Chemoattractant Receptor Cross-desensitization*

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Leukocytes participate in host defense by accumulating at local sites in response to inflammatory mediators where they may engulf foreign material and/or release toxic products that can cause substantial tissue damage. Agents of diverse chemical nature (short peptides, proteins, and lipids) have been identified as chemoattractants and stimulate leukocytes through G-protein-coupled receptors (1). Responses of leukocytes can be mediated by chemoattractants alone or modulated by other agents. For example, leukocytes that are attached to adhesion molecules respond to chemoattractants to elicit far greater cytotoxic responses than non-adherent cells. Leukocyte chemoattractant receptors are also subject to desensitization. Given that multiple mediators are present at sites of inflammation and that leukocytes contain receptors for many of them, their responses are likely to be cross-regulated. Although much has been learned about cellular activation and regulation by single receptors, mechanisms of receptor cross-regulation leading to priming or desensitization are only beginning to be unraveled.

Mechanism of Leukocyte Activation and Regulation

Chemoattractants such as the formylpeptide N-formylmethionine-nylleucophenylalanine (fMLP), a complement cleavage product (C5a), leukotriene B4 (LTB4), and platelet-activating factor (PAF) were identified years ago (2). More recently, a superfamily of related chemotactic cytokines (chemokines) and their receptors have been recognized with interleukin-8 (IL-8) being the best characterized among this group (3, 4). Of note, chemokine receptors CCR5 and CXCR4 participate with CD4 in the entry of human immunodeficiency virus (HIV) into cells (5). Chemoattractant receptors stimulate leukocytes via G-proteins that activate phospholipase C (2, 6). Most of these receptors are coupled to a pertussis-sensitive G-protein, presumably Gαi (6). Nonetheless, receptors for PAF and LTB4 activate a Gβγ-like G-protein as well as Gi. As chemoattractant receptors stimulate multiple responses, it is not yet clear whether selective G-protein usage mediates different responses. Although, by definition, all chemoattractants stimulate directed migration, at higher doses (about 20-fold) many also activate the opening of calcium channels and activate phospholipase D. These activities correlate with the onset of cytotoxic responses such as exocytosis and respiratory burst (7, 8). There is a hierarchy among chemoattractants for stimulation of cytotoxic responses with fMLP and C5a being more active than others, i.e. IL-8, PAF, and LTB4. These differences are likely related to the activation of shared pathways for chemotaxis and a distinct pathway for cytotoxic activation requiring prolonged receptor signaling (2, 7).

Cellular responses to chemoattractants can be up-regulated through priming and down-regulated by desensitization. This review will focus on the latter, although cellular models allow approaches to understanding both (9). Two types of desensitization termed homologous and heterologous have been described for G-protein-coupled receptors (10, 11). Homologous desensitization occurs in receptors in the agonist-occupied state and involves phosphorylation by G-protein-coupled receptor kinases. These phosphorylated receptors associate with members of the arrestin family of proteins resulting in a decreased affinity of the receptor for G-proteins and internalization. Heterologous desensitization occurs when a receptor loses its responsiveness following phosphorylation by second messenger-activated kinases (i.e. protein kinase A (PKA) or protein kinase C (PKC)), which have been activated by different receptors or signaling processes (10). Heterologous desensitization does not require agonist occupancy and does not lead to arrestin-mediated receptor internalization. Studies with leukocytes have demonstrated an additional level of complexity and the description of a new form of “heterologous” desensitization with selectivity for groups of chemoattractant receptors.

