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METACYCLOGENESIS OF *TRYPANOSOMA CRUZI* IN THE VECTOR *TRIATOMA INFESTANS*

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Many important aspects of the development of *Trypanosoma cruzi* in the vector are still unknown; in particular, our knowledge about the differentiation of the different forms are fragmentary. Therefore, to obtain more quantitative data we are investigating interactions of *Triatoma infestans* and two *T. cruzi* strains which belong to different zymodemes. Bugs and flagellates of our recently established system originate from the same village; *T. cruzi* is maintained in standardized cyclical passages in our laboratory (Böker & Schaub, 1984; Schaub, in press).

MATERIAL AND METHODS

In an initial investigation second instars were infected with 8,000 - 10,000 blood trypomastigotes/bug and fed on hens 3, 6 and 10 weeks p.i. During the first 12 weeks p.i. groups of 10 bugs were dissected at weekly intervals. Population densities of *T. cruzi* "Chile 7" (zymodeme 2) and *T. cruzi* "Chile 5" (zymodeme 1) were determined for the small intestine and rectum. After Giemsa staining, 100 flagellates from each region were classified into 18 forms. These included 5 intermediate stages to trypomastigotes: two drop-like forms originating from sphaeromastigotes (or epimastigotes) with a round posterior end and a kinetoplast either laterally adjacent to or posterior to the nucleus, but not subterminal; two slender forms shorter or longer than 30 µm, originating from epimastigotes; unequal divisions resulting in a trypomastigote and an epimastigote.

Similar measurements of population densities and the percentage of different stages were made at 14 weeks p.i. for the excretory system and the rectum of fifth instars of

T. infestans. Measurements were made on 10 unfed bugs and on 10 bugs 4 hours after feeding. These data were also determined for faeces and urine of the fed bugs. The bugs had been infected in the first instar (10,000 blood trypomastigotes/bug) and fed every 3 weeks (Schaub & Lösch, in press). All bugs were maintained at 26°C and 60-70% relative humidity.

RESULTS AND DISCUSSION

The first investigation above showed that in the small intestine the number of flagellates increased from about 30,000 one week p.i. to about 500,000 12 weeks p.i., *T. cruzi* "Chile 5" growing statistically significantly better than *T. cruzi* "Chile 7" ($P<0.01$). This difference was also evident for the rectal populations ($P<0.001$), which reached mean values of $1x10^6$ flagellates /bug for *T. cruzi* "Chile 7" and $1.5x10^6$ for *T. cruzi* "Chile 5". About 70-80% of the rectal population were attached to the rectal wall.

In the small intestine, transition forms to trypomastigotes occurred, but in both strains rarely exceeded 10% of the total populations (Table 1). Since we could evaluate in some weeks only smears from a low number of bugs, especially from the small intestine, the data should not be overinterpreted. However, some trends could be observed in both *T. cruzi* strains. During the first 7 to 8 weeks p.i. droplike forms dominated over slender transition stages and unequal divisions. Afterwards, the number of intermediate stages leading to trypomastigotes decreased, and these mainly originated from epimastigotes, often longer than 30 µm. Final trypomastigotes were found rarely, and only one

was longer than 30 µm. In bugs infected with *T. cruzi* "Chile 5", significantly more final trypomastigotes developed than in those infected with *T. cruzi* "Chile 7" ($P<0.01$).

In the rectum higher percentages of transition forms leading to trypomastigotes were found than in the small intestine, these changes occurring at the same time in both *T. cruzi* strains. In the first 6 weeks p.i. about 70% of them normally were unequal divisions, but 7 to 8 weeks p.i. they were mainly drop-like forms. The percentage of slender transition stages was less than 10% in the first 8 weeks, but increased to about 50% at 9-12 weeks p.i.

This increase of intermediate stages originating from short epimastigotes coincided with an enormous increase of final trypomastigotes; in the first 9 weeks less than 15% of *T. cruzi* "Chile 7" and 30% of *T. cruzi* "Chile 5" were final trypomastigotes, increasing afterwards to about 30% and 50%, respectively. The difference between the two strains over the course of infection was statistically highly significant ($p<0.001$).

