Interleukin-8 was originally discovered as one of the first chemokines activating neutrophil granulocytes (neutrophils) after secretion by lipopolysaccharide-stimulated monocytes. A wealth of information has been gathered concerning the intracellular events mediated by interleukin-8 and the role of interleukin-8 in numerous physiologic and pathophysiologic processes. We discuss recent advances in the understanding of the initial intracellular signals elicited by interleukin-8. Detailed investigation of these events has led to the identification of subtle but significant differences in the signal transduction processes evoked by interleukin-8 receptors. In particular, much has been learned concerning differences in the cellular mechanisms leading to desensitization, internalization, and recycling of interleukin-8 receptors, and functional consequences of interleukin-8 receptor diversity are now being unraveled. Curr Opin Hematol 2000, 7:178–182 © 2000 Lippincott Williams & Wilkins, Inc.

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Abbreviations

- f-MLP: N-formylmethionylleucylphenylalanine
- GRK-2: G protein–coupled receptor kinase-2
- IP₃: inositol 1,4,5-triphosphate

Tissue-derived endogenous chemotactic polypeptides (chemokines) are key mediators in migration of leukocytes to sites of inflammation, injury, and infection. Interleukin-8 is the prototypical chemokine attracting neutrophils [1,2]. It is involved in a wide variety of physiologic and pathophysiologic processes ranging from host defense against bacterial infections and phagocytosis of necrotic tissue to numerous autoimmune disorders, including rheumatoid arthritis and psoriasis, and to atherosclerosis [3,4]. The superfamily of chemokines, which comprises about 40 known members, can be divided into four subgroups, CXC, CC, C, and CX₃C, according to highly conserved cysteine motifs in their amino terminal domain [5]. They differ in their biologic activity on different leukocyte populations depending on the pattern of expression of their receptors. Many chemokines bind to more than one receptor, and most receptors recognize more than one ligand of the corresponding subgroup. Interleukin-8 activates with high affinity two receptors, CXCR-1, which also recognizes a second CXC chemokine, granulocyte chemotactic protein 2 (GCP-2), and CXCR-2, which can also be activated by several other CXC chemokines, including neutrophil-activating peptide 2 (NAP-2), growth-related oncogene α-γ (GROα-γ), and epithelial cell-derived neutrophil-activating protein 78 (ENA-78) [5,6]. Both CXCR-1 and CXCR-2 receptors are constitutively expressed in neutrophils [7,8].

Role of interleukin-8 in neutrophil signaling

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Interleukin-8-triggered intracellular signaling in neutrophils

It is widely accepted that most, if not all, cellular effects of interleukin-8 in neutrophils can be blocked by pretreatment with Bordetella pertussis toxin [9], which irreversibly inactivates heterotrimeric G proteins of the Gₛ subfamily [10]. One of the early intracellular events following activation of interleukin-8 receptors is an increase in the intracellular concentration of free Ca²⁺ ([Ca²⁺]ᵢ). Although some Ca²⁺-independent effects of interleukin-8 receptor activation have been suggested in a recent review by Bokoch [11], it is widely accepted that interleukin-8-triggered Ca²⁺ signals are major intracellular events responsible for many of the subsequent steps in the neutrophil activation process. Although it may sometimes be difficult to establish the role of rises in [Ca²⁺], for particular cellular functions following interleukin-8 receptor activation, Ca²⁺ dependence is generally accepted for the activation of β₂ integrins [12], for the phagosome-lysosome fusion process [13], and for exocytosis [14]. Controversy exists for the role of Ca²⁺ in
the regulation of the contractile apparatus involved in neutrophil shape change and motility [15]. Whereas chemotaxis clearly requires Ca\(^{2+}\)-dependent recycling of integrins in migrating neutrophils [16], shape change appears to be perfectly normal in Ca\(^{2+}\)-depleted cells [17]. Certain neutrophil or macrophage functions can still be found under conditions of high Ca\(^{2+}\) buffering [18] or depletion of internal stores after treatment with the Ca\(^{2+}\) ATPase inhibitor thapsigargin [14]. However, this does not inevitably mean that elevations in [Ca\(^{2+}\)]\(_i\) are not necessary for these functions. Depletion of Ca\(^{2+}\) storage organelles can induce Ca\(^{2+}\) influx in neutrophils, and in the case of experiments using high intracellular concentrations of Ca\(^{2+}\) chelators, the distance between the site of Ca\(^{2+}\) release and its effector molecule may be too short to allow complete binding of Ca\(^{2+}\). In neurons, eg, neurotransmitter release triggered by presynaptic action potentials is unequivocally Ca\(^{2+}\)-mediated but is largely insensitive to intracellular Ca\(^{2+}\) buffering with 1,2 bis-(2-aminoethoxyethan)-N,N,N\(_1\),N\(_1\)-tetraacetate (EGTA), [19]. Ca\(^{2+}\) binding to EGTA is just too slow to prevent its diffusion to the vesicle fusion apparatus.

