Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *87* (1): 81-86, jan./mar. 1992

LEISHMANIA BRAZILIENSIS SSP. IN THE NASAL MUCOSA OF GUINEA PIGS INOCULATED IN THE TARSI

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Two lots of 20 young male guinea pigs were inoculated subcutaneously in the tarsi with 10^4 amastigotes of Leishmania braziliensis braziliensis *or* L. b. guyanensis *to study the susceptibility of this Neotropical hystricomorph rodent the autochthonous parasites. Almost 50% of the animals showed lesions in the inoculation site and had parasitizations that were infective to hamsters, as shown by inoculating homogenates of the dermal lesion, of the spleen, of the liver, and of the nasal mucosa into hamsters at 20, 40, 60, and 120 days after inoculation of the guinea pig. Smears of the above organs showed the presence of amastigotes. Parasites inoculated into the tarsi were detected early in the skin, spleen, and liver of the guinea pig host. Blood cultures made by cardiopuncture on sacrifice of the guinea pigs were uniformly negative. The nasal mucosa of nearly all animals positive in the skin or viscera was invaded early by the parasites, although with greater frequency between 60 and 120 days post-inoculation.*

The use of this model for the study of mucocutaneous parasitism by L. braziliensis *is discussed, together with the phenomena of parasitism at a distance from the inoculation site, the temperature of the body regions affected, and the possible genetic influence on susceptibility of the guinea pig to* L. braziliensis.

Key words: experimental leishmaniasis – guinea pig – nasal mucosa involvement

The guinea pig *(Cavia cobayae)* is a hystricomorph rodent, the natural host of the sthenoxenic parasite *Leishmania enrietti* (Muniz & Medina, 1948). This animal model has served for the study of experimental infections by this flagellate during the 60's, and 70's (Bray & Bryceson, 1968; Bryceson & Turk, 1971; Bryceson et al., 1971).

Although there is information upon experimental leishmaniasis in guinea pigs, little has been published upon susceptibility of this rodent to other *Leishmania* species parasitizing man. The present paper reports on the dermomucosal dispersion of *L. braziliensis* spp. in the guinea pig, similar to the effects of this parasite in man.

MATERIALS AND METHODS

Parasites – Two isolates of *L. braziliensis*: *L. b. braziliensis* from a cutaneous lesion in a patient from Trujillo, Venezuela (MHOM/VEN/79/LA); *L. b. guyanensis* from the spleen of a naturally infected *Didelphis marsupialis* from the same area (MDID/VEN/82/Dm. 40). Both isolates were inoculated into the paws of male hamsters and also cultured in NNN + 199 medium. Cultures of the isolates were studied by Dr Nancy Saravia of CIDEIM in Cali, Colombia for isoenzymatic analysis and response to monoclonal antibodies.

Inoculation and study in guinea pigs – From non-ulcerated lesions of infected hamsters, suspensions of amastigotes in saline were made, counted by the technique of Stauber et al. (1958) to give a concentration of 10^5/ml of the parasites. Into the dorsal surface of the left tarsus of each of 20 male guinea pigs weighing 200-300 g, 0.1 ml of the suspension was injected. The animals were from a colony maintained more than 20 years in the Faculty of Medicine of the University of Los Andes. They were maintained on concentrated laboratory rat ration, fresh grass, and water *ad libitum*.

Twenty days post-inoculation the animals were examined at the inoculation site to detect

Received 23 July 1991.
Accepted 30 October 1991.

whatever hypertrophies, alopecias, erosions, ulcers, or combinations of these lesions might have appeared. Groups of five animals each were sacrificed at 20, 40, 60, and 120 days post-inoculation without taking into account the apperance of lesions at the sites of inoculation. Before death by anesthesia, cardiopuncture was made on each animal for three blood cultures per animal in NNN + 199 medium. At death, 20 mg of tissue was extracted from the dermis of the inoculation site, from the spleen, from the liver, and from the medial and inferior turbinates of the nasal mucosa. These tissue samples were homogenized separately with 0.5 ml saline, grinding in a mortar with 1.0 ml saline. A volume of 0.25 ml of these suspensions was injected with a 22 gauge needle into the hind paws of two hamsters: dermis into right hind paw and spleen in left hind paw of a hamster, liver into right hind paw and nasal mucosa in left hind paw of another hamster.

