Rev. Inst. Med. trop. São Paulo
29(6):385-387, *novembro-dezembro*, 1987

CUTANEOUS LEISHMANIASIS IN GERMFREE, GNOTOBIOTIC, AND CONVENTIONAL MICE. (Nota prévia).

Enio Cardillo VIEIRA (1), Jacques Robert NICOLI (1), Tasso MORAES-SANTOS (1), Marcelo Eustáquio SILVA (1), Carlos Alberto da COSTA (2), Wilson MAYRINK (2) & Eduardo Alves BAMBIRRA (3)

SUMMARY

Cutaneous leishmaniasis was much more severe in conventional than in gnotobiotic mice as revealed by macro and microscopic examination. An inoculum of **Leishmania mexicana amazonensis** was used.

KEY WORDS: Cutaneous leishmaniasis; Germfree mice; Gnotobiotic mice.

Germfree (GF) animals, when infected with pathogenic microorganisms, are either more resistant than their conventional (CV) counterparts, or less [4,7]. The GF status has been shown to modify immune parameters of host animals and to alter host-parasite relationship.

Leishmania mexicana amazonensis, strain IFLA/BR/67/PH8, was maintained **in vivo** by serial passages in hamster's noses. Pieces of the lesion were taken aseptically, 60 days after inoculation and put in test tubes containing a biphasic NNN/LIT medium. After 14 days of incubation at 23°C, the culture proved to be axenic. Promastigotes were counted in a Neubauer hemocytometer. Concentration of promastigotes was adjusted with 0.15M NaCl solution to the desired level.

A breeding nucleus of GF CFW(LOB) mice was obtained from Lobund Laboratory, University of Notre Dame, USA, and reared at the Department of Biochemistry and Immunology, University of Minas Gerais. The mice are free of all demonstrable microbes, except for a leukemogenic virus acquired prenatally which remains latent unless activated by radiation [8].

GF and CV 45 days old mice weighing approximately 25g each were distributed in four Trexler-type isolators. Four CV and six GF mice in isolators 1 and 2, respectively, were inoculated with $5x10^5$ promastigotes of **L. mexicana amazonensis.** Isolator 3 housed four GF mice inoculated with $2x10^5$ promastigotes. In isolator 4, six GF mice were inoculated with 10^5 promastigotes. Subcutaneous inoculation was performed 1 cm above the root of the tail.

In isolator 2, a monocontamination with an aerobic fungus was detected, ten days after inoculation. This fungus, identified as **Aspergillus sp**, grew at 25 but not at 37°C in thioglycollate broth and brain-heart infusion. Therefore, intestinal colonization could not be established. This constituted the gnotobiotic (GN) group. In isolator 3, a polycontamination was detected 40 days after the inoculation. The animals were conventionalized constituting the ex-GF group. Isolator 4 remained GF throughout the entire experimental period.

The GF status of the isolators was determined according to WAGNER [9]. After 114 days,

(1) Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais. Caixa Postal 2486. CEP 31271 Belo Horizonte, MG., Brasil.
(2) Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais. Caixa Postal 2486. Belo Horizonte, MG., Brasil.
(3) Departamento de Anatomia Patológica, Faculdade de Medicina, Universidade Federal de Minas Gerais. Caixa Postal 340, Belo Horizonte, MG., Brasil.

the animals were killed under ether anesthesia. Blood was drawn from the axillary plexus.

The lesions were examined macro and microscopically. In some animals, depilation was required to depict the lesion. The antibody titers were determined by an indirect immunofluorescence test. An anti-mouse immunoglobulin conjugate (F/P 4.5 ug/ml) (Pasteur Institute), was used.

All animals, regardless of their groups gained from 6.3 to 7.6 g during the experimental period. Results in Table I show that, for CV and ex-GF animals, in general, lesion size was bigger than in GN and GF groups. Results of macroscopic examination were confirmed by histology. GN and GF mice showed discrete cutaneous lesions restricted to the epidermis and the subcutaneous tissue. An inflammatory reaction consisting of focal mononuclear leukocytes was observed. Small nests of intracellular amastigotes were observed. In CV and ex-GF mice, on the other hand, the infection was more severe, reaching the skeletal muscles. There was an intense inflammatory reaction by mononuclear leukocytes. Areas of intense necrosis were observed. Numerous amastigotes were found in volumous intracellular nests. The aspect of the microscopy is shown in Figure 1. No visceral invasion was observed in all animals.

