Combatting Antibiotic-Resistant Bacteria: Immune Cell Dynamics Revealed by Single-Cell Profiling

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Abstract

Introduction: Bacterial pathogens are a significant cause of bloodstream infections, posing a persistent and deadly threat to both civilian and military populations. In military settings, the risk of infection is heightened due to trauma-related injuries, which increase susceptibility to opportunistic pathogens. Advances in transcriptional profiling at the single-cell level have provided a deeper understanding of cellular diversity in complex biological systems. To better understand the interplay between bacterial virulence strategies and host defense mechanisms that influence infection outcomes, single-cell analysis was employed to investigate immune responses to multiple bacterial pathogens.

Materials and Methods: A survivable 24h bacteremia murine model was employed to examine alterations in splenocyte transcriptomics due to infection. Mice were infected with *Staphylococcus aureus (Sa)*, *Klebsiella pneumoniae (Kp)*, or *Pseudomonas aeruginosa (Pa)* via tail vein injection. Animals were humanely euthanized at 24h post infection and splenocytes were collected. The splenocytes were revived, and single-cell analysis was performed using the 10X Genomics workflow, followed by sequencing. Data underwent rigorous quality control and subsequent analysis to ensure robust results.

Results: Gene expression analysis revealed significant transcriptional changes in immune cell populations following exposure to *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Differentially expressed genes (DEGs) were identified across all cell subtypes, with highest enrichment in macrophages followed monocytes, neutrophils and dendritic cells. The immune response varied significantly depending on the bacterial pathogen. With respect to number of differentially expressed genes, *Pseudomonas* aeruginosa infection showed robust response in T, B and dendritic cells. Neutrophils were strongly affected in all three bacterial infections.

Conclusions: This study maps immune cell responses in the spleen of mice infected with three bacterial pathogens, revealing key genes and pathways driving host-pathogen interactions. By defining the immune architecture at the single-cell level, it identifies critical pathways and therapeutic targets, paving the way for novel countermeasures against bacterial infections threatening military personnel.

Methods

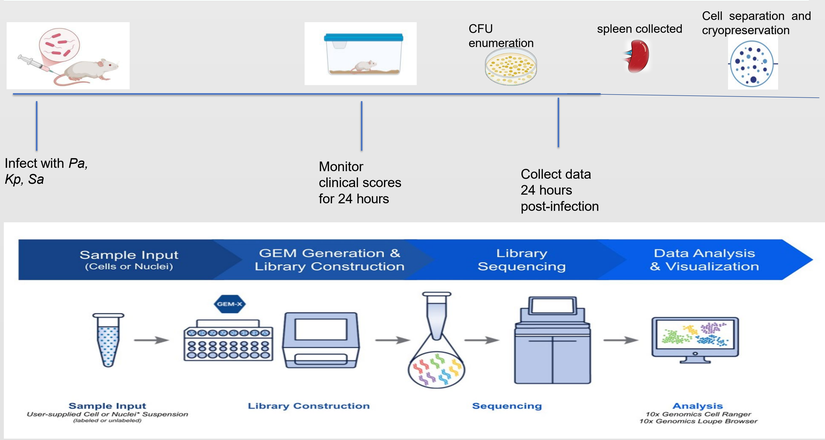


Figure 1- Outline for sample collection and processing. *Top panel* – Experiment using rodents for spleen collection. *Bottom panel*- Streamlined workflow for single cell RNAseq. Start with a single cell suspension of cells. Samples can be run with the standard workflow After gel bead-in-emulsion (GEM) generation, libraries can be constructed generating multiple readouts that can be linked back to the same single cell. Process data with cell ranger and visualize sample heterogeneity and continue with analysis and visualization. The image is obtained from https://www.10xgenomics.com

Cell Type Distribution via Single Cell Sequencing (sc RNA Seq) of Splenocytes

- Distribution of immune cell types (macrophages, neutrophils, monocytes, dendritic cells, T cells, B cells) is shown.
- Infection alters the proportions of various immune cell types, highlighting immune system remodeling in response to bacterial challenge.

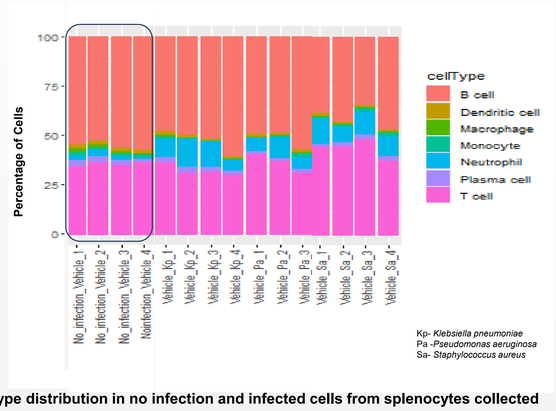


Figure 2- Bar chart representing cell type distribution in no infection and infected cells from splenocytes collected from each of the sample.

