

Special Section: From Insight to Modulation of CXCR4 and ACKR3 (CXCR7) Function – Minireview

Atypical Chemokine Receptor 3 (ACKR3): A Comprehensive Overview of its Expression and Potential Roles in the Immune System

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Received December 14, 2018; accepted April 24, 2019

ABSTRACT

Atypical chemokine receptor 3 (ACKR3), previously known as C-X-C chemokine receptor type 7 (CXCR7), has emerged as a key player in several biologic processes, particularly during development. Its CXCL11 and CXCL12 scavenging activity and atypical signaling properties, together with a new array of other nonchemokine ligands, have established ACKR3 as a main regulator of physiologic processes at

steady state and during inflammation. Here, we present a comprehensive review of ACKR3 expression in mammalian tissues in search of a possible connection with the receptor function. Besides the reported roles of ACKR3 during development, we discuss the potential contribution of ACKR3 to the function of the immune system, focusing on the myeloid lineage.

Introduction

This review focuses on the trio formed by C-X-C motif chemokine ligand (CXCL)12 and its two receptors, C-X-C chemokine receptor (CXCR)4 and CXCR7/ACKR3, with particular emphasis on the latter, which belongs to the atypical chemokine receptor subfamily. Since the 1990s, with CXCR4 being discovered as a coreceptor for HIV entry (Feng et al., 1996), the CXCL12/CXCR4 axis has been extensively studied in numerous homeostatic and pathologic settings, including organogenesis, leukocyte trafficking, and cancer. Atypical

chemokine receptor (ACKR)3 was first known as an orphan receptor named *receptor dog cDNA 1*, or RDC1 (Heesen et al., 1998), and was later adopted into the chemokine receptor family as CXCR7, the second receptor for CXCL12 (Balabanian et al., 2005) and also CXCL11 (Burns et al., 2006), before being renamed ACKR3 owing to its atypical non-G-protein-dependent signaling (Bachelier et al., 2014). Since then, compelling evidence has underscored the regulatory function of ACKR3 on the CXCL12/CXCR4 signaling axis. Initial studies in zebrafish models revealed that ACKR3 acts as a scavenger receptor that binds and internalizes CXCL12, thus indirectly modulating CXCR4 function by modifying chemokine bioavailability (Dambly-Chaudière et al., 2007; Valentin et al., 2007; Boldajipour et al., 2008; Donà et al., 2013). Additionally, ACKR3 may have direct functions in response to CXCL12 as a β -arrestin-biased signaling receptor (Rajagopal et al., 2010), although β -arrestin-mediated signaling downstream of ACKR3 remains to be demonstrated *in vivo*. Furthermore, with the identification of new nonchemokine ligands, including macrophage migration inhibitory factor (MIF) or intermediate opioid peptides, ACKR3 has emerged as a key player in homeostatic processes during embryogenesis and adult life but also in pathologic inflammatory and tumor contexts.

This mini-review is part of the mini-review series entitled “From Insight to Modulation of CXCR4 and ACKR3 (CXCR7) Function.” This research was funded by the European Union’s Horizon 2020 MSCA Program [Grant agreement 641833 ONCORNET] to all authors, by European Union’s Infect-Era project HPV-Motiva [ANR-15-IFEC-0004-0] to F.B. and G.S.-L., and by a PRC ANR Grant OSTEOVALYMPH [17-CE14-0019] coordinated by K.B. All authors are members of the LabEx LERMIT supported by ANR grant [ANR-10-LABX-33] under the program “Investissements d’Avenir” [ANR-11-IDEX-0003-01]. C.G. is beneficiary of a fellowship from Fondation de la Recherche Médicale (FRM).

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<https://doi.org/10.1124/mol.118.115329>

ABBREVIATIONS: ACKR3, atypical chemokine receptor 3; BM, bone marrow; CXCL11, C-X-C motif chemokine ligand 11; CXCL12, C-X-C motif chemokine ligand 12; CXCR4, C-X-C chemokine receptor type 4; CXCR7, C-X-C chemokine receptor type 7; EGFP, enhanced green fluorescent protein; ERK, extracellular signal-regulated kinase; HSC, hematopoietic stem cell; MIF, macrophage migration inhibition factor.

Here, we first summarize the state of the art on ACKR3 expression regarding human and rodent tissues and its role in development before discussing its potential contribution to the function of the immune system. In particular, we focus on myeloid cells, both at homeostasis and in pathologic settings, including inflammatory conditions and breast cancer.