**The phospholipase C\(\beta\)/inositol 1,4,5 triphosphate pathway**

In neutrophils, the initial interleukin-8-mediated Ca\(^{2+}\) signal is purely due to intracellular release, whereas a second, more prolonged signal appears to be mediated by Ca\(^{2+}\) influx, probably through Ca\(^{2+}\) release-activated Ca\(^{2+}\) channels [20,21•]. The classic pathway [22] leading to Ca\(^{2+}\) release from the endoplasmic reticulum involves activation of G\(_i\) proteins by seven-transmembrane receptors and the release of G protein G\(_{\beta\gamma}\) subunits, which activate phospholipase C\(\beta\) in a pertussis toxin–sensitive manner. Activation of phospholipase C\(\beta\) results in production of inositol 1,4,5 triphosphate (IP\(_3\)) and diacetyl-glycerol (DAG) leading to Ca\(^{2+}\) release and protein kinase C activation. Alternatively, phospholipase C\(\beta\) can be activated via G protein \(\alpha\)\(_i\) subunits, a mechanism that might account for a small pertussis toxin–insensitive component of interleukin-8-evoked Ca\(^{2+}\) release [23]. Recent reports indicate that even these initial interleukin-8-triggered events leading to Ca\(^{2+}\) signaling in neutrophils may be more complex than previously expected.

**Effector systems different from the phospholipase C\(\beta\)/IP\(_3\) pathway**

A phospholipase C\(\beta\)-/IP\(_3\)-independent pathway of chemoattractant-induced intracellular Ca\(^{2+}\) signaling has recently been suggested. Alemany et al. [24•] have used the bacterial tripeptide N-formylmethionylleucylphenylalanine (f-MLP) and the myeloidlike differentiated human leukemia cell line HL-60 to investigate chemoattractant-induced intracellular signaling. These authors have shown that stimulation of HL-60 cells with f-MLP resulted in the production of sphingosine 1-phosphate by sphingosine kinase via a pertussis toxin–sensitive G protein. Ca\(^{2+}\) signaling mediated by f-MLP could be completely prevented by two blockers of sphingosine kinase, \(\delta\)-threo dihydrospingosine and \(N,N\)-dimethyl-sphingosine. In HEK 293 cells, intracellular injection of sphingosine 1-phosphate induced a rapid Ca\(^{2+}\) mobilization, which could not be blocked by heparin in permeabilized cells, suggesting that IP\(_3\) receptor–coupled Ca\(^{2+}\) release channels were not involved [25]. Although it is not yet proven, it appears possible that a similar signal transduction process exists in neutrophils and can be activated by interleukin-8. Conversely, the existence of an interleukin-8-activated signal transduction pathway in human neutrophils involving the activation of phospholipase C\(\beta\) and Ca\(^{2+}\) release through IP\(_3\) receptor–coupled Ca\(^{2+}\) release channels has recently been confirmed [21•]. Furthermore, the production of IP\(_3\) in response to stimulation with interleukin-8 [26] and the existence of IP\(_3\)-induced Ca\(^{2+}\) release in human neutrophils [27] have repeatedly been demonstrated. It is therefore not known at present how these two pathways relate to each other.

