

# Overview and potential unifying themes of the atypical chemokine receptor family

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## ABSTRACT

**Chemokines modulate immune responses through their ability to orchestrate the migration of target cells. Chemokines directly induce cell migration through a distinct set of 7 transmembrane domain G protein-coupled receptors but are also recognized by a small subfamily of atypical chemokine receptors, characterized by their inability to support chemotactic activity. Atypical chemokine receptors are now emerging as crucial regulatory components of chemokine networks in a wide range of physiologic and pathologic contexts. Although a new nomenclature has been approved recently to reflect their functional distinction from their conventional counterparts, a systematic view of this subfamily is still missing. This review discusses their biochemical and immunologic properties to identify potential unifying themes in this emerging family. *J. Leukoc. Biol.* 99: 883–892; 2016.**

## Introduction

In the vertebrate immune system, the chemokine system consists of ~50 ligands and 20 dedicated GPCRs that mainly orchestrate leukocyte trafficking, both in physiologic and pathologic settings [1]. This system is characterized by great complexity, redundancy, and promiscuity. In fact, no chemokine is uniquely active on a given leukocyte population, and usually, a given leukocyte population has receptors for and responds to different molecules. Furthermore, most chemokines interact with >1 receptor, and the majority of receptors interacts with multiple chemokines.

Chemokine receptors can be categorized into 2 principal groups: conventional GPCRs and ACKRs. Conventional chemokine receptors represent the largest and best characterized group of chemokine receptors, including at least 19 members in humans, and their biology and biochemistry has been extensively reviewed recently [2–4]. These receptors are differentially expressed by leukocyte populations and many nonhematopoietic cells, including cancer cells, and this accounts for their role as chemotactic and immunoregulatory molecules. Responses evoked by binding

of chemokines to their respective conventional receptors rely on the balanced activation of the G protein and  $\beta$ -arrestin-dependent signal transduction modules, as the G protein-dependent signaling pathways that promote cell migration are tightly integrated with  $\beta$ -arrestins, which on one hand functionally uncouple G proteins and on the other, function as signalosome adaptor/scaffolding proteins, activating a plethora of intracellular signaling pathways involved in the control of different cellular functions [4–7]. Although all members share functional properties, membership is governed exclusively by structural criteria of chemokine bounded. In fact, these receptors are subdivided into 4 subgroups (CCR, CXCR, XCR, CX3CR), defined by which the chemokine subfamily they recognize (CCL, CXCL, XCL, CX3CL) [8]. In addition, these receptors can be classified further as shared or specific, according to the number of the chemokines they recognize, and as inflammatory or dual function, according to whether the chemokines they bind are mainly involved in emergency trafficking of leukocytes to sites of infection in response to an inflammatory challenge or also in immune system development and physiologic migration of leukocytes and their precursors under steady-state conditions [4, 8].

ACKRs constitute a smaller group of chemokine receptors, including, at present, 4 members [2, 9, 10]. Previously called decoys, interceptors, scavengers, or chemokine-binding proteins, the acknowledged term is now “atypical” as a consequence of their ability to use alternative signaling pathways compared with those observed for conventional receptors. Evidence indicates that apart from ACKR3 in selected conditions [11–15], usually ACKRs do not promote migration but rather, shape chemokine gradients by sequestering chemokines from the microenvironment [9], thus emerging as crucial regulatory components of chemokine networks in a wide range of developmental, physiologic, and pathologic contexts [16]. A new systematic nomenclature has been approved to reflect this functional distinction and at present, includes ACKR1 (Duffy antigen/chemokine receptor), ACKR2 (D6), ACKR3 (CXCR7), and ACKR4 (CCX-CKR) [2, 3]. Two other candidate ACKRs—ackr5 (CCRL2) and ackr6 (PITPNM3)—are awaiting functional confirmation [2, 3].

Abbreviations:  $-/-$  = deficient, 7TM = 7 transmembrane domain, ACKR = atypical chemokine receptor, AM = adrenomedullin, BAM22 = bovine adrenal medulla 22, GPCR = G protein-coupled receptor, MIF = macrophage migration-inhibitory factor

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In the last decade, a significant amount of data has been reported on expression and biochemical properties of ACKRs, opening an intriguing scenario about their functions in development and in inflammatory, infectious, and tumoral diseases [16]. ACKRs have been described to recognize distinct and complementary sets of chemokines and to be strategically expressed in different cellular contexts, preferentially non-hematopoietic cells rather than leukocytes, and have emerged as unified by their inability to initiate classic G protein-dependent signaling pathways after chemokine binding and by unique intracellular trafficking properties that support continuous chemokine uptake, transport, or presentation or chemokine receptor functionality [9, 16]. Conversely, although ACKRs display structural alterations at the C-terminal end, impacting on G protein and other coupling [17, 18], still, a detailed understanding of ACKR signaling properties is missing. Nevertheless, most of the information about ACKRs is limited to a single receptor and not extended to the other members. Therefore, differently from conventional counterparts, whose characterization, organization, and relationships between members are well established, several efforts are still required to identify potential unifying themes among ACKRs. In this review, we attempt a systematic description of ACKRs aimed at outlying the global organization of this family, identifying aspects deserving further investigations, and finally, drawing up a candidate ACKR profile that summarizes features required to a chemokine receptor to be classified as a member of the family.

## CLASSIFICATION OF ACKRs

### Structural classification

Consistent with their regulatory activity on a highly promiscuous system, all ACKRs have more than 1 ligand [9], and according to the number of ligands recognized, they can be categorized as large (ACKR1, ACKR2) or narrow (ACKR3, ACKR4) spectrum receptors. Furthermore, whereas ACKR2 and ackr5 specifically bind CC chemokines, and ACKR3 is selective for CXC chemokines, ACKR1 and ACKR4 show equal ability to bind chemokines belonging to CC and CXC subfamilies (see Fig. 1), whereas data available indicate no binding for XC and CX3C chemokines by ACKRs investigated [19–22]. Thus, differently from conventional chemokine receptors, ACKR binding specificity does not correlate with structural properties of their ligands (see Functional classification).

### Functional classification

Besides the structural criteria, chemokines may be categorized into several functional subgroups (inflammatory, homeostatic, and dual function), based on chemokine expression patterns [4, 8]. Noteworthy, this classification must be considered merely operational and not mutually exclusive. Similarly to their conventional counterparts, ACKRs can be categorized according to the functional properties of the chemokines they recognize (Fig. 1). ACKR1 and ACKR2 bind almost exclusively inflammatory chemokines [21, 23, 28, 29]. Both receptors also bind the homeostatic chemokine CCL14, but only its truncated form, generated by proteolytic cleavage, occurring under

	ACKR1	ACKR2	ACKR3	ACKR4	Ackr5
CCL1					
CCL2					
CCL3					
CCL3L1					
CCL4					
CCL5					
CCL7					
CCL8					
CCL11					
CCL13					
CCL22					
CCL24					
CCL26					
CCL28					
CXCL1					
CXCL2					
CXCL3					
CXCL5					
CXCL6					
CXCL7					
CXCL8					
CXCL9					
CXCL10					
CXCL11					
CXCL14					
CXCL16					
CXCL17					
CX <sub>3</sub> CL1					
CCL14					
CCL15					
CCL16					
CCL17					
CCL18					
CCL19					
CCL20					
CCL21					
CCL23					
CCL25					
CCL27					
CXCL4					
CXCL12					
CXCL13					
XCL1					
XCL2					
Chemerin					
MIF					
AM					
BAM22					

**Figure 1. Heat map of ACKR ligand binding.** Ligand-binding profiles of murine and/or human chemokines to ACKRs. Chemokines are divided into inflammatory (top) and dual function (both involved in inflammatory and homeostatic conditions; middle). (Bottom) Nonchemokine ligands. Green indicates evidence for binding, red indicates evidence for absence of binding, blue refers to controversial evidence, and white to absence of data. CCL3L1, CCL13, CCL14, CCL16, CCL18, CXCL6, and CXCL8 are present in humans but not in mice. CCL12 is found in mice but not in humans. Human but not murine ACKR2 binds to CCL7 and CCL8 [19, 23, 24], and human but not murine ACKR4 binds to CXCL13 [22, 25]. AM [26]; MIF [13]; BAM22 [27].

inflammatory conditions, is degraded efficiently by ACKR2 [30], whereas no information on relative affinity for full-length and truncated versions is available for ACKR1. The inflammatory being of ACKR1 is supported further by its ability to bind

CXCL4, which is stored in platelet  $\alpha$  granules [31]. The binding properties of ACKR1 and ACKR2 do not overlap with ACKR4, which conversely, preferentially binds homeostatic chemokines, even though CCL21 can also be produced under inflammatory conditions [22, 25]. ACKR3 only binds 2 chemokines: the homeostatic chemokine CXCL12 and the inflammatory chemokine CXCL11 [11, 20, 32]. It also has nonchemokine ligands, including MIF, a pleiotropic cytokine with chemokine-like functions involved in numerous inflammatory conditions [13], and an alternative, noncognate ligand for CXCR4 [33], the intermediate opioid peptide BAM22, and AM, involved in circadian glucocorticoid oscillation and in cardiac and vascular development, respectively [26, 27]. Interestingly, although ACKR3 binds all of these nonchemokine ligands with high affinity, the downstream effects are different, reflecting the importance of the biologic setting on dictating ACKR3 activity. In fact, ACKR3 acts as a rheostat for AM as the binding results in AM scavenging and dampening of ERK1/2 signaling, whereas the MIF/ACKR3 interaction contributed to MIF-triggered B cell migration and ERK1/2 activation, same as BAM22, which enhances adrenocorticotrophic hormone-induced ERK1/2 activation via  $\beta$ -arrestin1/2. Data on ackr5-binding properties are, at present, inconsistent. Conflicting data have been reported on the ability of this receptor, which is strongly induced by proinflammatory stimuli (LPS, IFN- $\gamma$ , TNF- $\alpha$ ) [34–37] and retinoic acid [38], to bind the homeostatic chemokine CCL19 and the inflammatory chemokine CCL5 [35, 39–41]. Moreover, the nonchemokine ackr5 ligand chemerin, which binds the receptor with high affinity, without triggering intracellular calcium mobilization and chemotaxis [42], is detected in healthy subject plasma and is up-regulated in many inflammatory conditions [38]. Taken together, results suggest that ACKR3 and ackr5 are involved in biologic functions outside of the chemokine system, similar to some conventional chemokine receptors [43].

