

TITLE: CHOLESTEROL CONSUMPTION BY *Mycobacterium smegmatis* DOES SIGNIFICANT MODULATION DURING *IN VITRO* INFECTION

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ABSTRACT: Tuberculosis is a disease caused by *Mycobacterium tuberculosis*, which infects alveolar macrophages, developing granulomas that compromise pulmonary physiology. The success of the infection is related with the accumulation of cholesterol in the host cell, conferring an alternative source of energy and carbon for the bacillus. Preliminary results from our research group revealed that the *in vitro* culture of *Mycobacterium smegmatis* (saprophytic species) in minimal medium (MM) induced the consumption of cholesterol by the bacillus, promoting a modulation of its physiology, cell wall architecture and consecutively the hydrophobic. These results suggest that adverse nutritional conditions may be part of the essential microbial adaptation to the microenvironment of the infection. In this way we propose to investigate the relevance of the mycobacteria culture in MM to provide macrophage infection and granuloma formation. *M. smegmatis* at early stationary phase of culture in *Middlebrook 7H9* or MM supplemented with cholesterol was used to infect J774.A1 macrophage cell line with multiplicity of infection of 100. We evaluated the infection by counting the colony forming units (CFU) of the intracellular bacilli, and the viability of macrophages by MTT method, after 12 hours of infection. The CFU and MTT data showed that the bacilli after culture in MM supplemented with cholesterol induced twice more phagocytosis resistance and lesser macrophage viability (< 30%) when compared with the macrophages infected with bacilli after culture in *Middlebrook 7H9*. After 12 hours of infection the cells were stained by Ziehl-Neelsen method and then showed the formation of structures like granuloma only when the macrophages were infected with *M. smegmatis* after culture in MM supplemented with cholesterol. Thus, our preliminary data suggest that the culture conditions in MM, which induces the consumption of cholesterol and changes the mycobacteria cell wall, does significant modulation of the bioactive molecules to induce the resistance and growth of bacilli, which helps to develop the granuloma, the hallmark of tuberculosis.

Keywords: tuberculosis, cell wall, cholesterol, granuloma

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