

Differences in virulence between subspecies of *Francisella tularensis* are reflected in different gene expression profiles of human neutrophils

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Aims: Neutrophils are the first line of cellular defense against pathogenic microorganisms, playing a crucial role in innate immunity. It is known that LVS is able to evade killing by human neutrophils. By in vitro infection of human granulocytes with *Francisella (F.) tularensis (t.)* strains of different virulence we aimed to identify cellular process within the granulocytes which are interfered by infection and to identify those interferences exclusively mediated by virulent strains of *F. t. tularensis* and *F. t. holarctica*.

Methods: Human neutrophils were isolated from heparinized venous blood of three healthy individuals using density gradient centrifugation. Five different *F. tularensis* strains (a highly virulent *F. t. tularensis* an attenuated *F. t. tularensis*, a virulent *F. t. holarctica*, an attenuated *F. t. holarctica* vaccine strain (LVS) and a *F. t. novicida*) were grown to exponential phase and used to infect the isolated neutrophils. RNA isolated 30 and 120 min post infection was employed for gene expression profiling using Human Genome U133 Plus 2.0 arrays (Affymetrix) according to standard protocols. The microarray data was validated by quantification of several rRNAs from these experiments by qPCR. Cytokine release was measured in culture supernatants using Luminex-based bead array technology.

Results: In general, the host-pathogen interaction resulted in modulation of transcriptional machinery, dysregulation of neutrophil bioenergetics, host immune responses and cell death processes. Differences in PMN gene expression induced by distinct strains could also be observed. The most striking difference was the lack of increased levels of IL-6, IL-8 and TNF α mRNA in neutrophils infected with the virulent strains of *F. t. tularensis* and *F. t. holarctica* respectively, compared to the increased levels in the lesser or avirulent *F. tularensis* strains (0 versus 1.7 to 6.45 fold change). Cytokine levels in cell culture supernatants support the micro array results.

Conclusions: We could find differences in the gene expression of human neutrophils infected with different *F. tularensis* strains of varying virulence. The lack of increased levels of IL-6, IL-8 and TNF α suggests that virulent *F. t. tularensis* and *F. t. holarctica* interfere with the inflammatory response of neutrophils, thereby possibly dysregulating processes as chemoattraction of neutrophils, respiratory response and apoptosis.