Study of cholesterol consumption by *Mycobacterium smegmatis* influencing sensitivity for stress factors

Jerson Marques de Araujo Costa [1], Isabella Luiza Dinis da Silva [1], Barbarella de Matos Macchi [2,3], Edilene Oliveira da Silva [1,5], José Luiz do Nascimento [2] and Chubert Bernardo Castro de Sena [1,3*]

[1] *Laboratory of Structural Biology, Institute of Biological Sciences, Federal University of Para, Belém, PA, Brazil*
[2] *Laboratory of Molecular and Cellular Neurochemistry, Institute of Biological Sciences, Federal University of Para, Belém, PA, Brazil*
[3] *National Institute of Science and Technology in Neuroimmunomodulation (INCT-NIM), Rio de Janeiro, RJ, Brazil*
[4] *National Institute of Science and Technology in Structural Biology and Bioimaging, Rio de Janeiro, RJ, Brazil*

**Correspondence should be addressed to Chubert Bernardo Castro de Sena; chubert@ufpa.br*

Mycobacterium smegmatis belongs to the genus of bacillary actinobacteria, known for its complex cell wall with several immunomodulatory constituents such as lipomannan (LM) and lipoarabinomannan (LAM). Frequently, *M. smegmatis* has been used as model organism for the study of tuberculosis, due to its fast growth, non-pathogenicity and high percentage of orthologous genes of the pathogenic specie *Mycobacterium tuberculosis*. The development of tuberculosis occurs when *M. tuberculosis* manages to multiply in alveolar macrophages after shutting down its microbicide activity. This event has been related to the accumulation of cholesterol in the host cell, being identified as an alternative source of carbon and energy for the bacillus that induces significant alterations in the architecture of the bacterial cell wall that have been related to the generation of granulomas *in vitro*. Thus, we propose to analyze the influence of cholesterol consumption by *M. smegmatis*, which is induced by *in vitro* culture in minimal medium (MM), to generate bacilli resistant to stressors such as pH, hydrogen peroxide (H2O2) and dodecyl sodium sulfate (SDS). *M. smegmatis* was cultured in complete medium *Middlebrook* 7h9 (7h9+Gly group) and MM, with cholesterol and without cholesterol supplementation (MM+Chol and MM groups, respectively) following of challenge to grow in those conditions with H_2O_2 (1mM and 10 mM), SDS (0.005%) and different pH (3.0 to 7.0). Bacilli resistant was monitory by spectrophotometer ($O.D._{600nm}$), and showed that culture in MM, with and without supplementation with cholesterol (MM+Chol and MM groups, respectively), develop bacilli resistant to H_2O_2, SDS and the pH of 3.0, when compared to culture without these stressors ($p<0.05$). This resistance profile was not evidenced when the bacilli were cultivated in complete Middlebrook 7h9 medium ($p<0.001$). because of the resistance against those stressors is related to changes in cell wall constituents, we analyzed the physicochemical integrity of the cell wall using Ziehl-Neelsen staining, which identifies bacilli resistant to alcohol and acid discoloration (BAAR+). All bacilli after culture in MM, regardless of supplementation (MM+Gly, MM+Chol and MM groups) showed some blue bacilli (BARR-), which were more frequent after cholesterol consumption (MM+Chol). Thus, our data suggest that nutritional deficiency, together with cholesterol consumption, infers changes that directly impact the physicochemical properties of the cell wall, enabling the survival of the bacillus in atypical conditions, contributing to the survival of the bacillus during host cell infection.

Key words: Tuberculosis, Cholesterol, Cell Wall, *Mycobacterium Smegmatis*

Estudo da Indução de consume de colesterol em *Mycobacterium smegmatis* influenciando a sensibilidade a fatores estressantes

Mycobacterium tuberculosis infecta macrófagos e acumula colesterol como fonte alternativa de energia. Isto ocorre *in vitro* pelo cultivo em meio mínimo (MM), alterando a arquitetura da parede celular. Nosso trabalho demonstra que estas alterações da parede celular propicia o desenvolvimento de bacilos resistentes a 1mM H_2O_2, 0.005% SDS e pH de 3,0. Nossos dados sugerem que as mudanças na arquitetura da parede celular bacteriana após a deficiência nutricional, associada com o consume de

colesterol, diretamente impactam as propriedades físico-químicas desta parede, possibilitando a sobrevivência do bacilo a condições atípicas, contribuindo para a sobrevivência do bacilo na célula hospedeira.

Palavras-chave: Tuberculose, Colesterol, *Mycobacterium Smegmatis*