Inflammatory chemokines play a minor role in normal development, but they orchestrate the immune response in several pathologic settings, from host defense to cancer development [44]. Conversely, homeostatic chemokines control stem cell migration during embryogenesis and therefore, are involved in development. The functional classification of ACKRs perfectly reflects the phenotypes exhibited by gene-targeted animals. ACKR1<sup>-/-</sup> and ACKR2<sup>-/-</sup> animals exhibit normal development and show defects only upon challenge with inflammatory stimuli [45, 46]. Ackr5<sup>-/-</sup> mice also develop normally to term, have a normal lifespan and no significant aberrations in lymphoid organs and circulating subsets, and do not show an overt phenotype under steady-state conditions but exert defects in dendritic cell migration to peripheral lymph nodes and mast cells responsible for reduced Th2 responses and cell-mediated contact hypersensitivity, respectively [42, 47], providing a further confirmation of the inflammatory profile of ackr5. Conversely, ACKR4<sup>-/-</sup> alters thymic homeostasis, resulting in the spontaneous development of autoimmune reactions [48]. To date, immune defects have not been described for ACKR3<sup>-/-</sup> animals, but its recently recognized role in normal embryonic development [49–52] explains the reported early lethality caused by severe cardiovascular defects [53, 54]. In conclusion, an

operational classification of these receptors as inflammatory or dual inflammatory/homeostatic can be proposed, with ACKRs, with a preferential role in constitutive leukocyte trafficking and development, typically narrow-spectrum receptors (ACKR3, ACKR4) and ACKRs implicated in the recruitment of leukocytes to inflamed tissues, considerably more promiscuous (large spectrum receptors: ACKR1, ACKR2, and potentially ackr5).

## EXPRESSION PROFILES OF ACKRs

### Nonhematopoietic compartment

According to their intrinsic inability to promote cell migration and their role in shaping the chemokine gradient, ACKRs are mainly expressed on endothelial and epithelial cells at barrier sites (i.e., skin, lung, gut, brain, placenta) rather than on migratory cells as their conventional counterparts [16]. Expression of ACKR3 and ACKR4 on stromal cells has been observed [55–57], and evidence of a complex expression pattern of other ACKRs, often related to the functional role of their ligands and the site in which they exert their activities, is emerging [16].

**Endothelium.** All ACKRs are expressed on endothelial cells. In particular, ACKR1 and ackr5 are specifically expressed on vascular endothelium (ACKR1 [29, 58–64]; ackr5 [36, 38, 65]), whereas ACKR2 and ACKR4 are on the lymphatic one (ACKR2 [66–68]; ACKR4 [69]). ACKR3 expression on vascular endothelium is well established, whereas data on its expression on lymphatic endothelium are controversial [26, 70–72]. The blood brain barrier is a crucial checkpoint for leukocyte entry in the CNS, and several homeostatic (CXCL12, CCL19, CCL20, CCL21) and inflammatory (CCL2, CCL5, CXCL1) chemokines are expressed on the vasculature and astrocytes within the brain parenchyma [73]. Noteworthy, all ACKRs except ACKR4 have also been found expressed at the blood brain barrier (ACKR1 [74]; ACKR2 [75, 76]; ACKR3 [77, 78]; ackr5 [34, 37, 79–81]). Transcript for ACKR4, whose ligands are highly expressed in the CNS, has been detected in murine brain [25], but further investigations are required to confirm its expression at the blood brain barrier.

**Epithelium.** All ACKRs are expressed on epithelial tissues of barrier organs (ACKR1 [60, 82, 83]; ACKR2 [84, 85]; ACKR3 [20, 86]; ACKR4 [48, 55, 56, 87–92]; ackr5 [93]) and on trophoblast cells, the epithelial cells of fetal origin that form the physical placental barrier between the mother and the developing conceptus (ACKR1 [94]; ACKR2 [94–97]; ACKR3 [97–100]; ACKR4 [25, 94]). No data are available on the expression of ackr5 in placenta, although elevated levels of its ligand chemerin have been reported recently [101]. Placental expression of ACKRs is also observed in other animal species, suggesting that they represent a highly specific and conserved regulatory mechanism at this anatomic site [94].

### Hematopoietic compartment

Differently from conventional counterparts, ACKRs are usually poorly expressed on cells of hematopoietic origin. ACKR1, ACKR2, and ackr5 have been reported in some leukocyte subsets—B lymphocytes (ACKR1 [102]; ACKR2 [24, 76, 103–106]; ackr5 [35, 39, 42, 47, 107, 108]) and alveolar

macrophages (ACKR2 [109]), in particular—whereas data on ACKR3 and ACKR4 are currently controversial as a result of different techniques and protocol used and of the absence of specific tools (i.e., species-specific antibodies; ACKR3 [10–12, 54, 110–115]; ACKR4 [22, 25, 56, 116]). ACKR1 is the only ACKR expressed on erythrocytes [117], whereas platelets express constitutively ACKR3 [118, 119] and ACKR2 upon systemic sclerosis [120]. Under specific conditions, ACKRs are coexpressed with conventional counterparts on leukocytes and fine tune the chemokine system, also interfering with the activation of conventional chemokine receptors through direct competition for cognate ligands. The best example is the CXCL12/CXCR4/ACKR3 “eccentric trio” [121], with ACKR3 coexpressed with its conventional counterpart CXCR4 in several leukocytes, including B and T lymphocytes [12, 111]. Coexpression of ACKR1 and CCR5 has been observed on primary endothelial cells [122], whereas ackr5 is coexpressed with its conventional counterpart CCR7 on B cells in a maturation stage-dependent manner and on dendritic cells upon stimulation with proinflammatory stimuli [47, 123]. ACKRs have also been reported to interfere with the signaling pathways of coexpressed conventional chemokine receptors, indicating that their regulatory activity may not be restricted to receptors with shared ligands but also applies to receptors coexpressed on the same cell. Examples are provided by ACKR2 interfering with CXCR5 signaling on B lymphocytes [24] and ACKR4 interfering with CXCR3 signaling on T lymphocytes [124]. Finally, it has to be noted that ACKR expression on different cell contexts may correspond to different functional properties, as exemplified by ACKR1, which acts as a sink on erythrocytes and as a transporter on endothelial cells [62]. In conclusion, each ACKR has a unique expression pattern, but their expression on endothelial and epithelial cells of barrier organs, including the blood brain barrier and placenta, and on some leukocyte subsets is now emerging as a common theme.

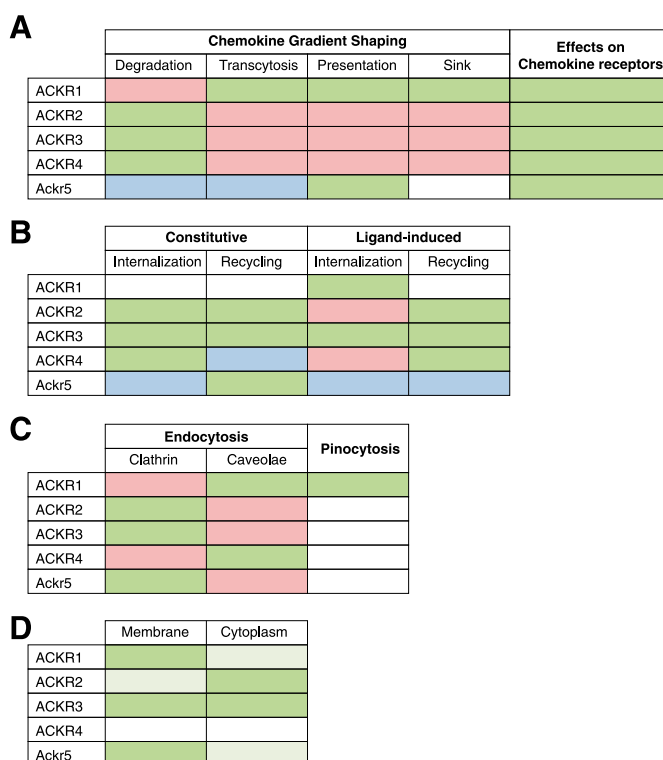
## MECHANISMS OF ACTION OF ACKRs

### Chemokine gradient shaping

Although as mentioned, the ability to shape the chemokine gradient is a common property, and different ACKRs use different mechanisms to fulfill this function, allowing ACKRs to be classified further into 3 “professional” categories: scavengers, transporters, and presenter [2] (**Fig. 2A**). ACKR2, ACKR3, ACKR4 and ackr5 share the ability to scavenge chemokines [41, 98, 125–127]. ACKR1 and ackr5 share the ability to transport or present (ACKR1 [58, 62, 74, 128, 129]; ackr5 [36, 38, 42, 130]) their ligands, suggesting that these 2 mechanisms may be strictly correlated, and when expressed on erythrocytes, ACKR1 also acts as a sink/reservoir for chemokines, thus working as a chemokine “buffering” receptor [45, 131]. As discussed in Signaling Properties of ACKRs, the mechanism used by a given ACKR to shape the chemokine gradient is strictly related on its trafficking properties.

### Effects on conventional chemokine receptor activity

The ability to interfere with conventional chemokine receptor activation represents an additional common theme among



**Figure 2. Heat maps of ACKR biologic and biochemical properties.** (A) The biologic functions of ACKRs, in terms of mechanisms involved in chemokine gradient shaping (degradation, transcytosis, presentation, sink) and effects on signaling of conventional chemokine receptors. (B) The biochemical properties of ACKRs, analyzed in terms of trafficking properties in constitutive and ligand-stimulated conditions. (C and D) Mechanisms of receptor internalization and cellular distribution are reported, respectively. Green indicates positive evidence, red indicates negative evidence, blue refers to controversial evidence, and white to absence of data. (D) Weak positivity indicated as light green results from the comparison between the 2 cellular compartments.

ACKRs, although the underlying molecular mechanisms are still largely undefined (**Fig. 2A**). All ACKRs but not ACKR4 have been demonstrated to be able to interfere with signaling properties of conventional receptors through direct binding of cognate ligands, and ACKR1, ACKR3, and ACKR4 have also been shown to form oligomers with conventional chemokine receptors [122, 124, 132, 133]. Coexpression of ACKR1 results in impaired CCR5-mediated signaling and chemotaxis properties [122], ACKR2 impairs CCR4-mediated chemotaxis [134], ackr5 impairs CCR7-dependent activation of B cells [123] and dendritic cell chemotaxis [47], and ACKR3 alters CXCR3 [135], as well as signaling properties of CD74 (the other MIF receptor) [13] and AM receptor [32]. Conversely, several reports have shown that ACKR3 improves CXCR4-dependent chemotaxis [54, 132, 136]. ACKR effects on conventional receptors goes beyond direct competition for cognate ligands, as it has been demonstrated that some ACKRs impair signaling activity of noncognate conventional chemokine receptors, such as ACKR2 and ACKR4, inhibiting CXCR5 on innate-like B cells and CXCR3 on T cells, respectively [24, 124]. As ACKR2 and ACKR4 activate  $\beta$ -arrestin-dependent signaling events (see  $\beta$ -Arrestin-dependent signaling



properties), it is tempting to speculate that interference with efficient coupling of conventional chemokine receptors to  $\beta$ -arrestins may be implicated. In conclusion, ACKRs appear to exert preferentially their regulatory activity by shaping the chemokine gradient when expressed on nonhematopoietic cells and by interfering with conventional chemokine receptor activation when coexpressed on hematopoietic cells (see this section for the biologic relevance of these mechanisms). As discussed in Trafficking Properties of ACKRs and Signaling Properties of ACKRs, different ACKRs exert these functions through different mechanisms related to their trafficking and signaling properties.

