PALADINS: PROTECTIVE APPROACHES LEVERAGING AD-APTIVE AND IN-NATE SYSTEMS

Identification Of New Host-interactomes During Cellular Anthrax

Michael Norris University of Florida Emerging Pathogens Institute **Andrew P. Bluhm** Department of Geography and Emerging Pathogens Institute, University of Florida **Abderrahmane Tagmount** Department of Physiological Sciences, University of Florida **Jason K. Blackburn** Department of Geography and Emerging Pathogens Institute, University of Florida **Christopher Vulpe** Department of Physiological Sciences, University of Florida

Background: At the cellular level, anthrax is caused by introduction of spores that undergo bacteremic outgrowth with concomitant secretion of toxin components that disturb cAMP levels and dysregulate kinase signaling in cells leading to cytotoxicity. During human anthrax, this is amplified systemically to the host level causing death. Cellular targets of edema and lethal toxins have been known for some time, however the totality of host-genes that contribute to cytotoxicity during infection by B. anthracis is unknown.

Purpose: Develop new therapeutics targeting host-genes required for anthrax pathogenesis.

Objective: Identify the complete cellular host-gene networks contributing to cell death by B. anthracis.
Rationale: By understanding the host-genes and gene interactions enabling anthrax pathogenesis we can target gene networks with high-likelihood gene therapeutics.

Relationship to other areas of study: Molecular medicine, CRISPR technology, host-targeted therapeutics

Methods: A whole genome CRISPR knockout library was created in RAW264.7 macrophages. Three replicate libraries were challenged with B. anthracis spores in a modified aminoglycoside spore internalization assay. Surviving macrophages were grown for 7 days and re-challenged. Cells at 7-, 10-, 14-, and 17-days post-infection were harvested, genomic DNA prepared, and sgRNAs sequenced. Sequence data was processed with MAGeCK and STRING analysis to identify the genes and genetic networks promoting cellular anthrax. Top targets were knocked out in macrophages to validate their roles.
Preliminary results: Our data indicate several components that regulate the mTOR pathway, and not mTOR itself, play direct roles in sensitivity to B. anthracis infection. Lysosomal ATPases and the ‘Ragulator’ complex (composed of Lamtor 1-4 genes) are significantly involved. The ‘Ragulator’ is involved in transducing signals that activate mTORC. Gene networks present in two or more timepoints with an FDR

Preliminary conclusions: CRISPR screening identified new genes and gene networks involved in cellular anthrax. There exists, within our data set, opportunities to further characterize the interactome of identified genes. Dual sgRNA libraries can be created to validate the inferred whole genome interactions in our network analysis. AAV-CRISPR therapeutics or PPMOs can be generated to knockdown genes as host-targeted pan-pathogen therapies. For example, metaxin-2 is involved in TNFa induced cell death and was identified in CRISPR screens from B. anthracis and B. thailandensis models and could ameliorate anthrax and melioidosis infections.

Impact to the DTRA mission and warfighter: New, transitory host-targeted therapeutics can be developed from targets identified in whole genome screens.