Expression and Function of Formyl Peptide Receptors on Human Fibroblast Cells

Scott E. VanCompernolle, Krista L. Clark, Kevin A. Rummel, and Scott C. Todd

The Journal of Immunology, 2003, 171: 2050–2056.

Foramylated peptides such as fMLP are generated by bacteria and are powerful chemoattractants that promote leukocyte migration into the site of infection (1, 2). Formylated peptides bind to the high affinity formyl peptide receptor (FPR) and/or the low affinity formyl peptide receptor-like-1 (FPRL1) (1). These receptors are seven transmembrane pertussis toxin-sensitive G protein-coupled receptors. Ligand binding to FPR activates a number of downstream effector enzymes including phospholipase C, catalyzing the cleavage of phosphatidylinositol 4,5-biphosphate into secondary messengers inositol 1,4,5-trisphosphate and diacylglycerol leading to calcium mobilization and activation of protein kinase C (PKC; Ref. 2). FPR ligation has also been shown to signal through the small G protein Cdc42 to activate Rac- and ARPA2/3-dependent pathways leading to actin nucleation (3, 4). The culmination of these signaling events triggers morphological and biochemical alterations including polarization of the actin cytoskeleton, activation of various integrins, and directed migration. FPR signaling also initiates production of superoxide and arachidonic acid metabolites and induces degranulation (5–9). These events allow phagocytic leukocytes to locate, sequester, and destroy invading microorganisms.

A search of the Human Expressed Sequence Tagged (EST) database reveals expression of FPR and FPRL1 in multiple tissues (Table I). We were surprised at the abundance of ESTs for FPR and FPRL1 in these nonlymphoid tissues. This nonleukocyte expression was observed by Becker et al. (10) who show staining of multiple tissues and cell types with rabbit polyclonal antisera against the C terminus of FPR.

We conducted this study to determine whether FPRs are expressed on fibroblasts and to evaluate the functional significance of these receptors on nonleukocyte cell types. Here we report the expression of FPRs on human fibroblasts. A variety of experimental approaches indicate that these receptors are able to induce intracellular signaling events leading to fibroblast motility. This first report of a functional FPR in cells of fibroblast origin suggests that these cells are able to respond directly to bacterial presence potentially contributing to innate immune responses.

Materials and Methods

Cell lines and reagents

HT-1080 (CCL-121) human fibrosarcoma cell line, MRC-5 (CCL-171) normal lung fibroblasts and WS1 (CRL-1502) normal skin fibroblasts (American Type Culture Collection, Manassas, VA) were cultured in DMEM with 10% FBS, 1% sodium bicarbonate buffer, and 1% HEPES buffer. HT-1080-GFP cells were generated by transfection with the pEGFP-NES-Neo plasmid (Clontech, Palo Alto, CA), and stably selected in G418. fMLP and phenylarsarsine oxide were purchased from Sigma-Aldrich (St. Louis, MO). Chelerythrin chloride and LY294002 were purchased from Biomol (Plymouth Meeting, PA). Trp-Lys-Tyr-Met-Val-o-Met-NH2 (WKYMVm) and BOC-Phe-Leu-Phe-Leu-Phe (BocFLFLF) were purchased from Phoenix Pharmaceuticals (Belmont, CA).

RT-PCR

RNA was isolated using an RNeasy Mini Kit (Qiagen, Santa Clarita, CA) and reverse transcribed with the Reverse Transcription System (Promega, Madison, WI). The cDNA was probed for the expression of FPR by PCR (1 min of hybridization at 62°C followed by a 1.5-min extension at 72°C for 32 cycles) with the sense primer 5'-CTGCCGTGGCTGACTTCTG-3' and the reverse primer 5'-CTTGTGCCTGAATCTCACCTGC-3' and for the expression of FPRL1 with the sense primer 5'-GAGAAAAATGCCTTTTGGCTG-3' and the reverse primer 5'-CATGCGGTGAACCTGAG-3'.

Program in Molecular Cellular and Developmental Biology, Division of Biology, Kansas State University, Manhattan, KS 66506

Received for publication September 18, 2003. Accepted for publication June 6, 2003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by American Cancer Society Grant RPG-00-338-01-CSM and by National Institutes of Health Center of Biomedical Research Excellence Grant P20 RR15563-01.

2 Address correspondence and reprint requests to Dr. Scott C. Todd, 232 Ackert Hall, Division of Biology, Kansas State University, Manhattan, KS 66506. E-mail address: stodd@ksu.edu.

3 Abbreviations used in this paper: FPR, formyl peptide receptor; PKC, protein kinase C; FPR1, FPR-like 1; FPR2, FPR-like 2; WKYMVm, Trp-Lys-Tyr-Met-o-Met-NH2; BocFLFLF, BOC-Phe-Leu-Phe-Leu-Phe; FNLPNTL-FL, formyl-norleucyl-Leu-norleucyl-Tyr-Lys-fluorescein; EST, expressed sequence tag; ECM, extracellular matrix; AM, acetoxymethyl ester.

