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Mechanisms regulating chemokine receptor activity

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Summary

Co-ordinated movement and controlled positioning of leucocytes is key to the development, maintenance and proper functioning of the immune system. Chemokines and their receptors play an essential role in these events by mediating directed cell migration, often referred to as chemotaxis. The chemotactic property of these molecules is also thought to contribute to an array of pathologies where inappropriate recruitment of specific chemokine receptor-expressing leucocytes is observed, including cancer and inflammatory diseases. As a result, chemokine receptors have become major targets for therapeutic intervention, and during the past 15 years much research has been devoted to understanding the regulation of their biological activity. From these studies, processes which govern the availability of functional chemokine receptors at the cell surface have emerged as playing a central role. In this review, we summarize and discuss current knowledge on the molecular mechanisms contributing to the regulation of chemokine receptor surface expression, from gene transcription and protein degradation to post-translational modifications, multimerization, intracellular transport and cross-talk.

Keywords: chemokine receptors; chemokines; regulation; immunity and infection

Chemokine receptor function and regulation

Chemokine receptors belong to the G protein-coupled receptor (GPCR) superfamily and are divided into four classes, named according to the type of chemokine (CC, CXC, CX_3C or XC) with which they interact.[1] Since the cloning of the interleukin-8 (CXCL8) receptor,[2] a total of 10 CC, seven CXC, one CX_3C and one XC receptors have been identified.[1,3] There is apparent redundancy in the system, as many chemokines bind multiple receptors of one class and more than one receptor can interact with each chemokine. However, some groups have found different receptor signalling and trafficking responses to individual chemokines, suggesting that this redundancy may not be as widespread as thought previously.[4,5]

Chemokine receptors have a wide range of biological functions and can be grouped as constitutive or inflammatory receptors depending on whether they play a role predominantly in development and homeostasis, or in host response to inflammation and infection.[6] They control the trafficking and positioning of leucocytes throughout the body by inducing directed cell movement towards the source of chemokine gradients (chemotaxis). In particular, inflammatory chemokine receptors have a significant role in host defence due to their ability to trigger leucocyte mobilization in response to chemokines secreted at sites of injury. Many chemokine receptors have been associated with various pathologies, including human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), cancer and inflammatory diseases. However, with the exception of HIV/AIDS, for which it is established that CXCR4 and CCR5 act as co-receptors for virus entry,[7–10] the molecular mechanisms by which chemokine receptors contribute to diseases are poorly understood. Work has been carried out in developing drugs targeting at least 10 of the known chemokine receptors. Although antagonists for several receptors are in clinical trials,[11–13] the only drug licensed to date is a CCR5 antagonist (Maraviroc) used in HIV therapy.[14] As CCR5 antagonism has failed to show clinical benefit with rheumatoid arthritis, it has been suggested that multiple chemokine receptor blockade may be more effective.[14,15] Consequently, much effort is currently put towards developing promiscuous antagonists to tackle the problem of redundancy/compensation,[12,13] but a greater understanding of the mechanisms regulating chemokine receptor

activity might also be required for the development of more efficient drugs.

The ability of cells to respond to chemokines can be modulated by mechanisms affecting either the chemokine or its receptor. Control can be exerted on the chemokine receptors to modulate the cellular levels of receptor molecules, or the presentation of functionally active receptors at the cell surface. Regulation of protein expression can be targeted at the level of gene regulation, mRNA and protein synthesis. However, these processes are too slow to be solely responsible for the changes required by individual cells to fine-tune their response according to the specific composition of the local environment.[16] Therefore, tight control of the presence of functional chemokine receptors at the cell surface is essential, and can be achieved by affecting the activation state, signalling ability and/or cellular localization of the receptor. This rapid control can be mediated in response to ligand binding but also as a consequence of cross-talk from other receptors.

A considerable amount of our knowledge regarding chemokine receptor biology comes from concepts uncovered for other GPCRs. However, a few chemokine receptors such as CXCR1, CXCR2, CXCR4, CCR2 and CCR5 have received much attention in the last two decades, leading to the discovery that as part of the desensitization process, chemokine-stimulated receptors are removed from the plasma membrane by endocytosis and transported within the cell.[5] Although the trafficking trend appears conserved between chemokine receptors, the mechanisms involved vary and thus cannot be considered generic. Understanding these mechanisms at the molecular and cellular levels could lead to new approaches to target chemokine receptors for disease therapy. In this review we summarize current knowledge about the various molecular mechanisms regulating the presence of functional chemokine receptors at the surface of cells.

