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Review

Modulation of chemokine receptor activity through dimerization and crosstalk

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Abstract. Chemokines are small, secreted proteins that bind to the chemokine receptor subfamily of class A G protein-coupled receptors. Collectively, these receptor-ligand pairs are responsible for diverse physiological responses including immune cell trafficking, development and mitogenic signaling, both in the context of homeostasis and disease. However, chemokines and their receptors are not isolated entities, but instead function in complex networks involving homo- and heterodimer formation as well as crosstalk with other signaling complexes. Here the functional consequences of chemokine receptor activity, from the perspective of both direct physical associations with other receptors and indirect crosstalk with orthogonal signaling pathways, are reviewed. Modulation of chemokine receptor activity through these mechanisms has significant implications in physiological and pathological processes, as well as drug discovery and drug efficacy. The integration of signals downstream of chemokine and other receptors will be key to understanding how cells fine-tune their response to a variety of stimuli, including therapeutics.

Keywords. Chemokine, chemokine receptor, dimerization, crosstalk, synergy, signal transduction.

Introduction

Chemokines are small chemoattractant cytokines best known for their role in directing immune cell migration [1]. Upon secretion, chemokines accumulate in localized areas by binding to cell surface carbohydrate-containing structures and extracellular matrix components, and recruit receptor-bearing leukocytes to sites of inflammation (inflammatory/inducible chemokines) or to secondary lymphoid organs during routine immune surveillance (homeostatic/constitutive chemokines) [2]. Chemokines and their receptors are also involved in many developmental processes, including central nervous system development, cardiogenesis and lymphopoiesis. In addition to their normal physiological roles, aberrant expression and/or regulation of chemokines and their receptors are associated with a many diseases, including inflammatory diseases, atherosclerosis, cancer and HIV [3].

The chemokine network consists of approximately 50 human ligands and 20 receptors. Many chemokines bind multiple receptors and most receptors bind multiple chemokines, suggesting the possibility of functional redundancy. However, emerging evidence indicates that there is specificity in many receptor-ligand interactions not only due to the ability of different ligands to induce different signals from a given receptor [4], termed functional selectivity [5], but also because of spatial and temporal control of their expression [6].

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Despite low sequence homology, chemokines have a highly conserved tertiary structure consisting of a disordered N-terminal region, three anti-parallel β-sheets and a C-terminal α-helix. Chemokines are also known to form dimers and higher order oligomers [7]. Although it has been demonstrated that chemokines bind and signal through their receptors as monomers, at least in the context of cell migration, the ability of chemokines to dimerize is critical for *in vivo* function. This is thought to be due, at least in part, to the role of oligomeric forms of chemokines in binding to cell surface glycosaminoglycans, which in turn facilitates their accumulation in localized areas, allowing them to function as directional cues for migrating cells [8]. In support of this hypothesis, it has been shown that oligomerization-deficient and GAG-binding-deficient chemokine variants result in impaired migration *in vivo* [8].

Chemokine receptors are seven transmembrane G protein-coupled receptors (GPCRs). When a chemokine agonist binds on the extracellular side of its receptor, it induces a conformational change of the receptor that is transmitted to heterotrimeric G proteins bound on the intracellular face. Upon activation of the heterotrimeric G proteins, the Gα subunit exchanges GDP for GTP and dissociates from the receptor and from the βγ subunits, and both G protein complexes go on to activate other downstream signaling events [4]. Classically, chemokine receptors and other GPCRs have been thought to function as monomers and have been studied as isolated systems to identify particular pathways activated by a given ligand/receptor. However, although GPCRs may activate G proteins as monomers [9–11], they are also known to homo- and heterodimerize. Furthermore, they can engage in direct and indirect crosstalk with other signaling receptors. Evidence for cooperative interactions between receptors and signaling pathways includes additivity, amplification and synergy in responses [12–14]. Adding another level of complexity to these signaling networks is the differential expression patterns of chemokines and their receptors that are both tissue specific as well as environmentally regulated. This allows chemokine receptors to partner with receptors and integrate into signaling pathways in a cell-dependent manner [6]. These added complexities create the possibility of unprecedented diversity and multifactorial responses that cast doubt on the concept of redundancy in the chemokine system.

There are many challenges in studying the occurrence and effects of chemokine receptor dimerization and crosstalk, and thus there is a limited understanding of these variations on the classic paradigm of GPCR activation involving one ligand, one receptor and one G protein per activation event. Nevertheless, a growing body of evidence supports the biological importance of dimerization and crosstalk, as discussed in this review.

Chemokine receptor oligomerization

Although members of the GPCR superfamily carry out a variety of functions, many commonalities exist both in the structure and activation mechanisms of these signal-transducing molecules. Accumulating evidence indicates that many GPCRs can form dimers or higher order oligomers, and while it has been shown that the β2-adrenergic receptor and rhodopsin can function as monomers with respect to G protein coupling, it has been suggested that dimers represent the basic functional unit of GPCRs [9–11, 15–19]. However, the functional relevance of GPCR oligomerization on GPCR activity is far from well understood [15–19]. Homo- and heterodimerization can have far-reaching implications with respect to agonist-induced activation and antagonist-induced inhibition mechanisms, G protein coupling and signaling, and internalization and desensitization of GPCRs. Therefore, it is critical to uncover the complexities of these signaling modules considering that GPCRs constitute a major target in pharmaceutical development and intervention [20], and that the efficacy of drugs directed against these receptors may be highly dependent on the interactome state of the receptor.

Currently, the strongest evidence for the functional importance of GPCR dimerization involves GPCR biosynthesis and export from the endoplasmic reticulum (ER) to the cell surface. Although there is some debate on how GPCR dimers and higher order oligomers are formed, it is generally thought to be a non-random process that occurs post synthesis and prior to translocation from the ER to the cell surface [18]. It has been suggested that in some cases, dimer formation stabilizes GPCRs, which in turn enables proper exportation from the biosynthetic machinery to the plasma membrane. One classic example of functionally dependent dimer formation involves the class C γ-aminobutyric acid $(GABA)_B$ receptor. $GABA_B$-R1 requires dimerization with $GABA_B$-R2 in the ER to help deliver it to the cell surface [21, 22]. Although $GABA_B$-R2 can be properly translocated to the plasma membrane independent of $GABA_B$-R1 binding, it is not functional unless paired with $GABA_B$-R1.

Methods to study GPCR oligomerization

One major hurdle in studying GPCR oligomerization is the inherent difficulty of recapitulating native cell

conditions that are physiologically relevant. Although current biochemical and biophysical techniques allow the study of GPCR oligomer formation in cells, it is challenging because of the potential for missing pertinent GPCR interactions, or alternatively, identifying artificial GPCR interactions because of overexpression. Despite these caveats, progress has been made and has evolved our understanding of how GPCRs function. Nevertheless, it is worth briefly reviewing some of the methods before describing examples of chemokine receptor homo- and heterodimerization.

