Defective $G_i$ Protein Coupling in Two Formyl Peptide Receptor Mutants Associated with Localized Juvenile Periodontitis*

Received for publication, July 16, 2001, and in revised form, August 30, 2001 Published, JBC Papers in Press, September 14, 2001, DOI 10.1074/jbc.M106621200

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The formyl peptide receptor (FPR) is a prototypical chemoattractant receptor expressed in neutrophils. It is well known that the FPR couples to $G_i$ proteins to activate phospholipase C, chemotaxis, and cytotoxic cell functions, but the in vivo role of the FPR in man has remained elusive. Recently, F110S and C126W mutations of the FPR have been associated with localized juvenile periodontitis. We studied FPR-F110S and FPR-C126W in comparison with wild-type FPR (FPR-WT) by coexpressing epitope-tagged versions of these receptors with the $G$ protein $G_{110S} \beta_2 \gamma_2$ in Sf9 insect cells. FPRs were efficiently expressed in Sf9 membranes as assessed by immunoblotting using the $\beta_2$-adrenoreceptor as a standard. FPR-C126W differed from FPR-WT and FPR-F110S in migration on SDS-polyacrylamide gels and tunicamycin-sensitive glycosylation. FPR-WT efficiently reconstituted high-affinity agonist binding and agonist- and inverse agonist-regulated guanosine 5'-O-(3-thiotriphosphate) (GTP$\gamma$S) binding to $G_{110S} \beta_2 \gamma_2$. In contrast, FPR-F110S only weakly reconstituted agonist-stimulated GTP$\gamma$S binding, and FPR-C126W was completely inefficient. Collectively, our data show almost complete and complete loss of $G_i$ protein coupling in FPR-F110S and FPR-C126W, respectively. The severe functional defects in FPR-F110S and FPR-C126W contrast with the discrete clinical symptoms associated with these mutations, indicating that loss of FPR function in host defense is, for the most part, readily compensated.

Neutrophils play an important role in host defense against bacterial infections and in the pathogenesis of various inflammatory diseases (1, 2). Neutrophils express GPCRs for the chemoattractants fMLP, complement C5a, interleukin-8, leukotriene B4, and platelet-activating factor (3–6). Chemoattractant receptors couple to $G_i$ proteins to activate phospholipase C, phosphoinositide 3-kinase- and 2AR, and $G_i$-regulated adenyl cyclase. However, although at cellular and molecular levels, the human FPR is one of the most extensively studied GPCRs (3–6, 8, 11), the in vivo role of the FPR in man has remained elusive.

LJP is a defined periodontal disease that begins at ~11–15 years of age, affects the permanent incisors and/or first molars, and is associated with the presence of Actinobacillus actinomycetemcomitans in subgingival pockets. LJP is not associated with other diseases, and there is evidence that LJP is caused by a genetic defect (12, 13). It has been known for a long time that neutrophils from LJP patients show reduced binding of the agonist fMLP and reduced fMLP-induced chemotaxis (14–16). Genetic analysis of 30 LJP patients revealed that 29 of those patients possess a F110S and/or C126W mutation in the FPR gene (17). In contrast, none of 31 patients with adult periodontitis and none of 20 control subjects possess a F110S or C126W mutation (17). The F110S mutation resides in the third transmembrane domain, whereas the C126W mutation resides in the second intracellular loop (Fig. 1). The second intracellular loop of the FPR is important for $G_i$ protein coupling (18), and a C126S mutation uncouples the FPR from $G_i$ proteins (19). Based on all these findings, we developed the hypothesis that the underlying cause of LJP is defective $G_i$ protein coupling of the FPR. To test this hypothesis, we engineered FLAG epitope-tagged FPR-F110S and FPR-C126W and analyzed coupling of these FPR mutants and FPR-WT to the $G_i$ protein $G_{110S} \beta_2 \gamma_2$ using Sf9 insect cells as the expression system. In previous studies, we already showed that Sf9 insect cells are a very sensitive system for studying $G_i$ protein coupling of chemoattractant receptors (20–22). Here we report that FPR-F110S and FPR-C126W show almost complete defect and a complete defect in $G_i$ protein coupling, respectively. The discrete clinical symptoms associated with the defective FPR indicate that the loss of FPR function in host defense is, for the most part, readily compensated.

