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Chapter 91

FPR Ligands

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ABSTRACT

Formyl peptide receptors (FPRs) belong to the classical G protein-coupled chemoattractant receptor family. They are mainly expressed in mammalian phagocytic leukocytes and play important roles in inflammatory and immune responses. N-formyl peptides produced by Gram-negative bacteria were among the first chemoattractants identified for two FPRs in human beings: FPR1 (originally termed FPR) and FPR2 (originally termed FPRL1). During the past few years, a variety of novel pathogen- and host-derived agonists as well as antagonists for the FPR family have been identified, indicating a broader spectrum of the biological significance of these receptors. Activation of FPRs in leukocytes by agonists induces cell chemotaxis, phagocytosis, release of proinflammatory mediators and gene transcription. Despite these new developments, the in vivo functions of FPRs and their ligands in disease states are not yet fully understood. This chapter summarizes the pharmacological characterization of FPR ligands and their implications in pathophysiological conditions.

INTRODUCTION

Inflammation is essentially a protective attempt by the organisms to remove the injurious stimuli and to initiate the healing process. The migration of leukocytes is crucial in the progression and resolution of inflammation and is tightly regulated. Leukocytes accumulate at the sites of inflammation by responding to pathogen- and host-derived chemotactic factors named chemoattractants. Numerous chemoattractants have been described including chemokines, activated complement fragments, lipid mediators, and pathogen-derived molecules.

Most chemoattractants use G protein-coupled receptors (GPCRs) classified as GPCRs for chemokines and for classical chemoattractants. Formyl peptide receptors (FPRs) belong to the family of classical GPCRs. These receptors are named based on their capacity to recognize the N-formyl methionine motif in synthetic neutrophil chemotactic peptides and in peptide fragments released into the culture supernatants by Escherichia coli. The FPR family members are coupled to pertussis toxin (PTX)-sensitive Gi proteins and mediate neutrophil and monocyte chemotaxis and activation. Although FPRs were identified and cloned in the early 1990s, the biological function of these receptors remains poorly understood. However, with the identification of a number of novel nonformylated and host-derived agonists for FPRs, it is becoming evident that these receptors and their peptide ligands play important roles in innate and adaptive immune responses.

FORMYL PEPTIDE RECEPTORS (FPRs)

The prototype human FPR was cloned from differentiated HL-60 myeloid leukemia cells. Two additional human FPRs were subsequently cloned and were named as FPR-like-1 (FPRL1) and FPRL2. Recently, these GPCRs were renamed as FPR1, FPR2/ALX (FPRL1), and FPR3 (FPRL2). FPR1 and FPR2 share 69% identity at the amino acid level, whereas FPR3 has 56% amino acid sequence identity to human FPR1 and 83% to FPR2. Both human FPR1 and FPR2 are expressed in many cell types, such as myeloid cells including monocytes/macrophages, neutrophils and immature dendritic cells (DCs), endothelial cells, hepatocytes, astrocytes, microglial cells, and fibroblasts. The expression of human FPR3 is more restricted and is detected in monocytes/macrophages and DCs but not in neutrophils. FPR3 is also expressed in plasmacytoid DCs and is upregulated on cell maturation. The signal transduction pathways of FPRs have been more extensively studied with FPR1 in phagocytic cells and in cell lines transfected with cloned receptor. The interaction of FPR1 with its agonist peptide fMLF uncouples trimeric G proteins to elicit a signaling cascade that activates phospholipase C (PLC), protein kinase C (PKC) and PI3 kinases (PI3Ks), which are translated into biological functions of receptor-bearing cells including chemotaxis and mediator release. FPR1 and FPR2 also crosstalk with some receptor tyrosine kinases, such as nerve growth factor (NGF) receptor TrkA and the epidermal growth factor receptor (EGFR).