## TRAFFICKING PROPERTIES OF ACKRs

Intracellular trafficking properties of ACKRs are of major relevance for their chemokine gradient-shaping functions. Constitutive trafficking has been interpreted as a mechanism allowing to cope rapidly with changes in cellular requirements and is a common feature to most scavenger ACKRs [98, 126, 127, 137–140], although information on ACKR1 is not available and currently debated for *ackr5* [39, 42] (Fig. 2B). However, of note, constitutive trafficking is not a unique feature of scavenger ACKRs, as increasing, emerging evidence indicates that several conventional chemokine receptors also undergo constitutive internalization and recycling in specific cell types and conditions (CXCR4 [141–143]; CXCR3 [144]; CCR7 [145]; CCR1 [146]). Interestingly, constitutive trafficking correlates with ACKR subcellular localization, as scavenger ACKRs are mainly located in recycling endosomes [138–140] [unpublished results], whereas transporter and presenter ACKRs are preferentially expressed on the cell membrane [42, 62, 82, 128, 129] (Fig. 2D). Whereas ligand stimulation promotes internalization of conventional chemokine receptors as a receptor desensitization mechanism, in the ACKRs case, it triggers mechanisms improving receptor activity (Fig. 2B), based on clathrin- or caveolin-dependent internalization routes in different ACKRs (Fig. 2C), resulting in improved receptor internalization for transporter ACKRs [127, 129] and accelerated recycling rate in scavenger ACKRs [126, 127, 138]. In conclusion, ACKR unique trafficking properties support the intracellular fate of their ligands as a key element for their ability to fine tune the chemokine gradient.

## SIGNALING PROPERTIES OF ACKRs

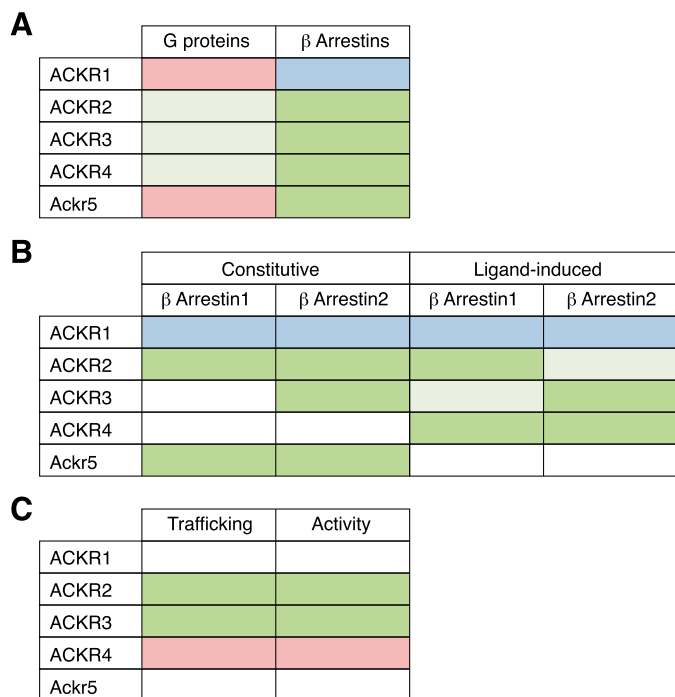
### Heterotrimeric $G\alpha$ protein-dependent signaling properties

Chemokine binding to its cognate receptor results in receptor structural rearrangements and activation of heterotrimeric G protein-dependent signaling pathways, ultimately leading to cell migration [147]. In particular, the  $G\alpha$  subunit inhibits adenylyl cyclase and decreases intracellular cAMP, whereas the  $G\beta\gamma$  subunit activates phospholipase C and induces calcium mobilization and diacylglycerol production [148]. A distinguishing feature of ACKRs, which initially allowed their classification as “silent” receptors, is their inability to sustain cellular migration [149], which is caused by absent or negligible ability to activate G

protein signaling [18] (see Fig. 3A). Direct measurements of ligand-dependent  $G\alpha_i$  activation has revealed no activity for ACKR1 [150] and ACKR3 [151], and measurements of intracellular cAMP levels have revealed no activity for ACKR3 [136] and only a minimal activity for ACKR2 [152]. Consistent with these findings, measurements of intracellular calcium mobilization as a second-messenger generation readout have reproducibly shown that all ACKRs tested are inactive, both in cells expressing the endogenous receptors [20, 24, 35, 42, 151], as well as in cell transfectants [19, 25, 32, 39, 42, 56, 125, 153–155]. One report has described that ACKR3 constitutively associates with  $G\alpha_i$  protein [132] and activates  $G\alpha_i$  signaling upon CXCL12 but not CXCL11 stimulation in rodent astrocytes and human glioma cell lines [14]. Noteworthy, although ACKR4 is unable to activate  $G\alpha_i$ ,  $G\alpha_s$ , or  $G\alpha_q$  subunits, uncoupling of constitutively associated  $G\alpha_i$  by pertussis toxin allows the receptor to slightly increase cAMP levels upon ligand engagement [156]. ACKRs share some alterations in sequence motifs responsible for  $G\alpha$  activation [18]. Changes are particularly evident for the DRYLAIV motif, located in the second intracellular loop, which is mutated very differently among ACKRs, with ACKR3 retaining the sequence most similar to the canonical one. However, as some conventional chemokine receptors support chemotaxis in the face of similar mutations in activation motifs [18], the definition of structural determinants responsible for the atypical  $G\alpha$  coupling properties of ACKRs deserves further detailed structure-activity relationship investigation. Taken together, these evidences indicate that with some exceptions in specific conditions and cell types, ACKRs are usually unable to activate the  $G\alpha$  protein-dependent signaling pathway, which is, in any case, dispensable for their chemokine regulatory functions.

### $\beta$ -Arrestin-dependent signaling properties

In recent years, it has become increasingly evident that the role of  $\beta$ -arrestins is not limited to 7TM internalization and desensitization. Conversely,  $\beta$ -arrestins function as adaptor proteins for different signaling proteins and thus, provide diversity and fine tuning of signaling activities [157, 158]. Although most 7TM receptors usually signal in a balanced fashion through the G protein and  $\beta$ -arrestin modules [159, 160], under specific conditions, some receptors have been shown to adopt G protein- or  $\beta$ -arrestin-biased signaling properties [158]. Evidence of biased signaling is rapidly accumulating also in the chemokine system [7, 161, 162], and in line with this emerging view, ACKRs have been proposed as constitutive  $\beta$ -arrestin-biased receptors [18]. Indeed, ligand-induced  $\beta$ -arrestin recruitment has been demonstrated for most scavenger ACKRs [156, 163] [unpublished results] (see Fig. 3A and B). Exceptions are represented by *ackr5*, which has not been investigated in this respect, and ACKR1, which has been shown to induce ERK1/2 phosphorylation in human airway smooth muscle cells whose expression of  $\beta$ -arrestins is debated [122, 164, 165]. Noteworthy, each ACKR shows a unique  $\beta$ -arrestin recruitment pattern, as ACKR2 and ACKR3 selectively recruit  $\beta$ -arrestin1 and  $\beta$ -arrestin2, respectively, whereas ACKR4 recruits both  $\beta$ -arrestins (see Fig. 3B). The structural reasons for this differential behavior have not been defined.  $\beta$ -Arrestin coupling correlates with receptor trafficking properties and is required for the scavenger function of ACKR2



**Figure 3. Heat maps of ACKR signaling properties.** (A) Ligand-induced activation of G proteins or  $\beta$ -arrestins signaling modules in murine and/or human ACKRs. (B) The selective recruitment of  $\beta$ -arrestin1 and  $\beta$ -arrestin2 isoforms under constitutive and ligand-induced conditions. (C) The involvement of  $\beta$ -arrestins on receptor trafficking and activity. Trafficking properties refers to internalization and recycling, whereas activity stands only for chemokine gradient shaping. Green indicates positive evidence, red indicates negative evidence, blue refers to controversial evidence, and white to absence of data. Weak positivity results, indicated as light green, have been defined according to the comparison of ACKR2 with CCR5 [152] (A), the observed activation of ACKR3 and ACKR4 only in selected settings [14, 156] (B), and the comparison between the 2  $\beta$ -arrestins' recruitments for ACKR2 and ACKR3 [unpublished results] [163].

[152] and ACKR3 [151, 166, 167], whereas it appears dispensable for ACKR4 internalization and ligand uptake [126] (see Fig. 3C). Analysis of the structural determinants involved in  $\beta$ -arrestin coupling has revealed that serine and threonine residues in the ACKR2 and ACKR3 intracellular carboxy-terminal tails are fundamental, although their effective phosphorylation status is debated for ACKR2 and unknown in the ACKR3 case [154, 168–170]. Some 7TMs, including chemokine receptors [146], adopt active conformations also in the absence of agonist binding, resulting in constitutive G protein activation and/or  $\beta$ -arrestin recruitment to the receptor and initiation of signal transduction pathways and receptor down-modulation [146, 171]. Constitutive interactions with  $\beta$ -arrestins have been reported for ACKR2, ACKR3, and ackr5 [39, 154, 170, 172] but not for ACKR1 [122], whereas no data have been reported for ACKR4 (Fig. 3B). For ACKR2 and ACKR3, constitutive association to  $\beta$ -arrestins is a key event for constitutive receptor trafficking and stabilizes receptor expression at the plasma membrane [154, 170, 172], whereas no biologic role for this constitutive recruitment has been proposed for ackr5. In conclusion,  $\beta$ -arrestin recruitment is emerging as a unifying

theme among ACKRs. ACKR internalization routes may dictate the involvement of  $\beta$ -arrestins in regulating biologic activities of the receptor. Taken together,  $\beta$ -arrestin coupling appears to be required for those ACKRs (ACKR2 and ACKR3) that sustain their biologic functions via clathrin-dependent internalization, whereas it is dispensable for the activity of ACKRs that use alternative, caveolae-dependent internalization routes (ACKR1 and ACKR4).

