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Experimental infections in Venezuelan lizards by *Trypanosoma cruzi*

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Summary

Virulent trypomastigotes of the Y strain of *Trypanosoma cruzi* were administered to *Tropidurus hispidus, Ameiva ameiva, Cnemidophorus lemniscatus, Polychrus marmoratus,* and *Phyllodactylus ventralis* (Sauria). Intraperitoneal and subcutaneous inoculations of lizards with mouse blood or with feces of infected *Rhodnius prolixus* (Reduviidae, Triatominae), as well as forced ingestion of triturated *Rhodnius*, produced no parasitaemias detectable either directly or by xenodiagnosis, while control mice became parasitized. Pretreatment with the immunosuppressive drug Fluocinolone acetonide led to establishing patent infections in inoculated lizards. Cryptic infections were established by inoculation of 1×10^6 parasites from Davis' medium, or by 95×10^3 parasites from lizard tissue culture. Parasites were not seen in tissues. Mice inoculated with blood or tissue homogenates from these lizards became parasitized. Parasites from Davis' medium inoculated into the peritoneal cavity of lizards were capable, to a very low degree, of penetrating the free peritoneal macrophages and changing into amastigotes. The factors possibly responsible for the natural resistance of poikilothermic vertebrates to *T. cruzi* are discussed.

Key words: Trypanosoma cruzi; Protozoa; hemoflagellate; trypomastigotes; lizards; Sauria; poikilotherms; *Ameiva ameiva; Cnemidophorus lemniscatus; Tropidurus hispidus; Polychrus marmoratus; Phyllodactylus ventralis;* Triatominae; *Rhodnius prolixus;* immunosuppressors; parasitaemia; intracellular development; peritoneal macrophages; Chagas' disease; natural resistance.

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Table 1. Experimental infections with virulent trypomastigotes of *Trypanosoma cruzi* derived from mouse blood or infected triatomids *(Rhodnius prolixus)*

Source of parasites (Trypomastigotes)	Inoculum	Route used	Spp. and no. lizards used	
Infected mouse blood	60×10^3/0.5 ml	i.p.	*T. hispidus*	6
			A. ameiva	2
			P. ventralis	2
Infected mouse blood	60×10^3/0.5 ml	s.c.	*T. hispidus*	6
			A. ameiva	2
			P. ventralis	2
Infected mouse blood	60×10^3/0.5 ml	both eyes, instillation	*T. hispidus*	6
			A. ameiva	2
			P. ventralis	2
Wild, naturally infected, bugs (feces)	50/ml	s.c.	*A. ameiva*	4
			P. ventralis	2
			C. lemniscatus	4
Triturated, experimentally infected, bugs	3–5	per os	*T. hispidus*	4
			A. ameiva	4
			P. ventralis	1
			C. lemniscatus	1

Control for each experiment: 3 male 30 g white mice; all were positive.
All experiments gave negative results by direct examination of fresh blood, Giemsa-stained blood smears, and by xenodiagnosis.

Introduction

Although poikilotherms are notoriously refractory to infection by *Trypanosoma cruzi* (Martins, 1968), Ryckman (1965) has reported experimental infections in 2 lizard species.

Of the many Venezuelan lizards, we (Urdaneta-Morales and McLure, 1972) have captured many specimens in peridomestic habitats where haematophagous triatomids abound. Although these bugs showed a high incidence of *T. cruzi* parasitization, examinations of the lizards revealed no patent infections. However, we were able to establish low *T. cruzi* infections in some lizards by inoculation from cultures and by immunosuppression.

Materials and methods

Animals. – Adults and juveniles of the following species were captured in the savannahs of northwest Guarico State, Venezuela (67° 15–20′ W, 9° 35′ N): 140 *Ameiva ameiva* and 34 *Cnemidophorus lemniscatus* (Teiidae), 405 *Tropidurus hispidus* and 4 *Polychrus marmoratus* (Iguanidae), and 30 *Phyllodactylus ventralis* (Gekkonidae). They were maintained in the laboratory on chopped hard-boiled egg and water.

Examination of animals. – Possible natural *T. cruzi* infections were ruled out as follows: 1.