In the mouse FPR gene family, there are at least eight members, Fpr1 and Fpr-rs1 to Fpr-rs7. Fpr1 encodes mFPR1, which is the ortholog of human FPR1 and binds fMLF with a relatively high affinity, whereas both Fpr-rs1 and Fpr-rs2 encode receptors most similar to human FPR2. The gene product of mouse Fpr-rs2, mFPR2, is a
low-affinity receptor for fMLF. The biological functions of other six mouse Fpr gene homologs have not been characterized. The high sequence divergence between species orthologs (~25–30% between human and mouse) indicates the complicated evolution of the FPR gene family. In vivo, mFPR1-KO mice were more susceptible to infection by Listeria monocytogenes, indicating the role of FPR1 in antibacterial defense. More recently, FPR1 (mFPR1) was shown to mediate trauma reaction in severe inflammatory response syndrome and neutrophil accumulation in the necrotic center of the liver injury, presumably by responding to N-formylated mitochondrial peptides released by ruptured cells. mFPR2-KO mice have also been generated recently and show diminished allergic airway inflammation and accompanying immune responses. FPR2 (mFPR2) expressed in human and mouse neutrophils interacts with an acute-phase protein serum amyloid A (SAA) produced by liver and also by melanoma cells to promote the release of the anti-inflammatory cytokine IL-10, which increases the interaction of neutrophils with invariant natural killer (NK) T cells. These NKT cells limit the immunosuppressive activity of neutrophils by decreasing IL-10, but increasing IL-12 production, thereby enhancing the anti-melanoma host responses. These new developments highlight the critical function of FPR1 and FPR2 in innate and adaptive host responses.

FPR AGONISTS

FPRs recognize many agonists including peptides and non-peptides. The peptides comprise three subtypes, pathogen-derived, host-derived and synthetic. The nonpeptides are either synthetic or derived from the host (Table 1).

| TABLE 1 The Agonists of FPRs |
|-----------------------------|
| **Agonists** | **Origin** | **Receptors** |
| **Peptides** | | |
| Bacteria-derived N-formyl peptides | | |
| N-formyl-MLF | *E. coli* | FPR1, FPR2 |
| N-formyl-MIFL | *S. aureus* | mFPR1 |
| N-formyl-MIVIL | *L. monocytogenes* | FPR1, FPR2 |
| N-formyl-MIGWI | *L. monocytogenes* | FPR1, FPR2 |
| N-formyl-MIVTLF | *L. monocytogenes* | FPR1, FPR2 |
| N-formyl-MIGWII | *L. monocytogenes* | FPR1 |
| N-formyl-MFEDAVAWF | *M. avium* | FPR1 |
| Mitochondria-derived N-formyl peptides | | |
| N-formyl-MMYALF | Mitochondria, ND6 | FPR1, FPR2 |
| N-formyl-MLKLIV | Mitochondria, ND4 | FPR1, FPR2 |
| N-formyl-MYFINILTL | Mitochondria, ND1 | FPR2 |
| N-formyl-MFADRW | Cytochrome c oxidase subunit | FPR1, FPR2 |
| N-formyl-Nle-LF-Nle-YK | Synthetic | FPR1, FPR2 |
| Mitocryptide-2 (MCT-2) | Mitochondria cytochrome b | FPR2 |
| **Microbe-derived peptides** | | |
| Hp (2–20) | *H. pylori* | FPR2, FPR3 |
| T20 (DP178) | HIV-1 gp41 aa. 643–678 | FPR1 |
| T21 (DP107) | HIV-1 gp41 aa. 558–595 | FPR2 |
| V3 peptide | HIV-1 gp120, V3 loop | FPR2 |
| N36 peptide | HIV-1 gp41 aa. 546–581 | FPR2 |
| F peptide | HIV-1 gp120 aa. 414–434 | FPR2 |
| gG-2p20 | Herpes simplex virus type 2 | FPR1 |
| N-formyl HKU-1 coronavirus peptide | Respiratory syndrome coronavirus | FPR1 |
| Agonists                      | Origin                           | Receptors                  |
|------------------------------|----------------------------------|----------------------------|
| **Host-derived peptides**    |                                  |                            |
| CKβ8–1 (human CCL23)         | Chemokine                        | FPR2, CCR1                 |
| SHAAGtide                    | CCL23 N-terminal 18 aa.          | FPR2, CCR1                 |
| Humanin (HN)                 | Neuroprotective peptide          | FPR2, FPR3                 |
| F2L                          | Heme-binding protein             | FPR2, FPR3                 |
| SAA                          | Acute-phase protein              | FPR2                       |
| Annexin 1 / lipocortin 1     | Glucocorticoid-regulated protein | FPR1                       |
| Ac1–25                       | Annexin 1                        | FPR1, FPR2, FPR3           |
| Ac2–26                       | Annexin 1                        | FPR1, FPR2, FPR3           |
| Ac9–25                       | Annexin 1                        | FPR1, FPR2                 |
| Antiflammin-2 (AF2)          | Annexin 1                        | FPR2                       |
| Aβ (1–42)                    | Amyloid precursor                | FPR2                       |
| D2D3                         | uPAR (88–274)                    | FPR2                       |
| SRSRYp                       | D2D3                             | FPR1                       |
| LL-37                        | Cathelicidin                     | FPR2                       |
| PrP (106–126)                | Prion protein                    | FPR2                       |
| Temporin A                   | *Rana temporaria*                | FPR2                       |
| PACAP27                      | Pituitary adenylate cyclase      | FPR2                       |

