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Review

New development in studies of formyl-peptide receptors: critical roles in host defense

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ABSTRACT

Formyl-peptide receptors are a family of 7 transmembrane domain, Gi-protein-coupled receptors that possess multiple functions in many pathophysiologic processes because of their expression in a variety of cell types and their capacity to interact with a variety of structurally diverse, chemotactic ligands. Accumulating evidence demonstrates that formyl-peptide receptors are critical mediators of myeloid cell trafficking in the sequential chemotaxis signal relays in microbial infection, inflammation, and immune responses. Formyl-peptide receptors are also involved in the development and progression of cancer. In addition, one of the formyl-peptide receptor family members, Fpr2, is expressed by normal mouse-colon epithelial cells, mediates cell responses to microbial chemotactic agonists, participates in mucosal development and repair, and protects against inflammation-associated tumorigenesis. These novel discoveries greatly expanded the current understanding of the role of formyl-peptide receptors in host defense and as potential molecular targets for the development of therapeutics. *J. Leukoc. Biol.* 99: 425–435; 2016.

Introduction

Leukocyte infiltration is a hallmark of inflammation, immune responses, and cancer and is critical for disease progression and resolution. Leukocyte trafficking and homing are mediated mainly by 2 families of GPCRs: 1 that recognizes chemokines, and 1 that recognizes classic chemoattractants derived from pathogens, damaged host tissues, and tumors [1–4]. FPRs belong to the family of classic chemoattractant GPCRs. Compared with other chemoattractant receptors, FPRs exhibit unique properties in the number of variants and the spectrum of ligands they interact with. The number and the sequences of genes coding for FPR members vary considerably among mammalian species. The human FPR family has 3 members, *FPR1*, *FPR2*, and *FPR3* (formerly *FPR*, *FPRL1*, and *FPRL2*, respectively) [5–8]. The mouse FPR (mFPR or Fpr) gene family consists of at least 8 members [9]. mFPR1, now officially termed *Fpr1*, is considered the mouse ortholog of human *FPR1*, whereas *Fpr2* is structurally and functionally most similar to human *FPR2* [10]. The other 6 murine *Fpr* genes are expressed in leukocytes, but their encoded receptors remain unknown [8].

A prominent feature of FPR family members is their ligand diversity, which includes a variety of structurally diverse ligands [8, 11]. Therefore, FPRs (Fprs) are also considered as a class of PRRs that interact with either pathogen-associated chemotactic ligands (chemotactic PAMPs), or DAMPs. With the availability of genetically engineered mouse strains deficient in one or more *Fprs*, the critical roles of FPRs (Fprs) in diseases are increasingly being recognized [8]. These receptors are found to not only mediate leukocyte trafficking in disease states but also promote myeloid cell differentiation, colon epithelial homeostasis, and cancer progression. Therefore, a better understanding of the biologic significance of FPRs should have important clinical relevance. This review will focus on some recent developments in FPR studies. The readers are recommended to refer to other excellent reviews for more aspects of FPRs [6, 8, 9, 12].

THE PRR PROPERTIES OF FPRs AND IMPLICATIONS IN HOST DEFENSE

FPR1 and *FPR2* [8, 9] were originally identified based on their capacity to recognize *N*-formyl peptides produced in nature by

Abbreviations: Aβ42 = amyloid β peptide 42, Anx A1 = annexin 1, BM = bone marrow, CCL = chemokine (C-C motif) ligand, CCR = C-C chemokine receptor type, CHIPS = chemotaxis inhibitory protein of *Staphylococcus aureus*, CRAMP = cathelin-related antimicrobial peptide, CX3CR = chemokine (C-X3-C motif) receptor, CXCL = chemokine (C-X-C motif) ligand, CXCR = C-X-C chemokine receptor type, DAMP = damage-associated

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the degradation of either bacterial or host cell mitochondrial proteins, such as *N*-formyl peptides, which represent a major byproduct of bacterial and mitochondrial metabolism [13, 14]. As such, these peptides are not only ubiquitous in the context of inflammation and infection but are highly diverse structurally and functionally [9, 15]. These peptides act as danger signals, capable of alerting the immune system to elevated levels of cell death or to exposure to pathogenic bacteria. This is supported by the findings that both *Fpr1*- and *Fpr2*-deficient mice display increased susceptibility to microbial, such as *Listeria monocytogenes* infection [16, 17].

In a mouse septic-syndrome model, *Fpr1* was critical in mediating neutrophil accumulation in response to circulating mitochondrial peptides [18], and in a liver sterile-injury model, it acted as an essential mediator of neutrophil accumulation, subsequent to chemokine GPCRs, in the necrotic center of the wound [19]. Consistent with its capacity to recognize bacterial chemotactic PAMPs, *Fpr1* acts as a major participant in the host–commensal interaction during dysbiosis, as demonstrated in acute *Toxoplasma gondii* gastrointestinal infection of mice in which the control of commensal outgrowth was a highly coordinated process involving both the host response and microbial signals. Notably, neutrophil infiltration into the intestinal lumen results in the generation of organized, intraluminal structures that encapsulate commensals and limit their contact with the epithelium. Formation of these "luminal casts" depends on *Fpr1* and, consequently, after infection, mice-deficient in *Fpr1* display increased microbial translocation, poor commensal containment, and increased mortality [20].

