Internship Proposal

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Project Title:
Copines: Novel players in the repair of plasma membrane damage triggered by pneumococcal toxins

Level:

Master Student

Project Summary:

CONTEXT: Plasma membrane (PM) is a selectively permeable structure that separates inside and outside cellular environments. PM disruption causes cell death and tissue inflammation. Several human pathogens produce pore-forming toxins (PFTs) that form PM pores, disrupting cell homeostasis and promoting bacterial dissemination. Streptococcus pneumoniae is the most common cause of deadly pneumonia. Its major virulence factor, the PFT pneumolysin (PLY), triggers overwhelming immune response and extensive tissue damage. At low PLY concentrations, cells recover from PLY-induced PM damage through understudied repair mechanisms.

AIM: We recently proposed that Copines are required for the repair of PLY pores. This project aims to identify Copine partners and validate their function in PM repair during PLY intoxication or pneumococcus infection.

Work to be developed by the student:

This project will include the following tasks and methodologies:

1)Establish molecular biology tools for the identification of Copines partners in PM repair (BioID-Copines construct, lung cell line that stably expresses the construct)

2)Intoxicate (PLY) or infect (pneumococcus) lung epithelial cells expressing BioID-Copines (cell culture, infection at BSL2 level)

3)Identify Copine partners (by complementary label-free quantitative mass spectrometry (MS)-based proteomics: IP-MS and BioID).

4)Validate best hits for their role in PM repair in lung cell monolayers and 3D lung models of

PLY intoxication or pneumococcal infection (immunoprecipitation, flow cytometry, microscopy).

This approach is expected to reveal new players involved in PM repair, acting in concert with Copines.

References:

1.Alves S, Pereira JM, Mayer RL, Goncalves ADA, Impens F, Cabanes D, Sousa S: Cells Responding to Closely Related Cholesterol-Dependent Cytolysins Release Extracellular Vesicles with a Common Proteomic Content Including Membrane Repair Proteins. Toxins 2022, 15(1):1-22.

2.Pereira JM, Xu S, Leong JM, and Sousa, S. "The Yin and Yang of Pneumolysin during Pneumococcal Infection". Frontiers Immunol. (2022)13: 878244. DOI: 10.3389/fimmu.2022.878244

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