EXPERIMENTAL PROCEDURES

Materials—The baculovirus encoding $G_{110S}$ was kindly provided by Dr. A. G. Gilman (Department of Pharmacology, University of Southwestern Medical Center, Dallas, TX). Recombinant baculovirus encoding the unmodified versions of the $G_i$ protein $G_{110S} \beta_2 \gamma_2$ subunits was a kind gift of Dr. P. Gierschik (Department of Pharmacology and Toxicology, University of Ulm, Ulm, Germany). CeH was obtained from Novartis (Basel, Switzerland). The generation of the baculovirus encoding FPR-WT (isoform 20) was described previously (20). fMLP, tunicamycin, and the anti-FLAG Ig (monoclonal antibody M1) were from Sigma. The anti-$G_{110S}$ Ig was from Calbiochem. [35S]GTP$\gamma$S (1100 Ci/mmol), [3H]dihydoraprenolol (85–90 Ci/mmol), and [3H]fMLP (56 Ci/mmol) were from PerkinElmer Life Sciences. Unlabeled GTP$\gamma$S and GDP were from Roche Molecular Biochemicals. All restriction enzymes and T4 DNA ligase were from New England Biolabs Inc. (Beverly, MA). Cloned Pfu DNA polymerase was from Stratagene (La Jolla, CA).

Construction of FLAG Epitope- and Hexahistidine-tagged FPR-F110S and FPR-C126W—A DNA sequence encoding the cleavable signal peptide from influenza hemagglutinin followed by the FLAG epitope, which can be recognized by the M1 antibody, was placed 5′ to the start codon of the DNAs of FPR mutants. We also added a hexa-
histidine tag to the C terminus to allow future purification of FPR mutants and to provide additional protection against proteolysis (20). FPR mutants were generated by sequential overlap-extension PCRs. To create FPR-F110S, in PCR-1A, the N-terminal portion of the DNA sequence encoding FPR-WT was amplified using a sense primer encoding the last 18 base pairs of the FLAG epitope and an antisense primer introducing the F110S mutation (accompanied by the creation of a new BspEI site) with pGEM-3Z-SF-FPR-WT26 as template. In PCR-1B, the C-terminal portion of the DNA sequence encoding FPR-WT was amplified using a sense primer introducing the F110S mutation and an antisense primer encoding the two C-terminal amino acids of FPR-WT, a hexahistidine tag, the stop codon, and an extra XhoI site with pGEM-3Z-SF-FPR-WT26 as template. In PCR-1C, the products of PCR-1A and PCR-1B were annealed in the region coding for the newly introduced F110S mutation and were amplified with the sense primer of PCR-1A and the antisense primer of PCR-1B. The resulting fragment was digested with AvaI and BseRI and cloned into pGEM-3Z-SF-FPR-WT26 digested with AvaI and BseRI. The DNA encoding FPR-F110S was cloned into the baculovirus expression vector pVL1392 using the EcoRI site at the 5′-end of the signal FLAG region of the receptor and the XhoI site at the 3′-end of the receptor. To create FPR-C126W, in PCR-2A, the N-terminal portion of the DNA sequence encoding FPR-WT was amplified using a sense primer introducing the last 18 base pairs of the FLAG epitope and an antisense primer encoding the two C-terminal amino acids of FPR-WT, a hexahistidine tag, the stop codon, and an extra XhoI site with pGEM-3Z-SF-FPR-WT26 as template. In PCR-2B, the C-terminal portion of the DNA sequence encoding FPR-WT was amplified using a sense primer introducing the C126W mutation and an antisense primer encoding the two C-terminal amino acids of FPR-WT, a hexahistidine tag, and an extra XhoI site with pGEM-3Z-SF-FPR-WT26 as template. In PCR-2C, the products of PCR-2A and PCR-2B were annealed in the region coding for the newly introduced C126W mutation and were amplified with the sense primer of PCR-2A and the antisense primer of PCR-2B. The resulting fragment was digested with AvaI and BseRI and cloned into pGEM-3Z-SF-FPR-WT26 digested with AvaI and BseRI. The DNA encoding FPR-C126W was cloned into the baculovirus expression vector pVL1392 using the SacI site at the 5′-end of the signal FLAG region of the receptor and the XhoI site at the 3′-end of the receptor. To create FPR-C126W, in PCR-2A, the N-terminal portion of the DNA sequence encoding FPR-WT was amplified using a sense primer introducing the last 18 base pairs of the FLAG epitope and an antisense primer introducing the C126W mutation (accompanied by the creation of a new BspEI site) with pGEM-3Z-SF-FPR-WT26 as template. In PCR-2B, the C-terminal portion of the DNA sequence encoding FPR-WT was amplified using a sense primer introducing the C126W mutation and an antisense primer encoding the two C-terminal amino acids of FPR-WT, a hexahistidine tag, the stop codon, and an extra XhoI site with pGEM-3Z-SF-FPR-WT26 as template. In PCR-2C, the products of PCR-2A and PCR-2B were annealed in the region coding for the newly introduced C126W mutation and were amplified with the sense primer of PCR-2A and the antisense primer of PCR-2B.