| **Agonists from peptide library** |                             |                            |
|----------------------------------|-----------------------------|----------------------------|
| WKYMVm                           | Peptide library             | FPR1, FPR2, FPR3           |
| WKYMVM                            | Peptide library             | FPR2, FPR3                 |
| MMK-1                             | Peptide library             | FPR2                       |
| MMWLL, formyl-MMWLL              | Peptide library             | FPR1                       |
| CGEN-855A                        | Peptide library             | FPR2                       |

| **Nonpeptides**                  |                             |                            |
|----------------------------------|-----------------------------|----------------------------|
| Lipoxin A4 and aspirin-triggered | Eicosanoids                 | FPR2, AhR                  |
| Lipoxins                         |                             |                            |
| Quinazolinone derivative (Quin-C1) | Combinatorial library     | FPR1, FPR2                 |
| Pyrazolone, 4-iodo-substituted, no. 43 | Combinatorial library | FPR1, FPR2                 |
| AG-14                             | Drug-like molecule library  | FPR1                       |
| Compound 1 and 2                 | Arylcarboxylic acid hydrazide derivatives | FPR2 |

| **Others**                       |                             |                            |
|----------------------------------|-----------------------------|----------------------------|
| PD168368                         | Gastrin-releasing           | FPR1, FPR2, FPR3           |
| PD176252                         | peptide/neuromedin B receptors (BB1/BB2) | FPR1, FPR2, FPR3 |
| Trp-and Phe-based analogs        | PD168368/ PD176252         | FPR1, FPR2, FPR3           |
| Related nonpeptide/nonpeptoid analogs | PD168368/ PD176252 | FPR1, FPR2                 |
| A-71623                          | Cholecystokinin-1 receptor agonist | FPR1, FPR2 |

**TABLE 1 The Agonists of FPRs—Cont’d**
Peptides

Microbial Peptides

Synthetic or E. coli-derived fMLF is most widely used to characterize the function of FPR1 and FPR2. The chemotactic activity of FMLF for neutrophils is increased by an oligoethylene glycol substituent. In addition to its chemotactic activity for myeloid cells, fMLF also induces intestinal epithelial cell migration and participates in epithelial restitution and wound closure. The activity of fMLF on intestinal epithelial cells is attributed to FPR1, which is located along actin filaments in lamellipodial and filopodial protrusions associated with activated PI3K, Rac1, and Cdc42.

In human mesenchymal stem cells, fMLF stimulates signaling pathways coupled to FPR1 that drive phospholipase C (PLC)/phospholipase D (PLD)-Ca2+-calmodulin-dependent kinase II-ERK-CREB during osteogenic differentiation. By contrast, fMLF inhibits the expression of PPARγ and suppresses adipocytic commitment during differentiation.

Thus, FPR1 and its agonist fMLF also display important functions in nonhematopoietic cells. In addition to fMLF, there are other N-formyl peptides derived from bacteria. For example, a pentapeptide fMIVIL from L. monocytogenes and a tetrapeptide fMIFL from Staphylococcus aureus preferentially activate mFPR1. fMFEDA V AWF derived from Mycobacterium avium also activates FPR1.

Although formylated peptides are believed to be mainly of bacterial origin, three hexapeptides fMLKLIV, fMMYALF, and fMFADRW corresponding to the N terminus of mitochondrial NADH dehydrogenase subunits 4 and 6 and cytochrome c oxidase subunit I are also potent chemotactic agonists for FPR1 and FPR2. Other mitochondrial N-formyl peptides (e.g. mitochondrial transcription factor A (TFAM) released during cell necrosis) also activate monocytes to release IL-8 (CXCL8), a neutrophil specific chemokine.

Mitochondria are considered to be evolutionary endosymbionts derived from bacteria and may thus contain bacterial molecular motifs. On cell injury, mitochondrial “damage”-associated molecular patterns (DAMPs) are released into the circulation and elicit neutrophil-mediated organ injury. The effect of mitochondrial DAMPs (MTDs) containing formyl peptides on neutrophils is mediated by FPR1, while mitochondrial DNA in MTDs activates Toll-like receptor (TLR) 9. Some functional “cryptic” peptides hidden in protein structures are termed “cryptides,” one of which, mitocryptide-2 (MCT-2) in mitochondrial cytochrome b, was a specific agonist for FPR2 and may play a role in inflammatory host responses.