Identification of a New Form of Chemoattractant Receptor Regulation

Early studies suggested a complexity of receptor cross-regulation beyond the classic concepts of homologous and heterologous desensitization (12–15). An approach to understanding “cross-desensitization” among chemoattractant receptors was provided by Didsbury et al. (16). They demonstrated that in HEK293 cells transiently coexpressing receptors for fMLP and C5a, activation of one receptor resulted in reduced activation of Ca2+ mobilization stimulated by the other. Cross-desensitization was specific for the chemoattractant receptors that activate phospholipase C (PLC) via a pertussis toxin-sensitive G-protein. Native α1-adrenergic receptors that activate PLC via a pertussis toxin-insensitive G-protein were not desensitized by fMLP and C5a and viceversa. This discovery led to the extensive characterization of specificity of this type of cross-regulation in neutrophils (17). For these studies, the chemoattractants fMLP, C5a, IL-8, PAF, and LTB4 and the purinoreceptor agonist ATP·γ·S were evaluated for their ability to cross-desensitize each other as measured by ligand-stimulated GTP·S binding to membranes or intracellular Ca2+ mobilization. It was shown that all receptors undergo effective homologous desensitization. In addition, fMLP, C5a, and IL-8 cross-desensitized Ca2+ mobilization to one another as well as to LTB4 and PAF (Table I) (17). PAF, LTB4, or ATP·γ·S did not, however, cross-desensitize the peptide chemoattractant receptors. The strength of receptors to desensitize Ca2+ mobilization to one another was fMLP > C5a > IL-8. In contrast, the susceptibility of peptide chemoattractant receptors to undergo cross-desensitization was reversed with IL-8 > C5a > fMLP. The ability of fMLP to induce a greater desensitization of Ca2+ mobilization by C5a and IL-8 was correlated with its ability to block C5a and IL-8-stimulated G-protein activation at the level of receptor/G-protein coupling. Surprisingly, neither C5a nor IL-8 inhibited fMLP-stimulated G-protein activation, although both blocked Ca2+ mobilization. Based on these studies it was postulated that chemoattractant receptor cross-regulation occurred at two levels, one at the level of receptor/G-protein coupling and another at a level distal to G-protein activation, resulting in a reduced activation of phospholipase C. Blackwood et al. (18) demonstrated that fMLP and C5a cross-regulate both chemotaxis and arachidonic acid release stimulated by each other. Although IL-8 desensitized chemotaxis stimulated by fMLP and C5a, it was less efficient in blocking arachidonic acid release by these chemoattractants. Campbell et al. (19), however, found that neutrophils displayed normal chemotactic responses to fMLP even after maximal stimulation with IL-8, but activation of neutrophils even with low concentrations of fMLP abrogated these
responses to IL-8. Nonetheless, in a murine pre-B cell line coexpressing fMLP receptor (FR) and an IL-8 receptor, CXCR2, both fMLP and IL-8 desensitized each other’s chemotactic responses although IL-8 was less effective in desensitizing Ca\(^{2+}\) mobilization by fMLP (20). These findings are consistent with a rank order of potency of chemoattractant receptor cross-regulation for Ca\(^{2+}\) mobilization (17). This further suggests that cross-regulation of chemoattractant-mediated biological responses such as adhesion, chemotaxis, Ca\(^{2+}\) mobilization, degranulation, and PLA\(_2\) activation occurs via the modulation of multiple steps in the signal transduction pathways.

**Mechanism of Chemoattractant Receptor Cross-desensitization**

**Role of Receptor Phosphorylation**—Study of the molecular mechanisms of chemoattractant receptor cross-regulation was facilitated by the use of a basophilic cell line, BBL-2H3, which could be transfected to express receptors singly or multiply. This cell line possesses the same complement of G-proteins as found in neutrophils and responds to chemoattractants to elicit a number of biochemical and biological responses in common with neutrophils (21). As with other G-protein-coupled receptors, agonist-stimulated phosphorylation of FR, C5aR, CXCR1, CXCR2, PAFR, and LTB\(_4\) receptor are associated with homologous desensitization (22–24). For all chemoattractant receptors studied, with the notable exception of FR, ligand-stimulated receptor phosphorylation is mediated via the activation of both G-protein-coupled receptor kinase and PKC (22–24). Although fMLP does activate PKC, its receptor is resistant to phosphorylation by this protein kinase. These findings provided a foundation for delineating some of the mechanisms involved in chemoattractant receptor cross-regulation as well as for explaining hierarchies of responses.