The existence of additional (or alternative?) messengers mediating Ca\(^{2+}\) release opens the possibility that other types of Ca\(^{2+}\) release channels apart from IP\(_3\) receptors exist in neutrophils (Fig. 1). Interestingly, ryanodine receptor–coupled Ca\(^{2+}\) release channels (ryanodine receptors), which have originally been thought to be restricted to electrically excitable cells, such as neurons and muscle cells, have meanwhile also been proposed in human neutrophils [29–32]. In electrically excitable cells, voltage-activated Ca\(^{2+}\) channels and Ca\(^{2+}\) influx through these channels can function as activators of ryanodine receptors [22]. The absence of voltage-activated Ca\(^{2+}\) channels in neutrophils suggests the existence of an intracellular messenger capable of coupling membrane receptors to Ca\(^{2+}\) release. cADP-ribose, which can evoke Ca\(^{2+}\) release from internal heparin-insensitive stores [33], might serve this function. Although the production of cADP-ribose in response to interleukin-8 has not yet been demonstrated in neutrophils, the existence of this pathway has been observed, eg, in natural killer cells [34•]. Ca\(^{2+}\) signals evoked in natural killer cells with an extract of natural killer cells pretreated with interleukin-8 and \(\beta\)-nicotinamide adenine dinucleotide (\(\beta\)-NAD) were insensitive to heparin but blocked with antibodies against ryanodine receptors and with ruthenium red, a blocker of ryanodine receptors [34•]. Future research will have to define the functional significance of sphingosine 1-phosphate and cADP-ribose as additional or alternative intracellular messengers in neutrophils.

**Differences in CXCR-1 and CXCR-2 receptor functioning**

Although both interleukin-8 receptors, CXCR-1 and CXCR-2, are seven-transmembrane receptors coupling
to the same G proteins [2], substantial evidence has accumulated in recent years that they differ in the functional consequences of their activation and in the cellular mechanisms mediating receptor desensitization, internalization, and reexpression.

**Receptor internalization and reexpression**

Repetitive or prolonged exposure of neutrophils to interleukin-8 as well as to other chemoattractants leads to a progressive attenuation of cellular responses. This progressive loss of function is called desensitization and represents a widespread phenomenon in cell biology. Two different forms of desensitization can be distinguished. Homologous (agonist-dependent) desensitization describes a loss of receptor function induced by binding of its own agonist, whereas heterologous (agonist-independent) desensitization refers to a loss of function of one receptor induced by agonists acting at different receptors [35].

Both CXCR-1 and CXCR-2 are subject to homologous and heterologous desensitization [36•]. Homologous desensitization by interleukin-8 involves internalization of agonist-occupied receptors, degradation of interleukin-8 by lysosomal enzymes, and reexpression of “recycled” receptors on the cell membrane [37]. Experiments with HEK 293 cells suggest differences in receptor internalization of the two interleukin-8 receptors. When these cells were transfected with CXCR-1, receptor internalization was observed only when the cells were cotransfected with the serine-threonin G protein–coupled receptor kinase-2 (GRK-2) and β-arrestin in addition to CXCR-1 [38••]. β-Arrestins are proteins that bind to phosphorylated G protein–coupled receptors and interfere with the receptor’s capability to interact with G proteins and direct agonist-occupied receptors to clathrin-coated pits, which then undergo dynamin-dependent endocytosis [39,40]. By contrast, CXCR-2 internalization could proceed without transfection of additional proteins [41••]. These results suggest the existence of a pathway leading to internalization of CXCR-2 independent of phosphorylation by GRK-2.