Non-formylated Peptides

Many peptides without N-formyl group have been identified as agonists for FPRs. A Helicobacter pylori peptide Hp (2–20) activates FPR2 and FPR3 and may contribute to the development of pyloritis by the recruitment of monocytes and basophils to the gastric mucosa in response to bacterial infection. Hp (2–20) also induces the migration, proliferation, and the expression of vascular endothelial growth factor (VEGF) in gastric epithelial cells, suggesting that this H. pylori peptide may promote gastric mucosal healing.

HIV-1 envelope proteins contain segments capable of interacting with either or both FPR1 and FPR2, including at least three domains in gp120, as well as two sequences from gp100. Although T20/DP178 from gp41 specifically activates human FPR1 in vitro and the murine FPR1 homolog mFPR1 in vivo, T21/DP107 from gp41 uses both FPR1 and FPR2 with higher efficacy on FPR2. N36 from gp41, which partially overlaps with T21/DP107, solely signals through FPR2. Two peptide domains in HIV-1 gp120 are potent chemoattractants and activators for FPR2, but not for FPR1, in human phagocytic leukocytes. One peptide domain, F peptide, consists of 20-amino acid residues and is located in the C4–V4 region of gp120 of the HIV-1 LAI strain. Another peptide of 33 amino acids (V3 peptide) is derived from a linear sequence of the V3 region of the HIV-1 MN strain. However, despite the existence of peptide domains in HIV-1 envelope proteins that interact with FPRs, it remains unclear whether such domains are released by enzymatic cleavage of the envelope proteins in vivo during HIV-1 infection.

In addition to peptides from HIV protein, there are also some other viral proteins containing sequences that act as FPR ligands. gG-2p20, derived from Herpes simplex virus type 2 (HSV-2), is a chemoattractant for both monocytes and neutrophils by activating FPR1. It is noteworthy that an HKU coronavirus peptide, MYVKWPWYVWL, is a potent antagonist, but N-formyl-MYVKWPWYVWL is a potent agonist for FPR1, suggesting the importance of N-terminal modification in the biological activity of a synthetic peptide.

Host-Derived Peptides

Peptides Associated with Amyloidogenic Diseases

At least 3 amyloidogenic polypeptides associated with chronic inflammation and amyloidosis have been identified as agonists for FPR2. They are serum amyloid A (SAA), β amyloid peptide Aβ42, and PrP106–126.

SAA, an acute-phase protein, increases in serum by as much as 1000-fold in inflammatory diseases, e.g. trauma, infection, and other environmental stress. Recombinant human SAA as the first mammalian host-derived peptide ligand identified for FPR2 is chemotactic for myeloid cells and T lymphocytes. In synovial tissues of patients with rheumatoid arthritis (RA), highly expressed SAA promotes the proliferation of human fibroblast-like synoviocytes (FLS)
and induces the expression of matrix metalloproteinases by FLS.\textsuperscript{32} SAA also protects RA FLS from apoptotic death induced by serum starvation, anti-Fas IgM, and sodium nitroprusside\textsuperscript{32} by interacting with FPR2. In addition, SAA stimulates CCL-2 production via FPR2 by human umbilical vein endothelial cells and monocytes contributing to the progression of atherosclerosis.\textsuperscript{21,22} SAA also stimulates M-CSF and CCL-2 expression in human and mouse hepatocellular carcinoma cells.\textsuperscript{23} SAA may interact with other receptors; the nucleotide receptor P2X7 was reported to mediate the protective role of SAA in human neutrophil apoptosis.\textsuperscript{1}

\(\text{A}_\beta_{42}\) is a 42-amino acid cleavage product of the amyloid precursor protein in the brain and a pathogenic factor for Alzheimer’s disease (AD).\textsuperscript{19} FPR2 mediates the migration and activation of mononuclear phagocytes, including macrophages and brain microglia, induced by \(\text{A}_\beta_{42}\). FPR2 also promotes the endocytosis of \(\text{A}_\beta_{42}\) by macrophages and microglia in the form of receptor and ligand complexes. If the exposure of macrophages to \(\text{A}_\beta_{42}\) is transient, the internalized \(\text{A}_\beta_{42}\) is degraded and FPR2 is rapidly recycled back to the cell surface. However, prolonged exposure results in accumulation of \(\text{A}_\beta_{42}\) and FPR2 complexes in macrophages, which culminates in progressive fibrillary aggregation of \(\text{A}_\beta_{42}\) and the macrophage death.\textsuperscript{3} These observations suggest that FPR2 not only mediates the proinflammatory activity of \(\text{A}_\beta_{42}\) but it may also play an important role in the fibrillary deposition of \(\text{A}_\beta_{42}\), a typical pathologic feature of AD.\textsuperscript{3}

