Single cell RNA sequencing reveals dynamic functions of myeloid cells during *Prevotella*-induced airway clearance of *Streptococcus pneumoniae*

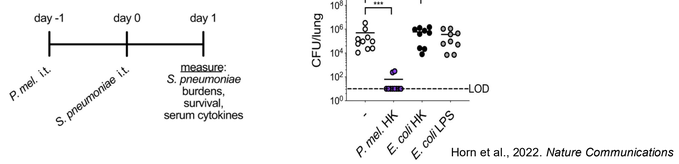
Sara Stoner, PhD[1]; Sam Fulte[1]; Steven Shaw, MS[1]; Eric Larson, PhD[2]; Sarah Clark, PhD[1]

[1]University of Colorado School of Medicine, Dept of Otolaryngology, Aurora, CO.

[2]University of Pennsylvania School of Dental Medicine, Dept of Basic and Translational Sciences, Philadelphia, PA.

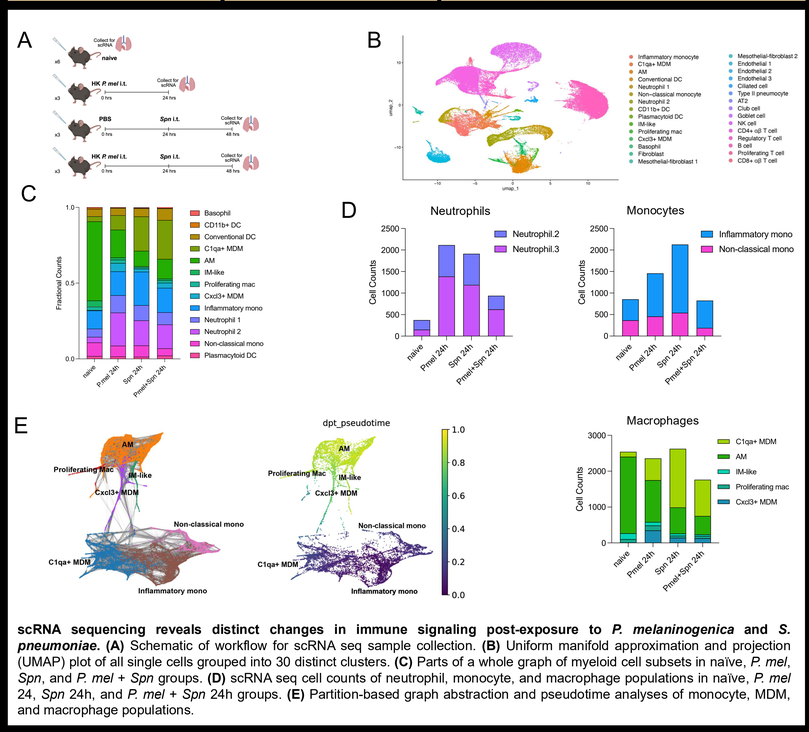
Introduction

- *Streptococcus pneumoniae* asymptomatically colonizes approximately 10-30% of healthy adults and 20-40% of children and is a leading cause of community-acquired pneumonia.
- The airway microbiota composition has been identified as a risk factor for pneumonia.
- *Prevotella* airway abundance is associated with reduced *S. pneumoniae* in the lungs.
- Our lab has previously demonstrated that *Prevotella* primes neutrophils to clear *S. pneumoniae* from the airway, however, **the mechanism by which *Prevotella* exposure alters neutrophil behavior, as well as the contribution of other immune cells to enhanced clearance, remains unknown.**



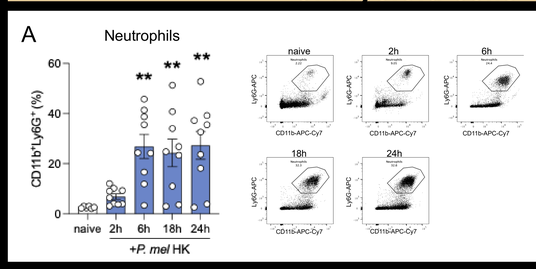
Horn et al., 2022. *Nature Communications*

