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The Human *N*-Formylpeptide Receptor. Characterization of Two cDNA Isolates and Evidence for a New Subfamily of G-Protein-Coupled Receptors[†,‡]

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ABSTRACT: Two variants of the human *N*-formylpeptide chemoattractant receptor have been isolated from a CDM8 expression library prepared from mRNA of human myeloid HL-60 cells differentiated to the granulocyte phenotype with Bt2cAMP. Both recombinant receptors, fMLP-R26 and fMLP-R98, are 350 amino acids long (M_r 38 420); they differ from each other by two residue changes at positions 101 and 346 and by significant differences in the 5′ and 3′ untranslated regions. Both clones were able to transfer to COS-7 cells the capacity to specifically bind a new and highly efficient hydrophilic derivative of *N*-formyl-Met-Leu-Phe-Lys, referred to as fMLPK-Pep12. Photolabeling experiments revealed that the glycosylated form of the fMLP receptor in COS cells has a molecular weight (M_r 50 000-70 000) similar to that observed for the native receptor in differentiated HL-60 cells. Northern blot analysis revealed a major transcript of 1.6-1.7 kb and two minor hybridization signals of 2.3 and 3.1 kb, suggesting a related family of receptors. The complex hybridization pattern obtained with restricted genomic DNA was consistent with either two genes encoding fMLP receptor isoforms or a single gene with at least one intron in the coding sequence. Sequence comparison established that the fMLP receptor belongs to the G-protein-coupled receptor superfamily. The structural similarities observed with RDC1, a receptor isolated from a dog thyroid cDNA library, which shares weak homologies with other members of the family, suggests that the fMLP receptor is representative of a new subfamily.

Polymorphonuclear neutrophils are phagocytic cells specialized in the destruction of microorganisms. To accomplish their essential role in host defense against bacterial and fungal infections, they emigrate from blood vessels to the sites of infection by active amoeboid movements. The directed locomotion of the neutrophils is triggered by specific substances, termed chemotactic factors, which include the platelet activating factor (PAF) (O'Flaherty et al., 1986), arachidonate metabolite leukotriene B4 (LTB4) (Goldman & Goetzl, 1982), anaphylatoxin complement fragment C5a (Shin et al., 1968; Chenoweth & Hugli, 1978), and interleukin 8 (IL-8/NAP-1) (Baggiolini et al., 1989), and a number of *N*-formylmethionyl peptides. The *N*-formylated peptides are believed to derive from bacterial protein degradation (Schiffman et al., 1975; Marasco et al., 1984) or to arise from mitochondrial proteins upon tissue damage (Carp, 1982).

In addition to the directed migration of the cell, PAF, C5a, LTB_4, IL-8, and fMLP[1] stimulate a variety of coordinated biochemical and cellular responses in neutrophil and macrophages, including aggregation, phagocytosis of particles,

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[1] Abbreviations: BSA, bovine serum albumin; fMLP, *N*-formylmethionine-leucine-phenylalanine (fMLP is the commonly used, but nonstandard, abbreviation, which incorrectly assigned the letter P for phenylalanine); fMLPK, *N*-formylmethionine-leucine-phenylalanine-lysine; fMLPK-Pep12, *N*-formyl-Met-Leu-Phe-N^{ϵ}[*m*-benzoyl-(3-*S*-Cys-Tyr-Asp-Lys-Leu-Phe-Ser-Leu-Ala-Gln-Asp-Ser-*N*-acetyl)dihydromaleimide]Lys; HBS, Hanks' balanced salt solution; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; Rt, retention time.

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production of cytotoxic and microbicidal superoxide radicals, release of granule proteolytic enzymes, and opening of ion channels [reviewed by Snyderman and Pike, (1984a,b), Snyderman et al. (1986), Snyderman and Uhing (1988) and Sha'afi and Molski (1988)]. Many of these responses are initiated via specific surface receptors coupled to a guanine nucleotide regulatory or G protein. It is now well established that the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP_2) by phospholipase C into the Ca^{2+} mobilizer inositol 1,4,5-trisphosphate (IP_3) and the protein kinase C activator 1,2-diacylglycerol (DG) represents an early biochemical event initiated by the ternary complex consisting of agonist, receptor, and regulatory G protein (Smith et al. 1985). The cellular responses are attenuated in neutrophils treated with the *Bordetella pertussis* toxin that ADP-ribosylates a 40–41-kDa protein with molecular properties similar to those of the α subunit of a regulatory G_i protein (Okajima & Ui, 1984; Lad et al., 1985).

Among the chemoattractant receptors, the *N*-formylmethionine peptide receptor of human neutrophils is the most studied [reviewed by Allen et al. (1988)]. In many respects, the *N*-formylpeptide–receptor system resembles hormone– and neurotransmitter–receptor systems: (i) Receptor–peptide interaction initiates the signal transduction and the affinity for the formylpeptide is regulated by guanine nucleotides and analogues (Snyderman & Uhing, 1988). (ii) The receptor is subsequently down-regulated, and repeated exposure to agonist results in desensitization of the transduction pathway (Seligman et al. 1982). Studies of *N*-formylpeptide binding to membranes of neutrophils or HL-60 cells differentiated in neutrophils suggested a model with two classes of independent receptors with high- and low-affinity binding sites (K_d 1 nM and 20–40 nM, respectively) (Koo et al., 1982; Gierschik et al., 1989). The difference in affinity did not seem to be due to intrinsically different receptors but rather to interactions between the receptor and the regulatory G protein. Thus, pretreatment of neutrophil or HL-60 plasma membrane with nonhydrolyzable analogues of GTP (e.g., GppNHp or GTPγS) reduces the number of high-affinity binding sites, and concomitantly, part of the high-affinity binding sites are converted into low-affinity binding sites (Snyderman & Uhing, 1988; Gierschick et al., 1989).

Through the use of photoaffinity labeling and cross-linking techniques, it has been established that the N-formylated peptide receptor in human neutrophils or differentiated myeloid HL-60 cells is a glycosylated protein with an apparent M_r of 55 000–70 000 that can be resolved into two isoforms with isoelectric points (p*I*) of 6.0 and 6.5 (Niedel et al., 1980a; Dolmatch & Niedel, 1983; Schmitt et al., 1983; Malech et al., 1985). Removal of the carbohydrates by treatment of the receptor with endo β-*N*-acetylglucosaminidase (endo F) leaves a 32 000-dalton protein backbone that conserves the ability to bind the ligand (Malech et al., 1985). Attempts to purify the receptor to homogeneity have been elusive, and only partial purifications have been reported (Hoyle & Freer, 1984; Huang, 1987).

The isolation of a cDNA, identified as coding for the human *N*-formylpeptide receptor, has been previously described (Boulay et al., 1990). However, the complete characterization of the recombinant receptor was not made. Here, we report the isolation of a major variant cDNA, referred to as fMLP-R26. We have characterized the recombinant receptor with regard to endocytosis of a rhodamine-derivatized hexapeptide (*N*-formyl-Nle-Leu-Phe-Nle-Tyr-Lys-RITC), binding parameters, photolabeling, and mRNA and genomic DNA analysis. We provide evidence for a related group of receptors within the G-protein-coupled superfamily.

Materials and Methods

Cells and Cell Culture. HL-60 and COS-7 cells were from the American Type Culture Collection (Rockville, MD). COS-7 monolayers and HL-60 promyelocytic cells were cultured and differentiated as previously described (Boulay et al., 1990).

Radioligand. The chemotactic tetrapeptide *N*-formyl-Met-Leu-Phe-Lys (fMLPK) was derivatized by coupling the hydrophilic dodecapeptide (*N*-acetyl-SDQALSFLKDYC) on the free amino group of the lysine and radioiodinated as previously described (Boulay et al., 1990). This new *N*-formylpeptide derivative was referred to as fMLPK-Pep12.

Binding Assays on HL-60 Cells and Transfected COS Cells. HL-60 cells grown for 72 h in the presence of Bt2cAMP were resuspended in HBS/HEPES/1% BSA at 2×10^6 cells/200 μL. Aliquots of 200 μL of cells were incubated for 2 h at 4 °C with increasing concentrations of ^{125}I-fMLPK-Pep12 with or without 4 μM fMLPK. Cell suspensions were layered over 500 μL of a chilled solution of sucrose (8% in HBS) and spun for 2 min in an Eppendorf centrifuge. The cell pellets were removed by cutting the tube with a knife blade, and the cell-associated radioactivity was determined by counting in a γ-counter.