Copyright © 2003 by The American Association of Immunologists, Inc.
Continuous resuspended in HBSS at 10^6 cells/ml, 1-ml aliquots were transferred to incubate for between 10 and 30 min in a 37°C water bath before assay. Cells were washed three times for 5 min each in PBS and resuspended in PBS with 2% paraformaldehyde. Cells were analyzed using a Varian Cary Eclipse 96-well fluorescent spectrophotometer. Alternatively 10^5 cells were removed from culture using nonenzymatic cell dissociation solution, harvested by centrifugation, resuspended in prewarmed DMEM with 10% serum, and allowed to equilibrate for 1 h at 37°C. fMLP (100 nM) was added to each sample for the indicated time and immediately fixed in PBS with 2% paraformaldehyde, 0.1% Triton X-100 for 1 h on ice. Cells were rinsed in PBS and stained in 100 µl of PBS with 1 U Alexa-488 phalloidin (Molecular Probes) for 1 h on ice. Cells were washed three times for 5 min each in PBS and resuspended in PBS with 2% paraformaldehyde. Cells were analyzed using a FACSCalibur flow cytometer.

**Chemotaxis assay**

HT-1080 cells or HT-1080-GFP cells were dissociated from culture by trypsinization, pelleted, and resuspended in serum-free DMEM supplemented with 0.1% BSA. To the top of 6.5-mm-diameter cell culture inserts of 8 µm pore size, 10^5 cells in 200 µl were added. In studies with matrix-coated cell culture inserts, inserts were incubated on the top and bottom surfaces with fibronectin (20 µg/ml) or collagen IV (20 µg/ml) overnight at 4°C, removed, washed with PBS, and allowed to dry before cells were added. The preloaded inserts were then placed in a 24-well culture plate containing 600 µl of serum-free DMEM with indicated concentrations of fMLP. After 2.5 h of incubation at 37°C, cells on the top surface were meticulously emancipated, and cells on the bottom surface were fixed with 2% paraformaldehyde in PBS and visually quantified per field of view using a Nikon Eclipse TE300 microscope (Nikon, Melville, NY). In some experiments, cells were quantified using CellTiter-Glo luminescent substrate according to the manufacturer's description (Promega) and detected with a PerkinElmer (Boston, MA) 1450 MicroBeta liquid scintillation and luminescence counter. Samples were read for 1 s with data represented as relative luminescent units or in some experiments converted to cell number using a standard curve. In Fig. 4B, cell migration was assessed using a Neuroprobe 96-well chemotaxis chamber (Neuroprobe, Gaithersburg, MD) according to the manufacturer's instructions. Briefly, 420 µl of serum-free DMEM was placed in the lower chamber with the indicated amount of fMLP and overlaid with an 8-µm pore size membrane. The device was sealed, and 10^5 cells in 200 µl of serum-free DMEM were added to the top chamber and allowed to migrate for 2.5 h at 37°C. The filter was removed, and nonmigrated cells were removed from the top by scraping. Migrated cells adhering to the bottom side of the filter were quantified using Cell-Titer-Glo and luminescence counting as above.

**Cell adhesion assay**

96-well flat-bottom E.I.A/R.I.A. high protein binding plates (Costar, Cambridge, MA) were precoated with BSA (2%), Fibronectin (20 µg/ml), or collagen IV (20 µg/ml) in carbonate buffer (500 mM sodium bicarbonate, pH 8.5) overnight at 4°C. Wells were washed twice and blocked with a solution of 2% BSA in PBS at 37°C for 1 h. HT-1080 were dissociated from culture by trypsinization, pelleted, and resuspended at 10^6 cells/ml in serum free DMEM supplemented with 0.1% BSA with the indicated drugs and were allowed to incubate at 37°C for 1 h. Cells were plated at a density of 2.5 × 10^4 cells/well and allowed to settle for 8 min at 37°C. To remove unbound cells, the plate was then submerged in a large volume of PBS and inverted followed by uniform washing. Then 200 µl of PBS were removed from each well followed by addition of 120 µl of CellTiter-Glo luminescent substrate and assayed on a luminescence counter.

**Results**

Fibroblasts express FPR and FPRL1

The human N-FPR gene cluster contains three genes (15–17). The high affinity FPR and the lower affinity FPRL1 are predominately expressed in phagocytic leukocytes (1, 15). The third gene, FPRL2, has not been reported to respond to N-formyl peptides (16, 18, 19). During a recent analysis of ESTs, we were surprised to find frequent cloning of FPR (Unigene Hs.753) and FPRL1 (Unigene Hs.99855) from nonlymphoid tissues (Table I). Although some historical data have been published that corroborates the nonlymphoid expression of these receptors, it is unclear which cell

Table 1. Nonlymphoid tissue expression of the FPR gene family (Unigene-clustered EST sequences)

| Tissue          | FPR Hs.753 | FPRL1 Hs.99855 |
|-----------------|------------|---------------|
| Colon           | X          |               |
| Eye             | X          |               |
| Kidney          | X          |               |
| Lung            | X          | X             |
| Heart           | X          |               |
| Liver           | X          |               |
| Ovary           | X          |               |
| Placenta        | X          |               |
| Colon X         | X          |               |
| Kidney X        | X          |               |
| Lung X          | X          |               |
| Liver X         | X          |               |
| Ovary X         | X          |               |
| Placenta X      | X          |               |
| Testis          | X          |               |
| Stomach         | X          |               |
| Colon X         | X          |               |

TCTCTGC-3' and for the expression of FPRL2 with the sense primer 5'-GAGAGAAAAATGGCCTTTTGCG-3' and the reverse primer 5'-CATTGCTTTGAACCTCGTCCCTC-3'. These primers were designed to amplify a specific fragment of each receptor and PCR products were sequenced to confirm that the PCR product represents the targeted gene.