The two hamsters injected with the biopsy material from each of the guinea pigs were confined in numbered cages, after marking by cutting the digits of the anterior paws. The hamsters were examined every 15 days for up to six months to detect tarsal lesions; when these appeared, the presence of amastigotes was investigated with Giemsa-stained smears. With tissue samples taken from the same sites in the guinea pigs, smears were made to be fixed in methanol and stained with Giemsa. For smears of dermis, spleen, and liver, 250 fields per slide were examined; for smears to the nasal mucosa, the whole slide was examined, requiring up to 10 h for each. Blood cultures were examined every 10 days for 50 days, in search of promastigotes.

Thermometry in guinea pigs – Three months post-inoculation, the temperature of the surviving animals was measured electronically by a lineal thermistor sensitive to 0.2 °C. Sites of measurement were intranasal, bucal, anal, axilar, and epidermal in the hind paws.

RESULTS

Tarsal inoculation of guinea pigs with 10^4 amastigotes of L. b. braziliensis *(L. A.) or* L. b. guyanensis *(Dm. 40)* – The first and only macroscopic examination of the tarsi of the guinea pigs, made 20 days post-inoculation, showed alterations whose frequency is presented in Table I. In both groups, almost half of the guinea pigs showed no apparent lesions, nor alopecias, in the same lapse of the time in which the hamsters inoculated with the same parasites, developed clearly visible histiocytomata.

TABLE I

Modifications of tarsal skin in guinea pigs inoculated with amastigotes of *Leishmania braziliensis* spp.

Isolate of *L. braziliensis*	LA	Dm 40
Normal	7	4
Alopecia	3	5
Light hypertrophy	4	2
Major hypertrophy	2	0
Ulcer	2	8
Alopecia with ulcer	2	1
Total	20	20

Blood cultures from cardiopuncture – Of 105 blood cultures, 76% remained aseptic during the whole period of observation. No parasite was seen in any of the blood cultures.

Subinoculation of hamsters with tissues or organs from guinea pigs infected with L. b. braziliensis *(L. A.)* – Of 20 guinea pigs inoculated, one died accidentally 96 days post-inoculation.

Homogenates of tarsal skin from eight of 10 guinea pigs inoculated 40 days previously were infectious to hamsters, producing lesions. This dermal parasitism in guinea pigs diminished progressively, to disappear in four months. The visceralization of parasites for spleen and liver, to judge by infectivity, is similar for both organs and also appears to diminish with time. The presence of parasites in the nasal mucosa of 7/19 guinea pigs (37%) was surprising. Fifty per cent of the guinea pigs showed or developed resistance to the infection (Table II). Parasitism was always discrete, with one to three amastigotes per 250 fields; parasites were abundant only in ulcerated tarsi. Direct observation of amastigotes was difficult in the spleen and liver: 1-2 parasites per 5,000 nuclei. Smears from the nasal mucosa of six guinea pigs whose homogenates were infective to hamsters were exhaustively searched; three showed parasites. In one slide, 45 min search showed four amastigotes in a macrophage; 4 h and 9 1/2 h search, respectively were

TABLE II

Infected/inoculated hamsters with inoculations of homogenates from organs of guinea pigs inoculated with *Leishmania braziliensis braziliensis* (L.A.)

Inoculations	Days	Sacrificed	Post-inoculation		Total
	20	40	60	120	
Skin	4/5	4/5	2/5	0/4	10/19
Spleen	2/5	1/5	1/5	1/4	5/19
Liver	1/5	2/5	2/5	0/4	5/19
Nasal mucosa	1/5	2/5	1/5	3/4	7/19
Infections/group of five guinea pigs	8/5	9/5	6/5	4/4	

TABLE III

Infected/inoculated hamsters with inoculations of homogenates from organs of guinea pigs inoculated with *Leishmania braziliensis guyanensis* (Dm 40)

Inoculations	Days	Sacrificed	Post-inoculation		Total
	20	40	60	120	
Skin	3/5	2/5	2/5	2/5	9/20
Spleen	1/5	0/5	0/5	0/5	1/20
Liver	1/5	2/5	1/5	0/5	4/20
Nasal mucosa	2/5	0/5	3/5	3/5	8/20
Infections/group of five guinea pigs	7/5	4/5	6/5	5/5	

needed to detect a single amastigote in the other two slides.