Subconfluent monolayers of COS cells in 60-mm tissue culture dishes were transfected by the DEAE-dextran method with 1 μg of CsCl-purified fMLP receptor cDNA. Seventy-two hours after transfection, monolayers were washed twice with chilled HBS/HEPES and once with HBS/HEPES/1% BSA and incubated in duplicate with increasing concentrations of ^{125}I-fMLPK-Pep12 diluted in 1.5 mL of an ice-cold solution of DME, 1% BSA, and 25 mM HEPES (pH 7.5) in the presence or absence of unlabeled fMLPK (4 μM). Incubations were performed at 4 °C for 2 h with gentle rocking. Monolayers were washed three times at 4 °C and solubilized in 1 M NaOH, and the bound radioactivity was counted in a γ-counter.

Membranes of transfected COS cells were prepared as described by Giros et al. (1989). Binding was assayed in HBS/HEPES/1% BSA at 4 °C as described above. To separate free and bound radioactive ligand, the membranes were centrifuged in Eppendorf tubes, and the pellets were rinsed once with 1 mL of buffer before the bound radioactivity was counted in a γ-counter.

Synthesis of a New Photoactivable Derivative of fMLPK and Photolabeling Experiments. Cells were photolabeled with a photoaffinity heterobifunctional derivative of fMLPK (fMet-Leu-Phe-N^{ϵ}-[N^{α}-[3-[[2-(*p*-azido-^{125}I-salicylamido)-ethyl]dithio]propionyl]biocytine]-Lys; referred to as fMLPK-B-^{125}I-SASD). The synthesis of this derivative was achieved as follows: the heterobifunctional moiety, N-$^{\alpha}$-[3-[[2-(*p*-azidosalicylamido)ethyl]dithio]propionyl]biocytine, was first prepared by mixing equimolar amounts (20 μmol) of sulfosuccinimido 3-[[2-(*p*-azidosalicylamido)ethyl]dithio]propionate (SASD) (from Pierce), biocytine (from Calbiochem), and triethylamine in 1 mL of dry dimethylformamide (DMF). The reaction was carried out overnight at room temperature in the dark, and the reaction products were separated by ascending chromatography on Whatman K6F silica gel plates, with a solvent system made of chloroform–methanol–water–acetic acid (55/20/3/3 v/v). Biocytine–SASD migrated with a R_f = 0.7. The purified precursor was dried under vacuum, resolubilized in anhydrous dimethyl sulfoxide (DMSO), and stored at −20 °C. Coupling to the chemotactic peptide fMLPK

was carried out by preparing the *N*-hydroxysuccinimido ester of biocytine–SASD as follows: 3 μmol of biocytine–SASD and 4 μmol of *N*-hydroxysulfosuccinimide were mixed with 4 μmol of dicyclohexylcarbodiimide in 200 μL of DMSO for 16 h at room temperature in the dark; 3 μmol of triethylamine and 2 μmol of fMet-Leu-Phe-Lys dissolved in 500 μL of DMF were added. The course of the coupling reaction was followed by HPLC with a Waters system equipped with a μBondapak C18 column (4.6 × 150 mm, 5 μm). The chemotactic derivative fMLPK-B-SASD, eluted with a 40-min linear gradient of 20–100% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 1 mL/min (Rt = 19 min), was dried under vacuum and stored in DMSO at −20 °C. Radioiodination was carried out on 1 nmol of the derivative as previously described. Carrier-free fMLPK-B-^{125}-SASD (≈4000 Ci/mmol) was separated from unlabeled derivative and free iodine by HPLC as described above (Rt = 20 min). On intact Bt2cAMP-treated HL-60 cells, the radioactive photoactivable derivative demonstrated a high affinity for the *N*-formylpeptide receptor with a K_d value of 1–2 nM (not shown).

In a typical photolabeling experiment, cells were resuspended in HBS/HEPES and preincubated with fMLPK-B-^{125}I-SASD (5 nM) in the presence or absence of 2 μM unlabeled peptide and then exposed to the light of a xenon lamp XBO 1000W/HS equipped with a parabolic reflector. Irradiation was operated at 0 °C for 5 min at 0.9 kW through a glass plate. The photolabeled cells were centrifuged through a 8% sucrose cushion, and the cell pellet was dissolved in Laemmli dissociation buffer in the presence of 5 mM dithiothreitol and protease inhibitors (PMSF, 2 mM; DFP, 1 mM; aprotinin, 0.23 unit/mL; leupeptin, 0.5 mM; chymostatin, 50 μg/mL). The solubilized material was analyzed on 9% polyacrylamide slab gels with a 5% stacking gel, prepared and run according to Laemmli (1970).

Nucleotide Sequencing. The inserts of clones 26 and 98 were sequenced by the dideoxy chain-termination method (Sanger et al., 1977) with the modified T7 polymerase (Sequenase, U.S. Biochemicals). Both strands of clones 26 and 98 were entirely sequenced.

Northern and Southern Blot Analysis. Total RNA was isolated from HL-60 and COS cells by the guanidinium isothiocyanate/phenol method at pH 4 as described by Chomczynski and Sacchi (1987). RNAs were fractionated on 1.2% agarose/formaldehyde gels (Maniatis et al., 1982) and transferred to nylon membranes (Hybond N from Amersham) according to the manufacturer's recommendations. Prior to hybridization, the filters were soaked in 5 mL of rapid hybridization buffer (Amersham) at 65 °C. Hybridization was performed in the same buffer for 3 h at 65 °C with the gel-purified restriction fragment *Hin*dIII–*Pst*I (1.1 kb) that encompasses the 5′ untranslated region and almost the entire coding region of clone fMLP-R 26. This probe was labeled with ^{32}P by random priming (Feinberg & Vogelstein, 1983). After hybridization, the nylon membrane was washed twice in 2× SSC/0.1% SDS at room temperature, once in 1× SSC/1% SDS for 15 min at 65 °C, and twice in 0.7× SSC/0.1% SDS for 15 min at 65 °C.

Genomic DNA was prepared from HL-60 cells according to Davis et al. (1986). Aliquots (20 μg) were digested with various restriction enzymes and electrophoresed on a 1% agarose gel, transferred to Hybond N, hybridized at 65 °C with the ^{32}P-labeled probe (*Hin*dIII–*Pst*I), and washed as described above.

Results

The difficulties encountered in purifying the *N*-formylpeptide receptor to homogeneity have thus far prevented the development of immunological and/or cDNA probes that could be used to clone and subsequently elucidate the primary structure of this receptor. Direct expression cloning in mammalian cells was therefore an appropriate strategy to isolate cDNA clones of the fMLP receptor. Our approach to expression cloning involved the transfection of simian COS cells with DNA from relatively small pools of cDNA clones and, several days, later the detection of positive pools by assaying the cells for endocytosis of a high-affinity ligand according to the procedure previously described for the cloning of the erythropoietin receptor (D'Andrea et al., 1989). Preliminary experiments with HL-60 cells showed that the uptake of ^{125}I-*N*-formylpeptide increased gradually during the first 60 min and then rapidly decreased (not shown). Hence, we assumed that the transfected COS cells expressing the fMLP receptor would be able to accumulate the radioactive formylpeptide as long as the uptake period is limited to 60 min. To optimize the detection assay, we synthesized a derivative of the chemoattractant *N*-formyl-Met-Leu-Phe-Lys, referred to as fMLPK-Pep12 (cf. Materials and Methods) by coupling to the ε-amino group of the lysine a hydrophilic dodecapeptide. This dodecapeptide represents the *N*-terminal peptide of the bovine mitochondrial ADP/ATP carrier, against which we had previously raised polyclonal antipeptide antibodies (Brandolin et al., 1989). The novel *N*-formylpeptide derivative retained its biological activity and proved to be a more potent ligand than its underivatized parent tetrapeptide (Boulay et al., 1990). Because of its hydrophilic character, this derivative interacted with lipid bilayers only to a small extent, compared to the currently used *N*-formylpeptides, i.e., *N*-formyl-Met-Leu-Phe and *N*-formyl-Nle-Leu-Phe-Nle-Tyr-Lys. Consequently, an excellent signal-to-noise ratio was obtained during the screening procedure.

