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Derivation of a panel of potential correlates of vaccine-induced protection against *Francisella tularensis* LVSR. De Pascalis¹, A. Y. Chou¹, K. L. Elkins¹¹Food and Drug Administration, Bethesda, United States

Aims: Ideally, protection against *Francisella tularensis* would be provided by the development of a vaccine that could be safely used on a large population to prevent tularemia. Our goal is the identification of a panel of immunologic and biologic correlates that are essential for predicting protection provided by immunization with any live attenuated *Francisella* strain. Such a panel might further be applied to *in vitro* screening of potential vaccines prior to more extensive animal studies. Ultimately, we wish to predict the outcome of vaccination in humans.

Methods: We have coupled an *in vitro* co-culture assay with genomic methodologies to identify biomarkers that correlate with vaccine efficacy. Using the *in vitro* assay, we compared LVS-infected bone marrow macrophages co-cultured with lymphocytes from naive control mice to those from LVS-vaccinated mice. The assessment of control of intramacrophage LVS growth provided correlations with vaccine efficacy, while analysis of purified mRNA from both recovered splenocytes and macrophages by real-time PCR provided information about genes involved in these immune interactions.

Results: Initially, we analyzed protein and gene expression of LVS-infected bone marrow macrophages to differentiate their contributions in controlling *in vitro* bacteria growth from the effects of splenocytes of vaccinated mice that were added to the culture. Macrophage expression of IL-6, IL-12 and TNF- α was partially independent of vaccine efficacy. In contrast, IFN- γ was produced entirely by splenocytes from LVS-vaccinated mice. Subsequently, we extended the analyses to a large panel of genes of immunologic interest. Comparisons of either total splenocytes or splenic T cells from LVS-vaccinated mice to those from non-vaccinated mice indicated that IFN- γ was up-regulated, as would be expected. However, other genes such as CCR3, TNF- α , IL-12R β 2, IL-9, IL-6, Gf1, T-box 21 and CXCR3 were reproducibly up-regulated as well. The most notable down-regulated genes were Spp1, IL-18R1, Tnfrsf8 and Tyk2.

Conclusions: Our model appears to be a valid tool for identifying genes that predict protection against *Francisella tularensis*. Ongoing studies are extending these data, comparing responses to LVS with good, fair, and poor lots and derivatives of LVS, as well as qualitatively different new *Francisella* vaccines.