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Macrophage screen identifies *Francisella* genes required for intracellular replication

A. Llewellyn¹, B. Napier¹, D. Weiss¹

¹Emory University, Emory Vaccine Center, Atlanta, United States

Aims: To determine which genes, previously identified as being required for *Francisella* pathogenesis *in vivo*, contribute to virulence by directly facilitating replication in macrophages.

Background: *Francisella tularensis* is a highly infectious bacterial pathogen that causes tularemia, a potentially life-threatening disease in humans. Due to the ease of aerosol dissemination of this organism and the minimal inoculum (≤ 10 bacteria) necessary to cause severe disease, *F. tularensis* has been weaponized for use in biowarfare. Unfortunately, we do not fully understand the genetic requirements for *F. tularensis* pathogenesis within the mammalian host. We recently employed a powerful global *in vivo* negative selection screen (TraSH) in mice to identify genes required for the pathogenesis of *F. novicida*, a subspecies of *Francisella* that is >95% identical to *F. tularensis* at the DNA level, encodes many of the same virulence genes, and causes a tularemia-like disease in mice but not humans. This approach resulted in the identification of 164 genes that are required for virulence *in vivo*, 44 of which encode novel hypothetical genes of unknown function. Since the ability to replicate within macrophages, thought to be the primary niche for replication *in vivo*, is a critical virulence attribute of *Francisella*, we set out to determine which of the 164 genes required for virulence *in vivo* are required for replication within macrophages or contribute to virulence through other processes.

Methods: We performed a bacterial replication screen in RAW264.7 macrophages, making use of a panel of transposon insertion mutants.

Results: Sixty-five genes were identified as being critical for bacterial replication in macrophages, including 20 of the 44 novel hypothetical genes. Many of the genes in the *Francisella* pathogenicity island were identified, validating the screen. Testing of a deletion mutant lacking one of the hypothetical genes demonstrated its role in replication in bone marrow-derived macrophages and in virulence in mice. Interestingly, 99 of the 164 genes were not required for intracellular replication in macrophages.

Conclusions: These results identify a panel of novel genes, which will be the basis for future study, as being critical for replication of *Francisella* in macrophages. The results also highlight the requirement in pathogenesis of a large number of genes that likely contribute to replication in other cell types or other processes during *in vivo* infection.