Regulation of protein expression

Long-term regulation of chemokine receptors is achieved by controlling the cellular levels of receptor molecules through changes in gene expression, mRNA stability and protein degradation. This can lead to both up- and down-regulation of a specific receptor, as reported for CXCR4.[17,18] With regard to leucocytes, the expression of chemokine receptors is tightly regulated on the different subtypes and changes through the processes of cell differentiation, activation and polarization.[19–24] This regulation is particularly important for inducible chemokine receptors such as CCR2 and CCR5 helping to recruit blood neutrophils, monocytes and activated T cells to sites of infection.[15,25] Host–pathogen interactions can also regulate chemokine receptor expression. For example, it was shown that bacterial lipopolysaccharide (LPS) interfered with CCL2-mediated recruitment of blood neutrophils and monocytes *in vivo* by down-regulating CCR2 expression.[26,27] LPS was found to act *in vitro* by affecting CCR2 mRNA stability,[28,29] as did the inflammatory cytokines interleukin-1 (IL-1), tumour necrosis factor (TNF-α) and interferon-γ (IFN-γ),[29,30] but with no major effect on CCR5 transcripts. In contrast, reactive oxygen intermediates produced by phagocytes for killing pathogens increased CCR2, CCR5 and CXCR4 mRNA expression and opposed the down-regulation induced by LPS.[31] Interestingly, chemokine receptor switch and modulation of mRNA expression has also been reported with *Mycobacterium tuberculosis* antigens and proposed to be part of a normal programme of cell co-ordination needed to contain infection.[32] Enhancing protein degradation independently of, or in combination with, a transcriptional control is also an efficient way to down-regulate chemokine receptor expression, as described for CXCR1 and CXCR2 on activated neutrophils or CCR2 during monocyte differentiation.[20,33] Significantly, changes in the regulation of chemokine receptor expression can contribute to pathological conditions such as Alzheimer's disease, where there is evidence for binding of the amyloid β protein to the receptor for advanced glycation end-products (RAGE) up-regulating CCR5 expression on brain endothelial cells causing T cell infiltration in the brain.[34]

Control of chemokine receptor functional activity

To be functionally active, cell surface chemokine receptors have to be coupled to a heterotrimeric G protein, presented in a conformation compatible with agonist binding, and ready to transmit intracellular signals. Other GPCRs are thought to reside in the plasma membrane in equilibrium between active and inactive states, depending on complex allosteric interactions and conformational changes affected by ligands as well as cell-specific parameters.[35–37] This is still relatively uncharted territory for chemokine receptors but, as will be discussed in detail later, experimental findings suggest that they may be subject to similar regulation. There is evidence for conformational heterogeneity in cell surface CXCR4 and CCR5 receptor populations sometimes related, but not always, to post-translational modifications of the proteins.[38–40] Indeed, sulphation and glycosylation have both been shown to influence ligand binding and signalling by CXCR4 and CCR5.[39,41] The membrane environment is another factor influencing the activation state of CXCR4 and CCR5, which require cholesterol and lipid rafts for chemokine binding and signalling.[42–44] However, if these parameters are important to maintain receptor integrity, whether or not they are accounting for their regulation remains unknown. One feature confirmed to impact on the functional regulation of many chemokine receptors is multimerization.

