**Background:** Coxiella burnetii (Cbx), a highly infectious and resilient intracellular bacterial pathogen, is the cause of Q fever, which can require months of antibiotic treatment. The current Cbx vaccine is approved in Australia alone and reactogenicity can occur in persons previously exposed to the bacterium. The Q-VaxCelerate consortium was assembled to develop an effective and less reactogenic Q-fever vaccine. Project objectives include multiparameter immune cell profiling to define a signature of immune protection in animal models of infection and in humans exposed to Cbx.

**Methods:** To characterize the immune response to Cbx infection or vaccination in both human and mouse samples we developed an approach utilizing Cytometry by Time Of Flight mass spectrometry (CyTOF) to measure >35 immune-parameters simultaneously. Donor samples from Oss, The Netherlands, which experienced an Q-fever outbreak from 2007-2011, were grouped by clinical history, anti-Cbx antibody titers, and IFNγ response to killed Cbx in vitro. PBMCs were incubated with killed Cbx, and analyzed by flow cytometry and mass cytometry (CyTOF). To assess the immune response to Cbx in mice, blood samples were collected prior to and after vaccination and following challenge. CyTOF analysis was conducted and bacterial load and spleen pathology assessed at sacrifice.

**Results:** In vitro stimulation with killed Cbx showed greater T-cell responses in seropositive donors. An ongoing study will characterize the immune responses from additional donors to profile across exposure status, clinical history, and HLA type. Initial analysis of murine samples indicates that vaccination results in an innate inflammatory response, a Th1-biased CD4 response and anti-Cbx antibody. Following challenge, unvaccinated mice exhibit enhanced inflammatory responses in both innate and T-cell populations as compared to vaccinated mice. Robust adaptive responses to challenge in vaccinated mice are evident in the increased expression of T-bet in B-cells and decreased bacterial burden and splenomegaly.

**Conclusions:** Analysis of murine data has provided insights into the immune response to vaccination and challenge. Continued analysis using semi-supervised algorithms will provide a more detailed description of the immune response to Cbx. Analysis from ongoing human studies will shed light on the various clinical outcomes of Q-fever. Insights gained from these studies will inform the design and assessment of candidate vaccines for Cbx. Together these data reveal novel hallmarks of immune responses to Cbx during and following infection and vaccination, with the potential to identify host-based biomarkers of protection against Q fever.

**Impact to the Mission and Warfighter:** Q fever has been of concern to the US Department of Defense because of high...
seroconversion rates detected among military personnel serving in Iraq. While the QVAX vaccine (CSL) provides protection from Cb infection, it cannot be given without pre-testing of vaccinees for prior exposure. There is thus clear need to develop a less reactogenic and potentially more efficacious vaccine for occupational and biodefense purposes. Data from these studies will inform the definition of immune signatures of Cb infection to facilitate testing and evaluation of candidate vaccines for Cb, and may provide insights on the acute and chronic clinical sequelae of Cb infection.

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