Mycobacterial Killing Assay in Raw 264. 7 Macrophages.

Charlcie Gatewood, Stephen Kotey, Xuejuan Tan and Yong Cheng
Department of Biochemistry and Molecular Biology, Oklahoma State University
Oklahoma Center for Respiratory and Infectious Diseases, Oklahoma State University, Stillwater, Oklahoma.

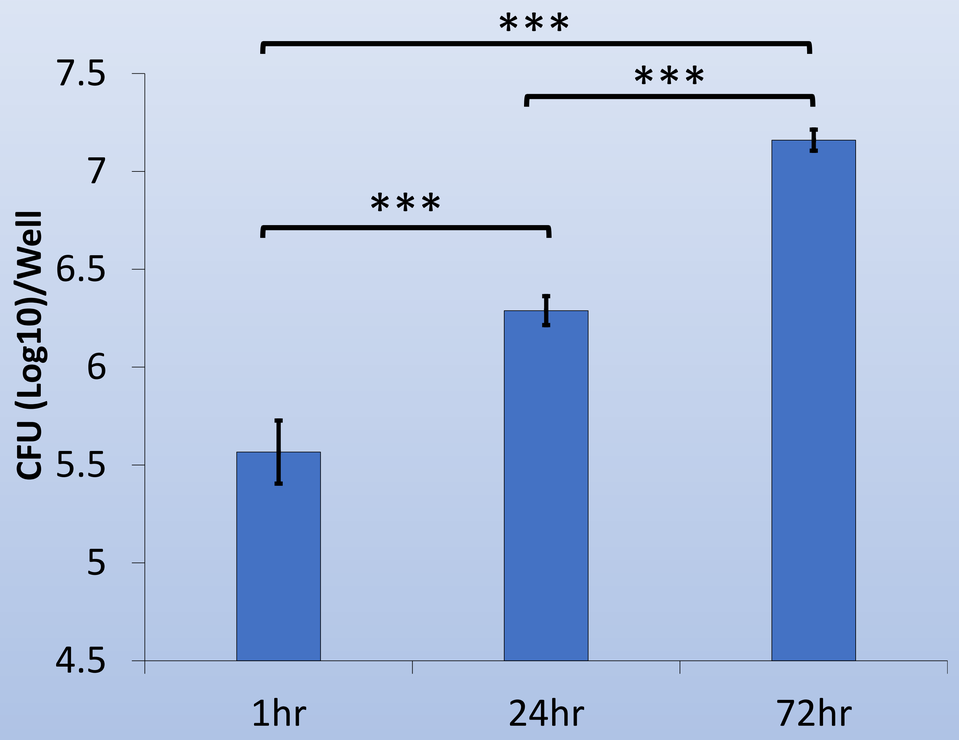
ABSTRACT

Mycobacterial killing assay is an important method to determine mycobacterial pathogenesis and antimycobacterial response in host cells. In our study, we analyzed the survival of *Mycobacterium abscessus* in mouse macrophage cell line, Raw 264.7 cells. *Mycobacterium abscessus* survival in macrophages is measured through a multiple step process: (1) Infection of the cells with *Mycobacterium abscessus* for 1 hour, 24 hours and 72 hours, (2) Plating cell lysates on agar plates, (3) Incubating plates at 37°C, and (4) Counting colonies on the plates. We study *Mycobacterium abscessus* survival in host cells to gain knowledge on this disease and how it affects patients with cystic fibrosis and chronic obstructive pulmonary disease. Prevalence of this disease has increased over the years, especially drug-resistant mycobacterial strains. It becomes important to understand the interaction between the host and Mycobacteria. In our mycobacterial killing assay, we observed that *Mycobacterium abscessus* can adapt to grow successfully in Raw 264.7 cells. Further studies are needed to halt and reduce *Mycobacterium abscessus* growth within host cells.

INTRODUCTION

Nontuberculous Mycobacterial infection is a human disease caused by germs and bacteria found in stagnated water and soil. There is reason to believe that the people most affected by NTM are those who already have a disease condition or any sort of lung damage. The infection is not contagious but can be very serious for those affected by it. Prevalence of these infections have increased over the years, and because it is drug resistant to most existing antibiotics and there is not an abundance of newly formulated drugs coming, it is important that research be done on this disease.

RESULTS



* = P≤ 0.05 ,** = P≤ 0.01, *** = P≤0.001

Fig 1: Graphical representation of the number of colonies formed 1hr, 24hr and 72hrs post-infection of Raw 264.7 macrophage. The data shows that *M. abscessus* proliferated successfully in the Raw 264.7 cells over time. Statistical analyses was performed using one tailed student T-test analyses in Microsoft excel.

METHODS

- Seeding 2 x 10^4 Raw 264.7 cells/well into 96-well plates overnight.
- Infect raw cells with *M. abscessus* at a multiplicity of infection of 5 for 1 hour.
- Discard growth medium and wash cells 3 times with fresh media.
- Replace media with fresh complete medium and incubate at 37 degrees Celsius and 5 percent CO2.
- After 1 hour, 24 hours and 72 hours, discard the media and wash cells with ice cold PBS.
- Lyse cells by adding 0.05% SDS.
- Make dilutions and plate on 7H10 agar plates.
- After 3-4 days, count colonies formed on plates.

CONCLUSION

- *M. Abscessus* can adapt and proliferate successfully in Raw 264.7 cells.
- There is need for further studies into mechanisms to halt and/or reduce *M. abscessus* growth in host-cells. These further studies will prove a useful tool in disease therapy and intervention

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