Table 1. Identified chemokine receptor homomers

Receptor	Formation	Methods	Cells		References
			Overexp.	Endogenous	
CCR2	Constitutive	BRET	HEK-293		133,134
	Inducible	IP	HEK-293	MM-1	48,135
CCR5	Constitutive	IP, Y2H, FLIM, BRET, FRET	HeLa, HEK-293, RBLs		57,76,78,133,136
	Inducible	IP	HEK-293		136–138
			L1.2		
CXCR1	Constitutive	Co-IP	HEK-293		56
		FRET			
		BRET			
CXCR2	Constitutive	IP	HEK-293	Neurons	56,139
		FRET			
		BRET			
		WB			
CXCR4	Constitutive	IP	HEK-293, HEK-tsA201		49,65,134,140
		FRET			
		BRET			
	Inducible	IP	MOLT4		47
DARC	Constitutive	BRET	HEK-293		141

BRET: bioluminescence resonance energy transfer; CO-IP: co-immunoprecipitation; DARC: duffy antigen receptor for chemokines; FLIM: fluorescence lifetime imaging; FRET: fluorescence resonance energy transfer; IP: immunoprecipitation; WB: Western blot; Y2H: yeast-2-hybrid.

Receptor multimerization

It is now accepted that GPCRs not only operate as single entities (monomers), but can also function as multimers regulated by allosteric mechanisms.[45,46] Chemokine receptors have been shown to form homomers as well as heteromers with other chemokine receptors, GPCRs or distinct types of cell surface receptors (Tables 1 and 2). Techniques commonly used to ascertain receptor–receptor interactions and demonstrate the presence of multimers in living cells include co-immunoprecipitation and fluorescence or bioluminescence resonance energy transfer (FRET or BRET; Tables 1 and 2). Note that many of the studies describing chemokine receptor multimers have been carried out on transfected cells where at least one of the interacting partners is over-expressed, and features of endogenous receptor complexes as well as their biological significance *in vivo* remain largely to be explored.

Early work has indicated that chemokine receptor dimerization was ligand-induced, as described for CXCR4 homodimers and CXCR4/CCR5 or CCR2/CCR5 heterodimers.[47–51] However, the current view is that chemokine receptor dimers are constitutively formed (Tables 1 and 2), and ligand binding stabilizes or reorganizes pre-existing complexes.[52–54] CXCR1 and CXCR2 exemplify this: a recent study revealed that CXCL8 binding stabilizes homodimers but alters heterodimers.[55] In fact, dimers are thought to assemble during biosynthesis prior to arriving at the cell surface, as shown for CXCR1/CXCR2 heterodimers[56] or for CCR5 homomers.[57] Other factors, such as the type of molecules complexed with the chemokine receptor or the cellular background, could affect where and how dimers form. For example, CXCR4 and the T cell receptor (TCR) only dimerize at the surface of T cells following CXCL12 stimulation,[50] while CXCR4 interacts with the tetraspannin CD63 in the biosynthetic pathway of B cells.[58–60] For CCR5, there are reports of constitutive intracellular interactions with CD4 in a monocytic cell-line[61] and stable cell surface CCR5/CD4 heteromers complexed with or without CXCR4 on transfected cells or blood-derived dendritic cells.[62–64] Another study described co-localized but independent monomeric CCR5 and CD4 molecules interacting upon binding of HIV-gp120 at the surface of transfected cells.[65–67] Pathogen-induced interaction has also been established for CXCR4 and the Toll-like receptor 2 (TLR-2).[68]

Importantly, multimerization impacts on the cell's biological response to chemokine exposure. Cross-talk within homomers or heteromers enables regulation of chemokine receptors in response to stimuli other than their own ligands. This process, called receptor or ligand-binding co-operativity, is known to occur within all types of GPCR dimers.[69] Positive binding co-operativity has been shown for the constitutive CXCR4/CXCR7 dimer in which CXCR7, a chemokine receptor unable to trigger G protein signalling,[70] enhances CXCR4-mediated signals following CXCL12 stimulation.[71] Positive co-operativity has also been described for the CXCR2/δ-opioid receptor (DOR) heterodimer, but in that case it is antagonism of CXCR2 that enhances the DOR response to ligand.[72] Nevertheless, dimers of chemokine receptors have been shown more often to exhibit negative binding co-oper-

