Differential Redox Mechanisms in Neutrophil Activation: A Comparative Proteomic Analysis

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Neutrophils are pivotal in host defense and inflammation, employing mechanisms such as granule content release, formation of neutrophil extracellular traps (NETs), and generation of reactive species. This study investigates redox mechanisms in neutrophils, specifically analyzing reactive species formation, and employs high-resolution proteomics to examine responses of neutrophils isolated from peripheral blood to phorbol-12-myristate-13-acetate (PMA) and ionomycin, a calcium ionophore. Neutrophils stimulated with PMA exhibited robust production of superoxide anion, hydrogen peroxide, and hypochlorous acid, characteristic of the neutrophil oxidative burst. In contrast, ionomycin stimulation did not significantly generate these reactive species, suggesting action mechanisms independent of reactive species generation. Comparative proteomics of the secretome from neutrophils activated by ionomycin or PMA revealed distinct profiles. The activation with PMA leads to neutrophil secretion of proteins derived from secretory vesicles and tertiary granules, with notable modifications including chlorination of tyrosine and tryptophan, and oxidation of methionine and cysteine residues. These modifications are attributed to reactive species generation, could affect pathogen elimination, and have signaling functions. Conversely, ionomycin activation showed enhanced secretion of proteins from azurophil and specific granules and uniquely promoted protein citrullination mediated by calcium-dependent peptidyl arginine deiminase 4 (PAD4). Protein citrullination may have a role in the cell signaling and autoimmune responses. This study enhances the understanding of neutrophil-mediated inflammatory responses, highlighting differential redox effects that influence infection resolution pathways.

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