Membrane proteins in human neutrophils

Identification and characterization of lipid rafts in subcellular organelles

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ABSTRACT

The human neutrophil is an important effector cell in acute inflammation and in the innate immune response against bacteria and fungi. When immune reactions occur in the tissue in response to antigen challenge, neutrophils are the first cells to enter the site of inflammation. The neutrophil is equipped with a vast amount of receptors that both interact with inflammatory mediators and host tissue as well as with the prey. These receptors are found on the cell surface but are also stored in different types of granules and vesicles in the cell. By mobilizing the granules and vesicles to various extents and thereby upregulate receptors to the plasma membrane, the mature neutrophil can modulate its communication with the environment. One granule type, the azurophil granules, primarily delivers a killing machinery to intracellular organelles containing a prey that has been engulfed. These granules have traditionally been regarded as classical lysosomes, but their membrane is so far largely uncharacterized.

The aim of this thesis was to elucidate details regarding azurophil granule membrane composition in order to further understand their role in neutrophil function. The studies led to identification of so-called lipid rafts in the azurophil and other granule membranes, and a detailed characterization of the azurophil granule lipid rafts with regard to protein composition was thus performed.

One of the proteins identified in azurophil granule membranes was stomatin. This protein was present also in other granule/vesicle membranes and the plasma membrane. Furthermore, the protein was localized to lipid rafts. Apart from stomatin, the azurophil granule membrane rafts contained a vast number of proteins, of possible importance for membrane structure/integrity and fusion. The fact that several cytoskeletal proteins also were identified, suggests that the granule membrane is organized in much the same way as the plasma membrane.

The thesis also includes studies on the neutrophil receptors for galectin-3, a potent activator of extravasated human neutrophils. Since granule mobilization is a prerequisite for galectin-3-induced activation of the cells, the receptors have been suggested to be granule localized. Here, galectin-3-binding proteins from specific/gelatinase granules were isolated, and among these, CD66a and CD66b were identified as the most plausible receptor candidates. The CD66b is a glycophosphatidyl inositol (GPI)-linked protein that was found to be localized to lipid rafts, suggesting that raft-associated signaling may be of importance for the galectin-3-induced neutrophil responses.

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I. The presence of stomatin in detergent-insoluble domains of neutrophil granule membranes
E. Feuk-Lagerstedt, M. Samuelsson, W. Mosegoeller, C. Movitz, Å. Rosqvist, J. Bergström, T. Larsson, M. Steiner, R. Prohaska, and A. Karlsson
J Leukoc Biol 2002; 72: 970-977

II. Lipid raft proteome of the human neutrophil azurophil granule
E. Feuk-Lagerstedt, C. Movitz, S. Pellmé, C. Dahlgren, and A. Karlsson
Submitted for publication, 2006

III. Identification of CD66a and CD66b as the major galectin-3 receptor candidates in human neutrophils
E. Feuk-Lagerstedt, E. T. Jordan, H. Leffler, C. Dahlgren, and A. Karlsson
J Immunol 1999; 163:5592-5598

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