PrP\textsubscript{106–126} is a prion protein fragment that is produced in human brains with prion disease.\textsuperscript{19} PrP\textsubscript{106–126} induces the migration and activation of human myeloid cells through FPR2 and may thus contribute to the inflammatory responses seen in the prion disease.

Although the interaction of FPRs with peptide agonists in general elicits proinflammatory responses, FPR2 may exert a neuroprotective effect by recognizing a small peptide, humanin (HN), which was identified through a functional expression screening based on its ability to suppress neuronal cell death seen in familial AD.\textsuperscript{32} HN is chemotactic for monocytes through the use of FPR2 and by competitive blockage of FPR2 abrogates the intracellular fibrillary aggregation of \(\text{A}_\beta_{42}\). In neuronal cells, HN protects the cells from apoptosis induced by \(\text{A}_\beta_{42}\). Thus, FPR2 may transduce life and death signals in neuronal cells depending on the nature of the agonists it encounters and may determine the outcome of AD. However, it has also been reported that HN-mediated protection of F11 neurohybrid cells is mediated through tyrosine kinases and STATs which are unlikely coupled to FPR2.\textsuperscript{32}

### Peptides Associated with Inflammatory and Antimicrobial Responses

Urokinase-type plasminogen activator\textsuperscript{18} is a serine protease known for its ability to regulate fibrinolysis. uPA is required for leukocyte trafficking to sites of inflammation \textit{in vivo}, and it indirectly activates FPR2 through the liberation of a chemotactic peptide D2D\textsubscript{388–274} from uPA receptor (uPAR) (CD87).\textsuperscript{19} Consistent with this, the presence of both uPAR and FPR2 on the cell surface is required for the chemotactic activity of uPA, whereas FPR2 alone is sufficient for the effect of D2D\textsubscript{388–274}. Thus, uPAR may facilitate fibrinolysis and serve as a source of chemotactic proinflammatory peptides necessary for host defense. SRSRY, a peptide corresponding to residues 88–92 in uPAR is an agonist of FPR1.\textsuperscript{32} In addition, uPAR\textsubscript{84–95} induces basophil migration by interacting with both FPR2 and FPR3 and mobilizes hematopoietic stem cells by activating FPR1 by desensitizing the chemokine receptor CXCR4.\textsuperscript{32}

FPRs interact with some bactericidal peptides contained in human neutrophil granules. LL-37, an enzymatic cleavage fragment of neutrophil granule protein cathelicidin and its mouse homolog CRAMP (see the Cathelicin Chapters in Bacterial/Antibiotic Peptides section of this book), activates FPR2 to promote myeloid cell chemotaxis.\textsuperscript{32} LL-37 also induces angiogenesis via FPR2 expressed on endothelial cells. In CRAMP-deficient mice, neovascularization is decreased during cutaneous wound repair.\textsuperscript{32} Another antibacterial neutrophil granule protein, cathepsin G, is a serine protease (see the Peptide Biosynthesis/Processing section of this book) and a specific agonist for FPR1.\textsuperscript{32} It is therefore conceivable that the capacity of antimicrobial neutrophil granule peptides to interact with FPRs may aid in the recruitment of phagocytic leukocytes to the sites of infection and thus accelerate the killing and clearance of the invading bacteria.

FPR2 has also been reported to interact with a chemoattractant peptide (CK\textsubscript{β8}) that activates phagocytic leukocytes. CK\textsubscript{β8} (CCL23/MPIF-1) uses a typical GPCR, CCR1, for its leukocyte chemotactic activity. However, an N-terminal truncation product of the CK\textsubscript{β8} splice variant CK\textsubscript{β8}-1 (22–137 aa) activates myeloid cells and FPR2-transfected cell lines at low nanomolar concentration range and is thus considered one of the most potent FPR2 agonists identified so far.\textsuperscript{3} However, the cell sources of CK\textsubscript{β8} and the circumstances for its release \textit{in vivo} remain unclear. Interestingly, an 18-amino acid peptide from CK\textsubscript{β8}, SHAAG, activates FPR2 activity but not CCR1, revealing a structural basis for the receptor specificity of CK\textsubscript{β8}.

