

Chemokine Decoy Receptors: Structure–Function and Biological Properties

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Abstract Chemokines induce cell migration through the activation of a distinct family of structurally related heterotrimeric G protein-coupled receptors (GPCR). Over the last few years, several receptors in this family that recognize chemokines but do not induce cell migration have been identified. These “atypical” chemokine receptors are unable to activate transduction events that lead directly to cell migration, but appear nonetheless to play a nonredundant role in the control of leukocyte recruitment at inflammatory sites and in tumors by shaping the

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chemoattractant gradient, either by removing, transporting, or concentrating their cognate ligands.

Abbreviations

GPCR	G-protein-coupled receptors
CCL	CC chemokine ligand
CXCL	CXC chemokine ligand
CCR	CC chemokine receptor
CXCR	CXC chemokine receptor
DARC	Duffy antigen receptor for chemokines
HCV	Hepatitis C virus
LPS	Lipopolysaccharide
MMP-9	Metallo protease 9

1 The Chemokine Universe

Cell migration is a key element in the ontogenesis of lymphoid tissues in normal and pathological conditions, in the patrolling of body compartments by leukocytes, and in the activation and orientation of innate and adaptive immunity. Different classes of soluble mediators can elicit directional migration of leukocytes. The main mediators of leukocyte trafficking are molecules that interact with rhodopsin-like, seven transmembrane domain, G-protein-coupled receptors (GPCR) and include bacteria-derived formyl-peptides, the complement fragments C5a and C3a, bioactive lipids (sphingosine, leukotrienes, and platelet-activating factor), and chemokines. Chemokines, whose name has in fact been derived by the condensation of *chemotactic cytokines*, are functionally related small secreted proteins structurally characterized by a conserved protein structure called chemokine scaffold, which is strictly dependent on the presence of two conserved disulfide bonds connecting cysteine residues. CC chemokines, which have the first two cysteine residues in adjacent position, and CXC chemokines, which have cysteine residues separated by a single intervening amino acid, account altogether for the large majority of molecules (25 and 15, respectively). The C subfamily, whose members have a single cysteine residue in the amino-terminus, and CX3C chemokines, with three residues separating the cysteine tandem, account only for a small minority of molecules (2 and 1, respectively) (Allen et al. 2007).

The structure-based classification of chemokines is reflected in the classification of chemokine receptors, which display in most cases significant ligand promiscuity among members of a defined subfamily, but are strictly restricted to members of

that given subfamily. Thus, the ten CC chemokine receptors (CCR1 to 10) and the six CXC chemokine receptors (CXCR1 to 6) recognize only CC and CXC chemokines, respectively. Similarly, the only receptors for C and CX3C chemokines (XCR1 and CX3CR1, respectively) are restricted to their respective ligands. All chemokine receptors are GPCR constituted by single polypeptide chain with three extracellular and three intracellular loops, an acidic amino-terminal extracellular domain involved in ligand binding, and a serine/threonine-rich intracellular carboxy-terminal domain. The external interface contributes to the ligand-recognition specificity, while conserved transmembrane sequences, the cytoplasmic loops, and the carboxy-terminal domain are involved in receptor signaling and internalization (Murphy et al. 2000).

The chemokine system is highly promiscuous, presumably to provide flexibility and specificity in leukocyte trafficking, and pleiotropic, with a given chemokine acting on different leukocyte populations to coordinate the recruitment of different but functionally related cells (Mantovani 1999). In general, polymorphonuclear neutrophils (PMN) are major target of ELR^+ -CXC chemokines acting through CXCR1 and CXCR2; monocytes are mainly recruited by CC chemokines acting through CCR1, CCR2, and CCR5; Th1 and natural killer (NK) cells, major players of type 1 inflammation, are mainly responsive to ELR^- -CXC chemokines through CXCR3 and to CX3CL1 acting through CX3CR1; Th2 and eosinophils, associated to type 2 inflammatory responses, are attracted through the action of CCR3 and CCR4 agonists.

It is important to realize that though chemokines' major function is the coordination of leukocyte recruitment in physiologic and pathologic conditions, they also mediate other biological activities, including regulation of cell differentiation and proliferation, survival, and senescence. This is of particular relevance considering that chemokine receptors are expressed in several normal and malignant non-leukocyte cell types (Bonecchi et al. 2009; Charo and Ransohoff 2006).

2 Regulation of the Chemokine System

Chemokine's biological activities are regulated at several levels. At the ligand level, chemokines may be classified according to their production in homeostatic (i.e., produced constitutively) and inflammatory (i.e., produced in response to inflammatory or immunological stimuli) conditions (Mantovani 1999). Chemokines are also target of post-translational modifications, which influence their functional properties, including processing at the amino- and carboxy-terminus by proteases (Proost et al. 2006) and cytrullination (Proost et al. 2008).

The action of different chemokines can also be controlled at the receptor level. Chemokine receptor function has been shown to be context-dependent. Under concomitant exposure to pro- and anti-inflammatory stimuli, such as lipopolysaccharide (LPS) and IL-10, inflammatory chemokine receptors (such as CCR2) can undergo uncoupling from G proteins and maintain the ability to

internalize and degrade the ligand, both *in vitro* and *in vivo* (D'Amico et al. 2000). Under these conditions, chemokine receptors in fact act as “false receptors,” in that they are structurally identical to signaling receptors but behave as decoys. Indeed, chemokine receptors mediate significant ligand sequestration as part of their normal signaling function, as demonstrated by the increase in chemokine levels observed in chemokine receptors knock-out animals (Cardona et al. 2008).