The first evidence for GPCR dimerization and higher order oligomerization came from atomic force microscopy studies revealing the *para*-crystalline organization of rhodopsin in native retinal disks [23]. Since then, many other studies have illustrated the homo- and heterodimerization or higher order oligomerization of several GPCRs [24], including chemokine receptors [17]. Biochemical techniques that have traditionally been used to probe the existence of GPCR dimers include separation by SDS-polyacrylamide gels, cross-linking experiments, and coimmunoprecipitation (CoIP) of tagged-GPCRs followed by Western blot analysis [25]. However, in the past few years there has been a shift towards utilizing fluorescence resonance energy transfer (FRET) techniques for studying these protein-protein interactions [25, 26]. In particular, applications of FRET and bioluminescence resonance energy transfer (BRET) methods possess distinct advantages over more traditional techniques because they can detect both intracellular and cell surface expressing oligomers in living cells. FRET and BRET are based on the non-radiative transfer of energy from a donor molecule to an acceptor molecule that is in close proximity (less than 100 Å) and have overlapping excitation and emission spectra [16]. The distance restraints required for resonance energy transfer make FRET and BRET particularly advantageous for the detection of GPCR interactions in intact living cells [16, 25, 26] even though they are unable to distinguish between dimers and higher order oligomers [18, 19]. For both techniques, tagged receptors are coexpressed in heterologous expression systems, such as HEK293T cells. Such studies have expanded our knowledge concerning the biological relevance of GPCR dimerization and elucidated details of dimerization, biogenesis and cellular localization.

However, it is important to consider that the variable methodology and manipulation of heterologous cell systems to study particular GPCRs can impact the observed results. For example, while application of a membrane impermeable chemical cross-linking agent to cells is one way to stabilize oligomers prior to solubilization, there is also the risk of inducing non-native interactions. Furthermore, when using a heterologous expression system as in BRET or FRET, overexpression is a common occurrence that can sometimes result in unwanted artifacts [15]. In addition to the observation of oligomerization that is caused by artificially high levels of transfected receptor, overexpression can also result in signaling artifacts by facilitating non-native interactions such as coupling to low-affinity G proteins [14]. Adding fluorescent moieties to receptors may also interfere with their function. Therefore, prior knowledge of the technical limitations of specific methods is key for promoting valid conclusions and understanding potential sources of discrepancies between related studies. For more comprehensive reviews of the potential advantages and disadvantages of the various approaches used to study GPCR oligomerization, see [16, 25, 27].

Chemokine receptor homo- and heterodimerization

Of the ~20 chemokine receptors currently known, nearly half have been reported to physically associate with another chemokine receptor, either through homo- or heterodimerization. The chemokine receptor homodimers that have been identified so far include CXCR1, CXCR2, CXCR4, CCR2, CCR5, and Duffy antigen/receptor for chemokine (DARC). In addition to forming homodimers, several chemokine receptors can physically associate with different chemokine receptors to form heterodimers. Table 1 outlines the chemokine receptor homo- and heterodimers identified to date. In some instances high sequence homology between receptors is thought to be a good indicator of the capacity for heterodimer formation. However, heterodimers can also form between chemokine receptors with lower sequence identity, and across the CC and CXC subclasses.

CCR2/CCR5 oligomerization

The homo- and heterodimerization of CCR2 and CCR5 has been well established [28–32], and is not surprising given their close sequence identity. However, the functional consequences of this interaction in terms of ligand involvement and signaling effects have been controversial. On the one hand, it has been reported that ligand stimulation is required for CCR2 and CCR5 dimerization [31–33], while others have demonstrated that dimer formation is ligand independent and formed constitutively prior to ER translocation [28–30]. CCR2/CCR5 interactions in HEK293T cells and peripheral blood mononuclear cells (PBMCs) were initially described by Mellado et al. [31] as ligand dependent, implying that dimer formation was occurring at the cell surface in response

Table 1. Summary of chemokine receptor dimers.

Homodimers	Constitutive or inducible	
CCR2	Constitutive [29, 37], inducible [31, 33]	
CCR5	Constitutive [28, 30, 115], inducible [32]	
CXCR1	Constitutive [39]	
CXCR2	Constitutive [38, 39, 43]	
CXCR4	Constitutive [37, 41–43], inducible [116]	
DARC	Constitutive [52]	
Heterodimers	**Constitutive or inducible**	**Functional effect**
CCR2/CCR5	Constitutive [29], inducible [31]	Transinhibition of ligand binding [29], altered signaling [31]
CXCR1/ CXCR2	Constitutive [39]	None
CXCR4/CCR2	Constitutive [37, 51]	Transinhibition of chemotaxis and calcium response, antagonist transinhibition of ligand binding [51]
CXCR4/ CXCR7	Constitutive [49]	Delayed ERK activation, enhanced calcium response [49]
CXCR4/CCR5	Constitutive [47]	T cell costimulation and alternative signaling [47]
DARC/CCR5	Constitutive [52]	Transinhibition of chemotaxis and calcium response [52]

to ligand stimulation. Specifically, costimulation of cells expressing both CCR2 and CCR5 with CCL2 and CCL5 resulted in CCR2/CCR5 heterodimerization. Interestingly, CCR2/CCR5 heterodimers required lower chemokine concentrations for activity, and the heterodimer complexes signaled through $G_{q/11}$, likely mediating cell adhesion but not chemotaxis. In contrast to the findings by Mellado et al., others have detected constitutive homo- and heterodimerization of CCR2 and CCR5 [28–30]. For example, through a combination of BRET and CoIP studies, El-Asmar et al. [29] found that CCR2 and CCR5 oligomerize, and display an equivalent propensity for homo- and heterodimer formation. Furthermore, their data did not suggest any effect of ligand addition on receptor dimer formation or altered signaling activity; instead they found ligand-induced transinhibition of ligand binding for the other protomer in the CCR2/CCR5 complex. This competitive cross inhibition of the CCR2/CCR5 heterodimer occurred upon addition of either CCR5-specific or CCR2-specific ligands. The discrepancies between these two studies may be a result of the different methodologies used to study these complexes [17]. Mellado et al. used a chemical cross-linking agent following the addition of chemokine, but prior to cell lysis and immunoprecipitation in an effort to stabilize receptor interactions, whereas El-Asmar et al. did not. The addition of the cross-linking agent could potentially have induced non-native associations or addition of chemokine could have enhanced the cross-linking efficiency, explaining the apparent differences in these studies. In summary, it has been clearly demonstrated that CCR2 and CCR5 form constitutive homo- and heterodimers that can be modulated by cross inhibition upon ligand stimulation [29]; however, the role of the ligand in inducing dimerization is not entirely clear. The discrepancies also reinforce the experimental challenges in dissecting the relevant interactions and functional consequences in such systems.