### Annexin I (Anx A1) and its N-Terminal Peptides

It is intriguing that some FPR agonists have dual roles in inflammatory responses. Anx A1, also named lipocortin I, is a glucocorticoid-regulated, phosphorylated-binding protein possessing both proinflammatory and anti-inflammatory activity mediated in part by FPR1. Expressed in a variety of cell types, Anx A1 is particularly abundant in neutrophils where it is located on the outer cell surface and serves to inhibit neutrophil transendothelial migration. At low
Temporin A (TA) is a frog-derived antimicrobial peptide that was found to induce the migration of human monocytes, neutrophils, and macrophages. Characterization of the signaling characteristics of TA in monocytes and the use of receptor transfected HEK293 epithelial cell line revealed that this peptide uses FPR2 as a receptor. TA is also chemotactic in vivo, because it elicited infiltration of neutrophils and monocytes into the injection site in mice. Another temporin peptide Rana-6 is chemotactic for human phagocytes also by using FPR2. Thus, frog-derived temporins have the capacity to chemoattract phagocytes through human FPR2 and the mouse homolog mFPR2, suggesting the participation of amphibian antimicrobial peptides in host innate immunity. Because the expression of FPR homolog(s) has not been reported in species other than mammals, it will be interesting to clarify whether such receptor exists in amphibians and other species such as insects, which normally rely on the secretion of antimicrobial peptides as a natural host defense.

Other Host-Derived Peptides

It is important that highly efficacious agonist peptide for FPR3 has been isolated from spleen extracts. F2L, an acetylated amino-terminal 21-amino acid peptide, is derived from the cleavage of the human heme-binding protein, an intracellular tetrapyrolle-binding protein. F2L chemoattracts and activates monocyte-derived DCs, thus seems to be a novel and unique natural chemoattractant peptide for FPR3 in DCs and monocytes, in agreement with the selective expression of FPR3 in these cells, suggesting its role in linking innate and adaptive immune responses by activating antigen-presenting FPR3+ DCs, which express reduced levels of FPR1 and FPR2. In mice, mouse F2L activates neutrophils by using mFPR2, a homolog of human FPR2, because neutrophils from mFPR2−/− mice lost all responsiveness to F2L. These results suggest that mFPR2 in mice is likely a homolog of both human FPR2 and FPR3.

Agonists from Peptide Library

Random small peptide libraries are rich sources of FPR agonists and have yielded a number of potent chemotactic stimulants for leukocytes. For instance, WKYMVm, a hexapeptide representing a modified sequence isolated from a random peptide library, was initially reported to be an efficacious stimulant of human B lymphocytes, monocytic cell lines, as well as peripheral blood neutrophils. It was subsequently found that WKYMVm uses both FPR1 and FPR2, and is by far the most potent peptide agonist for FPR2 to chemoattract and activate human phagocytic cells. The WKYMVm analog, produced by converting the D-amino acid methionine at the C-terminus into to an L-amino acid, becomes a more selective agonist of FPR2 and also a weaker activator of FPR3. Another peptide, MMK-1, which is also derived from a random peptide library, is a potent and very selective chemotactic agonist for FPR2. Although FPR agonists identified in random peptide libraries seem not to be pathophysiologically relevant to diseases, they nevertheless constitute useful pharmacological tools for the studies of FPR signaling pathways, structural basis of the ligands versus receptors, and identification of receptor antagonists that could be valuable in conditions resulted from receptor overactivation.

Nonpeptides

Host-derived Nonpeptide FPR Agonists

LXA4, biosynthesized from arachidonic acid metabolism, is the first described endogenous lipid ligand for FPR2. However, despite the original observation of LXA4 being a chemotactic agonist for FPR2, this lipid metabolite has been shown to mainly exert inhibitory activities on a variety of inflammatory responses. LXA4 inhibits the production of inflammatory cytokines by epithelial cell lines in response to TNFα and LPS, while it simulates the expression of the anti-inflammatory cytokine IL-10. In DCs, LXA4/ATL activates the suppressor of cytokine signaling (SOCS)-2 and
in T cells, LXA4 attenuates TNFα release by impairing ERK signaling. LXA4 also prevents renal fibrosis by inhibiting PDGF-induced TGF-β production in mesangial cells. In addition, LXA4 attenuates inflammation-induced pain and displays proresolution properties in various disease models, including dermal inflammation, ischemia/reperfusion injury, peritonitis, colitis, cystic fibrosis, asthma, parasitic infection and glomerulonephritis. The multiplicity of the anti-inflammatory activity of LXA4 raises the question of how this lipid mediator shares FPR2 with other peptide agonists that trigger a proinflammatory signaling pathway. One plausible explanation was that LXA4 binds to an FPR2 domain, which is different from the domains utilized by peptide agonists. However, because LXA4 has been reported to interact with multiple receptors depending on the cell types, it remains to be clarified whether the biological function of LXA4 is the consequence of interaction with more than one receptor.

