Cross-desensitization Among Receptors for Platelet Activating Factor and Peptide Chemoattractants

EVIDENCE FOR INDEPENDENT REGULATORY PATHWAYS

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Ricardo M. Richardson‡§, Bodduluri Haribabu‡, Hydar Ali‡, and Ralph Snyderman¶

From the Departments of ‡Medicine and ¶Immunology, Duke University Medical Center, Durham, North Carolina 27710

Cross-desensitization among receptors for peptide chemoattractants have been shown to involve two independent processes, receptor phosphorylation and inhibition of phospholipase C (PLC) activation. Receptors for lipid chemoattractants, i.e. platelet activating factor (PAF) and leukotriene B, did not inhibit the responses of peptide chemoattractant receptors, suggesting distinct signaling pathways. To examine cross-desensitization between receptors for lipid and peptide chemoattractants, cDNA encoding the PAF receptor (PAFR) was co-expressed into RBL-2H3 cells with cDNAs encoding receptors for either formylated peptides (FR), a product of the fifth component of complement (C5aR) or interleukin-8 A (IL-8RA). PAFR was homologously phosphorylated and desensitized by PAF, and cross- phosphorylated and desensitized by fMet-Leu-Phe, C5a, and IL-8. In contrast, the receptors for peptide chemoattractants were neither cross-phosphorylated nor cross-desensitized by PAF. Stauroporine blocked cross-phosphorylation and cross-desensitization of the PAFR by peptide chemoattractants. Truncation of the cytoplasmic tail of PAFR (mPAFR) abolished its homologous and cross-phosphorylation. mPAFR was also resistant to cross-desensitization by peptide chemoattractants at the level of PLC activation. Interestingly, mPAFR mediated a sustained Ca\(^{2+}\) mobilization in response to PAF and was more active in inducing GTPase activity, phosphoinositide hydrolysis, secretion, and phospholipase D activation than the wild type PAFR. In contrast to PAFR, stimulation of the mPAFR cross-phosphorylated and cross-desensitized responses to IL-8RA. As expected, FR, which is resistant to cross-phosphorylation by C5aR and IL-8RA, was not phosphorylated by mPAFR. However, unlike C5aR and IL-8RA, mPAFR did not inhibit the ability of FR to activate PLC. Blocking Ca\(^{2+}\) influx inhibited mPAFR-mediated sustained Ca\(^{2+}\) response, phospholipase D activation and secretion, but not phosphoinositide hydrolysis and cross-phosphorylation and cross-desensitization of IL-8RA. The data herein suggest that cross-desensitization of PAFR by peptide chemoattractants is solely due to receptor phosphorylation. The PAFR and the peptide chemoattractant receptors do not cross-regulate each other at the level of PLC, suggesting distinct regulatory pathways.

Phagocytic leukocyte accumulation and activation are regulated in part by chemoattractants released by bacteria or produced by the host. Chemoattractants include products of bacterial protein synthesis (\(\text{fMLP}\), i.e. \(\text{fMLP}\)), a cleavage product of the fifth component of complement (C5a), interleukin-8 (IL-8); and the lipid chemoattractants, platelet activating factor (PAF) and leukotriene B, (1). Chemoattractants activate leukocytes via cell surface receptors which are coupled to guanine nucleotide regulatory proteins (G protein) to activate phospholipase C (PLC) (1–3). Like other members of this family, chemoattractant receptors become desensitized upon agonist exposure, resulting in a loss of cellular responsiveness (1, 4). Receptor desensitization has long been categorized as homologous or heterologous, the former being restricted to the agonist-occupied form of the receptor, mediated by phosphorylation by a receptor-specific kinase (GRK) (4–6). Heterologous desensitization occurs independently of receptor occupancy by ligand and involves phosphorylation of the receptor by second messenger activated kinases such as protein kinase C (PKC) (5) or protein kinase A (PKA) (4, 5). Chemoattractant-mediated leukocyte functions have been shown to be also regulated by a third type of desensitization designated as “class desensitization” (7, 8). This type of desensitization was demonstrated among receptors for peptide chemoattractants (FR, C5aR, and IL-8RA) (9), which utilize the same G protein (\(G_{\alpha i}\)) to activate a common pool of phospholipase C (PLC\(\beta_2\)) (10, 11). This form of cross-desensitization is less specific than homologous (involves unoccupied receptor) but more specific than heterologous desensitization in that only a class of chemoattractant receptors appeared to be involved (8, 9). Studies in neutrophils also demonstrated that whereas the responses to the lipid chemoattractants PAF and leukotriene B, were cross- desensitized by all the peptide chemoattractants, the lipid chemoattractants did not cross-desensitize responses to any of the peptide chemoattractants (8). This finding further supported the notion of distinct classes of chemoattractant receptors with different mechanisms of regulation.