Prevotella alters the myeloid cell response to pneumococcal pneumonia

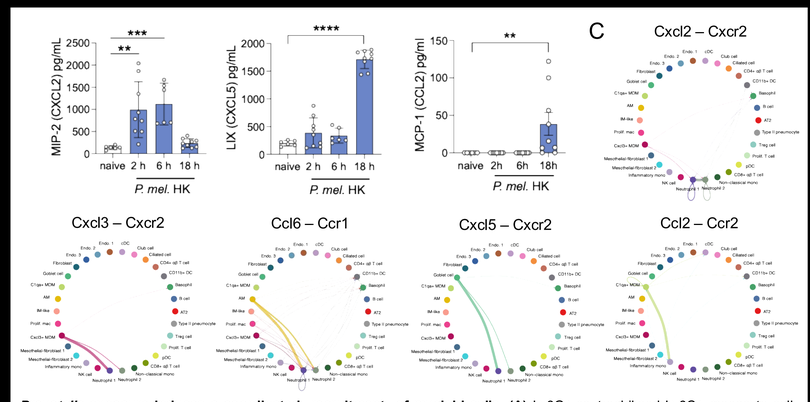


scRNA sequencing reveals distinct changes in immune signaling post-exposure to *P. melaninogenica* and *S. pneumoniae*. (A) Schematic of workflow for scRNA seq sample collection. **(B)** Uniform manifold approximation and projection (UMAP) plot of all single cells grouped into 30 distinct clusters. **(C)** Parts of a whole graph of myeloid cell subsets in naïve, *P. mel*, *Spn*, and *P. mel* + *Spn* groups. **(D)** scRNA seq cell counts of neutrophil, monocyte, and macrophage populations in naïve, *P. mel* 24, *Spn* 24h, and *P. mel* + *Spn* 24h groups. **(E)** Partition-based graph abstraction and pseudotime analyses of monocyte, MDM, and macrophage populations.

Prevotella exposure induces a coordinated recruitment of myeloid cells

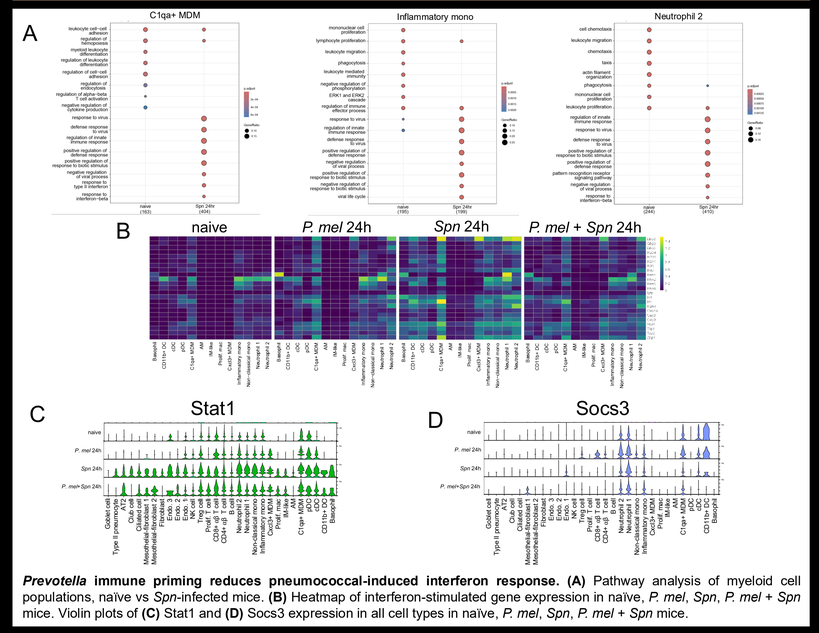


Prevotella exposure induces a coordinated recruitment of myeloid cells (continued)



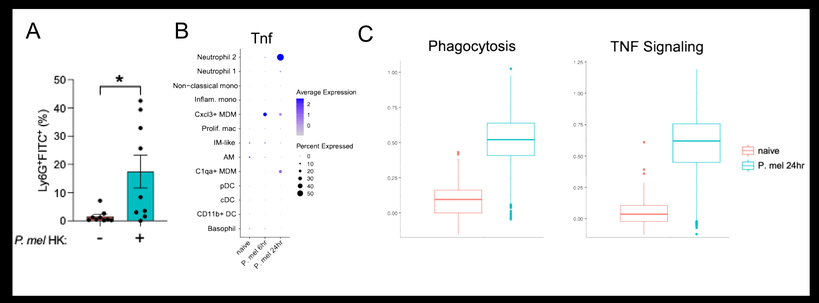
***Prevotella* exposure induces a coordinated recruitment of myeloid cells. (A)** Ly6G+ neutrophil and Ly6C+ monocyte cell counts in lung tissue were quantified 0, 2, 6, 18, and 24 hours after intratracheal exposure to *P. mel* HK. **(B)** CXCL2, CXCL5, and CCL2 production measured via ELISA at 0, 2, 6, and 18 hours post-exposure to HK *P. mel*. **(C)** CellChat analysis of CXCL2, CXCL3, CCL6, CXCL5, and CCL2 signaling (naïve, *P. mel* 24h, *Spn* 24h, *P. mel* + *Spn* 24h conditions combined). ******p<.01, *******p<.001, ********p<.0001