**Generation of Recombinant Baculoviruses, Cell Culture, and Membrane Preparation—**Sf9 cells were cultured in 250-ml disposable Erlenmeyer flasks at 28 °C under rotation at 125 rpm in SF 900II medium (Life Technologies, Inc.) supplemented with 5% (v/v) fetal calf serum (BioWhittaker, Inc., Walkersville, MD) and 0.1 mg/ml gentamycin. Cells were maintained at a density of 1.0–6.0 × 10⁶ cells/ml. Cells were infected with 1:100 dilutions of high-titer baculovirus stocks encoding GPCRs, Go₁₂, and β₂γ₂ complex. At this time, we added tunicamycin (10 μg/ml) to some cultures to inhibit N-glycosylation of GPCRs (23). Cells were cultured for 48 h before membrane preparation. Sf9 membranes were prepared as described (24) using 1 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 10 μg/ml benzamidine, and 10 μg/ml leupeptin as protease inhibitors. Membranes were suspended in binding buffer (12.5 mM MgCl₂, 1 mM EDTA, and 75 mM Tris-HCl, pH 7.4) and stored at −80 °C until use.

**Binding Assays—**[³H]Dihydroalprenolol saturation binding to determine the expression of the β₂-adrenoceptor (β₂AR) was carried out as described (24). For [³H]MLP saturation binding, Sf9 membranes were thawed, centrifuged at 15,000 × g for 15 min at 4 °C, and suspended in binding buffer. Reaction mixtures (500 μl) contained Sf9 membranes (50–75 μg of protein/tube) in binding buffer supplemented with [³H]MLP (0.2–30 nM). Nonspecific binding was determined in the presence of 10 μM unlabeled MLP. Incubations were conducted for 60 min at 25 °C with shaking at 200 rpm. Aliquots of 200 μl (containing 15–40 μg of protein) were withdrawn at different time points. Nonspecific [³H]MLP binding was determined from free [³H]MLP by filtration through GF/C filters, followed by three washes with 2 ml of binding buffer (4 °C). Filter-bound radioactivity was determined by liquid scintillation counting. For GTP·S binding time course studies, Sf9 membranes were suspended in 1500 μl of binding buffer supplemented with 0.05% (w/v) bovine serum albumin, 1 nM [³S]GTP·S, 9 nM unlabeled GTP·S, and 1 μM GDP in the absence and presence of 15 μM GDP (10 μM) or CaH (5 μM). Reactions were conducted at 25 °C with shaking at 200 rpm. Aliquots of 200 μl (containing 50–150 μg of protein) were withdrawn at different time points. Nonspecific [³S]GTP·S binding was determined in the presence of 10 μM unlabeled GTP·S and was <0.1% of total binding. Bound [³S]GTP·S was separated from free [³S]GTP·S by filtration through GF/C filters, followed by three washes with 2 ml of binding buffer (4 °C). Filter-bound radioactivity was determined by liquid scintillation counting. For GTP·S saturation experiments, reaction mixtures (500 μl) contained Sf9 membranes (15–30 μg of protein/tube), 0.5–2 nM [³S]GTP·S plus unlabeled GTP·S to achieve final GTP·S concentrations of 0.5–20 nM, 1 μM GDP, and 0.05%/w/v bovine serum albumin in the presence and absence of fMLP (10 μM) or CaH (3 μM). Reactions were conducted for 60 min at 25 °C with shaking at 200 rpm. For studying the effect of NaCl on GTP·S binding, reaction mixtures (500 μl) contained Sf9 membranes (15–30 μg of protein/tube), 0.4 nM [³S]GTP·S, 1 μM GDP, 0.05%/w/v bovine serum albumin, and NaCl at various concentrations in the absence and presence of fMLP (10 μM). Reactions were conducted for 60 min at 25 °C with shaking at 200 rpm.