Finally, a nonredundant role in tuning chemokine's biological properties is mediated by “atypical” chemokine receptors (Mantovani et al. 2006), which are considered chemokine decoy receptors being unable to directly mediate cell migration, but tune signaling receptors' activity by clearance, transport, or presentation of the ligand.

3 Chemokine Decoy Receptors

Chemokine decoy receptors recognize distinct and complementary sets of ligands and are strategically expressed in different cellular contexts (Fig. 1). On the basis of the absence of evidence of signaling properties, these receptors were initially called “silent” (Mantovani et al. 2001). More recent evidence has shown that, despite being unable to activate conventional signaling events, they can activate poorly characterized signaling pathways that lead to ligand internalization, degradation, or transport (Mantovani et al. 2006). Detailed structure–function analysis of this

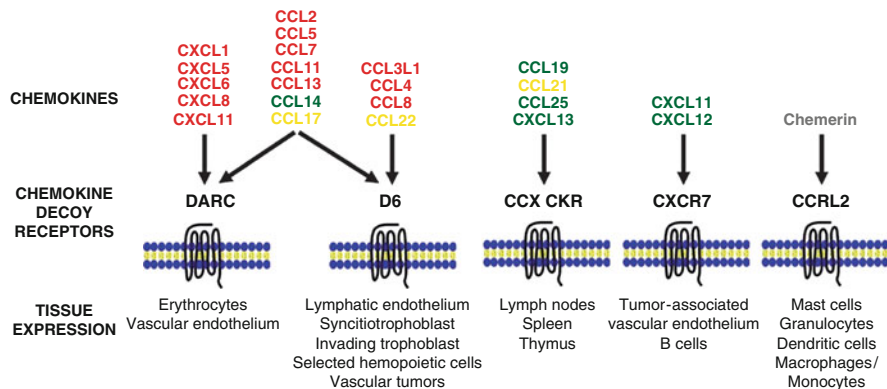


Fig. 1 Chemokine decoy receptors ligand specificity and tissue distribution. D6 and DARC are mainly expressed on the endothelium (lymphatic and vascular, respectively); DARC is also expressed by erythrocytes, and some evidence of D6 expression by leukocytes has also been provided. CCX-CKR is expressed by various tissues. CXCR7 is expressed by lymphocytes and by tumor-associated vascular endothelium. CCRL2 is expressed by myeloid-derived leukocytes. Chemokines are color-coded as pro-inflammatory (red), homeostatic (green), and those with mixed function (yellow)

receptor subfamily is not available, but it is interesting to note that structural determinants supporting $G_{\alpha i}$ activation, a key signaling event in cell migration, are not conserved in these receptors. Other general characteristics that distinguish this class of receptors from other chemokine receptors are unusual expression patterns and broad binding profiles.

The subfamily of chemokine decoy receptors includes D6 (Bonini et al. 1997; Nibbs et al. 1997b), Duffy Antigen Receptor for Chemokines (DARC) (Horuk et al. 1993), CCRL2 (Fan et al. 1998), CCX CKR (Gosling et al. 2000), and CXCR7 (Boldajipour et al. 2008).

3.1 D6

D6 has been the first atypical chemokine receptor functionally identified as a decoy (Fra et al. 2003). Cloned from placenta (Bonini et al. 1997) and hematopoietic stem cells (Nibbs et al. 1997b), it is located in the CCR cluster within the 3p21.3 region of the human genome (Maho et al. 1999). D6 binds most of the inflammatory CC chemokines (agonists of CCR1 through CCR5, see Fig. 1). Nevertheless, D6 has some binding selectivity, in that it does not recognize homeostatic CC chemokines (Nibbs et al. 1997b) and, among inflammatory CC chemokines, it degrades the active forms of CCL22 and CCL14 but not their amino-terminal CD26-processed inactive forms (Bonecchi et al. 2004; Savino et al. 2009). D6 is expressed at high levels by endothelial cells of lymphatic afferent vessels in the skin, gut, and lungs (Nibbs et al. 2001), and in the placenta, where it is present on invading trophoblast cells and on the apical side of syncytiotrophoblast cells (Martinez de la Torre et al. 2007). D6 is also expressed at very low levels by circulating leukocytes (McKimmie and Graham 2006). Following initial results to the contrary (Nibbs et al. 1997a), it is now clear that neither the human nor the murine D6 sustain signaling and functional activities that are typically observed after chemokine receptor triggering by ligand binding, such as calcium fluxes and chemotaxis (Fra et al. 2003; Martinez de la Torre et al. 2005). In all cells tested so far, including the physiologically relevant milieu of lymphatic endothelium (Fra et al. 2003) and trophoblast cells (Martinez de la Torre et al. 2007), D6 does not mediate chemokine transfer through the cell monolayer and instead mediates chemokine degradation. D6^{-/-} mice have been generated, and the data obtained in different animal models are consistent with the role of D6 as a chemokine scavenger *in vivo*. D6^{-/-} mice develop exacerbated inflammatory responses in different experimental diseases sustained by increased levels of inflammatory CC chemokines detected both locally and in draining lymph nodes, which may also result in some conditions in a defective-specific immune response and inflammation-driven tumor promotion (see below). In conclusion, *in vitro* and *in vivo* data strongly support a decoy function for D6, which controls tissue inflammation by acting as a chemokine scavenger on lymphatic vessels.