Table 2. Infections with culture-derived virulent trypomastigotes of *T. cruzi*, intraperitoneally injected

Trypomastigote established source	Inoculum[a] parasites /0.1 ml	Pretreatment	Spp. and no. lizards used		Infection established by		
					direct[b] exam.	xeno[c]	inoc.[d]
Davis[e]	1×10^6	none	*T. hispidus*	5	–	4	4
			C. lemniscatus	5	–	–	–
			P. ventralis	3	–	1	1
Davis	1×10^6	Fluocinol.[f]	*T. hispidus*	4	1	1	1
		5 mg/kg*	*C. lemniscatus*	2	1	1	1
Davis	1×10^6	Fluocinol.	*T. hispidus*	4	–	–	–
		0.5 mg/kg**	*C. lemniscatus*	2	1	1	1
Tissue[g] culture ...	95×10^3	none	*T. hispidus*	3	–	1	1
			C. lemniscatus	1	–	–	–
			P. ventralis	2	–	–	–
			P. marmoratus	1	–	1	1
Davis	50×10^3	Paraffin[h]	*T. hispidus*	5	peritoneal macro-exam.[i]		

[a] Controls: identical inocula to 3 male 30 g white mice; all were positive
[b] Fresh blood and Giemsa-stained smears; positives – trypomastigotes seen
[c] positives – metatrypomastigotes seen feces of *R. prolixus* engorged on lizards
[d] Inoculation of blood/tissue from positive lizards; positives – trypo- and amastigotes in mice
[e] Trypomastigotes inoculated from 20-day old Davis' medium culture
[f] Fluocinolone acetonide injected daily for 5 days before and 7 days after *T. cruzi* inoculation (Kumar et al., 1970) * 200 μg ** 20 μg
[g] Trypomastigotes inoculated from 7-day old *T. hispidus* liver cell culture
[h] Injection of liquid paraffin 48 h before *T. cruzi* inoculation
[i] Giemsa-stained smears of peritoneal exudate taken 3, 6, 24, 48, and 72 h after *T. cruzi* inoculation; positives – amastigotes (all 5 *T. hispidus*)

examination of fresh blood, taken by Scorza's technic (1971), under phase contrast at 400×; 2. examination of blood smears fixed 1′ in methanol and stained 45′ in 5% Giemsa solution in phosphate buffer, pH 7.2; and 3. xenodiagnosis. Groups of 10 2nd-stage *Rhodnius prolixus* from our laboratory strain were allowed to engorge on each lizard. The feces of the bugs were examined for parasites 30, 45, and 60 days after engorgement.

Parasites. – The Y strain of *T. cruzi* (Silva and Nussenzweig, 1953) was used. It was maintained by serial passage in: 1. white mice, with intraperitoneal inoculation of a new host every 10 days; 2. in Davis' medium; and 3. in lizard tissue culture (Lugo, 1972), with biweekly transfers in both culture media.

Experimental infections. – Tables 1 and 2 describe experimental infections attempted on the 5 lizard species. Parasite counts in blood, triatomid feces, or culture media were made with a haemocytometer. Certain of the lizards were treated with the immunosuppressive drug Fluocinolone acetonide (Table 2). Doses were 200 μg or 20 μg/day for 5 days before and 7 days after *T. cruzi* intraperitoneal inoculation.

Lizards showing parasites in the blood were autopsied and portions of heart, liver, and skeletal

muscle were fixed in Bouin, sectioned, and stained with haematoxylin-eosin. Sections were examined under oil immersion at 1250×. Blood and tissue homogenates of these organs from infected lizards were suspended in sterile saline and inoculated intraperitoneally into groups of 5 white mice each. Blood from the mice was examined for parasitaemias, beginning 3 days after the inoculation.

The possible development of intraperitoneally inoculated parasites in the free macrophages of the lizard peritoneal cavity was investigated. Peritoneal exudate was stimulated in 5 *T. hispidus* by intraperitoneal injection of 0.2 ml sterile liquid paraffin/kg body weight. 24, 48, 72, and 96 h after injection, 0.05 ml samples of peritoneal fluid were taken (Akiyama and Taylor, 1970). Each sample was spread on a slide to an area of 1 cm^2, Giemsa-stained, and its macrophages counted. Macrophage counts increased 24 h after paraffin injection and declined after 72–96 h. Thus, a group of lizards (Table 2) was treated with paraffin 48 h before inoculation with *T. cruzi*, and samples of peritoneal fluid taken 3, 6, 24, 48, and 72 h afterward. Smears of the fluid were stained with Giemsa and examined for parasites in the macrophages.

Results

No trypanosomes were found in any lizard examined before experimental infection, thus ruling out the presence of patent natural infections in lizards from an endemic zone of *T. cruzi*. Infections by microfilariae, haemogregarines, and *Plasmodium (Sauramoeba) tropiduri* were seen in *T. hispidus*.

