Elucidating the role of Interleukin 34 during bacterial infections

Project leader: Prof. Dr. Samuel Huber and Prof. Dr. Hans-Willi Mittrücker

Affiliation: I. Medizinische Klinik und Poliklinik and Institut für Immunologie
Universitätsklinikum Hamburg-Eppendorf

Background and preliminary data:

Inflammation is fundamental in the host's defence against invading pathogens. Not only the type of pathogen and its equipment of virulence factors determine severity of the infection but also host factors can aggravate or ameliorate the course of disease. Recently, interleukin 34 (IL-34) has been identified as the second ligand for the Colony Stimulating Factor 1 receptor (CSF-1R). Interestingly, IL-34 seems to play a crucial role in the differentiation and proliferation of macrophages, which express CSF-1R. Previous studies further indicate that IL-34 is upregulated in various infectious diseases including bacterial infections. However, these studies were mostly based on correlative analyses and *in vitro* experiments. Functional *in vivo* experiments have not been performed so far.

Therefore, in a first step, we analyzed the IL-34 expression in mice under steady state conditions and in models of local inflammation. We found that IL-34 is expressed in various organs and by many cell types such as dendritic cells, neutrophils, granulocytes and macrophages during homeostasis. However, during inflammation, non-hematopoietic cells seem to be the most relevant source. In a second step, we analyzed IL-34 expression in mice upon infection with *L. monocytogenes* and found upregulation of IL-34 in spleen and liver. However, functional experiments addressing the relevance of this finding have not been done so far. Thus, and to study the role of IL-34 on a functional level, we have generated and validated *Il34-deficient* mice.

Hypothesis:
Based on our preliminary data and the published literature, we hypothesize that non-immune cell derived IL-34 plays a protective role in bacterial infections by acting on macrophages

Aims and Work Programme:
We want to elucidate the role of IL-34 in the pathogenesis of bacterial infections. To this end, we will use *Il34*-deficient mice and mouse models for bacterial infections (e.g. *L. monocytogenes*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Citrobacter rodentium*). First, we will measure the expression level and cellular source of IL-34 in infection. Second, we will use *Il34-deficient* mice to study the functional role of IL-34 in these infections. We will assess disease severity and control of the bacteria. We will further evaluate innate and adaptive immune response of infected mice in terms of cellular phenotype and cytokine profile of immune cells using flow cytometry. We will use established sets of antibodies to determine the response of different cell types (epithelial cells, T and B cells as well macrophages and dendritic cells). Additionally, we will perform an unsupervised approach by CITEseq of total cells isolated from colon of wildtype mice with or without infection. With this strategy, we minimize a potential bias due to purification protocols designed for specific cell population. Third, we aim to identify the target cells of IL-34. Based on our preliminary data, B cells and macrophages are potential IL-34 targets. To this end, we will test the *in vitro* response of defined cell subsets to IL-34 stimulation. We will analyse the proliferation and cytokine profile of these cells. Furthermore, as CSF-1R is the known receptor of IL-34, we have crossed *Csf1r* conditional knockout mice with *LysM*-cre (macrophage) and *CD19*-cre (B cell) mice in order to identify relevant target cells of IL-34. Finally, we aim to translate our findings to human biology. Thus, we will measure IL-34 levels in patients during the course of bacterial infections and correlate its expression with clinical parameters. Taken together, this study will identify the role of IL-34 in infection and thereby build the basis for future immunotherapies designed on targeting this cytokine.

Project-related publications: (max. 5)

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2. Baghdadi M, Umeyama Y, Hama N, Kobayashi T, Han N, Wada H, Seino KI. Interleukin-34, a comprehensive review. J Leukoc Biol. 2018 Nov;104(5):931-951.
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