3.2 DARC

Originally described as the erythrocyte receptor for malaria parasites (Miller et al. 1976), DARC was later identified as the erythrocyte receptor for the chemokine CXCL8 (Horuk et al. 1993). It was subsequently demonstrated that DARC has a promiscuous chemokine-binding profile, interacting with 11 inflammatory chemokines of both the CXC and the CC subfamilies but not with homeostatic chemokines (Gardner et al. 2004), and among CXC chemokines, DARC selectively binds angiogenic ELR⁺ chemokines (CXCL1, CXCL3, CXCL5, CXCL6, and CXCL8) but not angiostatic ELR⁻ chemokines (CXCL9 and CXCL10). The Fy gene that encodes DARC is located outside the CCR and CXCR clusters in the 1q22–q23 region of chromosome 1 (Pruenster and Rot 2006) and has likely evolved independently from other chemokine receptors. Indeed, at the structural level, DARC shows only a minor sequence homology with other chemokine receptors (40% similarity) and lacks structural determinant required for G-protein coupling (Chaudhuri et al. 1993). Consistently with this, after chemokine binding, it does not support ligand-induced signaling or migration (Neote et al. 1994), although cells expressing DARC can internalize the ligand (Peiper et al. 1995).

DARC expression in red blood cells decreases during maturation, being higher on reticulocytes than on older cells in peripheral blood (Liu et al. 2010). DARC function is also necessary for osteoclast differentiation (Edderkaoui et al. 2007). Most individuals of African descent (>95% Africans in malaria endemic regions, 70% of African-Americans) lack expression of DARC by erythrocytes, which was presumably the result of a selective advantage provided by resistance against certain forms of parasite infection (Miller et al. 1976). The lack of DARC in these individuals is the principal genetic determinant for the benign ethnic leukopenia (primarily neutropenia) (Reich et al. 2009), suggesting that DARC function is necessary for a correct hematopoiesis.

Irrespective of ethnicity and expression by erythrocytes, DARC is expressed by endothelial cells (Peiper et al. 1995), suggesting that this molecule has a role in vascular biology. DARC-expressing endothelial cells line postcapillary venules and veins in many organs, including high endothelial venules in lymph nodes. Venular expression of DARC has been reported in diverse normal tissues, such as skin, kidney, lung, brain, thyroid, and spleen, as well as in inflamed tissues, such as the rheumatoid joint synovium, psoriatic skin, various kidney diseases, and lungs with suppurative pneumonia (Hadley and Peiper 1997; Rot 2005).

DARC appears to regulate chemokine bioavailability and, consequently, leukocyte recruitment through two distinct mechanisms: when expressed in endothelial cells, it sustains the abluminal to luminal transcytosis of tissue-derived chemokines and their subsequent presentation to circulating leukocytes (Pruenster et al. 2009); when expressed in erythrocytes, it acts as a sink of circulating chemokines.

3.3 *CCRL2*

CCRL2 is located in the CCR cluster on chromosome 3p21–23 (Samson et al. 1996) and shares over 40% amino-acid identity with CC chemokine receptors. Like other decoy receptors, it lacks a conserved DRY motif (Fan et al. 1998). This receptor is expressed by monocytes, neutrophils, and DCs, and, in all cell types, it is highly upregulated by LPS-driven stimulation (Migeotte et al. 2002). One study has reported functional activities (chemotaxis and calcium fluxes) after CCRL2 engagement by CCL2, CCL5, CCL7, and CCL8, although no evidence for a direct ligand–receptor interaction was provided (Biber et al. 2003). Using CCRL2-transfected cells, we and others have failed in confirming CCRL2 recognition of these or other CC and CXC chemokines (Bonecchi et al. unpublished data). CCRL2 has been recently shown to bind the chemoattractant protein chemerin with high affinity (Zabel et al. 2008), but unlike the other known chemerin receptor, ChemR23 (Wittamer et al. 2003), it neither induce cell migration nor is it internalized after ligand engagement. Using truncated forms of chemerin, the authors demonstrated that CCRL2 binds the N-terminal domain of chemerin, a portion of the molecule that is not involved in binding and activation of ChemR23. Furthermore, CCRL2-expressing cells preloaded with chemerin induced functional responses in cells transfected with ChemR23, indicating that chemerin is still functional after CCRL2 binding. These results have led to the proposal of CCRL2 as a receptor presenting its chemoattractant ligand to functional receptors.