ACKR3 Expression in Mammalian Tissue

To address the question of ACKR3 expression, mice have been genetically modified to investigate in which cells and tissues the *Ackr3* promoter is active (Table 1). These mouse models include replacement of the endogenous *Ackr3* coding region by either a β -galactosidase (*LacZ*) reporter (Gerrits et al., 2008) or an enhanced green fluorescent protein (EGFP) sequence (Cruz-Orengo et al., 2011). These models also include the *Ackr3-EGFP* bacterial artificial chromosome mouse model, where an *Ackr3* promoter-EGFP fusion sequence was inserted into a random location in the genome. In this case, EGFP expression is driven by *Ackr3* promoter activity while leaving the endogenous

Ackr3 locus intact (Gong et al., 2003; Sánchez-Alcañiz et al., 2011).

In parallel, several groups have investigated *Ackr3* expression at the transcriptional level by means of northern blot, real-time polymerase chain reaction, or in situ hybridization (Table 2) and at the protein level by means of immunofluorescence, immunohistochemistry, or flow cytometry (Table 2). *Ackr3* mRNA is mostly detected in mouse heart, kidney, spleen, lung, and brain (Table 2) and is transiently expressed during embryogenesis, in accordance with different reporter mouse models (Table 1). To summarize, by combining various techniques, ACKR3 mRNA and protein have been detected 1) in mesenchymal stromal cells; 2) in brain-resident cells, including astrocytes, glial, and neuronal cells, 3) in cells of the vascular system and, more specifically, cells from vascular smooth muscle and venous endothelium; and of particular interest 4) in immune cell populations. In the immune system, ACKR3 mRNA is detected using transcriptomic approaches in hematopoietic lineages, in both lymphoid (e.g., B cells) and myeloid (e.g., macrophages) cells; however, in EGFP reporter mouse models, the *Ackr3* promoter-dependent signal cannot be distinguished from background in any of the studied

TABLE 1
Genetically modified mouse models to study *Ackr3* expression in vivo and associated phenotypes

Model	Genetic Background	Description	References
Constitutive deletion <i>Ackr3</i> ^{-/-}	C57Bl/6	Mice carrying loxP-flanked <i>Ackr3</i> exon 2 crossed with <i>Deleter</i> -Cre mice Phenotype: Perinatal death of >95% <i>Ackr3</i> ^{-/-} mice. Thickened semilunar valves. Normal development of B cells and granulocytes. Altered neuron migration during embryonic development.	Sierro et al. (2007), Sánchez-Alcañiz et al. (2011), Wang et al. (2011), Yu et al. (2011), Trousse et al. (2015)
<i>Ackr3</i> ^{-/-}	129 Sv/Ev x C57Bl/6	Knock-in of LacZ reporter in <i>Ackr3</i> exon 2 (IRES-LacZ/PGK-Neo cassette) Phenotype: Perinatal death of 70% <i>Ackr3</i> ^{-/-} mice. Nonviable mice: Myocardial degeneration, fibrosis, and cardiac hyperplasia. No defects in semilunar valves. Surviving mice: Cardiac hyperplasia in 25%. Normal lifespan, no hematopoietic or hematologic defects, no reproductive defects.	Gerrits et al. (2008)
Conditional (tissue- or cell type-specific) deletion <i>Tie2-Cre; Ackr3</i> ^{fllox/-}	C57Bl/6	Endothelium-specific deletion. Phenotype: Mice survive to adulthood and are fertile. Cardiac hypertrophy, thickened ventricular walls, and thickened semilunar valves in 40% of the mice.	Sierro et al. (2007), Yu et al. (2011)
<i>Dlx5/6-Cre; Ackr3</i> ^{fllox/fllox}	C57Bl/6	GABAergic neuron-specific deletion. Phenotype: Mice survive to adulthood. Altered migration of neurons during embryonic development (E16.5).	Sánchez-Alcañiz et al. (2011)
<i>Dlx112b-Cre; Ackr3</i> ^{fllox/-}	C57Bl/6 CD1	GABAergic neuron-specific deletion. Phenotype: Neuron positioning defects similar to those of constitutive <i>Ackr3</i> ^{-/-} mice.	Wang et al. (2011)
<i>Emx1-Cre; Ackr3</i> ^{fllox/-}	Not given	Glutamatergic neuron-specific deletion. Phenotype: No effect on the position of cortical projection neurons.	Wang et al. (2011)
<i>Tbr2-Cre; Ackr3</i> ^{fllox/fllox}	Not given	Glutamatergic neuronal progenitor-specific deletion. Phenotype: Altered migration of neurons in adulthood.	Abe et al. (2018)
<i>Dbx1-Cre; Ackr3</i> ^{fllox/fllox}	C57Bl/6	Cajal-Retzius neuron progenitor-specific deletion. Phenotype: Defects in the positioning of a subpopulation of Dbx1-expressing neurons during embryonic development (E14.5).	Trousse et al. (2015)
Conditional-inducible (cell type-specific inducible) deletion <i>Scl-Cre^{ERT}; Ackr3</i> ^{fllox}	C57Bl/6	Tamoxifen-inducible deletion of <i>Ackr3</i> from Scl-expressing cells (HSC, myeloid lineage, endothelium and regions in the central nervous system) in adult mice. Findings: ~35% increase in CXCL12 plasma levels. No other apparent phenotype described.	Stacer et al. (2016)
Reporter gene <i>Ackr3</i> ^{*/EGFP} <i>Ackr3-EGFP</i>	C57Bl/6 CD1	Replacement of <i>Ackr3</i> exon 2 by EGFP. No phenotype is described. BAC insertion of the <i>Ackr3</i> promoter fused to an EGFP coding region. Endogenous <i>Ackr3</i> locus remains intact. No secondary effects.	Cruz-Orengo et al. (2011) The Gene Expression Nervous System Atlas (GENSAT) Gong et al. (2003)

