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IFN-mediated reprogramming of human plasmacytoid dendritic cells in antibacterial immunity

Salmonella enterica are Gram-negative intracellular bacteria that infect humans and animals, posing a major public health threat as a leading cause of foodborne illness worldwide. From self-limiting gastroenteritis to deadly bacteremia, salmonellosis leads to 1.3 billion new cases of non-typhoidal *Salmonella* infections[(1)] and 20 million cases of enteric fever from typhoidal *strains* globally every year[(2)]. The rising incidence of these infections highlights the urgent need for novel therapeutic strategies.

Plasmacytoid dendritic cells (pDC) are immune innate cells renowned for their role in antiviral responses, owing to their exceptional capacity to produce huge amounts of type I IFN[(3)]. However, their contribution to defense against bacteria has been underestimated and remains poorly understood. Recently, we demonstrated that *Salmonella* not only infect human pDC but directly drive their maturation, activation, pro-inflammatory cytokine, and type I interferon (IFN) secretion in a SLAMF7 and SLAMF8-co-dependent manner[(4)]. This finding opens new avenues to explore pDC function in bacterial immunity.

Our preliminary results show that both type I and II IFN enhance SLAMF7/8 expression on pDC. This suggests that IFNs may act as priming signals that sensitize pDC to bacterial infection by modulating SLAM receptor expression and activation state. However, whether these cytokines also influence the expression of costimulatory molecules or the production of inflammatory cytokines by pDC remains unknown.

Human pDCs will be isolated from peripheral blood mononuclear cells of healthy donors using magnetic sorting. In parallel, we will use the CAL-1 cell line, a human pDC-like leukemic cell line that recapitulates many phenotypic and functional characteristics of primary pDCs. Both primary pDCs and CAL-1 cells will be in vitro infected with WT *Salmonella enterica* Typhimurium (MOI 25) as previously performed, and stimulated with recombinant human **IFN-α2a**, **IFN-β1a**, **IFN-γ**, or a

combination for 6, 12, and 24 hours. Unstimulated cells will serve as controls. After stimulation, surface expression of SLAMF7, SLAMF8, and costimulatory molecules (**CD80**, **CD86**, **CD40**) will be analyzed by flow cytometry. Intracellular cytokine staining will be performed to quantify **IFN-α** and **TNF-α** production at the single-cell level. In addition, total RNA will be extracted to assess SLAMF7/8 gene expression by RT-qPCR. Supernatants will be collected to confirm cytokine release using ELISA or multiplex bead-based assays. Recent findings highlight the notion that metabolism underlie the functionality of immune cells. We will then assess the metabolic status of infected pDC cell lines in the presence or absence of IFNs. This will be done using SCENITH[5], a powerful method to study single-cell energetic metabolism by profiling translation inhibition using flow cytometry. This combined approach will enable us to validate interferon-driven responses in both primary and model pDC systems, providing robust insights into the mechanisms of pDC activation.

Evaluating the effect of IFN subtypes on pDC activation will provide insights into how these cells are functionally reprogrammed in inflammatory contexts, particularly during bacterial infections such as salmonellosis. This could help uncover new immunomodulatory mechanisms and support the development of pDC-based vaccination strategies or immunotherapies.

References

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