Attempts at infecting lizards with blood from infected mice, with feces from infected bugs, or by forced feeding on ground-up infected bugs were all unsuccessful (Table 1). All control mice infected by the same routes showed parasitaemias 7 days after infection.

Parasitaemias of *T. cruzi* were seen only in lizards treated with the immunosuppressive drug Fluocinolone acetonide and inoculated with trypomastigotes harvested from Davis' medium (Table 2). The parasitaemia, detected 5–7 days after inoculation, was always so scanty and intermittent that it could not be followed daily. The blood trypomastigotes were slender and of the C- or S-form (Hoare, 1972). No amastigotes were seen in the sections of the visceral organs from the lizards showing parasitaemia.

Lizards inoculated with parasites from Davis' medium (with or without previous glucocorticoid treatment), or from *T. hispidus* liver cell culture gave positive xenodiagnoses when these were made 1 or 2 weeks after inoculation. Mice inoculated with blood or tissue homogenates from these lizards showed parasites in the blood and tissues (Table 2).

A very few intracellular parasites were seen within the free peritoneal macrophages of *T. hispidus* previously treated with paraffin and inoculated with trypomastigotes from Davis' medium (Table 2). In those animals infected 24 h previously, they showed the typical morphology of the amastigote stages of *T. cruzi*.

Discussion

T. cruzi, in contrast to other Stercoraria, has a low vertebrate host specificity but seems to be endemic only in New World mammals (Hoare, 1972). Natural resistance of these hosts is species and strain variant, probably involving humoral and cellular mechanisms (Hanson, 1977). Poikilotherm resistance may be due to intense lysis and phagocytosis of intraperitoneally inoculated parasites (Rubio, 1956a), but the action of macrophages on *T. cruzi* in non-susceptible vertebrates is unknown (Teixeira, 1979). In our experiments, extracellular parasites were seen in the peritoneal fluid 3–24 h after inoculation and intracellular parasites were found after 24 h, but only 5% of free peritoneal macrophages contained parasites 48–72 h later. Those few trypomastigotes, either invasive or phagocytized (Dvorak, 1976), had changed to amastigotes 24 h after inoculation, but apparently could not multiply within lizard macrophages, suggesting an inappropriate intracellular environment.

Rubio (1956b) reports the in vitro trypanolytic action of fresh batrachian sera, but also mentions survival for several days of trypomastigotes in blood and tissue of certain experimental animals. We have seen virulent trypomastigotes of *T. cruzi* for up to 2 weeks in some inoculated lizards, which may explain the findings of Ryckman (1965).

Mechanisms of natural resistance to *T. cruzi* are obscure. Tissue affinities of the parasite are well known (Bice and Zeledón, 1970). Host cell size, age, and type may influence intracellular parasite density in culture and in vivo (Neva et al., 1961; Dvorak, 1976). The senior author (Urdaneta-Morales, 1979) found monkey cell cultures (Vero fibroblasts) inoculated with *T. cruzi* from blood or culture medium to give twice the percentage of infected cells and twice the densities of amastigotes and trypomastigotes as fish cell cultures *(Pimephales promelas).* Differences in penetrability and susceptibility of becoming infected between poikilotherm and homoiotherm cells have not been determined. The refractoriness of birds to *T. cruzi* infections, which is not dependent on natural antibodies (Nery-Guimares and Lage, 1972), has been demonstrated in vitro to be due to complement-mediated lysis by serum (Kierszenbaum et al., 1976).

Differences in membrane components or in superficial receptors of blood-dwelling *T. cruzi* trypomastigotes have been invoked to explain the varying ability of strains to invade mouse tissue (Melo and Brener, 1978). Particular receptors in the membrane of the phagocyte may influence the ingestion of blood- and culture-derived parasites in the mouse peritoneal cavity (Alcantara and Brener, 1979).

Since, in our experiments, parasitaemias were observed by direct methods only in lizards previously treated with an immunosuppressive drug, immune response in the suppression of parasitaemia needs further study.

Experimental lizard infections by the trypomastigotes harvested from reptile tissue culture suggest a preadaptation by selection of the parasite to poikilo-

therm cells through previous maintenance in such cells in vitro. On the other hand, the infections seen in lizards without immunosuppressive treatment but inoculated with relatively high numbers of parasites from Davis' medium are in accord with the findings of Silva and Nussenzweig (1953), who emphasize that the virulence of this same strain in mice is directly related to the number of parasites inoculated.

It may be that the well-known refractoriness of poikilotherms to *T. cruzi*, in comparison to mammals, is due to qualitative and/or quantitative differences in their cell membranes, as well as differences in immune response (Trischmann et al., 1978).

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