To better define the role of desensitization in the regulation of the inflammatory response, this laboratory has developed a model system using a rat basophilic leukemia cell line (RBL-2H3) into which multiple chemoattractant receptors can be expressed and stimulated to elicit cellular responses similar to those observed in neutrophils (9, 12–14). Using this system, receptors for peptide chemoattractants were co-expressed and

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\(\text{fMLP}\), \text{fMLP}, \text{C5aR}, \text{IL-8RA}, \text{PAFR}, \text{PLC}\,

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it was recently demonstrated that in addition to receptor phosphorylation and uncoupling of receptors from G protein activation, inhibition of downstream effector activity, presumably phospholipase C, plays an important role in the class desensitization of peptide chemoattractants (9). In the present work, the nature of cross-regulation among peptide and lipid chemoattractant receptors was investigated. To this end, receptors for PAFR were co-expressed in RBL-2H3 cells with those for FR, C5aR, or IL-8-RA to study cross-desensitization among receptor classes. A PAFR mutated to express a truncated and phosphorylation deficient carboxyl terminus was also co-expressed with the peptide chemoattractant receptors in these cells. The data presented in this work show that cross-desensitization of PAFR by the peptide chemoattractants is a consequence of PAFR cross-phosphorylation and does not involve inhibitory effects on the activation of PLC.

**EXPERIMENTAL PROCEDURES**

**Materials**—[32P]Orthophosphate (8,500–9,120 Ci/mmol), myo-[3-3H]Inositol (24.4 Ci/mmol), [3H]Myristic acid (11.2 Ci/mmol), [3P]GTP (6000 Ci/mmole), and [32P]GTP-S (1300 Ci/mmol) were purchased from DuPont NEN. 125I-IL-8 was from Amersham. IL-8 (monocyte derived) was purchased from Genzyme. Geneticin (G418) and all tissue culture reagents were purchased from Life Technologies, Inc. Monosonal 12CA5 antibody, protein G-agarose, and proteases inhibitors were from Boehringer Mannheim. [32P]GTP, Indo-I acetoxymethyl ester, phorbol 12-myristate 13-acetate (PMA), and pluron 12C5 antibody, which specifically binds to the epitope tag. The identity of the phosphorylated immunoprecipitated with 12CA5 antibody, which specifically binds to the epitope tag. The identity of the phosphorylated immunoprecipitated with 12CA5 antibody, which specifically binds to the epitope tag. The identity of the phosphorylated immunoprecipitated with 12CA5 antibody, which specifically binds to the epitope tag.
FIG. 1. Phosphorylation of epitope-tagged chemokine receptors expressed in RBL-2H3 cells. A, [32P]‐labeled double transfected RBL-2H3 cells (3 × 10^6/60-mm plate) expressing epitope-tagged receptors for either FMLP and PAF (FR-PAFR), C5a and PAF (C5aR-PAFR), or IL-8 and PAF (IL8R-PAFR) were incubated for 5 min with (lanes 2, 3, 5, 6, 8 and 9) or without (lanes 1, 4, and 7) stimulants. Cells were lysed, immunoprecipitated with 12CA5 antibody, and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. This experiment has been repeated 3 times with similar results. B, RBL-2H3 cells expressing either epitope-tagged PAFR and native C5aR (*C5aR-PAFR) (lanes 10–12) or epitope‐tagged C5aR and native PAFR (*PAFR-C5aR) (lanes 13–15) were stimulated with or without PAF and C5a and receptor phosphorylation were assessed as described above. This experiment has been repeated twice with similar results.