**Equilibrium binding assay**

Cells were removed from culture by trypsinization, pelleted, and resuspended in HBSS with 1% BSA and 10 µM phenylalanine oxide to inhibit endocytosis of bound FPRs (11, 12). After equilibration at 37°C for 30 min, cells were placed in FACS tubes and incubated for 1 h in the dark at 25°C under constant rotation with increasing doses of N-formyl-norleucyl-Leu-Phe-norleucyl-Tyr-Lys-fluorescein (FNLPNL-FL) (Molecular Probes, Eugene, OR). Stocks of FNLPNL-FL were made in DMSO and diluted to a final concentration of less than 0.01% DMSO in each assay. At concentrations of 0.1–10 nM FNLPNL-FL, a flow cytometer effectively discriminates between ligand that is associated with cells and ligand that is in solution surrounding cells (13). A FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) calibrated with FITC-labeled beads (Quantum 24-FTIC) (Bangs, Fishers, IN) was used to quantify fluorescence binding per cell. Non-specific binding was determined in the presence of 10 µM fMLP and subtracted from total binding to give specific binding. A conversion of 1.22 FNLPNL-FL equivalents/fluorescein equivalents was used as determined by Fay et al. (14). Nonlinear regression was used to determine receptor number per cell and K_d.

**Calcium flux assay**

HT-1080, MRC-5, or WS1 cells were removed from culture using nonenzymatic cell dissociation solution (Sigma-Aldrich), harvested by centrifugation, and resuspended in prewarmed HBSS containing 0.1% BSA red acetoxyethylmethyl ester (AM) and 4.43 µM fluo-3 AM (Molecular Probes, Eugene, OR) and incubated at 37°C for 45 min. Cells were centrifuged and resuspended in HBSS at 10^5 cells/ml, 1-ml aliquots were transferred to 1.5-ml tubes, and drug treatments were added. Samples were allowed to incubate for between 10 and 30 min in a 37°C water bath before assay. Continuous fluorescent measurements of calcium-bound and free Fura red and fluo-3 were made using a FACSCalibur flow cytometer with excitation at 488 nm. Calcium-bound Fura red was read using the FL-3 (650 nm) channel, fluo-3 using FL-1 (520 nm). fMLP was added to samples at indicated time points. Data are represented as the ratio of fluo-3 to Fura red fluorescence. In the calcium dose titration experiments, 2.5 × 10^5 cells per well were seeded in a Costar black-walled 96-well assay plate (Corning, Corning, NY) and allowed to adhere for 24 h. Cells were then incubated in HBSS containing 4.43 µM fluo-4 AM (Molecular Probes) and incubated at 37°C for 45 min. Cells were then washed three times in HBSS and incubated before analysis at 25°C. After determining baseline fluorescence of each well, fMLP was added, and the peak fluorescence intensity was read at 1 s (this duration was predetermined to give the peak response) using a Varian Cary Eclipse fluorescent spectrophotometer (Palo Alto, CA) with an excitation of 488 nm and emission at 515 nm.

**Actin polymerization assay**

HT-1080, MRC-5, or WS1 cells were removed from culture using nonenzymatic cell dissociation solution, harvested by centrifugation, resuspended in DMEM with 10% serum and 2.5 × 10^5 cells per well were plated in a Costar 96-well black-walled plate and allowed to adhere 24 h. In some experiments, cells were preincubated with the FPR antagonist BocFLFLF at 10 µM for 5 min. fMLP (50 nM) or WKYMVM (50 nM) was added to each sample for the indicated time and immediately fixed in PBS with 2% paraformaldehyde, 0.1% Triton X-100 for 1 h on ice. Cells were rinsed in PBS and stained in 100 µl of PBS with 1 U Alexa-488 phalloidin (Molecular Probes) for 1 h on ice. Cells were washed three times for 5 min each in PBS and resuspended in PBS with 2% paraformaldehyde. Cells were analyzed using a FACSCalibur flow cytometer.

**Results**

The human N-FPR gene cluster contains three genes (15–17). The high affinity FPR and the lower affinity FPRL1 are predominately expressed in phagocytic leukocytes (1, 15). The third gene, FPRL2, has not been reported to respond to N-formyl peptides (16, 18, 19). During a recent analysis of ESTs, we were surprised to find frequent cloning of FPR (Unigene Hs.753) and FPRL1 (Unigene Hs.99855) from nonlymphoid tissues (Table I). Although some historical data have been published that corroborates the nonlymphoid expression of these receptors, it is unclear which cell
types may express these proteins and, more importantly, whether such receptors are functional on nonleukocyte populations. To study the expression of FPRs on human fibroblasts, we used RT-PCR. Normal human fibroblasts from lung (MRC-5) and skin (WS1) as well as a human fibrosarcoma cell line (HT-1080) were used to generate cDNA. Primers specific for FPR and FPRL1 but not FPRL2 (not shown) amplified a product of the correct size (851 bp for FPR and 791 bp for FPRL1) from the fibroblast cDNA in each case (Fig. 1A). The RT-PCR results shown in Fig. 1 are representative of three different experiments (Fig. 1A). PCR products were sequenced to verify specific amplification of FPR and FPRL1 (not shown).