Chemokine receptor oligomerization associated with HIV-1 infection

CCR5 is a particularly interesting chemokine receptor to study considering its role as a principle coreceptor in M-tropic human immunodeficiency virus (HIV-1) [34, 35]. The importance of CCR5 for HIV-1 entry into cells has been clearly demonstrated through a small population of individuals possessing the allelic truncation variant, CCR5Δ32, which is retained in the ER and confers resistance to HIV-1 infection [35, 36]. The mechanism for protection of homozygous individuals is clear, since the receptor never makes it to the cell surface. However, in heterozygous individuals, the CCR5 truncation mutant oligomerizes with intracellular wild-type (WT) CCR5, thereby causing retention of WT CCR5 in the ER, and thus resistance to HIV.

An additional chemokine receptor polymorphism that has also received significant attention involves

the CCR2 Val64Ile mutation (CCR2V64I), which is linked to delayed disease progression [34]. However, the mechanism by which CCR2V64I exhibits protective effects is unknown. It was initially shown that CXCR4 and CCR5 could heterodimerize with CCR2V64I, providing a potential explanation for the effects of the variant [35]; however, Percherancier et al. later found that CXCR4 heterodimerization was indistinguishable with WT CCR2 compared to the mutant [37]. Lee et al. [34] surveyed several possible mechanisms for the CCR2V64I delayed disease correlation, including decreased levels of surface CCR2V64I expression, decreased HIV-1 co-receptor surface expression levels (*i.e.*, CXCR4, CCR5), or decreased co-receptor activity involving viral fusion, all of which were unchanged in comparison to WT CCR2 activity. Instead, calcium flux data indicated that CCR2V64I caused heterologous desensitization of CCR5 and CXCR4 when stimulated by the CCR2 ligand, CCL2. However, WT CCR2 also caused desensitization and thus these effects could not explain the protective effect of the mutant.

CXCR1 and CXCR2 oligomerization

CXCR1 and CXCR2 have been reported to form constitutive oligomers independently and in complex with one another, although some discrepancies exist in the literature [38, 39]. CoIP studies of CXCR2-transfected HEK293 cells by Trettel et al. [38] provided the first evidence for the constitutive formation of functional CXCR2 dimers, although coexpression studies with CXCR2 and CXCR1 revealed no interactions. However, it was later demonstrated that CXCR1 and CXCR2 did form constitutive heterodimers and dimer formation was independent of the addition of their mutual high-affinity ligand, CXCL8 (IL-8) [39]. To identify CXCR1/CXCR2 heterodimer formation in the latter study, a variety of techniques were employed including CoIP, FRET, BRET and ER trapping [39]. This comprehensive analysis demonstrated several key features regarding CXCR1 and CXCR2 interactions. Saturation BRET assays suggested that the propensity for CXCR1 and CXCR2 heterodimerization was comparable to CXCR1 and CXCR2 homodimerization independent of ligand. Also, ER trapping experiments showed that CXCR1/CXCR2 dimer formation occurred intracellularly and prior to receptor export to the cell surface. ER trapping required the attachment of the 14-residue ER retention motif derived from the C-terminal tail of the α_{2C}-adrenoreceptor [40] to the C terminus of CXCR1 (CXCR1-ER). Coexpression of CXCR1-ER with WT CXCR2 resulted in a marked decrease in the surface levels of CXCR2 and CXCR1, clearly demonstrating that CXCR1 homo– and heterodimers contribute to their own GPCR assembly and cell surface delivery in a ligand-independent manner.

CXCR4 oligomerization

CXCR4 is another chemokine receptor that has been shown to form constitutive homodimers [37, 41, 42], and, with some contention as to whether ligand-induced FRET signals were just the result of receptor conformational changes or not, CXCR4 may dimerize in response to ligand [32, 43]. It was demonstrated by FRET analysis that homodimers of CXCR4 exist at the cell surface and intracellularly in HEK293 and HeLa cells. Furthermore, it was shown through FRET photobleaching and cholesterol depletion studies that CXCR4 dimers are present in lipid rafts, which are important signaling microdomains [42]. A synthetic peptide of the CXCR4 transmembrane helix 4 (TM4), proposed to be involved in dimerization based on the rhodopsin receptor dimer, was able to reduce the FRET signal, suggesting disruption of dimer formation. This synthetic peptide also had dramatic inhibitory effects on CXCL12-induced chemotaxis and actin polymerization. However, the TM4 peptide only reduced the FRET signal by ~20–25%, yet it abrogated migration of SupT1, Hut78 and PM1 cells, significantly reduced migration of monocytes, and abolished actin polymerization in HeLa cells. Thus, it is still uncertain what contribution the dimer plays in the context of migration and actin polymerization, or whether the functional results are simply due to altered conformations induced by the peptide [42]. In this regard, Percherancier et al. also reported the presence of constitutive CXCR4 homodimers and examined the functional implications of a different peptide against TM4 of CXCR4, X442. Based on BRET studies, X442 peptide was found to inhibit CXCL12-induced conformational changes of CXCR4 homodimers and abolished CXCL12-induced calcium release, but did not interfere with the constitutive dimer BRET signal. Theses data suggest that CXCL12 induces conformational changes in the CXCR4 homodimer and that disruption of this conformational change inhibits function, at least in the context of calcium mobilization [37].

Another piece of evidence for CXCR4 dimerization involves the "warts, hypogammaglobulinemia, infections and myelokathexis" (WHIM) syndrome. WHIM syndrome is a rare immunodeficiency disease that has been linked in many cases to mutations in the C terminus of CXCR4 that result in truncated variants [44]. The truncated receptors are expressed, and thus likely coexist with WT receptors in heterozygous individuals. The truncated receptors exhibit enhanced signaling activity and fail to desensitize and internalize upon CXCL12 stimulation [45]. Interestingly, it was

demonstrated that cells coexpressing WT and mutant CXCR4 also exhibit enhanced chemotaxis and ERK1/2 signaling responses, and that CXCL12 failed to induce internalization not only of the mutant receptor, but also of WT CXCR4 in these cells [46]. It was later demonstrated by CoIP and BRET studies that the mutant and WT CXCR4 form constitutive heterodimers, suggesting a mechanism whereby mutant CXCR4 can alter the function of the WT receptor in heterozygous WHIM leukocytes by preventing their internalization [45].

CXCR4 heterodimerization

In addition to CXCR4 forming homodimers, there is some evidence for CXCR4 heterodimer formation, which can lead to alternative G protein coupling besides Gi. Contento et al. [47] provide evidence to suggest that CXCR4 and CCR5 recruitment to the immunological synapses (IS) of T cells, and subsequent receptor association, promote chemokine-induced costimulation of T cells. Interestingly, CXCR4/CCR5 heterodimers were shown to couple to G_q and/or G_{11} and generate stimulatory signals that can enhance T cell activation, thus providing a mechanism for modulating T cell behavior [47, 48]. While other reports have not demonstrated CXCR4 and CCR5 interactions [37, 41], this may reflect certain cell type and environmental dependencies of CXCR4 and CCR5 associations.