[Calculated text]

| Cells Agonists | % Cross-desensitization |
|----------------|-------------------------|
| FR-PAFR | FMLP → PAF | 47 ± 12.3^a |
| C5aR-PAFR | C5a → PAF | 80 ± 7.9^b |
| C5aR-PAFR | PAF → C5a | 3 ± 2.7 |
| IL8R-PAFR | IL-8 → PAF | 31 ± 14^c |
| C5aR-PAFR | PAF → IL-8 | 1 ± 3.7 |

^a p < 0.05; ^b p < 0.01.
single cell cloning was utilized to isolate double transfectants. Competition binding using \[^{3}H\]WEB 2086 and Scatchard analysis of wild type versus the mutant indicated that deletion of the carboxyl tail had no significant effect on the affinity of the receptor (Kd 10 and 19 nM for wild type and mutant PAFR, respectively). Clones expressing similar receptor numbers (2.8-3.3 \times 10^6 receptors/cell) were utilized to determine the functional properties of the mutant PAFR versus the wild type receptor.

Time course of both PAFR- and mPAFR-mediated \[^{35}S\]GTP\(_{\gamma}\)S binding in membranes were similar, with half-maximal binding at \(-4\) min (data not shown). Maximum binding after 20 min of reactions were 0.13 \pm 0.02 and 0.12 \pm 0.016 pmol of \[^{35}S\]GTP\(_{\gamma}\)S bound/mg of protein for mPAFR and PAFR, respectively. However, GTPase activity in membranes (Fig. 4A), phosphoinositide hydrolysis (Fig. 4B), and \(\beta\)-hexosaminidase release (Fig. 4C) in intact cells revealed that mPAFR was more active than the wild type PAFR in mediating cellular responses. Peaks of intracellular Ca\(^{2+}\) mobilization in response to PAF (10 nM) were similar for both, wild type (598 nM) and mutant PAFR (624 nM) (Fig. 4D). However, a more sustained response was obtained with mPAFR as compared to the wild type receptor (Fig. 4D).

The ability of mPAFR and wild type PAFR to stimulate PLD activation was also determined and compared to that of IL-8RA in RBL-2H3 cells co-expressing IL-8RA and either PAFR (IL8R-PAFR) or mPAFR (IL8R-mPAFR). As shown in Fig. 5, IL-8-induced PLD activation was similar in both cell lines, 0.61 \pm 0.014- and 0.53 \pm 0.04-fold over basal, for IL8R-PAFR and IL8R-mPAFR cells, respectively (Fig. 5). In contrast, PAF-induced PLD activity was \(\approx 5\)-fold greater in cells expressing IL8R-mPAFR (1.43 \pm 0.07) than IL8R-PAFR (0.26 \pm 0.02).

Cross-phosphorylation of mPAFR by fMLP and IL-8—\(^{32}P\). Labeled RBL-2H3 cells co-expressing the epitope-tagged mPAFR and either FR (mFR), or IL8R (IL8R-mPAFR) were treated with different ligands (PAF, 100 nM; fMLP, 1 \mu M; or IL-8, 100 nM) and immunoprecipitated with 12CA5 antibody. As shown in Fig. 6, mPAFR (\(-42\) kDa) was resistant to phosphorylation by PAF (lanes 2 and 5) and cross-phosphorylation by either fMLP (lane 3) or IL-8 (lane 6). FR (\(-65\) kDa) (lane 3) and IL-8RA (\(-70\) kDa) (lane 6) were homogenously phosphorylated by fMLP and IL-8, respectively. While FR was resistant to cross-phosphorylation, stimulation of the mutant PAFR resulted in IL-8RA cross-phosphorylation (lane 5). The extent of mPAFR-mediated cross-phosphorylation of IL-8RA was similar to the extent of phosphorylation of IL-8RA by PMA (Fig. 2, lane 13). In addition, IL-8RA cross-phosphorylation by mPAFR was inhibited by staurosporine (100 nM) (data not shown).

