Activation of professional phagocytes with emphasis on formyl peptide receptors

Akademisk avhandling
som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs universitet kommer att offentligen försvaras i Föreläsningssalen, våning 3, Guldhedsgatan 10A, Göteborg.

Torsdagen den 28 maj 2009 kl. 9.00
av
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Fakultetsopponent: Professor Mikael Rhen
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Avhandlingen baseras på följande arbeten:

I. Karlsson J., Fu H., Boulay F., Bylund J., Dahlgren C.
The peptide Trp-Lys-Tyr-Met-Val-D-Met activates neutrophils through the formyl peptide receptor only when signaling through the formylpeptide receptor like 1 is blocked. A receptor switch with implications for signal transduction studies with inhibitors and receptor antagonists.
Biochem Pharmacol. 2006 May 14;71(10):1488-96

II. Karlsson J., Stenfeldt A., Rabiet M.J., Bylund J., Forsman H., Dahlgren C.
The FPR2 specific ligand MMK-1 activates the neutrophil NADPH-oxidase, but triggers no unique pathway for opening of plasma membrane calcium channels.
Cell Calcium 2009. In Press

III. Karlsson J., Bylund J., Movitz C., Björkman L., Forsman H., Dahlgren C.
A methodological approach to studies of desensitization of the formyl peptide receptor: Role of the read out system, reactive oxygen species and the specific agonist used to trigger neutrophils
Submitted

IV. Bellner L.*, Karlsson J.*, Fu H., Boulay F., Dahlgren C., Eriksson K., Karlsson A.
A monocyte-specific peptide from herpes simplex virus type 2 glycoprotein G activates the NADPH-oxidase but not chemotaxis through a G-protein coupled receptor distinct from the members of the formyl peptide receptor family
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Activation of professional phagocytes with emphasis on formyl peptide receptors

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Phagocytic cells such as neutrophil granulocytes and monocytes are an essential part of our innate immune system and play an important role in the battle against pathogens. G-protein coupled receptors (GPCRs) and more specifically chemoattractant receptors are a vital part in guiding phagocytes towards the site of infection. Chemoattractant receptors are also involved in an effective activation of these cells.
This thesis investigates activating ligands and signalling properties of three different G-protein coupled receptors (GPCR) involved in innate immunity. Where the first two belongs to the formyl peptide receptor (FPR) family of chemoattractant receptors and the third is a non-chemotactic receptor expressed on monocytes.
The first paper describes the selective activation of the two receptors formyl peptide receptor 1 (FPR1) and formyl peptide receptor 2 (FPR2) by a synthetically derived hexapeptide with the sequence WKYMVm. We show that WKYMVm binds to both receptors but signal through FPR1 only when FPR2 is blocked. In paper number two we add the peptide MMK-1 to the list of FPR2 binding activators of the NADPH-oxidase. We also showed that calcium signalling induced by both FPR1 and FPR2 is dependent of release from intracellular stores and a subsequent opening of store operated calcium channels (SOCs) in the plasma membrane. Desensitization of chemotactic receptors is of importance for the termination of proinflammatory activities acted out by phagocytes. The third paper is a methodological study with the aim of solving problems associated with oxidation of stimulus in *in vitro* desensitization studies where intracellular calcium is measured. The solution put forward was to add serum proteins in the reaction mixture or to use a flow cytometry based method where the amount of reactive oxygen species (ROS) produced in the bulk could be reduced. In the fourth paper we identify a monocyte activating peptide, gG-2p19, derived from the secreted portion of the Herpes simplex virus type 2 (HSV-2) glycoprotein G. Monocytes produced ROS in response to stimulation with gG2p19 while neutrophils did not. The receptor for gG2p19 was shown to be a GPCR by its sensitivity to pertussis toxin, but the peptide could not induce chemotaxis through this receptor. It was determined that the receptor responsible for activation did not belong to the FPR family, but still share at least one common signalling pathway with FPR2.

Keywords: Phagocyte, G-protein coupled receptor, chemoattractant, formyl peptide receptor, reactive oxygen species, calcium

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