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Intracellular killing of *Mycobacterium avium* by activated murine macrophages: The role of nitric oxide

Non-Tuberculosis Mycobacteria (NTM), specifically *Mycobacterium 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 267-4. One million RAW cells were infected with *M avium* at MOI 10:1 in the presence and absence of 100 U/ML of IFNγ. In addition, Greiss Assay and colony-forming units (CFU) respectively monitored NO levels and mycobacteria growth at 24 and 48 hours.

Our results showed that at 4 hours of infection (day 0), (2.05×10^5 CFU) was counted, whereas (4.32×10^5 CFU) was measured at 48 hours (day 2), meaning that *M. avium* was not eliminated inside un-stimulated macrophages, but rather persisted and grew. At the later time point, the levels of NO in infected macrophages were in the range of 32.3 μM when compared to 1.83 μM observed in uninfected macrophages. Furthermore, *M. avium* growth was shown to be substantially inhibited when macrophages were treated with IFNγ (1.01×10^5 CFU). The levels of NO increased up to 56.3 μM. We did not detect arginase activity in the infected cultures nor in uninfected macrophages.

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. These data also illustrate that NO can promote or inhibit mycobacterial growth and that there is a delicate equilibrium that underlies its production. 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. The effect of NO and mycobacterial growth in other strains of the *M. avium* complex and its effect, needs to be further evaluated.