3.4 *CCX-CKR*

The chemokine receptor CCX-CKR is located in the CCR cluster at position 3q22.1. It is widely expressed in several tissues, T cells, and immature dendritic cells (DC). It binds the CC chemokines CCL19 and CCL21, and weakly binds also CXCL13 (Gosling et al. 2000). As other decoy receptors, CCX-CKR presents modifications just after the DRY motif in the second intracellular loop and does not transduce conventional signaling activity after ligand engagement (Mantovani et al. 2001; Townson and Nibbs 2002). Conversely, cells transfected with CCX-CKR degrade CCL19 with very high efficiency (Comerford et al. 2006). Binding their functional receptors, the ligands of CCX-CKR mediate trafficking of naïve T cells, DC, B cells, and follicular helper T cells to and within lymphoid organs, and have a major role in the ontogeny of lymphoid organs and extranodal lymphoid tissues, which characterize chronic autoimmune disorders, such as rheumatoid arthritis and multiple sclerosis (Muller and Lipp 2003). Indeed, although CCL19, CCL21, and CXCL13 are homeostatic chemokines secreted constitutively by DC and monocytes, the production of CCL19 and CCL21 is also augmented by inflammatory signals and CXCL13 expression is induced by the anti-inflammatory cytokine IL-10 (Perrier et al. 2004; Sallusto et al. 1999).

Thus, it is tempting to speculate that CCX-CKR may play a role in the homeostatic and, perhaps more importantly, in the regulated trafficking of lymphocytes and DC in inflammatory and autoimmune conditions in which lymphoid neogenesis occurs. Information from gene-modified mice will be required to put this hypothesis to a test.

3.5 CXCR7

The CXCR7 chemokine receptor, previously known as RDC1, possesses high sequence similarity with other known chemokine receptors, and its gene is located on chromosome 2 in close proximity to the *CXCR1*, *CXCR2*, and *CXCR4* genes (Infantino et al. 2006) in the human genome. CXCR7 binds the chemokines CXCL12 and CXCL11 (Balabanian et al. 2005), and conflicting results on its ability to induce conventional signaling have been published. It was proposed that CXCR7 is functional only when it dimerizes with other chemokine receptor partners such as CXCR4, enhancing (Hartmann et al. 2008; Sierro et al. 2007) or inhibiting CXCL12-induced signaling (Levoye et al. 2009), but recent publications demonstrate that CXCR7 in several cell types is a signaling receptor as evidenced by phosphorylation of MAPKp42/44 (Hartmann et al. 2008) or Akt (Wang et al. 2008). In this respect, it is interesting to note that important functional signatures of signaling chemokine receptor, such as a DRY motif at the boundary of third transmembrane helix and the second intracellular loop, a CxNPxxY sequence in the seventh transmembrane domain, and four conserved cysteine residues in the extracellular segments, are present in CXCR7.

CXCR7 is poorly expressed on normal somatic cells, but it is elevated on transformed cells and during embryonic development in both human and murine tissues (Thelen and Thelen 2008). During development, CXCR7 is expressed on emerging blood vessels in mice, and CXCR7^{-/-} mice are born with ventricular septal defects and semilunar heart valve malformation that lead to perinatal lethality while their hematopoiesis is normal (Sierro et al. 2007). In zebrafish, CXCR7 expression has been detected in somatic cells, and a major function of CXCR7 in this context appears to be to internalize and sequester CXCL12, thus enhancing the dynamics of CXCL12 concentration changes required for proper migration of primordial germ cells (Boldajipour et al. 2008). In the hematopoietic system, CXCR7 has been reported to be expressed on PMN, monocytes, and B cells, while its expression on T lymphocytes is still debated (Balabanian et al. 2005; Hartmann et al. 2008; Infantino et al. 2006; Sierro et al. 2007). Now it is clear that, interfering with CXCL12 activity, CXCR7 plays a nonredundant role in development and in tumors (see below). However, the mechanism of action of CXCR7 needs to be further elucidated to understand if it acts as a CXCL12 scavenger or it interferes with CXCR4 signaling through heterodimerization or competition with signaling molecules (Maksym et al. 2009).

4 Chemokine Decoy Receptors Mechanism of Action

A major role in the biological properties of chemokine decoy receptors relies on their intracellular trafficking properties, which drive continuous chemokine uptake, transport, or concentration.

4.1 Receptor Internalization

Chemokine decoy receptors have been described to internalize through both clathrin-coated pits and caveolae (Fig. 2). The clathrin-mediated pathway has been demonstrated for D6 through a mechanism that is dynamin I-, Rab5- (Bonecchi et al. 2008), and β -arrestin-dependent (Galliera et al. 2004), and for CXCR7 (Borroni unpublished observations). Conversely, overexpression of wild-type caveolin-1 strongly suggests that CCX-CKR endocytosis uses caveolae (Comerford et al. 2006) through a pathway that, while requiring dynamin, does not need β -arrestins- or clathrin-coated pits. DARC is also targeted into caveolae after being internalized into polarized cells (Pruenster et al. 2009).