To measure FPR protein levels at the cell surface, we used an equilibrium binding assay. HT-1080 or MRC-5 cells were incubated with increasing doses of the fluorescein-labeled chemotactic peptide FNLPNTL-FL and allowed to reach equilibrium. Flow cytometric analysis was used to quantify bound FNLPNTL-FL peptides per cell (Fig. 1B). A simplified one-site binding nonlinear regression analysis was used to fit a curve and determine $K_d$ and receptor number per cell. Total receptor number and $K_d$ for HT-1080 are 583 ± 82 and 0.67 ± 0.2, respectively. The receptor number and $K_d$ for MRC-5 are 1600 ± 100 and 0.2 ± 0.004, respectively (Fig. 1B). These data are representative of three independent experiments. The concentrations of FNLPNTL-FL used in the binding assay (<2 nM) reflect binding to the higher affinity FPR and binding to the low affinity FPRL1 would not be significant at these concentrations. The $K_d$ values are similar to published $K_d$ values for FPR in other cell types; however, the total receptor numbers per cell are 50-fold less than that found on neutrophils (13, 20, 21). Despite the low level of FPR expression, it is reasonable to consider this expression to have potential biological significance based on studies in neutrophils that demonstrate that occupancy of as few as 50 receptors results in signaling (13, 21).

\section*{FPRs ON FIBROBLAST CELLS}

\section*{FMLP stimulates pertussis toxin-sensitive intracellular calcium release}

In neutrophils, FPR couples to pertussis toxin-sensitive G$_\alpha$ proteins, which activate PLC leading to the breakdown of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-phosphate resulting in intracellular calcium mobilization (6, 22, 23). To determine whether ligand binding to FPRs induces similar signal transduction in fibroblast cells, we examined the stimulated release of calcium from intracellular stores. Cells were loaded with the fluorescent calcium indicators Fluo-3 and Fura red before stimulation with fMLP. Calcium release was determined by the ratio of fluro-3 to Fura red fluorescence measured in real time using flow cytometry. Cells were measured for 30 s to establish baseline cytosolic calcium levels followed by different doses of fMLP. Each experiment was internally controlled by addition of ionomycin, a G protein-independent calcium ionophore at the end of the run to verify adequate loading of the indicators and responsiveness of the cells (not shown). A 5 nM dose of fMLP stimulates intracellular calcium release from HT-1080, leaving the cells responsive to a 100 nM dose 60 s later (Fig. 2A). High doses of fMLP have been shown to desensitize FPR via receptor phosphorylation and internalization (21, 24–26). Doses of fMLP above 100 nM are sufficient to desensitize HT-1080 to a subsequent dose at 90 s (Fig. 2A). Calcium flux was also measured on adherent fibroblasts, HT-1080, MRC-5, and WS1, using a fluorescence plate reader (Fig. 2B). Consistent with the results from HT-1080 cells and flow cytometry, MRC-5 and WS1 cells trigger Ca$^{2+}$ release in response to fMLP stimulation (Fig. 2B). Dose-response curves indicate that all three cell lines respond within a similar molar range of fMLP (Fig. 2B).

In leukocytes FPR and FPRL1 couple to pertussis toxin-sensitive G$_\alpha$ proteins. To determine whether FPR and FPRL1 are coupled to pertussis toxin-sensitive G$_\alpha$ proteins in fibroblasts, we treated HT-1080 cells with pertussis toxin before fMLP stimulation and calcium flux analysis. A dose of 50 ng/ml was sufficient to inhibit calcium release (Fig. 2A) in HT-1080 cells.

\section*{FMLP stimulates a transient increases in and reorganization of F-actin in HT-1080}

Activation of neutrophil FPR with fMLP stimulates disassociation of G$_\beta\gamma$, signaling a phosphatidylinositol 3-kinase and Rac-dependent increase in F-actin and redistribution of the cytoskeleton leading to polarization and formation of lamellipodia and filopodia (3, 4, 27, 28). To determine whether fMLP induces actin polymerization in fibroblasts, cells were treated with fMLP for 0–60 s, fixed, stained for F-actin, and assayed by fluorescence spectrophotometry. Treatment with fMLP induces rapid actin polymerization in all three cell lines (Fig. 3A). To demonstrate that fMLP induces actin polymerization by interacting specifically with FPRs, we used the FPR antagonist BocFLFLFL. Cells preincubated with 10 $\mu$M BocFLFLFL failed to polymerize actin in response to fMLP stimulation (Fig. 3A). A similar response in F-actin levels is seen when HT-1080 cells are nonenzymatically removed from the culture plate, placed in suspension before treatment, and analyzed by flow cytometry (not shown).