Table 2. Identified chemokine receptor heteromers and their functional outcomes

Receptors	Formation	Methods	Cells		Co-operativity (assays)	References
			Overexp.	Endogenous		
Chemokine receptors						
CXCR1/CXCR2	Constitutive	Co-IP, FRET BRET	HEK-293		No	55,56
CXCR4/CXCR7	Constitutive	Co-IP, FRET	HEK-293	IM-9	Positive (Ca^{2+} flux)	71
CXCR4/CCR2	Constitutive	BRET	CHO-K1 HEK-293		Negative (binding, chemotaxis)	74
CXCR4/CCR5	Constitutive	Co-IP	NIH 3T3		Positive (chemotaxis)	63,130
CXCR4/CCR2/CCR5	Constitutive	BRET	HEK-293		Negative (binding, chemotaxis)	73
CCR2/CCR5	Inducible	Co-IP	HEK-293	PBMCs	Positive (Ca^{2+} flux)	48
	Constitutive	Co-IP, BRET	CHO-K1 HEK-293	$CD4^+$ T cells	Negative (binding)	133
DARC/CCR5	Constitutive	Co-IP, BRET	HEK-293		Negative (chemotaxis, Ca^{2+} flux)	141
GPCRs						
CCR5/C5aR	Constitutive	Co-IP, BRET	RBLs HEK-293		Negative (co-internalization)	76
CXCR2/DOP	Constitutive	Co-IP, FRET BRET	HEK-293		Positive (G protein activation)	72
CXCR4/DOP	Constitutive	Co-IP, FRET	HEK-293	MM-1 Monocytes	Negative (chemotaxis, adhesion, Ca^{2+} flux)	142
CCR5/opioid receptors	Constitutive	Co-IP	CHO	CEMx174	Negative (chemotaxis)	132,143
Others						
CXCR2/AMPA GluR1	Constitutive	Co-IP	HEK-293	Neurons	Negative (chemotaxis)	144
CXCR4/CD4	Inducible (HIV)	Co-IP		PBMCs	N.D.	145,146
CXCR4/TCR	Inducible	Co-IP, FRET	Jurkat T	PBMCs T cells	Positive (Ca^{2+} flux)	50
CXCR4/IGF-R1	Constitutive	Co-IP		MCF-7 MDA-MB-231	Positive (chemotaxis)	147
CXCR4/CD63	Inducible	Co-IP	HEK-293		N.D.	60
CCR5/CD4	Constitutive	FRET BRET, Co-IP	HEK-293 CHO K1		N.D.	61,64,148
	Inducible (HIV)	FRET	HEK-293	DCs	N.D.	66

AMPA GluR1: a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate-type glutamate receptor 1; BRET: bioluminescence resonance energy transfer; C5aR: complement component 5a receptor; CO-IP: co-immunoprecipitation; DARC: duffy antigen receptor for chemokines; DCs: dendritic cells; DOP: δ-opioid receptor; FRET: fluorescence resonance energy transfer; IGF-R1: insulin-like growth factor-1 receptor; N.D.: not determined; PBMCs: peripheral blood mononuclear cells.

ativity, where binding of an agonist to one receptor inhibits ligand binding to the other.[53] Antagonist binding to one chemokine receptor has also been shown to cross-inhibit the other chemokine receptor in the pair, both *in vitro* and *in vivo*.[73,74] Although a few publications have shown that binding co-operativity within a dimer can involve co-internalization of receptors,[75,76] it is not considered to be the rule. As for other GPCRs, it is thought that both negative and positive co-operativity are mediated through allosteric changes in receptor conformation following ligand binding.[45,53,77] A 'cigar bundle' model has been proposed recently for chemokine receptors

whereby clusters of dimers are packed at the cell surface, with the potential for allosteric cross-talk between neighbouring dimers to affect more distant receptors in a domino effect.[54]

The physiological relevance of chemokine receptor oligomerization was highlighted initially with CCR5, when a naturally occurring truncation (Δ32) of this receptor leading to retention of wild-type CCR5/CCR5Δ32 heterodimers in the endoplasmic reticulum was found to confer resistance to HIV-1 infection.[78,79] More recently, it was shown using blood cells from CCR5Δ32-expressing individuals that CCR2/CXCR4/CCR5 heteromers accounted for a negative ligand-binding co-operativity, which inhibited leucocyte recruitment *in vitro* and *in vivo*.[73] Overall, multimerization is emerging as an additional level of regulation providing cell and tissue specificity to fine-tune chemokine receptor activity *in vivo*.