One of the recent progresses in studies of FPRs is the identification of many host-derived agonist peptides as chemotactic DAMPs for FPRs, including SAA, Aβ42, LL-37, and a neutrophil granule protein, cathepsin G [21–26]. SAA is an acute-phase protein, which, by interacting with *FPR2*, converts neutrophils from a protumor to an antitumor phenotype [27, 28]. Aβ42 is a major causative factor of brain inflammation in Alzheimer disease [24, 29]. LL-37 and its mouse homolog CRAMP are antibacterial and also have alarmin activity. Recognition of LL-37 by *FPR2* on tumor cells promotes angiogenesis by recruiting BM mesenchymal stem cells into the stroma of human ovarian cancer xenografts [30]. The biologic significance of cathepsin G as an *FPR1* ligand remains unclear.

Another chemotactic DAMP, originally reported as an *FPR1* ligand, Anx A1, was initially identified as an anti-inflammatory protein because of its capacity to retain neutrophils in blood vessels during inflammatory responses. However, Anx A1 was also found to increase the invasiveness of certain tumor cells by interacting with both *FPR1* and *FPR2* [31]. In addition, Anx A1 was shown as a major chemoattractant released by necrotic human GBM cells to activate *FPR1* on live GBM cells [21]. **Table 1** is an as-yet-incomplete list of important chemotactic PAMPs and DAMPs reported to date for FPRs (Fprs). The list also includes an FPR3 (FPRL2) ligand, F2L, which is a chemotactic peptide fragment derived from heme-binding protein that chemoattracts DCs [38], with a biologic role in vivo that is yet to be defined. Unlike *FPR1* and *FPR2*, the expression of *FPR3* is rather limited to monocytes and DCs and is highly phosphorylated and is more localized to small intracellular vesicles. This suggests that *FPR3* rapidly internalizes after binding its ligands and, thereby, may serve as a "decoy" receptor [40, 41]. The mouse counterpart of *FPR3* is not very clear at this point. Mouse neutrophils were chemoattracted by F2L. However, the cells from *Fpr2* knockout mice totally lost chemotactic response to F2L, suggesting that mouse *Fpr2* may function as both human *FPR2* and *FPR3* [39].

Human *FPR2* and its mouse counterpart *Fpr2* have also been reported to interact with lipid-mediator LXA4 to either exert a proinflammatory or resolving activity in host responses in stress. The exact mechanistic basis for the dual role of LXA4 in host defense is not well established because the mediator has not been unanimously confirmed to be a bona fide FPR2 (Fpr2) agonist, and it has also been reported to interact with other cell surface or intracellular receptors, such as leukotriene receptors and aryl hydrocarbon receptor. Because of its unique physicochemical nature, some laboratories have not been able to verify LXA4 as an agonist for FPR2 or Fpr2 either for proinflammatory or anti-inflammatory activity. Therefore, despite the initial definition of *FPR2* as ALX to indicate its identity as a LXA4 receptors, the controversy persists and should require a cooperative and rigorous effort to definitely confirm or disqualify LXA4 as an FPR2 (Fpr2) agonist.

It is conceivable that with the progress of more rigorous studies, additional chemotactic PAMPs and DAMPs are likely to be added to the list to better explain the complexity of the nature of FPRs.

PARTICIPATION OF FPRs IN ORCHESTRATED LEUKOCYTE TRAFFICKING

It has been realized that both physiologic and pathologic trafficking of leukocytes in vivo is mediated by >1 sequentially expressed chemoattractant GPCRs on the cell surface regulated by differentiation or maturation stimulants in the microenvironment. Recent studies with genetically engineered mice in a variety of disease models have positioned FPRs (Fprs) in the process of leukocyte sensing of chemotactic cues, established either by chemotactic PAMPs or DAMPs, in bacterial infection, immune responses, and wound healing.

Fpr1/Fpr2–CXCR2-mediated neutrophil recruitment in the livers of mice infected with *Listeria monocytogenes*

Listeria species is an opportunistic pathogen that causes severe infections in immunocompromised individuals [42]. The incidence of listeriosis in human is low [43, 44], but the lethality

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molecular pattern, DC = dendritic cell, EGF = epidermal growth factor, F2L = formyl-peptide receptor (FPR)-like (FPRL)-2 ligand, FPR = formyl-peptide receptor, FPRL = formyl-peptide receptor-like, GBM = glioblastoma multiforme, GC = gastric cancer, GPCR = Gi–protein-coupled receptors, LL-37 = human cathelicidin, LLC = Lewis lung cancer, LTB4 = lipid chemoattractant leukotriene B4, LXA4 = lipoxin A4, OVA = ovalbumin, PAMP = pathogen-associated molecular pattern, PRR = pattern recognition receptor, SAA = serum amyloid A, TAM = tumor-associated macrophage, UC = ulcerative colitis, WT = wild type

TABLE 1. The ligand promiscuity of FPRs

Source agonists	Diseases	FPR1 (Fpr1)	FPR2 (Fpr2)	FPR3 (Fpr2)	References
Bacteria					
fMLF (*E.coli*)	Infection	++++	++		[5, 9] H and M
Listeria peptides	Infection	++++	+++		[17, 32] M
Helicobacter pylori (2–20)	Stomach cancer		+++	++	[8, 9] H
Host					
Mitochondrial PEP	Sepsis, injury		+++	++++	[13, 14, 18] H and M
LL37 (mouse CRAMP)	Host defense		+++		[25] H, [26, 33] M
Cathepsin G	Host defense	+++			[22] H
SAA	Inflammation		++++		[23] H, [28] M
$A\beta_{42}$	Alzheimer disease		++++		[24] H, [29] M
Prion protein 106–126	Prion dis		+++		[24] H
Anx A1	Wound healing	++	+++	++	[21, 31, 34] H, [35, 36] M
uPAR fragment	Coagulation		+++		[37] H
F2L	DC migration		++	+++	[38] H, [39] M