We further tested the ability of another FPR agonist, WKYMVm, to trigger actin polymerization in fibroblasts (29). Treatment of fibroblasts with 50 nM WKYMVm results in actin polymerization similar to that seen with fMLP (Fig. 3B).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Fibroblast cells express FPRs. A, RT-PCR analysis of FPR expression in HT-1080, MRC-5, and WS1 cells. Bands are the correct size for each amplified product and sequence verified to be FPR and FPRL1. Control lanes C1 and C2 were run without template cDNA. B, HT-1080 and MRC-5 cells were analyzed using an equilibrium binding assay. Fluorescence data were converted to number of bound complexes per cell and corrected for nonspecific binding. Each data point represents the mean of 10,000 cells. Complexes per cell and $K_d$ were determined by one site binding nonlinear regression analysis.}
\end{figure}
fMLP stimulates directed migration of fibroblast cells

HT-1080-GFP cells were seeded onto polycarbonate cell culture inserts with increasing concentrations of the chemotactic peptide fMLP in the lower chamber. Baseline migration of cells in serum-free medium was low with ~2 cells/20× field of view. fMLP stimulates a 5-fold increase in cell migration (Fig. 4A). The response to fMLP increases in a dose-dependent manner with a maximum response seen at 100 nM for HT-1080 cells. MRC-5 and WS1 primary fibroblast cells also migrate in response to fMLP. Maximal migration occurs at 400 nM fMLP for MRC-5 and at 50 nM for WS1 (Fig. 4B). Similar results were obtained in six different experiments using both 8 μM cell culture inserts and a Neuprobe chemotaxis chamber (see Materials and Methods). As is characteristic of a chemotactic response, higher doses of fMLP cause a decrease in migration because high, receptor-saturating doses obscure ability of the cell to detect the chemotactic gradient.

We next evaluated whether fMLP stimulates proliferation of fibroblast cells. Cells treated with doses of fMLP ranging from 4 to 1000 nM for 72 h did not exhibit increased incorporation of [3H]thymidine (MRC-5 averaged 2252 ± 264 cpm, and WS1 averaged 809 ± 114 cpm) relative to controls (2158 ± 95 cpm and 1098 ± 262 cpm, respectively).

fMLP-stimulated HT-1080 adhesion and migration is enhanced in the presence of fibronectin

Transient adhesive interactions between integrins and extracellular matrices (ECM) mediate cellular migration. Because signaling through FPRs results in activation of integrins responsible for neutrophil adhesion and migration (30–34), we investigated the effect of fMLP treatment on fibroblast cell adhesion to specific ECM. HT-1080 cells were seeded into 96-well plates precoated with different ECM proteins and allowed to adhere for 8 min. After extensive washing, adherent cells were quantified. fMLP treatment (100 nM) caused a 5-fold increase in adhesion to fibronectin (Fig. 5A). This adhesion was inhibited by 1 μM chelerythrine chloride, a specific inhibitor of PKC, and 50 μM LY294002, an inhibitor of phosphatidylinositol 3-kinase (Fig. 5B). In comparison, fMLP treatment caused only a modest increase in adhesion above background levels on collagen (Fig. 5A).

To assay migration on different ECM, HT-1080-GFP were seeded onto polycarbonate cell culture inserts coated on the top and bottom face with collagen or fibronectin and allowed to migrate for 8 h. Migrated cells were visualized and quantified by microscopy. Baseline migration of unstimulated HT-1080 was enhanced on fibronectin-coated inserts compared with collagen-coated or uncoated inserts. fMLP treatment resulted in increased migration on fibronectin, collagen IV, or BSA but the effect was most pronounced on fibronectin (Fig. 5C).

Discussion

We have shown that human fibroblasts express functional FPRs. Stimulation of these fibroblasts with fMLP causes calcium mobilization, actin polymerization, adhesion, and directed migration similar to that of leukocytes. This is the first report of functional FPR expression in either transformed or normal fibroblast cells.

Although a preponderance of functional data for these receptors has focused on phagocytic leukocytes, some research suggests that other cell types may have functional FPRs. Expression of FPR by transfection in Chinese hamster ovary cells, Xenopus oocytes, or U937 cells confers responsiveness to fMLP stimulation resulting in calcium mobilization, adhesion, and migration (35–37). Furthermore, FPR and FPRL1 have been shown to be functionally expressed on a human astrocytoma cell line, where FPR signaling is proposed as a mechanism of host defense in the brain, and in cardiac smooth muscle, where fMLP treatment produces contraction of arterial tissues (38–40). Functional FPR expression in the human liver cell line HepG2 was shown to regulate hepatic acute phase genes (41).

The image of phagocytic cells chasing and capturing bacteria by following a trail of formylated peptides is a dramatic illustration of...
the innate immune response. The molecular events that enable leukocyte chemotaxis toward bacteria have been extensively studied. The existence of FPRs on nonleukocyte cells may have been disregarded as aberrant gene expression or simply not studied because of the initial question: why would a fibroblast chase a bacterium? Of course, this question is based on assumptions that may be false. Fibroblast motility is unlikely to have the objective of capturing bacteria; rather, fibroblasts remodel damaged tissues. In response to tissue damage, fibroblasts migrate into inflammatory sites along a cross-linked fibrin and fibronectin matrix. This migration allows fibroblasts to repopulate wounds, remodel the provisional fibrin matrix via the deposition of collagen, and provide a new fibrous attachment for the growing tissue (42, 43). Functional FPRs expressed on fibroblasts might enable motility in response to formyl peptides produced by bacteria at the wound site.

