RESEARCH ABSTRACT

SWINE HEALTH

Title: Utilization of Neutrophil Extracellular Trap DNA by *Mycoplasma hyopneumoniae*, **NPB 15-147**

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Scientific Abstract:

Mycoplasma hyopneumoniae infection of swine is a major disease problem for producers world-wide. Vaccines have had some success in reducing disease, but colonization of swine herds continues unabated with the potential for severe disease in naïve animals and exacerbation of disease associated other pathogens. Few antibiotics are effective against this organism because of structural and molecular differences from other bacteria. New intervention targets could be associated with the process of macromolecular precursor acquisition, which is necessary for growth. One such target is the membrane DNase that is thought to participate in the acquisition of DNA precursors. Our hypothesis is that neutrophils are recruited to areas of infection in the lung, where they release extracellular chromatin called NETs (neutrophil extracellular traps) that serve to entrap bacteria while additional host immune responses can be mounted against the pathogen. According to our hypothesis, these NETs would be degraded by mycoplasma surface nucleases and the released purines and pyrimidines taken up through membrane transporters for use in DNA replication. By demonstrating the flow of nucleotides from NETs to the mycoplasma, we can begin to consider these nucleases as viable intervention targets either through pharmacological approaches or vaccination.

The overall approach was to take THP-1 cells labeled with a modified nucleic acid base, induce extracellular traps (METs), add mycoplasmas and then monitor the destruction of the METs with concomitant uptake of the labeled nucleotide and its incorporation into the mycoplasma DNA. Our initial attempt was to incorporate Cy3-labeled nucleotides onto DNA and monitor its uptake into mycoplasmas by fluorescence microscopy. We could produce Cy3-labeled PCR products and demonstrate their degradation by *M. hyopneumoniae* whole cells. We could also show uptake of Cy3-labeled nucleotides into mycoplasma DNA. We also optimized the conditions needed for induction of the THP-1 extracellular traps. However, we were unable to label THP-1 DNA with Cy3-labeled nucleotides despite repeated attempts. Thus, we modified our approach by substituting BrdU for Cy3-labeled nucleotides. BrdU is a smaller modification and can be monitor by antibody binding. Detection of BrdU requires fixation of cells, permeabilization to allow for antibody penetration, DNase I treatment to expose the incorporated BrdU to the antibody, and then fluorescence microscopy. Despite success in incorporating BrdU into THP-1 DNA, we failed to visualize the BrdU in the METs due to the need for DNase treatment to expose the label, which degraded the METs prior to adding *Mhyo*. Finally, we tested the thymidine analogue 5-ethynyl-2'-deoxyuridine (EdU). EdU can be detected by treating labeled DNA with a fluorescent azide through a Cu(I)-catalyzed cycloaddition reaction also referenced as "click" chemistry. We first demonstrated *Mhyo* can incorporate EdU into its DNA by incubating *Mhyo* with EdU, reacting the cells with an Alexa555-labeled azide, and detecting fluorescence via confocal microscopy. In addition, we validated *Mhyo*'s membrane nuclease function and its ability to degrade extracellular DNA. Next, the THP-1 cells were incubated with EdU and induced with phorbol 12-myristate 13-acetate (PMA) to produce EdU-labeled METs. *Mhyo* was then incubated with the induced THP-1 cells for a period of time to determine whether the bacterium was able to degrade the METs and integrate EdU into its own genomic DNA. Visualizing the resulting *Mhyo* via fluorescent confocal microscopy exhibited Alexa555 fluorescence indicating that EdU was incorporated into *Mhyo* nucleic acids. These findings showed *Mhyo* could procure free nucleotide bases from NET-like structures in this monocytic cell line by nuclease action and incorporate them into its genome, supporting our hypothesis that *Mhyo* can utilize extracellular traps as a source of nucleic acid precursors.