Like the chemokine receptors CXCR3 and CXCR4 and the viral chemokine receptors US28 and ORF74, D6 and CCX-CKR undergo constitutive ligand-independent

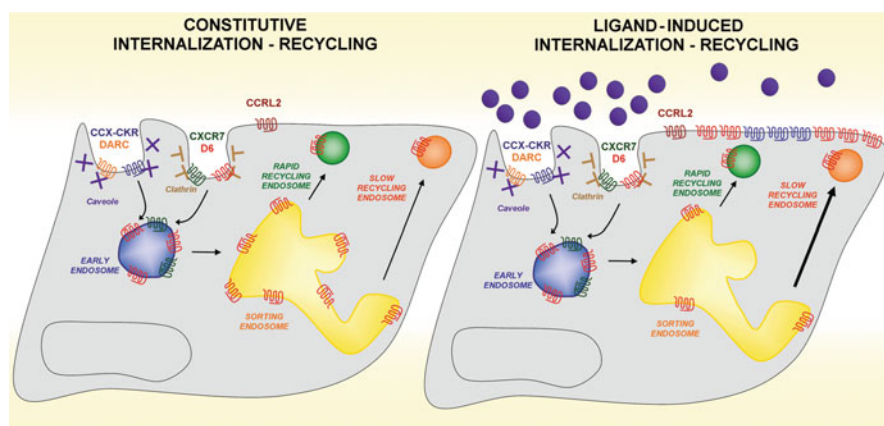


Fig. 2 Chemokine decoy receptors trafficking. D6 and CXCR7 internalize through clathrin-coated pits while DARC and CCX-CKR internalize through caveolae. D6 and CCX-CKR undergo a constitutive internalization followed by recycling in the absence of ligand (*left*). CCRL2 is not internalized even after ligand engagement. After being internalized, receptors may be recycled to the plasma membrane through the rapid (Rab4) and slow (Rab11) recycling endosomes. Detailed information about intracellular pathways are available only for D6 that after being internalized into clathrin-coated pits vesicles are transported to Rab5-positive early endosomes through a dynamin-dependent process. After chemokine engagement, D6 and CCX-CKR upregulate their expression on the cell membrane (*right*)

internalization (Comerford et al. 2006; Galliera et al. 2004). Owing to constitutive internalization, D6 is mainly located in intracellular endosomes and barely detectable on cell surface (Blackburn et al. 2004). Also, CXCR7 has been found predominantly in intracellular compartments colocalized with LAT, a lymphocyte signaling adaptor enriched in the inner leaflet of the plasma membrane and partially colocalized with the early endosomal marker EEA1 (Hartmann et al. 2008). CXCR7 is internalized after CXCL12 or CXCL11 binding in lymphocytes (Balabanian et al. 2005). Conversely, CCRL2 is predominantly expressed on cell surface and a minor pool is present within the cytoplasm. This receptor is not internalized either in the absence or in the presence of the ligand (Zabel et al. 2008).

4.2 Recycling

D6 is constitutively associated with both early (Rab4/5) and recycling endosomes (Rab11) (Bonecchi et al. 2008) but not with lysosomes (Weber et al. 2004). Differently from signaling chemokine receptors, after chemokine engagement, D6 does not decrease its membrane expression but optimizes its degradative activity by increasing its expression on cell surface through a Rab11-dependent mechanism (Bonecchi et al. 2008). Once internalized, the chemokine dissociates from the receptor, and it is targeted to degradation while the receptor is recycled back to the plasma membrane through both rapid and slow recycling pathways, with mechanisms that are strictly dependent on cytoskeleton dynamics (Borroni unpublished observation). A similar ligand-dependent upregulation has been described for CCX-CKR (Comerford et al. 2006), though in this case the redistribution mechanism was not elucidated. Conversely, CXCR7, which is mainly detected in early endosomes (Hartmann et al. 2008), decreased its membrane expression after chemokine stimulation. Thus, it is tempting to speculate that some signal events activated upon ligand engagement might be a prominent sign for receptor cycling events rather than the sign of the typical activation of a chemokine receptor.

4.3 Structural Motifs and Trafficking Adapters

Conventional chemokine receptors use ligand-driven signals to direct occupied receptors to the endocytic machinery through the interaction with β -arrestins, a response accompanied by reduced surface receptor levels and desensitization of remaining surface receptors to further stimulation (Shenoy and Lefkowitz 2003). As mentioned, chemokine decoy receptors have dispensed with signaling due to the presence of altered structural determinants. Nevertheless, association with β -arrestins seems to play a major role in receptor internalization and recycling (Galliera et al. 2004). CXCR7 interacts with β -arrestin in basal conditions, and CXCL11 or

CXCL12 engagement significantly enhanced this interaction that is maintained on endosomes and other intracellular vesicular compartments. CXCR7 interaction with β -arrestin 2 is necessary for CXCL12 uptake from the extracellular space (Luker et al. 2009). The association of D6 with β -arrestin in the absence of ligand is still debated. Galliera et al. proposed that, as for CXCR7, D6 retains the ability to associate β -arrestin 1 and 2 in basal condition (Galliera et al. 2004), and this interaction is required for its constitutive internalization. Conversely, McCulloch et al. demonstrated that relocalization of β -arrestins is not required for D6 internalization but the receptor uses both β -arrestins (McCulloch et al. 2008). Despite the differences, both groups agree that D6 has the potential to constitutively drive the relocalization of β -arrestins within the cytoplasm through a mechanism that is still unknown.

