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Transcriptome analysis of human immune responses following live vaccine strain (LVS) *Francisella tularensis* vaccination

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Abstract

The live vaccine strain (LVS) of *Francisella tularensis* is the only vaccine against tularemia available for humans, yet its mechanism of protection remains unclear. We probed human immunological responses to LVS vaccination with transcriptome analysis using PBMC samples from volunteers at time points pre- and post-vaccination. Gene modulation was highly uniform across all time points, implying commonality of vaccine responses. Principal components analysis revealed three highly distinct principal groupings: pre-vaccination (−144 h), early (+18 and +48 h), and late post-vaccination (+192 and +336 h). The most significant changes in gene expression occurred at early post-vaccination time points (≤48 h), specifically in the induction of pro-inflammatory and innate immunity-related genes. Evidence supporting modulation of innate effector function, specifically antigen processing and presentation by dendritic cells, was especially apparent. Our data indicate that the LVS strain of *F. tularensis* invokes a strong early response upon vaccination. This pattern of gene regulation may provide insightful information regarding both vaccine efficacy and immunopathogenesis that may provide insight into infection with virulent strains of *F. tularensis*. Additionally, we obtained valuable information that should prove useful in evaluation of vaccine lots as well as efficacy testing of new anti-*F. tularensis* vaccines.

Keywords: Human; Gene regulation; Vaccination; Bacterial; Molecular biology

1. Introduction

Francisella tularensis, the causative agent of tularemia, is a gram-negative, facultative, intracellular bacterium, first isolated in 1911 in association with a plague-like disease among squirrels in Tulare County, California (McCoy and Chapin, 1912; McLendon et al., 2006). *F. tularensis* is a CDC Category A threat organism due to its high infectivity rate after exposure to low numbers of organisms (10–50 bacteria), the ease of dispersal, and its potential to cause high morbidity and mortality rates among aerosol-exposed individuals (Isherwood et al., 2005; McLendon et al., 2006; Oyston and Quarry, 2005). Human tularemia presents in ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, and septic forms, and is spread through blood-feeding arthropod bites or exposure to infected vermin, soil, or water (Dennis et al., 2001). Most forms of tularemia cause mild acute symptoms of an undifferentiated febrile illness and are treatable by broad-spectrum antibiotics (Dennis et al., 2001). Primary pneumonic tularemia is rarely seen in naturally occurring cases; however, the intentional deployment of weaponized or genetically modified/antibiotic-resistant strains of *F. tularensis* presents a public health hazard estimated to result in high incapacitating casualty and mortality rates (Dennis et al., 2001). The economic impact of such an event is believed to be upwards of $6.4 billion for every 100,000 persons exposed (Kaufmann et al., 1997; Dennis et al., 2001).

The only tularemia vaccine available in the United States is an investigational new drug (IND). It is a live attenuated vaccine, comprised of the live vaccine strain (LVS) of *F. tularensis*

Abbreviations: LVS, Live vaccine strain; PCA, Principal components analysis

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(Eigelsbach and Downs, 1961). The vaccine is administered by scarification of the volar surface of the forearm and creates a persistent papular/pustular lesion at the inoculation site similar to infection with virulent *F. tularensis* infection (Burke, 1977). The vaccine, in use for 50 years, has substantially lowered the number of laboratory-acquired incidents of tularemia (Oyston and Quarry, 2005; Isherwood et al., 2005; Burke, 1977).

Relatively little is understood regarding protection against tularemia in humans. Much of the data regarding *F. tularensis* immunopathogenesis, as well as the mechanism of protection afforded by the vaccine, comes from murine models. Humoral immunity was previously believed to be important as passive protection against LVS challenge was demonstrated in mice given immune serum from LVS-vaccinated humans (Drabick et al., 1994). However, vaccine-induced humoral responses to LVS may play no role in protection against human pathogenic *F. tularensis* strains (Tarnvik, 1989). Instead, as with most intracellular pathogens, cell-mediated responses are thought to be critical in long-lasting protective immunity (Waag et al., 1992, 1996; Tarnvik et al., 1985; Surcel et al., 1991; Sjostedt et al., 1992). Protection of mice from lethal challenge with LVS develops as early as 2–3 days after intradermal vaccination (Elkins et al., 1992). Similarly, intranasal infection of mice with lethal doses of LVS results in rapid NK cell recruitment and activation in the lungs (Lopez et al., 2004). Recent studies in our laboratory suggest that early innate responses (<48 h) occur in humans after LVS vaccination and may correlate with protective immunity (Fuller et al., 2006). Rapid development of immunity after vaccination in mice also suggests that protective mechanisms are partly attributable to strong initial innate immune responses. The cellular components of these early immune responses, such as NK cells, neutrophils, and DCs, have only recently become the focus of attention in murine and human studies of LVS vaccination (Telepnev et al., 2005; Sjostedt et al., 1994; Malik et al., 2006; Isherwood et al., 2005; Fuller et al., 2006; Elkins et al., 2003; Cole et al., 2006; Barker et al., 2006).

