Introduction

Non-Tuberculosis Mycobacteria (NTM), specifically Mycobacterium avium (M. avium) is a rod-shaped microorganism that causes lung infections in patients with weakened immune systems or underlying lung diseases such as cystic fibrosis, COPD, and HIV. Previous in vitro studies in our laboratory have shown that nitric oxide (NO) plays a critical role in controlling M. tuberculosis growth inside macrophages. Others have reported that Mycobacterium avium can survive and multiply within non-activated cultured macrophages. Still, the antimicrobial mechanism of NO within activated macrophages to control M. avium growth remains to be clarified. This study is aimed to investigate whether the production of nitrogen-reactive species (NO) is a critical factor in the control of M. avium infection in murine-activated macrophages.

Our hypothesis is “that activation of M. avium infected macrophages with IFNγ will significantly increase NO production, then improving infection clearance.”

To test our hypothesis, we used the murine macrophage cell line RAW 264.7. One million RAW cells were infected with M. avium at MOI 10:1 for 4 h and rinsed twice with PBS to eliminate non-adhered mycobacteria. The infected macrophages were cultured in the presence or absence of 100 U/ML of IFNγ for 24 and 48 h. Greiss Assay and colony-forming units (CFU) respectively monitored NO levels and intracellular mycobacteria growth at each time point. After protein quantitation of 50 μg of cellular extracts were tested for arginase activity by an enzymatic assay.

Hypothesis & Methods

**Hypothesis:**

“Activation of M. avium infected macrophages with IFNγ will significantly increase NO production, then improving infection clearance.”

**Methods:**

1. One million RAW cells were plated into 6 well plates and then infected with 10:1 MOI of M. avium in the presence and absence of IFNγ for 24 and 48 hrs.
2. At each time point supernatants were collected to determine nitrite production using the Greiss Reagent. Cellular extract were prepared to test for mycobacteria growth by CFU; NOS2 protein by Western blot and arginase activity by enzymatic assay.
3. Statistical analysis: The results are expressed as the mean ± SEM from 3 different experiment for each condition. Statistical analysis were performed by two-tailed t test using the PRIZM software.

Results

**Arginase expression in M. avium infected macrophages**

**Nitrites production & NOS2 protein expression**

**Nitrites production in different conditions**

**Mycobacterium growth in agar**

**Future Plans**

1. While this hypothesis worked for the M. avium strain, determine if there is a significant inhibitory effect of NO on intracellular growth of other NTM strains (M. abscessus and M. intracellulare) commonly found in Louisiana.
2. Since L-arginine is the substrate for the activation of NOS2 we will like to assess the role of L-arginine-rich environment in facilitating the extracellular spreading of NTM strains.

Conclusions

1. We conclude that the increased resistance of M. avium was associated with and dependent on IFNγ stimulation that kills several mycobacteria in a dependent generation of reactive nitrogen intermediates (NO).
2. These data also illustrate that NO can promote or inhibit mycobacterial growth and that there is a delicate equilibrium that underlies its production.
3. The opposite effects of NO on the resistance to M. avium emphasize the distinct nature of the strategies used by M. avium to survive the host’s antimicrobial machinery.
4. The effect of NO and mycobacterial growth in other strains of the M. avium complex and its effect, needs to be further evaluated.