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Autor: Neal, R.A. / McHardy, N.

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Protozoology Department, The Wellcome Research Laboratories, Beckenham, Kent, U.K.

Comparison of infectivity of *Trypanosoma cruzi* blood stream trypomastigotes and metacyclic trypomastigotes from *Rhodnius prolixus*

R. A. Neal, N. McHardy

Summary

Four strains of *Trypanosoma cruzi (Y, BG, M* and *Peru)* retain their ability to infect *Rhodnius prolixus* and to produce virulent infections in mice for from 12 to 39 years. About 60 or more metacyclic trypomastigotes were consistently lethal to mice. The mean number of metacyclics per bug ranged from 1.2 to 17.3×10^3. Comparative studies of virulence of metacyclics and blood trypomastigotes showed the blood forms to be slightly more virulent. The route of injection was shown to be more significant in varying the host response to infection, subcutaneous inoculation being the preferred route.

Introduction

Laboratory studies on the control of *T. cruzi* by chemotherapy or immunization usually require confirmation using the natural mode of transmission involving development of metacyclic trypomastigotes in the rectum of a triatome bug. The present investigation compares the subcutaneous and intraperitoneal routes of inoculation of both bug-derived metacyclics, and blood-derived trypomastigotes in mice. Also included are data on the numbers of metacyclic trypanosomes found in the bugs.

Material and methods

Preparation of metacyclic trypomastigote suspension

Rhodnius prolixus was an old laboratory colony of Venezuelan origin (Mshelbwala and Ormerod, 1973). Mixed age populations were maintained and fed on anaesthetised guinea-pigs. All insects were maintained at 27–28° C and 50–60% r.h. Third or 4th stage instars were fed on heavily

Correspondence: Dr. R. A. Neal, Protozoology Department, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K.

infected mice showing approximately 1×10^7 trypomastigotes/ml in the circulating blood. Ten bugs were fed on each mouse. Unfed bugs were discarded and destroyed.

Following the data of Mshelbwala and Ormerod (1973) examination of the bugs was delayed until the 4th week since these authors found maximum metacyclics at this time. To deal with large batches of 60–100 bugs a simplified technique was developed which involved cutting the rear portion of the abdomen containing the rectum and grinding in a hand operated all glass grinder (Griffiths tube) in saline pH 7.0. The large particles of insect origin were allowed to settle and the supernatant containing the flagellates separated. After two washes, the suspension was counted by haemacytometer. A differential count for trypomastigotes was made with a fresh preparation with phase microscopy, using criteria of shape, undulating membrane, flagellum and motility to distinguish between trypomastigotes and epimastigotes (Mshelbwala and Ormerod, 1973). Sphaeromastigotes (Brack, 1968) were not observed.

Preparation of blood trypomastigote suspension

Blood was withdrawn from infected mice by cardiac puncture, using heparin as anticoagulant. The number of trypomastigotes was counted using a haemacytometer. The blood was diluted with physiological saline so that the infective dose was contained in 0.2 ml.

Injection of mice

Subcutaneous (s.c.) injections were placed between the scapulae, while intraperitoneal (i.p.) injections were delivered into the left upper abdomen.

Strains of T. cruzi and mice

Most work was carried out with the standard laboratory strain originally isolated in São Paulo, Brazil, strain *Y*. Other strains used were *Peru* and *BG* (≡ *BH*). These strains are documented by Andrade (1974). A fourth strain used was strain *M* isolated in Argentina in 1962. All strains were maintained as acute infections in mice by passaging to normal mice at weekly intervals.

Outbred lines of mice were used, strain CD-1 being used for the majority of this work. Other outbred lines were Evans and TO.

Assessment of infection

Fresh tail blood from each mouse was examined by preparation of smears approximately 1 red blood cell thick. In the infectivity of metacyclic trypomastigote experiments the blood was examined daily for about 2 weeks, then three times per week. The number of trypomastigotes observed in 25 microscope fields ($\times 10$ ocular, $\times 40$ objective) was determined. The mean survival time was calculated from the days of death of mice. This figure is biased since there is a cut-off time at 42 days. All experiments were observed for a maximum of 42 days.

Results

Infectivity of metacyclic trypomastigotes to mice

Most complete data was obtained with strain *Y*. The infectivity to strain CD-1 mice is shown in Table 1.