While a FDA-approved vaccine for *F. tularensis* may be on the horizon, the methods used to determine the mechanism of protective immunity have not been re-visited in decades. Here, we examined PBMC samples from vaccinees at varying time points in an effort to delineate early immune responses upon which long lasting protection is built. Using modern transcriptome analysis following vaccination, we gained a better understanding of the immunology of LVS vaccination and perhaps the responses necessary for long-lasting immunity. These data will aid in evaluating host response to virulent *F. tularensis* and because LVS is a uniquely live vaccine, the data offer a seldom-observed study of human response to bacterial infection.

2. Materials and methods

2.1. Collection and preparation of PBMCs

Volunteers were recruited from U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) personnel at risk of laboratory exposure to *F. tularensis*. A human use clinical protocol to collect peripheral blood samples was approved by institutional review boards at USAMRIID (Human Use Committee FY04-16). Donors provided informed consent and met eligibility criteria. Six healthy adults (three males and two females, 22–54 years of age) received a primary LVS vaccination (*F. tularensis* vaccine, NDBR 101, Lot 4) and donated peripheral blood, 6 days prior to vaccination, 18 h after vaccination, 48 h after vaccination, 8 days after vaccination, and 14 days after vaccination. Inoculum consisted of a single 0.6-ml drop of LVS vaccine (2×10^9 colony-forming units (cfu) per ml) delivered intradermally by bifurcated needle. All vaccinees had positive "takes" (initial formation of small papule which then ulcerates). Vaccinations were deemed clinically successful by microagglutination assays (titers ranged from 1:20 to 1:1280) at day +28(Massey and Mangiafico, 1974). Blood was collected into heparinized syringes under sterile conditions at the medical clinic and transferred to a biosafety level (BSL)-2 laboratory for processing. PBMCs were purified from whole blood by Ficoll-Paque Plus (GE Healthcare, Piscataway, NJ, USA) density gradient centrifugation.

2.2. RNA isolation

The cell pellet was resuspended in 1 ml TRIzol (Invitrogen, Carlsbad, CA, USA) solution and pipetted multiple times to dissolve all clumps and break up the cellular DNA. An additional 2 ml of TRIzol was added to the cell lysate and mixed well. One milliliter of TRIzol-lysed solutions were then was dispensed into 1.5 ml Eppendorf centrifuge tubes and snap frozen in a bath of 95% ethanol and dry ice, then transferred to −70 °C until RNA was purified. Total cellular RNA was isolated according to the manufacturer's specifications followed by purification with an affinity resin column (Qiagen, Inc., Valencia, CA, USA). The quality and concentration of the RNA were determined by measuring the absorbance at 260 and 280 nm. RNA integrity was confirmed by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). All plastic materials used in the lysing procedure were certified RNAse- and DNAse-free and all samples were handled with latex glove-covered hands in a BSL-2 laminar flow cabinet to minimize deposition of environmental RNAse onto or into sample tubes.

2.3. Transcriptome/microarray analysis

RNA samples used in this study were checked for evidence of degradation and integrity and had a minimum A260/A280 ratio of > 1.9 and a minimum 28S/18S ratio of > 1.6 (2100-Bioanalyzer, Agilent Technologies, Palo Alto, CA, USA). The Human Genome U133+2 array, which consists of 54,654 probe sets representing approximately 33,000 human genes, was used (Affymetrix, Inc., Santa Clara, CA, USA). GeneChip analysis was performed with Microarray Analysis Suite (MAS) 5.0, Data Mining Tool (DMT) 2.0, and Microarray Database software (Affymetrix, Inc., Santa Clara, CA, USA). All of the probe sets represented on the GeneChip arrays were globally normalized and scaled to a signal intensity of 100.