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Mycobacterium avium-Macrophage Infection Model Reveals Multiplicity of Infection-Dependent Morphological Transitions and Innate Immune Activation

Giulia Bianchi[1], Hugo Mateus[2], David Pires[1,3], Paulo JG Bettencourt[1,2]

[1]Center for Interdisciplinary Research in Health, Universidade Católica Portuguesa, 2635-631 Rio de Mouro, Portugal.
[2]Faculty of Medicine, Universidade Católica Portuguesa, 2635-631 Rio de Mouro, Portugal.
[3]Global Health and Tropical Medicine, GHTM, Instituto de Higiene e Medicina Tropical, IHMT, Universidade Nova de Lisboa, 1349-008, Lisboa, Portugal.

Introduction

• *Mycobacterium avium* (Mav) is an opportunistic species of non-tuberculous mycobacteria (NTM) associated with the development of Mav complex lung disease (MAC-LD), primarily affecting immunocompromised individuals.

• The global incidence of NTM infections is steadily increasing, with pulmonary cases rising by approximately 4% annually.

• Mav has emerged as a growing public health concern, particularly among populations with weakened immune systems or underlying pulmonary disorders such as chronic obstructive pulmonary disease (COPD) and bronchiectasis.

• Although treatment options are available, they demonstrate only modest efficacy, and no vaccine currently exists.

Objectives

• To establish an *in vitro* infection model of Mav in human macrophages to better understand host-pathogen interactions and the innate immune responses induced by Mav infection.

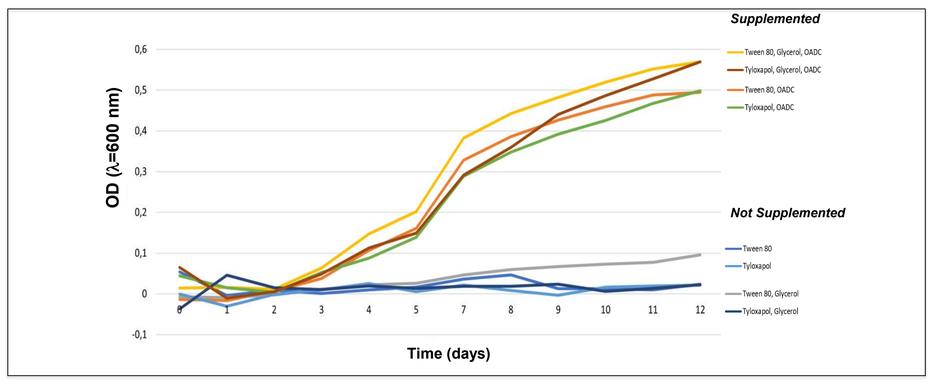
Methods

• To improve existing infection methodologies, we optimized the liquid culture conditions for Mav growth, assessed its intracellular survival in THP-1 human macrophages under various multiplicities of infection (MOI 0.1, 10 and 100), and times post-infection (0, 24, 72, 120h), and characterized 3 morphotypes, as well as the resulting innate immune responses using RT-qPCR, ELISA, and flow cytometry, based on references 1-3.

Results

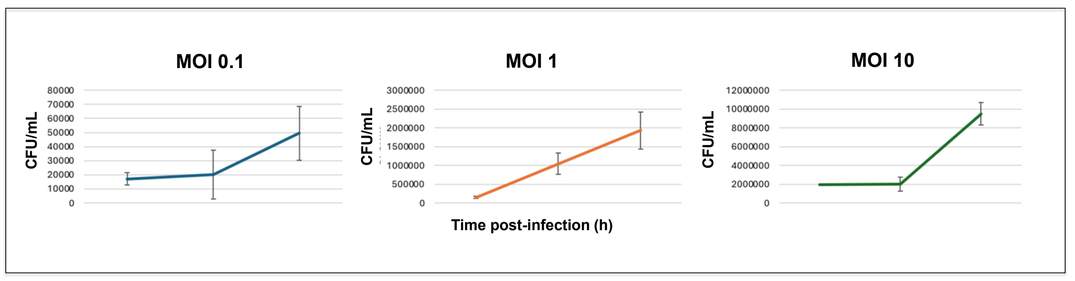
1. Optimization of liquid culture conditions for Mav growth