Early fibroblast action may also facilitate the entry, movement, and exit of leukocytes from the inflamed site. Recently, the human lung epithelial cell line A549 was shown to have functional FPR expression that when stimulated lead to the synthesis of the acute phase protein fibrinogen (44). The idea that fibroblasts and perhaps other nonleukocyte cells possess the ability to respond to bacterial peptides may broaden our views of the innate immune response. That formyl peptides generate signal transduction and promote fibroblast motility is now demonstrated, but the next question is what else might they cause? The possibility that fibroblasts recognizing formyl peptides are triggered to secrete specific cytokines or inflammatory mediators should be the subject of future investigation.

Another possibility that should be considered is that there may be other ligands for FPRs and that the biological role for FPRs on fibroblasts relates to these nonformylated ligands. There is some evidence that the antimigratory protein annexin I (lipocortin 1) interacts with FPR (44, 45). In this regard, cathelicidin LL-37 has been reported to bind FPRL1. Cathelicidin LL-37 is an antimicrobial peptide secreted by neutrophils and by some epithelial cells in response to infection (46). Additionally, HIV envelope gp41 and
gp120 (47), serum amyloid A (48), and the prion peptide fragment 106–126 (49) have been reported to bind to FPRL1. The promiscuity of FPRL1 for multiple ligands, including some of endogenous origin, coupled with the poorly understood function of FPRL2 and the diverse cellular and tissue expression of the formyl peptide receptors, may suggest an unidentified function for the receptor family.

Because FPRs on fibroblasts could respond in vivo to these ligands, it is tempting to speculate an innate immune role for fibroblasts or other cell types that have functional FPRs. The prevalence of microbial flora on the skin and lungs would make available a formyl peptide signal in most wound sites. In this case, formyl peptides might serve as an additional signal to attract lung or skin fibroblasts to the wound to begin the repair process. Another plausible mechanism would be the migration of fibroblasts to sites of infection to contain or slow the infection through collagen deposition and tissue remodeling. This report demonstrates that FPRs of infection to contain or slow the infection through collagen dep- 

References

1. Le, Y., B. Li, W. Gong, W. Shen, J. Hu, N. M. Dunlop, J. J. Oppenheim, and J. M. Wang. 2000. Novel pathophysiological role of classical chemotactic peptide receptors and their communications with chemokine receptors. Immunol. Rev. 177:185.

2. Honeycutt, P. J., and J. E. Niedel. 1986. Cytochalasin B enhancement of the diacylglycerol response in formyl peptide-stimulated neutrophils. J. Biol. Chem. 261:15900.

3. Belisle, B., and A. Abo. 2000. N-Formyl peptide receptor ligation induces receptor-dependent actin reorganization through Gp130 subunits and class la phosphoinosi- 

tide 3-kinases. J. Biol. Chem. 275:26223.

4. Glogauer, M., J. Hartwig, and T. Stossel. 2000. Two pathways through Cdc42 couple the N-formyl receptor to actin nucleation in permeabilized human neutrophils. J. Cell Biol. 150:785.

5. Dillon, S. B., M. W. Vergheese, and R. Snyderman. 1988. Signal transduction in cells following binding of chemotactic peptides to membrane receptors. Virchows Arch. B Cell Pathol. Incl. Mol. Pathol. 55:65.

6. Panaro, M. A., and V. Mitolo. 1999. Cellular responses to FMLP challenging: a mini-review. Immunopharmacol. Immunotoxicol. 21:397.

7. Rossi, F. 1986. The O2−-forming NADPH oxidase of the phagocytes: nature, mechanisms of activation and function. Biochim. Biophys. Acta 853:65.

8. Sandberg, R. R., and J. E. Smolen. 1988. Early biochemical events in leukocyte activation. Lab. Invest. 59:360.

9. Seifert, R., and G. Schultz. 1991. The superoxide-forming NADPH oxidase of phagocytes: an enzyme system regulated by multiple mechanisms. Rev. Physiol. Biochem. Pharmacol. 117:1.

10. Becker, E. L., F. A. Forouhar, M. L. Grunnet, F. Boulay, M. Tardif, B. J. Bornman, D. Sodja, R. D. Ye, J. R. Woks, Jr., and P. M. Murphy. 1998. Broad immunocytochemical localization of the formylpeptide receptor in human organs, tissues, and cells. Cell Tissue Res. 292:129.

11. Low, D. A., J. B. Baker, W. C. Kooch, and D. D. Cunningham. 1981. Released protease-nexin regulates cellular binding, internalization, and degradation of serine proteases. Proc. Natl. Acad. Sci. USA 78:2340.

12. Tolley, J. O., G. M. Omann, and A. J. Jesaitis. 1987. A high yield, high-purity elution method for preparing human granulocytes demonstrating enhanced experimental life-times. J. Leukocyte Biol. 42:43.