A cDNA library from HL-60 cells that were differentiated into granulocytes with dibutyryl adenosine cyclic monophosphate (Bt2cAMP) was constructed in the high-efficiency COS cell expression vector CDM8 (Aruffo & Seed, 1987). Poly (A^+) mRNA was isolated 54 h after induction by Bt2cAMP and after checking for expression of the receptor by radioactive *N*-formylpeptide binding. The yield of full-length clones was optimized by size-selecting cDNA above 1.3 kb before ligation into the CDM8 vector. This library proved to be a rich source of fMLP-receptor cDNA clones. Plasmid DNA from 184 pools, each containing approximately 700 independent bacterial colonies, was used to transfect subconfluent COS cell monolayers. The low level of nonspecific ^{125}I-fMLPK-Pep12 binding to COS transfectants and the relatively small standard deviation from a negative pool to the next (1800 ± 350 dpm) allowed the unambiguous identification of 12 positive pools, yielding signals from 7000 to 20000 cpm. Two cDNA variants referred to as fMLP-R26 and fMLP-R98 were identified as coding for the *N*-formylpeptide receptor. Their characterization is fully described hereafter.

cDNA Characterization and Sequence Comparison of fMLP-R26 and fMLP-R98 Receptors. The inserts in the cDNA clones were excised by *Xba*I digestion and analyzed by agarose gel electrophoresis. Clone fMLP-R98 had a longer insert (1.9 kb) than clone fMLP-R26 (1.35 kb) (Figure 1A). Nucleotide sequencing revealed the presence of a large open reading frame (ORF) of 1050 bp in both inserts. The putative translated regions were found to be collinear except for two single, nonsilent base differences. In both sequences, the first ATG codon is not in a context that fully corresponds to the consensus sequence (CCRCCATGG) typical of initiation sites

of eukaryotic mRNAs (Kozak, 1987). Nevertheless, with an A in position -3 and the periodic occurrence of G in positions -6 and -9, this ATG codon emerges as a potential initiation site (Kozak, 1987). Furthermore, since both cDNA clones encode a functional protein with respect to cell surface localization, binding, and internalization of radioactive or fluorescent *N*-formylpeptide derivatives, they must include the entire coding region of the receptor. We assume, therefore, that the first ATG codon is used to initiate the translation (Figure 1B).

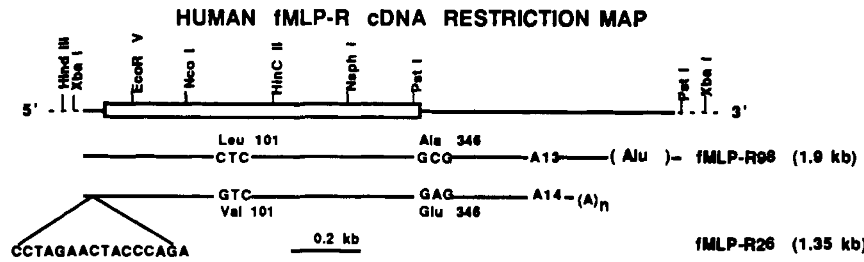
For both cDNAs, the predicted translation product is 350 amino acids long, with a calculated M_r of 38 420. Both translated products contain two potential N-linked glycosylation sites in the amino-terminal region (Asn-Ser-Ser and Asn-Ile-Ser, at positions 4 and 10) and a third potential site (Asn-Phe-Ser) at position 179. However, previous biochemical investigations that used endoglycosidase F digestion have provided evidence for only two N-linked oligosaccharides on the receptor expressed in HL-60 cells (Malech et al., 1985). The coding sequence of fMLP-R26 cDNA was found to differ slightly from that of fMLP-R98. The difference consisted in two base substitutions, namely, C → G at position 301 and C → A at position 1037, resulting in the replacement of Val^{101} and Glu^{346} in fMLP-R26 by Leu and Ala in fMLP-R98 (Figure 1A). Additional changes were observed in the 5′ and 3′ untranslated regions. Compared to the cDNA of fMLP-R26, fMLP-R98 cDNA showed a 16-bp deletion at position -39 and an A was absent at position 1175. Furthermore, the 3′ noncoding sequences diverged at positions 1219 and 1220. While the 3′ untranslated sequence of fMLP-R26 ended with poly(A) tail downstream from the consensus hexanucleotide (AATAAA) polyadenylation signal, that of fMLP-R98 extended for an additional set of 661 bases containing a sequence homologous to the "Alu" family of repetitive elements (nucleotides 1535–1835) (Jelinek & Schmid, 1982). In view of the limited differences throughout the nucleotide sequences, it seems likely that these two cDNA clones reflect an allelic variation of the same gene rather than two different genes.

Binding and Endocytosis of a Rhodamine-Labeled Hexapeptide by the Recombinant fMLP Receptor Expressed in COS-7 Cells. To further confirm the identity of the isolated cDNA clones with the fMLP receptor, we incubated transfected COS cells with the tetramethylrhodamine-labeled *N*-formyl-Nle-Leu-Phe-Nle-Tyr-Lys (TMR peptide). This rhodamine derivative is a powerful chemoattractant for human neutrophils and differentiated HL-60 cells and has previously been used to study the cellular distribution and internalization of the chemotactic receptor (Niedel et al., 1979, 1980b). As the fluorescent derivative has no chemical group that may be cross-linked with amino acid residues at the binding site, the fluorescence staining rapidly became diffuse during the fixation step. TMR–peptide binding was therefore directly observed on living cells. As shown by fluorescence microscopy, 20%–30% of the COS cells transfected with fMLP-R26 cDNA presented surface staining when cells were incubated with 1 nM TMR–peptide for 1 h at 4 °C (Figure 2A). No staining was observed when the rhodamine derivative was added to the cells in the presence of 2 μM fMLPK (data not shown). Incubation of transfectant cells for 1 h at 37 °C indicated further that the receptor was capable of mediating endocytosis. Most of the ligand was present in vacuoles that punctuate the cytoplasm and/or accumulate in the perinuclear region, presumably lysosomes (Figure 2B); relatively little staining was observed on the plasma membrane, indicating that most of the surface receptors had been internalized. Essentially the same results were obtained with COS cells transfected with fMLP-R98 cDNA (not shown).

Binding Parameters of the Recombinant fMLP Receptor Expressed in COS Cells. We next tested whether binding of ^{125}I-fMLPK-Pep12 to the recombinant fMLP receptor expressed in COS cells was similar to binding to the endogenous fMLP receptor in differentiated HL-60 cells. This was examined by steady-state binding techniques on intact cells either in suspension or adherent to tissue culture plates, and the formylpeptide binding isotherms were analyzed by the graphic method described by Rosenthal (1967). Surprisingly, the binding parameters of the ligand to the transfected COS cell monolayers were found to be different from those observed in differentiated HL-60 cells, as seen in Figure 3. While Bt2cAMP-differentiated HL-60 cells displayed a single class of binding sites with a K_d value of 1.5–2.0 nM, COS cells transfected with fMLP-R26 cDNA exhibited high- and low-affinity binding sites ($K_{d1} \approx 0.7$–1 nM, $K_{d2} \approx 20$–40 nM), as illustrated by the Scatchard analyses in the insets of Figure 3. Essentially the same binding characteristics were demonstrated with cells transfected with fMLP-R98 cDNA with, nevertheless, a slightly better K_d value ($K_{d2} \approx 5$–10 nM) for the low-affinity class of binding sites (Boulay et al., 1990). In a number of independent experiments (data not shown), ^{125}I-fMLPK-Pep12 binding to COS cells in suspension was tested to determine whether the low-affinity state was an artifact inherent in the binding methodology applied to adherent cells. The same binding isotherms were found with cells in suspension, indicating that adherence did not alter the binding characteristics of the recombinant receptor. Thus, although the fMLP receptor cDNA encodes a single polypeptide that is believed to contain a single binding site for N-formylated peptides, both high- and low-affinity binding sites are demonstrated on intact transfected COS cells. This result contrasts with the single class of binding sites found in intact neutrophils or viable macrophages (Tennenberg et al., 1988; Snyderman et al., 1984) and Bt2cAMP-treated HL-60 (this study; Chaplinsky & Niedel, 1982). However, data obtained with membrane preparations of neutrophils or differentiated HL-60 clearly support the existence of two classes of binding sites. It is now clear that GTP or its nonhydrolyzable analogues (but not the corresponding adenosine compounds) act on these membranes via a G protein (Okajima & Ui, 1984; Lad et al., 1985) to cause the conversion of the fMLP receptor from a high-affinity state to a low-affinity state (Snyderman & Uhing, 1988; Gierschick et al., 1989).

