Modulation of Neutrophil Extracellular Trap Formation in Health and Disease

Ava Hosseinzadeh
Modulation of Neutrophil Extracellular Trap Formation in Health and Disease

Abstract
The critical prompt innate immune response is highly built upon the influx of neutrophils from the blood stream to the site of infection. In the battlefield, neutrophils sense pathogen-associated molecular patterns (PAMPs) through their pattern-recognition receptors (PRRs) to launch a number of responses with the goal to defeat the invading pathogen. Neutrophils' wide spectrum of responses ranges from reactive oxygen species production (ROS), phagocytosis, cytokine and chemokine secretion, and neutrophil extracellular trap (NET) formation. The NET scaffold is composed of nuclear chromatin which is armed with antimicrobial proteins. DNA traps are able to ensnare and kill microbes in the extracellular space and NET release concurs with cell death of the neutrophil. An increasing body of literature describes that NETs impose deleterious effects on the host itself in addition to their antimicrobial activity. These hazardous effects mainly stem from pro-inflammatory and tissue-destructive activity of NETs. These two diverse outcomes of NETs result in a series of effects on both host and pathogen. Therefore, it seems rational that NET formation is tightly regulated and not happening spontaneously. The opportunistic fungal pathogen Candida albicans captured and killed by NETs. This fungus has the remarkable ability to grow as budding yeast or as filamentous hyphae, and reversely alternate between these morphotypes. Hyphae are the tissue-destructive, invasive and pro-inflammatory form of C. albicans, whereas yeast is the proliferative, non-invasive form. Hence, it is important to find out how neutrophils discriminate between distinct growth forms of C. albicans and how NET release is regulated in this regard.

To assess neutrophils responses towards each growth form of C. albicans, the mere ratio of each fungal morphotypes is an insufficient measure to describe comparable amounts used in infection experiments; we therefore used dry mass of fungal cells to serve as a common denominator for amounts of fungal cells with different morphotypes. As assessment of dry mass is laborious, we developed a quick correlative method, which quantified fungal metabolic activity corresponding to the actual dry mass. We applied this method in consecutive studies investigating the neutrophil responses specific to different morphotypes of C. albicans.

Positive and negative regulators of NET formation were investigated for this thesis in a mechanistic fashion. To identify how NET release is negatively regulated during C. albicans infection we focused on anti-inflammatory receptors on neutrophils. We observed that adenosine signals via adenosine receptor reduces the amount of NETs exclusively in response to C. albicans hyphae, the invasive, pro-inflammatory form. We identified adenosine receptor A3 as the responsible receptor suggesting that targeting of adenosine A3 would be a promising approach to control invasive fungal infection, since particularly during immune reconstitution invasive mycoses are frequently accompanied by hyperinflammation which additionally worsens the patient’s state.

As unbalanced inflammation is harmful to the host, a situation reflected in autoimmune diseases, such as systemic lupus erythematosus, we aimed to find molecules, which are able to inhibit NET formation. Thus, we introduced the non-toxic agent tempol. During ROS-dependent stimulation of NET formation via C. albicans and phorbol esters, the stable redox-cycling nitroxide tempol efficiently blocked NET induction. We therefore proposed tempol as a potential treatment during inflammatory disorders where NET formation is out of balance. In quest for positive regulators of NET formation we found the major addictive component of tobacco and electronic cigarettes, nicotine, as compelling direct inducer of NET release. Interestingly, nicotine is associated with exacerbated inflammatory diseases exerting its pro-inflammatory activity via acetylcholine receptor by targeting protein kinase B (known as Akt) activation with no effect on NADPH oxidase complex in a ROS independent fashion. In consideration of neutrophils role in smoking-related diseases we propose targeting Akt could lower the undesirable effect of NET.

In conclusion, this thesis identified new modulators of NET formation in response to fungal infection and more broadly to other NET-inducing stimuli, which might have implications in forthcoming therapies.