## CONCLUDING REMARKS

Proteins belonging to a given family usually share structural features but often fulfill different functions. Likewise, members of the ACKR family share the role of master regulators of the chemokine system by shaping the chemokine gradient and interfering with signaling properties of the conventional counterparts, but mechanisms used to exert these regulatory activities are different among the different members. According to the mechanism adopted, ACKRs may be classified as committed scavenger receptors (ACKR2, ACKR3, and ACKR4), transporter/presenter receptors (ACKR1), and receptors that exert both functions (ackr5). The structural determinants underlying different functions are still largely unknown—as poorly defined are those dictating the specific chemokine-binding profile of different ACKRs. Nonetheless, promiscuity in chemokine binding, expression on endothelial/epithelial cells of barrier organs, specific trafficking properties, and  $\beta$ -arrestin-biased signaling pathways are emerging as ACKR-unifying themes and may be considered the profile that identifies members of this family.

## AUTHORSHIP

E.M.B. and A.V. drafted the manuscript. M.L. critically revised the manuscript.

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## DISCLOSURES

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## REFERENCES

1. Bonecchi, R., Galliera, E., Borroni, E. M., Corsi, M. M., Locati, M., Mantovani, A. (2009) Chemokines and chemokine receptors: an overview. *Front. Biosci. (Landmark Ed.)* 14, 540–551.
2. Bachelier, F., Graham, G. J., Locati, M., Mantovani, A., Murphy, P. M., Nibbs, R., Rot, A., Sozzani, S., Thelen, M. (2014) New nomenclature for atypical chemokine receptors. *Nat. Immunol.* 15, 207–208.

3. Bachelierie, F., Graham, G. J., Locati, M., Mantovani, A., Murphy, P. M., Nibbs, R., Rot, A., Sozzani, S., Thelen, M. (2015) An atypical addition to the chemokine receptor nomenclature: IUPHAR Review 15. *Br. J. Pharmacol.* **172**, 3945–3949.
4. Bachelierie, F., Ben-Baruch, A., Burkhardt, A. M., Combadiere, C., Farber, J. M., Graham, G. J., Horuk, R., Sparre-Ulrich, A. H., Locati, M., Luster, A. D., Mantovani, A., Matsushima, K., Murphy, P. M., Nibbs, R., Nomiyama, H., Power, C. A., Proudfoot, A. E., Rosenkilde, M. M., Rot, A., Sozzani, S., Thelen, M., Yoshie, O., Zlotnik, A. (2014) International Union of Basic and Clinical Pharmacology. [corrected]. LXXXIX. Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors. *Pharmacol. Rev.* **66**, 1–79.
5. Thelen, M., Stein, J. V. (2008) How chemokines invite leukocytes to dance. *Nat. Immunol.* **9**, 953–959.
6. Rajagopal, S., Bassoni, D. L., Campbell, J. J., Gerard, N. P., Gerard, C., Wehrman, T. S. (2013) Biased agonism as a mechanism for differential signaling by chemokine receptors. *J. Biol. Chem.* **288**, 35039–35048.
7. Corbisier, J., Galès, C., Huszagh, A., Parmentier, M., Springael, J. Y. (2015) Biased signaling at chemokine receptors. *J. Biol. Chem.* **290**, 9542–9554.
8. Zlotnik, A., Yoshie, O. (2012) The chemokine superfamily revisited. *Immunology* **36**, 705–716.
9. Bonecchi, R., Savino, B., Borroni, E. M., Mantovani, A., Locati, M. (2010) Chemokine decoy receptors: structure-function and biological properties. *Curr. Top. Microbiol. Immunol.* **341**, 15–36.
10. Graham, G. J., Locati, M., Mantovani, A., Rot, A., Thelen, M. (2012) The biochemistry and biology of the atypical chemokine receptors. *Immunol. Lett.* **145**, 30–38.
11. Balabanian, K., Lagane, B., Infantino, S., Chow, K. Y., Harriague, J., Moepps, B., Arenzana-Seisdedos, F., Thelen, M., Bachelierie, F. (2005) The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes. *J. Biol. Chem.* **280**, 35760–35766.
12. Humpert, M. L., Pinto, D., Jarrossay, D., Thelen, M. (2014) CXCR7 influences the migration of B cells during maturation. *Eur. J. Immunol.* **44**, 694–705.
13. Alampour-Rajabi, S., El Bounkari, O., Rot, A., Muller-Newen, G., Bachelierie, F., Gawaz, M., Weber, C., Schober, A., Bernhagen, J. (2015) MIF interacts with CXCR7 to promote receptor internalization, ERK1/2 and ZAP-70 signaling, and lymphocyte chemotaxis. *FASEB J.* **29**, 4497–4511.
14. Odemis, V., Lipfert, J., Kraft, R., Hajek, P., Abraham, G., Hattermann, K., Mentlein, R., Engele, J. (2012) The presumed atypical chemokine receptor CXCR7 signals through G(i/o) proteins in primary rodent astrocytes and human glioma cells. *Glia* **60**, 372–381.
15. Zhao, D., Zhu, Z., Li, D., Xu, R., Wang, T., Liu, K. (2015) Pioglitazone suppresses CXCR7 expression to inhibit human macrophage chemotaxis through peroxisome proliferator-activated receptor  $\gamma$ . *Biochemistry* **54**, 6806–6814.
16. Nibbs, R. J., Graham, G. J. (2013) Immune regulation by atypical chemokine receptors. *Nat. Rev. Immunol.* **13**, 815–829.
17. Daiyasu, H., Nemoto, W., Toh, H. (2012) Evolutionary analysis of functional divergence among chemokine receptors, decoy receptors, and viral receptors. *Front. Microbiol.* **3**, 264.
18. Cancellieri, C., Vacchini, A., Locati, M., Bonecchi, R., Borroni, E. M. (2013) Atypical chemokine receptors: from silence to sound. *Biochem. Soc. Trans.* **41**, 231–236.
19. Nibbs, R. J., Wylie, S. M., Yang, J., Landau, N. R., Graham, G. J. (1997) Cloning and characterization of a novel promiscuous human beta-chemokine receptor D6. *J. Biol. Chem.* **272**, 32078–32083.
20. Burns, J. M., Summers, B. C., Wang, Y., Melikian, A., Berahovich, R., Miao, Z., Penfold, M. E., Sunshine, M. J., Littman, D. R., Kuo, C. J., Wei, K., McMaster, B. E., Wright, K., Howard, M. C., Schall, T. J. (2006) A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. *J. Exp. Med.* **203**, 2201–2213.
21. Gardner, L., Patterson, A. M., Ashton, B. A., Stone, M. A., Middleton, J. (2004) The human Duffy antigen binds selected inflammatory but not homeostatic chemokines. *Biochem. Biophys. Res. Commun.* **321**, 306–312.
22. Gosling, J., Dairaghi, D. J., Wang, Y., Hanley, M., Talbot, D., Miao, Z., Schall, T. J. (2000) Cutting edge: identification of a novel chemokine receptor that binds dendritic cell- and T cell-active chemokines including ELC, SLC, and TECK. *J. Immunol.* **164**, 2851–2856.
