AN *IN VIVO* ATLAS OF HOST-PATHOGEN TRANSCRIPTOMES DURING *STREPTOCOCCUS PNEUMONIAE* COLONIZATION AND DISEASE

Adonis D'Mello[1], Ashleigh N. Riegler[2], Eriel Martínez[2], Sarah M. Beno[2], Tiffany Ricketts[2], Ellen F. Foxman[3], Carlos J. Orihuela* & Hervé Tettelin[1]*
*These authors contributed equally

[1]Department of Microbiology and Immunology, Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, United States of America
[2]Department of Microbiology, The University of Alabama at Birmingham, Birmingham, AL, United States of America.
[3]Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT 06520, USA.

Introduction: We detail host-pathogen interaction gene expression profiles of *Streptococcus pneumoniae* (*Spn)* and its infected mouse model in the blood, heart, kidneys, lungs and nasopharynx using dual RNA-Seq.

Methods:

- To capture the host and bacterial transcriptomes during infection, mice were challenged with each of 3 *Spn* strains (TIGR4, D39, and 6A-10) and tissue/organs collected from the nasopharynx, lungs, blood, and kidneys (the latter lacking 6A-10 samples). Heart and *in vitro* data (TIGR4 only) were included from our previously published study [1].
- RNA was isolated, sequenced and mapped to each respective genome. *Spn* analyses were performed on core genes without paralogs shared among the 3 *Spn* strains. Differential gene expression was performed with the nasopharyngeal samples as the baseline for *Spn,* and respective uninfected tissues for the host.

Results:

- Pneumococcal burden inferred from the proportion of *Spn* to mouse reads varied in a strain and site-specific manner.

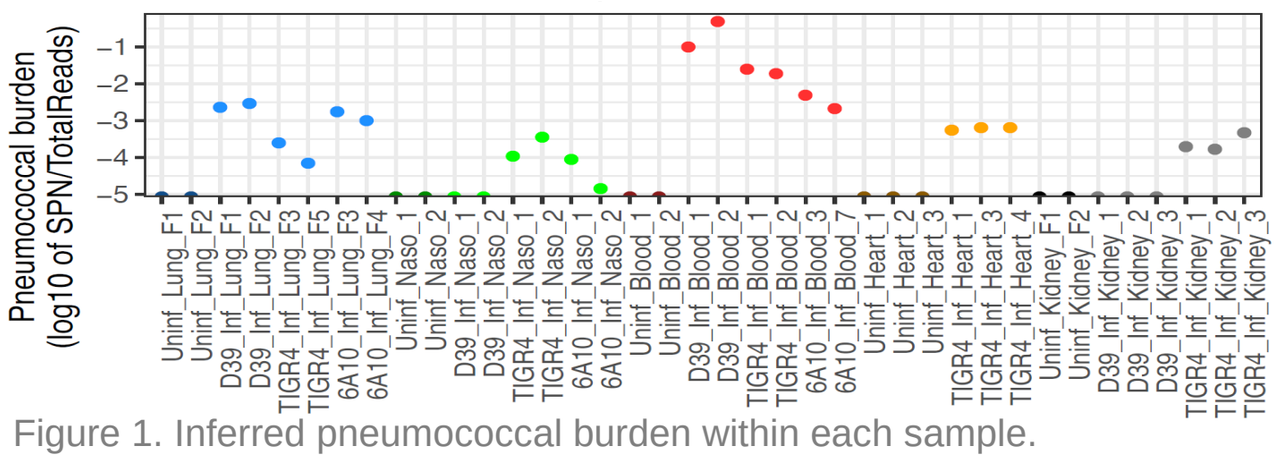

Figure 1. Inferred pneumococcal burden within each sample.

- The *in vivo Spn* and host transcriptomes demonstrated differences in gene expression profiles between colonization and infection, and are strikingly different from *in vitro* data.