**Agonists from Small Compound Library**

In addition to recognizing peptide agonists and the lipid agonist LXA4, nonpeptide small compounds may also be recognized by FPRs. For instance, a quinazolinone derivative Quin-C1 was reported as a highly selective agonist for FPR2. High-throughput screening of compound library has yielded compound 43 that reduces inflammation in an ear swelling model in mice through FPR2, but it was later shown as a preferential agonist for FPR1 that activates neutrophils. In addition, AG-14 was found to activate neutrophils by using FPR1. Compounds 1 and 2, arylcarboxylic acid hydrazide derivatives, are agonists for FPR2 that induce the production of TNFα by macrophages.

**Others**

PD168368 and PD176252, antagonists of gastrin-releasing peptide/neuromedin B receptors (BB1/BB2), are potent agonists of both FPR1 and FPR2. Their Trp-and Phe-based analogs and related nonpeptide/nonpeptoid analogs are also agonists of FPRs. In addition, the cholecystokinin-1 receptor agonist A-71623 activates both FPR1 and FPR2. These physiologically relevant nonpeptide agonists for FPRs may contribute to the regulation of inflammation and immune responses. The similarities and divergence of their binding domains in FPRs and signal transduction pathways as compared with that of peptide agonists will be of great pathophysiological and pharmacological interests.

**FPR ANTAGONISTS**

The importance of FPRs in microbial infection and host immune responses suggests that these GPCRs may be targets of pharmacological intervention to attenuate complications associated with undesirable receptor overactivation. This consideration leads to the search and development of FPR antagonists, which have yielded many molecules as listed in Table 2.

**Microbial FPR Antagonists**

Cyclosporin H (CsH) produced by a fungus is an inverse agonist (negative agonist) that suppresses the constitutive activity of FPR1. Similar to CsH, CsA also inhibits fMLF-induced neutrophil degranulation and cell activation, although the inhibitory effect is less potent than CsH. PDGF-inhibitor protein (FLIPr), a staphylococcal anti-inflammatory protein, directly binds to FPR1 and FPR2 and attenuates fMLF-induced cell activation. At low concentrations, FLIPr selectively inhibits the binding of some FPR2 agonists including MMK-1, WKYMVM, PrP (106–126), and Apβ_12. At high concentrations, it reduces fMLF binding to FPR1. Iodinated chemotaxis inhibitory protein of S. aureus (CHIPS) is a potent peptide antagonist of FPR1. LDDL is an immunosuppressive hexapeptide derived from retroviral trans-membrane envelope protein p15E and is a specific FPR1 antagonist. Peptides derived from the membrane proximal region of fusion proteins from HIV 1 and 2, Coronavirus 229 E, severe acute respiratory syndrome (SARS) coronavirus and Ebola virus are all potent antagonists of FPR1. These observations suggest the capacity of microbia to produce molecules that inhibit FPR-mediated function of host cells that are directly involved in antimicrobial responses.

**Host-derived Antagonists**

Several “endogenous” FPR antagonists have been reported. Deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) identified as antagonists for FPR1 with unclear pathophysiological significance. CDCA also partially inhibits FPR2 agonist-induced myeloid cell activation. Spinorphin (LVVYPWT), which belongs to the family of hemorphins, is a peptide antagonist for FPR1. In addition, Quin-C7, a synthetic nonpeptide compound, is a highly selective antagonist for FPR2. Interestingly, Quin-C7 is derived from FPR2 agonist Quin-C1 through chemical modification.

**Synthetic Peptide Antagonists**

Boc1 (t-Boc-MLF) and Boc2 (t-Boc-FLFLF) were originally found as antagonists for FPR1. However, a recent study shows that at low micromolar concentrations, both Boc1 and Boc2 are selective for FPR1; at high micromolar concentrations, Boc2 also partially inhibits the function of FPR2. The WRW4 obtained from hexapeptide library is a more selective antagonist for FPR2. Further, a cell permeable peptide PBP10, which is derived from a PIP2-binding
domain of the cytoskeleton protein gelsolin, displays FPR2 antagonist activity by its ability to penetrate cell membrane to act on the intracellular domains in FPR2.  