FPRs recognize many pathogen- and host-derived chemotactic peptides associated with inflammation and cancer. +, relative potency of interaction; H, human FPRs; M, mouse Fprs; PEP, phosphoenolpyruvate; uPAR, urokinase plasminogen activator receptor.

rate is as high as 30% in infected patients [45, 46]. *Listeria* sp. enters a variety of mammalian cells in which the bacteria replicate and spread from one cell to the next to escape host immune surveillance [47–54]. The resistance to *Listeria* sp. infection is dependent on mobilization of immune responses, in particular neutrophils, into the infected site as the first and key step of host defense [50, 55, 56]. A classic paradigm defines one of the PRRs, TLR2, as a key mediator of host resistance by interacting with bacteria lipoprotein to directly promote the transcription of proinflammatory cytokines and chemokines through inflammasome pathways in immune cells to initiate host responses, in which chemokines CXCL1/2 are believed to be responsible for rapid neutrophil accumulation at the site of infection (**Fig. 1**) [57].

It is, therefore, perplexing and surprising that deficiency in either *Fpr1* or *Fpr2* exacerbated the severity of *Listeria* sp. infection of mice [17]. A recent study aimed at elucidating the role of *Fprs* in neutrophil accumulation in *Listeria* infection reveals that neutrophil accumulation in the liver of WT mice with i.v. administration of *Listeria* initiates at as early as <30 min after infection and reaches its maximum at 4 h. In contrast, the neutrophil-specific chemokines CXCL1/2, which are implicated in the TLR2-mediated proinflammatory cascade [58–65] in listerial infection, are barely detectable in the liver 30 min after infection. The appearance of CXCL1/2 in the liver is detected starting at 4 h after infection, a time point far beyond the appearance of neutrophils in the liver. Interestingly, in *Fpr*-deficient mice, although the production of CXCL1/2 in *Listeria*-infected mouse liver showed kinetics and magnitude similar to that in WT mice, the early phase neutrophil accumulation is markedly reduced in either *Fpr1*- or *Frp2*-deficient mice and is almost completely absent in mice-deficient in both Fprs. Further studies show that *Listeria* produces chemotactic agonists for both Fpr1 and Fpr2, consistent with earlier findings that synthetic peptides based on the putative *Listeria* product sequences are potent neutrophil chemoattractants by interacting with both human and mouse FPRs [32]. Therefore, *Fpr*-deficient mice suffer from increased bacterial load in the liver and markedly reduced production of H_2O_2 by neutrophils in response to the bacteria, in association with compromised bacterial killing and markedly accelerated mortality.

Therefore, Fprs antecede CXC chemokines in sensing *Listeria*-derived chemotactic PAMPs to rapidly mediate neutrophil recruitment into the infected liver. This process should be important for bringing in many neutrophils to enable their responses to *Listeria* lipoproteins, potent PAMPs for TLR2. Thus, Fprs belong in the paradigm of host resistance to *Listeria* infection as one of the frontline sentinels responding to the invading pathogen (Fig. 1). However, whether this revised paradigm is applicable to infection by species other than *Listeria* is unclear. Considering the capacity of FPRs to recognize a broad range of chemotactic PAMPs (Table 1.), it is not surprising to note that recent studies have also implicated FPRs in infection models of pneumococcal meningitis [66] by recruiting early neutrophil infiltration in response to bacteria chemoattractants.

CCR2-Fpr2–CCR7 participates in the stepwise trafficking of DCs in allergic airway inflammation

DCs are critical in airway inflammatory responses [67, 68]. During viral infection, allergen challenge, or endotoxin inhalation, $CD11b^+$ monocyte-derived DCs are rapidly recruited from the circulation into the airway. These cells are of monocytic origin [68, 69], which initiates and accounts for the severity of allergic airway inflammation. In mice, $Ly6C^{high}$ conventional monocytes are $CX3CR1^{low}$, $CCR2^+$, $CD62L^+$, and $CCR5^-$, and under inflammatory conditions, they differentiate into inflammatory DCs and acquire the capacity to prime T cell–mediated immune responses in draining lymph nodes [70]. In the lung, inflammatory stimuli, such as TLR PAMPs or exposure to environmental pollutants, trigger the production of chemokines that have been implicated in recruitment of inflammatory DCs in a CCR2-dependent manner [68, 69, 71, 72], via interaction with endogenous CCL2. However, other chemokine receptors have also been implicated in the recruitment of monocyte-derived inflammatory DCs into the lung, as evidenced by observations showing that *CCR5*- and *CCR6*-deficient mice have reduced

