[PS2.37]

Nitric oxide inhibits *mglA* expression and phagosomal escape of intracellular *Francisella tularensis*

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Francisella tularensis is a highly virulent intracellular bacterium capable of rapid multiplication in phagocytic cells. Previous studies have revealed that activation of macrophages infected with *F. tularensis* leads to control of infection, and reactive nitrogen and oxygen species are important for the bacterial killing.

We investigated the effects of adding: 1) SNAP, which generates nitric oxide or 2) SIN-1, a donor of peroxynitrite, to J774 murine macrophage-like cells infected with the *F. tularensis* live vaccine strain. Irrespective of treatment, no killing was observed during a 6 h period. However, addition of SNAP significantly increased colocalization between LAMP-1 and bacteria at 2h, indicating a phagosomal containment of *F. tularensis*.

At the same time, a specific inhibitory effect on bacterial transcription was observed since the level of a constitutively expressed gene, *tul4*, was inhibited 5-fold whereas the gene encoding the global regulator MglA was inhibited 100-fold.

F. tularensis-infected J774 cells are incapable to secrete TNF-α in response to *E. coli* LPS. Addition of SNAP almost completely reversed the *F. tularensis*-mediated suppression of TNF-α secretion. Similarly, infection with an MglA mutant did not inhibit the LPS-induced TNF-α secretion.

In summary, we demonstrate that addition of SNAP- while not affecting the viability of intracellular *F. tularensis* - led to a phagosomal containment of the bacteria. We suggest that this containment may be caused by the concomitant inhibition of *mglA* expression. The results further indicate that the *F. tularensis*-mediated suppression of TNF-α secretion may require a cytosolic localization of the bacterium.

Keywords: Francisella, nitric oxide, MglA, phagosome