**Analysis of FPR and Go₁₂ Expression in Sf9 Membranes—**Sf9 membranes were analyzed by immunoblotting using the M1 monoclonal antibody (1:1000), which recognizes the N-terminal FLAG epitope of the chemooattractant receptors (20, 24), and anti-Go₁₂ Ig (1:1000) (21). The acrylamide concentration in gels was 10%/w/v. Proteins were transferred onto Immobilon-P transfer membranes (Millipore Corp., Bedford, MA). Proteins were visualized with peroxidase-coupled sheep antirabbit IgG (anti-Go₁₂ Ig), respectively, using o-dianisidine and H₂O₂ as substrates.
Different Electrophoretic Mobility and Glycosylation of FPR-C126W Compared with FPR-WT and FPR-F110S—The expression of FLAG epitope-tagged FPR-WT and FPR mutants in Sf9 membranes was examined by immunoblotting with the M1 monoclonal antibody. As a standard, we used FLAG epitope-tagged β2AR expressed at a level of 3.9 pmol/mg as assessed by agonist saturation binding. In this way, the expression of FPR-WT and FPR mutants could be determined without relying on the [3H]fMLP binding assay. This was important for two reasons. First, the [3H]fMLP binding assay, as other agonist binding assays (25), largely underestimates the actual GPCR expression level (21). Second, high-affinity [3H]fMLP binding depends on intact FPR/Gi protein coupling (19, 20, 26), but we assumed that Gi protein coupling is defective in FPR-F110S and FPR-C126W.

Fig. 2A compares the electrophoretic mobility of FPR-WT, FPR mutants, and the β2AR and analyzes the effect of tunicamycin treatment on electrophoretic mobility. Fig. 2B allows for direct comparison of the electrophoretic mobility of fully glycosylated FPR-WT and FPR mutants. The β2AR migrated as a doublet of 50–52 kDa, representing differently glycosylated forms of the GPCR (24). As reported before (20), FPR-WT migrated as a series of broad bands of 38–45 kDa, reflecting the fact that this GPCR is more extensively glycosylated than the β2AR. Although the different shapes of the β2AR and FPR-WT bands render a precise comparison difficult, one can nonetheless estimate that FPR-WT was expressed at a level of ~6–8 pmol/mg.

To examine the glycosylation of FPR-WT in more detail, we treated Sf9 cells with an inhibitor of N-glycosylation, tunicamycin (23), during the protein expression period. In membranes from tunicamycin-treated Sf9 cells, FPR-WT migrated as a single 38–39-kDa band. This size corresponds well to the predicted molecular mass of unmodified FPR-WT (27), indicating that FPR-WT is exclusively N-glycosylated. However, the intensity of the FPR-WT band in membranes from tunicamycin-treated Sf9 cells was much lower than the band intensities in control membranes, suggesting that N-glycosylation is important for proper membrane targeting of FPR-WT. The importance of N-glycosylation for membrane targeting has been reported for other GPCRs (28, 29).

In terms of expression level, apparent molecular mass, glycosylation pattern, and tunicamycin sensitivity of glycosylation, FPR-F110S was indistinguishable from FPR-WT. The data also indicate that membrane targeting of FPR-WT and FPR-F110S is similar. Moreover, there was no evidence for structural instability of FPR-F110S since such a property would have resulted in decreased expression levels relative to FPR-WT (30).

The expression level of FPR-C126W seemed to be in a range similar to that of the expression levels of FPR-WT and FPR-F110S, but it was difficult to quantify FPR-C126W expression precisely because the migration of FPR-C126W on SDS-polyacrylamide gels was very different from the migration of FPR-WT and FPR-F110S. Specifically, in addition to the multiple 38–45-kDa bands that were observed for FPR-WT and FPR-F110S, we also observed multiple bands in the 28–32-kDa region and, less prominently, in the 45–100-kDa region. Although the majority of FPR-C126W migrated as 28–32-kDa proteins, i.e. well below the expected molecular mass of nonglycosylated FPR-WT (38–39 kDa), this discrepancy does not automatically imply that FPR-C126W represents an aggregated GPCR. Abnormal migration on SDS-polyacrylamide gels was also observed for wild-type GPCRs (23). The 38–45-kDa bands in Sf9 membranes expressing FPR-C126W were less intense than the corresponding bands in membranes expressing FPR-WT and FPR-F110S. In membranes from tunicamycin-treated Sf9 cells, the size of the 28–32-kDa proteins decreased somewhat; but overall, tunicamycin had little effect on the migration of FPR-C126W. In contrast to the data obtained with FPR-WT and FPR-F110S, tunicamycin did not have an inhibitory effect on the expression of FPR-C126W. Taken together, our data clearly demonstrate that the C126W mutation has a profound impact on electrophoretic mobility and glycosylation of the FPR, whereas the F110S mutation is without effect.