CXCR4 was originally thought to be the sole receptor of CXCL12. Recently, however, another chemokine receptor, CXCR7, was identified and found to bind CXCL12 with high affinity. CXCR7 has since been shown to also bind CXCL11 (ITAC) with lower affinity, although both ligands fail to stimulate calcium flux or chemotaxis in CXCR7-expressing cells [49, 50]. To evaluate the possible function of CXCR7, FRET analyses were performed with HEK293 cells coexpressing labeled CXCR4 and CXCR7 [49]. Constitutively formed CXCR4/CXCR7 heterodimers were detected in these cells. Ligand-independent dimer formation was further probed by CoIP experiments performed on untransfected HEK293 cells (possessing endogenous CXCR4), stably expressing CXCR7 HEK293 cells and IM-9 cells expressing endogenous CXCR4 and low levels of CXCR7. The HEK293 cells (parental control and stably expressing CXCR7 lines) were then used to identify the functional impact of CXCR4/CXCR7 heterodimerization. Specifically, it was found that CXCL12 stimulation of the coexpressing cells caused enhanced calcium flux in comparison to the cells expressing CXCR4 only. Ligand-induced CXCR4/CXCR7 signaling also demonstrated delayed ERK activation compared to CXCR4 signaling alone [49]. The lack of classical chemokine receptor signaling by CXCR7 suggests a distinct role for this receptor. Although unknown, CXCR7 activity may include signaling through alternative pathways or perhaps modulating CXCL12-induced CXCR4 activity by physical association.

As mentioned previously, dimers can also exist between weakly homologous chemokine receptors [47, 51]. For instance, although CXCR4 and CCR2 share only 34 % sequence identity, they have been shown to form constitutive heterodimers by BRET analysis using HEK293T cells coexpressing both receptors [37, 51]. To determine the effect of CXCR4/CCR2 heterodimerization on binding and signaling, CHO-K1 cell lines expressing CXCR4 and/or CCR2 as well as primary $CD4^+$ T cells were used in functional assays [51]. Overall, competition binding assays on cells coexpressing CXCR4 and CCR2 demonstrated that addition of the CXCR4-specific ligand, CXCL12, decreased CCL2 (MCP-1) binding to CCR2, and *vice versa.* This cross-inhibition mechanism is considered allosteric in nature whereby agonist binding of one receptor in a dimer complex results in a conformational change affecting the agonist binding ability to the other receptor. An additional, although unexpected, consequence of CXCR4/CCR2 heterodimerization in heterologous expression systems was the transinhibition of CCR2 agonist binding upon the addition of a specific CXCR4 small-molecule antagonist, AMD3100. Similarly, a CCR2-specific antagonist, TAK-779, also antagonized CXCL12 binding to CXCR4. In terms of calcium flux, there was no evidence for additive or synergistic effects induced by CXCL12 or CCL2 in CXCR4/CCR2-expressing cells *versus* cells expressing either receptor individually. However, consistent with the competitive binding data, AMD3100 and TAK-779 inhibited CXCR4 and CCR2 agonist-mediated calcium mobilization. Migration assays in human T cells similarly demonstrated cross-inhibition of CXCR4 and CCR2 agonist-induced chemotaxis by TAK-779 and AMD3100, respectively. Inhibitory effects were also demonstrated in $CD4^+$ T cells, expressing native CXCR4 and CCR2, supporting the biological relevance of these interactions [51]. Together, the antagonist-mediated cross-inhibition of the CXCR4/CCR2 dimer complex is an intriguing mechanism for the modulation of chemokine receptor activity because it suggests additional consequences beyond a simple specific antagonist-receptor interaction, which has significant implications for therapeutics.

DARC oligomerization

Recently, a novel mechanism for regulation of chemokine receptor signaling involving dimerization of DARC was reported [52]. DARC is a unique chemo-

kine receptor because it has been shown to bind many chemokines of the CC and CXC family with high affinity [53]. However, DARC lacks the conserved DRY motif common to class A GPCRs, which is considered important for G protein coupling, and indeed, there is no evidence to support ligand-induced signaling by DARC [53]. Instead, DARC is thought to dampen the immune response by scavenging chemokines without signaling [54]. BRET analysis has revealed that DARC forms constitutive homodimers, as well as constitutive DARC/CCR5 heterodimers [52]. Addition of ligand did not significantly affect homo- or hetero-dimerization of DARC, supporting constitutive oligomerization. The functional consequences of DARC/CCR5 heterodimerization included inhibition of calcium flux and cell migration in response to stimulation with the CCR5 ligand, CCL5 (RANTES). However, there was no effect on ligand-induced CCR5 internalization. Furthermore, saturating the DARC receptor by pre-treatment of DARC/CCR5-coexpressing cells with ligands that bind DARC but not CCR5 failed to restore the CCL5/CCR5 calcium flux and migration signaling [52]. These observations suggest that the inhibition of CCL5/CCR5 signaling was related to the heterodimerization as opposed to DARC simply sequestering CCL5 ligand from CCR5. These data provide evidence for a novel mechanism for DARC function, suggesting that its ability to dampen inflammatory responses may extend beyond the role of acting as a sink for excess chemokines to an additional means of inhibiting chemokine-induced signaling through CCR5 and perhaps other chemokine receptors as well. The biological relevance of this dimer complex was also supported by studies performed on primary endothelial cells expressing CCR5 and DARC levels within the physiological range [52].

Summary

GPCR oligomers are thought to constitutively form early on in the biosynthetic pathway. Although it is still too early to tell whether we can generalize these observations to chemokine receptors, current evidence on chemokine receptor oligomerization suggests that this is a strong possibility. The majority of work presented here demonstrates constitutive, ligand-independent dimer formation of chemokine receptors prior to cell surface delivery (see Table 1). As suggested for other GPCRs, dimerization may be important not only for the stability it provides during GPCR synthesis, but also as an additional mechanism for modulating receptor function. Specifically, dimer formation can affect ligand binding as well as G protein coupling and signaling. For example, agonists/antagonists of one receptor can influence the activation of an interacting receptor. Interestingly, current structural models of GPCRs, based on the crystal structure of rhodopsin, indicate that the intracellular surface area of a dimer may provide a better footprint for interacting with the multisubunit G protein [55, 56]. However, further evidence demonstrating the relationship between chemokine receptor oligomerization and its functional consequences is necessary for ultimately determining the extent of the impact of oligomerization on chemokine receptor activity.

Chemokine receptor crosstalk and synergy

The extracellular milieu is comprised of numerous proteins, ions, sugars and metabolites that influence cellular function and the coordination of cells within tissues. These signals are received by the cell and converted to intracellular signals that ultimately determine a cellular response. Given the vast array of extracellular signaling molecules and target receptors, the potential for interactions between different networks, whether it be to amplify, inhibit, or alter a response, is significant. Nevertheless, there is a high degree of selectivity in crosstalk events, not only in terms of which receptors or signals may interact, but also in terms of cell-type specificity in the occurrence and degree of certain crosstalk events. The protein composition of different cell types is not the same; they have varying levels of G proteins, cytosolic tyrosine kinases, and other similar signaling molecules may dramatically affect crosstalk interactions and functional response.