23. Nibbs, R. J., Wylie, S. M., Pragnell, I. B., Graham, G. J. (1997) Cloning and characterization of a novel murine beta chemokine receptor, D6. Comparison to three other related macrophage inflammatory protein-1alpha receptors, CCR-1, CCR-3, and CCR-5. *J. Biol. Chem.* **272**, 12495–12504.
24. Hansell, C. A., Schiering, C., Kinstrie, R., Ford, L., Bordon, Y., McInnes, I. B., Goodyear, C. S., Nibbs, R. J. (2011) Universal expression and dual function of the atypical chemokine receptor D6 on innate-like B cells in mice. *Blood* **117**, 5413–5424.
25. Townson, J. R., Nibbs, R. J. (2002) Characterization of mouse CCX-CKR, a receptor for the lymphocyte-attracting chemokines TECK/mCCL25, SLC/mCCL21 and MIP-3beta/mCCL19: comparison to human CCX-CKR. *Eur. J. Immunol.* **32**, 1230–1241.
26. Klein, K. R., Karpnich, N. O., Espenschied, S. T., Willcockson, H. H., Dunworth, W. P., Hoopes, S. L., Kushner, E. J., Bautch, V. L., Caron, K. M. (2014) Decoy receptor CXCR7 modulates adrenomedullin-mediated cardiac and lymphatic vascular development. *Dev. Cell* **30**, 528–540.
27. Ikeda, Y., Kumagai, H., Skach, A., Sato, M., Yanagisawa, M. (2013) Modulation of circadian glucocorticoid oscillation via adrenal opioid-CXCR7 signaling alters emotional behavior. *Cell* **155**, 1323–1336.
28. Bonini, J. A., Martin, S. K., Dralyuk, F., Roe, M. W., Philipson, L. H., Steiner, D. F. (1997) Cloning, expression, and chromosomal mapping of a novel human CC-chemokine receptor (CCR10) that displays high-affinity binding for MCP-1 and MCP-3. *DNA Cell Biol.* **16**, 1249–1256.
29. Kashiwazaki, M., Tanaka, T., Kanda, H., Ebisuno, Y., Izawa, D., Fukuma, N., Akimitsu, N., Sekimizu, K., Monden, M., Miyasaka, M. (2003) A high endothelial venule-expressing promiscuous chemokine receptor DARC can bind inflammatory, but not lymphoid, chemokines and is dispensable for lymphocyte homing under physiological conditions. *Int. Immunol.* **15**, 1219–1227.
30. Savino, B., Borroni, E. M., Torres, N. M., Proost, P., Struyf, S., Mortier, A., Mantovani, A., Locati, M., Bonecchi, R. (2009) Recognition versus adaptive up-regulation and degradation of CC chemokines by the chemokine decoy receptor D6 are determined by their N-terminal sequence. *J. Biol. Chem.* **284**, 26207–26215.
31. Szabo, M. C., Soo, K. S., Zlotnik, A., Schall, T. J. (1995) Chemokine class differences in binding to the Duffy antigen-erythrocyte chemokine receptor. *J. Biol. Chem.* **270**, 25348–25351.
32. Proost, P., Mortier, A., Loos, T., Vandercappellen, J., Gouwy, M., Ronse, I., Schutse, E., Put, W., Parmentier, M., Struyf, S., Van Damme, J. (2007) Proteolytic processing of CXCL11 by CD13/aminopeptidase N impairs CXCR3 and CXCR7 binding and signaling and reduces lymphocyte and endothelial cell migration. *Blood* **110**, 37–44.
33. Pawig, L., Klasen, C., Weber, C., Bernhagen, J., Noels, H. (2015) Diversity and inter-connections in the CXCR4 chemokine receptor/ligand family: molecular perspectives. *Front. Immunol.* **6**, 429.
34. Hamby, M. E., Coppola, G., Ao, Y., Geschwind, D. H., Khakh, B. S., Sofroniew, M. V. (2012) Inflammatory mediators alter the astrocyte transcriptome and calcium signaling elicited by multiple G-protein-coupled receptors. *J. Neurosci.* **32**, 14489–14510.
35. Hartmann, T. N., Leick, M., Ewers, S., Diefenbacher, A., Schraufstatter, I., Honczarenko, M., Burger, M. (2008) Human B cells express the orphan chemokine receptor CRAM-A/B in a maturation-stage-dependent and CCL5-modulated manner. *Immunology* **125**, 252–262.
36. Monnier, J., Lewén, S., O'Hara, E., Huang, K., Tu, H., Butcher, E. C., Zabel, B. A. (2012) Expression, regulation, and function of atypical chemerin receptor CCRL2 on endothelial cells. *J. Immunol.* **189**, 956–967.
37. Zuurman, M. W., Heeroma, J., Brouwer, N., Boddeke, H. W., Biber, K. (2003) LPS-induced expression of a novel chemokine receptor (L-CCR) in mouse glial cells in vitro and in vivo. *Glia* **41**, 327–336.
38. Gonzalez-Feo, S., Del Prete, A., Pruenster, M., Salvi, V., Wang, L., Sironi, M., Bierschenk, S., Sperandio, M., Vecchi, A., Sozzani, S. (2014) Endothelial cell-derived chemerin promotes dendritic cell transmigration. *J. Immunol.* **192**, 2366–2373.
39. Leick, M., Catusse, J., Follo, M., Nibbs, R. J., Hartmann, T. N., Veelen, H., Burger, M. (2010) CCL19 is a specific ligand of the constitutively recycling atypical human chemokine receptor CRAM-B. *Immunology* **129**, 536–546.
40. Biber, K., Zuurman, M. W., Homan, H., Boddeke, H. W. (2003) Expression of L-CCR in HEK 293 cells reveals functional responses to CCL2, CCL5, CCL7, and CCL8. *J. Leukoc. Biol.* **74**, 243–251.
41. Del Prete, A., Bonecchi, R., Vecchi, A., Mantovani, A., Sozzani, S. (2013) CCRL2, a fringe member of the atypical chemoattractant receptor family. *Eur. J. Immunol.* **43**, 1418–1422.
42. Zabel, B. A., Nakae, S., Zúñiga, L., Kim, J. Y., Ohyama, T., Alt, C., Pan, J., Suto, H., Soler, D., Allen, S. J., Handel, T. M., Song, C. H., Galli, S. J., Butcher, E. C. (2008) Mast cell-expressed orphan receptor CCRL2 binds chemerin and is required for optimal induction of IgE-mediated passive cutaneous anaphylaxis. *J. Exp. Med.* **205**, 2207–2220.
43. Tillmann, S., Bernhagen, J., Noels, H. (2013) Arrest functions of the MIF ligand/receptor axes in atherogenesis. *Front. Immunol.* **4**, 115.
44. Griffith, J. W., Sokol, C. L., Luster, A. D. (2014) Chemokines and chemokine receptors: positioning cells for host defense and immunity. *Annu. Rev. Immunol.* **32**, 659–702.
45. Dawson, T. C., Lentsch, A. B., Wang, Z., Cowhig, J. E., Rot, A., Maeda, N., Peiper, S. C. (2000) Exaggerated response to endotoxin in mice lacking the Duffy antigen/receptor for chemokines (DARC). *Blood* **96**, 1681–1684.
46. Jamieson, T., Cook, D. N., Nibbs, R. J., Rot, A., Nixon, C., McLean, P., Alami, A., Lira, S. A., Wiekowski, M., Graham, G. J. (2005) The chemokine receptor D6 limits the inflammatory response in vivo. *Nat. Immunol.* **6**, 403–411.
47. Otero, K., Vecchi, A., Hirsch, E., Kearley, J., Vermi, W., Del Prete, A., Gonzalez-Feo, S., Garlanda, C., Azzolino, O., Salogni, L., Lloyd, C. M.,