Effect of mPAFR-mediated Cross-phosphorylation of IL-8RA on Receptor/G Protein Coupling—The ability of IL-8RA to stimulate \[^{35}S\]GTP\(_{\gamma}\)S binding in membranes from cells expressing IL8-RA and mPAFR was determined. As shown in Fig. 7, pretreatment of the cells with IL-8 caused \(-67\%\) homologous desensitization of IL-8-induced response compared to control (untreated cells). There was no effect on mPAFR. Membranes from cells pretreated with PAF (100 nM) was resistant to homologous desensitization but showed a \(-50\%\) decrease of the response to IL-8RA. Simultaneous pretreatment of the cells with staurosporine (100 nM) blocked significantly both the homologous (\(-40\%\)) and cross- (\(66\%\)) desensitization of IL-8RA-induced \[^{35}S\]GTP\(_{\gamma}\)S binding.

Cross-desensitization of mPAFR-induced Ca\(^{2+}\) Mobilization by fMLP and IL-8—It was determined whether the Ca\(^{2+}\) mobilization in response to PAF was cross-desensitized by either fMLP or IL-8 in RBL-2H3 cells expressing FR-mPAFR or IL8R-mPAFR. PAF-induced Ca\(^{2+}\) mobilization was resistant to cross-desensitization by pretreatment of the cells with either

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**TABLE II**

| Amino acid sequences of the carboxyl-terminal tails of the wild type PAF receptor (PAFR) and the mutant PAF receptor (mPAFR) construct |
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| Bold and underlined serines and threonines residues are potential phosphorylation sites of the wild type receptor which have been either removed by deletion or substituted by alanine or glycine in the mutant PAFR. |

**Fig. 4. Functional characterization of phosphorylation-deficient PAF receptor mutant (mPAFR) versus PAFR.**
A, for GTPase activity, membranes were prepared from RBL-2H3 cells expressing either the mutant PAFR or the wild type PAFR and assayed at different concentrations of PAF. Data shown are representative of one of three experiments performed in triplicate. B, for the generation of inositol phosphates, cells were cultured overnight in the presence of \[^{3}H\]inositol (1 \mu Ci/ml). Cells were preincubated (10 min, 37°C) with a HEPES-buffered saline containing 10 \mu M LiCl in a total volume of 200 \mu l and stimulated with different concentrations of PAF for 10 min. \[^{3}H\]Inositol phosphates release was determined. Data are represented as fold stimulation over basal, which was 475 \pm 30 cpm for mPAFR and 347 \pm 49 for PAFR. The experiment was repeated three times with similar results. C, cells were treated with different concentrations of PAF and \(\beta\)-hexosaminidase release in the medium was assessed. Data are represented as percentage of total which were 0.56 \pm 0.003 (PAFR) and 0.47 \pm 0.006 (mPAFR). The experiment was repeated twice with similar results. D, RBL-2H3 cells (3 \times 10^6 cells assay) were loaded with Indo-1 and PAF (10 nM)-induced Ca\(^{2+}\) mobilization was measured.
fMLP or IL-8 (Table III). However, while pretreatment of cells with a first dose of PAF had no effect on fMLP-induced Ca\(^{2+}\) mobilization, response to IL-8 was desensitized by \(-60\%\) (Table III). In contrast to PAFR and mPAFR, Ca\(^{2+}\) mobilization in response to FR was cross-desensitized by both C5aR (Table III) and IL-8RA (data not shown) despite FR resistance to cross-phosphorylation (9).