We have already discussed the occurrence of homo- and heterodimerization interactions between different chemokine receptors as well as some of the functional consequences of such interactions. Yet, chemokine receptors not only dimerize with each other, but can heterodimerize with other types of GPCR and non-GPCR receptors [57–59]. Receptor heterodimerization and indirect mechanisms of receptor crosstalk confer another level of complexity in the signaling network and diversify the functional effects of chemokines. Although the occurrence of receptor crosstalk has long been established [60, 61], the importance and consequences of crosstalk for chemokine receptor signaling and function is only now becoming more appreciated both in the context of normal cellular function and in disease.

Receptor crosstalk refers to the ability of a particular receptor to influence the signaling and function of another receptor. Different mechanisms of crosstalk include the following:

– Physical association between receptors (oligomerization)
– Activation of cytosolic tyrosine kinases that transactivate/inhibit signaling of other receptors
– Induction of ligand levels
  · Transcriptional regulation
  · Post-transcriptional (*e.g.*, mRNA stability) regulation
  · Metalloprotease cleavage of tethered ligands
– Localization of receptors in specific membrane microdomains (*e.g.*, lipid rafts)
– Amplification of downstream signaling molecules (*e.g.*, calcium flux)

The panels of Figure 1 summarize different mechanisms by which crosstalk can occur, examples of which will be presented in this section. Chemokine receptor crosstalk can influence the response a cell has to receptor agonists including the amplitude and duration of the signaling, rates of desensitization of receptors, and receptor trafficking [60]. Appreciating the mechanisms and consequences of such crosstalk may help in understanding many nuances in cellular signaling and could prove to be especially important in the context of pharmaceutical development.

Chemokine receptor oligomerization with non-chemokine receptors

Chemokine receptors can physically associate with other chemokine receptors as well as non-chemokine receptor signaling molecules, providing an additional mechanism for modulating chemokine receptor activity. For example, receptor transinhibition and transactivation are two commonly encountered crosstalk mechanisms that can occur from physical association and may alter ligand binding, signaling and internalization of the receptors involved.

Given the involvement of CXCR4 in development, tumor metastasis, and its function as an HIV coreceptor, CXCR4 has been a major focus of many chemokine receptor structure-function studies. In addition to forming homo- and heterodimers with chemokine receptors, CXCR4 is capable of forming dimers with a number of non-chemokine receptors. For example, CXCR4 has been reported to associate with CD26 [62], CD4 [63, 64], the T cell receptor (TCR) [65], the κ-opioid receptor [66] and the δ-opioid receptor (DOR) [67]. In the case of T cells, CXCL12-mediated CXCR4 signaling resulted in prolonged activation of ERK [68, 69]. At the time, the specific mechanism for prolonged ERK activation was unknown; however, it was later shown that CXCL12 triggers heterodimer association between CXCR4 and the TCR on the cell surface of T cells. Kumar et al. [65] identified a novel mechanism for CXCL12-induced activation of CXCR4 on T cells, involving ZAP-70, a tyrosine kinase, as well as the immunoreceptor tyrosine-based activation motif (ITAM) domains present on the TCRs. In T cells, ITAM domains of TCRs are phosphorylated when strong agonists bind, causing subsequent recruitment of ZAP-70 that, upon activation, can trigger downstream signaling cascades. In the case of CXCR4, CXCL12 stimulation is sufficient to cause dimerization, phosphorylation of the ITAM domains and signaling through ZAP-70. Together, these events cause prolonged ERK signaling as well as increased calcium release and enhanced AP-1 transcriptional activity, providing a novel mechanism in CXCR4 signaling.

Recently, CXCR4 was also found to heterodimerize with DOR. In addition to their roles in analgesia and mood, opioid receptors are involved in general immune function. Coexpression of CXCR4 and DOR in MM-1 monocytic cells resulted in inhibition of activation upon simultaneous addition of agonists (CXCL12 and [D-Pen2, D-Pen5]Enkephalin) despite the ligand-binding competency of the coexpressed receptors. Interestingly, the CXCR4/DOR heterodimer was stabilized in its 'inactive state' and was blocked from desensitization. In addition to CXCR4, CCR5 has been shown to interact with the μ-, κ-, and δ-opioid receptors [70–72]. These crosstalk interactions affect ligand-induced receptor signaling by heterologous desensitization of either receptor [70–72]. The ability of chemokine receptors to associate with receptors outside of their own family is a testament to the complicated role that they play in immune trafficking and regulation.

Receptor transinhibition

Transinhibition (or transinactivation) is an important component of crosstalk that can influence receptor signaling and sometimes account for deviations in expected cell responses [73]. Often transinhibitory responses are related to physical association and inhibition of signaling, as demonstrated with the aforementioned inhibition of CCR5 through DARC [52], heterologous desensitization of CCR5 and CXCR4 by CCR2 and CCR2V64I [34] and allosteric transinhibition of CCR2/CXCR4 heterodimers by antagonists [51].

However, indirect mechanisms of transinhibition (independent of physical association) also exist. For example, anti-CD3 antibody stimulation of the TCR in Jurkat cells was found to inhibit CXCL12 (SDF-1)-induced chemotaxis, in part by reducing CXCR4 surface expression. Reciprocally, CXCL12 stimulation resulted in reduced phosphorylation of TCR substrates including ZAP-70, SLP-76, and LAT (linker for activation of T cells). Taken together, these data