Effect of Nonhydrolyzable Nucleotides on ^{125}I-fMLPK-Pep12 Binding. To investigate further the underlying mechanisms that determine the expression of high- and low-affinity binding sites in COS transfectants, we tested the possible effect of guanine nucleotides on the binding parameters of the recombinant receptor. Membrane preparations of COS cells, transfected with either fMLP-R26 cDNA or fMLP-R98 cDNA, were assayed for ^{125}I-fMLPK-Pep12 binding in the presence of nonhydrolyzable GTP or ATP analogues. While the nonhydrolyzable adenine nucleotide compound (AppNHp) had little effect on the amount of bound ligand, a 50% decrease was observed when the incubation medium was supplemented with GTP-γ-S (not shown). This decrease is mostly likely the result of a GTP-γ-S-induced transition of high-affinity binding sites to low-affinity sites, suggesting that a GTP-binding protein present in the COS cells interacts with the recombinant fMLP receptor. Similar observations were recently described in the case of the D2 dopamine receptor expressed in COS cells (Giros et al., 1989). However, additional mechanisms that

A



B

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  -61  CCCAGACCTAGAACTACCCAGAGCAAGACCACAGCTGGTGAACAGTCCAGGAGCAGACAAG
    1  ATG GAG ACA AAT TCC TCT CTC CCC ACG AAC ATC TCT GGA GGG ACA CCT GCT GTA TCT GCT
       met glu thr asn ser ser leu pro thr asn ile ser gly gly thr pro ala val ser ala   20
   61  GGC TAT CTC TTC CTG GAT ATC ATC ACT TAT CTG GTA TTT GCA GTC ACC TTT GTC CTC GGG
       gly tyr leu phe leu asp ile ile thr tyr leu val phe ala val thr phe val leu gly   40
  121  GTC CTG GGC AAC GGG CTT GTG ATC TGG GTG GCT GGA TTC CGG ATG ACA CAC ACA GTC ACC
       val leu gly asn gly leu val ile trp val ala gly phe arg met thr his thr val thr   60
  181  ACC ATC AGT TAC CTG AAC CTG GCC GTG GCT GAC TTC TGT TTC ACC TCC ACT TTG CCA TTC
       thr ile ser tyr leu asn leu ala val ala asp phe cys phe thr ser thr leu pro phe   80
  241  TTC ATG GTC AGG AAG GCC ATG GGA GGA CAT TGG CCT TTC GGC TGG TTC CTG TGC AAA TTC
       phe met val arg lys ala met gly gly his trp pro phe gly trp phe leu cys lys phe  100
  301  GTC TTT ACC ATA GTG GAC ATC AAC TTG TTC GGA AGT GTC TTC CTG ATC GCC CTC ATT GCT
       val phe thr ile val asp ile asn leu phe gly ser val phe leu ile ala leu ile ala  120
  361  CTG GAC CGC TGT GTT TGC GTC CTG CAT CCA GTC TGG ACC CAG AAC CAC CGC ACC GTG AGC
       leu asp arg cys val cys val leu his pro val trp thr gln asn his arg thr val ser  140
  421  CTG GCC AAG AAG GTG ATC ATT GGG CCC TGG GTG ATG GCT CTG CTC CTC ACA TTG CCA GTT
       leu ala lys lys val ile ile gly pro trp val met ala leu leu leu thr leu pro val  160
  481  ATC ATT CGT GTG ACT ACA GTA CCT GGT AAA ACG GGG ACA GTA GCC TGC ACT TTT AAC TTT
       ile ile arg val thr thr val pro gly lys thr gly thr val ala cys thr phe asn phe  180
  541  TCG CCC TGG ACC AAC GAC CCT AAA GAG AGG ATA AAT GTG GCC GTT GCC ATG TTG ACG GTG
       ser pro trp thr asn asp pro lys glu arg ile asn val ala val ala met leu thr val  200
  601  AGA GGC ATC ATC CGG TTC ATC ATT GGC TTC AGC GCA CCC ATG TCC ATC GTT GCT GTC AGT
       arg gly ile ile arg phe ile ile gly phe ser ala pro met ser ile val ala val ser  220
  661  TAT GGG CTT ATT GCC ACC AAG ATC CAC AAG CAA GGC TTG ATT AAG TCC AGT CGT CCC TTA
       tyr gly leu ile ala thr lys ile his lys gln gly leu ile lys ser ser arg pro leu  240
  721  CGG GTC CTC TCC TTT GTC GCA GCA GCC TTT TTT CTC TGC TGG TCC CCA TAT CAG GTG GTG
       arg val leu ser phe val ala ala ala phe phe leu cys trp ser pro tyr gln val val  260
  781  GCC CTT ATA GCC ACA GTC AGA ATC CGT GAG TTA TTG CAA GGC ATG TAC AAA GAA ATT GGT
       ala leu ile ala thr val arg ile arg glu leu leu gln gly met tyr lys glu ile gly  280
  841  ATT GCA GTG GAT GTG ACA AGT GCC CTG GCC TTC TTC AAC AGC TGC CTC AAC CCC ATG CTC
       ile ala val asp val thr ser ala leu ala phe phe asn ser cys leu asn pro met leu  300
  901  TAT GTC TTC ATG GGC CAG GAC TTC CGG GAG AGG CTG ATC CAC GCC CTT CCC GCC AGT CTG
       tyr val phe met gly gln asp phe arg glu arg leu ile his ala leu pro ala ser leu  320
  961  GAG AGG GCC CTG ACC GAG GAC TCA ACC CAA ACC AGT GAC ACA GCT ACC AAT TCT ACT TTA
       glu arg ala leu thr glu asp ser thr gln thr ser asp thr ala thr asn ser thr leu  340
 1021  CCT TCT GCA GAG GTG GAG TTA CAG GCA AAG TGAGGAGGGAGCTGGGGGACACTTTCGAGCTCCCAGCTC
       pro ser ala glu val glu leu gln ala lys
 1090  CAGCTTCGTCTCACCTTGAGTTAGGCTGAGCACAGGCATTTCCTGCTTATTTTAGGATTACCCACTCATCAGAAAAAAA
 1168  AAAAAAAGCCTTTGTGTCCCCTGATTTGGGGAGAATAAACAGATATGAGTTT(A)n        3'  fMLP-R26
                                                       ATTATTGACTTCTTTTTTGATTTTGG
 1246  ACCTCAGCCTCGGGTGGTCAGGGTGGGAAATGATAGGAAGAAGCTGTCATCTGCATCCTAGTTTGCCTGAAATGAACCC
 1325  AAATAATACCCATTATTATTAGTCCTGAATTATGAGTAGTGAATGATACCCATCATTCTGGCATCATGATGAGTAGTGT
 1404  CCACTTCCATTCTGAAAAGTGCCCTGCTGTGAAAAATAAATTATATAGTCATCCTAGGTAAATGAAGGAGGAGGGAGAA
 1483  GTGTGAAAGAGTATGGCTTAAATCAGACAAGATATACAAGAAGATACTTTATATAGGGCAGGAGCGGTGGCTCATGCCT
 1562  GTAATCCCAGCACTTTGGGAGGCCGAGGCAGGCGGATCACCAGAGGTCAGGAATTCGAGAACAGCCTGGCCAACATGGT
 1641  GAAACCCTGTCTCTACTAAAAATACAAAAATTAGCTGGGCGTAGTGGCAGGCTCCCGTAATCCCAGCTACTCAGGAGAC
 1720  CGAGGCAGGAGAATCGCTTGGACCTGGAAGGCGGAGGTTGTAGTGAGCCAAGAAAACGCCACTACACTCCAGCCTGGGT
 1799  GACAGAGAGAGACTCCGGCTCAGAAAAAAAAAAAAAA                            3'  fMLP-R98
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FIGURE 1: Human *N*-formylpeptide receptor cDNA variants. (A) Schematic representation and restriction endonuclease cleavage map of the inserts of two independent cDNA clones of the human fMLP receptor. The coding region is illustrated by an open box. The 5′ and 3′ untranslated regions are represented by a thick line and the vector polylinker regions by dotted lines. (B) Combined nucleotide sequences of fMLP-R26 and fMLP-R98 cDNAs and predicted amino acid sequence from fMLP-R26 translated region. The numbers on the left side indicate positions of nucleotides, and numbers on the right side refer to the amino acid sequence. The potential N-glycosylation sites (Asn-X-Ser/Thr) are boxed. Double-underlined nucleotides from position −55 to −39 and at position 1175 indicate the base deletions in clone fMLP-R98. The two circled amino acids at positions 101 and 346 denote the differences between fMLP-R26 and fMLP-R98. The consensus AATAAA polyadenylation signal and the Alu sequence are underlined. The nucleotide sequence data reported will appear in GenBank under the accession number M33538 for fMLP-R26 and M33537 for fMLP-R98.

