MOI Project 6

Characterization of the tertiary GBP1-LPS-CASP4 complex

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The chemokines CCL17 and CCL22 are mainly secreted by antigen presenting cells and regulate the recruitment of T cells and other immune cells. They signal via the receptor CCR4 which is expressed on a variety of immune cells, including T cells, basophils, mast cells, macrophages and dendritic cells (DCs). Although both ligands interact with the same receptor, CCL17 exerts a more immunostimulatory role in allergies or inflammatory diseases, whereas CCL22 is associated with immunosuppressive functions in the tumor environment. This might be caused by different binding properties of the chemokines to CCR4, thereby inducing different signaling pathways or efficacies, a phenomenon called biased agonism. The function of CCL17 and CCL22 in infectious diseases is not well understood, yet. Here, we want to analyze the role of CCL17 and CCL22 after chronic infection of mice with an attenuated *Salmonella* Typhimurium (STM) strain, which allows not only to focus on early host defense functions but also to analyze adaptive immune responses. We could already show that CCL17 and CCL22 are upregulated after acute infection with STM in mouse models, but their interference with T cell responses during chronic infections is so far unclear. Therefore we want to elucidate the contribution of the chemokines CCL17 and CCL22 in the induction of T cell responses in chronic infection with STM. Obesity is one of the biggest health problems worldwide and increases the likelihood of type II diabetes or cardiovascular disease, but is also a risk factor for infections. Obesity causes a weak, chronic inflammatory response which does not only alter metabolic responses but also influences immune responses. These changes affect adipose tissue, but also other organs such as lung and intestine and can enhance the susceptibility for infections. We found that naive CCL17- and CCL22- double deficient female mice possess a higher body weight than wild-type animals. Since we were also able to detect the expression of CCL17 in adipose tissue, it is possible that CCL17 and CCL22 play a previously unknown role in the homeostasis and/or differentiation of adipose tissue. Therefore, we want to characterize the function of the chemokines after chronic infection with STM, as well as in metabolic syndrome after high-fat diet and elucidate the host defense response to STM infection in animals with metabolic syndrome.

Background: Several bacterial pathogens thrive in the cytosol of host epithelial cells. Pyroptosis, the inflammatory cell death, constitutes an efficient cell-intrinsic immune defense by fulfilling two functions: firstly, the programmed cell death deprives intracellular bacteria of their replication niche, and secondly, release of proinflammatory alarmins and cytokines attracts professional immune cells to the site of infection to further restrict the bacterial invader. Excessive pyroptotic cell death and resulting proinflammatory immune responses can however lead to sepsis, which still cannot be causally treated. To develop novel therapies against bacteria-induced sepsis, understanding in detail the host-pathogen interactions that lead to pyroptosis is essential. Interferon-inducible guanylate-binding proteins (GBPs) are key players in innate immunity and promote pyroptosis in response to intracellular bacteria through accelerated activation of the non-canonical inflammasome caspase-4 (CASP4)[1-5].

Own previous work: We showed that two human GBPs, GBP1 and GBP2, bind and aggregate the bacterial surface molecule lipopolysaccharide (LPS), a potent inducer of pyroptosis, and that these GBP-LPS complexes significantly increase the activity of the CASP4 inflammasome[6,7].

Aim of the project: This project aims to characterize the postulated tertiary GBP-LPS-CASP4 complex using cell-based and cell-free interaction and structure-function studies to understand the mechanism of non-canonical inflammasome activation leading to pyroptosis on a molecular level.

Work program:

Structure-function studies identifying interacting domains:

- Transduction of CRISPR/CAS9 generated cell lines deficient in GBP and CASP4 with vectors stably expressing GBP-CASP4 chimeric proteins
- Transfection of the generated cell lines with LPS, monitoring hall marks of pyroptosis (cell death, cytokine secretion) with fluorescence-based methods
- Infection of the generated cell lines with bacterial pathogens monitoring pyroptosis and bacterial survival with fluorescence- and luminescence-based methods

Biochemical interaction studies characterizing kinetics and affinities of the tertiary complex:

- Purification of recombinant-expressed wild type proteins and protein chimeras with liquid chromatography methods
- Labeling of proteins with fluorescence-dyes
- Fluorescence-based interaction studies (FRET, SPR, MST)

Structural studies, characterizing the tertiary complex on a molecular level:

- Crystallization and X-ray structural resolution of the complex
- Transmission electron microscopy of the complex

References

1 Pilla, D.M. *et al. Proc Natl Acad Sci U S A* **111**, 6046-6051 (2014).
2 Meunier, E. *et al. Nature* **509**, 366-370 (2014).
3 Finethy, R. *et al. mBio* **8**, e01188-01117 (2017).
4 Lagrange, B. *et al. Nat Commun* **9**, 242 (2018).
5 Fisch, D. *et al. Elife* **8** (2019).
6 Kutsch, M. *et al. EMBO J* **39**, e104926 (2020).
7 Dickinson, M.S. *et al. Proc Natl Acad Sci U S A* **120**, e2216028120 (2023).