5 Chemokine Decoy Receptors in Action

5.1 Role in Inflammation

Despite the fact that chemokine decoy receptors are structurally unable to support cell migration, it is now well established that they play a nonredundant role in inflammatory responses. They actively participate in the formation of chemotactic gradients removing, transcytating or concentrating chemokines, controlling leukocyte extravasation from the blood vessels to the inflamed tissue, and leukocyte traffic to lymph nodes (Fig. 3). Attenuation of the severity of inflammation by means of chemokine scavenging was demonstrated *in vivo* for D6 by the use of various animal models in several organs. Compared to wild-type mice, D6^{-/-} mice developed exaggerated inflammation, characterized by aberrant leukocyte infiltration and neovascularization due to increased levels of inflammatory CC chemokines, in the skin after phorbol ester application (Jamieson et al. 2005) or complete Freund's adjuvant subcutaneous injection (Martinez de la Torre et al. 2005). In placenta, D6 expressed by syncytiotrophoblast cells reduced inflammation-induced fetal loss in mice (Martinez de la Torre et al. 2007), and loss in D6 immunoreactivity was observed in arresting vs. viable littermate attachment sites in porcine uterus (Wessels et al. 2007). D6 controls inflammation also in the liver, as demonstrated by murine model of acute injury by toxic agents (Berres et al. 2009). In agreement with this murine experimental system, a significant correlation was found between two single nucleotide polymorphisms and liver inflammation in a cohort of HCV-infected patients, even if the functional relevance of these D6 variants was not investigated (Wiederholt et al. 2008). Concerning colon inflammation, conflicting results were published. Bordon et al. using the dextran sodium sulfate-induced model of colitis have found that D6^{-/-} mice are protected. Unexpectedly, they found that this protection is not due to differences in chemokine levels but due to enhanced production of IL-17A secreted by gamma delta T cells in the lamina

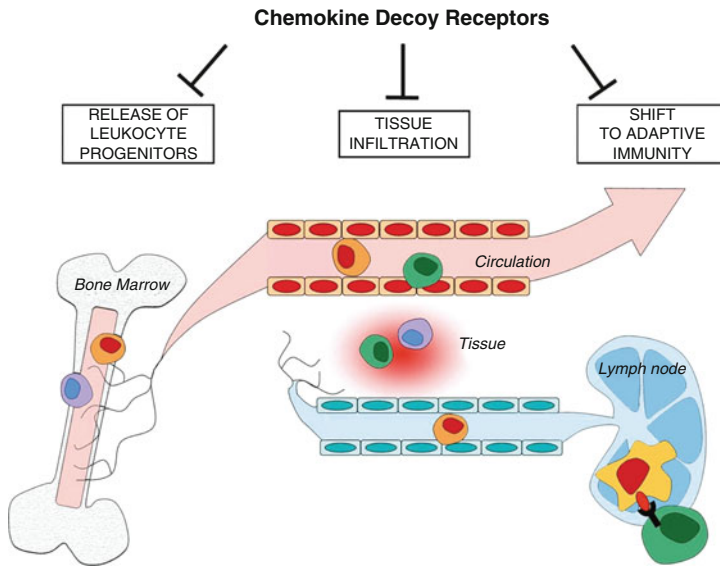


Fig. 3 Chemokine decoy receptors functions in peripheral tissues. Chemokine decoy receptors expressed on blood and lymphatic vessels cooperate in a coordinated action for the control of local inflammatory reactions and adaptive immunity negatively acting on bone marrow leukocyte recruitment, leukocyte extravasation, and trafficking to lymph nodes

propria of $D6^{-/-}$ compared to wild-type mice (Bordon et al. 2009). On the contrary, using the same experimental system, Vetrano et al. found that $D6^{-/-}$ mice display higher levels of several pro-inflammatory chemokines compared to wild-type mice, resulting in increased inflammation. By the use of bone marrow cells' adoptive transfer, they have demonstrated that the protective effect is exerted by D6 expressed by stromal/lymphatic cells (Vetrano et al. 2010). In spite of drawing opposite conclusions from the murine model, both groups found that D6 is expressed in the resting colon predominantly by stromal cells and it is up-regulated in colitic mice and in human colon samples of inflammatory bowel disease patients. D6 was also found abundantly expressed by lymphatic endothelial cells in the lung (Nibbs et al. 2001), and in an allergen-induced airway disease model, $D6^{-/-}$ mice showed increased inflammation compared to wild-type mice (Whitehead et al. 2007). Similarly, $D6^{-/-}$ mice challenged with intranasal administration of low doses of *Mycobacterium tuberculosis* rapidly die because of a strong local and systemic inflammatory response that give rise to liver and kidney damage (Di Liberto et al. 2008). Interestingly, in certain conditions, the uncontrolled local inflammation observed in $D6^{-/-}$ mice has been shown to impair the development of an appropriate specific immune response. In an encephalomyelitis model based on subcutaneous immunization with the myelin oligodendrocyte glycoprotein-derived peptide 35–55 in complete Freund's adjuvant, the absence of D6 led to an increased tissue inflammation, with local "trapping" of $CD11c^{+}$ dendritic-like

cells causing a blunted adaptive immune response (T-cell proliferation and IFN- γ production) and protection from disease development. However, D6 $^{-/-}$ mice showed increased susceptibility to disease when the impairment in adaptive immune response was by-passed by effector lymphocytes' adoptive transfer (Liu et al. 2006).