**Effect of Extracellular Calcium on mPAFR-mediated Signal Transduction**—The effect of Ca\(^{2+}\) influx on mPAFR-mediated cellular responses as well as cross-phosphorylation and cross-desensitization of IL-8RA was assessed using the cation chelator EGTA to block extracellular calcium influx. EGTA had no effect in mPAFR-mediated PI hydrolysis (Fig. 8A) However, EGTA blocked mPAFR-induced PLC activation (Fig. 8B), secretion (Fig. 8C), and the sustained Ca\(^{2+}\) mobilization in response to PAF (Fig. 8, D and E). EGTA had no significant effect on mPAFR-mediated cross-desensitization of IL-8RA induced Ca\(^{2+}\) response (Fig. 8, D and E) and cross-phosphorylation of IL-8RA (Fig. 9).

**DISCUSSION**

Previous studies have indicated that chemotactant receptors trigger cellular activities by two related but distinguishable pathways. The first via the heterotrimeric G protein-mediated activation of PLC, the second via activation of PLD (1). Whereas all chemoattractants (peptides and lipids) initiate the former equally well, peptide chemotactants are far more active in stimulating PLD, which is required for the cytotoxic actions of phagocytes (i.e. respiratory burst and exocytosis) (1, 15–17). While chemoattractant receptors for peptides and lipids all activate PLC via G proteins, the difference in their regulation has been suggested to be a consequence of their utilizing different G proteins and PLC isozymes (10, 18–20). This laboratory has been investigating the regulation and cross-regulation of leukocyte chemoattractant receptors and has developed cellular models allowing genetic analysis of receptor functions. Using these strategies, it has been shown that peptide chemotactant receptor desensitization occurs at two sites, the first involving receptor phosphorylation leading to diminished G protein activation. The second involves the inhibition of the activation of PLC (9). Data in neutrophils demonstrated differences in cross-regulation among receptors for peptide versus lipid chemotactants (8), suggesting disparate signaling and regulatory pathways. However, the molecular mechanism underlying this phenomenon of cross-regulation among different classes of receptors has not been studied.

A goal of this study was to better define the regulation and...

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**FIG. 5. PAFR- and mPAFR-mediated PLD activation.** RBL-2H3 cells expressing IL8R-PAFR or IL8R-mPAFR were labeled with \([35S]\)GTP\(\gamma\)S and stimulated with the indicated ligand. The data shown are the means of three different experiments. The percentages of inhibition of PLD activation were determined. Values are represented as percentage of inhibition of PLD activation, which is defined as the maximal increase above basal [35S]GTP\(\gamma\)S bound to control membranes (untreated cells) after 10 min of reaction. Basal activities were 0.2 \(\pm\) 0.015 pmol of [35S]GTP\(\gamma\)S bound/mg of protein. Maximum net stimulation was determined. Values are represented as percentage of inhibition (cross-desensitization) of the Ca\(^{2+}\) response elicited in the absence of pretreatment (PAF, 595 \(\pm\) 41 nM, fMLP, 532 \(\pm\) 28 nM, IL-8, 533 \(\pm\) 17 nM, and C5a, 487 \(\pm\) 17 nM). Data are the means \(\pm\) S.E. of three different experiments.

**FIG. 6. Cross-phosphorylation of mPAFR by FR and IL-8R.** RBL-2H3 cells co-expressing FR-mPAFR or IL8R-mPAFR were \(\alpha\)-labeled and stimulated for 5 min with (lanes 2, 3, 5, and 6) or without (lanes 1 and 4) agonists. Receptor phosphorylation was assessed as described in the legend to Fig. 1. This experiment has been repeated three times with similar results.

**TABLE III**

Cross-desensitization of Ca\(^{2+}\) mobilization in cells expressing FR-mPAFR and IL8R-mPAFR

| Cells               | Agonists | % Cross-desensitization |
|---------------------|----------|-------------------------|
| FR-mPAFR            | fMLP → PAF | 7 \(\pm\) 0.3           |
|                     | PAF → fMLP | 2 \(\pm\) 0.26          |
| IL8R-mPAFR          | IL-8 → PAF | 8 \(\pm\) 1            |
|                     | PAF → IL-8 | 61 \(\pm\) 4.7*         |
| FR-C5aR             | fMLP → C5a | 60 \(\pm\) 9*           |
|                     | C5a → fMLP | 50 \(\pm\) 10*          |