All three experiments show that 100% mortality occurred when about 60 or more metacyclics were inoculated though 2/6 mice died after inoculation with a theoretical 0.2 of a metacyclic. Each infected mouse died with a high parasitaemia. The increase of numbers of parasites inoculated was correlated with decrease of survival time and prepatent period. Every mouse in which

Table 1. Infectivity of metacyclic trypomastigotes of *T. cruzi* strain *Y* from experimentally infected *Rhodnius prolixus* to mice strain CD-1

Experiment no.	No. of metacyclics inoculated	Proportion of mice infected	Proportion of mice died	Mean prepatent period ± s.e. days	Mean survival time ± s.e. days (maximum = 42 days)
1	5500	5/5	5/5	7.8 ± 0.37	20 ± 2.7
	2000	5/5	5/5	7.0 ± 0.32	16 ± 0.37
	200	5/5	5/5	10.2 ± 0.73	23 ± 2.4
	20	4/4	4/4	10.8 ± 0.75	21 ± 0.48
2	250	6/6	6/6	11 ± 0.22	21 ± 1.3
	125	6/6	6/6	10 ± 0.33	19 ± 0.45
	63	6/6	6/6	11 ± 0.49	20 ± 0.75
	31	5/6	5/6	16 ± 1.6	26 ± 3.9
	16	5/6	5/6	12 ± 0.33	27 ± 3.3
3	200	6/6	6/6	15 ± 1.6	24 ± 2.3
	20	5/6	5/6	21 ± 4.4	28 ± 3.0
	2	2/6	2/6	34 ± 5.1	36 ± 3.9
	0.2	2/6	2/6	31 ± 4.9	39 ± 2.6
	0.02	0/6	0/6	>42	>42
	0	0/6	0/6	>42	>42

Table 2. Number of metacyclic trypomastigotes recovered from experimentally infected *Rhodnius prolixus*

Strain of *T. cruzi* (experiment)	Number of bugs used	Mean number of metacyclics/bug $\times 10^3$	Proportion of metacyclics to total flagellates (%)
Y (a)	31	3.9	22
(b)	59	17.3	34
(c)	36	3.6	43
(d)	67	15.0	32
Peru (a)	19	5.2	26
(b)	6	1.2	10
BG (a)	20	6.0	24
(b)	6	2.9	26
M (a)	6	6.3	28

trypomastigotes were detected microscopically, died within the observed period of 42 days.

Evidence on infectivity of other strains of *T. cruzi* is less well documented owing to smaller numbers of mice in each group. With strain *Peru*, 3/3 mice inoculated with either 1.5 or 3.1×10^3 metacyclics/mouse died within 17 days

Table 3. Comparison of the infectivity of metacyclic and blood trypomastigotes of *T. cruzi* strain *Y* to mice, strain CD-1

Inoculum	Route of injection	Proportion of mice died	Mean survival time ± s.e. days (maximum = 42 days)
Experiment 1			
2×10^4 bug metacyclics	s.c.	10/10	19.5 ± 0.27
2×10^4 blood trypomastigotes	s.c.	10/10	14.9 ± 1.28
Experiment 2			
2×10^4 bug metacyclics	s.c.	5/5	16.0 ± 0
2×10^4 bug metacyclics	i.p.	3/5	28.2 ± 5.69
2×10^4 blood trypomastigotes	s.c.	5/5	14.2 ± 1.11
2×10^4 blood trypomastigotes	i.p.	2/5	35.0 ± 4.63

Table 4. Comparison of the intraperitoneal and subcutaneous route of infection with blood trypomastigotes of *T. cruzi* strain *Y* in mice strain CD-1

Route of infection	Inoculum	Proportion of mice died	Mean survival time ± s.e. days (maximum = 42 days)
Experiment 3			
Subcutaneous	2×10^6	10/10	8.8 ± 1.10
	2×10^5	10/10	14.1 ± 1.44
	2×10^4	10/10	15.5 ± 0.16
	2×10^3	10/10	19.0 ± 2.47
	2×10^2	10/10	25.7 ± 1.56
Intraperitoneal	2×10^6	10/10	12.6 ± 1.35
	2×10^5	10/10	15.1 ± 2.11
	2×10^4	9/10	22.1 ± 2.36
	2×10^3	7/10	31.8 ± 2.74
	2×10^2	7/10	31.2 ± 1.46
Experiment 4			
Subcutaneous	2×10^4	10/10	14.3 ± 0.37
	2×10^3	10/10	21.8 ± 2.20
	2×10^2	10/10	20.8 ± 0.49
	2×10^1	5/10	35.1 ± 2.48
	2×10^{-1}	0/9	>42.0

and strain *BG* infected 4/4 mice inoculated with 2.8 or 5.7×10^3 metacyclics. The latter mice died within 21 days. All 4 mice inoculated with either 4.2, 8.4 or 16.8×10^3 metacyclics of strain *M* were infected. However, only 2 mice died while the other 2 animals, given the lowest dose of metacyclics, survived 42 days when the experiment was discontinued. From these limited experiments, it is clear that these strains of *T. cruzi* were highly virulent to mice of Evans and TO lines.