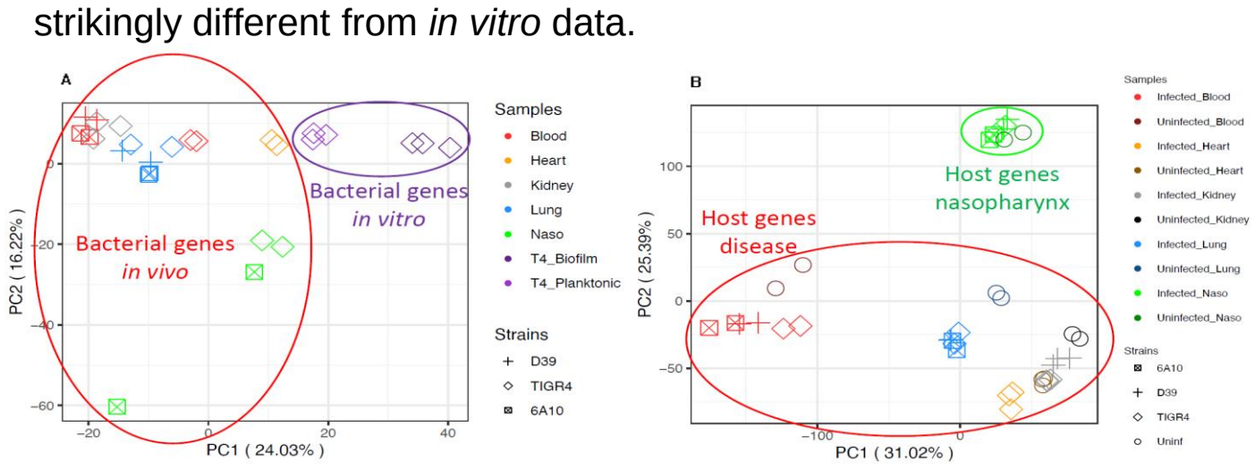

Figure 2. A. Principal Component Analysis (PCA) of infected samples with the addition of *in vitro* grown planktonic and biofilm TIGR4 samples from our previous study [1]. B. *In vivo* PCA of host gene expression profiles across samples colonized or infected with *Spn*, and uninfected.

- *Spn* and its host differentially regulated unique gene subsets in a site-specific manner.

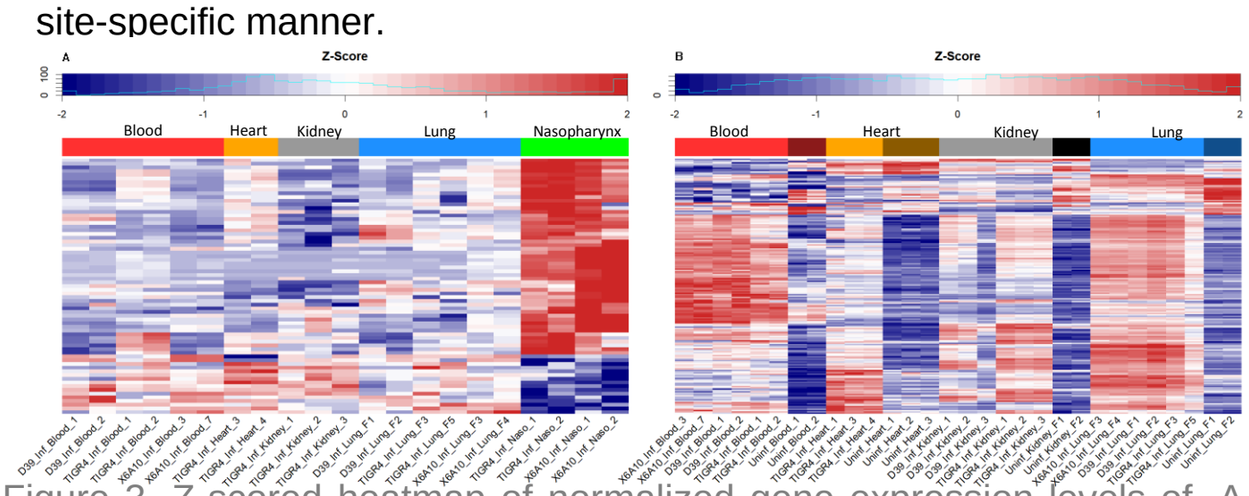

Figure 3. Z-scored heatmap of normalized gene expression levels of, A. 69 *Spn* differentially expressed (DE) genes shared across all infected organ vs. nasopharynx comparisons and, B. 190 host DE genes shared across all infected vs. uninfected organ comparisons except the nasopharynx, *i.e.* shared across disease anatomical sites.

Acknowledgments: Supported with funds from: i) Merck, Sharpe & Dohme, Corp. Merck Investigator Studies Program award IISP ID#: 57329 – Pneumovax entitled: "Dual RNA-seq for characterization of the *Streptococcus pneumoniae* and host transcriptomes during bacteremia/invasive disease", and ii) NIH/NIAID R01AI114800 award entitled: "Cardiac microlesion formation during invasive pneumococcal disease." We acknowledge essential support provided by members of the Institute for Genome Sciences' Genomics Resource Center, Genome Informatics Core, and High-Performance Computing Core.

- Site specific highly, or differentially expressed (or both) *Spn* genes were selected for generation of *Spn* TIGR4 knockouts. Deletions of these genes lowered *Spn* burden at the corresponding anatomical site.
- Cross infection experiments in which genes highly expressed in nasopharynx − Δ*ula* and ΔSP_1675 − were tested in the model of lower respiratory tract infection with bacteremia. Genes highly expressed in disease anatomical sites − Δ*pepO* and Δ*zmpB* − were tested with the nasopharynx model of colonization.