In neutrophils and in BBL-2H3 cells expressing different combinations of chemoattractant receptors, peptide chemoattractants (fMLP, C5a, IL-8) desensitized Ca\(^{2+}\) mobilization to one another and to PAF (25, 26). In contrast, PAF did not desensitize Ca\(^{2+}\) mobilization stimulated by any of these peptide chemoattractants. Studies with BBL-2H3 provided an understanding for the unidirectional desensitization of PAF-mediated responses. It was shown that PAFR was cross-phosphorylated upon activation of FR, C5aR, or CXCR1. This correlated with cross-desensitization of G-protein activation in membranes as well as Ca\(^{2+}\) mobilization in intact cells. The demonstration that phorbol ester also caused phosphorylation of PAFR and that a PKC inhibitor blocked PAFR phosphorylation by fMLP, C5a, and IL-8 indicates that the susceptibility of PAFR to cross-desensitization is due at least in part to PKC-mediated phosphorylation of PAFR. This contention was extended by the finding that when a phosphorylation-deficient, truncated PAFR (mPAFR) was coexpressed in BBL-2H3 cells with either FR or CXCR1, neither fMLP nor IL-8 cross-desensitized PAF-mediated responses (26). Interestingly, mPAFR, which activates cellular responses of greater magnitude and for longer duration than PAFR, resulted in cross-phosphorylation and desensitization of CXCR1 but not FR (Table I). Also, mPAFR generated a signal downstream of R/G coupling to desensitize its own Ca\(^{2+}\) mobilization response but did not cross-desensitize the response to fMLP (27). These findings demonstrate that the ability of fMLP, C5a, and IL-8 to unidirectionally desensitize PAF-mediated responses is exclusively because of PKC-mediated phosphorylation of the PAFR, and the downstream component is not affected. The inability of PAFR to induce phosphorylation of C5aR and CXCR1 is likely because of its own rapid phosphorylation and desensitization. Although PAFR is resistant to regulation of its downstream component by FR, C5aR, or IL-8R, neither it nor the highly active mPAFR provides a signal for downstream modification to regulate the peptide chemoattractant receptors. Given the susceptibility of FR, C5aR, and CXCR1 and the resistance of PAFR and the phosphorylation-deficient mutant (mPAFR) to inhibition by pertussis toxin, the lack of downstream cross-regulation between these groups of receptors may reflect their distinct G-protein usage (Fig. 1).

Receptor phosphorylation plays an important but not an exclusive role in desensitization among chemoattractant receptors. For example, activation of FR resulted in the cross-phosphorylation and cross-desensitization of G-protein activation and Ca\(^{2+}\) mobilization stimulated by C5a and IL-8 (25). C5a and IL-8 also cross-phosphorylated and cross-desensitized responses to each other. However, receptor phosphorylation cannot explain the ability of C5aR to desensitize Ca\(^{2+}\) mobilization and IP\(_3\) generation by FR and by a phosphorylation-deficient mutant of CXCR1 (M2-CXCR1) despite a lack of both cross-phosphorylation and suppression of G-protein activation (28). This indicates that the ability of peptide chemoattractant receptor receptors to cross-desensitize Ca\(^{2+}\) mobilization to one another is mediated via two processes: a PKC-mediated receptor cross-phosphorylation, to which FR and M2-CXCR1 are resistant, and a downstream component, which is shared by some but not other receptors, resulting in decreased activation of PLC. The effects of these two modifications appear to be additive. Accordingly, the inability of FR to undergo receptor cross-phosphorylation probably results in its relative resistance to cross-desensitization by other chemoattractants in neutrophils. In contrast, the greater susceptibility of IL-8-induced response reflects a higher susceptibility of its receptor to cross-phosphorylation in addition to inhibition of the downstream component (Table II, Fig. 1). PAFR, which couples to a G\(_\alpha\)i-like G-protein, may have an independent downstream regulatory component. Evidence for this comes from homologous desensitization of calcium mobilization by mPAFR, which is resistant to receptor phosphorylation (27).

**Evidence for the Role of PLC\(_\beta\)s Modification in “Downstream” Desensitization**—The downstream component whose modification results in the cross-regulation of a select group of chemoattractant receptors has not yet been identified, but clearly, it results in
Minireview: Chemoattractant Receptor Cross-desensitization

Cross-regulation of leukocyte chemoattractant receptors. Peptide chemoattractants (FMLP, C5a, IL-8) activate signaling pathways through Gq and bg subunits to activate PLCbg and adenyl cyclase (AC) and produce IP3, diacylglycerol (DAG), and cAMP. The PKC-activated via diacylglycerol provides Signal 1 to cross-phosphorylate susceptible chemoattractant receptors. PKA provides Signal 2 to phosphorylate PLCbg to specifically inhibit bg-mediated PLCbg activation. As yet unidentified signal generated from Go, or bg may provide an additional signal (Signal 2') to specifically inhibit Gbg signaling. Both Signals 2 and 2' do not inhibit signaling through Gbg-like G-protein activated by PAFR. PAFR does not generate the inhibitory signals (Signals 2 or 2') for downstream desensitization of Gbg-coupled receptors.