13. Sklar, L. A., D. A. Finney, Z. G. Oades, A. J. Jesaitis, R. G. Painter, and C. G. Cochrane. 1984. The dynamics of ligand-receptor interactions: real-time analyses of association, dissociation, and internalization of an N-formyl peptide and its receptors on the human neutrophil. J. Biol. Chem. 259:5661.

14. Fay, S. P., R. G. Posner, W. N. Swann, and L. A. Sklar. 1991. Real-time analysis of the assembly of ligand, receptor, and G protein by quantitative fluorescence flow cytometry. Biochemistry 30:5066.

15. Durst, A., M. J. Gao, H. L. Tiffany, D. McDermott, and P. M. Murphy. 1994. Differential expression of members of the N-formylpeptide receptor gene cluster in human phagocytes. Biochim. Biophys. Acta 201:174.

16. Gao, J. L., H. Chen, J. D. Filer, C. A. Koza, and P. M. Murphy. 1998. Differential expansion of the N-formylpeptide receptor gene cluster in human and mouse. Genomics 51:270.

17. Murphy, P. M., T. Ozcelik, R. T. Kenney, H. L. Tiffany, D. McDermott, and U. Francke. 1992. A structural homologue of the N-formyl peptide receptor characterization and chromosomal mapping of a peptide chemoattractant receptor family. J. Biol. Chem. 267:7637.

18. Betten, A., J. Blylund, T. Cristophe, F. Boulay, A. Romero, K. Hellstrand, and C. Dahlgren. 2001. A proinflammatory peptide from Helicobacter pylori activates monocytes to induce lymphocyte dysfunction and apoptosis. J. Clin. Invest. 108:1221.

19. Christophe, T., A. Karlsson, C. Dugave, M. J. Rabiet, F. Boulay, and C. Dahlgren. 2001. The synthetic peptide Trp-Lys-Tyr-Met-Val-Met-NH2 specifically activates neutrophils through FPRL1/lipoxin A4 receptors and is an agonist for the orphan monocyte-expressed chemotactant receptor FPR2L. J. Biol. Chem. 276:21585.

20. Quennenerger, O. E. R. Prossnitz, S. L. Cavanagh, C. G. Cochrane, and R. D. Ye. 1993. Multiple domains of the N-formyl peptide receptor are required for high-affinity ligand binding: construction and analysis of chimeric N-formyl peptide receptors. J. Biol. Chem. 268:18167.

21. Hoffman, J. F., J. J. Linderman, and G. M. Omann. 1996. Receptor up-regulation, internalization, and interconversion: critical components of a quantitative description of N-formyl peptide-receptor dynamics in the neutrophil. J. Biol. Chem. 271:18394.

22. Bennett, T. A., T. A. Key, V. V. Gurevich, R. Neubig, E. R. Prossnitz, and L. A. Sklar. 2001. Real-time analysis of G protein-coupled receptor reconstitution in a solubilized system. J. Biol. Chem. 276:22453.
23. Seifert, R., and K. Wenzel-Seifert. 2001. Defective Gi protein coupling in two formyl peptide receptor mutants associated with localized juvenile periodontitis. *J. Biol. Chem.* 276:42943.

24. Ali, H., R. M. Richardson, E. D. Tomhave, J. R. Didsbury, and R. Snyderman. 1993. Differences in phosphorylation of formyl peptide and C5achemoattractant receptors correlate with differences in desensitization. *J. Biol. Chem.* 268:24247.

25. Liu, L., O. Harbecke, H. Elwing, P. Pollin, A. Karlsson, and C. Dahlgren. 1998. Desensitization of formyl peptide receptors is abolished in calcium ionophore-primed neutrophils: an association of the ligand-receptor complex to the cytoskeleton is not required for a rapid termination of the NADPH-oxidase response. *J. Immunol.* 160:2465.

26. Tardif, M., L. Mercier, I. Bouchon, and F. Boulay. 1993. Agonist-dependent phosphorylation of N-formylpeptide and activation peptide from the fifth component of C (C5a) chemotactic receptors in differentiated HL60 cells. *J. Immunol.* 150:3534.

27. Lauffenburger, D. A., and A. F. Horwitz. 1996. Cell migration: a physically integrated molecular process. *Cell* 84:359.

28. Lofgren, R., J. Ng-Sikorski, A. Sjolander, and T. Andersson. 1993. $\beta_2$ integrin engagement triggers actin polymerization and phosphatidylinositol trisphosphate formation in non-adherent human neutrophils. *J. Cell Biol.* 123:1597.

29. Dahlgren, C., T. Christophe, F. Boulay, P. N. Madianos, M. J. Rabiet, and A. Karlsson. 2000. The synthetic chemotactic receptor Trp-Lys-Tyr-Met-Val-o-Met activates neutrophils preferentially through the lipoxin $\Lambda_5$ receptor. *Blood* 93: 1810.

30. Volz, A. 1993. Regulation of CD18 expression in human neutrophils as related to shape changes. *J Cell Sci.* 106:493.

31. Dereian, C. K., R. J. Santulli, P. E. Rao, H. F. Solomon, and J. A. Barrett. 1995. Inhibition of chemotactic peptide-induced neutrophil adhesion to vascular endothelium by cAMP modulators. *J. Immunol.* 154:308.

