Understanding multi-scale innate immune defences against bacterial pathogens at the single-cell level

Mammals have innate defence systems that resist infection by a range of bacteria. However, the activation of immune responses is inherently a heterogenous process, resulting in different and probabilistic outcomes of the host-pathogen interactions at the single cell level. This poses a fundamental systems biology and immunology question; how do robust immune responses emerge from this heterogeneous, apparently random, single cell behaviour?

In this proposal, we will study direct interactions between innate immune macrophages and the important food-borne bacteria *Listeria monocytogenes* that causes significant levels of human morbidity and mortality. Our data demonstrate that interactions between *L. monocytogenes* and macrophages, a key event controlling the overall infection critically rely on a small fraction of 'successful' pathogens, which can effectively replicate and spread within the infected host cell population. We hypothesise that while *L. monocytogenes* aims to subvert the antibacterial signalling responses in the host, the overall protective immune responses emerge from multi-scale coordination of heterogenous single cell responses and noisy paracrine signalling trough quorum-like sensing and temperature adaptation.

OBJECTIVES: The main aim of the project is to quantitatively understand mechanisms involved in the muti-scale coordination of innate immune responses against bacterial pathogens at the single cell level. In contrast to previous studies, we will focus on events occurring at low physiological multiplicities of infection and physiological febrile temperature range, which give rise to heterogenous and stochastic effects in the infected population. Our main objectives are:

1) Elucidate how pathogen threats are encoded in dynamical responses of NF-κB/STAT/IRF systems upon *L. monocytogenes* infection. We will dissect the host signalling responses in infected cells, as a function of density and temperature and understand how they relate to different (and seemingly probabilistic) outcomes of single-cell host-pathogen interactions.

2) Understand the population-level host strategies that underpin successful immune response to infection. We will investigate how paracrine signalling in the infected and bystander population and temperature adaptation coordinate the overall population level inflammatory responses. We will specifically investigate role of TNFα, IL1β, IL6 and IFNα/β signalling in the process.

3) Determine whether the host and pathogen variability are co-regulated as a fundamental immune mechanism. We will use image genomics (smFISH) and immunostaining to measure single-cell effector responses in host macrophages as well as monitor activation of the major PrfA regulon in *L. monocytogenes* at the single bacterium resolution.

4) Develop predictive mathematical models of innate immune response to *L. monocytogenes* using genetic and chemical perturbations to validate modelling and manipulate infection outcomes.

METHODS: The heterogeneous and dynamic nature of the host-pathogen interactions cannot be resolved in typical population-level analyses. In this project, we will use systems biology approaches including live-cell imaging, smFISH and mathematical modelling to dissect host-pathogen interactions at the single cell level. Confocal microscopy will be used to monitor the activation of NF-κB/STAT/IRF in individual host cells in real-time together with *L. monocytogenes* growth and PrfA virulence. These will be combined with multiplex single molecule fluorescent in situ hybridisation (smFISH) and immunostaining to understand target effector responses in different subpopulations of cells. Stochastic and deterministic single cell and agent-based population models of signalling responses to *Lm* will be used to mechanistically dissect host defence strategies and provide experimentally testable predictions.

IMPACT/SIGNIFICANCE: This project will provide a new mechanistic understanding of the interactions between host macrophages and *L. monocytogenes*, well-studied pathogen model that serves as a paradigm for understanding responses to other pathogens at the single cell level. The comprehensive mathematical models will allow to elucidate new innate immune mechanisms involved in antibacterial responses. Developed tools and experimental approaches will be broadly applicable to other pathogens and single cell gene expression systems, e.g., development and cancer.