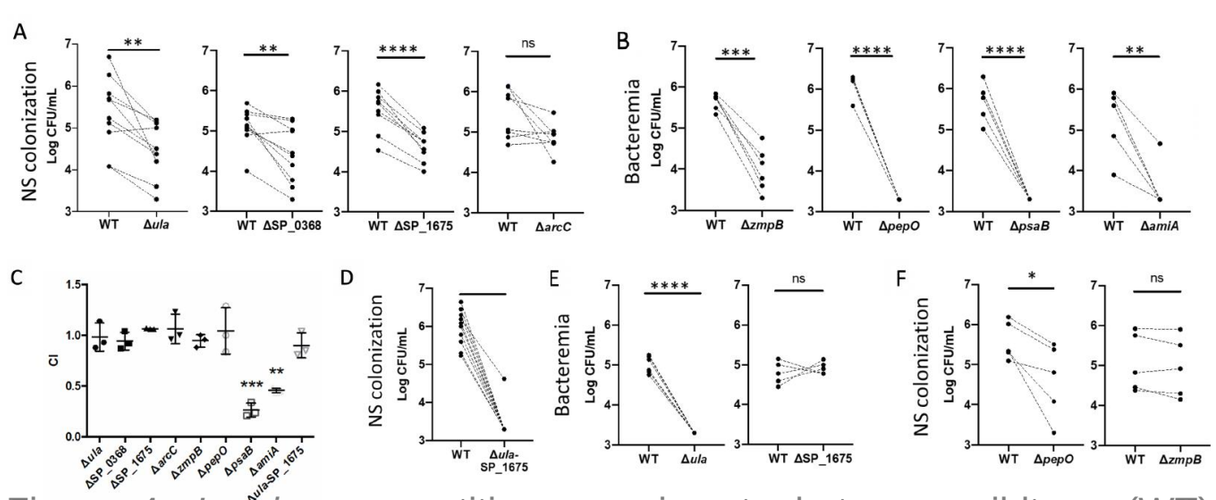

Figure 4. *In vivo* competition experiments between wildtype (WT) TIGR4 and isogenic mutants (Dots with a connecting dashed line = paired samples). A. Bacterial titers in nasal lavage fluid of colonized mice 5 days post-inoculation. B. Bacterial titers in the blood of mice 1 day after intratracheal inoculation. C. Competitive index values of mutants tested in panels A and B tested for overall fitness. D. A double mutant was tested versus TIGR4 WT in the nasopharynx model of colonization. E,F. Cross infection experiments.

- Host gene signatures revealed induction of the interferon pathway during invasive disease. Induction of the interferon response in mice using poly(I:C) was protective of *Spn* infection.

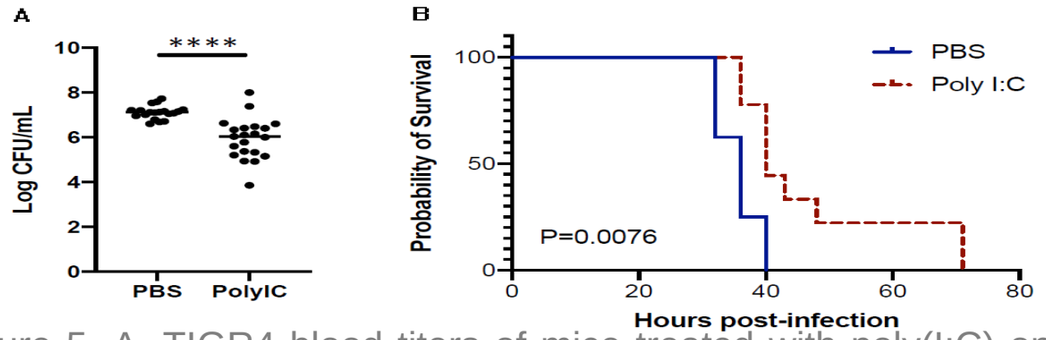

Figure 5. A. TIGR4 blood titers of mice treated with poly(I:C) one day prior to intraperitoneal TIGR4 challenge versus control 24h post-infection. B. Kaplan-Meier survival plot of mice after poly(I:C) treatment and challenge.

Conclusion:

- Different *Spn* strains were more adept at growth at certain anatomical sites.
- *In vitro Spn* gene expression profiles were not representative of *in vivo Spn.*
- *Spn* nasopharyngeal colonization had a different gene expression profile relative to *Spn* diseased tissues/organs.
- Host response to nasopharyngeal colonization was minimal and starkly distinct from *Spn* diseased tissues/organs.
- *Spn* upregulated specific genes/operons within the nasopharynx related to nutrient uptake, such as the *ula* operon.
- *Spn* upregulated different genes/operons in diseased tissues related to virulence such as *zmpB*.
- 52 *Spn* genes were determined to be highly expressed at all sites including the nasopharynx. These consisted of 2 known potential vaccine candidates *zmpB* and *pspA*.
- Deletion *Spn* mutants generated based on highly/differentially expressed genes validated their importance for *in vivo Spn* survival, as well as importance of some genes only for colonization or disease.
- Host response to nasopharyngeal colonization revealed significantly fewer DE genes than in diseased tissues, perhaps due to tolerance of *Spn* colonization.
- All host diseased tissues upregulated 190 genes enriched for genes involved in the host interferon pathway.
- Stimulation of the host interferon pathway prior to *Spn* challenge was shown to be protective, validating it's role in *Spn* infection.

1. A. T. Shenoy *et al.*, Streptococcus pneumoniae in the heart subvert the host response through biofilm-mediated resident macrophage killing. *PLoS Pathog* **13**, e1006582 (2017).