Subinoculation of hamsters with homogenates of tissues and organs of guinea pigs with L. b. guyanensis *(Dm. 40)* – All guinea pigs survived. In half the animals inoculated, once the non-specific inflammation due to the injection has disappeared, lesion appeared (11/12). However, although there were slightly fewer animals showing lesions in the skin, the parasitism persisted during the whole period of observation. Visceralization appeared to be early: 4/10 animals showed parasites in the viscera at 40 days, and homogenates were infective for hamsters. Invasion of the nasal mucosa, as with the other strain of the parasite, appeared in 60% of the animals at 40 days, while appearing in only 20% previously (Table III).

All blood cultures were negative, indicating absence or extremely low parasitemia. It may be that the parasites spread to other organs either very early or are disseminated by another route than the blood stream.

Thermometry of infected guinea pigs – Once the presence of amastigotes in the nasal mucosa was confirmed, in animals also showing parasitized lesions in the tarsi and also parasites in the spleen and liver in lower numbers, the body temperature of three guinea pigs was measured 120 days post-inoculation. Table IV gives the results. The skin of the paws, as also the mucosa of the nasal cavity at the level of the inferior turbinate, where parasites had been detected directly or indirectly, had similar temperatures (30-31 °C); this was lower than the bucal and anal cavities, which showed temperatures between 35 and 42 °C. The temperature of the axilas was equal to or higher than the bucal and anal cavities.

TABLE IV

Local morning temperatures in three male guinea pigs inoculated three months previously with *Leishmania braziliensis* spp (°C)

Animal	1	2	3
Nasal orifice	31-32	31-30.5	31
Mouth (palate)	41	42	42
Axila	41	43-42	44-42
Hind paws			
right	31	30	31
left	31	31	31
Rectum	35	39	39

DISCUSSION

Pessoa & Barretto (1944) have discussed extensively the mechanism of invasion or dissemination in order to explain the appearance of oronasal lesions in mucocutaneous leishmaniasis. They discarded the hypothesis of autoinoculation for the assumption that, in the beginning of the disease there is a fugitive invasion of the blood stream which carries the parasites to the mucosa; the parasites later disappearing from the blood.

The finding of amastigotes of *L. braziliensis* spp. in the mucosa of guinea pigs inoculated at a distant site, in the skin of the hind paws, together with the amastigotes from these animals infecting hamsters, establishes two salient facts: the susceptibility of the guinea pig to *L. braziliensis* and the dissemination of this parasite toward the viscera and, more consistently, to the nasal mucosa.

Leishmania braziliensis naturally infects man and other animals in close association. Recently, Bonfante et al. (1981) and Aguilar et al. (1986) have signalled the role of the donkey as a domestic reservoir. In these cases, an autocthonous Neotropical parasite has been able to infect mammals introduced to the continent, showing the possibility of a greater infectivity than parasites such as *L. mexicana,* which has not yet been detected in dogs or donkeys.

The eventual infectivity of *L. braziliensis* for an autocthonous rodent such as the guinea pig, reported in this paper, widens the spectrum of infectivity already demonstrated in natural infections of other autocthonous animals confined to this continent, such as the hystrichomorph and cricetid rodents, the didelphids, the edentates, the procyonids and the simians (Lainson & Shaw, 1987). This suggest the presence of common genes for histocompatibility or susceptibility to peripyloric parasites in Neotropical metatherians and eutherians.