Number of metacyclic trypomastigotes recovered from R. prolixus

The data collected in Table 2 shows that all strains of *T. cruzi* were equally infective to *R. prolixus* and yielded similar numbers of metacyclics ranging from $1.2–17.3 \times 10^3$/bug. Since the bugs were handled by the batch method there is no information on the individual variation between insects.

Infectivity of metacyclic and blood trypomastigotes in mice by the subcutaneous and intraperitoneal routes of inoculation

This work used *T. cruzi* strain *Y*, and mice, strain CD-1. The infective dose was 2×10^4 organisms, injected either s.c. or i.p. In two experiments (Table 3), the infection which developed following s.c. injection of blood trypomastigotes was more rapidly fulminating than with bug metacyclics.

In a third experiment (Table 4), a comparison was made between the s.c. and i.p. route of inoculation, using blood trypomastigotes, strain *Y*, in CD-1 mice. Groups of 10 mice were injected with doses of blood trypomastigotes from 2×10^2 to 2×10^6 by each route. There was a clear correlation between the number of organisms in the inoculum, and the time to death of the mice. The s.c. route again gave a more rapidly-developing infection, and the i.p. route gave rather more variable results. In a further experiment, also included in Table 4, the number of blood trypomastigotes of strain *Y* was extended down to 2×10^{-1} organisms. None of the nine mice were killed by this inoculum, although infections were diagnosed in six by blood examination. Inoculation of 2×10^1 trypomastigotes killed 5/10 mice, and infection was detected in the surviving five.

Discussion

Comparison of metacyclic trypomastigotes and blood trypomastigotes shows that one passage through *R. prolixus* did not materially alter the virulence to mice. Both types of parasite killed all mice down to the 200 organism dose level, while 20 and 2 inocula gave a variable infectivity and mortality. The lowest limit of the lethal dose may be lower than 200 organisms, since experiments with metacyclics showed 100% mortality down to 20 organisms.

The conclusion from the above experiments that as few as 20 metacyclic trypomastigotes can be lethal to mice could have considerable epidemiological significance. However, it must be remembered that the strains of *T. cruzi* were of maximum virulence which was shown to be unchanged by one passage through

Rhodnius prolixus. Field strains are known to have much lower pathogenicity. Mazzotti (1940) for example observed a non-lethal infection in 6 mice inoculated with metacyclic trypomastigotes from wild *Triatoma barberi.* The numbers of parasites he used were from 800 to 8000, but he did not observe a graded response in the mouse infection. Carvalheiro and Collares (1965) concluded that one passage of strain *Y* through *Triatoma infestans* did not change the virulence. However, their data were difficult to compare with the present study, since they did not quantitate the numbers of trypanosomes in their experiments.

A further interesting observation is that individual *R. prolixus* harbour about 1 to 17×10^3 metacyclic trypomastigotes. Again this must represent near the maximum possible number since the bugs were fed on very heavy mouse infections. There is no information on the number of metacyclics excreted with the faecal droplet and further, the number of metacyclics which succeed in penetrating into the host is not known. Thus the present data cannot give information on the field challenge with *T. cruzi* but it does indicate maximal challenge.

The above data extends the observation of Brener et al. (1974) that the strains of *T. cruzi* remain infective to bugs after prolonged maintenance in mice. In the present study, the strains *Peru, M, Y* and *BG* had been maintained in mice for 12, 13, 22 and 39 years respectively.

The interpretation of the above experiments has assumed that metacyclic trypomastigotes from the bug were the infectious form. Mshelbwala and Ormerod (1973) considered that this was true, but considered that the amastigote (= sphaeromastigote) might also be infectious to the vertebrate host. Surprisingly, no amastigotes were observed in the *Rhodnius* experiments reported above.

The greater infectivity of the blood trypomastigote over the bug metacyclic trypanosome following syringe inoculation can probably be explained by the fact that the blood trypomastigotes were derived from mice in which the infection had been syringe-passaged for many years. Selection of a population following this method of passage may, therefore, have occurred whereas the bug derived metacyclic trypomastigotes cannot have been selected.

The more uniform and virulent infections after subcutaneous inoculation of *T. cruzi* must involve many factors. In addition to the mouse adaptation of the blood trypomastigotes noted above, the intraperitoneal site is recognized for the prompt cellular response to «foreign» material and could affect the less well adapted metacyclics more than the blood trypomastigotes. The conclusions from these experiments support and extend Mshelbwala and Ormerod's (1973) observation that the preferred route of inoculation of *T. cruzi* is by subcutaneous injection.

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