TABLE 2
ACKR3 mRNA and protein detection in mammalian tissue

System	Cell Type	Origin	Mouse/Rodent		Human		References
			mRNA	Protein	mRNA	Protein	
Haemato-poietic system	B cells	Peripheral blood, BM, and/or lymphoid organs	RNAseq	FC	Transcriptomics, RT-PCR	FC, IF	Infantino et al. (2006), Siervo et al. (2007), Heng et al. (2008), Tarnowski et al. (2010), Bjaajoux et al. (2012), Wang et al. (2012), Melo et al. (2014), Alampour-Rajabi et al. (2015)
	T cells (CD4+, regulatory, helper, memory)		RNAseq		Transcriptomics, RT-PCR	FC, IF	Balabanian et al. (2005), Infantino et al. (2006), Siervo et al. (2007), Heng et al. (2008), Tarnowski et al. (2010), Bjaajoux et al. (2012), Melo et al. (2014)
	Innate lymphoid cells		RNAseq		Transcriptomics, RT-PCR		Heng et al. (2008)
	NK cells		RNAseq		Transcriptomics, RT-PCR		Infantino et al. (2006), Siervo et al. (2007)
	Dendritic cells		RNAseq		Transcriptomics, RT-PCR	FC, IF	Infantino et al. (2006), Siervo et al. (2007), Heng et al. (2008)
	Monocytes		RNAseq		RT-PCR	FC, IF	Infantino et al. (2006), Tarnowski et al. (2010), Chatterjee et al. (2015)
	Neutrophils		RNAseq			FC	Heng et al. (2008)
	Macrophages		RNAseq			FC	Heng et al. (2008), Chatterjee et al. (2015)
	Basophils		RT-qPCR		RT-PCR	FC	Infantino et al. (2006)
	Mesenchymal stem cells	BM			Transcriptomics, RT-PCR	FC	Kim et al. (2016), Liu et al. (2018), Kuçi et al. (2019)
Circulatory / lymphatic system	ND	Whole (heart)	Lac-Z reporter, northern blot		Northern blot		Burns et al. (2006), Gerrits et al. (2008), Berahovich et al. (2014)
	Venous endothelium	Digestive tract, heart, kidney, lung, liver, lymphoid organs	Lac-Z reporter (except liver)			IHC	Berahovich et al. (2014)
	Vascular smooth muscle cells	Heart, nervous system, kidney, digestive tract, skeletal muscle, lymphoid organs	RT-PCR			IHC	Neusser et al. (2010), Rajagopal et al. (2010), Berahovich et al. (2014)
	Sinusoidal cells	Spleen		IHC		IHC	Berahovich et al. (2014)
	Endothelial cells	Kidney				IHC	Neusser et al. (2010)
Nervous system	ND	Kidney lymphatic vessels	RT-qPCR			IF	Neusser et al. (2010)
	ND	Vena cava	RT-qPCR				Klein et al. (2014)
	ND	Thoracic duct					Klein et al. (2014)
	ND	Brain (whole)	Lac-Z reporter, ISH, northern blot		Northern blot		Burns et al. (2006), Gerrits et al. (2008), Thelen and Thelen (2008), Yu et al. (2011), Berahovich et al. (2014)
	ND	Brain vasculature	EGFP reporter, ISH			FC (intracellular)	Schönemeier et al. (2008), Cruz-Orengo et al. (2011)
Respiratory system	Astrocytes	Brain		IHC			Calatuzzolo et al. (2011), Puchert et al. (2017)
	ND	Lung (whole)	RT-PCR, northern blot	IF	Northern blot		Burns et al. (2006), Berahovich et al. (2014), Cao et al. (2016), Konrad et al. (2017), Ngamsri et al. (2017)
	ND	Lung vessels	Lac-Z reporter				Gerrits et al. (2008)

(continued)

