

# Intracellular Radicals in Neutrophils

## Processing and Functional Implications

### Akademisk avhandling

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av

**Halla Björnsdóttir**

Fakultetsopponent:

Donald J. Davidson, senior lecturer

University of Edinburgh

Avhandlingen baseras på följande arbeten:

- I. Björnsdóttir H, Granfeldt D, Welin A, Bylund J, Karlsson A. Inhibition of phospholipase A2 abrogates intracellular processing of NADPH-oxidase derived reactive oxygen species in human neutrophils. *Experimental Cell Research* 2013. 319: 761-74.
- II. Björnsdóttir H, Welin A, Michaëlsson E, Osla V, Berg S, Christenson K, Sundqvist M, Dahlgren C, Karlsson A, Bylund J. Neutrophil NET formation is regulated from the inside by myeloperoxidase-processed reactive oxygen species. *Submitted manuscript*.
- III. Björnsdóttir H, Welin A, Stylianou M, Christenson K, Urban C, Forsman H, Dahlgren C, Karlsson A, Bylund J. Cytotoxic peptides from *Staphylococcus aureus* induce ROS-independent neutrophil cell death with NET-like features. *Manuscript*.
- IV. Wekell P\*, Björnsdóttir H\*, Björkman L, Sundqvist M, Christenson K, Osla V, Berg S, Fasth A, Welin A, Bylund J, Karlsson A. Neutrophils from patients with SAPHO syndrome show no signs of aberrant NADPH-oxidase dependent production of intracellular reactive oxygen species. *Submitted manuscript*. \*Joint first authorship



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# Intracellular Radicals in Neutrophils

## Processing and Functional Implications

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### ABSTRACT

Neutrophils are the most abundant leukocyte in human blood and essential components of our defense against microbial pathogens. These cells can neutralize microbial pathogens by phagocytosis, which involves engulfment and degradation of microbes intracellularly, as well as by the formation of neutrophil extracellular traps (NETs), which are structures released from neutrophils made up of DNA and proteins that capture microbes extracellularly. One characteristic of neutrophils is that they can produce massive amounts of reactive oxygen species (ROS) upon activation of a specialized enzyme system, the NADPH oxidase. The ROS can be produced at different cellular sites, inside the phagosome, intracellularly inside granules, and at the plasma membrane leading to the release of ROS extracellularly. Whereas ROS produced inside phagosomes are crucial for microbial killing, much less is known about intracellular ROS produced inside granules, which is therefore in focus in this thesis.

Neutrophils contain multiple types of granules that are storage organelles for soluble proteins, receptors, and effector molecules. Part of the NADPH oxidase is found in granule membranes and upon activation, ROS can be produced inside granules where they may be processed by myeloperoxidase (MPO) to yield other types of ROS. In **paper I**, MPO-processing of intracellular ROS was shown to be dependent on phospholipase A<sub>2</sub> (PLA<sub>2</sub>) activity. However, PLA<sub>2</sub> was not directly involved in the processing but rather indirectly by mediating the fusion of different granule types, which enables the ROS and MPO to meet inside the cell. It has previously been suggested that the autoinflammatory disorder SAPHO syndrome, characterized by neutrophil dermatosis and typically sterile inflammation of the bone, is associated with neutrophils lacking the production of intracellular ROS. In **paper IV**, four patients with SAPHO syndrome were investigated with respect to ROS production and other neutrophil functions. All patients, however, produced normal amounts of intracellular ROS demonstrating that decreased intracellular ROS production is not a general feature of SAPHO syndrome.

In **paper II** and **III**, the role of intragranular ROS for the formation of NETs was studied. **Paper II** demonstrates that intragranular ROS are essential to drive active NET formation and that intracellular processing of these ROS by MPO is a critical step. **Paper III** shows that NETs are not only the result of an active process but can also be induced by alternative means, e.g., by cytotoxic peptides released from bacteria. Unlike the process described in the literature and in **paper II**, this type of NET formation was not dependent on ROS or MPO.

In conclusion, the processing of intracellularly produced ROS in neutrophils has been characterized and both production and processing were found to be essential for active NET formation. Further, an alternative mechanism of NET formation was described that is independent of ROS production.

**Keywords:** neutrophil, reactive oxygen species, neutrophil extracellular traps

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