotes to 10-30%, and epimastigote recovery was reduced when eluted with PSG I = 0.181 and I = 0.217. The highest epimastigote recovery featured PSG with an ionic strength of 0.234 and 0.253 (Table II). Lysis of a high percentage of epimastigotes occurred in a column eluted with PSG I = 0.234 to I = 0.389, and a smaller percentage of lysis occurred with PSG I = 0.181 to I = 0.253. The opposite occurred with trypomastigotes which showed high percentage of lysis in PSG I = 0.181 to I = 0.253 and low percentage in I = 0.234 to I = 0.389 (Table II). Eighty percentage of the trypomastigotes from irradiated mice was collected in 2 fractions, but a less compact distribution was found for trypomastigotes grown in normal mice (42% recovery in 3 fractions) (Table II). See Fig. 1.

Distribution profiles with different strains — Epimastigotes and trypomastigotes of Colombiana had a charge distribution pattern similar to that of the Y strain. The main difference was that the bloodstream trypomastigotes of the Colombiana strain were eluted with PSG of higher ionic strength than that used to elute epimastigotes. Elutions of CL trypomastigotes were similar to those of the Y strain, but there was that the bloodstream trypomastigotes of ionic strenght used (Table III).

DISCUSSION

The DEAE cellulose chromatography method used previously to purify trypomastigotes was employed successfully here to indicate cell surface charge differences between fraction of the trypomastigote and of epimastigote populations (LANHAM [14]; LANHAM & GODFREY [15]; GUTTERIDGE et al. [10]; MERCADO & KATUSHA [16]; VILLALTA & LEON [22]; SOUSA [19]; AL-ABBASSY et al. [1]). SOUSA [19] showed that in **T. cruzi**, different strains have characteristic cell surface charge distribution profiles, and she was able to separate slender and stout forms of trypomastigote subpopulations. Our modification of the method improved the possibility of recovery for 80% or more of the total trypomastigote populations.

Our results showed that epimastigotes have a broader surface charge distribution than trypomastigotes. The large variation found for epimastigotes may be due to a mixture of cells in different developmental phases, but may not be true for trypomastigotes which, according to HOARE & WALLACE [11], are not reproducing forms. Epimastigotes in the stationary and logarithmic growth phases have broader electrophoretic mobilities than those of bloodstream trypomastigotes (De SOUZA et al. [7]).

Irradiated immunossuppressed mice were used to determine if the homogenous nature of the trypomastigotes was the result of selection by immunologic action on a heterogenous population, or a characteristic already existent at the time of formation. Low levels of specific antibodies were produced in 4-7 days after infection of the mice, but these levels can be avoided by irradiation (GUTTERIDGE et al. [10]). Since there is little variation in the trypomastigote population obtained from immunossuppressed mice, the trypomastigotes probably originated as a homogeneous population rather than as the result of selection of a heterogeneous population.

Absorption of a relatively thick layer of host blood components coat on the surface of