- Facchetti, F., Mantovani, A., Sozzani, S. (2010) Nonredundant role of CCRL2 in lung dendritic cell trafficking. *Blood* **116**, 2942–2949.
48. Bunting, M. D., Comerford, I., Seach, N., Hammett, M. V., Asquith, D. L., Körner, H., Boyd, R. L., Nibbs, R. J., McColl, S. R. (2013) CXCR-CCR deficiency alters thymic stroma impairing thymocyte development and promoting autoimmunity. *Blood* **121**, 118–128.
  49. Dambly-Chaudière, C., Cubedo, N., Ghysen, A. (2007) Control of cell migration in the development of the posterior lateral line: antagonistic interactions between the chemokine receptors CXCR4 and CXCR7/RDC1. *BMC Dev. Biol.* **7**, 23.
  50. Donà, E., Barry, J. D., Valentin, G., Quirin, C., Khmelinskii, A., Kunze, A., Durdu, S., Newton, L. R., Fernandez-Minan, A., Huber, W., Knop, M., Gilmour, D. (2013) Directional tissue migration through a self-generated chemokine gradient. *Nature* **503**, 285–289.
  51. Valentin, G., Haas, P., Gilmour, D. (2007) The chemokine SDF1 $\alpha$  coordinates tissue migration through the spatially restricted activation of Cxcr7 and Cxcr4b. *Curr. Biol.* **17**, 1026–1031.
  52. Venkiteswaran, G., Lewellis, S. W., Wang, J., Reynolds, E., Nicholson, C., Knaut, H. (2013) Generation and dynamics of an endogenous, self-generated signaling gradient across a migrating tissue. *Cell* **155**, 674–687.
  53. Gerrits, H., van Ingen Schenau, D. S., Bakker, N. E., van Disseldorp, A. J., Strik, A., Hermens, L. S., Koenen, T. B., Krajnc-Franken, M. A., Gossen, J. A. (2008) Early postnatal lethality and cardiovascular defects in CXCR7-deficient mice. *Genesis* **46**, 235–245.
  54. Sierro, F., Biben, C., Martínez-Muñoz, L., Mellado, M., Ransohoff, R. M., Li, M., Woehl, B., Leung, H., Groom, J., Batten, M., Harvey, R. P., Martínez-A, C., Mackay, C. R., Mackay, F. (2007) Disrupted cardiac development but normal hematopoiesis in mice deficient in the second CXCL12/SDF-1 receptor, CXCR7. *Proc. Natl. Acad. Sci. USA* **104**, 14759–14764.
  55. Lucas, B., White, A. J., Ulvmar, M. H., Nibbs, R. J., Sitnik, K. M., Agace, W. W., Jenkinson, W. E., Anderson, G., Rot, A. (2015) CCRL1/ACKR4 is expressed in key thymic microenvironments but is dispensable for T lymphopoiesis at steady state in adult mice. *Eur. J. Immunol.* **45**, 574–583.
  56. Heinzel, K., Benz, C., Bleul, C. C. (2007) A silent chemokine receptor regulates steady-state leukocyte homing in vivo. *Proc. Natl. Acad. Sci. USA* **104**, 8421–8426.
  57. Marquez-Curtis, L. A., Qiu, Y., Xu, A., Janowska-Wieczorek, A. (2014) Migration, proliferation, and differentiation of cord blood mesenchymal stromal cells treated with histone deacetylase inhibitor valproic acid. *Stem Cells Int.* **2014**, 610495.
  58. Lee, J. S., Frevert, C. W., Wurfel, M. M., Peiper, S. C., Wong, V. A., Ballman, K. K., Ruzinski, J. T., Rhim, J. S., Martin, T. R., Goodman, R. B. (2003) Duffy antigen facilitates movement of chemokine across the endothelium in vitro and promotes neutrophil transmigration in vitro and in vivo. *J. Immunol.* **170**, 5244–5251.
  59. Patterson, A. M., Siddall, H., Chamberlain, G., Gardner, L., Middleton, J. (2002) Expression of the Duffy antigen/receptor for chemokines (DARC) by the inflamed synovial endothelium. *J. Pathol.* **197**, 108–116.
  60. Hadley, T. J., Lu, Z. H., Wasniowska, K., Martin, A. W., Peiper, S. C., Hesselgeser, J., Horuk, R. (1994) Postcapillary venule endothelial cells in kidney express a multispecific chemokine receptor that is structurally and functionally identical to the erythroid isoform, which is the Duffy blood group antigen. *J. Clin. Invest.* **94**, 985–991.
  61. Woolley, I. J., Hotmire, K. A., Sramkoski, R. M., Zimmerman, P. A., Kazura, J. W. (2000) Differential expression of the Duffy antigen receptor for chemokines according to RBC age and FY genotype. *Transfusion* **40**, 949–953.
  62. Pruenster, M., Mudde, L., Bombosi, P., Dimitrova, S., Zsak, M., Middleton, J., Richmond, A., Graham, G. J., Segerer, S., Nibbs, R. J., Rot, A. (2009) The Duffy antigen receptor for chemokines transports chemokines and supports their promigratory activity. *Nat. Immunol.* **10**, 101–108.
  63. Middleton, J., Patterson, A. M., Gardner, L., Schmutz, C., Ashton, B. A. (2002) Leukocyte extravasation: chemokine transport and presentation by the endothelium. *Blood* **100**, 3853–3860.
  64. Rot, A. (2005) Contribution of Duffy antigen to chemokine function. *Cytokine Growth Factor Rev.* **16**, 687–694.
  65. Orecchioni, S., Gregato, G., Martin-Padura, I., Reggiani, F., Braidotti, P., Mancuso, P., Calleri, A., Quarna, J., Marighetti, P., Aldeni, C., Pruneri, G., Martella, S., Manconi, A., Petit, J. Y., Rietjens, M., Bertolini, F. (2013) Complementary populations of human adipose CD34<sup>+</sup> progenitor cells promote growth, angiogenesis, and metastasis of breast cancer. *Cancer Res.* **73**, 5880–5891.
  66. Lee, K. M., McKimmie, C. S., Gilchrist, D. S., Pallas, K. J., Nibbs, R. J., Garside, P., McDonald, V., Jenkins, C., Ransohoff, R., Liu, L., Milling, S., Cerovic, V., Graham, G. J. (2011) D6 facilitates cellular migration and fluid flow to lymph nodes by suppressing lymphatic congestion. *Blood* **118**, 6220–6229.
  67. McKimmie, C. S., Singh, M. D., Hewit, K., Lopez-Franco, O., Le Brocq, M., Rose-John, S., Lee, K. M., Baker, A. H., Wheat, R., Blackburn, D. J., Nibbs, R. J., Graham, G. J. (2013) An analysis of the function and expression of D6 on lymphatic endothelial cells. *Blood* **121**, 3768–3777.
  68. Nibbs, R. J., Kriehuber, E., Ponath, P. D., Parent, D., Qin, S., Campbell, J. D., Henderson, A., Kerjaschki, D., Maurer, D., Graham, G. J., Rot, A. (2001) The beta-chemokine receptor D6 is expressed by lymphatic endothelium and a subset of vascular tumors. *Am. J. Pathol.* **158**, 867–877.
  69. Immunological Genome Project Consortium. (2012) Transcriptional profiling of stroma from inflamed and resting lymph nodes defines immunological hallmarks. *Nat. Immunol.* **13**, 499–510.
  70. Berahovich, R. D., Zabel, B. A., Lewén, S., Walters, M. J., Ebsworth, K., Wang, Y., Jaen, J. C., Schall, T. J. (2014) Endothelial expression of CXCR7 and the regulation of systemic CXCL12 levels. *Immunology* **141**, 111–122.
  71. Neusser, M. A., Kraus, A. K., Regele, H., Cohen, C. D., Fehr, T., Kerjaschki, D., Wüthrich, R. P., Penfold, M. E., Schall, T., Segerer, S. (2010) The chemokine receptor CXCR7 is expressed on lymphatic endothelial cells during renal allograft rejection. *Kidney Int.* **77**, 801–808.
  72. Watanabe, K., Penfold, M. E., Matsuda, A., Ohyanagi, N., Kaneko, K., Miyabe, Y., Matsumoto, K., Schall, T. J., Miyasaka, N., Nanki, T. (2010) Pathogenic role of CXCR7 in rheumatoid arthritis. *Arthritis Rheum.* **62**, 3211–3220.
  73. Williams, J. L., Holman, D. W., Klein, R. S. (2014) Chemokines in the balance: maintenance of homeostasis and protection at CNS barriers. *Front. Cell. Neurosci.* **8**, 154.
  74. Minten, C., Alt, C., Gentner, M., Frei, E., Deutsch, U., Lyck, R., Schaeren-Wiemers, N., Rot, A., Engelhardt, B. (2014) DARC shuttles inflammatory chemokines across the blood-brain barrier during autoimmune central nervous system inflammation. *Brain* **137**, 1454–1469.
  75. Fouillet, A., Mawson, J., Suliman, O., Sharrack, B., Romero, I. A., Woodroffe, M. N. (2012) CCL2 binding is CCR2 independent in primary adult human astrocytes. *Brain Res.* **1437**, 115–126.
  76. Neil, S. J., Aasa-Chapman, M. M., Clapham, P. R., Nibbs, R. J., McKnight, A., Weiss, R. A. (2005) The promiscuous CC chemokine receptor D6 is a functional coreceptor for primary isolates of human immunodeficiency virus type 1 (HIV-1) and HIV-2 on astrocytes. *J. Virol.* **79**, 9618–9624.
  77. Cruz-Orengo, L., Holman, D. W., Dorsey, D., Zhou, L., Zhang, P., Wright, M., McCandless, E. E., Patel, J. R., Luker, G. D., Littman, D. R., Russell, J. H., Klein, R. S. (2011) CXCR7 influences leukocyte entry into the CNS parenchyma by controlling albulinal CXCL12 abundance during autoimmunity. *J. Exp. Med.* **208**, 327–339.
  78. McKimmie, C. S., Graham, G. J. (2010) Astrocytes modulate the chemokine network in a pathogen-specific manner. *Biochem. Biophys. Res. Commun.* **394**, 1006–1011.
  79. Brouwer, N., Zuurman, M. W., Wei, T., Ransohoff, R. M., Boddeke, H. W., Biber, K. (2004) Induction of glial L-CCR mRNA expression in spinal cord and brain in experimental autoimmune encephalomyelitis. *Glia* **46**, 84–94.
  80. Zhao, P., Yang, Y., Feng, H., Zhao, L., Qin, J., Zhang, T., Wang, H., Yang, S., Xia, X. (2013) Global gene expression changes in BV2 microglial cell line during rabies virus infection. *Infect. Genet. Evol.* **20**, 257–269.
  81. Douglas, R. M., Chen, A. H., Iniguez, A., Wang, J., Fu, Z., Powell, Jr., F. L., Haddad, G. G., Yao, H. (2013) Chemokine receptor-like 2 is involved in ischemic brain injury. *J. Exp. Stroke Transl. Med.* **6**, 1–6.
  82. Chaudhuri, A., Nielsen, S., Elkjaer, M. L., Zbrzezna, V., Fang, F., Pogo, A. O. (1997) Detection of Duffy antigen in the plasma membranes and caveolae of vascular endothelial and epithelial cells of nonerythroid organs. *Blood* **89**, 701–712.
  83. Liu, X. F., Li, L. F., Ou, Z. L., Shen, R., Shao, Z. M. (2012) Correlation between Duffy blood group phenotype and breast cancer incidence. *BMC Cancer* **12**, 374.
  84. Choi, W., Byun, Y. J., Jung, E., Noh, H., Hajrasouliha, A. R., Sadrai, Z., Chang, E., Lee, J. H., Lee, H. K. (2015) Chemokine decoy receptor D6 mimicking trap (D6MT) prevents allosensitization and immune rejection in murine corneal allograft model. *J. Leukoc. Biol.* **97**, 413–424.
  85. Singh, M. D., King, V., Baldwin, H., Burden, D., Thorat, A., Holmes, S., McInnes, I. B., Nicoll, R., Shams, K., Pallas, K., Jamieson, T., Lee, K. M., Carballido, J. M., Rot, A., Graham, G. J. (2012) Elevated expression of the chemokine-scavenging receptor D6 is associated with impaired lesion development in psoriasis. *Am. J. Pathol.* **181**, 1158–1164.
  86. Yu, H., Zhang, L., Liu, P. (2015) CXCR7 signaling induced epithelial-mesenchymal transition by AKT and ERK pathways in epithelial ovarian carcinomas. *Tumour Biol.* **36**, 1679–1683.
  87. Rode, I., Boehm, T. (2012) Regenerative capacity of adult cortical thymic epithelial cells. *Proc. Natl. Acad. Sci. USA* **109**, 3463–3468.
  88. Ohigashi, I., Takahama, Y. (2014) CCRL1 marks heterogeneity in cortical and medullary thymic epithelial cells. *Eur. J. Immunol.* **44**, 2872–2875.
  89. Ribeiro, A. R., Meireles, C., Rodrigues, P. M., Alves, N. L. (2014) Intermediate expression of CCRL1 reveals novel subpopulations of medullary thymic epithelial cells that emerge in the postnatal thymus. *Eur. J. Immunol.* **44**, 2918–2924.
  90. Bai, J., Smock, S. L., Jackson, Jr., G. R., MacIsaac, K. D., Huang, Y., Mankus, C., Oldach, J., Roberts, B., Ma, Y. L., Klappenbach, J. A.,