***Mycobacterium avium* Growth Curves**

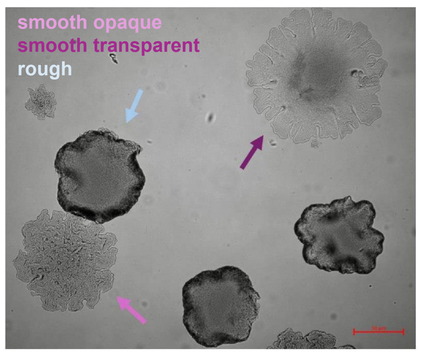


2. Intracellular survival of Mav in THP-1 macrophages

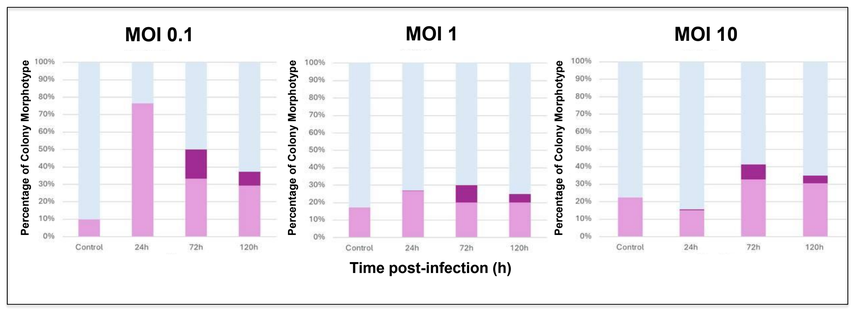
***Mycobacterium avium* Intracellular Growth in THP-1 Macrophages**



3. Morphotype characterization

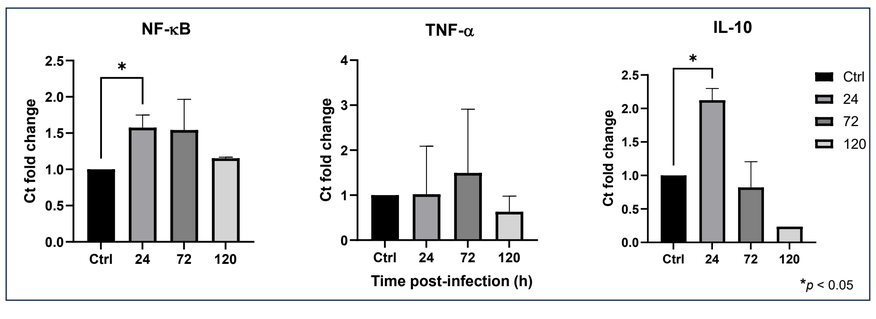


***Mycobacterium avium* Morphotype Quantification**



4. Activation of the NF-κB signaling pathway

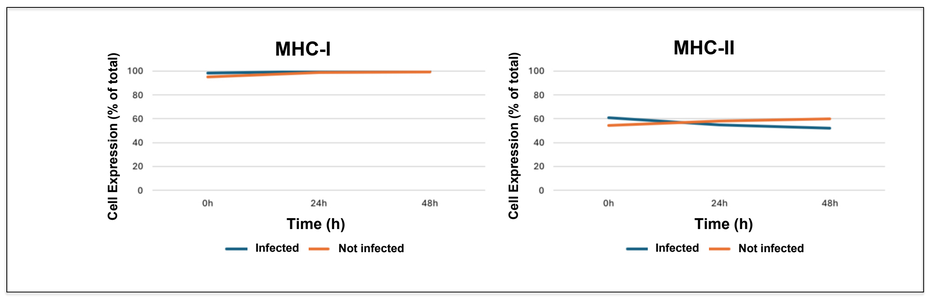
Induction of NF-κB, TNF-α and IL-10



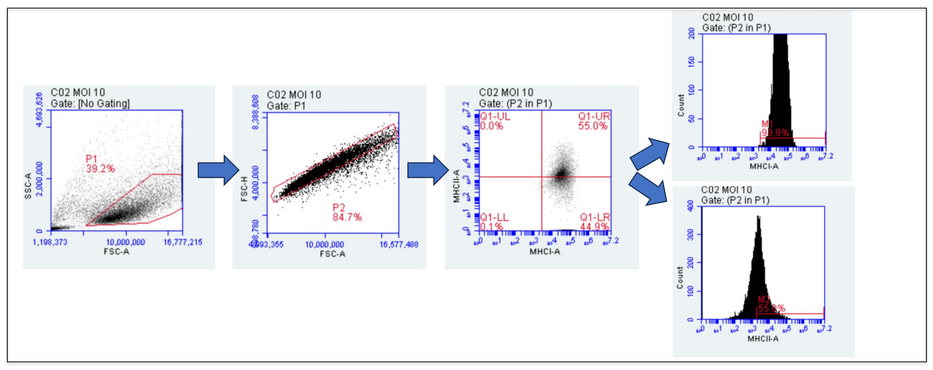
Rough morphotypes, prevalent in liquid culture, reverted to smooth opaque variants following macrophage uptake at low, but not high MOIs. Smooth transparent colonies expanded intracellularly, at later time-points, but were absent in liquid culture.

5. Mav Infection of macrophages resulted in expression of MHC-I and MHC-II

MHC-I and MHC-II Expression in *Mycobacterium avium*-infected THP-1 Macrophages



Flow cytometry gating strategy



Conclusions

Collectively, these results provide a detailed map of the innate immune response to Mav under varying experimental conditions, contributing to a deeper understanding of how this emerging pathogen interacts with human host cells.

References

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