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Research Note

DEAD *MYCOBACTERIUM LEPRAE* INHIBITS PHAGOCYTOSIS BY INFLAMMATORY MACROPHAGES *IN VIVO*. PARTICIPATION OF THE BACTERIA CELL LIPIDS IN THE PHENOMENON

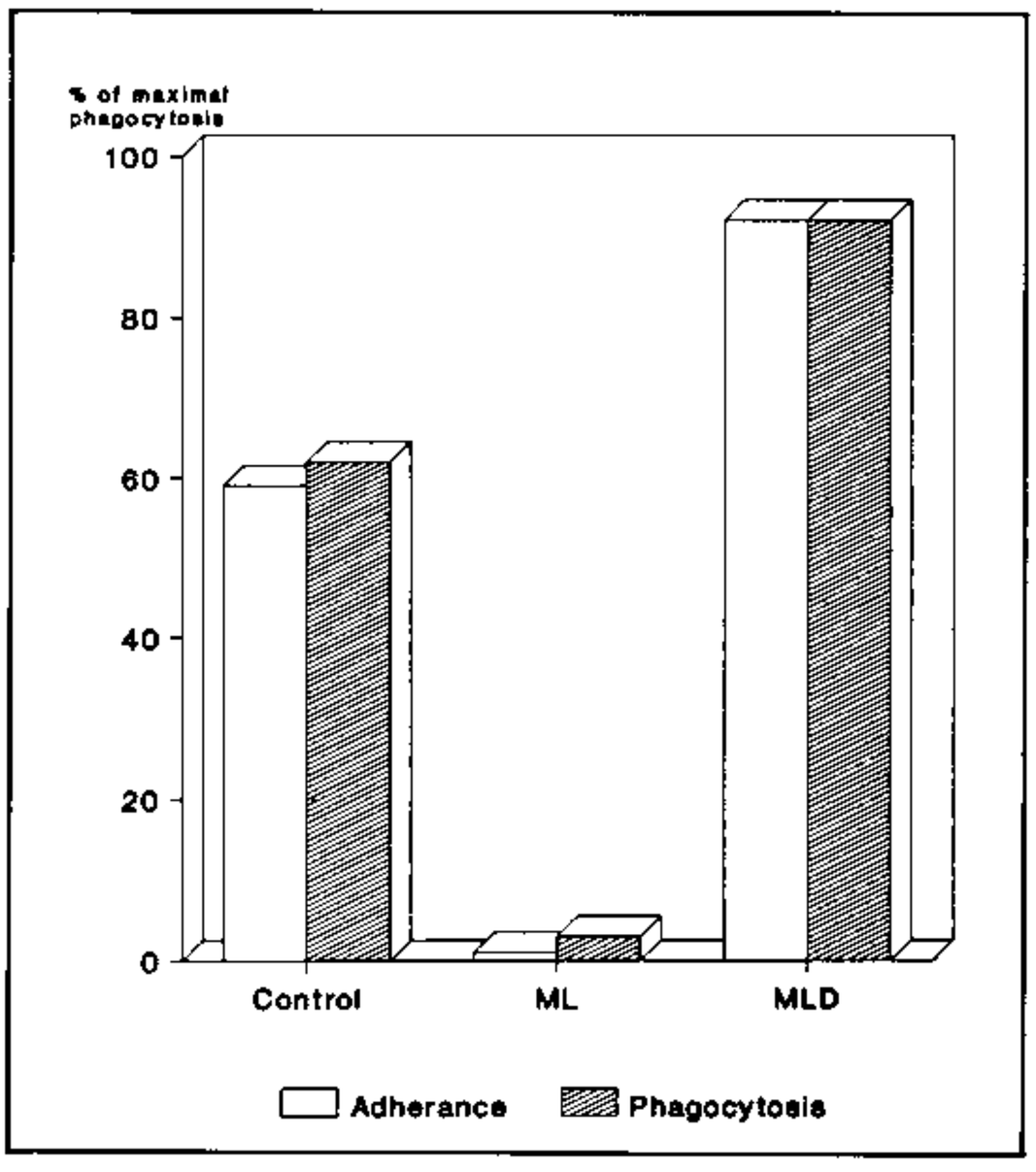
A. C. N. MOURA/[+] & M. MARIANO

Departamento de Imunologia, Instituto de Ciências Biomédicas, USP, Av. Prof. Lineu Prestes, 2415, 05508 São Paulo, SP, Brasil

The "immune-repulsion" concept postulated by R. M. Fauve et al. (1974, *Proc. Natl Acad. Sci.* USA, *71:* 4052-4056) is based on the fact that some parasites and tumor cells instead of triggering an inflammatory response in the host tissues, induce an inhibitory effect on the process. This concept – different from that known for immune-supression – centres the parasite adapting mechanisms in the inhability of the inflammatory reaction to process the antigen.

Evidences that dead *Mycobacterium leprae* (ML) induce immune-repulsion in Balb/c mice were obtained considering that the injection of the bacilli into the animals foot-pad leads to mild inflammatory reaction (8% in foot-pad increase) and that the inflammatory response evoked by BCG in a site previously injected with dead ML was significantly diminished. Conversely, when we delipidated the ML by petroleum ether (MLD) and previously injected in the foot-pad, no influence was observed on the foot-pad thickness obtained after BCG inoculation. These results suggest that the lipid bacterial contents play a role in the phenomenon. It was also shown that DTH reaction to bacilli antigens obtained with MLD is greater than that observed with ML (A. C. N. Moura & M. Mariano, unpublished results).

In order to investigate *in vivo* whether or not macrophages were involved in the phenomenon the following experiments were done. Round glass cover-slips, 12 mm in diameter, were inserted into the subcutaneous tissue of Balb/c mice, removed after 5 days and, the phagocytic ability of macrophages stuck on the glass surface analysed as previously described (M. Mariano et al., 1977, *J. Path., 3:* 27-34). Briefly, cover-slips removed after 5 days of implantation were incubated with sheep red blood cells sensitized with a rabbit IgG. After one hour, cover-slips were washed in saline, fixed in 2% glutharaldehyde and examined under phase contrast microscopy. The percentage of cells with five or more phagocytosed erythrocytes was estimated after counting two hundred cells. In other group of animals a suspension of 8×10^6 purified dead *M. leprae* obtained from infected armadillo (kindly supplyed by Dr Rees, Leprosy Centre of Mill Hill, WHO) were injected into the pocket where cover-slips were implanted. A third group of animals was equally treated with the bacilli previously delipidated by petroleum ether according to the technic proposed by H. Bloch (1950, *J. Exp. Med., 91:* 197-201).



Influence of purified Mycobacterium leprae (ML) and dead, purified and delipidated *M. leprae* (MLD) on the phagocytic ability of inflammatory macrophages *in vivo*.

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The results showed that about 50% of macrophages from the control group phagocytosed more than 5 erythrocytes. On the other hand, no phagocytosis was observed by macrophages removed from lesions injected with intact *M. leprae.* Conversely, the injection of delipidated bacteria into the cover-slip pocket, overhelmed the phagocytic index of the cells on the glass surface to 92% (Figure). More than 93% of those inflammatory macrophages exposed or not to ML or to MLD were viable as evaluated by Trypan blue test.

These data suggest that macrophages are involved in immune-repulsion induced by dead *M. leprae* and that the lipidic components of the bacteria cell wall play a central role in the phenomenon.

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