decreased activation of PLCbg as IP3 production is depressed. The finding that PLCbg is phosphorylated by PKA and that this is associated with the inhibition of PLC-mediated responses stimulated by Gbg but not Gq, or Gbg, suggested a role for PLCbg phosphorylation on cross-desensitization (29). This idea is supported by the finding that FMLP, C5a, and IL-8 but not PAF stimulate cAMP formation in neutrophils and in transfected RBL cells (30, 31). It is possible that PKA-mediated phosphorylation of PLCbg by a group of chemoattractant receptors selectively inhibits activation by Gbg but not by Gq, or Gbg, or Gbg. This would provide a mechanism for cross-regulation of chemoattractant receptors at the downstream level. Studies by Ali et al. (27, 30) in RBL-2H3 cells showed that FMLP but not PAF stimulated cAMP production. A membrane-permeable cAMP analog resulted in inhibition of both phosphoinositide hydrolysis and exocytosis stimulated by FMLP but not PAF. In addition, both phosphoinositide hydrolysis and exocytosis by FMLP but not PAF were greatly enhanced by a PKA inhibitor. The inhibitory effect of cAMP on FMLP-mediated responses likely involves phosphorylation of PLCbg by PKA. As evidence, both FMLP and a membrane-permeable cAMP analog caused phosphorylation of PLCbg, the only PLC isozyme expressed in this cell line. Furthermore, the purified catalytic subunit of PKA phosphorylated PLCbg immunoprecipitated from this cell line, and preincubation of cells with FMLP but not PAF blocked in vitro phosphorylation of PLCbg by PKA. C5a also stimulates cAMP formation in RBL-2H3 cells, and CAMP regulates the function of this receptor as it does for FMLP. These findings are consistent with the hypothesis that receptor-stimulated cAMP production and the subsequent phosphorylation of PLCbg by PKA cross-desensitizes receptors that activate PLCbg by the same mechanism. This contention is supported by the finding that α and bg subunits of G-proteins activate PLCbg by interacting at distinct sites (32).

Evidence against the Role of PLCbg Modification in “Downstream” Desensitization—The role of PLCbg phosphorylation in downstream peptide chemoattractant receptor desensitization remains to be tested directly. Recent data raise doubts that this is the sole mechanism for the downstream effect. A major site for phosphorylation of PLCbg by PKA has recently been identified as Ser-1105 (33). Phosphorylation of PLCbg at this site by PKA blocked PAF and other Gbg-coupled receptor-mediated responses in RBL-2H3 and COS cells coexpressing the receptor and Gbg. This is in contrast to neutrophils, differentiated HL-60 cells, transfected RBL-2H3 cells, and COS cells where PLCbg phosphorylation by PKA leads to inhibition of FMLP but not PAF-mediated phosphoinositide hydrolysis and Ca2+ mobilization (30). The reason for this discrepancy may be related to the overexpression of Gbg in the studies of Yue et al. (33) because PKA did not effect PAF responses in RBL cells not transfected with Gbg (27). Recent studies by Richardson et al. (34) also questioned the role PLCbg phosphorylation as the sole determinant of downstream chemoattractant receptor cross-regulation. CXC2, which stimulated Ca2+ mobilization and caused PLCbg phosphorylation similar in magnitudes to those stimulated by CXC1, did not cross-desensitize the response to FMLP or C5a although CXC1 did. However, a phosphorylation-deficient mutant of CXC2 (331T), which induced a greater cell activation for a longer duration, resulted in cross-desensitization of both FMLP and C5a-induced responses, indicating that a downstream component was inhibited by 331T-CXC2. Nonetheless, wild-type and phosphorylation-deficient receptors both phosphorylated PLCbg to a comparable extent, and Ca2+ mobilization to both was inhibited by exogenous cAMP (34). The findings were taken as evidence that PLCbg phosphorylation may be necessary but not sufficient for chemoattractant receptor cross-desensitization.

Role of Signal Length on Chemoattractant Receptor Cross-desensitization

Studies in neutrophils showed that IL-8 is not only the most susceptible chemoattractant receptor to undergo cross-desensitization but it also provides the weakest signal for cross-desensitization of other chemoattractant receptors (17, 19). In neutrophils, responses to IL-8 are mediated via the activation of both CXC1 and CXC2 (35). Although CXC1 cross-desensitizes responses to other peptide chemoattractants, CXC2 did not. Richardson et al. (34) suggested that IL-8 provides the weakest desensitizing signal because of the brief receptor signaling. For example, CXC2, which did not produce a cross-desensitizing signal, is rapidly phosphorylated and internalized upon ligand stimulation so that >95% of the surface receptors were lost within 5 min (36, 37). In contrast, the phosphorylation-deficient mutant 331T-CXC2 was resistant to internalization (<5% internalization after 30 min) and generated a signal for cross-desensitization presumably because of greater G-protein turnover (34, 38). The sustained production of second messengers likely activates inhibitory pathways to cause both phosphorylation of susceptible receptors and modification of downstream components to diminish the activation of PLCbg by certain chemoattractant receptors (Table III). As receptor phosphorylation leads to G-protein uncoupling and internalization the hierarchy of chemoattractant receptors to generate cytotoxic signals as well as their susceptibility to cross-desensitization and the ability to cross-desensitize other receptors is likely regulated by receptor phosphorylation sites and, as a consequence, signaling time.

