

S 2-3

A genetic screen identifies novel *Francisella tularensis* ssp. *novicida* genes involved in modulating the macrophage innate immune responseJ. Jones¹, D. Monack¹¹Stanford University, Microbiology and Immunology, Stanford, United States

Within the host, *Francisella* is engulfed by host macrophages where it escapes phagosomal degradation and replicates to high numbers in the cytosol. The host response to *Francisella* involves coordination of both vacuolar and cytosolic sensing of the pathogen. Sensing in the vacuole is dependent on TLR2 and the adapter MyD88, which leads to production of TNF- α , and pro IL-1 β . Cytosolic bacteria are recognized by a cytosolic surveillance pathway, which leads to the production of type-I interferons (IFN), including IFN- β . These type-I IFNs act in an autocrine and paracrine fashion to initiate a signaling cascade resulting in inflammasome activation and the release of mature pro-inflammatory cytokines (IL-1 β and IL-18) and macrophage cell death. We conducted a genome-wide forward genetic screen to identify *Francisella* genes involved in modulating the host innate immune response in macrophages. We identified 72 gene insertions that result in lower induction of the innate immune response. These include known virulence factors such as genes located in the *Francisella* Pathogenicity Island (FPI), which contains homologs of type VI secretion systems. These mutants showed decreased intracellular replication, and induced lower levels of IFN- β and host cell death relative to a wild-type strain. Additionally, 188 gene insertions were identified that resulted in increased induction of pro-inflammatory responses. These include genes involved in lipid A modification and LPS and O-antigen synthesis. Targeted deletions of FTN_1212, wbtA, and lpcC resulted in strains that induced a hyper inflammatory response indicated by increased kinetics of TNF- α , IFN- β , and IL-1 β release as well as host cell death relative to a wild-type strain. This phenotype was not due to increased intracellular replication. Additionally, this hyper induction of pro-inflammatory cytokines was still dependent on the FPI genes, as LPS-FPI double mutants failed to induce the cytosolic response. Furthermore, TLR2 and Myd88/Trif signaling contribute to the increased inflammatory response to LPS mutants. Taken together these data suggest that genes in the FPI are critical for intracellular replication and induction of macrophage innate immune responses, and that *Francisella* uses lipid A modification to mask ligands that can be recognized by TLR2 as well as other intracellular pattern recognition receptors.