Chemokine receptor desensitization

Chemokine receptors are coupled to heterotrimeric G proteins and undergo conformational changes following ligand binding. The G protein dissociates into guanosine triphosphate (GTP)-bound Gα and the Gβ/γ complex, which activate second messengers and stimulate effector proteins leading to intracellular signalling.[80] It has emerged that GPCRs can also elicit G protein-independent signals through interaction with the scaffolding proteins β-arrestins, linking activated receptors to various signalling pathways that act independently of, in synergy with or in opposition to, G protein-mediated signals.[81] However, β-arrestins are best known for their pivotal role in the regulation of GPCR signals via the process of desensitization, a feedback mechanism protecting cells from overstimulation. In this section we consider what is called homologous desensitization only affecting agonist-activated receptors (Fig. 1).[82] Briefly, following agonist binding, signalling receptors become rapidly phophorylated on their cytoplasmic tail, usually by one member of the G protein receptor kinase (GRK) family, which uncouples the G protein from the receptor and prevents further activation. Phosphorylated receptors interact with one of the β-arrestins acting as a scaffold targeting receptors for internalization, leading to a permanent or transient loss of cell surface receptors due to degradation or subsequent recycling of internalized molecules, respectively.[5] The ability of a chemokine receptor to interact with β-arrestins can influence its fate in multiple ways. First, the strength and stability of receptor/β-arrestins interactions seem critical in determining whether or not an agonist-activated chemokine receptor is internalized, as described for CCR7 and CCR2.[83–85] Secondly, the affinity of these interactions can influence the destiny of receptors once internalized. Indeed, GPCRs that rapidly recycle (Class A) preferentially bind β-arrestin 2 with low

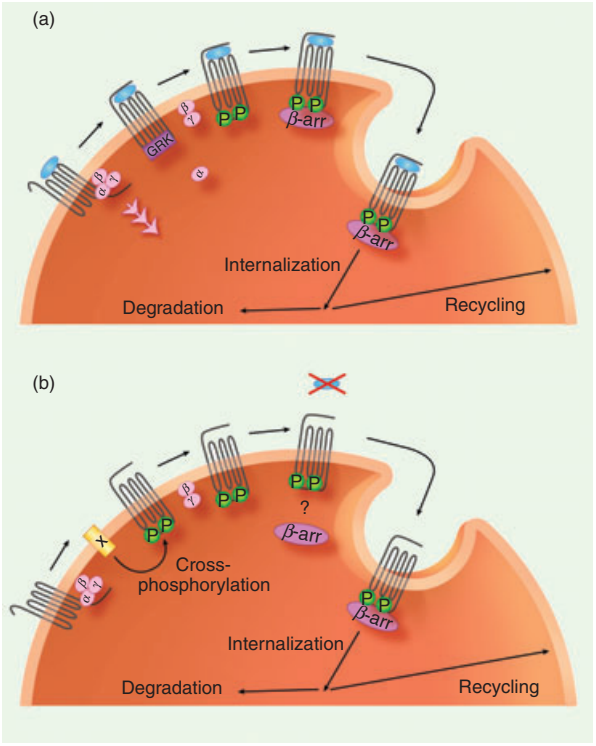

Figure 1. Agonist-dependent (a) and independent (b, heterologous) chemokine receptor desensitization. (a) Following agonist binding and G protein mediated signalling, the chemokine receptor cytoplasmic tail is rapidly phosphorylated, usually by a G protein receptor kinase (GRK); this uncouples the G protein, which dissociates into guanosine triphosphate (GTP)-bound Gα and the Gβγ complex, and enables interaction with a β-arrestin, which acts as a scaffold targeting the receptor for internalization. Once internalized, the receptor follows recycling or degradation pathways. (b) Receptor X mediates cross-phosphorylation of the chemokine receptor, which may involve protein kinase C (PKC), leading to inhibition of chemokine-induced signalling and in some cases internalization of the receptor.

affinity and dissociate from it upon internalization, whereas those that slowly recycle or are degraded (Class B) bind both β-arrestins with high affinity and remain β-arrestin-bound inside the cell.[86] To date, only class B chemokine receptors have been described, with evidence for β-arrestins binding to agonist-treated CXCR4, CCR2 and CCR5 in internal compartments[87–89] (see Fig. 2).