Defective High-affinity [3H]fMLP Binding to FPR-F110S and FPR-C126W—As already stated above, high-affinity [3H]fMLP binding to FPR depends on the expression of Gi proteins (19, 20, 26). In previous studies on various GPCRs including the FPR, we already showed that Goi2 is expressed at high levels (~200–450 pmol/mg) in Sf9 membranes (21, 22, 31). Using Sf9 membranes expressing Goi2 at ~300 pmol/mg and FPR-WT as a standard, we confirmed that Goi2 was expressed at similar levels in membranes expressing FPR-F110S and FPR-C126W (Fig. 2C). Thus, we can estimate that there is an ~40–50-fold excess of FPR-WT and FPR mutants relative to Goi2 in Sf9 membranes. This ratio should provide excellent conditions for detecting GPCR/G protein coupling in terms of high-affinity agonist binding and GDP/GTP exchange (22).

As reported before (20), in membranes expressing FPR-WT, we readily detected high-affinity [3H]fMLP binding. FPR-WT bound [3H]fMLP with a Kd of 3.2 ± 1.0 nm and a Bmax of 0.9 ± 0.10 pmol/mg (Fig. 3A). The Kd for high-affinity [3H]fMLP binding to FPR-WT in Sf9 membranes agrees with the Kd values obtained for the FPR expressed in native systems (32, 33). It should also be noted that nonglycosylated [3H]fMLP binding in Sf9 membranes expressing FPR-WT was <10% of total [3H]fMLP binding, ensuring high sensitivity for detecting high-affinity agonist binding. However, despite the sensitivity of Sf9 membranes for analyzing high-affinity [3H]fMLP binding (Fig. 3A), the [3H]fMLP binding was undetectable for FPR-F110S and FPR-C126W.
Inefficiency of FPR-F110S and FPR-C126W in Stimulating GTP\(\gamma\)S Binding to Gi Proteins—The results of the \([\text{3H}]\)fMLP binding studies strongly suggest that G\(_{i,2}\) protein coupling is defective in FPR-F110S and FPR-C126W. However, these studies cannot rule out the possibility that fMLP binds to the FPR mutants with low affinity and that this low-affinity fMLP binding induces a conformational change in GPCRs that enables them to promote GDP/GTP exchange. Indeed, even for FPR-WT, low-affinity fMLP binding to the FPR results in efficient stimulation of GDP/GTP exchange (20, 21). Additionally, FPR-WT is constitutively active; i.e., even agonist-free FPR-WT efficiently stimulates GDP/GTP exchange at G\(_{i,2}\) proteins. This constitutive activity of FPR-WT is unmasked by strong inhibitory effects of the inverse agonist CsH on basal GDP/GTP exchange (20, 21).

To address the possible activation of FPR mutants by low-affinity fMLP binding and to determine the constitutive activity of FPR-F110S and FPR-C126W, we analyzed GDP/GTP\(\gamma\)S exchange. In addition to its hydrolysis resistance, GTP\(\gamma\)S exhibits an almost complete G\(_{i,2}\) protein coupling defect and that FPR-C126W exhibits a complete defect.

In an effort to enhance the relative stimulatory effects of fMLP on GTP\(\gamma\)S binding in membranes expressing FPR-F110S and FPR-C126W, we studied the effect of NaCl on GTP\(\gamma\)S binding. Na\(^+\) stabilizes the FPR and other chemoattractant receptors in an inactive state. As a result, Na\(^+\) reduces basal GTP\(\gamma\)S binding and substantially enhances the relative stimulatory effect of chemoattractants (20, 22, 34). In membranes expressing FPR-WT, NaCl strongly reduced basal GTP\(\gamma\)S binding and enhanced the relative stimulatory effect of fMLP from \(~1.5\)-fold in the absence of NaCl up to almost 6-fold with 150 mM NaCl (Fig. 5, A and B). In contrast, even at concentrations as high as 200 mM NaCl, NaCl had minimal inhibitory effect on basal GTP\(\gamma\)S binding and no enhancing effect on GTP\(\gamma\)S binding in the presence of fMLP in membranes expressing FPR-F110S (Fig. 5, C and D) or FPR-C126W (E and F). These data corroborate the notion that FPR-F110S and FPR-C126W exhibit defective G\(_{i,2}\) protein coupling and are not constitutively active.