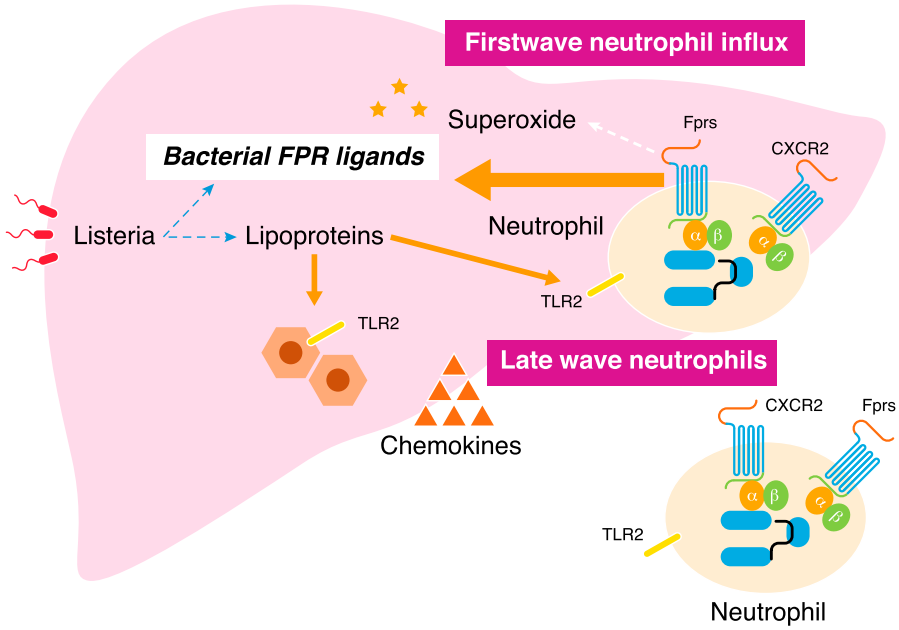

Figure 1. FPRs control the first wave neutrophil infiltration in *Listeria*-infected liver. Both Fpr1 and Fpr2 in mice sense bacterial chemoattractants to directly mediate a rapid (within 30 min) neutrophil influx into *Listeria*-infected liver. Listerial lipoproteins activate TLR2 expressed by hepatocytes and leukocytes to enhance the production of CXCR2-specific chemokines to elicit a late wave (after 4 h) neutrophil recruitment in the liver. Neutrophils activated by bacterial FPR ligands produce superoxide critical for bacteria elimination.

cigarette smoke inhalation–induced airway inflammation [73, 74], suggesting a more complicated DC traffic pattern based on the context of the causes of the inflammatory syndrome. After trafficking of DCs precursors into the inflammatory airway, TLR agonist PAMPs or DAMPs rapidly down-regulate the function of chemokine GPCRs expressed on DCs [75], and further cell trafficking to the lymphatic tissues is mediated by then up-regulated CCR7 [76]. Thus, chemokine GPCR-mediated inflammatory DC trafficking has been accepted as the means by which DCs are recruited and directed to complete their journey from an innate arm to the adaptive arm of immune responses. Once DCs are mature and express CCR7, they can migrate from sites of innate inflammation to draining lymph nodes to stimulate T cells and to initiate adaptive immunity.

However, the model of chemokine GPCRs alone in DC trafficking in allergic airway inflammation is inadequate and has recently had to incorporate one of the FPRs, Fpr2, as an indispensible link in the chain of events. This has been shown by greatly reduced severity of OVA-elicited allergic airway inflammation in *Fpr2*-deficient mice [77], in association with a marked reduction of infiltration of $Ly6C^{+}$ monocyte-derived inflammatory DCs in the small airways and a subsequent lack of DCs in the T cell zones in the draining mediastinal lymph nodes. These observations raise the possibility that Fpr2 might be an active participant in the sequential chemoattractant signal relay required for the trafficking of $Ly6C^{+}$ monocyte-derived inflammatory DCs in the inflamed lung. In fact, the role of CCR2, Fpr2, and CCR7 in DC trafficking is tightly orchestrated from circulation via airways to the lymph nodes [33]. This updated DC trafficking model illustrates the necessity for CCR2 to mobilize $Ly6C^{high}$ monocytic DC precursors from the BM into the circulation [78, 79], where the cells undergo extravasation into the perivascular regions of inflamed lung and become immature DCs upon exposure to DAMPs present in the airway. The immature DCs lose the expression of functional CCR2, but gain high-level expression of Fpr2, which guides the cells into the peribronchiolar regions in response to a host-derived chemotactic DAMP—CRAMP [33]—which not only forms a chemotactic gradient cue to mediate DC trafficking but also is capable of promoting DC maturation stimulated by TLR ligand PAMPs [80]. A shift of the chemoattractant GPCR expression occurs again as DCs mature; the Fpr2 is down-regulated, but the DC homing chemokine GPCR, CCR7, is highly elevated and enables the matured DCs to be directed into lymphatic organs. Thus, DC trafficking in the inflammatory airway consists of a fine-tuned functional relay of chemoattractant GPCRs on the cell surface, progressing from CCR2, with Fpr2 as an intermediate, and CCR7 as the final player to complete the last segment of homing (**Fig. 2**). These discoveries, therefore, are the basis for a modified model of DC trafficking in airway inflammation in which CCR2, Fpr2, and CCR7 sequentially respond to their respective endogenous ligands resulting in the initiation, amplification, and resolution of the host responses. Despite the high homology of Fpr1 with Fpr2, Fpr1 does not share with Fpr2 the endogenous chemotactic DAMP peptide CRAMP; therefore, its role in allergic airway inflammation remains unclear and requires further investigation.

In addition to sequential chemoattractant GPCR expression induced by microenvironmental stimulants, DC trafficking in vivo may use multiple models that also include the potential involvement of GPCR heterologous desensitization, in which activation of one GPCR may cause a temporary unresponsiveness in another GPCR, despite its normal-level expression on the cell surface and the presence of its cognate ligand or ligands [81, 82]. It is, therefore, important to study the pattern of GPCR requirement for DC trafficking in individual disease states to avoid generalization of a single model. In this context, use of mice-deficient in single or multiple receptors should be most