Chemokine receptors can be internalized via clathrin- or caveolin-dependent endocytosis, although other independent pathways have also been reported.[5] Interestingly, CCR2 and CCR5 have been shown to follow both clathrin-dependent and caveolin-mediated pathways and the route of endocytosis could be cell-type dependent.[42,90–93] The intracellular path followed by a chemokine receptor determines the fate of this receptor, i.e. being sent for degradation (down-regulation) or being sequestered intracellularly before returning to the cell surface (resensitization). Receptors can follow one path exclusively, such as

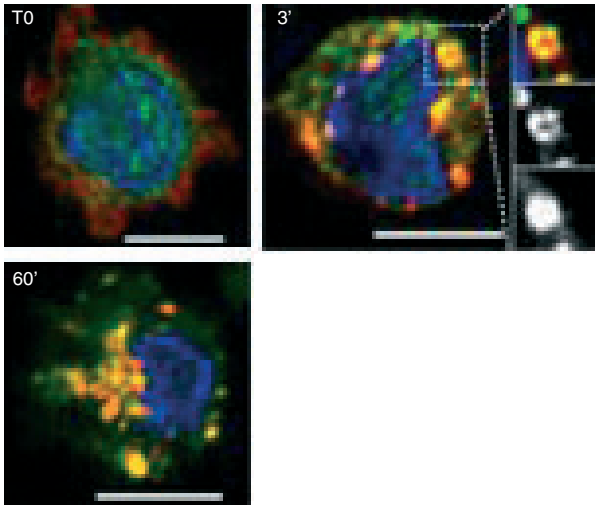

Figure 2. Intracellular transport of β-arrestin-bound CCR5 receptors following CCL5-treatment. Isolated human blood monocytes were treated with 100 nM CCL5 for the indicated time-period. Cells were fixed and permeabilized before labelling for CCR5 (red) and β-arrestins (green), as described previously.[88] Scale bar 5 μm.

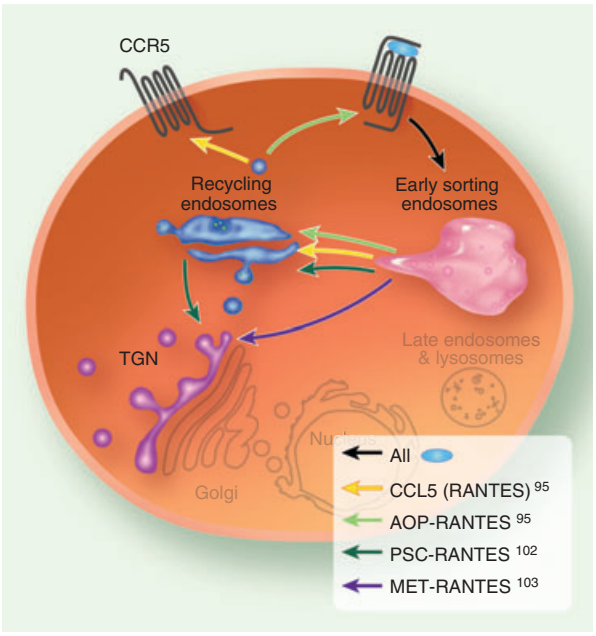

Figure 3. Different trafficking routes proposed for agonist-treated CCR5. Following agonist-stimulation, internalized CCR5 receptors are transported through the early endocytic pathway towards recycling and avoiding degradation. However, there are suggestions that the route followed by CCR5 may be ligand-dependent, as summarized here for the chemokine CCL5 and three of its derivatives.

CCR5 or CXCR3 sent for recycling or degradation, respectively.[94–98] Alternatively, they can enter either pathway depending on the cell-type and duration of ligand treatment, as reported for CXCR2 and CXCR4.[99–101] Note that the agonist itself can impact upon the fate of a receptor. For instance, with CCR5, any agonist-stimulated receptors seem to follow the recycling route but the distribution of receptors along the pathway could be agonist-specific (Fig. 3). Following internalization, CCR5 receptors treated with the natural chemokine CCL5 [regulated upon activation normal T cell expressed and secreted (RANTES)] are located in recycling endosomes (RE) before re-accumulating in the plasma membrane.[95] In contrast, they keep cycling back from the cell surface to the RE after exposure to the chemically modified aminooxypentane (AOP)-RANTES,[95] become trapped in the trans-Golgi network (TGN) after passage through RE with Nα-(n-nonanoyl)-des-Ser1-[l-thioproline2, l-α-cyclohexyl-glycine3] PSC-RANTES,[102] and appear to bypass the RE to accumulate in the TGN with methionine MET-RANTES.[103]