While the role of D6 as a chemokine scavenger and negative regulator of inflammation is well assessed, the role of DARC in inflammation is still a matter of debate, possibly because this receptor may exert different functions in different cellular contexts. It is well assessed that DARC expressed on erythrocytes modulates chemokine bioavailability by acting as a chemokine scavenger (Darbonne et al. 1991) and as a long-term reservoir of chemokines that prevents their loss from blood into distant organs and tissues (Schnabel et al. 2010). In agreement with this, in a murine model of lung inflammation, DARC expressed by erythrocytes limits lung injury, controlling the distribution and presentation of chemokines that bind CXCR2 (Reutershan et al. 2009), and chemokines disappear from the circulation more rapidly when injected into DARC $^{-/-}$ mice as compared to wild-type animals (Darbonne et al. 1991; Hadley and Peiper 1997). Furthermore, using a systemic endotoxemia model followed by erythrocytes transfusion, it was demonstrated that expression of DARC by red cells reduce lung inflammation (Mangalmurti et al. 2009). Conversely, DARC over-expression on endothelial cells in a transgenic mouse model resulted in increased leukocyte extravasation *in vivo* (Horton et al. 2007), and DARC expression on cell monolayer results in enhanced chemokine-induced leukocyte transmigration *in vitro*. However, the proinflammatory role of DARC as a chemokine transporter is still lacking strong genetic evidence, and the use of DARC $^{-/-}$ mice lacking expression on both erythrocytes and endothelial cells has given rise to conflicting results. LPS treatment resulted in increased neutrophil infiltrate in DARC $^{-/-}$ mice (Dawson et al. 2000), while a different group reported opposite results in a similar experimental setting (Luo et al. 2000). In models of acute renal failure, DARC $^{-/-}$ mice have better renal function than the wild-type littermates due to reduced PMN infiltrate (Zarbock et al. 2007), while in models of prolonged renal inflammation, DARC $^{-/-}$ have increased inflammation at early time points and similar renal injury at later time points (Vielhauer et al. 2009). Finally, Duffy negative individuals (with DARC $^{-}$ erythrocytes and DARC $^{+}$ endothelial cells) have a lower mean white blood cell and PMN count (Reich et al. 2009) that correlated with a survival advantage in HIV-infected persons (Kulkarni et al. 2009), and have delayed graft function and increased graft failure following kidney transplantation (Mange et al. 2004), suggesting that DARC expressed by erythrocytes may be protective for kidney inflammation.

The role of CCRL2 in inflammation has been investigated in an atopic allergy model using CCRL2 $^{-/-}$ mice, which showed reduced inflammation compared to wild-type littermates when sensitized with a low dose of antigen-specific IgE (Zabel et al. 2008). Experiments using mast cell-deficient mice engrafted with either wild-type or CCRL2-null mast cells showed that the defect was due to the lack of CCRL2 expression by mast cells. These data indicate that CCRL2 expressed on mast cells play a proinflammatory role, possibly acting as glycosaminoglycans

(Proudfoot 2006) in the concentration and presentation of ligands to conventional signal-transducing receptors.

5.2 *Role in Tumor Biology*

Chemokines are a key component of cancer-related inflammation and are downstream of genetic events that cause neoplastic transformation and affect tumor progression in multiple pathways (Mantovani et al. 2010). Expression of chemokine decoy receptors in the tumor might have profound consequences on their biology, affecting the amounts of intra-tumoral chemokines. Here the preliminary data available in the tumor biology context for DARC, D6, and CXCR7 are reported.

DARC binds both angiogenic ELR⁺-CXC chemokines, important in tumor angiogenesis, and inflammatory CC chemokines that recruit immune cells that sustain tumor growth. DARC is expressed by several human tumors, including erythroleukemia (Horuk et al. 1994), glioblastoma (Desbaillets et al. 1997), hemangiosarcoma (Tang et al. 1998), and breast carcinoma, in which a negative relationship was found between DARC expression and lymph nodes metastasis, estrogen receptor status, and poor survival (Wang et al. 2006). Experimental tumor models using tumor cell lines over-expressing DARC have shown increased tumor necrosis and decreased angiogenesis and metastasis, associated with decreased CCL2 and MMP-9 levels in the implanted tumors (Addison et al. 2004). Similarly, when melanoma tumor cell lines were injected in transgenic mice over-expressing DARC in endothelial cells, their growth was inhibited (Horton et al. 2007). Explanted tumors displayed enhanced leukocyte infiltration but reduced vascularization, while over-expression of CXCR2 had the reverse effect on tumor angiogenesis and growth. Since prostate cancer has a significantly higher incidence in African-Americans, which in great majority lack DARC erythroid expression (Lentsch 2002), and because clinical data indicate that angiogenic ELR⁺-CXC chemokines contribute to the pathogenesis of prostate cancer, the role of DARC in this tumor has been the object of intense investigation (Waugh et al. 2008). In a mouse model of spontaneous prostate cancer, the absence of DARC did not modify tumor incidence but was correlated with enhanced tumor growth and levels of angiogenic chemokines (Shen et al. 2006), suggesting that DARC clears angiogenic CXC chemokines from the prostate tumor microcirculation. The molecular mechanism by which DARC exerts its protective effect on tumor growth has been suggested to be mediated by a DARC interactor, the KAI1/CD82 tetraspanin protein (Bandyopadhyay et al. 2006). KAI1/CD82 is down-regulated in several tumors, and its down-modulation in epithelial cells is associated with poor prognosis and metastatic progression, revealing that it is a metastasis-suppressor protein (Hemler 2005). Melanoma cell lines transfected with KAI1/CD82 have a reduced ability to metastasize to the lung when injected in wild-type animals but not DARC^{-/-} mice. Interaction with endothelial DARC transmits a senescence signal

