

Mitsuda reaction in armadillos *Dasybus novemcinctus* using human and armadillo derived antigens

*A Reação de Mitsuda em tatus *Dasybus novemcinctus* utilizando antígeno humano e antígeno derivado de tatus¹*

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Abstract

The armadillo has been an important experimental model for leprosy, besides it is still an important resource for bacilli. Despite the innumerable studies about armadillos of the Genus *Dasybus*, little is known about the real susceptibility of this species to the Hansen's bacillus after experimental infection with *M. leprae*. Many authors have reported that 80% of the inoculated animal will develop the disease. In Brazil, positive inoculation of this species was obtained only twice, being raised the hypothesis that these animals are more resistant to experimental infection. In the present study the response to the Mitsuda antigen was used as an indicative of cellular immune response to *M. leprae* in armadillos. Twenty one animals were tested with two Mitsuda antigen preparations, human derived ($4,2 \times 10^9$ bacilli/ml) and armadillo derived ($1,6 \times 10^8$ bacilli/ml) antigens. Response after 28 days of intradermal testing showed that most of the animals presented an infiltrate composed by grouped macrophages with vacuolated cytoplasm and rare lymphocytes. This response resembles lepromatous leprosy in humans and suggests that these animals would be susceptible to development of disseminated leprosy when successfully inoculated. Bacilloscopy in these animals varied from 3+ to 4+ according to Ridley's scale (1966). Two animals developed a

granulomatous reaction with borderline pattern and bacilloscopy varying from 1+ to 3+.

Key-words: Mitsuda reaction; *Dasybus novemcinctus*; Mitsuda antigen.

Introduction

The susceptibility of the armadillo *Dasybus novemcinctus* to *Mycobacterium leprae* infection was demonstrated in 1971 by Kircheimer and Storrs¹. The large amounts of *M. leprae* recovered from infected animals allowed a better understanding of the biological characteristic of the bacillus once it has not yet being cultivated in cell free media. In the United States, both in the Laboratory of the Hansen's disease Center in Louisiana and in Florida where Dr Storrs worked, nearly 80% of the animals developed disease after experimental infection with *M. leprae* bacilli^{2,3}.

In South America, however, the same did not happen. Convit⁴, in Venezuela, admitted having difficulties to infect armadillos of the species *Dasybus novemcinctus* and the animals in which he successfully reproduced the disease were smaller armadillos, the *Dasybus sabanicola*, proved to be inappropriate for such studies. Convit suggested that armadillos of the species *D. novemcinctus* in South America were more resistant than North American armadillos to the infection with *M. leprae*.

In the armadillo facility at the Institute "Lauro de Souza Lima", in Bauru, Brazil, the reproduction of the disease was accomplished at two different occasions a few years ago⁵. Unfortunately, the need, at that time, to eliminate the armadillo specimens from the colony hampered the *M. leprae* inoculation studies and we could not infect a large number of

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animals. With the repopulation of the colony years later, the armadillos started to be studied again. One of the priority matters to unveil was to demonstrate resistance or not of South American animals of the genus *Dasypos* to experimental reproduction of leprosy. The evaluation of the Mitsuda test would be the first step in such direction.

The aim of this paper is to demonstrate the results obtained with the Mitsuda testing of armadillos of the species *D. novemcinctus* captured in the region of Bauru, with human and armadillo derived antigens.

Material and Methods

The Mitsuda test was performed in 21 armadillos of the species *D. novemcinctus*, adults, males and females, weighting 3.5 to 5.0 Kg. None of the animals were previously inoculated with *M. leprae*.

For antigen inoculation, animals were restrained in an appropriate cage, the abdomen was carefully washed with soap and water, dried and the site of inoculation cleaned with 70% alcohol without excessive rubbing.

Two antigen preparations were used, a human origin antigen (H) and an armadillo derived antigen (A). The antigen H contained 4.2×10^9 *M. leprae* bacilli/ml and the antigen A contained 1.6×10^8 *M. leprae* bacilli/ml. Animals were intradermally inoculated in the right and left lower abdominal regions, respectively, with 0.1 ml of the antigen A and H. A round elevation was observed at the inoculation site and its limits were marked with small needle punctured and application of India ink at the site. The antigen tubes were thoroughly homogenized before each inoculation to assure uniform suspension of bacilli.

After 21 days the site of inoculation was examined for presence of induration (nodules or papules) and biopsied with a 4 mm punch. Tissue fragments were fixed in 10% buffered formalin. In the absence of induration, biopsies were taken from the center of the tattooed area of the skin. The skin fragments were embedded in paraffin, stained by hematoxylin-eosin and Fite-Faraco for alcohol acid resistant bacilli staining. The bacilloscopic index varied from 0+ to 6+, by Ridley's scale⁶. The size of the infiltrates was described according to Job's (1982). Small infiltrates occupied up to 20% of the area of the tissue section, medium size infiltrates occupied 50% of the area and large infiltrates occupied more than 50% of the area⁷.