Sorting of internalized chemokine receptors to the recycling or degradative pathways requires complex interactions with the machinery mediating movement of molecules between intracellular compartments. Endocytic adaptors recognize specific determinants in the cytoplasmic domains of the receptors, mainly small sorting motifs and post-translational modifications.[5,104] Two of these determinants, the PDZ ligand motif and ubiquitination, have received much interest recently, and were shown to support recycling or degradation of chemokine receptors, respectively. At least 12 chemokine receptors have been identified as containing potential PDZ ligand motifs in their extreme C-terminal cytoplasmic tail.[5] The PDZ ligand motifs are presumed to interact with PDZ domain containing proteins of the sorting machinery, but only a few of these interactions have been unveiled. CCR5 post-endocytic sorting to the recycling pathway is dependent on its PDZ ligand motif,[94] which has been shown to interact with a protein implicated in receptor recycling called EBP50/NHERF-1.[105] For CXCR2 that can be both recycled following short ligand exposure and degraded following more prolonged ligand treatment,[99] the PDZ ligand motif serves to delay degradation by preventing lysosomal sorting, due probably to interaction with an as yet unknown PDZ-containing protein.[106] Ubiquitination has emerged as an important modification for sending the chemokine receptor CXCR4[107] and other GPCRs[104] to degradation. For CXCR4, CXCL12 stimulation leads to ubiquitination of cell surface receptors as well as ubiquitin-dependent endocytosis and trafficking of ubiquitinated CXCR4 to lysosomes.[108,109] However, ubiquitination does not seem to be required for the degradation of all chemokine receptors.[98,106]

Cross-talk and heterologous regulation

In addition to co-operativity within chemokine receptor multimers, various examples for regulation by indirect

cross-talk with other receptors, without evidence of physical interactions but occurring through interconnectivity of cellular signalling networks, have been described.[53] Note that such regulation can be bidirectional, although here we consider only cases of cross-talk towards chemokine receptors. The cross-talk can be targeted at the receptor itself, the heterotrimeric G protein it is coupled to or downstream signalling components, resulting in trans-inhibition or -activation of chemokine receptor activity.

Trans-inhibition results from a negative pathway of cross-talk leading to desensitization of chemokine receptors or the down-regulation of their expression, as discussed in an earlier section. Here we are considering agonist-independent (heterologous) desensitization involving inactivation and/or down-modulation of cell surface chemokine receptors. As for the other mechanisms of regulation presented in this review, the pathways of heterologous desensitization are undoubtedly receptor- and cell-type dependent. Heterologous desensitization often implies rapid signalling inactivation of surface chemokine receptors, inhibiting chemokine-induced intracellular calcium mobilization. It happens whether the cross-talk comes from another chemokine receptor such as for CXCR1 and CXCR2 with CCR5 in transfected cells,[110] or CXCR4 with CCR5 in human pre-B and -T cells,[111,112] another GPCR as for CXCR1 with the N-formyl peptide (FPR) and C5a receptors,[113] or an unrelated surface receptor such as the TcR with CXCR4 in immortalized cell lines.[114] In many reports, the inactivation has been linked to rapid cross-phosphorylation of the chemokine receptor, with some studies identifying protein kinase C (PKC) as the point of convergence between the different receptor pathways.[110,113,115,116] Alternatively, receptor inactivation can result from indirect effects as reported for CXCR4 either in pre-B cells, where CD24 altered its distribution in membrane lipid rafts by changing cholesterol levels,[117] or in leukaemia cells, where an oncoprotein has been shown to highjack kinases of the CXCR4-dependent calcium pathway.[118] Signalling inactivation can be, but is not always, followed by the down-modulation of cell surface chemokine receptors.[116,119,120] Conversely, heterologous down-modulation can occur without prior desensitization of chemokine-mediated signalling, as we uncovered with the cross-regulation of CC chemokine receptors 1, 2 and 5 by TLR-2 on human blood monocytes.[88] In this instance, we found that activation of TLR-2 triggered relatively slow phosphorylation and removal of cell surface CCR5 molecules by activating the machinery used to support chemokine-dependent endocytosis.[88]