- Crackower, M. A., Alves, S. E., Hayden, P. J. (2015) Phenotypic responses of differentiated asthmatic human airway epithelial cultures to rhinovirus. *PLoS One* **10**, e0118286.
91. Kriegova, E., Tsyrlunyk, A., Arakelyan, A., Mrázek, F., Ordeltova, M., Petzmann, S., Zatloukal, J., Kolek, V., du Bois, R. M., Popper, H., Petrek, M. (2006) Expression of CXCR2 in pulmonary sarcoidosis. *Inflamm. Res.* **55**, 441–445.
92. Meurens, F., Berri, M., Auray, G., Melo, S., Levast, B., Virlogeux-Payant, I., Chevalere, C., Gerdt, V., Salmon, H. (2009) Early immune response following *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* infection in porcine jejunal gut loops. *Vet. Res.* **40**, 5.
93. Oostendorp, J., Hylkema, M. N., Luinge, M., Geerlings, M., Meurs, H., Timens, W., Zaagsma, J., Postma, D. S., Boddeke, H. W., Biber, K. (2004) Localization and enhanced mRNA expression of the orphan chemokine receptor L-CCR in the lung in a murine model of ovalbumin-induced airway inflammation. *J. Histochem. Cytochem.* **52**, 401–410.
94. Wessels, J. M., Linton, N. F., van den Heuvel, M. J., Cnossen, S. A., Edwards, A. K., Croy, B. A., Tayade, C. (2011) Expression of chemokine decoy receptors and their ligands at the porcine maternal-fetal interface. *Immunol. Cell Biol.* **89**, 304–313.
95. Madigan, J., Freeman, D. J., Menzies, F., Forrow, S., Nelson, S. M., Young, A., Sharkey, A., Moffett, A., Graham, G. J., Greer, I. A., Rot, A., Nibbs, R. J. (2010) Chemokine scavenger D6 is expressed by trophoblasts and aids the survival of mouse embryos transferred into allogeneic recipients. *J. Immunol.* **184**, 3202–3212.
96. Martínez de la Torre, Y., Buracchi, C., Borroni, E. M., Dupor, J., Bonicchi, R., Nebuloni, M., Pasqualini, F., Doni, A., Lauri, E., Agostinis, C., Bulla, R., Cook, D. N., Haribabu, B., Meroni, P., Rukavina, D., Vago, L., Tedesco, F., Vecchi, A., Lira, S. A., Locati, M., Mantovani, A. (2007) Protection against inflammation- and autoantibody-caused fetal loss by the chemokine decoy receptor D6. *Proc. Natl. Acad. Sci. USA* **104**, 2319–2324.
97. Teoh, P. J., Menzies, F. M., Hansell, C. A., Clarke, M., Waddell, C., Burton, G. J., Nelson, S. M., Nibbs, R. J. (2014) Atypical chemokine receptor ACKR2 mediates chemokine scavenging by primary human trophoblasts and can regulate fetal growth, placental structure, and neonatal mortality in mice. *J. Immunol.* **193**, 5218–5228.
98. Naumann, U., Cameron, E., Pruenster, M., Mahabaleswar, H., Raz, E., Zerwas, H. G., Rot, A., Thelen, M. (2010) CXCR7 functions as a scavenger for CXCL12 and CXCL11. *PLoS One* **5**, e9175.
99. Tripathi, V., Verma, R., Dinda, A., Malhotra, N., Kaur, J., Luthra, K. (2009) Differential expression of RDC1/CXCR7 in the human placenta. *J. Clin. Immunol.* **29**, 379–386.
100. Schanz, A., Baston-Bust, D., Krussel, J. S., Heiss, C., Janni, W., Hess, A. P. (2011) CXCR7 and syndecan-4 are potential receptors for CXCL12 in human cytotrophoblasts. *J. Reprod. Immunol.* **89**, 18–25.
101. Carlino, C., Trotta, E., Stabile, H., Morrone, S., Bulla, R., Soriani, A., Iannitto, M. L., Agostinis, C., Mocchi, C., Minozzi, M., Aragona, C., Perniola, G., Tedesco, F., Sozzani, S., Santoni, A., Gismondi, A. (2012) Chemerin regulates NK cell accumulation and endothelial cell morphogenesis in the decidua during early pregnancy. *J. Clin. Endocrinol. Metab.* **97**, 3603–3612.
102. Świątkowski, W., Rahnema, M., Kocki, J., Świątkowska, A. (2013) Impact of Duffy antigen receptor for chemokine gene expression on mandibular bone density in menopausal women. *Int. J. Oral Maxillofac. Surg.* **42**, 411–416.
103. McKimmie, C. S., Fraser, A. R., Hansell, C., Gutiérrez, L., Philipsen, S., Connell, L., Rot, A., Kurowska-Stolarska, M., Carreno, P., Pruenster, M., Chu, C. C., Lombardi, G., Halsey, C., McInnes, I. B., Liew, F. Y., Nibbs, R. J., Graham, G. J. (2008) Hemopoietic cell expression of the chemokine decoy receptor D6 is dynamic and regulated by GATA1. *J. Immunol.* **181**, 8171–8181.
104. Ford, L. B., Cerovic, V., Milling, S. W., Graham, G. J., Hansell, C. A., Nibbs, R. J. (2014) Characterization of conventional and atypical receptors for the chemokine CCL2 on mouse leukocytes. *J. Immunol.* **193**, 400–411.
105. Hajrasouliha, A. R., Sadrai, Z., Lee, H. K., Chauhan, S. K., Dana, R. (2013) Expression of the chemokine decoy receptor D6 mediates dendritic cell function and promotes corneal allograft rejection. *Mol. Vis.* **19**, 2517–2525.
106. Rot, A., McKimmie, C., Burt, C. L., Pallas, K. J., Jamieson, T., Pruenster, M., Horuk, R., Nibbs, R. J., Graham, G. J. (2013) Cell-autonomous regulation of neutrophil migration by the D6 chemokine decoy receptor. *J. Immunol.* **190**, 6450–6456.
107. Migeotte, I., Franssen, J. D., Goriely, S., Willems, F., Parmentier, M. (2002) Distribution and regulation of expression of the putative human chemokine receptor HCR in leukocyte populations. *Eur. J. Immunol.* **32**, 494–501.
108. Yoshimura, T., Oppenheim, J. J. (2011) Chemokine-like receptor 1 (CMKLR1) and chemokine (C-C motif) receptor-like 2 (CCL2R2); two multifunctional receptors with unusual properties. *Exp. Cell Res.* **317**, 674–684.
109. Bazzan, E., Saetta, M., Turato, G., Borroni, E. M., Cancellieri, C., Baraldo, S., Savino, B., Calabrese, F., Ballarin, A., Balestro, E., Mantovani, A., Cosio, M. G., Bonicchi, R., Locati, M. (2013) Expression of the atypical chemokine receptor D6 in human alveolar macrophages in COPD. *Chest* **143**, 98–106.
110. Berahovich, R. D., Zabel, B. A., Penfold, M. E., Lewén, S., Wang, Y., Miao, Z., Gan, L., Pereda, J., Dias, J., Slukvin, I. I., McGrath, K. E., Jaen, J. C., Schall, T. J. (2010) CXCR7 protein is not expressed on human or mouse leukocytes. *J. Immunol.* **185**, 5130–5139.
111. Hartmann, T. N., Grabovsky, V., Pasvolsky, R., Shulman, Z., Buss, E. C., Spiegel, A., Nagler, A., Lapidot, T., Thelen, M., Alon, R. (2008) A crosstalk between intracellular CXCR7 and CXCR4 involved in rapid CXCL12-triggered integrin activation but not in chemokine-triggered motility of human T lymphocytes and CD34+ cells. *J. Leukoc. Biol.* **84**, 1130–1140.
112. Infantino, S., Moepps, B., Thelen, M. (2006) Expression and regulation of the orphan receptor RDC1 and its putative ligand in human dendritic and B cells. *J. Immunol.* **176**, 2197–2207.
113. Melo, R. D., Longhini, A. L., Bigarella, C. L., Baratti, M. O., Traina, F., Favaro, P., de Melo Campos, P., Saad, S. T. (2014) CXCR7 is highly expressed in acute lymphoblastic leukemia and potentiates CXCR4 response to CXCL12. *PLoS One* **9**, e85926.
114. Wang, H., Beatty, N., Chen, S., Qi, C. F., Masiuk, M., Shin, D. M., Morse III, H. C. (2012) The CXCR7 chemokine receptor promotes B-cell retention in the splenic marginal zone and serves as a sink for CXCL12. *Blood* **119**, 465–468.
115. Humpert, M. L., Tzouros, M., Thelen, S., Bignon, A., Levoye, A., Arenzana-Seisdedos, F., Balabanian, K., Bachelier, F., Langen, H., Thelen, M. (2012) Complementary methods provide evidence for the expression of CXCR7 on human B cells. *Proteomics* **12**, 1938–1948.
116. Comerford, I., Harata-Lee, Y., Bunting, M. D., Gregor, C., Kara, E. E., McColl, S. R. (2013) A myriad of functions and complex regulation of the CCR7/CCL19/CCL21 chemokine axis in the adaptive immune system. *Cytokine Growth Factor Rev.* **24**, 269–283.
117. Cutbush, M., Mollison, P. L. (1950) The Duffy blood group system. *Heredity (Edinb)* **4**, 383–389.
118. Rath, D., Chatterjee, M., Borst, O., Müller, K., Langer, H., Mack, A. F., Schwab, M., Winter, S., Gawaz, M., Geisler, T. (2015) Platelet surface expression of stromal cell-derived factor-1 receptors CXCR4 and CXCR7 is associated with clinical outcomes in patients with coronary artery disease. *J. Thromb. Haemost.* **13**, 719–728.
119. Chatterjee, M., Borst, O., Walker, B., Fotinos, A., Vogel, S., Seizer, P., Mack, A., Alampour-Rajabi, S., Rath, D., Geisler, T., Lang, F., Langer, H. F., Bernhagen, J., Gawaz, M. (2014) Macrophage migration inhibitory factor limits activation-induced apoptosis of platelets via CXCR7-dependent Akt signaling. *Circ. Res.* **115**, 939–949.
120. Codullo, V., Baldwin, H. M., Singh, M. D., Fraser, A. R., Wilson, C., Gilmour, A., Hueber, A. J., Bonino, C., McInnes, I. B., Montecucco, C., Graham, G. J. (2011) An investigation of the inflammatory cytokine and chemokine network in systemic sclerosis. *Ann. Rheum. Dis.* **70**, 1115–1121.
121. Thelen, M., Thelen, S. (2008) CXCR7, CXCR4 and CXCL12: an eccentric trio? *J. Neuroimmunol.* **198**, 9–13.
122. Chakera, A., Seeber, R. M., John, A. E., Eidne, K. A., Greaves, D. R. (2008) The Duffy antigen/receptor for chemokines exists in an oligomeric form in living cells and functionally antagonizes CCR5 signaling through hetero-oligomerization. *Mol. Pharmacol.* **73**, 1362–1370.
123. Catusse, J., Leick, M., Groch, M., Clark, D. J., Buchner, M. V., Zirlik, K., Burger, M. (2010) Role of the atypical chemoattractant receptor CCR4 in regulating CCL19 induced CCR7 responses in B-cell chronic lymphocytic leukemia. *Mol. Cancer* **9**, 297.
124. Vinet, J., van Zwam, M., Dijkstra, I. M., Brouwer, N., van Weering, H. R., Watts, A., Meijer, M., Fokkens, M. R., Kannan, V., Verzijl, D., Vischer, H. F., Smit, M. J., Leurs, R., Biber, K., Boddeke, H. W. (2013) Inhibition of CXCR3-mediated chemotaxis by the human chemokine receptor-like protein CXCR-CKR. *Br. J. Pharmacol.* **168**, 1375–1387.
125. Fra, A. M., Locati, M., Otero, K., Sironi, M., Signorelli, P., Massardi, M. L., Gobbi, M., Vecchi, A., Sozzani, S., Mantovani, A. (2003) Cutting edge: scavenging of inflammatory CC chemokines by the promiscuous putatively silent chemokine receptor D6. *J. Immunol.* **170**, 2279–2282.
126. Comerford, I., Milasta, S., Morrow, V., Milligan, G., Nibbs, R. (2006) The chemokine receptor CXCR-CKR mediates effective scavenging of CCL19 in vitro. *Eur. J. Immunol.* **36**, 1904–1916.
127. Luker, K. E., Steele, J. M., Mihalko, L. A., Ray, P., Luker, G. D. (2010) Constitutive and chemokine-dependent internalization and recycling of CXCR7 in breast cancer cells to degrade chemokine ligands. *Oncogene* **29**, 4599–4610.
128. Middleton, J., Neil, S., Wintle, J., Clark-Lewis, I., Moore, H., Lam, C., Auer, M., Hub, E., Rot, A. (1997) Transcytosis and surface presentation of IL-8 by venular endothelial cells. *Cell* **91**, 385–395.
129. Zhao, Y., Mangalmurti, N. S., Xiong, Z., Prakash, B., Guo, F., Stolz, D. B., Lee, J. S. (2011) Duffy antigen receptor for chemokines mediates chemokine endocytosis through a macropinocytosis-like process in endothelial cells. *PLoS One* **6**, e29624.

