

Granulocyte activation by danger signals and blocking of receptor responses

Akademisk avhandling

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av

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Avhandlingen baseras på följande arbeten

- I. **A.-L. Stenfeldt and C. Wennerås.** Danger signals derived from stressed and necrotic epithelial cells activate human eosinophils.
Immunology 2004, 112:605-614
- II. **A.-L. Stenfeldt, C. Wennerås and C. Dahlgren.** Epithelial cell components as danger signals to human neutrophils.
In manuscript
- III. **A.-L. Stenfeldt, J. Karlsson, C. Wennerås, J. Bylund, H. Fu and C. Dahlgren.** Cyclosporin H, Boc-MLF and Boc-FLFLF are antagonists that preferentially inhibit activity triggered through the formyl peptide receptor.
Inflammation 2007, *In Press*
- IV. **A.-L. Stenfeldt, J. Karlsson, C. Wennerås, J. Bylund, H. Fu and C. Dahlgren.** The non-steroidal anti-inflammatory drug piroxicam blocks ligand binding to the formyl peptide receptor but not the formyl peptide receptor like 1.
Biochemical Pharmacology 2007, 74:1050-1056



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Granulocyte activation by danger signals and blocking of receptor responses

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Granulocytes are the most abundant cells in the peripheral blood. They serve to eliminate invading microbes and parasites and release anti-microbial agents. In the event of injury, granulocytes are recruited to damaged tissues. Formerly it was thought that only foreign microorganisms and molecules could induce an immune response, but later it was proposed that the immune system can react to any molecule, endogenous or exogenous, that is perceived as dangerous to the body (danger signals). This thesis will focus on granulocytes and how these cells can be directly activated by danger signals. It will also discuss possible ways to block receptors that may contribute to the activation of granulocytes by damaged cells.

The effect of damaged and stressed cells on granulocytes was evaluated by studying different classical activation markers such as the release of granule content, expression of the surface marker CD11b and the production of superoxide radicals. Potential danger signals were induced from epithelial cells that were disintegrated by freeze-thawing, and freeze-pressing, and were stressed by heat treatment. The results show that disintegrated epithelial cells can directly activate granulocytes. This finding may change the view of these cells role in inflammatory reactions.

Molecules from damaged tissue cells have been suggested to orchestrate the immune response through pattern recognition receptors (PRRs). PRRs is a group of highly conserved receptors that have developed during evolution. There are at least two subpopulations of PRRs and the formyl peptide receptor (FPR) family is one of them. In order to properly interpret receptor inhibition experiments, the precise receptor specificities of the employed antagonists are of crucial importance. Lately, a great number of agonists for various formyl peptide receptors (FPR) have been identified using a selection of antagonists. There is, however, some confusion about the receptor specificities for many of these antagonists. To investigate the specificity of FPR antagonists the FPR specific agonist *N*-formyl-Met-Leu-Phe (fMLF), the formyl peptide receptor like 1 (FPRL1) specific agonist Trp-Lys-Tyr-Met-Val-L-Met-NH₂ (WKYMVM) and an agonist that binds to both these receptors, Trp-Lys-Tyr-Met-Val-D-Met-NH₂ (WKYVMm), were used as neutrophil stimuli. The inhibition of neutrophil responses was investigated by the addition of the antagonists tert-butylloxycarbonyl-Met-Leu-Phe (Boc-MLF also termed Boc-1), tert-butylloxycarbonyl-Phe-Leu-Phe-Leu-Phe (Boc-FLFLF also termed Boc-2), cyclosporin H, Trp-Arg-Trp-Trp-Trp-Trp (WRWWW) and the non-steroidal anti-inflammatory drug piroxicam. These experiments show that the neutrophil responses triggered through FPR were inhibited by low concentrations of the antagonists cyclosporin H, Boc-MLF and Boc-FLFLF. Higher concentrations of the Boc peptides also partially inhibited the signaling through FPRL1. The non-steroidal anti-inflammatory drug piroxicam inhibits the neutrophil responses triggered through FPR but not through FPRL1. This inhibition is due to a reduced binding of fMLF to its receptor.

Keywords: granulocytes, neutrophils, eosinophils, danger signals, danger theory, formyl peptide receptors, antagonists, inhibitors