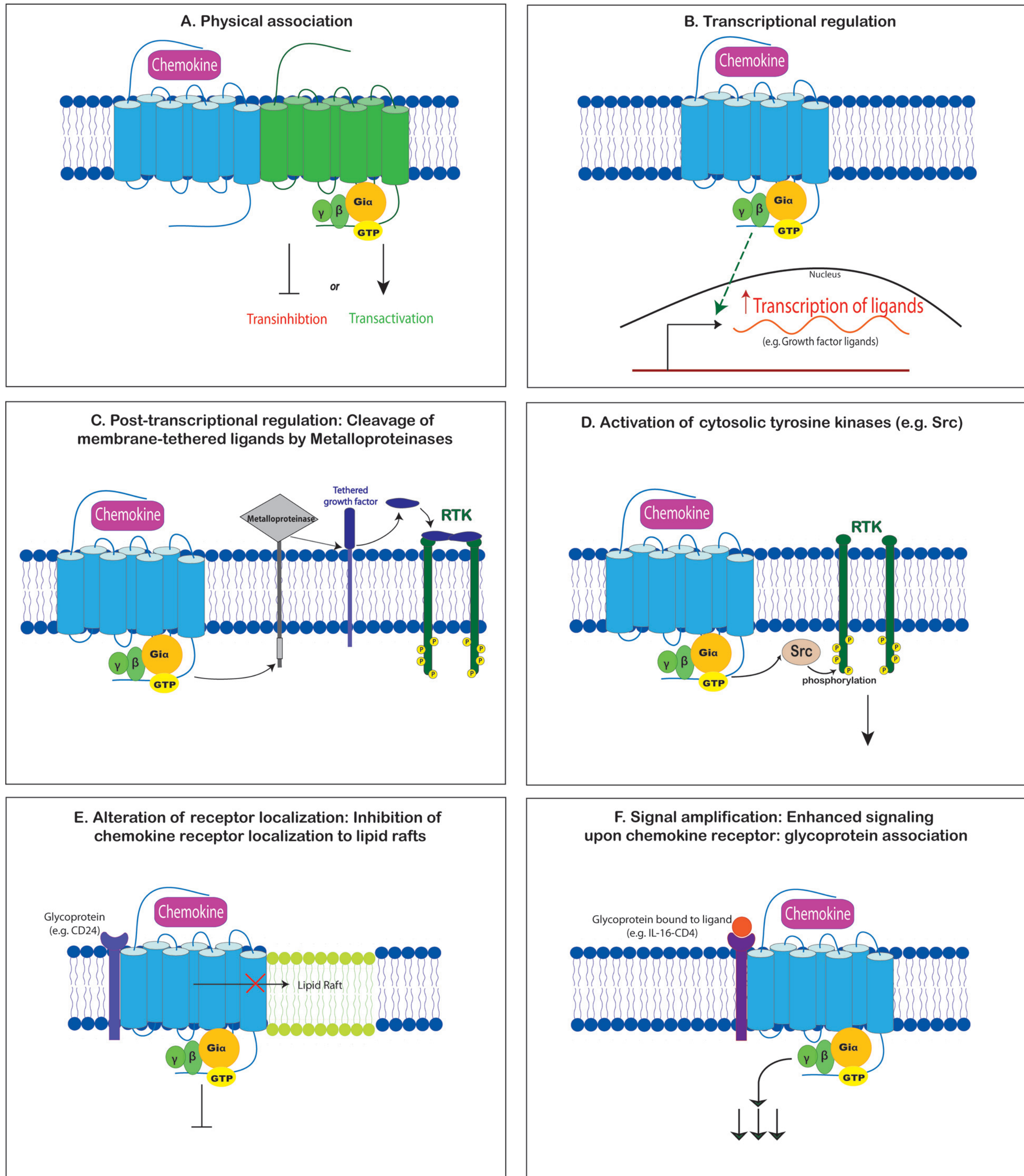

Figure 1. Potential mechanisms of chemokine receptor crosstalk allowing transactivation or transinhibition of involved receptors. (A) Physical association with another receptor (oligomerization). (B) Transcriptional regulation of ligands. (C) Post-transcriptional regulation of ligand levels: metalloproteinase cleavage and release of tethered ligands (depicted), altered rates of degradation or translation/mRNA stability (not depicted). (D) Transactivation of receptors *via* activation of cytosolic tyrosine kinase signaling. (E) Trafficking of receptors to/away from signaling microdomains of the plasma membrane (*e.g.*, lipid rafts). (F) Downstream signaling amplification (synergy).

provide evidence for negative crosstalk and feedback modulation of the activity between CXCR4 and the TCR [74].

While reducing the levels of receptors or ligands are potential means of achieving transinhibition, another mechanism includes disrupting or altering receptor localization in signaling microdomains of the plasma

membrane, such as lipid rafts. For instance, the cell surface glycoprotein, CD24, inhibited CXCL12/CXCR4-induced signaling in pre-B lymphocytes and MDA-MB-231 breast cancer cells by reducing the association of CXCR4 in membrane localized lipid rafts, potentially by altering cholesterol levels ($CD24^{-/-}$ 18H18 pre-B cells had higher levels of cholesterol than the counterpart $CD24^{+/+}$ cells) [75].

Disease states may also influence chemokine receptor signaling in a unique manner. For example, the presence of the BCR/ABL fusion oncoprotein common in leukemia cells, but not in normal lymphocytes, was found to crosstalk and interfere with CXCL12/CXCR4 signaling and function [76]. It has been shown that BCR/ABL can block CXCL12/CXCR4-induced calcium flux, chemotaxis and phosphorylation of p70S6K, PI3 kinase and Lyn [76, 77]. Since CXCL12/CXCR4 signaling is important for the retention of immature immune cells in the marrow, transinhibition of CXCR4 function by BCR/ABL may contribute to the ability of chronic myelogenous leukemia (CML) cells (and potentially other BCR/ABL leukemia cells) to escape from the marrow and accumulate in the blood [76]. Ptasznik et al. demonstrated that phosphorylated BCR/ABL was able to bind to an Src-related kinase, Lyn, and that this association resulted in the constitutive activation of Lyn and PI3 kinase. As a result, there was a complete loss of responsiveness to CXCL12 in terms of migration, as shown by BCR/ABL gene transfer studies in HL-60 cells, and in terms of phosphorylation of Lyn and PI3 kinase in CML cells, which could be rescued by the BCR/ABL inhibitor, STI-571. It was postulated that through the constitutive activation of these pathways, BCR/ABL could tie up signaling molecules needed for CXCR4-induced chemotaxis and calcium mobilization, thereby disrupting the ability to respond to CXCL12 [76]. This provides a unique example of how a crosstalk event can be specifically associated with a disease state and have major functional implications in the context of disease.

Many other examples of transinhibitory signaling by chemokines exist including the ability of CCL2 (MCP-1) to negatively regulate insulin signaling [78], CCL4 (MIP-1β) and CXCL9 (MIG) to reduce IL-4-mediated STAT6 activation [79], CXCL12 to inhibit integrin signaling in a Lyn-dependent manner [80], and the ability of many CXC chemokines lacking the ELR (Glu-Leu-Arg) motif near the N terminus to inhibit vascular endothelial growth factor (VEGF), bovine fibroblast growth factor (bFGF), and ELR^{+} chemokine-induced angiogenic signaling [81, 82]. Often these inhibitory effects of chemokine signaling on other pathways are ligand and/or cell-type specific.

Receptor transactivation

Although signaling pathways activated by a particular ligand-receptor interaction have classically been described and depicted in a linear manner, it is clear that there are often deviations from this type of clear-cut relationship. Transactivation, the ability of one receptor to evoke the signaling response of another receptor, is one type of mechanism responsible for such deviations [83, 84].

In particular, transactivation of receptor tyrosine kinases (RTKs) by GPCR signaling is a well-established phenomenon [14, 61, 84, 85]. RTKs are single-pass transmembrane receptors that contain intracellular tyrosine kinase domains important for their signaling and regulation [86]. RTKs are commonly activated by growth factors, which induce dimerization of the receptors, resulting in trans- and autophosphorylation events that provide docking sites for the recruitment of signaling machinery [86]. Transactivation of both upstream and downstream signaling components of growth factor-induced RTK pathways by active G proteins have been described and often account for the growth and proliferation-inducing properties of many GPCRs [73, 84]. Like thrombin, angiotensin, endothelin, acetylcholine and lysophosphatidic acid (LPA) receptors [85, 87], certain chemokine receptors can also transactivate a variety of RTKs [88–93].