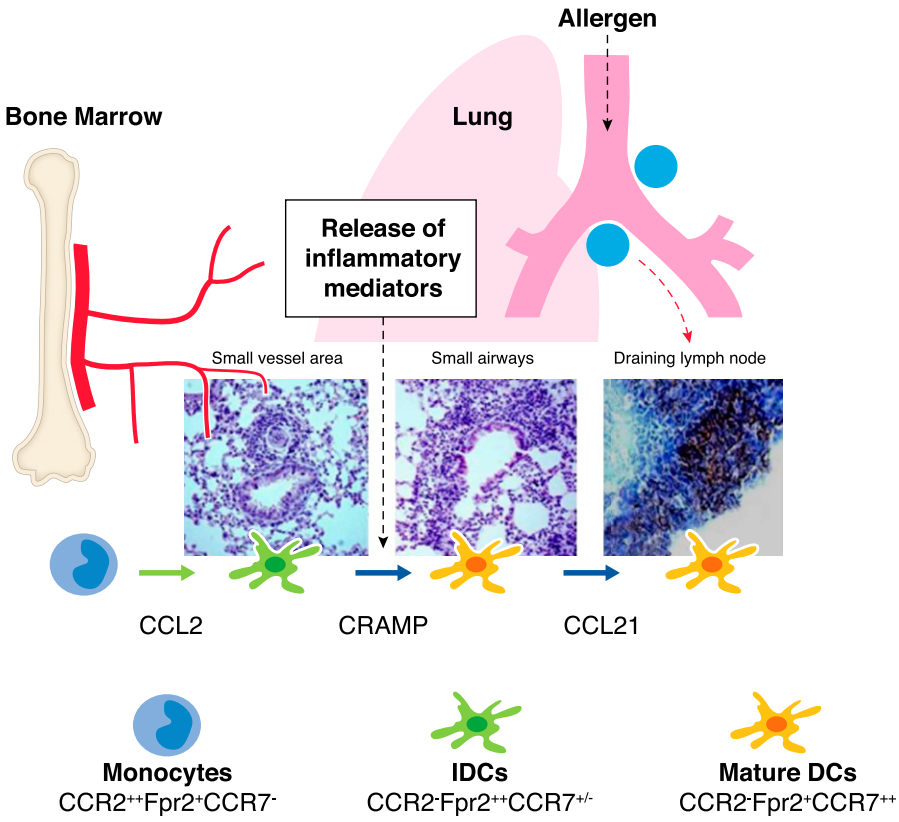

Figure 2. Sequential sensing of chemotactic cues in the tissue by Fpr2 and chemokine GPCRs by inflammatory DCs in allergic airway inflammation. The chemokine receptor CCR2 mobilizes inflammatory DC precursors from the circulation to the perivascular region of the inflamed lung in response to the cognate ligand CCL2. Once in the perivascular regions, DCs express high levels of Fpr2 with down-regulation of CCR2, presumably in response to environmental inflammatory stimuli, such as PRR ligands and cytokines. Fpr2 then mediates DC trafficking to the peribronchiolar areas in response to the endogenous Fpr2 ligand CRAMP, which is increased in the lung during inflammatory responses. In the peribronchiolar areas, inflammatory DCs undergo full maturation in association with the expression of high levels of the chemokine GPCR CCR7 for further homing into the draining lymph nodes to elicit adaptive immune responses.

helpful, but caution is needed concerning whether deletion of one GPCR gene may affect the expression and function of another GPCR.

Cooperation of Fpr2 with other GPCRs in wound healing

Neutrophil recruitment from blood to extravascular sites of sterile or infectious tissue injury is a hallmark of early innate immune responses, and the molecular events leading to cell exit from the bloodstream have not been well-defined [67, 68]. Once outside the vessel, individual neutrophils often show strikingly coordinated chemotaxis and cluster formation, simulating the swarming behavior of insects [69–76, 83]. A 2-photon, intravital microscopy study recently revealed the molecular nature of the players that mediate neutrophil responses on the levels of a single cell and a population within the complex space of inflamed skin tissue in a mouse ear. This defines a critical role for intercellular signal networking among neutrophils mediated by LTB4, which rapidly amplifies acute inflammatory responses to local cell death in laser beam–inflicted wounds, resulting in an enhanced radius of highly directed, interstitial neutrophil recruitment. Integrin receptors are dispensable for long-distance cell migration [84] but have a role in maintaining dense cellular clusters when neutrophil congregates rearrange the collagen-fiber network in the dermis to form a collagen-free zone at the wound center. In this newly formed environment, integrins in concert with neutrophil-derived LTB4 and other chemoattractants, presumably CRAMP and CXCL2 that use Fpr2 and CXCR2 promote local neutrophil accumulation while forming a tight wound seal whose borders cease growing in concert with late recruitment of monocytes and macrophages at the edge of the displaced collagen fibers. These observations identify the factors that contribute to neutrophil swarming in the extravascular space of damaged skin tissue and reveal how local events are propagated over large distances. Consequently, sequential signaling mediated by chemoattractant GPCRs—Ltb4r1, Fpr2, and CXCR2—coordinates an organized neutrophil swarming that isolates the injured site from surrounding viable tissue [85], which may be important for the initiation of a healing process.

In another skin-wound healing model, Fprs seem to act as the first player in the chemotaxis signal relay, resulting in rapid neutrophil infiltration [86]. The study shows that, in normal mice, neutrophils infiltrate the dermis in the wound before the production of neutrophil-specific CXC chemokines by the injured tissue. In contrast to normal mice, the early neutrophil infiltration is markedly reduced in mice-deficient in both *Fprs* in association with delayed wound closure. The critical role of Fprs in wound healing is based on the findings that skin wound tissues contain chemotactic DAMPs (Table 1.) that chemoattract FPR expressing neutrophils. Therefore, Fprs are critical for normal healing of the sterile skin wound by mediating neutrophil infiltration [86].

Overall, these observations suggest the complexity of the contribution of FPRs (Fprs) to the healing of wounds at different

anatomic sites and that careful consideration is required to target these receptors to either accelerate the healing or to prevent excessive inflammatory responses, which may cause greater damage to vital organs [87].

