Project X: Two-Pore channels as Host regulators of Bacterial Infection and Inflammation

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Background and preliminary data:

Bacterial infections remain a major clinical challenge, and in the face of rising antibiotic resistance, alternative therapeutic strategies that modulate host immune pathways are of increasing importance. Inflammation is a fundamental component of the host's defense against invading pathogens. Not only do the type of pathogen and its virulence factors determine the severity of infection, but also host signaling pathways can impact the course of disease. Calcium release through two-pore channels (TPCs) by nicotinic acid adenine dinucleotide phosphate (NAADP) regulates the trafficking and function of endo-lysosomal compartments [1]. TPCs are thus involved in vesicle fusion and maturation, processes that determine both pathogen clearance and immune cell activation. Pathogens have evolved strategies to exploit these signaling pathways. In *Salmonella enterica*, NAADP–TPC2–mediated calcium is critical for the maturation of the Salmonella-containing vacuole (SCV), thereby sustaining bacterial survival in macrophages [2]. Similar pathways have been implicated in *Shigella flexneri* and *Citrobacter rodentium* infections. *Staphylococcus aureus*, a major Gram-positive pathogen, also engages NAADP–TPC signaling but via a distinct mechanism. *S. aureus* can enter host cells through unconventional endocytic routes and secrete pore-forming toxins that trigger localized calcium release [3]. In these studies, pharmacological blockade of the NAADP–TPC pathway reduced bacterial entry and intracellular persistence; however, these results have not been validated *in vivo*.

At the molecular level, NAADP–TPC is critical for formation of Ca^{2+} microdomains at endo-lysosomal membranes. These microdomains couple localized Ca^{2+} release to the activation of calcineurin, dynamin, and Transcription Factor EB (TFEB). These spatial signals enable immune cells and epithelia to execute rapid vesicle trafficking decisions, directly impacting pathogen survival and host defense.

Preliminary data on T cells indicate the functional relevance of TPCs in the T-cell-mediated tolerance. Blockade of NAADP signaling impairs TCR-dependent cell activation and proliferation, and promotes the transdifferentiation of effector cells into regulatory cells, resulting in reduced T cell-mediated immune damage[4]. Moreover, deletion or pharmacological blockade of TPCs promotes Treg development and is protective in murine models of inflammatory bowel disease[5]. These findings highlight a potential immunomodulatory function of TPCs in adaptive immunity. However, expression analyses reveal that TPCs are more strongly expressed in innate immune cells such as macrophages, dendritic cells, and epithelial cells. This suggests that TPC signaling may play a more fundamental role in innate immune control of bacterial infections.

Hypothesis:

Two-pore channels (TPCs) regulate NAADP-dependent endosomal Ca^{2+} signaling that controls vesicle trafficking in host cells. Intestinal pathogens (*Salmonella*, *Citrobacter/Shigella*) and systemic pathogens (*Staphylococcus aureus*) exploit this pathway to promote entry and intracellular survival. Disruption of TPC function will therefore impair bacterial pathogenesis and modulate host immune responses.

Aims and Work Programme:

1. Analysis of the role of two pore channels in bacterial infections
2. *In vivo* dissection of two-pore channels in specific subsets of immune and epithelial cells

Aim 1 – Analysis of the role of two pore channels in bacterial infections

We will begin by using global knockout mice lacking TPC1, TPC2, or both channels, and then infect them with *Salmonella enterica*, *Citrobacter rodentium*, and *Staphylococcus aureus*. These experiments will provide the first direct evidence of whether loss of TPC signaling alters the course of infection in

vivo. To assess immune responses, we will use established antibody panels to examine different cell types, including epithelial cells, T and B lymphocytes, macrophages, and dendritic cells. To distinguish whether the critical contribution of TPCs derives from hematopoietic cells or from non-immune tissue compartments, we will generate bone marrow chimeras. Finally, we will take an unbiased approach by performing single-cell sequencing on total cells isolated from intestines and livers of wildtype and knockout mice, both with and without infection.

Aim 2 – *In vivo* dissection of TPC function in infection and inflammation

Building on the in vivo studies, we will proceed with conditional knockout models. For both two-pore channels, conditional knockout mouse lines are available. Using Villin-Cre, we will target intestinal epithelial cells. To study the innate immune system, we will generate conditional knockouts in macrophages and neutrophils (LysM-Cre), dendritic cells (CD11c-Cre), and in lymphocytes (CD4-Cre or CD19-Cre). These studies will elucidate the role of TPCs in bacterial survival and dissemination, as well as in shaping host responses, thereby linking in vitro findings directly to infection outcomes in vivo.

In parallel, we will combine genetic approaches with pharmacological modulation using established TPC agonists and antagonists. These experiments will clarify how TPC-dependent trafficking supports bacterial entry and persistence in both epithelial and innate immune cells. Ultimately, this project will define the role of two-pore channel–mediated calcium signaling in the immune response against bacterial pathogens and pave the way toward new therapeutic strategies aimed at modulating immune function in the context of infection.

Project-related publications: (max. 5)

[1] Y. Sakurai *et al.*, “Two-pore channels control Ebola virus host cell entry and are drug targets for disease treatment,” *Science (1979)*, vol. 347, no. 6225, pp. 995–998, 2015, doi: 10.1126/science.1258758.

[2] M. Najibi, H. H. Honwad, J. A. Moreau, S. M. Becker, and J. E. Irazoqui, “A novel NOX/PHOX-CD38-NAADP-TFEB axis important for macrophage activation during bacterial phagocytosis,” *Autophagy*, vol. 18, no. 1, p. 124, 2021, doi: 10.1080/15548627.2021.1911548.

[3] M. Rühling *et al.*, “A Novel Rapid Host Cell Entry Pathway Determines Intracellular Fate of Staphylococcus aureus,” *Elife*, vol. 13, Jul. 2025, doi: 10.7554/ELIFE.102810.2.

[4] M. Nawrocki *et al.*, “Trans-Ned 19-Mediated Antagonism of Nicotinic Acid Adenine Nucleotide-Mediated Calcium Signaling Regulates Th17 Cell Plasticity in Mice,” *Cells*, vol. 10, no. 11, Nov. 2021, doi: 10.3390/CELLS10113039.

[5] T. He *et al.*, “ Inhibition of two-pore channels in antigen-presenting cells promotes the expansion of TNFR2-expressing CD4 + Foxp3 + regulatory T cells ,” *Sci Adv*, vol. 6, no. 40, p. eaba6584, Sep. 2020, doi: 10.1126/sciadv.aba6584.