Staphylococcus infection affects gene expression in different cell types in splenocytes

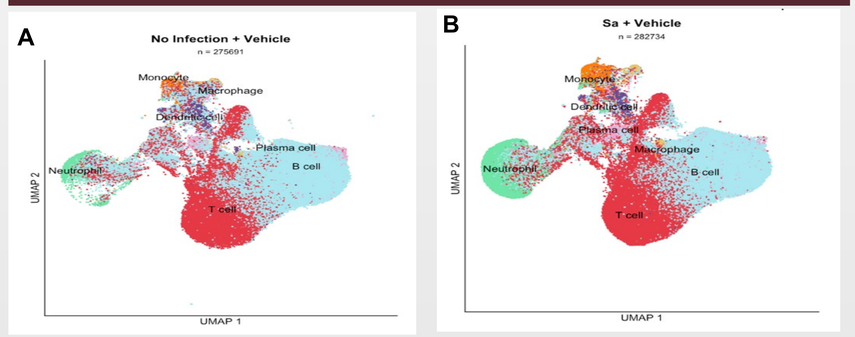


Figure 3- UMAP (Uniform Manifold Approximation and Projection) dimension reduction method that projects high-dimensional gene expression data A) without infection and B) with infection with Sa

Bacterial infections lead to specific gene expression of immune cells in splenocytes

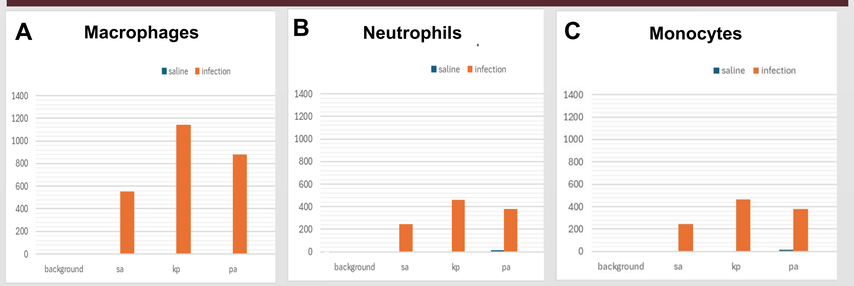
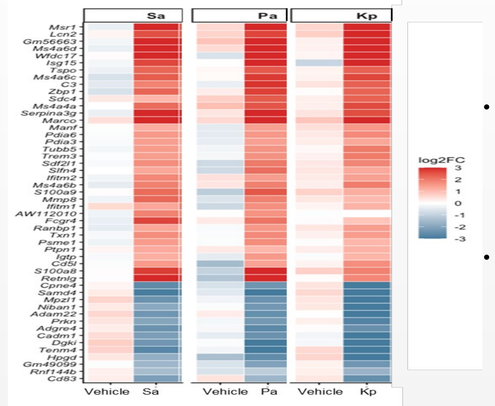


Figure 4- Differentially expressed genes False Discovery Rate (FDR) corrected p value of <0.05 for A) macrophages B) Neutrophils c) Monocytes. Each cell type exhibits a unique set of gene expression changes post-infection, with macrophages showing the highest enrichment.

Heatmap with Gene expression changes in Macrophages



- Heatmap Identifies a core set of genes involved in the macrophage response to diverse bacterial pathogens.
- Based on gene expression profile, we can corelate the findings from clinical outcomes.

Figure 5- Top 50 common differentially gene expression changes across different bacterial infections in macrophages

Specific pathways modulated by each of bacterial pathogens

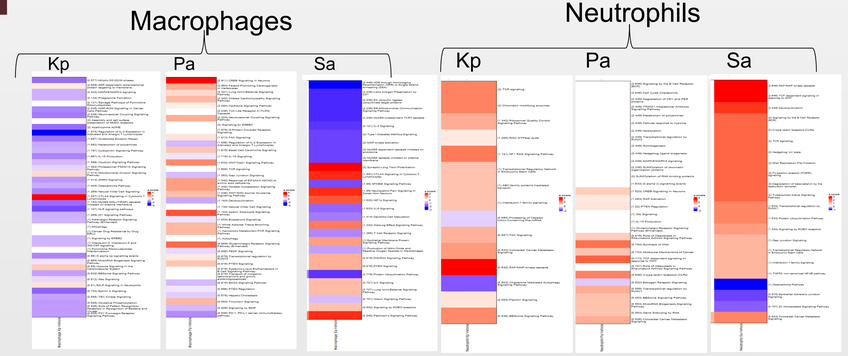


Figure 6- Pathway Analysis conducted by Ingenuity pathway analysis for identifying bacterial infection specific signals in macrophages and neutrophils specific differentially expressed genes.

Conclusions

- **Single-cell RNA sequencing** of splenocytes from mice infected with three major bacterial pathogens (Sa, Kp, Pa) reveals profound and cell type-specific immune responses.
- **Macrophages** show the most significant transcriptional changes, followed by monocytes, neutrophils, and dendritic cells.
- **Neutrophil**s are strongly affected in all infections.
- **Pathway analysis** identifies both common and pathogen-specific immune signaling pathways, providing insight into the molecular mechanisms of host defense
- **Mapping the immune landscape** at single-cell resolution uncovers critical genes and pathways that could serve as therapeutic targets.

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