THE ROLE OF FPR2 IN THE MAINTENANCE OF COLON MUCOSAL HOMEOSTASIS AND PROTECTION AGAINST INFLAMMATION-ASSOCIATED TUMORIGENESIS

Although FPRs were first identified on phagocytic leukocytes, these receptors are also expressed by many nonhematopoietic cells, including intestinal epithelial cells and have important roles in colon mucosal homeostasis, inflammatory responses, and tumorigenesis.

UC is associated with an elevated risk for colorectal cancer [88]. It is believed that the chronic inflammatory process associated with UC is responsible for the neoplastic transformation of the intestinal epithelium [89]. Proinflammatory cytokines and chemokines, as well as matrix-degrading enzymes, growth factors, and reactive oxygen species present in the tissue microenvironment of UC, enhance epithelial cell proliferation, abnormal cell turnover, leukocyte infiltration, and angiogenesis, culminating in mutagenesis and tumor formation [90, 91]. The association of UC with cancer involves inflammation of the submucosa of the colon, induced by direct contact with the intestinal microorganisms that promote tumor growth in the overlaying epithelium [92]. Therefore, the capacity of the intestinal epithelial layer to cope with the intestinal microbiota is important not only for limiting inflammation but also for preventing tumorigenesis in the colon.

FPR1 has been shown to localize along the lateral membrane of crypt epithelial cells in the human colon. Activation of FPR1 by bacterial fMLF promotes epithelial growth and restoration of the mucosal integrity [93]. In mice, some chemotactic PAMPs and DAMPs, such as Anx A1, fMLF, and viable *Lactobacillus rhamnosus* GG, stimulate Fpr1 on intestinal epithelial cells, and this leads to the generation of reactive oxygen species via enterocyte NADPH oxidase 1, resulting in rapid phosphorylation of focal adhesion kinase and ERK/MAPK [34]. Activated FPR1 also mediates the migration and proliferation of enterocytes adjacent to colonic wounds [94]. Intestinal crypts of the *Fpr1*-deficient mice contain increased number of proliferating epithelial cells and show slower migration along the crypt-villus axis, despite their normal intestinal tissue architecture, suggesting that Fpr1 may be important in maintaining the homeostasis of the intestinal epithelia with yet unclear mechanistic basis [93]. In addition, in the mouse colon, commensal bacterial lysates and fMLF activate ERK/MAPK pathway in an FPR-dependent manner [95], without clear identity of the receptor subtypes that respond to commensal products.

To elucidate the identity of individual Fprs involved in commensal bacteria interaction in the colon, a recent study [96] using *Fpr* single- or double-deficient mice revealed that Fpr2, rather than Fpr1, appears to have a more prominent role in maintaining the normal growth of colonic epithelial cells. Fpr2 is found to be expressed on the apical and lateral membranes of mouse colon-crypt epithelial cells and mediates fMLF-dependent epithelial-cell proliferation and renewal. Moreover, colonic epithelial cells in *Fpr2*-deficient mice displayed defects in commensal bacterium-dependent homeostasis, shown by the absence of responses to fMLF stimulation, shortened colon crypts, reduced acute inflammatory responses to dextran sulfate sodium challenge, delayed mucosal restoration after chronic injury, and increased azoxymethane-induced tumorigenesis. In contrast to Fpr2, although Fpr1 also mediates fMLF-stimulated colon epithelia chemotaxis and activation, the crypt length in the colon of *Fpr1*-deficient mice is normal when compared with that of the WT mice. In addition, the colon of *Fpr1* and *Fpr2* double-deficient mice showed shortened crypts comparable to that observed in *Fpr2* single-deficient mice, suggesting a phenocopy of the *Fpr2* single deficiency. Furthermore, bacteria RNA sequencing suggests "dysbiosis" in the colon of *Fpr2*-deficient mice, with increased abundance of microbiome. These results confirm a critical role of Fpr2 in the homeostasis, inflammation, and epithelial repair processes in the colon, potentially mediated by interaction with microbiome products. These observations, consistent with the critical role of Fprs in *Listeria* resistance, confirm the important position of FPRs in the coevolution of mammals with microbiome, which is mutually beneficial in the homeostatic conditions but may err once the balance of the commensals vs. host is disrupted.

THE DUAL ROLE OF FPRS IN CANCER PROGRESSION

Under physiologic conditions, FPRs expressed by normal cells are essential for host defense against microbial infection, as well as for control of inflammation, immune responses, and epithelial homeostasis. However, malignant cells also express FPRs and respond to bacteria or endogenous agonists. For instance, in human GC cells, aberrantly expressed FPRs, upon activation, mediates epithelial–mesenchymal transition, proliferation, migration, and cell resistance to apoptosis [97]. However, it is intriguing that, in xenograft experiments, GC cells with silenced FPR1 formed more rapidly growing tumors in immune-compromised mice. Mechanistic studies showed that tumors formed by GC cells with silenced FPR1 contain higher vascular density, suggesting FPR1 may promote the production of antiangiogenic factors by GC cells, thus reducing the blood supply to the tumor.

