Internship Proposal

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Project Title:
Recycle2Repair: Role of recycling endosome in repairing plasma membrane damage induced by pneumococcal infection

Level:

Master Student

Project Summary:

CONTEXT: The plasma membrane (PM) is a selectively permeable barrier that separates the internal and external cellular environments. Its disruption can lead to cell death and tissue inflammation. Many human pathogens secret pore-forming toxins that create pores in the host cell PM, thus disturbing cell homeostasis and aiding pathogen spread. Streptococcus pneumoniae, the leading cause of pneumonia, relies on its pore-forming toxin pneumolysin (PLY) to trigger an overwhelming immune response and severe lung tissue damage. At low PLY concentrations, likely found at early steps of infection, cells can recover from PM damage by activating PM repair mechanisms, which remain poorly understood.
AIM: We have preliminary data showing that the master regulator of the recycling endosome, Rab11a, is recruited to the cell PM upon damage induced by PLY. This project aims to explore the role of Rab11a in PM repair during PLY intoxication and pneumococcus infection.
PUBLIC HEALTH IMPACT: Data generated will contribute to our understanding of host survival responses to S. pneumoniae infection and provide the molecular basis for the development of therapies targeting PM repair. Given the urgent need for better treatments to overcome the widespread antibiotic resistance in S. pneumoniae strains, this work is critical for public health.

Work to be developed by the student:

This project will include the following tasks and methodologies:
1)Intoxicate (PLY) or infect (S. pneumoniae) lung epithelial cells depleted of Rab11a (cell culture, infection at BSL2 level);

2)Assess PM damage by permeability to propidium iodide or Draq7 assays (flow cytometry or high-content microscopy);

3)Assess recovery of PM integrity by permeability assays after microbial washout or elimination by specific antimicrobial drugs;

4)Evaluate the assembly of repair events such as the accumulation of Gp96 and MyosinIIA at PM damage sites (live cell imaging, immunofluorescence, colocalization assays), PM blebbing and vesicular shedding (electron microscopy, nanoparticle tracking);

5)Interaction of Rab11 with Gp96 or MyosinIIA (immunoprecipitation);

This approach is expected to reveal how the Rab11a-regulated recycling endosome contributes to PM repair during PLY-driven S. pneumoniae infection.

References:

1.Mesquita FS, Brito C, Mazon Moya MJ, Pinheiro JC, Mostowy S, Cabanes D, Sousa S. “Endoplasmic reticulum chaperone Gp96 controls actomyosin dynamics and protects against pore-forming toxins.” EMBO Rep. (2017) 18:303-318. DOI: 10.15252/embr.201642833.

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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