32. Sheikh, S., and G. B. Nash. 1996. Continuous activation and deactivation of $\alpha_5\beta_1$ integrin is not required for a rapid termination of the NADPH-oxidase response. *J. Exp. Med.* 182:207.

33. Derian, C. K., R. J. Santulli, P. E. Rao, H. F. Solomon, and J. A. Barrett. 1995. F-Met-Leu-Phe and complement C5a receptors are expressed in liver cells and mediate hepatic acute phase gene regulation. *J. Exp. Med.* 182:207.

34. Tardif, M., L. Mery, L. Brouchon, and F. Boulay. 1993. Agonist-dependent phosphorylation of N-formylpeptide and activation peptide from the fifth component of C (C5a) chemotactic receptors in differentiated HL60 cells. *J. Immunol.* 150:3534.

35. Seifert, R., and K. Wenzel-Seifert. 2001. Defective Gi protein coupling in two formyl peptide receptor mutants associated with localized juvenile periodontitis. *J. Biol. Chem.* 276:42943.

36. Ali, H., R. M. Richardson, E. D. Tomhave, J. R. Didsbury, and R. Snyderman. 1993. Differences in phosphorylation of formyl peptide and C5achemoattractant receptors correlate with differences in desensitization. *J. Biol. Chem.* 268:24247.

37. Liu, L., O. Harbecke, H. Elwing, P. Pollin, A. Karlsson, and C. Dahlgren. 1998. Desensitization of formyl peptide receptors is abolished in calcium ionophore-primed neutrophils: an association of the ligand-receptor complex to the cytoskeleton is not required for a rapid termination of the NADPH-oxidase response. *J. Immunol.* 160:2465.

38. Lauffenburger, D. A., and A. F. Horwitz. 1996. Cell migration: a physically integrated molecular process. *Cell* 84:359.

39. Lofgren, R., J. Ng-Sikorski, A. Sjolander, and T. Andersson. 1993. $\beta_2$ integrin engagement triggers actin polymerization and phosphatidylinositol trisphosphate formation in non-adherent human neutrophils. *J. Cell Biol.* 123:1597.

40. Dahlgren, C., T. Christophe, F. Boulay, P. N. Madianos, M. J. Rabiet, and A. Karlsson. 2000. The synthetic chemotactic receptor Trp-Lys-Tyr-Met-Val-o-Met activates neutrophils preferentially through the lipoxin $\Lambda_5$ receptor. *Blood* 93: 1810.

41. Volz, A. 1993. Regulation of CD18 expression in human neutrophils as related to shape changes. *J Cell Sci.* 106:493.

42. Dereian, C. K., R. J. Santulli, P. E. Rao, H. F. Solomon, and J. A. Barrett. 1995. Inhibition of chemotactic peptide-induced neutrophil adhesion to vascular endothelium by cAMP modulators. *J. Immunol.* 154:308.

43. Sheikh, S., and G. B. Nash. 1996. Continuous activation and deactivation of integrin CD11b/CD18 during de novo expression enables rolling neutrophils to immobilize on platelets. *Blood.* 87:5040.

44. Rainier, G. E., C. Buckley, D. L. Simmons, and G. B. Nash. 1997. Cross-talk between cell adhesion molecules regulates the migration velocity of neutrophils. *Curr. Biol.* 7:316.

45. Anderson, S. I., N. A. Hotchin, and G. B. Nash. 2000. Role of the cytoskeleton in rapid activation of CD11b/CD18 function and its subsequent downregulation in neutrophils. *J. Cell Sci.* 113:2737.

46. Coats, W. D., Jr., and J. Navarro. 1990. Functional reconstituation of $\beta$-receptor activity in Xenopus laevis oocytes. *J. Biol. Chem.* 265:5964.

47. Kew, R. R., T. Peng, S. J. DiMartino, D. Madhavan, S. J. Weinman, D. Cheng, and E. R. Prossnitz. 1997. Undifferentiated U937 cells transfected with chemoattractant receptors: a model system to investigate chemotactic mechanisms and receptor structure/function relationships. *J. Leukocyte Biol.* 61:329.

48. Rainier, G. E., C. Buckley, D. L. Simmons, and G. B. Nash. 1997. Cross-talk between cell adhesion molecules regulates the migration velocity of neutrophils. *Curr. Biol.* 7:316.

49. Seifert, R., and K. Wenzel-Seifert. 2001. Defective Gi protein coupling in two formyl peptide receptor mutants associated with localized juvenile periodontitis. *J. Biol. Chem.* 276:42943.

50. Lofgren, R., J. Ng-Sikorski, A. Sjolander, and T. Andersson. 1993. $\beta_2$ integrin engagement triggers actin polymerization and phosphatidylinositol trisphosphate formation in non-adherent human neutrophils. *J. Cell Biol.* 123:1597.

51. Dahlgren, C., T. Christophe, F. Boulay, P. N. Madianos, M. J. Rabiet, and A. Karlsson. 2000. The synthetic chemotactic receptor Trp-Lys-Tyr-Met-Val-o-Met activates neutrophils preferentially through the lipoxin $\Lambda_5$ receptor. *Blood* 93: 1810.