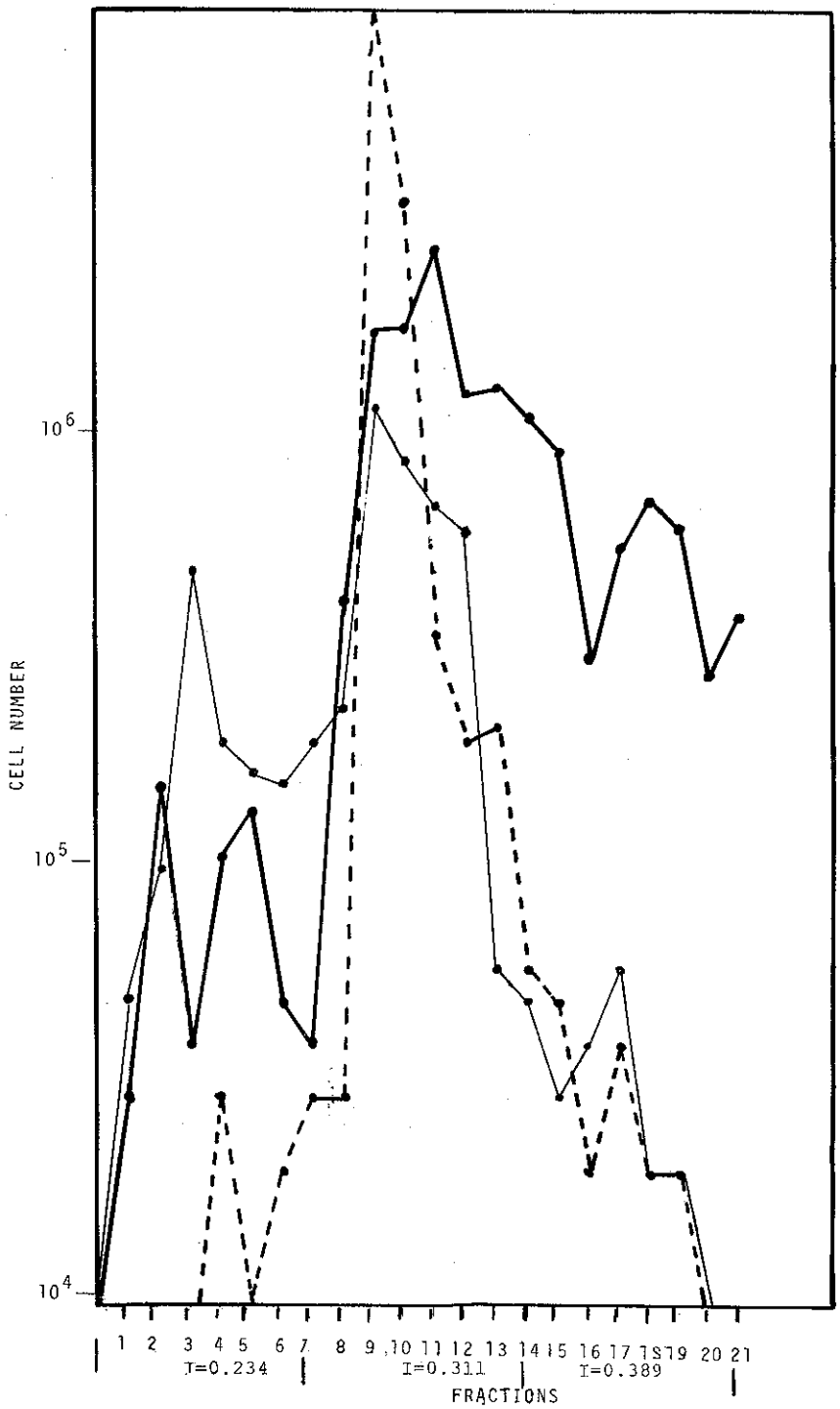

Fig. 1 — Charge distribution profiles of T. cruzi Y strains cells. Cells were passed through a DEAE-cellulose column eluted with seven 10 ml-fractions each at PSG I = 0.234; 0.311; and 0.389. Epimastigotes (——); trypomastigotes (——); trypomastigotes obtained from irradiated mice (- - - -)

the trypomastigotes, as demonstrated by KRETTLI & NUSSENZWEIG[13], and DE SOUZA et al.[8], could explain the cell charge homogeneity of the population observed here. If the homogeneity of cell charge could be atributed to specific membrane components exposed in the trypomastigote surface, the chances for the immune defense system to produce specific antibodies

T A B L E III

Cell distribution in epimastigote and trypomastigote population in Colombiana and CL strains expressed in cell recovery percentage

Parasite suspensions	I=0.181	I=0.217	I=0.234	I=0.253	I=0.311	I=0.389	Total recovery	Resin retained	Lysis
Colombiana epimastigotes	9	14		31			54	42	4
Colombiana trypomastigotes	9	4		4			17	58	25
Colombiana epimastigotes			1		8	5	14	—	—
Colombiana trypomastigotes	—	—	—	—	24(10 tubes)	58(10 tubes)	82	0	18
CL epimastigotes			0		0	1	1		
CL epimastigotes	0	0		1			1		
CL trypomastigotes	13	66		2			81		

able to eliminate these parasites would be high, but this is not what happens. We suggest that this coat functions as a defense mechanism against the immunologic action of the host.

RESUMO

Trypanosoma cruzi: **Distribuição de cargas celulares**

Diferenças na carga celular entre populações de epimastigotas e de tripomastigotas foram comparadas nas cepas Y, CL e Colombiana do **T. cruzi.** As populações de tripomastigotas mostraram-se mais homogêneas quanto à carga celular do que os epimastigotas. Esta maior homogeneidade de cargas não foi decorrente da seleção de sub-populações de tripomastigotas por ação do sistema imunológico do hospedeiro, mas talvez esta se deva à capa superficial formada por componentes sangüíneos dos hospedeiros.

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