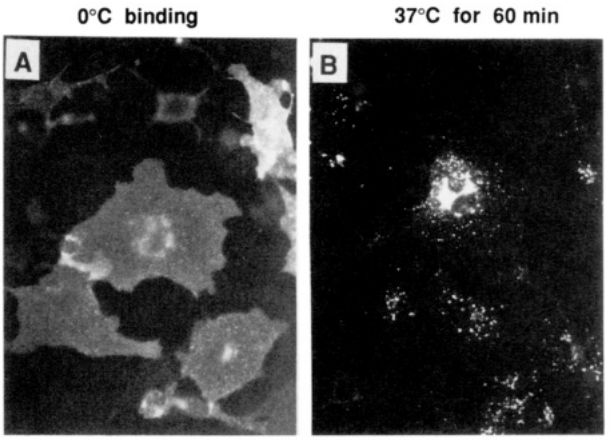

FIGURE 2: Binding and endocytosis of a fluorescent *N*-formylpeptide derivative by COS cells transiently expressing fMLP receptor. COS-7 cells were electroporated in PBS with fMLP-R26 cDNA as described by Gearing et al. (1989). After 3 days, the transfected COS cells were incubated with 1 nM tetramethylrhodamine-labeled *N*-formyl-Nle-Leu-Phe-Nle-Tyr-Lys (Molecular Probes Inc., Eugene, OR) in HBS/HEPES/1% BSA for 1 h at 4 °C (picture A) or 37 °C (picture B). Surface-bound and intracellular ligand was visualized on the living cells.

are not mutually exclusive may also modulate the affinity of the fMLP recombinant receptor: (i) Due to the overexpression of the receptor in transfected COS cells and the possible saturation of the exocytic pathway, differential carbohydrate processing may occur, generating receptors glycosylated to different extents and endowed with distinct affinities. This view is supported by the altered binding characteristics of the nonglycosylated receptor in tunicamycin-treated HL-60 cells (Heiman et al., 1986). (ii) The high density of receptors expressed on the plasma membrane may cause aggregation, resulting in modification of the binding affinity. (iii) Post-translational modifications such as phosphorylation, methylation, or acylation may be responsible for altered binding parameters.

Photolabeling of the fMLP Receptor Expressed in COS Cells and Differentiated HL-60 Cells. To characterize the fMLP receptor recombinant further, we compared its molecular size with that of the receptor expressed in differentiated HL-60 cells following photoaffinity labeling with an azido derivative of the tetrapeptide fMet-Leu-Phe-Lys. This compound, referred to as fMLPK-B-SASD, is a bifunctional derivative that is able to react covalently via its photoactivable azido group on the one hand and to interact with avidin or streptavidin via its biotin moiety on the other hand. This derivative was designed for further studies aimed at isolating regions of the polypeptide chain interacting with the N-formylated peptide.

As observed by others (Niedel et al., 1980a; Schmitt et al., 1983; Malech et al., 1985; Allen et al., 1986), the glycosylated form of the fMLP receptor in human neutrophils and differentiated HL-60 cells migrated in polyacrylamide gel electrophoresis as a broad band with an apparent M_r of 55 000–70 000. Two additional, sharper bands, with M_r values of 38 000 and 40 000, were also consistently detected in spite of the presence of protease inhibitors (Figure 4, lane 2). The labeling was specific, as evidenced by the total lack of radiolabeling when an excess of unlabeled formylpeptide was simultaneously added (lanes 1 and 3). Essentially the same pattern, with a broad radioactive band of similar molecular weight, was observed when photolabeling was performed with COS cells transfected with either fMLP-R26 (lane 4) or fMLP-R98 cDNA (not shown). Thus, the formylpeptide

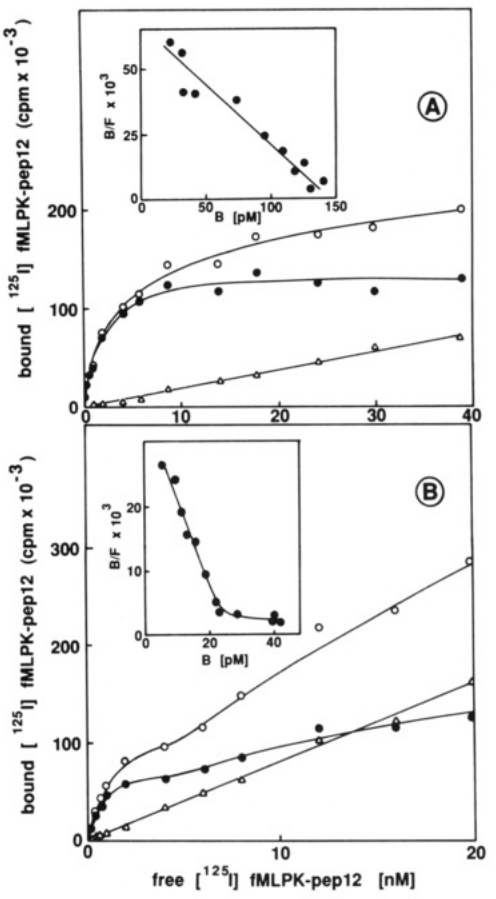

FIGURE 3: Binding characteristics of the fMLP receptor expressed in Bt2cAMP-treated HL-60 cells and in transfected COS cells. To determine whether binding of ^{125}I-fMLPK-Pep12 to the recombinant fMLP receptor was similar to binding to the endogenous receptor in differentiated HL-60 cells, transfected COS and differentiated HL-60 cells were incubated for 2 h at 4 °C in medium containing the radioactive ligand at the indicated concentrations. Binding was determined in the presence and absence of 4 μM fMLPK. Specific binding (●) was determined by subtracting the amount of radioactive ligand to the cells in the presence of fMLPK (△) from the amount of radioactive ligand bound in absence of competitor (○). Scatchard representations of specific ^{125}I-fMLPLK-Pep12 binding are shown in the insets. (A) Binding was assayed on HL-60 cells in suspension 72 h after differentiation with 500 μM Bt2cAMP; 2 × 10^6 cells were used per point. (B) Binding was assayed on COS cell monolayers, containing 10^5 subconfluent cells/plate, 72 h after transfection with fMLP-R26 cDNA (B = bound; B/F = bound/free).

receptor recombinant appears to be glycosylated in a similar manner as the natural formylpeptide receptor. However, in contrast to HL-60 cells, the COS transfectants did not display the two minor bands at 38 000 and 40 000.

mRNA and Genomic DNA Analysis. RNA from Cos-7 cells and differentiated or undifferentiated HL-60 cells was screened by Northern blotting for the presence of fMLP-receptor mRNA transcripts. Equal amounts of total RNA were electrophoresed in denaturing agarose and transferred to nylon (Figure 5). Hybridization of the transferred RNA from Bt2cAMP- or DMSO-treated HL-60 cells with a 1.1-kb restriction fragment (*Hin*dIII–*Pst*I from fMLP-R26) revealed a prominent 1.6–1.7-kb species, as well as two minor species of 2.3 and 3.1 kb when poly(A+) RNA from Bt2cAMP-treated cells was used. A very faint signal was detectable in RNA from untreated HL-60 cells. This latter result is consistent with a previous study demonstrating that a subpopulation of cells spontaneously displays the fMLP receptor in the absence of inducers (Niedel et al., 1980b).