This is further supported by experiments with the rat basophil leukemia 2H3 (RBL-2H3) cell line. When these cells were transfected with a mutant CXCR-2 receptor that lacks the intracellular carboxyl terminus necessary for phosphorylation, receptor internalization could still be observed [42]. The latter finding indicates that additional or alternative mechanisms exist for internalization of CXCR-2 as compared with that of CXCR-1. This is also in agreement with the finding that internalization following heterologous desensitization, which is GRK-2 independent [36•] and protein kinase C dependent [43], is different in the two interleukin-8 receptor subtypes. In these experiments, stimulation with the bacterial tripeptide f-MLP of human neutrophils led to desensitization of CXCR-1 and CXCR-2. However, only CXCR-2 was internalized, whereas CXCR-1 surface expression remained unchanged [44], which again suggests a phosphorylation-independent mechanism of receptor internalization.

Interestingly, dynamin appears to be involved not only in GRK-2- and β-arrestin-dependent CXCR-1 internalization, but also in GRK-2- and β-arrestin-independent CXCR-2 internalization. Transfection with a dominant negative mutation of dynamin (dynamin I K44A) dramatically slowed interleukin-8-induced internalization of both CXCR-1 and CXCR-2 [41••]. It is at present not known how agonist-occupied CXCR-2 receptors are directed to the clathrin-coated pits.

Other unresolved issues include the contribution of certain subtypes of GRKs to CXCR phosphorylation of agonist-occupied CXCR. Six subtypes of GRKs are meanwhile known [45] and it is not clear which subtypes are responsible for CXCR phosphorylation in neutrophils. Furthermore, the cellular mechanisms leading to activation of GRKs are only incompletely understood. Most data have been gathered for the β2 adrenergic receptor kinase (GRK-2). For this kinase, it has been shown that several prerequisites must be fulfilled to allow phosphorylation of β2 adrenergic receptors. To become phosphorylated, these receptors must be activated by a ligand and binding of Gβγ subunits, and phosphatidylinositol 4,5-biphosphate (PIP2) to the β2 adrenergic receptor kinase is required for membrane association and kinase activity in vitro [46].

**Functional implications**

Receptor internalization not only is a mechanism to limit cellular responses following agonist binding but also appears, in combination with reexpression, to be a fundamental step for chemotaxis. In RBL-2H3 cells expressing M2CXCR-1, a phosphorylation-deficient mutant CXCR-1, both interleukin-8-induced chemotaxis and receptor internalization were significantly reduced or even absent. By contrast, exocytosis and other interleukin-8-evoked granulocyte activities were normal or even enhanced [47]. Further evidence comes from experiments with HEK 293 cells. When transfected with CXCR-2, these cells exhibit chemotaxis, but when they are also transfected with the dominant negative mutant dynamin I K44A, not only receptor internalization but also chemotaxis are markedly attenuated [41••]. Because the two interleukin-8 receptors differ in their molecular mechanisms of receptor internalization, we might speculate that they also induce different chemotactic behavior.

Such a possibility is supported by the finding that CXCR-1 and CXCR-2 differ in the concentration
dependence of receptor internalization. Because desensitization and internalization of CXCR-2 are faster and occur at lower interleukin-8 concentrations, it has been suggested that this subtype might be functionally dominant at sites distant from the inflammation, whereas CXCR-1 might be more important at the center of an inflammation [48]. In this context it is interesting to note that generation by interleukin-8 of the respiratory burst, which—from a functional point of view—appears most efficient when evoked only at the center of an inflammation, is solely mediated by CXCR-1 [49]. Although both receptors couple to the same G proteins in neutrophils, activation of phospholipase D and the subsequent generation of the respiratory burst by interleukin-8 cannot be evoked by CXCR-2 [49]. CXCR-2 is too rapidly desensitized and internalized to mediate phospholipase D activation and superoxide anion production, which require prolonged receptor activation [50]. The molecular mechanisms of interaction of interleukin-8 with its different receptors are now being studied with the use of mutated forms of interleukin-8 [51]. These peptides will help to clarify the role of the two interleukin-8 receptors for the different processes of interleukin-8-mediated neutrophil responses and will help to identify potential indications for interleukin-8 receptor antagonists, e.g., in certain (auto-)immunologic dysfunctions.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• Of special interest

** Of outstanding interest


Hematopoietic growth factors