To allow comparison with previous studies from our laboratory (20–22), we conducted the time course studies of GTP\(\gamma\)S binding with a high GTP\(\gamma\)S concentration (10 nM), whereas the experiments investigating the effects of NaCl were conducted with a subsaturating concentration of GTP\(\gamma\)S (0.4 nM) (see “Experimental Procedures”). When comparing the GTP\(\gamma\)S binding values in the absence of NaCl in the two types of experiments, we noticed that the maximum values with fMLP in the time course experiments were similar for all three FPRs (Fig. 4); but in the NaCl experiments, the maximum GTP\(\gamma\)S binding values for FPR-F110S and FPR-C126W were much lower than those for FPR-WT (Fig. 5). Considering that the expression levels of G\(_{i,2}\) were similar for all three FPRs (Fig. 2C), these findings suggested the hypothesis that G\(_{i,2}\) exhibits a higher GTP\(\gamma\)S affinity in membranes expressing FPR-WT than in membranes expressing FPR-F110S and FPR-C126W.

To address this hypothesis, we conducted GTP\(\gamma\)S saturation binding studies. fMLP stimulated GTP\(\gamma\)S binding to G\(_{i,2}\) in membranes expressing FPR-WT with a \(K_d\) of 0.9 ± 0.3 nM (Fig. 6A), whereas the \(K_d\) for fMLP-stimulated GTP\(\gamma\)S binding to G\(_{i,2}\) in membranes expressing FPR-F110S was 3.5 ± 0.5 nM. These data indicate that agonist-occupied FPR-WT stabilizes a conformation in G\(_{i,2}\) that confers an \(~4\)-fold higher GTP\(\gamma\)S affinity for the G protein than the G\(_{i,2}\) conformation stabilized by fMLP-occupied FPR-F110S. GPCR-specific regulation of the GTP\(\gamma\)S affinity of G\(_{i,2}\) was reported before for the \(\beta_{2}\)AR and several wild-type chemoattractant receptors (20–22, 31). Thus, our data indicate that the higher GTP\(\gamma\)S affinity of G\(_{i,2}\) in membranes expressing FPR-WT
relative to membranes expressing FPR-F110S and FPR-C126W accounts for the higher fMLP-stimulated GTPγS binding values in the former system when a subsaturating GTP concentration is used (Fig. 5, A–C).

The GTPγS saturation binding studies also allowed us to answer the question of how many G protein subunits a single FPR molecule activates. Recent studies (21, 22) have shown that chemotactic receptor activity is lost in the FPR-WT because fMLP-occupied FPR-WT undergoes a conformational change that results in a decrease in agonist affinity of the GPCR that is not mimicked by other agonists (e.g. A23187, leukotriene B4, or sodium ionophore A23187). To obtain the relative stimulatory effects of fMLP on GTPγS binding in Sf9 cell membranes expressing FPR-WT, FPR-F110S, or FPR-C126W plus Gi, i2 was prepared. [35S]GTPγS binding experiments in membranes expressing FPR-WT plus Gi, i2 were carried out as described under “Experimental Procedures.” Membranes were incubated for the periods of time indicated in the presence of solvent (basal; ○), 10 μm fMLP (●), or 3 μM CsH (▲). The total GTPγS concentration was 10 nM (1 nM [35S]GTPγS plus 9 nM unlabeled GTPγS). Reaction mixtures also contained 1 μM GDP. Data shown are the means ± S.D. of three experiments performed in triplicates. Binding data were analyzed by nonlinear regression and were best fitted (F test) to monophasic saturation curves.