TABLE 1

Effect of **Leishmania mexicana amazonensis** infection on conventional (CV), monocontaminated with a fungus (GN), conventionalized (ex-GF), and germfree (GF) mice, 114 days after inoculation

Group	No. of animals	Size of the inoculum	General macroscopic aspect of the lesion at the site of inoculation	General microscopic aspect of the tissues at the site of inoculation	Average antibody titer
CV	4	$5x10^6$	big closed nodule	lesion reaching the skeletal muscles. Intense inflammatory reaction and necrosis	1:320
GN	6	$5x10^5$	small closed nodule	lesions restricted to epidermis and subcutaneous tissue. Inflammatory reaction consisting of focal mononuclear leukocytes	1:320
ex-GF	4	$2x10^5$	big closed and ulcerated nodule	similar to CV group	1:320
GF	6	10^5	very small nodule or no lesion	similar to GN group	1:320

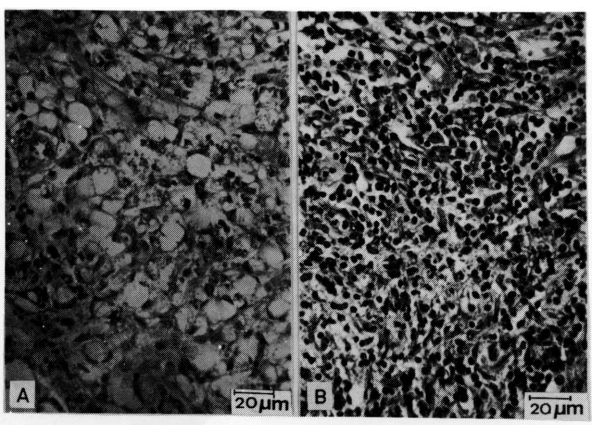

Fig. 1 — Cutaneous leishmaniasis. Aspect of the tissue at the site of inoculation of **Leishmania mexicana amazonensis.** Germfree (A) — Scarse inflammatory cell infiltrate with many large macrophages containing leishmanias. Conventional (B) — The dense inflammatory cell infiltrate is comprised by macrophages, lymphocytes, and plasma cells. Some of the macrophages contain leishmanias.

The levels of antibodies were the same for all groups. The cellular immunity was not evaluated.

Different mice strains exhibit a broad range of susceptibility to different species of **Leishmania** [3,6]. ANDRADE et al. reported similar levels of antibody for a resistant (A/J) and a susceptible (BALB/c) strain of mice [1]. A depression of cell mediated immunity was observed during the development of the disease in mice [2].

The less severe disease in GN and GF mice when compared with CV and ex-GF mice may be due to the milder cellular response of the former [5]. Germs from the skin may also be responsible for this difference. In CV animals, a secondary infection can enhance the invasion of **Leishmania.** Further experiments are

under way at this laboratory when the cellular immunity will be evaluated.

RESUMO

Leishmaniose tegumentar em camundongos isentos de germes, gnotobióticos e convencionais

A leishmaniose tegumentar foi muito mais grave em camundongos convencionais e convencionalizados do que em seus pares isentos de germes ou gnotobióticos. Usou-se um inóculo de **Leishmania mexicana amazonensis.**

ACKNOWLEDGEMENTS

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Financiadora de Estudos e Projetos (FINEP). The technical help of Francisco Abílio Nascimento, Ronilda Maria de Paula and Roberto Teodoro da Costa is greatly acknowledged.

REFERENCES

1. ANDRADE, Z. A.; REED, S. G.; ROTERS, S. B. & SADIGURSKY, M. — Immunopathology of experimental cutaneous leishmaniasis. **Amer. J. Path.**, 114: 137-148, 1984.

2. BARRAL, A.; PETERSEN, E. A.; SACKS, D. L. & NEVA, F. A. — Late metastatic leishmaniasis in the mouse. A model for mucocutaneous disease. **Amer. J. trop. Med. Hyg.**, 32: 277-285, 1983.

3. CHILDS, G. E.; LIGHTNER, L. K.; MCKINNEY, L.; GROVES, M. G.; PRICE, E. E. & HENDRICKS, L. D. — Inbred mice as model hosts for cutaneous leishmaniasis. I. Resistance and susceptibility to infection with **Leishmania brasiliensis, L. mexicana,** and **L. aethiopica. Ann. trop. Med. Parasit., 8:** 25-34, 1984.

4. GORDON, H. A. & PESTI, L. — The gnotobiotic animal as a tool in the study of host microbial relationship. **Bact. Rev.**, 35: 390-429, 1971.

5. OLSON, G. B. & WOSTMANN, B. S. — Cellular and humoral immune response of germfree mice stimulated with 7SHGG or **Salmonella typhimurium. J. Immunol.,** 97: 275-286, 1966.

6. PÉREZ, H.; LABRADOR, F. & TORREALBA, J. W. — Variations in the response of five strains of mice to **Leishmania mexicana. Int. J. Parasit.**, 9: 27-32, 1979.

7. PLEASANTS, J. R. — Gnotobiotics. In: MELBY JR., E. C. & ALTMAN, N. H., ed. — **CRC Handbook of Laboratory Animal Science.** Cleveland, CRC Press, 1974. p. 119-174.

8. POLLARD, M. — Viral status of "germfree" mice. **Nat. Cancer Inst. Monogr.**, 20: 167-172, 1965.

9. WAGNER, M. — Determination of germfree status. **Ann. N.Y. Acad Sci.**, 78: 89-101, 1959.

Recebido para publicação em 08/12/1986.