Transcriptome Sequencing Data Sets of Human Lung Epithelial Cells in the Course of Francisella tularensis Infection

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ABSTRACT Francisella tularensis is a highly infectious intracellular bacterium representing the causative agent of tularemia, a severe disease which requires prompt antibacterial intervention for mitigating its potential high mortality. Inhaled bacteria interact with lung cells belonging to various subpopulations, including those of the epithelium. As of today, the host epithelial response to inhalational infection with F. tularensis is poorly understood. Here, we announce host transcriptome data sets which systematically address the human epithelial response to F. tularensis at different time points postinfection.

The facultative intracellular Gram-negative zoonotic pathogen Francisella tularensis is the causative agent of tularemia, a severe bacterial infectious disease of humans that is endemic in some parts of North America and Eurasia (1, 2). Due to its unusually high infectivity and the respiratory character of tularemia, F. tularensis has been classified as a category A select agent potentially associated with bioterrorism use (3–7). Previous in vivo studies have identified the epithelium cells as an important population affected in the course of F. tularensis infection (8, 9). However, little is known about the survival and replication of F. tularensis in these cells and about the creative strategies they use to maneuver their host cells. Here, we present transcriptome sequencing (RNA-seq) data of A549 human lung epithelial cells during F. tularensis infection.

Francisella tularensis subsp. holarctica strain LVS (ATCC 29684) expressing green fluorescent protein (GFP) was used for the infection of A549 (ATCC CCL-185) cells. Bacterial cultures were grown at 37°C to mid-log phase in TSBC (tryptic soy broth [Difco] supplemented with 0.1% cysteine [BD, France]). The bacteria were washed with phosphate-buffered saline (PBS) and resuspended in Dulbecco modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum. A549 cells were seeded 20 h prior to infection in 6-well microtiter plates at a density of 10⁶ cells per well. The cells were infected, in biological duplicates, with the overnight LVS culture to produce a multiplicity of infection of 20,000, centrifuged at 600 × g for 5 minutes, and incubated at 37°C for 0.5, 1, 3, 6, 12, and 24 hours. Following incubation, the 30-minute and 1-hour time point cultures were washed three times with PBS and subjected to RNA extraction using the RNeasy kit (Qiagen), while residual DNA was digested using RNase-free DNase (Qiagen). For subsequent time points, 1 hour postinfection, the medium was replaced with DMEM supplemented with 50 μg/ml gentamicin to kill extracellular bacteria. Thirty minutes later, cells were washed three times with PBS and left with DMEM supplemented with 10% FBS. At the indicated time points (3, 6, 12, and 24 hours postinfection), total RNA was similarly extracted.

RNA-seq was performed at the JP Sulzberger Columbia Genome Center (New York, NY). Libraries were generated using the Illumina TruSeq stranded mRNA kit according to the manufacturer’s instructions. Polyadenylated RNA enrichment was performed. Sequencing of the 100-bp paired-end reads was performed on the Illumina NovaSeq 6000 system. Pseudalignment to a kallisto index created from transcriptomes (human
GRCh38) was performed using kallisto (0.44.0). For RNA-seq quality control, we used fastQC v0.11.5, checked for per-base sequence quality, per-sequence quality scores, and adapter content. We verified that each sample reached at least 75% of the target read goal, and we checked for adequate sequence alignment percentages. An analysis of differentially expressed genes under various conditions was performed using the R package DESeq2 v1.18.1, with default parameters.

Sequencing yielded 19.6 million to 27 million reads per sample (Table 1), resulting in the identification of 21,066 transcripts. Differential expression along infection was demonstrated for 9,375 genes with more than 150 uniquely aligned reads of RNA.

This study represents the first detailed transcriptome analysis of human epithelial response to Francisella tularensis infection and enables the identification of specific host factors involved in the pathophysiology of tularemia.

Data availability. The transcriptomic data have been deposited to the NCBI database, and their SRA and GEO accession numbers are provided in Table 1.

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**TABLE 1** Summary of transcriptome samples submitted<sup>a</sup>

| Sample description<sup>b</sup> | No. of reads | % mapped reads<sup>c</sup> | SRA accession no. | GEO accession no. |
|-------------------------------|--------------|----------------------------|-------------------|-------------------|
| A549 mock_rep1                | 20,678,853   | 88.2                       | SAMN15775158      | GSM4718274        |
| A549 0.5 hpi_rep1             | 25,554,304   | 87.1                       | SAMN15775157      | GSM4718275        |
| A549 1 hpi_rep1               | 27,287,012   | 89.7                       | SAMN15775156      | GSM4718276        |
| A549 3 hpi_rep1               | 21,438,963   | 88                         | SAMN15775115      | GSM4718277        |
| A549 6 hpi_rep1               | 19,664,127   | 87.6                       | SAMN15775151      | GSM4718278        |
| A549 12 hpi_rep1              | 20,477,312   | 88.5                       | SAMN15775150      | GSM4718279        |
| A549 24 hpi_rep1              | 22,983,964   | 88                         | SAMN15775149      | GSM4718280        |
| A549 mock_rep2                | 22,330,500   | 89.2                       | SAMN15775144      | GSM4718283        |
| A549 0.5 hpi_rep2             | 20,860,054   | 87.4                       | SAMN15775145      | GSM4718282        |
| A549 1 hpi_rep2               | 21,68,131    | 88.5                       | SAMN15775143      | GSM4718284        |
| A549 3 hpi_rep2               | 20,751,390   | 86.3                       | SAMN15775142      | GSM4718285        |
| A549 6 hpi_rep2               | 25,684,370   | 85.6                       | SAMN15775141      | GSM4718286        |
| A549 12 hpi_rep2              | 23,009,615   | 87.5                       | SAMN15775140      | GSM4718287        |
| A549 24 hpi_rep2              | 25,669,488   | 87.4                       | SAMN15775140      | GSM4718287        |

<sup>a</sup>The GEO accession number of the transcriptome series is GSE155970, and the SRA BioProject number is PRJNA656244. The GEO title of the project is “Transcriptome sequencing data sets for human epithelial cells response during Francisella tularensis infection.”

<sup>b</sup>Biologically duplicated samples are denoted as rep1 or rep2.

<sup>c</sup>Percentage of reads mapped to the reference genome (human GRCh38).