Results

The type and size of cellular infiltrates observed after inoculation of the two types of antigens are demonstrated in Table 1, as well as the results of bacilloscopy.

From the 21 animals, only one animal presented an indurated nodule at the site of inoculation with both antigens after 21 days. In the remaining animals there was no visible or noticeable nodules at palpation, therefore the biopsies were guided by tattoos.

In most of the sections examined, from animals inoculated with antigen A and H, showed the same type of cellular infiltrate, with some quantitative differences in respect to size of infiltrates and amount of bacilli found.

The majority of the animals showed medium size infiltrates presented in a few or multiple foci of perivascular and perineural infiltrates in the superficial and/or deep dermis (Figure 1). The infiltrates were constituted by mononuclear cells, with predominance of macrophages with abundant vacuolated cytoplasm, clear or dense nucleus. In some cases a reduced number of such cells were noticed around vessels and in other animals a moderate number of grouped macrophages were observed occupying 20 to 30% of the area of the section, scattered with lymphocytes (Figure 2). Mast cells were are markedly present in some sections, they were numerous and best visible with the Fite-Faraco staining. The bacilloscopic indexes of the biopsies varied from 3+ to 4+ and the amount of bacilli was proportional to the number of cells of the infiltrate (Figure 3). Sometimes there were isolated mononuclear cells with bacilli penetrating collagen fibers. The animals with this response pattern corresponded to the lepromatous type reaction to the Mitsuda antigen.

Extensive, diffuse, granulomatous histiocytic infiltrates occupying the superficial and deep dermis were observed in two animals (animals ILSL 32 and ILSL 33). These infiltrates were constituted by vacuolated macrophages, with abundant cytoplasm, central nucleus and dense or vacuolated nucleus (Figure 4). In some areas epithelioid cells and rare giant cells could be seen; there were a few foci of lymphocytes (Figure 5). These cells occupied more than 50% of the dermis. In the animal ILSL 33 several areas of necrosis were observed. The bacilloscopic index varied from 2+ to 3+ in these two animals (Figure 6). The described pattern resembles a borderline response.

Table 1. Characteristics of the reaction in armadillos after intradermal testing with different Mitsuda antigen preparations.

Identification of animal	Human Mitsuda antigen (H)			Armadillo Mitsuda antigen (A)		
	Size of infiltrate*	Cells**	BI***	Size of infiltrate*	Cells**	BI***
ILSL27	Small	M	-	Medium	M	2+/3+
ILSL29	Medium	M	4+	Medium	M	4+
ILSL30	Medium	M,L	4+	Medium	M,	4+
ILSL31	Medium	M	4+	Medium	M	3+
ILSL32	Large	M,L,E	3+	Large	M,L,E,CG	3+
ILSL33	Medium	M,L,E	2+	Large	M,L,E,CG	1+/2+
ILSL34	Medium	M	3+	Medium	M,L	3+
ILSL35	Small	M	2+	Small	M	2+
ILSL36	Medium	M	4+	Small	M	-
ILSL37	Medium	M	4+	Medium	M	4+
ILSL38	Medium	M	4+	Medium	M	3+
ILSL39	Medium	M	4+	Small	M	1+
ILSL40	Medium	M,L	4+	Medium	M,L	3+
ILSL41	Small	M	4+	Small	M	3+
ILSL42	Small	M	3+	Small	M	3+
ILSL43	Medium	M,L	4+	Medium	M,L	4+
MAN1	Medium	M,L	4+	Small	M	-
MAN4	Medium	M,L	4+	Medium	M,L,N	4+
MAN5	Medium	M,L	2+/3+	Small	M	1+
MAN6	Medium	M,	4+	Medium	M	4+
MAN8	Medium	M,	4+	Medium	M	4+

M= macrophages, L= lymphocytes, E= epithelioid cells, CG= giant cells, N=neutrophils, BI=bacilloscopic index

Discussion

Job *et al.*⁷ (1982) were the first to study the response of armadillos to the Mitsuda antigen. In the first experiment, 14 animals were tested with human derived lepromin containing 3.8×10^7 *M. leprae*.

In human beings several authors described the Mitsuda reaction as an indicative of cellular immune response. Positive Mitsuda reactions occurred in individuals capable to respond,

therefore, more resistant to infection or with a tendency to develop tuberculoid leprosy. The negative Mitsuda responses occurred in individuals with low ability to respond or with a specific immune suppression, and when infected they would develop less resistant leprosy forms (borderline and lepromatous type infection)⁸.