Hybridization of the *Hin*dIII–*Pst*I cDNA probe with ge-

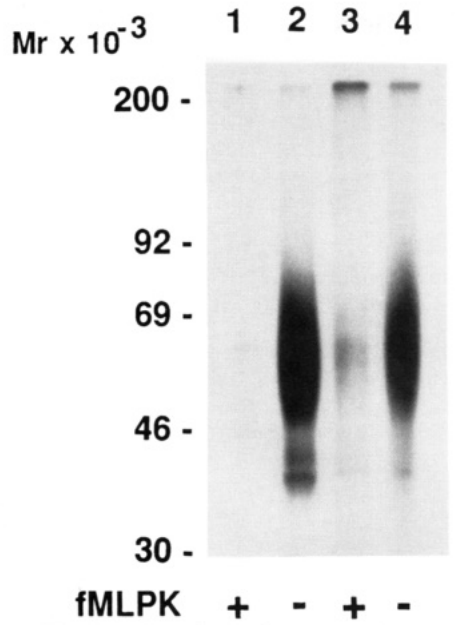

FIGURE 4: Photolabeling of the fMLP receptor expressed in Bt2cAMP-treated HL-60 cells and in transfected COS cells. Approximately 4×10^6 HL-60 cells treated for 72 h with 500 μM Bt2cAMP (lanes 1 and 2) and 5×10^5 transfected COS cells (lanes 3 and 4) were resuspended in HBS/HEPES (pH 7.5) and incubated for 1 h at 0 °C in the dark with the photoactivable derivative fMLPK-B-^{125}I-SASD at the concentration of 5 nM. Cells were allowed to bind the photoactivable probe in the presence of 2 μM fMLPK (lanes 1 and 3) or its absence (lanes 2 and 4), and then irradiated for 5 min through a glass plate. Gel electrophoresis was carried out on a 9% polyacrylamide Laemmli gel under denaturing and reducing conditions with molecular weight markers (Pharmacia). The autoradiograph was exposed for 24 h.

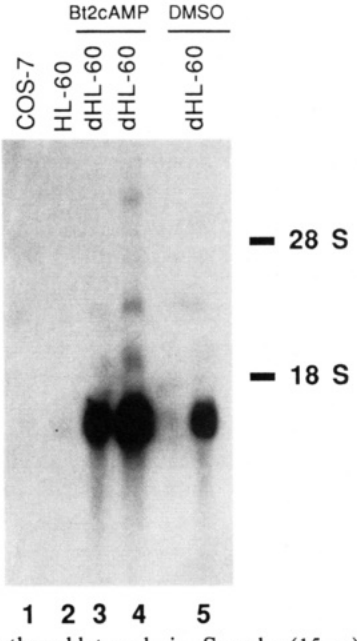

FIGURE 5: Northern blot analysis. Samples (15 μg) of total RNA from COS cells (lane 1), promyelocytic HL-60 cells, either undifferentiated (lane 2) or differentiated for 48 h with 500 μM Bt2cAMP (lane 3), 1 μg of the same poly(A^+)mRNA preparation that was used for the library construction (lane 4), and 15 μg of total RNA from HL-60 cells differentiated in 1.2% DMSO for 4 days were electrophoresed in a denaturing formaldehyde/agarose gel and transferred to Hybond N. RNAs were hybridized to a 1.1-kb ^{32}P-labeled restriction fragment (*Hin*dIII–*Pst*I) of fMLP-R26, as indicated under Materials and Methods. The nylon membrane was washed and exposed for 60 h to a 3M X-ray film.

nomic DNA, digested with various restriction enzymes, under stringent conditions gave a relatively complex pattern (Figure

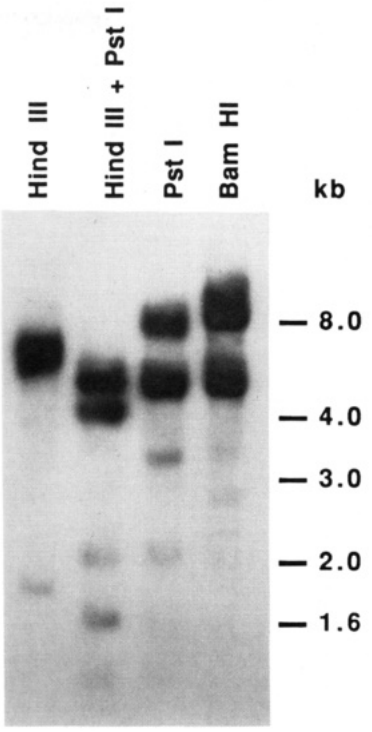

FIGURE 6: Southern analysis of human fMLP receptor genomic sequence. Twenty micrograms of high molecular weight genomic DNA was digested with *Hin*dIII (lane 1), *Hin*dIII plus *Pst*I (lane 2), *Pst*I (lane 3), or *Bam*HI (lane 4), separated by agarose gel electrophoresis, transferred to nylon, and hybridized under stringent conditions with a ^{32}P-labeled restriction fragment (*Hin*dIII–*Pst*I) of fMLP-R26. The nylon filter was subsequently washed and exposed for 12 h to a 3M X-ray film. The sizes of the fragments were estimated from a 1-kb DNA ladder.

6). In *Pst*I and *Bam*HI digests, two large DNA fragments (8 and 5 kb) and additional weakly hybridizing bands of smaller size were detected. The pattern of hybridization detected in the *Hin*dIII digest was less complicated; a major band of 6.0–6.5 kb that appeared as a doublet in short exposure and a faintly hybridizing band of smaller size (1.8 kb) were detected. When a double digestion with *Pst*I and *Hin*dIII was performed, the 8-kb *Pst*I and 6.0–6.5-kb *Hin*dIII fragments disappeared, whereas a strong hybridization signal corresponding to a 4-kb DNA fragment was observed. Taken together, these results suggest either the presence of two genes encoding fMLP receptor isoforms or a single gene with introns in the coding sequence. The weakly hybridizing bands may correspond to intron–exon fragments or, most probably, to novel receptors related to the fMLP receptor.

Structural Similarities to Other Receptors. A possible relationship of the fMLP receptor with a human neutrophil protein of M_r 24000 that binds the photoactivable *N*-formylpeptide derivative (fMLPK-SASD) and cosediments with specific granules has been recently proposed by Allen et al. (1989). It is of interest to note that we do not observe any amino acid sequence similarity between the fMLP receptor, described here, and the N-terminal sequence of the 24000-Da protein. Thus, the 24000-Da protein identified by Allen et al. (1989) is not a proteolytic fragment of the fMLP receptor as suggested.

The hydropathy profile of the *N*-formylpeptide receptor determined by the method of Kyte and Doolittle (1982) indicated seven highly hydrophobic regions, a pattern remarkably similar to the profile of bacteriorhodopsin and rhodopsine-type receptors, such as β-adrenergic and muscarinic receptors, or other members of the G-protein-linked receptor family (Zucker