The promoting activity of FPR1 in human glioma and other tumors

In contrast to observations with GC cells, in some circumstances, an opposing effect of FPRs was observed malignant tumors. In human glioma, FPR1 was found to be selectively expressed by cells of the more highly malignant GBM [98]. By interacting with ligand DAMPs (Table 1.) released into the tumor microenvironment, FPR1 transactivated the receptor for EGF, and the 2 receptors cooperated to enhance the survival, invasiveness, and production of angiogenic factors by GBM cells [98–102]. The contribution of FPR1 to GBM progression was demonstrated in studies showing that small interfering RNA targeting of *FPR1*

markedly reduced the tumorigenic capacity of GBM cells in nude mice. Moreover, FPR1 may be involved in the establishment of GBM, because the receptor is expressed by CD133/Nestin$^+$ glioma stem-like cells [103], which, upon implantation in nude mice, form more rapidly growing tumors and produce higher levels of the angiogenic factors as a consequence of FPR1 activation. More recently, Anx A1 released by necrotic GBM cells was identified as an activator of FPR1 expressed by live GBM cells which, in turn, exacerbated their malignant phenotype. These studies established a paracrine/autocrine FPR1/Anx A1 axis in the GBM microenvironment that provides critical signals for tumor progression [15] (**Fig. 3**). The clinical relevance of FPR1 in GBM was shown by the observation that, in human surgically resected glioma specimens, FPR1 and Anx A1 are both expressed by more highly malignant tumors with an inverse correlation with patient survival [21, 98].

FPR1 and 2 are also expressed by human breast cancer cells, and activation with Anx A1 enhances tumor cell proliferation [104]. In addition, consistent with findings in human GBM, human liver cancer cells express FPR1, which promotes the chemotaxis, invasion, proliferation, and production of angiogenic factors by cancer cells. Silencing FPR1 markedly reduced the tumorigenic capacity of human liver cancer cells in immunocompromised mice. These observations suggest that FPRs may be used by a variety of malignant tumors to exacerbate their progression. In this context, FPRs in malignant tumors may be considered plausible targets in designing novel therapeutics. As a support of this notion, treatment of mice bearing human GBM with an FPR1 antagonist, CHIPS was shown to prolong mouse survival [105].

M1 macrophage polarization supported by Fpr2

Despite their potential tumor-promoting activities in a number of cancer cells, Fpr2 in macrophages has been shown to sustain M1 polarization to benefit antitumor host defense by down-regulating the content of TAMs, a hallmark of cancer-associated inflammation [106]. TAMs are believed to be recruited by chemokines, and the cells can either impede tumor growth by producing cytotoxic mediators or promote tumor progression by producing growth-inducing and angiogenic factors [107, 108]. Blood-derived monocytes enter tumor tissues and differentiate into macrophages followed by further development into M1 or M2 polarized subtypes, which differ in their patterns of cytokine secretion and function [106]. The "classically activated" M1 macrophages contribute to tumor rejection through type I cytokine production and antigen presentation [108, 109]. The "alternatively activated" M2 macrophages enhance angiogenesis and tissue remodeling through type II cytokines [110, 111]. In >80% of cancers, TAMs mostly exhibit an M2 phenotype [112]. Tumor- and stroma-produced mediators, including a variety of chemokines, promote the recruitment and activation of TAMs, which contribute to tumor cell proliferation, migration, angiogenesis, and metastasis [113].

Experimental and clinical studies have shown that MCP-1 (also known as CCL2) is most frequently expressed by tumor cells, and its concentration is correlated with the level of TAMs in tumors [112, 114]. In a LLC implantation model, *Fpr2*-deficient mice bearing subcutaneously implanted LLC cells exhibited significantly shortened survival than did normal mice because of more rapidly growing tumors. In contrast, in transgenic mice overexpressing *Fpr2*, subcutaneously implanted LLC tumors grew more slowly than those in WT littermates. Investigation of tumor tissues revealed more TAMs in tumors grown in *Fpr2*-deficient mice. Macrophages derived from *Fpr2*-deficient mice also showed a more-potent chemotactic response to LLC-derived supernatant, which could be neutralized by an anti-CCL2 antibody, indicating CCL2 as a major chemoattractant for TAMs in LLC tumor. The increased chemotaxis of *Fpr2*-deficient mouse macrophages in response to LLC supernatant was due to their higher expression of CCR4, a chemokine GPCR that is also recognized by the ligand

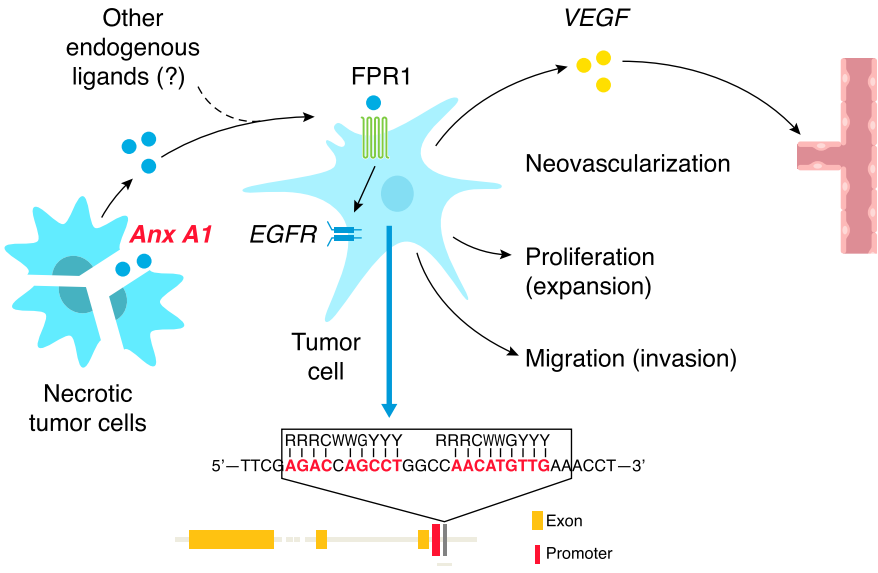

Figure 3. FPR1 promotes the progression of human glioblastoma. FPR1 is selectively expressed by highly malignant human glioblastoma cells. The transcription of FPR1 in glioblastoma cells is promoted by overactivated NF-κB, which competitively binds to the FPR1 promoter. Demethylation of P53 increases its competitive binding to the FPR1 promoter and promotes the differentiation of glioblastoma cells accompanied by reduced expression of FPR1. Upon activation by the ligand Anx A1 released by necrotic tumor cells, FPR1 mediates glioblastoma cell chemotaxis, invasion, and production of proangiogenic factors, such as vascular endothelial cell growth factor and CXCL8. FPR1 in glioblastoma cells also transactivates the receptor for EGF via a G-protein–SRC kinase pathway, and 2 receptors cooperate to exacerbate the malignant phenotype of the tumor cells.