Growth factors, such as epidermal growth factor (EGF), type 1 insulin-like growth factor (IGF-1), and platelet-derived growth factor (PDGF) bind to receptors of the RTK family to elicit signaling pathways involved in cell growth and proliferation [94]. Interestingly, chemokine-induced activation of cell proliferation pathways such as ERK1/2 are sometimes a result of transactivation of growth factor receptors as opposed to a direct result of G protein signaling [73, 95, 96]. In fact, a dependency on epidermal growth factor receptor (EGFR) signaling for chemokine-induced ERK1/2 phosphorylation and mitogenesis has been demonstrated for CXCR4 signaling in an ovarian cancer line and by CCR3 signaling in bronchial epithelial cells, as specific inhibitors of EGFR abolish CXCL12/CXCR4 and CCL11/CCR3 induced ERK1/2 activation and cell proliferation in these cells, respectively [88, 96]. Many different mechanisms for RTK transactivation have been described, including increases in the presence of soluble growth factor ligands and induction of receptor phosphorylation by cytosolic tyrosine kinases such as Src [89, 91, 97].

Chemokine receptor crosstalk is certainly not limited to interactions with receptor tyrosine kinases. A variety of other pathways involving, but not limited to, other GPCRs, neurotrophin receptors, surface glycoproteins, immune cell receptors, and nucleotide

receptors can crosstalk and integrate with chemokine receptor signaling pathways [74, 75, 98–101]. However, since crosstalk events between chemokine receptors and RTKs are the most well established, they are the focus of this section of the review. Some of the signaling interactions between chemokine receptors and other receptors, in the context of synergy, are also discussed.

Modulation of growth factor levels by chemokine receptor signaling

One mechanism whereby chemokine signaling can transactivate RTK growth factor receptors is by transcriptional regulation of the growth factors themselves. Enhanced rates of growth factor transcription and subsequent increases in the levels of secreted protein can have both paracrine and autocrine signaling implications. For example, CXCL12/CXCR4 signaling was shown to induce mRNA and protein levels of VEGF in an Akt-dependent manner in MDA-MB-231 human breast cancer cells [102]. Such mechanisms of crosstalk between CXCR4 and VEGF receptor (VEGFR) to promote angiogenesis has important implications in the realm of cancer research [102, 103]. Post-transcriptional regulation is another mechanism by which chemokine receptor signaling can modulate growth factor levels. Although this can encompass mRNA stability and enhanced rates of translation, a more common mechanism of post-transcriptional modulation of growth factor levels involves cleavage of pro-growth factors by activated metalloproteinases. CCL20 (MIP-3α) was shown to induce cell proliferation of colonic epithelial cells (Caco-2 cells), which has implications for inflammatory bowel disease (IBD) [97]. The dose-dependent increase in cell proliferation, in response to CCL20 signaling through CCR6, was determined to be dependent on the activation of ERK1/2 MAPK downstream of EGFR transactivation. Further investigation of the mechanism by which EGFR was transactivated revealed a metalloproteinase-dependent release of the EGFR ligand, amphiregulin. Amphiregulin is present in a pro-form tethered to the membrane; cleavage by TACE/ADAM-17 metalloproteinase releases amphiregulin to its soluble, active form. Activation of EGFR by amphiregulin was necessary for induction of ERK activation and the mitogenic effects of CCL20. Interestingly, CCL20 induced a transient increase in EGFR phosphorylation compared to sustained phosphorylation observed with EGF stimulation, suggesting potential differences in signal regulation and duration through the transactivation of EGFR [97].

Ligand-independent transactivation of RTKs by cytosolic tyrosine kinases

Although the examples provided in the previous section described mechanisms of RTK transactivation by inducing levels of active, soluble ligand, there is also evidence for transactivation of the RTK receptors in ligand-independent manners. One example involves the transactivation of HER2 by CXCL12/CXCR4 signaling in breast cancer and prostate cancer cells [89, 104]. In both instances, this transactivation event was found to be Src kinase dependent and may have implications on growth, survival, and invasive potential of these cancer cells [89, 104]. However, a potential HER2 ligand has yet to be identified and so HER2 may in general become activated in a ligand-independent manner [104]. Nevertheless, there are other examples of chemokine receptor signaling inducing ligand-independent transactivation of RTKs for which there are known ligands. For one, the transactivation of VEGFR2 by CXCL8 (IL-8), was shown to occur in a VEGF-independent manner [91]. Although a mechanism of VEGFR transactivation by CXCL12/CXCR4-induced VEGF transcription was mentioned earlier in this review, Pretreaca et al. [91] describe a separate instance of Src-dependent phosphorylation and transactivation of VEGFR2 by CXCL8. Effects of CXCL8 on endothelial permeability were determined to be dependent on this crosstalk event.

Activation of chemokine receptors by RTKs

In a reciprocal manner, it has become increasingly evident that a variety of RTKs have the ability to transactivate GPCRs [87]. The ability of growth factor signaling through RTKs to transactivate chemokine receptor signaling appears to have important implications particularly in the context of migration [83]. In a similar manner to the reverse phenomenon, transactivation of chemokine receptors can occur through a variety of mechanisms including transcription and post-transcriptional regulation of chemokine and chemokine receptor levels and through direct physical associations.

The ability of insulin-like growth factor-1 (IGF-1) to induce cell migration of MDA-MB-231 breast cancer cells was shown to depend on transactivation of CXCR4 [105]. The migratory effects of IGF-1 stimulation were partially inhibited by pretreatment with pertussis toxin (a toxin that specifically inhibits signaling through Gi, the predominant G protein activated by chemokine receptors) and by CXCR4 RNAi. These migratory effects of IGF-1 were shown to be subsequent to or independent of IGF-1 receptor (IGF-1R) phosphorylation since it was not affected by CXCR4 RNAi or pertussis toxin. Transactivation of

CXCR4 by IGF-1 occurred independently of its ligand, CXCL12. However, CoIP experiments demonstrated the presence of a constitutive complex of CXCR4, IGF-1R, Giα and Gβ, suggesting a physical interaction mechanism for this transactivation event. Additionally, the combined effect of CXCL12 and IGF-1 stimulation of MDA-MB-231 migration was additive [105].

Interestingly, another breast cancer cell line, MCF-7 cells, did not migrate toward CXCL12, but did migrate toward IGF-1 despite similar CXCR4 expression on the two cell types. It is also interesting to note that, as observed in the MDA-MB-231 cells, the CXCR4/IGF-1R complex also coimmunoprecipitated in the MCF-7 cells. However, there were much lower levels of G protein associated with the CoIP in MCF-7 cells *versus* the MDA-MB-231 cells. Akekawatchai et al. [105] reported that IGF-1-induced migration in the MCF-7 cells was CXCR4 independent and pertussis toxin insensitive, suggesting an alternative mechanism for the migration effects of IGF-1 stimulation in these cells. This emphasizes the point that most crosstalk events are probably cell type dependent and/or involve different chemokine receptors, contributing to the complexity of the signaling network.