130. Baekkevold, E. S., Yamanaka, T., Palframan, R. T., Carlsen, H. S., Reinhold, F. P., von Andrian, U. H., Brandtzaeg, P., Haraldsen, G. (2001) The CCR7 ligand eIC (CCL19) is transcytosed in high endothelial venules and mediates T cell recruitment. *J. Exp. Med.* **193**, 1105–1112.
131. Darbonne, W. C., Rice, G. C., Mohler, M. A., Apple, T., Hébert, C. A., Valente, A. J., Baker, J. B. (1991) Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin. *J. Clin. Invest.* **88**, 1362–1369.
132. Levoe, A., Balabanian, K., Baleux, F., Bachelier, F., Lagane, B. (2009) CXCR7 heterodimerizes with CXCR4 and regulates CXCL12-mediated G protein signaling. *Blood* **113**, 6085–6093.
133. Luker, K. E., Gupta, M., Luker, G. D. (2009) Imaging chemokine receptor dimerization with firefly luciferase complementation. *FASEB J.* **23**, 823–834.
134. Bonecchi, R., Locati, M., Galliera, E., Vulcano, M., Sironi, M., Fra, A. M., Gobbi, M., Vecchi, A., Sozzani, S., Haribabu, B., Van Damme, J., Mantovani, A. (2004) Differential recognition and scavenging of native and truncated macrophage-derived chemokine (macrophage-derived chemokine/CC chemokine ligand 22) by the D6 decoy receptor. *J. Immunol.* **172**, 4972–4976.
135. Singh, A. K., Arya, R. K., Trivedi, A. K., Sanyal, S., Baral, R., Dormond, O., Briscoe, D. M., Datta, D. (2013) Chemokine receptor trio: CXCR3, CXCR4 and CXCR7 crosstalk via CXCL11 and CXCL12. *Cytokine Growth Factor Rev.* **24**, 41–49.
136. Décaillot, F. M., Kazmi, M. A., Lin, Y., Ray-Saha, S., Sakmar, T. P., Sachdev, P. (2011) CXCR7/CXCR4 heterodimer constitutively recruits beta-arrestin to enhance cell migration. *J. Biol. Chem.* **286**, 32188–32197.
137. Royle, S. J., Murrell-Lagnado, R. D. (2003) Constitutive cycling: a general mechanism to regulate cell surface proteins. *Bioessays* **25**, 39–46.
138. Bonecchi, R., Borroni, E. M., Anselmo, A., Doni, A., Savino, B., Miolo, M., Fabbri, M., Jala, V. R., Haribabu, B., Mantovani, A., Locati, M. (2008) Regulation of D6 chemokine scavenging activity by ligand- and Rab11-dependent surface up-regulation. *Blood* **112**, 493–503.
139. Hoffmann, F., Müller, W., Schütz, D., Penfold, M. E., Wong, Y. H., Schulz, S., Stumm, R. (2012) Rapid uptake and degradation of CXCL12 depend on CXCR7 carboxyl-terminal serine/threonine residues. *J. Biol. Chem.* **287**, 28362–28377.
140. Weber, M., Blair, E., Simpson, C. V., O'Hara, M., Blackburn, P. E., Rot, A., Graham, G. J., Nibbs, R. J. (2004) The chemokine receptor D6 constitutively traffics to and from the cell surface to internalize and degrade chemokines. *Mol. Biol. Cell* **15**, 2492–2508.
141. Futahashi, Y., Komano, J., Urano, E., Aoki, T., Hamatake, M., Miyauchi, K., Yoshida, T., Koyanagi, Y., Matsuda, Z., Yamamoto, N. (2007) Separate elements are required for ligand-dependent and -independent internalization of metastatic potentiator CXCR4. *Cancer Sci.* **98**, 373–379.
142. Pelekanos, R. A., Ting, M. J., Sardesai, V. S., Ryan, J. M., Lim, Y. C., Chan, J. K., Fisk, N. M. (2014) Intracellular trafficking and endocytosis of CXCR4 in fetal mesenchymal stem/stromal cells. *BMC Cell Biol.* **15**, 15.
143. Zhang, Y., Foudi, A., Geay, J. F., Berthebaud, M., Buet, D., Jarrier, P., Jalil, A., Vainchenker, W., Louache, F. (2004) Intracellular localization and constitutive endocytosis of CXCR4 in human CD34+ hematopoietic progenitor cells. *Stem Cells* **22**, 1015–1029.
144. Meiser, A., Mueller, A., Wise, E. L., McDonagh, E. M., Petit, S. J., Saran, N., Clark, P. C., Williams, T. J., Pease, J. E. (2008) The chemokine receptor CXCR3 is degraded following internalization and is replenished at the cell surface by de novo synthesis of receptor. *J. Immunol.* **180**, 6713–6724.
145. Schaeuble, K., Hauser, M. A., Rippl, A. V., Bruderer, R., Otero, C., Groettrup, M., Legler, D. F. (2012) Ubiquitination of the chemokine receptor CCR7 enables efficient receptor recycling and cell migration. *J. Cell Sci.* **125**, 4463–4474.
146. Gilliland, C. T., Salanga, C. L., Kawamura, T., Trejo, J., Handel, T. M. (2013) The chemokine receptor CCR1 is constitutively active, which leads to G protein-independent, beta-arrestin-mediated internalization. *J. Biol. Chem.* **288**, 32194–32210.
147. Qin, L., Kufareva, L., Holden, L. G., Wang, C., Zheng, Y., Zhao, C., Fenalti, G., Wu, H., Han, G. W., Cherezov, V., Abagyan, R., Stevens, R. C., Handel, T. M. (2015) Structural biology. Crystal structure of the chemokine receptor CXCR4 in complex with a viral chemokine. *Science* **347**, 1117–1122.
148. Thelen, M. (2001) Dancing to the tune of chemokines. *Nat. Immunol.* **2**, 129–134.
149. Mantovani, A., Bonecchi, R., Locati, M. (2006) Tuning inflammation and immunity by chemokine sequestration: decoys and more. *Nat. Rev. Immunol.* **6**, 907–918.
150. Horuk, R., Colby, T. J., Darbonne, W. C., Schall, T. J., Neote, K. (1993) The human erythrocyte inflammatory peptide (chemokine) receptor. Biochemical characterization, solubilization, and development of a binding assay for the soluble receptor. *Biochemistry* **32**, 5733–5738.
151. Rajagopal, S., Kim, J., Ahn, S., Craig, S., Lam, C. M., Gerard, N. P., Gerard, C., Lefkowitz, R. J. (2010) Beta-arrestin- but not G protein-mediated signaling by the “decoy” receptor CXCR7. *Proc. Natl. Acad. Sci. USA* **107**, 628–632.
152. Borroni, E. M., Cancellieri, C., Vacchini, A., Benureau, Y., Lagane, B., Bachelier, F., Arenzana-Seisdedos, F., Mizuno, K., Mantovani, A., Bonecchi, R., Locati, M. (2013) beta-Arrestin-dependent activation of the cofilin pathway is required for the scavenging activity of the atypical chemokine receptor D6. *Sci. Signal.* **6**, ra30.1–11, S1–S3.
153. Neote, K., Mak, J. Y., Kolakowski, Jr., L. F., Schall, T. J. (1994) Functional and biochemical analysis of the cloned Duffy antigen: identity with the red blood cell chemokine receptor. *Blood* **84**, 44–52.
154. Galliera, E., Jala, V. R., Trent, J. O., Bonecchi, R., Signorelli, P., Lefkowitz, R. J., Mantovani, A., Locati, M., Haribabu, B. (2004) beta-Arrestin-dependent constitutive internalization of the human chemokine decoy receptor D6. *J. Biol. Chem.* **279**, 25590–25597.
155. Galligan, C. L., Matsuyama, W., Matsukawa, A., Mizuta, H., Hodge, D. R., Howard, O. M., Yoshimura, T. (2004) Up-regulated expression and activation of the orphan chemokine receptor, CCRL2, in rheumatoid arthritis. *Arthritis Rheum.* **50**, 1806–1814.
156. Watts, A. O., Verkaar, F., van der Lee, M. M., Timmerman, C. A., Kuijter, M., van Offenbeek, J., van Lith, L. H., Smit, M. J., Leurs, R., Zaman, G. J., Vischer, H. F. (2013) beta-Arrestin recruitment and G protein signaling by the atypical human chemokine decoy receptor CCX-CKR. *J. Biol. Chem.* **288**, 7169–7181.
157. Gurevich, V. V., Gurevich, E. V. (2013) Structural determinants of arrestin functions. *Prog. Mol. Biol. Transl. Sci.* **118**, 57–92.
158. Shukla, A. K., Xiao, K., Lefkowitz, R. J. (2011) Emerging paradigms of beta-arrestin-dependent seven transmembrane receptor signaling. *Trends Biochem. Sci.* **36**, 457–469.
159. Rajagopal, K., Lefkowitz, R. J., Rockman, H. A. (2005) When 7 transmembrane receptors are not G protein-coupled receptors. *J. Clin. Invest.* **115**, 2971–2974.
160. Schwartz, T. W., Frimurer, T. M., Holst, B., Rosenkilde, M. M., Elling, C. E. (2006) Molecular mechanism of 7TM receptor activation—a global toggle switch model. *Annu. Rev. Pharmacol. Toxicol.* **46**, 481–519.
161. Steen, A., Sparre-Ulrich, A. H., Thiele, S., Guo, D., Frimurer, T. M., Rosenkilde, M. M. (2014) Gating function of isoleucine-116 in TM-3 (position III:16/3.40) for the activity state of the CC-chemokine receptor 5 (CCR5). *Br. J. Pharmacol.* **171**, 1566–1579.
162. Zidar, D. A. (2011) Endogenous ligand bias by chemokines: implications at the front lines of infection and leukocyte trafficking. *Endocr. Metab. Immune Disord. Drug Targets* **11**, 120–131.
163. Luker, K. E., Gupta, M., Steele, J. M., Foerster, B. R., Luker, G. D. (2009) Imaging ligand-dependent activation of CXCR7. *Neoplasia* **11**, 1022–1035.
164. Al-Alwan, L. A., Chang, Y., Rousseau, S., Martin, J. G., Eidelman, D. H., Hamid, Q. (2014) CXCL1 inhibits airway smooth muscle cell migration through the decoy receptor Duffy antigen receptor for chemokines. *J. Immunol.* **193**, 1416–1426.
165. Pera, T., Hegde, A., Deshpande, D. A., Morgan, S. J., Tiegs, B. C., Theriot, B. S., Choi, Y. H., Walker, J. K., Penn, R. B. (2015) Specificity of arrestin subtypes in regulating airway smooth muscle G protein-coupled receptor signaling and function. *FASEB J.* **29**, 4227–4235.
166. Lee, E., Han, J., Kim, K., Choi, H., Cho, E. G., Lee, T. R. (2013) CXCR7 mediates SDF1-induced melanocyte migration. *Pigment Cell Melanoma Res.* **26**, 58–66.
167. Odemis, V., Boosmann, K., Heinen, A., Küry, P., Engele, J. (2010) CXCR7 is an active component of SDF-1 signalling in astrocytes and Schwann cells. *J. Cell Sci.* **123**, 1081–1088.
168. Canals, M., Scholten, D. J., de Munnik, S., Han, M. K., Smit, M. J., Leurs, R. (2012) Ubiquitination of CXCR7 controls receptor trafficking. *PLoS One* **7**, e34192.
169. Ray, P., Mihalko, L. A., Coggins, N. L., Moudgil, P., Ehrlich, A., Luker, K. E., Luker, G. D. (2012) Carboxy-terminus of CXCR7 regulates receptor localization and function. *Int. J. Biochem. Cell Biol.* **44**, 669–678.
170. McCulloch, C. V., Morrow, V., Milasta, S., Comerford, I., Milligan, G., Graham, G. J., Isaacs, N. W., Nibbs, R. J. (2008) Multiple roles for the C-terminal tail of the chemokine scavenger D6. *J. Biol. Chem.* **283**, 7972–7982.
171. Seifert, R., Wenzel-Seifert, K. (2002) Constitutive activity of G-protein-coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **366**, 381–416.
172. Coggins, N. L., Trakimas, D., Chang, S. L., Ehrlich, A., Ray, P., Luker, K. E., Linderman, J. J., Luker, G. D. (2014) CXCR7 controls competition for recruitment of beta-arrestin 2 in cells expressing both CXCR4 and CXCR7. *PLoS One* **9**, e98328.

## KEY WORDS:

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