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Review

Basic characteristics of the neutrophil receptors that recognize formylated peptides, a danger-associated molecular pattern generated by bacteria and mitochondria

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ABSTRACT

Proper recruitment and activation of neutrophils to/at sites of infection/inflammation relies largely on the surface expression of chemoattractant receptors of which a formyl peptide receptor (FPR1) was the first to be cloned and characterized in more detail. This receptor displays high affinity for bacterial- or mitochondrial-derived peptides that contain a formylated methionine in the N-terminus. The neutrophil chemoattractant receptors belong to the group of 7-transmembrane domain receptors that signal through activation of heterotrimeric G proteins. These receptors have been shown to be important in host defense against microbial intruders and in regulating inflammatory reactions. The two FPRs (FPR1, FPR2) expressed in neutrophils share significant sequence homology and bind many structurally diverse activating (agonistic) and inhibiting (antagonistic) ligands, ranging from peptides to lipopeptides containing peptide sequences derived from intracellular regions of the FPRs. Recent structural and functional studies of the two neutrophil FPRs have generated important information for our understanding of general pharmacological principles, governing regulation of neutrophil function and inflammation and increased knowledge of more general G-protein coupled receptor features, such as ligand recognition, biased signaling, allosteric modulation, and a unique receptor cross-talk phenomenon. This article aims to summarize recent discoveries and pharmacological characterization of neutrophil FPRs and to discuss unmet challenges, including recognition by the receptors of diverse ligands and how biased signals mediate different biological effects.

Contents

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1. A brief historical overview on the pattern recognition of formyl peptides

1.1. Neutrophil functions directed by N-formylated peptides

More than 40 years ago, it was anticipated that peptides containing a formylated methionine (fMet) in their N-terminus could constitute a "molecular pattern" recognized by cells of our innate immune system. The rational for this is that bacterial protein synthesis starts with fMet, a residue sequentially cleaved off by peptide deformylase to generate mature proteins during bacterial growth. Immune cells, equipped with proper recognition structures (receptors), should be able to approach through chemotactic migration, an infected tissue containing formylated peptides [1]. Accordingly, fMet-containing peptides were identified as chemoattractant for phagocytic cells of our innate immune system [1]. Such peptides were also generated and released by growing bacteria [2], and the production of formyl peptides could be further enhanced by an addition of a peptide deformylase inhibitor to growing Escherichia coli bacteria [3]. Binding studies with radiolabeled peptides delivered additional strong and direct evidence for the presence of formyl peptide-specific receptors with binding characteristics very similar to that of the insulin- and β-adrenergic receptor [4]. These formyl peptide receptors (FPRs) were highly expressed on the cell surface of phagocytes, but not on red blood cells, lymphocytes, platelets, or brain cell membranes [5]. The FPRs together with a number of other known neutrophil chemoattractant receptors are involved in the recruitment of cells from the bone marrow to the blood stream and from the blood stream to inflammatory sites [2,6,7]. At the functional level, formyl peptides typically induce not only chemotactic migration, but also mobilization of receptors and adhesion molecules from intracellular storage granules, secretion of proteolytic enzymes, and reactive oxygen species (ROS) generated by a specialized electron transporting system, the phagocyte NADPH-oxidase [8,9]. The fact that the fMet peptides are recognized as a microbial pathogen associated molecular pattern (PAMP), suggests that the PAMP recognition concept, as yet mainly associated with Toll-like receptors [10], is applicable also to the FPRs. The presence of certain amino acids and their spatial arrangement have been shown to be more important for recognition than the precise amino acid sequence, and the FPRs can possibly recognize more than 10^5 distinct formyl peptides originating from bacteria [11]. In addition, proteins coded for by mitochondrial host cell DNA are translated in a process resembling that in bacteria. As a consequence fMet peptides are recognized not solely as a microbial pathogen associated molecular pattern (PAMP), but also as an endogenous danger/damage-associated molecular pattern (DAMP). The fact that this molecular pattern is associated not only with bacterial infections, but also to the release of danger signals from damaged host cells/tissues, suggests important roles for the FPRs in the inflammatory responses initiated by an established microbial infection and in aseptic inflammatory reactions initiated by host specific events.

1.2. The family of formyl peptide receptors

A receptor expressed in human phagocytes that recognizes formylated peptides (the formyl peptide receptor 1; FPR1) was the first neutrophil receptor to be cloned [12,13]. Soon after the cloning of FPR1, two additional FPR1-like receptors, FPR2 (previously FPRL1) and FPR3 (previously FPRL2), were cloned from a promyelocyte cDNA library, using low-stringency hybridization with the FPR1 cDNA as a probe [12,14]. The three FPRs (FPR1–3) belong to a family of chemoattractant G-protein coupled receptors (GPCRs; [15]), that includes also receptors for the complement component C5a (C5aR), the lipid metabolite platelet activating factor (PAFR) and the chemokine IL-8 (CXCR1/2) [16,17]. Neutrophils express several other GPCRs, including the ones that recognize ATP and UTP ($P2Y_2R$), short free fatty acids (FFAR), and CXCL12 (CXCR4). These receptors comprise a single 350–370 amino acid long polypeptide chain (Fig. 1) that spans the cell membrane seven times. The parts of the receptors facing the cell exterior are believed to interact with activating/inhibiting ligands, while the parts facing the cytosolic side of the membrane are important for signal transduction to downstream functions [18,19]. The transmembrane regions and the signaling domains of the chemoattractant GPCRs share certain sequence similarities, whereas the degree of sequence similarity is less obvious in the extracellular domains supposed to contain the ligand recognition site [20].

Following the initial *in vitro* studies showing that human and rabbit neutrophils recognize and respond functionally to formylated peptides, more genetically oriented studies were conducted, using key reagents, such as cDNA probes and specific antibodies. The results showed genes encoding orthologs of the human FPRs

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