to cancer cells expressing KAI1/CD82, whereas cells that lack KAI1 can proliferate, potentially giving rise to metastases (Bandyopadhyay et al. 2006). Collectively, these data indicate that DARC expression on tumor cells or on endothelial cells plays a negative role in tumor progression: in the first case, through the control of angiogenic and inflammatory chemokines, and in the second, transmitting a senescence signal to tumor cells through interaction with KAI1/CD82.

In line with its recognized role of D6 in the control of the inflammatory responses, recent data show that D6^{-/-} mice have increased susceptibility to tumor development in a phorbol ester-induced skin carcinogenesis model (Nibbs et al. 2007) and in the azoxymethane/sodium dodecylsulphate model of colon cancer (Vetrano et al. 2010), suggesting its relevance also in inflammation-induced tumorigenesis. In both models, a significant increase in chemokine levels and inflammatory cell infiltration was demonstrated. Moreover, transgenic D6 expression in keratinocytes confers significant protection from phorbol ester-induced papilloma formation (Nibbs et al. 2007). D6 is also expressed by large granular lymphocyte leukemia cells (Daibata et al. 2004), malignant vascular tumors (Nibbs et al. 2001), Kaposi's sarcoma spindle cells (our unpublished observation), choriocarcinoma cell lines (Martinez de la Torre et al. 2007), and breast cancer cells (Wu et al. 2008). In human breast, cancer D6 expression is inversely correlated with clinical stages and lymph nodes metastasis, but positively with disease-free survival rate in patients (Wu et al. 2008). Over-expression of D6 in breast cancer cell lines resulted in decreased CC chemokine levels, vessel density, tumor-associated macrophage recruitment, and metastasis. In a similar manner, over-expression of D6 in a Kaposi sarcoma cell line resulted in decreased growth when cells were xenografted in nude mice (Savino et al. manuscript in preparation). These observations indicate that D6 expressed by tumor cells or lymphatic vessels of tumor stroma acts as a tumor suppressor gene by negative regulation of chemokine availability.

CXCR7 is frequently expressed by transformed cells and not their normal counterparts (Wang et al. 2008). CXCR7⁺ cells show *in vitro* increase cell survival and adhesiveness, suggesting a constitutive signaling activity of this receptor (Burns et al. 2006), and CXCR7 over-expression in prostate cancer cells resulted in transcriptional modifications in molecules involved in adhesion (cadherin-11 and CD44) and angiogenesis (CXCL8 and VEGF) (Wang et al. 2008). In human specimens, CXCR7 was found in breast tumoral cells, as well as in tumor-associated blood vessels (Miao et al. 2007) and in Kaposi's sarcoma-associated HHV-8-infected endothelial cells (Raggio et al. 2005). CXCR7 was also found expressed by prostate carcinoma, where its expression correlated with tumor aggressiveness (Wang et al. 2008), and in non-small cell lung carcinoma, being more expressed in patients with post-operative metastatic recurrence (Iwakiri et al. 2009). From a molecular point of view, the CXCR7 gene has been found to be rearranged in lipomas (Broberg et al. 2002; Miao et al. 2007) and to be a direct target of Hypermethylated in Cancer 1 (H1C1), a tumor suppressor gene early inactivated during tumorigenesis (Van Rechem et al. 2009). Finally, in experimental models, treatment of tumor-bearing mice with a selective CXCR7 antagonist or RNA interference for CXCR7 resulted in smaller and poorly organized masses without any vascularization (Burns et al. 2006).

Collectively, *in vitro* and *in vivo* data indicate that, unlike DARC and D6, CXCR7 behaves as a tumor-promoting gene, whose expression induces genes involved in cell adhesion and proliferation and enhanced tumorigenesis.

6 Concluding Remarks

Initially described as “silent” receptors by virtue of their inability to directly induce cell migration, chemokine decoy receptors are now emerging as a new family of molecules with heterogeneous structural and biochemical properties fulfilling the common scope to shape chemokine gradients. Mainly expressed by non-hematopoietic cell types, these receptors transport, remove, or concentrate complementary sets of chemokines, allowing the creation, maintenance, and regulation in time of chemokine gradients in the tissue. Thus, they play a complementary function to canonical chemokine receptors, which are required to recognize the gradient and direct leukocyte recruitment. The use of knock out mice has demonstrated that chemokine decoy receptors have a non-redundant function in inflammation regulating leukocyte extravasation from the blood vessels to the inflamed tissue and their traffic to lymph nodes (Fig. 3). A role in leukocyte bone marrow mobilization is very likely because they target chemokines previously reported to have a myelosuppressive and/or mobilizing effect on hematopoietic stem cells. Though detailed structure–function analysis are not available yet, emerging evidences also suggest that chemokine decoy receptors are not “silent,” but they activate G protein-independent signaling pathways, which control their internalization and intracellular trafficking required to fulfill their biological activities.

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