\(\ast p < 0.05\).
The approach undertaken was to co-express, in RBL-2H3 cells, PAFR, or a phosphorylation deficient PAFR with the receptors for fMLP, C5a, or IL-8. The data presented herein demonstrate that PAFR was cross-phosphorylated upon activation of all three peptide chemoattractant receptors (Fig. 1) and that this correlated with the cross-desensitization of the receptor as measured by GTP$\gamma$S binding in membranes (Fig. 3) and Ca$^{2+}$ mobilization in intact cells (Table I). The results suggest a role for phosphorylation mediated by PKC in PAFR cross-desensitization since both peptide chemoattractant- and PMA-mediated phosphorylation were inhibited by the PKC inhibitor staurosporine (Fig. 2).

Like many members of the G-protein coupled receptor family, PAFR possesses a serine/threonine-rich cytoplasmic tail (5 serines and 6 threonines), which contains potential sites for agonist-dependent and -independent phosphorylation (21, 22). The carboxyl terminus plays a key role in the desensitization and regulation of several G-protein-coupled receptors (23–28). Elimination of phosphorylatable residues in the carboxyl terminus of the guinea pig PAFR, by either alanine substitution or truncation, diminished agonist-induced receptor desensitization (29). Based on these observations and, in an attempt to assess the role of receptor phosphorylation on PAFR regulation, a phosphorylation deficient human PAFR mutant containing a truncated carboxyl terminus was constructed (Table II). When co-expressed with FR and IL-8RA, this mutant was resistant to cross-phosphorylation and, importantly, cross-desensitization both at the level of G protein activation and Ca$^{2+}$ mobilization (Fig. 6 and Table III). This indicates that, unlike the peptide chemoattractant receptors, cross-desensitization of PAFR by the peptide chemoattractants is solely due to phosphorylation of the receptor by a PKC-dependent process and does not involve the direct downstream inhibition of PLC activation. This latter observation further supports the concept of class desensitization as a form of cellular regulation involving group of receptors sharing similar activation pathways (7–9) and suggests that receptors for lipid chemoattractants are regulated differently.

Studies in intact neutrophils indicated that whereas peptide chemoattractants desensitized the responses to lipid chemoat-

**Fig. 8.** Effect of EGTA on mPAFR-mediated cellular responses. A, inositol phosphates (IP) generation; B, phospholipase D activation; C, $\beta$-hexosaminidase release; and D and E, Ca$^{2+}$ mobilization in the presence and absence of EGTA (5 mM) were determined as described in the legend to Fig. 4 and Table I. The experiments were repeated twice with similar results.

**Fig. 9.** Effect of EGTA on mPAFR-mediated cross-phosphorylation of IL-8RA. RBL-2H3 cells expressing IL8R-mPAFR were $^{32}$P-labeled and stimulated for 5 min with (lanes 3–6) or without (lanes 1 and 2) agonists in the presence (lanes 2, 4, and 6) and absence (lanes 1, 3, and 5) of 5 mM EGTA. Receptor phosphorylation was assessed as described in the legend to Fig. 1. This experiment has been repeated three times with similar results.
When this was blocked by EGTA, PLD activation did not occur, allowing for the opening of a channel allowing Ca²⁺ influx and activation of PLD. Data from Figs. 4 and 5 show that greater activation of G proteins by the receptor protein activation mediated by a single dose of ligand. This more-phosphorylation and, thus, desensitization. The mPAFR is in-proteins apparently due to resistance of the receptor to undergo PLD (Figs. 4 and 5). The mechanism for this enhanced receptor activity appears to be a consequence of greater turnover of G proteins apparently due to resistance of the receptor to undergo phosphorylation and, thus, desensitization. The mPAFR is in-structive in that it shows that the cytoplasmic tail of the PAFR, presumably through phosphorylation, regulates the extent of G protein activation mediated by a single dose of ligand. It more-over shows that greater activation of G proteins by the receptor allows for the opening of a channel allowing Ca²⁺ influx (30). When this was blocked by EGTA, PLD activation did not occur, demonstrating the requirement of Ca²⁺ influx for the activation of this enzyme. Stimulation of the mPAFR (Fig. 6), unlike PAFR (Fig. 1), led to cross-phosphorylation of IL-8RA. This cross-phosphorylation was dependent on PLC but not on PLD activation, since EGTA which blocked the latter, but had no effect on IL-8RA phosphorylation (Fig. 9). This suggests that the level of PLC activation by PAFR determines its ability to cross-phosphorylate and cross-desensitize peptide chemo-attractant receptors. Under normal conditions, given the level of receptors expressed in neutrophils or in the studies here, the PAFR apparently does not activate enough PLC to mediate cross-phosphorylation.