**Other Antagonists**

The nonsteroidal anti-inflammatory drug (NSDAID) piroxicam, a nonselective cyclooxygenase (COX)-inhibitor, can reduce neutrophil superoxide production induced by FPR1 agonists but had no significant effect on FPR2-mediated responses. However, piroxicam inhibits WKYMVm-induced neutrophil intracellular calcium mobilization and superoxide release when the drug was combined with the FPR2 specific antagonist WRW4. The inhibitory effect of piroxicam is dependent on its binding to the receptor, therefore seems to be FPR specific.

**PERSPECTIVES**

In this chapter, we attempted to provide a comprehensive overview of FPR ligands including agonists and antagonists. It is now clear that FPRs play important roles in the regulation of inflammatory and immune responses. The process of understanding of the biological roles of FPRs has been

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**TABLE 2 FPR Antagonists**

| Antagonists                        | Origin                      | Receptors |
|-----------------------------------|-----------------------------|-----------|
| **Microbial antagonists**         |                             |           |
| Chemotaxis inhibitory protein of S. aureus (CHIPS) | *Staphylococcus aureus*     | FPR1      |
| FPR1-inhibitor protein (FLIPr)    | *Staphylococcus aureus*     | FPR1, FPR2|
| Cyclosporin A (CsA)               | Fungi                       | FPR1      |
| Cyclosporin H (CsH)               | Fungi                       | FPR1      |
| HKU coronavirus peptides          | Coronavirus                  | FPR1      |
| Coronavirus 229E peptides          | Coronavirus                  | FPR1      |
| SARS coronavirus peptides         | Coronavirus                  | FPR1      |
| HIV-1 peptides                    | HIV-1                       | FPR1      |
| HIV-2 peptides                    | HIV-2                       | FPR1      |
| Ebola peptide                     | Ebola virus                 | FPR1      |
| LDLLDL                             | Retrovirus                  | FPR1      |
| **Synthetic peptide antagonists** |                             |           |
| i-Boc-MLF                         | Synthetic peptide           | FPR1      |
| t-Boc-MLF (Boc1)                   | Synthetic peptide           | FPR1      |
| t-Boc-FLFLF (Boc2)                 | Synthetic peptide           | FPR1, FPR2|
| Trp-Arg-Trp-Trp-Trp (WRW4)         | Random peptide library      | FPR1      |
| PBP10                              | Gelsolin                    | FPR2      |
| **Host-derived antagonists**      |                             |           |
| Spinorphin                        | Bovine spinal cord          | FPR1      |
| Chenodeoxycholic acid (CDCA)      | Endogenous (bile acid)      | FPR1, FPR2|
| Deoxycholic acid (DCA)            | Endogenous (bile acid)      | FPR1      |
| **Others**                        |                             |           |
| Piroxicam                         | Nonsteroid anti-inflammatory drugs (NSDAIDs) | FPR1      |
| SDS                                | Amphiphile                  | FPR1      |
considerably prompted by the identification of a variety of ligands from different sources, in particular, from microbes and human. In addition to the evidence that the FPRs are involved in antimicrobial defense and inflammation, they are also implicated in the progression of cancer. For example, FPR1 is selectively expressed by highly malignant human glioma cells and increases the motility, growth, and angiogenesis of human glioblastoma by interacting with host-derived agonist(s) released by necrotic tumor cells.\textsuperscript{35} An effort to identify potential FPR1 agonist(s) in glioma cell supernatant revealed Anx A1 as a major component.\textsuperscript{31} Therefore, FPR1 and Anx A1 axis may constitute targets for novel antiglioma therapy. Human FPR2 is also expressed in astrocytoma cell line,\textsuperscript{9} hepatocellular carcinoma,\textsuperscript{25} and breast cancer\textsuperscript{17} with yet unclear pathophysiological implications.

It is intriguing that FPR1 and FPR2 exhibit a promiscuity to interact with a broad range of ligands that do not share significant primary or tertiary structure similarities. How a single FPR could bind such a diverse array of ligands, from small peptides to nonpeptide compounds, remains to be elucidated, possibly through classical receptor chimera approaches and site-directed mutagenesis. Further investigation should not only benefit better understanding of the role of FPRs and their ligands in disease states but also the identification of pharmacological targets for the development of therapeutic agents.

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