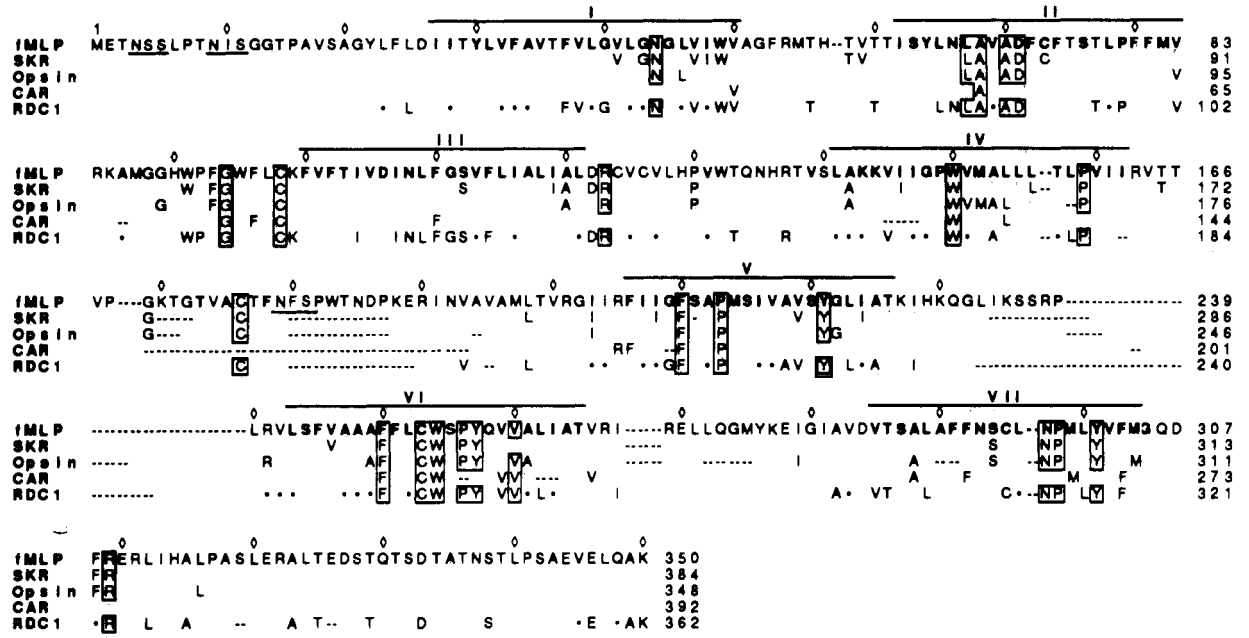

FIGURE 7: Sequence similarities with some members of the G-protein-coupled receptor family. The amino acid sequence of fMLP-R26 was manually aligned with those of bovine substance K receptor (SKR), bovine opsin, cAMP chemoattractant receptor from *D. discoideum* (CAR) and RDC1 from dog thyroid by using conserved residues of homology units of G-protein-linked receptors (from Masu et al. (1988), and Libert et al. (1989); the residue gaps (--) introduced by these authors to optimize the alignment between SKR, opsin, CAR, and RDC1 were conserved). It was necessary to introduce only four gaps in the fMLP receptor sequence (one at His^{57}, one at Leu^{156}, and two at Pro^{168}) to maintain the alignment between the hydrophobic segments I and V. Since loop V–VI is much smaller in the fMLP receptor and little homology occurs in this region, the remaining sequence of the fMLP receptor was aligned separately by using the conserved consensus block (FxxCWxPY) as starting point. To maintain homology, three gaps were introduced at Ile^{268} and one at Leu^{296}. Positions of the putative transmembrane domains were assigned on the basis of the hydropathy profile and comparison with other G-protein-coupled receptors. The potential N-glycosylation sites are underlined. Amino acids that appear in more than three of the aligned sequences are boxed. Conservative replacements between fMLP receptor and dog thyroid RDC1 protein are indicated by dots.

et al., 1985; Dohlman et al., 1987; Peralta et al., 1987; Masu et al., 1987). The sequence comparisons revealed in the fMLP receptor some amino acid sequence features that are common to the G-protein-coupled receptor superfamily. Figure 7 illustrates the amino acid sequence alignment of fMLP-R26 receptor with bovine substance K receptor (SKR), bovine rhodopsin, cyclic AMP chemoattractant receptor (CAR) from *Dictyostellium discoideum*, and RDC1, isolated from a dog thyroid cDNA library [Masu et al. (1987), Nathans and Hogness (1983), Klein et al. (1988), and Libert et al. (1989), respectively]. The latter protein is a potential G-protein-coupled receptor of unknown function, which shows a weak similarity to all other receptors of the family (Libert et al., 1989). Groups of residues distributed throughout the sequences appear to be highly conserved in all these receptors. In most instances, the regions of greatest sequence similarity are concentrated in the hydrophobic segments, whereas the amino- and carboxy-terminal regions, and the loops between the hydrophobic regions IV, V, and VI are widely different in length and in amino acid composition. Interestingly, the fMLP receptor shows a much higher degree of similarity with RDC1 than with the other receptors. For instance, while the third hydrophobic segment of SKR, rhodopsin, or CAR shows low similarity to that of the fMLP receptor, RDC1 and the fMLP receptor share a seven-residue feature (INLFGS·F) in this hydrophobic domain. In addition, the carboxy-terminus regions of fMLP and RDC1 receptors show significant similarities; three amino acids of the last five are matched, and in the overall C-terminus region, 10 out of 40 residues are identical if two gaps are introduced in RDC1 for an optimal alignment. Over the 350 residues of the amino acid sequence, the fMLP receptor shows 23.7% amino acid identity with RDC1, and the percentage of matched residues increases to 40% if conservative replacements are included. The degree of strict sequence identity is only 14%, 13%, and 6% for fMLPR–SKR, fMLPR–opsin, and fMLPR–CAR comparisons, respectively.

On the basis of the structural similarities with the G-protein-coupled receptors, and by analogy with the proposed models for the disposition of bacteriorhodopsin and rhodopsin in the membrane (Henderson & Unwin, 1975; Ovchinnikov, 1982, Hargrave, 1982), we suggest, as illustrated in Figure 8, that the polypeptide chain of the *N*-formylpeptide chemoattractant receptor is arranged with seven α-helices spanning the plasma membrane, the N-glycosylated amino-terminal region being exposed to the extracellular medium and the carboxy-terminal end to the cytosol.

It is worth noting that the third intracellular loop between the hydrophobic segments V and VI is only 16 amino acids long, with a potential phosphorylation site (KSSR) for protein kinase A. It is probably important for G protein interaction to occur, as suggested by mutagenesis experiments with the β-adrenergic receptor (Dixon et al., 1987). The carboxyl terminus is also serine and threonine rich; five serine and six threonine residues out of 40 amino acids are scattered throughout the carboxyl end. Like rhodopsin and β-adrenergic receptor, the fMLP receptor might be phosphorylated by a specific kinase.

Discussion

Methodological Aspects of the Cloning Strategy. Two full-length cDNAs, fMLP-R26 and fMLP-R98, encoding closely related forms of the human receptor for *N*-formylpeptide chemoattractants, have been isolated by an expression cloning strategy in simian COS cells. Similar approaches based on the use of COS cells have been recently described to isolate cDNAs encoding growth factor receptors (Sims et al., 1988; Yamasaki et al., 1988), Fc receptors (Stengelin et

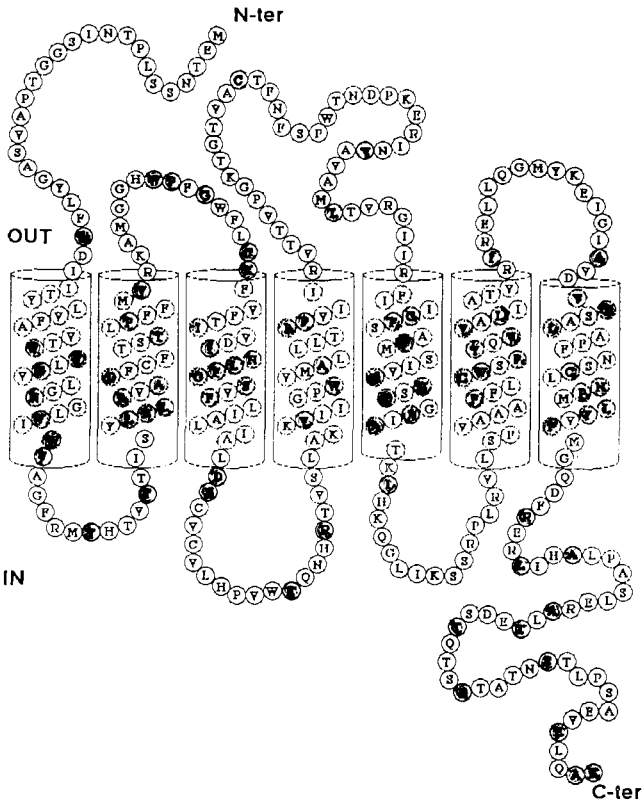

FIGURE 8: Schematic representation of the human fMLP receptor in the plasma membrane. The folding of the polypeptide chain was predicted by hydropathy plotting by the method of Kyte and Doolittle (1982), and the model shown here is reasonably similar to the models established for other members of the G-protein-coupled receptor superfamily. The conserved amino acids in the human fMLP receptor (fMLP-R26) and the dog RDC1 receptor are represented in grey.

al., 1988), lymphocyte cell surface antigens (Aruffo & Seed, 1987; Seed & Aruffo, 1987), erythropoietin and granulocyte–macrophage colony-stimulating factor (GM-CSF) receptors (D'Andrea et al., 1989; Gearing et al., 1989), and the murine interferon γ receptor (Munro & Maniatis, 1989).

