STORAGE ORGANELLES THAT ARE DISTINCT FROM THE CLASSICAL GRANULES IN HUMAN NEUTROPHILS

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The thesis is based on the following papers:

I. Pellmé S, Mörgelin M, Tapper H, Mellqvist U-H, Dahlgren C and Karlsson A. Localization of human neutrophil interleukin-8 (CXCL-8) to organelle(s) distinct from the classical granules and secretory vesicles
Journal of Leukocyte Biology (2006); 79:564-73

II. Pellmé S, Dahlgren C and Karlsson A. The two neutrophil plasma membrane markers alkaline phosphatase and HLA Class I antigen do not co-localize completely in granule deficient cytoplasts. An ideal plasma membrane marker in human neutrophils is still lacking.
Submitted for publication

III. Pellmé S, Nordenfelt P, Lönnbro P, Johansson V, Dahlgren C, Karlsson A and Tapper H. Phagosomes form and mature without involvement of the endoplasmic reticulum in neutrophil-like HL-60 cells
In manuscript

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ABSTRACT

The human neutrophil is a crucial participant in acute inflammation. Appropriate immune response is dependent on rapid direction of phagocytic cells through the tissue, towards the inflammatory focus. Such movement is governed by chemoattractants, e.g., interleukin-8 (IL-8/CXCL-8). Circulating neutrophils are packed with granules that contain effector molecules used for different cell activities. Upon activation, the neutrophil mobilizes the granules to the plasma membrane and the forming phagolysosome, thereby exposing new receptors and releasing substances to the extracellular milieu or into the phagolysosome. The classical neutrophil granules are well-defined and one of them, the secretory vesicle, has been suggested to contain the CXCL-8 of resting neutrophils. However, using fractionation techniques and immunogold labeling, we show that neutrophils store CXCL-8 in an organelle distinct from the granules and secretory vesicles. In neutrophil cytoplasts, we found partial colocalization of CXCL-8 and calnexin, a marker for the endoplasmic reticulum (ER), suggesting that a proportion of CXCL-8 is localized to the ER or ER-like structures in the neutrophil.

The identification of specific markers for individual subcellular compartments is crucial to neutrophil research. HLA class I (HLA-I) has been proposed as an ideal marker for the plasma membrane, much due to the fact that it is un-influenced by stimulation. By the use of detailed fractionation protocols, we found that HLA-I not only colocalizes with the plasma membrane but is also present in other organelles of slightly higher densities. Moreover, the mixed enzyme-linked immunosorbent assay (MELISA), used to detect the β_2-microglobulin (β_2m)/HLA-I complex, proved to be negatively affected by uncomplexed β_2m, making it difficult to use HLA-I as a marker during, for example, phagolysosome formation.

The involvement of the ER in macrophage phagocytosis is a matter of debate. The classical dogma that mature neutrophils are poor producers of protein and that they contain ER of very limited amounts has essentially precluded these cells from the discussion. However, neutrophils do produce proteins, such as CXCL-8, upon stimulation, suggesting a functional ER in these cells. We studied calnexin and CXCL-8 in the context of phagocytosis, using the promyelocytic cell line HL-60, known to carry out phagocytosis in much the same way as neutrophils do. CXCL-8 and calnexin did not colocalize during phagocytosis, and calnexin was not detected on the phagosomal membrane. We conclude that phagocytosis does not involve ER fusion in HL-60 cells and neutrophils, and that these cells differ from macrophages in this respect.

Key words: neutrophil, HL-60 cells, cytoplasts, granules, CXCL-8, ER, phagocytosis, HLA-I, subcellular fractionation, immunogold

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