Cross-talk can also lead to trans-activation of chemokine receptors and a potentiation of their functional activity, but few studies have been able to identify the mechanisms involved.[53] Potentiation of calcium signalling has been reported for CXCR2 upon co-stimulation of another GPCR, the PY_2 nucleotide receptor, and suggested ligand-induced synergy between the two receptors.[121] Activation of the neurokinin 1 receptor has also been shown to potentiate the effect of CXCL8 on human neutrophils and was proposed to have a priming effect on CXCR1 and CXCR2.[122] The chemokinetic effect of cytokines is thought to prime cells to increase their migratory response to chemokines, as found with IL-5-enhancing eosinophil chemotaxis in response to CCL11.[123] Furthermore, potentiation and synergy between different chemokine receptors has been involved in the migration of primary cells. For example, CXCL8 has been shown to increase monocyte migration towards CCL2 and CCL7,[124] while CCL2 and CCL7 can stimulate neutrophil chemotaxis towards CXCL8.[125] An intriguing finding came from the study of cross-talk between CCR1 and the high-affinity IgE receptor FcεRI in transfected cells, whereby engagement of FcεRI inhibited CCL3-mediated chemotaxis but engagement of both CCR1 and FcεRI had a synergistic effect on cell degranulation.[126] This would suggest that receptor cross-talk can take place at multiple levels and could have a relatively complex bearing on cell response to chemokine stimulation.

The impact of receptor cross-talk on how immune cells adapt their behaviour to specific situations is undeniable. Combinations of chemokines, cytokines and growth factors act synergistically to amplify inflammatory responses, and this is thought to be due to integration of multiple signalling pathways.[123,124] Cross-talk initiated from non-chemokine receptors is also emerging as an important and complex phenomenon used to enhance or modulate innate immune responses to pathogens. Synergy between CCR2 and FPR agonists has recently been shown to co-operate with TLR-4 for production of the inflammatory chemokine CXCL8 upon LPS stimulation, which in turn synergizes with CCL2 to mediate CXCR1/CXCR2-dependent chemotaxis of human monocytic cells.[127] In addition, heterologous desensitization between TLR-2 and the CC chemokine receptors 1, 2 and 5 or CXCR2 has been shown to take place *in vivo*, affecting the migration and homing of mouse monocytes and neutrophils.[128,129] Furthermore, synergy and cross-talk may have therapeutic implications, as illustrated with some HIV-related studies. Synergy between CXCR4 and CCR5 was recently shown to enhance human monocyte and T cell chemotaxis and to completely block infection by a dual tropic HIV-1 strain,[130] while cross-desensitization of CCR5 by the opioid receptor specifically decreased the susceptibility of peripheral blood mononuclear cells (PBMCs) and macrophages to HIV-1 R5 viruses.[131] However, it remains to be ascertained whether these are pure cross-talk situations or involve receptor multimerization.[63,132]

Conclusion

Advances in our understanding of chemokine receptor biology have highlighted the fact that a controlled regulation of their activity is probably more important than their activation *per se*, certainly in the context of the immune system for both homeostasis and inflammatory responses. It is becoming apparent that individual receptors are subject to different mechanisms of regulation depending upon the type of cells on which they are expressed, the cell differentiation and activation status, as well as the microenvironment. We have learnt that some of the molecular mechanisms involved in the regulation are shared among chemokine receptors while others are purely receptor-specific, with either transient or permanent consequences on cell responsiveness to chemokine stimulation. Overall, we can conclude that the complexity of the regulation process confers specificity to what is an apparently redundant chemokine/chemokine receptor system. Nevertheless, much more research is needed to appreciate the ins and outs of this regulation, evaluate the true relevance of individual mechanisms *in vivo* and establish how the chemokine system integrates with the rest of the immunoregulatory machinery.

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Disclosures

The authors declare having no conflicts of interest.

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