Our screening procedure, based on the endocytosis of a specific radioactive ligand by positively transfected cells, was markedly facilitated by the use of a novel, hydrophilic *N*-formylpeptide derivative. In addition to a high affinity and specificity for the fMLP receptor, i.e., K_d in the 1 nM range and full displacement by unlabeled *N*-formylpeptide, this derivative has the advantage of interacting with the lipid bilayer only to a small extent. An excellent signal-to-noise ratio could therefore be obtained in the screening assay. Although relatively small pools were used in this study, further experiments have shown that a sufficient signal could be obtained with primary pools of $3–4 \times 10^3$ recombinants. Thus, the uptake of a radioactive ligand should allow the screening of a relatively large number of independent clones and may, in some cases, be a good alternative to the detection assay by direct microscopic autoradiography with photographic emulsion. This latter procedure, recently used by Gearing et al. (1989) to isolate a cDNA clone encoding the GM-CSF receptor, allows the screening of larger pools of clones (2×10^4), but it necessitates the covalent cross-linking of the ligand to the receptor. This requirement is not easily fulfilled when small ligands with few functional groups are involved. The strategy used for the fMLP receptor should be applicable to the cloning of other oligopeptide receptors.

Origin of the Two N-Formylpeptide Receptor Variants. The complete sequencing of the fMLP-R26 and fMLP-R98 cDNAs revealed several nucleotide differences in both the translated and the untranslated regions. These two cDNAs are likely to represent allelic variations of the same gene. Both cDNAs are, in fact, equipotent in transferring an elevated ^{125}I-peptide uptake capacity to transfected COS cells. According to the criteria used in this study, i.e., correct cell surface localization, binding, and endocytosis of *N*-formylpeptide derivatives, the replacements of Val^{101} and Glu^{346} in fMLP-R26 with Leu and Ala in fMLP-R98 have no incidence on the functional expression of the fMLP receptor. Further studies, with more refined techniques, will be needed to determine whether these two receptors display differential interactions with regulatory G protein(s), thus stimulating different cellular responses.

Several observations have led us to the conclusion that the 1.35-kb fMLP-R26 cDNA is the most abundant species coding for the *N*-formylpeptide receptor in human neutrophils. First, the cDNA appears to be fully processed, with a poly(A) tail 13 bases downstream from the consensus AATAAA polyadenylation signal. Second, RNA blot analysis (Figure 5) reveals the presence of an abundant 1.6–1.7-kb transcript that can only correspond in size to the fMLP-R26 cDNA. The origin of the 1.9-kb fMLP-R98 cDNA is unclear. Whether fMLP-R98 reflects an incomplete splicing or arises from the minor 2.3- or 3.1-kb mRNA species is difficult to answer. On the one hand, the former possibility is supported by the fact that fMLP-R98 contains the some polyadenylation signal as fMLP-R26 at position 1201. On the other hand, the absence of a poly(A) tail suggests that a portion of the 3′ noncoding region is missing. This suggestion is supported by the fact that the 3′ noncoding region contains an incomplete human Alu sequence of repeated elements (Jelinek & Schmid, 1982). Therefore, it is not excluded that the 1.9-kb cDNA derives from the minor, but consistently detected, 2.3-kb transcript.

Our Northern blot data do not agree with data recently published by another group (Murphy et al., 1990). Using injection of *Xenopus* oocytes with size-fractionated mRNA from Bt2cAMP-treated HL-60 cells, Murphy et al. (1990) provided evidence that a 2.0-kb transcript directed the expression of a receptor responsible for fMLP-dependent calcium mobilization in oocytes. None of the transcripts detected in our Northern blot analyses has a 2.0-kb size. Therefore, this raises the question whether this 2.0-kb messenger encodes a receptor identical with or related to the fMLP receptor we have cloned. On the one hand, because the fMLP receptor mediates calcium movement in phagocytic cells, it is reasonable to assume that the receptor encoded by the 2.0-kb transcript in the oocytes (Murphy et al., 1990) is an authentic fMLP receptor. On the other hand, the receptor encoded by fMLP-R26 or fMLP-R98 behaves, in many respects, as an authentic *N*-formylpeptide receptor: (I) It exhibits high affinity (1 nM range) and specificity (full displacement by fMLPK or fMLP) for different *N*-formylpeptides derivatives (fMLPK-Pep12 and TMR-hexapeptide); [^{3}H]fMLP binds to the cloned expressed receptor with an affinity of 2–4 nM and is fully displaced with 2 μM fMLP (data not shown). (ii) The detection of the 1.6–1.7-kb transcript in Bt2cAMP- or DMSO-treated HL-60 cells parallels the detection of ^{125}I-*N*-formylpeptide binding sites (not shown). (iii) The photolabeling pattern with transfected COS cells is the same as that observed with differentiated HL-60 cells. (iv) The predicted protein belongs to the G-protein-coupled receptor superfamily, as expected from physiological and biochemical studies. A definite answer should be obtained by cloning the cDNA encoding the fMLP receptor responsible for calcium movement in RNA-injected oocytes.

Are Several fMLP Receptors Involved in the Activation of Phagocytic Cells? Although there is no pharmacological evidence for the existence of fMLP receptor subtypes, biochemical studies have shown that the fMLP receptor could be resolved into two isoforms of M_r 50 000 and 60 000, with isoelectric points of 6.0 and 6.5, respectively (Schmitt et al., 1983; Malech et al., 1985). Our present data also suggest the existence of isoforms. First, two sharp bands of M_r 38 000 and 40 000 were consistently detected by photolabeling in differentiated HL-60 cells (Figure 4, lane 2). Since they are detected even in the presence of protease inhibitors, they may represent the nonglycosylated forms of two isoreceptors rather than proteolytic fragments of the polypeptide chain. Secondly, the analysis of genomic DNA with a cDNA probe revealed a complex pattern consistent with the presence of either two genes encoding the fMLP receptor or a single gene with introns in the coding sequence. In the case of the D_2 dopamine receptor, it is now clear that alternative splicing directs the expression of two isoforms (Giros et al., 1989; Monsma et al., 1989). Whether such a mechanism occurs in the case of the fMLP receptor is an important issue to settle. The existence of multiple isoreceptors exhibiting different affinities for the ligand, and possibly being coupled to different G proteins, might explain the diversity of responses associated with the activation of the fMLP receptor. For instance, the directed locomotion of the cell appears at doses of fMLP substantially lower (<10–50-fold) than those required to stimulate the release of lysosomal enzymes or trigger the production of superoxide radicals (Snyderman & Pike, 1984b).

The N-Formylpeptide Receptor Belongs to a New Subfamily of G-Protein-Coupled Receptors. An unexpected result of this study is that the fMLP receptor we have cloned shares striking similarities with the RDC1 protein isolated from a dog cDNA library. At present, RDC1 is considered as a potential G-protein-linked receptor of unknown function, expressed in heart, kidney, and the thyroid gland (Libert et al., 1989). Although this type of comparison cannot predict either the nature of the ligand or the function of RDC1, the similarities of RDC1 with the fMLP receptor suggest that they are both members of a new subfamily of G-protein-coupled receptors. This view is supported by our Northern and Southern blot data demonstrating, under stringent conditions, several weakly hybridizing bands that may represent other proteins related to the fMLP receptor. It is tempting to speculate that the members of this subfamily are all acting via the same transduction pathway. This hypothesis is appealing, since other receptors of the neutrophil plasma membrane, including LTB4, C5a, PAF, and Il-8 receptors, stimulate the same cellular functions and seem to share with the fMLP receptor a common pool of G_i regulatory protein(s) (Devreotes & Zigmond, 1988).

Concluding Remarks. The availability of specific cDNA clones of the fMLP receptor should make it possible to address more directly a number of questions on molecular and biochemical aspects concerning the structure and function of this receptor. Is the receptor phosphorylated on serine and threonine residues of the cytoplasmic tail upon activation of the transduction pathways, by what kinase, and what would be the functional changes linked to phosphorylation? Are there specialized forms of the receptors able to stimulate the different function of the neutrophil? Which G protein(s) is required for chemotaxis? How does the receptor interact with the cytoskeleton? Is the receptor recycled or degraded in lysosomes after endocytosis? The cDNA clones will facilitate the development of valuable molecular and immunological tools for answering these questions. In addition, the availability of nucleotide sequences common to the fMLP receptor and RDC1 should allow the selective amplification and cloning of related receptors.

Acknowledgments

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