The second investigation of the influence of blood uptake by the bug on the populations of *T. cruzi* "Chile 5" in the excretory system and rectum (Schaub & Lösch, in press) provided further information concerning the behaviour of *T. cruzi*. In unfed bugs, dissected 14 weeks p.i. and 6 weeks after last feeding, about 70% of the rectal population were attached to the rectal wall with an obvious preference for the rectal pads. The three rectal populations (rectal lumen, anterior and posterior rectal wall) contained 10-15% transition forms leading to trypomastigotes. Unequal div-

isions were seen only occasionally, but nearly identical percentages of drop-like and slender stages were found (with a slight dominance of drop-like forms in the lumen). In the course of defaecation and urination after feeding, the percentage of sphaeromastigotes (and their drop-like transition stages) and epimastigotes decreased. Some drops of urine contained exclusively final trypomastigotes and slender intermediate stages. Dissection of these bugs 4 hours after feeding showed that the percentage of the trypomastigotes on the rectal wall had decreased. Therefore, the metacyclics in the urine must originate from the rectal wall, especially from the posterior part. This seems to be caused by a higher detachment rate of trypomastigotes compared to epimastigotes. After feeding, a higher percentage of intermediate stages to trypomastigotes appeared, these originating mainly from epimastigotes.

Summarizing both investigations, it must be emphasized that metacyclic trypomastigotes in the bug originate from three sources: sphaeromastigotes, epimastigotes and unequal divisions. In the course of an infection higher percentages of unequal divisions appeared in the growth phase, not only in the rectum, but also in the small intestine. The percentage of drop-like forms originating from sphaeromastigotes normally varied between 30-60% of the intermediates, but decreased dramatically in the rectum after a blood uptake by the bug. However, this induced metacyclogenesis of epimastigotes that were attached to the rectal wall. Their slender transition forms also dominated in the rectum in the stationary phase of *T. cruzi* development. These results

indicate that different factors should induce metacyclogenesis in the different forms of *T. cruzi*.

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TABLE I

Quantitation of the different transition stages to trypomastigotes of *T. cruzi* "Chile 7" and *T. cruzi* "Chile 5" in the small intestine and rectum of *T. infestans* at different times p.i.

		T. cruzi "Chile 7"					*T. cruzi* "Chile 5"				
	weeks p.i.	n	% total Σ	% drop	% slen.	% div.	n	% total Σ	% drop	% slen.	% div.
s.i.	1	6	3 19	32	32	37	4	1 2	100	0	0
	2	7	3 21	81	5	14	6	7 44	46	48	7
	3	4	8 29	48	14	38	5	8 31	90	7	3
	4	4	10 40	73	20	8	7	3 24	100	0	0
	5	2	14 34	53	15	32	8	10 119	67	26	8
	6	10	16 251	55	19	26	9	11 128	30	39	31
	7	8	8 146	52	20	28	6	0 7	0	100	0
	8	5	7 35	0	100	0	4	1 3	100	0	0
	9	7	2 14	0	100	0	3	2 5	0	100	0
	10	4	9 36	14	78	8	4	2 9	11	89	0
	11	4	2 8	75	25	0	3	6 19	16	79	5
	12	6	8 48	40	58	2	3	1 4	0	100	0
re.	1	0	- -	-	-	-	5	2 9	33	0	67
	2	9	5 46	57	2	41	8	4 34	24	3	74
	3	10	5 53	26	2	72	10	8 76	18	7	75
	4	8	12 97	25	0	75	10	14 143	27	0	73
	5	10	15 151	31	1	68	9	9 84	32	1	67
	6	10	8 81	26	10	64	10	10 95	23	10	67
	7	10	3 25	96	4	0	10	3 26	96	4	0
	8	8	27 213	76	8	17	10	13 125	58	29	14
	9	8	9 71	25	58	17	10	9 89	24	69	8
	10	10	13 220	35	54	11	10	9 187	21	71	8
	11	7	9 98	30	55	15	9	6 100	40	40	20
	12	9	13 120	43	32	26	9	9 79	66	22	13

n = number of bugs (In some bugs smears could not be evaluated because of low flagellate densities); % total = mean of intermediate stages to trypomastigotes as a percentage of the total population; Σ = total number of intermediate stages to trypomastigotes in all evaluated bugs; % drop = drop-like transition stages as a percentage of Σ; % slen. = slender transition stages as a percentage of Σ; % div. = division stages as a percentage of Σ; s.i. = small intestine; re. = rectum.