Role of G-protein Modification on Chemoattractant Receptor Cross-desensitization

The signal for Gbg-coupled receptors is initially mediated by Gbg, and therefore, modification of these proteins may regulate PLCbg activation. Studies on isoprenylation and carboxymethylation of the bg subunit of G-protein (Gbg) indicate that this regulates Gbg-mediated responses in neutrophils. Isoprenylation and carboxymethylation of Gbg allow it to localize to the plasma membrane where it activates PLCbg (39). In vitro reconstitution studies showed that carboxymethylated Gbg was 10-fold less effective in activating PLCbg (40). FMLP stimulated Gbg carboxymethylation in
neutrophils (41). Inhibition of carboxymethylation blocked the fMLP-induced respiratory burst. Phosphorylation of G_{Y12} by PKC substantially blocked the ability of the G_{Y12} to activate effector enzymes (42). Thus, modification of G_{Y} by carboxymethylation and/or phosphorylation could be involved in cross-desensitization (Fig. 1). This may explain the finding of Pike and Snyderman (43) that inhibition of carboxymethylation in leukocytes depresses chemotactic function.

A newly described family of proteins known as regulators of G-protein signaling (RGS) reduces the length of G-protein signaling by enhancing its GTPase activity, thus making less G protein available (44). RGS could therefore play a role in chemotactic receptor cross-desensitization by regulating signal length. In this regard, transient overexpression of RGS1, RGS3, and RGS4 but not RGS2 was found to inhibit chemotactic receptor-mediated motility in a transfected lymphoid cell line (45).

**Concluding Comments**

Prior exposure of cells to some signals enhances or depresses subsequent responses to others. Study of chemotactic receptor cross-regulation has been facilitated by the development of cellular systems allowing genetic and biochemical manipulation. These investigations have disclosed a previously unrecognized complexity of receptor cross-regulation and indicate that this occurs by at least two distinct mechanisms. First, the level of receptor signaling is mediated through phosphorylation by kinases activated by second messengers. A second, downstream regulatory site also controls the activation of classes of chemotactic receptors. Cross-regulation via this site inhibits the activation of PLC and appears to be shared by groups (classes) of receptors using the same G protein. Chemotactic receptor cross-regulation appears to be related to the length of signaling which in turn is regulated by receptor phosphorylation as well as the rate of internalization. Of interest, chemotactic receptors for the same or similar ligands (i.e. IL-8) appear to be differentially regulated solely by their signal length, which endows the receptor with different biological activities (i.e. migration versus cytotoxicity) and abilities to cross-desensitize other receptors.

The concepts currently being developed in leukocyte receptor cross-regulation may well be of more general significance. For example, receptors with both shared ligands and signal transduction pathways mediate the biological effects of a large number of chemokines. Among other activities, chemokines regulate the migration and homing of T and B lymphocytes and also act as coreceptors for HIV infection (46, 47). As these cells contain multiple receptors for chemokines, their migration as well as their susceptibility to infection may be subject to receptor cross-regulation. Recent studies indicate that defects in host defense in opiate abuse may be because of cross-regulation and inhibition of chemokine receptors (48). Although the molecular mechanisms are not clear, there is already evidence that leukocyte adhesion molecules and chemotactic receptor cross-regulate each other’s function in coordinating the transmigration (49). Here again, some of the general principles of chemotactic receptor cross-regulation appear significant as both receptor phosphorylation-dependent- and -independent mechanisms are likely involved (50, 51). In mammals the sense of smell is mediated through thousands of G-protein-coupled receptors that undergo rapid desensitization (52). Selective receptor cross-regulation may form a basis for rapid desensitization to similar odors without affecting the sense of smell to others. The recognition that receptor cross-regulation is an important way to fine tune the cellular responses should allow greater attention to this area of research and a more precise understanding of cross-regulatory mechanisms.

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