Prevotella immune priming reduces pneumococcal-induced interferon response

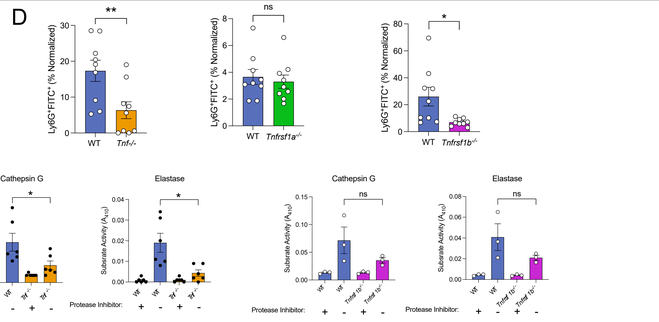


***Prevotella* immune priming reduces pneumococcal-induced interferon response. (A)** Pathway analysis of myeloid cell populations, naïve vs *Spn*-infected mice. **(B)** Heatmap of interferon-stimulated gene expression in naïve, *P. mel*, *Spn*, *P. mel* + *Spn* mice. Violin plots of **(C)** Stat1 and **(D)** Socs3 expression in all cell types in naïve, *P. mel*, *Spn*, *P. mel* + *Spn* mice.

TNFα signaling drives enhanced neutrophil-mediated clearance of *S. pneumoniae* after immune priming by *Prevotella*

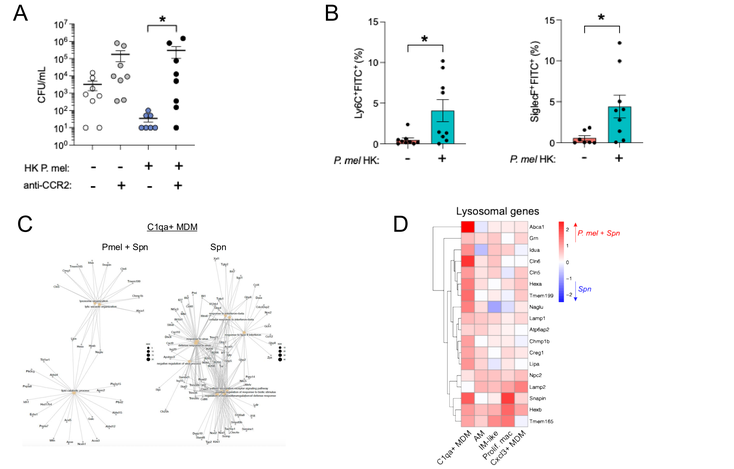


TNFα signaling drives enhanced neutrophil-mediated clearance of *S. pneumoniae* after immune priming by *Prevotella* (continued)



TNFα signaling drives enhanced neutrophil-mediated clearance of *S. pneumoniae* after immune priming by *Prevotella*. (A) FITC-labeled HK Spn was administered intratracheally to mice with or without 24 hours exposure to HK P. mel. After 3 hours, flow cytometry was used to quantify FITC+ neutrophils. **(B)** Dot plot of Tnf expression in myeloid cells of naïve, P. mel 6h, and P. mel 24h mice. **(C)** Box plot of Neutrophil 2 Phagocytosis and TNF Signaling module scores for naïve vs P. mel 24h mice. **(D)** Neutrophils were isolated from WT, *tnf*$^{-/-}$, *tnfr1*$^{-/-}$, and *tnfr2*$^{-/-}$ mice after 24 hours of exposure to HK *P. mel* and incubated with pre-opsonized heat-killed FITC-labeled *Spn* for 30 minutes at 37°C. Flow cytometry was then used to quantify FITC^{+} neutrophils. Unpaired t-test. **(E)** Serine protease activity for cathepsin G and elastase + /– protease inhibitor cocktail detected by substrate cleavage for purified WT and *Tnf*$^{-/-}$ lung neutrophils. Student's t-test. *p<.05, ***p<.001

Monocytes and macrophages play a role in enhanced clearance of *S. pneumoniae* after *Prevotella* exposure



***Immune priming by Prevotella alters macrophage activation state.* (A)** Lung tissue *Spn* burden 24 hpi in mice with or without HK P. mel exposure, with or without anti-CCR2 antibody treatment for CCR2+ monocyte depletion. **(B)** FITC-labeled HK *Spn* was administered intratracheally to mice with or without 24 hours exposure to HK *P. mel*. After 3 hours, flow cytometry was used to quantify FITC+ monocytes and alveolar macrophages. **(C)** GO Network analysis of enriched pathways in C1qa+ MDM population, *Spn* vs *P. mel* + *Spn* mice. **(D)** Log fold change heatmap of lysosomal genes in macrophage populations.

Conclusions

- *Prevotella* exposure alters myeloid cell composition and recruitment during pneumococcal pneumonia.
- Interferon-stimulated gene expression is lower in various lung cell types in mice exposed to *Prevotella*.
- TNFα signaling is required for enhanced phagocytosis of *S. pneumoniae* and serine protease production by neutrophils.
- *Prevotella* exposure enhances phagocytosis of *S. pneumoniae* by monocytes and macrophages, contributing to enhanced clearance of infection.

Acknowledgments

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