Job *et al.*⁷ observed in armadillos three different responses to the Mitsuda antigen:

1) a lepromatous lepromin reaction, in which the

inflammatory infiltrate was small and occupied around 20% of the dermis. The infiltrate was composed by macrophages with oval or round nucleus, abundant cytoplasm and marked vacuolization which resulted in a foamy aspect to the cell. Rare lymphocytes were present and the Faraco-Fite staining showed numerous bacilli inside macrophages. Eleven animals out of 14 presented this histopathological aspect.

- 2) Another type of response was observed in one armadillo and it was considered a borderline reaction. In such case, there were large numbers of macrophages with round or oval nucleus, vacuolated cytoplasm occupying 50% of the section and innumerable bacilli. There were areas with a larger number of lymphocytes and less bacilli.
- 3) The third type corresponded to a tuberculoid type reaction in two animals. There were epithelioid cells, giant cells and lymphocytes occupying 30% of the area of the section. Rare bacilli were found in those cases⁷. From the 11 animals presenting lepromatous type reaction to lepromin, 10 developed disseminated leprosy infection after 13 to 66 months. The animals with tuberculoid type response did not develop disease. In face of these results, the authors suggest that by using the lepromin test it is possible to discriminate susceptible from resistant animals.

In a later study, Job *et al.*⁹ (1987), showed that the histopathological aspect of the Mitsuda reaction in armadillos could vary in tuberculoid, borderline tuberculoid, borderline lepromatous and lepromatous type patterns. From 102 armadillos tested with antigen A containing 1.6×10^8 *M. leprae*/ml, nine presented positive Mitsuda reaction, five of those with indurated visible nodules. Job *et al.* discussed that similarly to humans, the histological picture of the Mitsuda reaction will reproduce the histopathological aspect of the disease, suggesting further that armadillos would be able to develop the tuberculoid form of leprosy.

We did not observe a well defined tuberculoid type reaction in the sections presently examined. There were two cases with reaction that resembled the borderline pattern, all the others would be lepromatous types. The animals presenting the borderline type reaction are suggested to be more resistant to infection, and the animal with the lepromatous type reaction would be more susceptible to *M. leprae* infection.

In respect to the two Mitsuda antigen preparations, A and H, the results showed few differences. A previous study also done in armadillos did not show significant differences in

the response to armadillos or nude mice (*nu/nu*) derived antigens for the tuberculoid type, as well as in the lepromatous type response patterns¹⁰. The bacilloscopic indexes and size of the infiltrate variations detected in the present study seem to be related more to uniformity of the inoculated *M. leprae* suspension than to immune response of the animals once the cellular pattern did not vary and the proportional amount of bacilli was identical in both groups.

As Convit⁴, the researchers at the Instituto Lauro de Souza Lima followed the recommended protocol for armadillo inoculation proposed by Kircheimer and Storrs¹ in 1971. Natural resistance of South American armadillos to infection by *M. leprae* may exist and this would be unlinked to the Mitsuda antigens. The Mitsuda test shows that the reaction we will observe in a successful infection, nevertheless other factors may be involved in the natural resistance to leprosy, similarly to what happens in human beings.

Resumo

O tatu foi um modelo experimental importante para o estudo da hanseníase, além de ser ainda uma importante fonte para coleta de bacilos. Apesar dos inúmeros relatos de estudos em tatus da espécie *Dasybus novemcinctus*, pouco se sabe sobre a real susceptibilidade desta espécie ao bacilo de Hansen após inoculação experimental com o *M. leprae*. Alguns autores relatam que cerca de 80% dos animais desenvolveriam a doença quando infectados. No Brasil, a inoculação experimental desta espécie resultou em inoculações positivas em apenas dois momentos, tendo até sido levantada a hipótese de estes animais serem mais resistentes à infecção experimental. No presente estudo, utilizou-se a resposta ao antígeno de Mitsuda como um indicador de resposta imune celular de tatus ao *M. leprae*. Para tanto foram utilizados 21 animais testados com antígenos de Mitsuda derivados de humanos ($4,2 \times 10^9$ bacilos/ml) e antígeno produzido a partir de tatus ($1,6 \times 10^8$ bacilos/ml). A resposta após 28 dias mostrou que a maior parte dos animais apresentava, no exame histológico, infiltrado constituído por macrófagos com citoplasma vacuolado formando vários agrupamentos, entremeados por raros linfócitos. Esta resposta imita a hanseníase virchoviana em seres humanos e sugere que os animais seriam susceptíveis a desenvolverem a forma disseminada da doença frente a inoculações bem sucedidas. A baciloscopia nestes animais variou de 3+ a 4+, pela escala de Ridley (1966). Dois tatus desenvolveram reação granulomatosa de padrão dimórfico com baciloscopia variando de 1+ a 3+.

Palavras-chave: reação de Mitsuda; *Dasybus novemcinctus*; antígeno de Mitsuda.

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