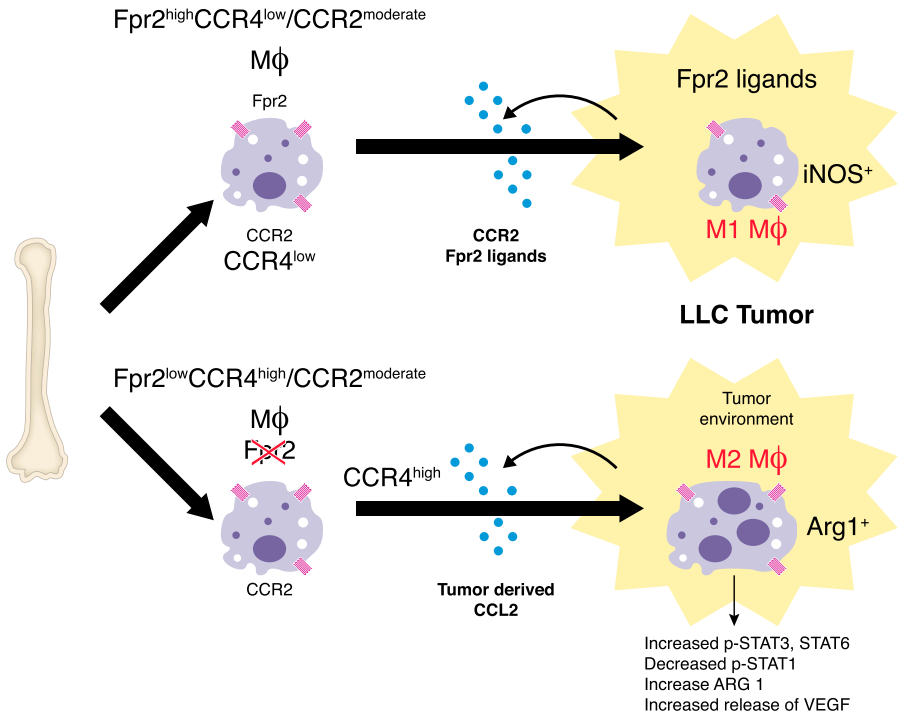

Figure 4. Fpr2 promotes antitumor host defense. Mouse macrophages expressing both CCR2 and Fpr2 infiltrate transplanted LLC where macrophages undergo M1 polarization in response to tumor-derived Fpr2 ligands. Macrophages-deficient in *Fpr2* express high levels of CCR4, which synergizes with CCR2 to recruit TAM in response to tumor-derived CCL2, followed by polarization into M2 cells in response to an as-yet-undefined tumor microenvironment factor.

CCL2 [115, 116]. Interestingly, treatment with Fpr2 antagonists increased the chemotactic response of WT mouse macrophages to CCL2, in association with increased expression of CCR4; LLC tumor cell supernatant and Fpr2 agonists were capable of polarizing WT macrophages toward an M1 phenotype [117]. Therefore, Fpr2 appears to increase host defense against implanted LLC by favoring macrophages with an M1 phenotype with more-potent antitumor activities (**Fig. 4**). However, a caveat may exist in generalizing this LLC model because not all tumors produce copious levels of CCL2, and the nature of LLC-derived Fpr2 agonist DAMPs is unknown. More studies are required to elucidate the precise mechanisms by which Fpr2 promotes macrophages polarization into an M1 subtype to limit tumor growth and whether this property of Fpr2 could be exploited to develop antitumor drugs.

CONCLUDING REMARKS

FPRs, as a subfamily of classic chemoattractant GPCRs, have one of the most diverse collections of ligands, and they are expressed by a great variety of cell types, including cancer cells. Recent studies have shown an essential role of FPRs in critical pathophysiologic conditions, not only in leukocyte trafficking but also in epithelial homeostasis and tumorigenesis. One of most notable features of FPRs in these pathophysiologic processes is that they act as key players in the sequential sensing of chemoattractant gradients by inflammatory cells, in particular neutrophils and DCs, in a tissue microenvironment that dynamically regulates the expression of chemoattractant GPCRs to guide the cells to their destination where efficient host responses are mounted. Another important, paradigm-changing discovery is the role of Fpr2 in protecting colon mucosal homeostasis and inflammation and antitumor defense by interaction with commensal products. These novel findings greatly expanded the scope of FPR biology whose pathophysiologic relevance had remained obscure for decades since their discovery. However, much more remains to be learned, with rigorous exploration of the participation of FPRs in more disease models, which, fortunately, has become increasingly feasible with the generation of genetically engineered mice with altered expression of FPR genes. It is thus plausible that future studies focusing on the regulation, signal transduction, and structural basis for ligand recognition, and importantly, on participation in pathophysiologic processes in humans, should yield novel therapeutic targets for diseases.

AUTHORSHIP

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DISCLOSURES

The authors declare no conflicts of interest.

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