The infection of approximately half the guinea pigs with *L. braziliensis* and the retention of the parasites for at least three months suggests that these animals possess alleles for susceptibility and resistance (S-R), similar to those studied by Roberts (1988) on chromosome 12 of mice for the susceptibility or resistance to *L. mexicana.*

Visceralization or, more properly, invasion or localization of the parasites in the nasal mucosa, did not occur in all of the animals that developed a lesion at the inoculation site. This suggests the existance, in the guinea pig, of genes of the H-2 type, like those that intervene and regulate the visceralization of the leishmanias of the *L. donovani* and *L. mexicana* type in the mouse model (Blackwell & Roberts, 1987).

We wish to emphasize, not only the bare facts that laboratory-reared young male guinea pigs show differential susceptibility and resistance to different subspecies of *L. braziliensis,* but also the significance of the secondary mucosal localization of these parasites in our model.

Leishmania b. braziliensis of the piedmont of the northwest cordillera of Venezuela produces mucosal lesions in a varying percentage of the susceptible human population. In the suburbs of the city of the Trujillo, we have found mucosal lesions in 3 of 342 cutaneous cases that we have treated (Scorza et al., 1988). In the north central region of the country, Aguilar (1985) has found mucosal parasites in four out of 16 cases, in an epidemic outbreak in a village, apparently due to an infected donkey. It should be noted in these four patients that the nasal mucosa was invaded early in the infection, being found before, during, and after specific treatment with an antimonial. The early finding of parasites in an apparently healthy nasal mucosa, and its synchronicity with cutaneous lesions, has been well documented by Pessoa & Barretto (1944).

We hope that these results may furnish something of a tool, not only for explanation of the mucosal dissemination of *L. braziliensis* in a laboratory model, but also to elucidate something of the genetics of susceptibility and dissemination of *L. braziliensis,* keeping in mind that this parasite infects only the highly susceptible hamster and BALB/c mice (El-On & Hamburger, 1987). Incidentally, the susceptibility of the guinea pig is to parasites of the *"braziliensis"* complex; though we have not worked with parasites unequivocally identified as belonging to the *"mexicana"* complex, we have been unable to infect guinea pigs with two isolates which, by the morphology of the amastigotes, and by the extensive and dissemi-

nated lesions in hamsters, appear to be of the *"mexicana"* type.

Noriega del Aguilar (1919) mentioned that Antonio Laverán inoculated two guinea pigs in the testicles with amastigotes of *L. tropica* from homogenate of the testicle of a mouse which had been infected with promastigotes from culture. The same author describes lesions in the testicular albuginea of a guinea pig inoculated in the testicle three months previously with the scrapings from a leishmanial lesion of the glans of a patient of the hospital "Dos de Mayo" in Lima, this patient showing extensive mucosal ulceration. The parasites, confirmed by Giemsa staining, were probably *L. braziliensis.* Pereira de Castro (1960), postulating that *L. enrietti* in the guinea pig tended to produce metastatic lesions in the extremities and those parts of the body having lower temperatures, was able to confirm that lesions in the body regions having a temperature of 35 °C were smaller and tended to regress spontaneously. Zeledon & Blanco (1965), with parasites considered to be *L. braziliensis,* inoculated hamsters with promastigotes in various regions of the body; inoculations into the dorsal and ventral regions, with temperature of 36.7 and 36.8 °C respectively, failed. Inoculations into the muzzle, paws, and tail, where the temperatures ranged from 27.2 to 29.9 °C, produced lesions that developed in as little as two weeks. In our experiments, guinea pigs inoculated in the hind tarsi showed parasites at a distance in the nasal mucosa; they were found more frequently in this site than in the viscera. Although we did not search for parasites in other regions or organs, we suppose that the metastasis of the tarsal lesions to the mucosa was mediated by the similar temperature of these regions, although this does not explain the presence of the parasites in the viscera. We are inclined to believe that the presence of the parasites in the spleen and the liver represents a process of sequestration, rather than of colonization. In any case, the parasitization of the nasal mucosa, for both isolates, was less than in the tarsi, but greater than in the viscera.

ACKNOWLEDGMENTS

To Dr N. Saravia and to the Misses Ana Holguin and Claudia Navas for the analytical studies and for the identification of the parasites isolated. To Mr Ian McLure for English translation and Miss Leyla Abchi for typewritting of manuscript.

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