Mira et al. [83] also investigated IGF-1-induced migration of MCF-7 cells. Although contradictory results to Akekawatchai et al. were demonstrated in terms of the pertussis toxin sensitivity of the chemotaxis, both groups similarly found the migration to be independent of CXCR4. Mira et al. [83] demonstrated that transactivation of CCR5 was important for the migratory effects of IGF-1 on the MCF-7 cells, as a dominant negative CCR5 ER-retention mutant (KDELccr5delta32) was able to abolish IGF-1-induced chemotaxis. Surprisingly, the stimulation of the MCF-7 cells with CCR5 ligands induced only low levels of migration, while IGF-1 induced significant chemotaxis that was pertussis toxin sensitive. Interestingly, one of the mechanisms by which the IGF-1-mediated migration was achieved was through transcriptional induction of the CCR5 ligand, CCL5 (RANTES). However, the low level of migration to CCL5 alone suggests the involvement of a more complicated crosstalk mechanism than simple transcriptional up-regulation of CCR5 ligands. A biphasic model for the IGF-1-induced CCR5 transactivation and chemotaxis was proposed: a fairly rapid (15-min) induction of CCL5 by message stabilization and increased translation, and a more delayed (120-min) transcriptional up-regulation of CCL5. Although CCR5 can bind multiple ligands, the IGF-1 effect on transactivation of CCR5 appeared to be specifically related to the induction of CCL5 since neutralizing antibodies against CCL5, but not other CCR5 ligands diminished the IGF-1 migration response [83]. A similar mechanism was observed with bFGF-dependent transactivation of CCR2 in bovine aortic endothelial cell chemotaxis [83].

Synergy in chemokine signaling

Sometimes crosstalk signaling not only results in transactivation or additive integration of signaling pathways, but can actually induce synergistic effects on signaling and function [14, 106]. Recent studies have shown that some combinations of chemokines, cytokines and growth factors can produce synergistic effects on pathways, invoking responses much greater than the sum of the components [14, 106]. However, synergy among and between chemokines and other signals is a subject that has received relatively little attention. Yet it is important to consider the potential consequences of crosstalk since cells are exposed to a milieu of factors that act together *in vivo*, and combinations of these factors may not lead to simple linear or additive responses.

A number of accounts of synergy between chemokines and with other types of cytokines have been reported, particularly in the context of calcium signaling, inflammation and the recruitment of immune cells [107–109]. Many examples of synergy between and among these networks have been reviewed in detail by Gouwy et al. [107]. Therefore, in this section of the review, we focus on a few more recent and/or separate reports of crosstalk resulting in synergy.

One interesting example of synergistic crosstalk involves the interaction between the cell surface glycoprotein, CD4, and the chemokine receptor CCR5. Crosstalk between CD4 and CCR5 results in a reduction in CCL4 (MIP-1β) ligand binding affinity to CCR5 by 3.5-fold, yet promotes enhanced signaling in human osteocarcinoma cells (HOS) [99]. Although IL-16/CD4 signaling does not independently induce G protein activation, an increase in G protein signaling was observed upon stimulation with CD4 ligand, IL-16, in the CCR5-CD4 coexpressing HOS cell membranes. This crosstalk was suggested to involve allosteric regulation of binding and signaling of CCR5 by CD4 [99].

There are also a few dramatic examples of synergy in which some chemokine receptors produce little or no effect until the cell is costimulated with another ligand. One example is with CXCL13 (BCA-1)/CXCR5 and CCL19 (MIP-3β)/CCR7 working in concert, but not independently, to render apoptotic resistance to B cell acute lymphocytic leukemia (B-ALL) and B-cell chronic lymphocytic leukemia (B-CLL) [110]. Together these chemokines induce a significant resistance to TNF-α-mediated apoptosis in part through the up-regula-

tion of PEG10, a member of the inhibitor of apoptosis (IAP) family [110].

Synergy has also been demonstrated between CXC chemokines (CXCL8/IL-8 or CXCL12/SDF-1) and CC chemokines (CCL2/MCP-1 or CCL7/eotaxin) in the context of cell migration, where one chemokine could significantly enhance the migration of cells toward suboptimal doses of the second chemokine [106]. These synergistic effects on monocyte chemotaxis and ERK1/2 activation were found to depend on the combination of a CC chemokine with a CXC chemokine, as synergy did not occur with the combination of two CC or two CXC chemokines [106]. The evidence presented suggested that these particular examples of synergy were most likely a result of integration of signaling pathways and not due to receptor dimerization. Along these lines, it is interesting to note that transfection of CCR2 and CXCR4 into CHO cells, which do not express any endogenous chemokine receptors, was insufficient for recapitulating the ERK1/2 synergy, and so the precise mechanism remains incomplete [106]. However, this result again illustrates the dependence on cell background and suggests that the non-hematopoietic cells might be missing essential mediators that allow synergy to occur.

It is important to keep in mind that protein ligands are not the only stimuli that can synergize with chemokine-induced signaling pathways. Uridine triphosphate (UTP) binding to the P2Y2 nucleotide receptor was found to crosstalk and synergize with CXCR2 to induce enhanced levels of calcium signaling [100]. Therefore, more than just the protein composition of the extracellular milieu needs to be considered when evaluating how signals crosstalk and integrate into complex signaling networks.

Summary

Although just a few of the many possible examples have been described here, we have tried to present a representative spectrum of different mechanisms and consequences of crosstalk events related to chemokine receptor signaling. It is clear that homo- and heterodimerization of chemokine receptors and their crosstalk with other proteins has implications in both normal physiology and disease; however, it is unclear how these various crosstalk events are altered in the transition between healthy and disease states and this may be an area of interest for understanding mechanisms of disease and for considering beneficial pharmacological targets.

Conclusions

Here we have summarized a variety of physical as well as indirect interactions between chemokine receptors and other molecules. These interactions appear to have striking implications on the signaling and function of chemokines, emphasizing the importance of signals working in concert and not in isolation. Although there is significant value to investigating the properties of specific ligand-receptor interactions, it is clear that crosstalk with other signaling networks may alter the traditional responses of chemokines and their receptors. Additionally, chemokine receptors may be exploited by other networks, completely independent of chemokine involvement. Nevertheless, these crosstalk interactions are likely selective and may be cell-type and tissue dependent. Moreover, it is possible that disease states may alter the initiation and consequences of crosstalk.

As mentioned earlier, there is significant potential for functional redundancy within the chemokine network due to some overlap in the expression patterns and function of chemokines and their receptors. Yet complete redundancy or functional overlap has not been the case [6]. Instead, it appears that chemokines and their receptors exhibit directional and specific activation in a variety of circumstances. And now, it is becoming clear that crosstalk interactions add another level of specificity for the fine-tuning of cellular responses during homeostasis and disease states.

Although much has been studied in the way of chemokine/receptor binding, it seems there is still much to uncover regarding the complex nature of chemokine receptors, with their ability to interact with other non-chemokine receptors, couple intracellularly to G protein subtypes other than Gi [111–113], and signal through G protein-independent pathways [48, 114]. Considering all of these complexities will be fundamental to the elucidation of the multifaceted functions of the chemokine network. Delineating how chemokines and chemokine receptors integrate with other components of the extracellular milieu could profoundly impact the rationale design of drug therapy for the treatment of particular diseases.

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