Conclusions—Our data show that the F110S mutation in the third transmembrane domain of the FPR and the C126W mutation in the second intracellular loop of the FPR (Fig. 1) result in a fundamental alteration in the overall structure and process-
Membranes expressing FPRs plus G proteins; but most likely, FPR-F110S/C126W exhibits a complete Gi protein coupling defect, too. Again, there is no evidence that the C126W mutation, with FPR-F110S is sufficient to result in LJP (17). This is consistent with the fact that even partial inactivation of Gi protein coupling of the FPR constitutes the molecular basis for the clinical symptoms in LJP patients such as increases in cytosolic calcium concentration and lysosomal enzyme release are robust (15, 16, 38). These fMLP-induced responses in neutrophils from LJP patients such as A. actinomycetemcomitans, a bacterium involved in the pathogenesis of LJP, showed grossly altered electrophoretic mobility and glycosylation (15). Therefore, it is likely that defective Gi protein coupling in FPR-C126W was complete (Figs. 2–5). A Gi protein coupling defect in FPR-C126W was not unexpected since the second intracellular loop is involved in Gi protein coupling (18) and since FPR-C126S exhibits a similar functional phenotype as FPR-C126W (Figs. 2–6) (19).

FPR-F110S and FPR-C126W have been identified in a number of LJP patients, but not in patients with adult periodontitis or in control subjects (17). Therefore, it is likely that defective Gi protein coupling of the FPR constitutes the molecular basis for the clinical symptoms in LJP patients with the F110S and C126W mutations. Our present data, together with clinical data (12, 13, 17), suggest that the human FPR plays an essential role in host defense against A. actinomycetemcomitans, a bacterium involved in the pathogenesis of LJP. Possibly, A. actinomycetemcomitans produces chemotactic receptor antagonists that prevent activation of compensatory GPCRs.

Apparently, an ~90% loss of Gi protein coupling as observed with FPR-F110S is sufficient to result in LJP (17). This is consistent with the fact that even partial inactivation of Gi proteins by pertussis toxin is sufficient to efficiently block most fMLP-induced responses in neutrophils (36, 37). In agreement with these data, there is no evidence that the C126W mutation, leading to a complete loss of Gi protein coupling (Figs. 2–5), results in more severe clinical symptoms than the F110S mutation (17). We did not study the F110S/C126W double mutation; but most likely, FPR-F110S/F110S exhibits a complete Gi protein coupling defect, too. Again, there is no evidence that the double mutation is associated with more severe clinical symptoms than either of the single mutations (17).

Although our present data provide an explanation for LJP in patients with proven F110S and/or C126W mutations of the FPR (17), these mutations cannot be the only cause of LJP. Specifically, in certain LJP patients, high-affinity [3H]fMLP binding is reduced by only 50% (14), whereas with FPR-F110S and FPR-C126W, high-affinity agonist binding is abolished (Fig. 2). In addition, several fMLP-induced responses in neutrophils from LJP patients such as increases in cytosolic calcium concentration and lysosomal enzyme release are robust (15, 16, 38). These fMLP responses are incompatible with an almost complete loss and a complete loss of Gi protein coupling in FPR-F110S and FPR-C126W, respectively. Thus, it is possible that additional, as yet unidentified FPR mutations, resulting in less severe defects in Gi protein coupling than the F110S and C126W mutations, exist in certain LJP patients.

Compared with FPR-WT and FPR-F110S, FPR-C126W showed grossly altered electrophoretic mobility and glycosylation (Fig. 2, A and B). Altered electrophoretic mobility of the FPR from an LJP patient relative to the FPR from a control subject was observed previously (15). Thus, it is tempting to speculate that the LJP patient studied by Perez et al. (15) expressed FPR-C126W. However, the electrophoretic results obtained by Perez et al. and us cannot be directly compared because FPR glycosylation in Sf9 cells and neutrophils is intrinsically different (20, 39).

It is somewhat surprising that severe Gi protein coupling defects of the FPR result only in a very localized infectious disease that is not associated with susceptibility to infectious diseases in general (13). These data indicate that the human organism readily compensates, to a very large extent, for the loss of FPR function. It is possible that the formyl peptide-like receptor that binds fMLP with low affinity (11, 40, 41) takes over fMLP functions. In addition, the complement C5a receptor, interleukin-8 receptor, leukotriene B4 receptor, and platelet-activating factor receptor, all of which are expressed in neutrophils and induce similar cellular responses as the FPR, may contribute to FPR substitution (3–6).
Acknowledgment—We acknowledge the help of C. Houston with the immunoblots.

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J. Biol. Chem. 2001, 276:42043-42049.
doi: 10.1074/jbc.M106621200 originally published online September 14, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M106621200

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