The extent of mPAFR-mediated cross-phosphorylation of IL-8RA was minor as compared to the extent of cross-phosphorylation of wild type PAFR by IL-8RA (Figs. 6 and 9 versus Figs. 1 and 2). Nonetheless this phosphorylation of the IL-8RA was significant in that it resulted in a 50% and 61% cross-desensitization of IL-8RA-induced GTPγS binding and intracellular Ca²⁺ mobilization, respectively. Furthermore, staurosporine blocked both the cross-phosphorylation and cross-desensitization of IL-8RA indicating a role for PKC in this process. It has been shown that homologous phosphorylation of rhodopsin, β-adrenergic receptors, and muscarinic receptors by receptor specific kinases (rhodopsin kinase and β-adrenergic receptor kinases) do not cause receptor desensitization unless specific protein such as arrestin or β-arrestin are present (35–38). However, heterologous phosphorylation of these receptors by PKC and PKA (although to lower extents than homologous) caused 35–50% inhibition of receptor/G protein coupling (4, 5, 37). This suggests that receptor desensitization at the level of receptor/G protein coupling for a number of receptors, including those for IL-8, may be due to the phosphorylation of a small number of specific residue(s) involved in such interaction.

Another finding of note is that neither the PAFR nor the mPAFR at the dose tested (EC₅₀ versus EC₁₀₀) generated the signal which results in downstream inhibition of PLC activation by peptide chemoattractant receptors, since neither PAFR nor mPAFR inhibited Ca²⁺ mobilization by FR (Tables I and III). Thus, not only are PAFR not subject to regulation at the level of PLC activation by peptide chemoattractant receptors, they do not produce the signal(s) sufficient to mediate this activity. Since mPAFR mediates substantial activation of PLC, Ca²⁺ influx, and PLD, it can be presumed that second messengers produced by these pathways are not sufficient to produce class-desensitization of peptide chemoattractants. More likely, a component(s) unique to the peptide chemoattractant recep-tors pathways is involved in its susceptibility to regulation of PLC activation. The nature of this signal(s) or second messenger(s) remains to be determined. A clue may be found in the differences in G proteins and PLCs used by PAFR versus peptide chemoattractant receptors. Whereas the latter interact preferentially with G₃ (pertussis toxin (Ptx)-sensitive) to stimulate PLCβ₂ via Gβγ (10, 11, 19), PAFR is thought to couple to members of the Gq family (Ptx-insensitive) of G proteins to activate PLCγ via Go (13, 19, 31–34). Thus the Gβγ of Gq may be a site for cross-regulation of chemoattractant receptors for peptide.

In all, these studies put the utility of cellular and genetic models to elucidate the complexities of receptor regulation and cross-regulation. While all the chemoattractant receptors studied are effective mediators of directed cellular migration, they are likely to play different physiological roles in other aspects of phagocyte regulation. The PAFR clearly is subject to different regulatory processes than the peptide che-moattractant receptors as reflected in its resistance to inhibition at the level of PLC activation and its inability to affect PLC activation by the peptide chemoattractant receptors. These data are likely to reflect the distinct usage of G proteins and PLC isozymes by the different groups of receptors and indicates that receptor cross-regulation involves mechanisms beyond receptor phosphorylation.

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