TABLE 2—Continued

System	Cell Type	Origin	Mouse/Rodent		Human		References
			mRNA	Protein	mRNA	Protein	
Secretory system	ND	Kidney (whole)	Northern blot		RT-qPCR, northern blot	IHC	Burns et al. (2006), Neusser et al. (2010), Maishi et al. (2012), Berahovich et al. (2014), Mazzinghi et al. (2008)
	Renal multipotent progenitors	Patient-derived healthy kidney tissue			RT-qPCR	IF	
	ND	Kidney tubules	Lac-Z reporter			IHC	Neusser et al. (2010), Berahovich et al. (2014)
	ND	Kidney glomeruli	Lac-Z reporter				Gerrits et al. (2008)

BM, bone marrow; FC, flow cytometry; IF, immunofluorescence; ISH, in situ hybridization; IHC, immunohistochemistry; ND, not determined; NK, natural killer; RNaseq, RNA sequencing; RT-PCR, reverse transcription polymerase chain reaction; RT-qPCR, real time quantitative polymerase chain reaction.

immune cell subsets in steady state (i.e., CD45⁺, CD19⁺ B cells, CD4⁺ and CD8⁺ T cells, CD11b⁺ and CD11c⁺ myeloid cells) (Cruz-Orengo et al., 2011). This was confirmed in LacZ mouse models (Berahovich et al., 2014). Furthermore, assessing ACKR3 protein expression in native conditions poses a technical challenge from its constitutive recycling between the membrane and the endosomal compartment, leading to a predominant intracellular localization. This is a limitation for antibody generation and validation (Berahovich et al., 2010a) and a potential source of discrepancies reported in studies related to ACKR3 expression and function. For instance, ACKR3 protein was not detected either in human and mouse leukocyte subsets in peripheral blood (Berahovich et al., 2010); however, ACKR3 protein was detected in human secondary lymphoid organ-derived B cells and dendritic cells (Infantino et al., 2006).

ACKR3 Function: From Genetically Modified Mice to Nonchemokine Ligands

Determining when and where ACKR3 is expressed has led to greater understanding of the functions that ACKR3 might be exerting. In particular, the expression pattern of ACKR3 may hint toward a functional role in such cells or tissues. These functions could have a direct effect on ACKR3-expressing cells either through noncanonical signaling pathways or modulation of CXCR4 functions or in a paracrine way via the modulation of CXCL12 and CXCL11 levels, impacting nearby-cell function through CXCR4 and CXCR3, respectively. Valuable information about the function of ACKR3 was first provided by an analysis of the effects after constitutive and cell-type conditional *Ackr3* gene deletion in various mouse models (Table 1). Subsequently, identification of other nonchemokine ligands has broadened our understanding of ACKR3 biology, particularly in terms of the role of ACKR3 beyond the chemokine system. In the following section, we discuss how the study of knockout mouse models and nonchemokine ligands has led to further insights into the functions of ACKR3.

Lessons from Constitutive and Conditional Knockout Models. Most *Ackr3*^{-/-} mice develop normally in early embryonic stages but die either perinatally or in utero in late developmental stages, usually from embryonic gestation day E17.5, owing to cardiovascular complications (Sierra et al., 2007; Yu et al., 2011; Trouse et al., 2015). Accordingly, it seems that ACKR3, similarly to CXCR4 and CXCL12, is essential to normal mouse development and physiology and that it plays a complementary or nonredundant role with regard to CXCR4; however, the lethal phenotype obtained in the C57Bl/6 background is less severe on a mixed genetic background (129 Sv/Evbrd x C57Bl/6) with a survival rate of approximately 30% (Gerrits et al., 2008). This might be linked to CXCL11-associated ACKR3 functions because expression of this chemokine ligand is absent in C57Bl/6 mice (Sierra et al., 2007). Considering the cardiovascular and cerebral defects observed in *Ackr3*-deficient mice, a large body of work has focused on ACKR3 contribution to heart and brain physiology. During both heart and brain development, *Ackr3* undergoes a change in expression pattern after E14.5,

which coincides with the onset of mouse death (Sierro et al., 2007; Gerrits et al., 2008; Sánchez-Alcañiz et al., 2011; Wang et al., 2011; Yu et al., 2011).

Two studies suggest that a link may exist between *Ackr3* expression and the control of cell proliferation in heart tissue. For example, in constitutive *Ackr3*^{-/-} mice, increased cell proliferation prevented heart-valve thinning that leads to a lethal cardiovascular phenotype (Yu et al., 2011). Moreover, in another study, 25% of surviving adult *Ackr3*^{-/-} mice suffered from cardiac hyperplasia (Gerrits et al., 2008). Of note, migration and apoptosis of semilunar valve mesenchymal cells remained normal from E14 to E18.5 in *Ackr3*^{-/-} mice. In contrast, during brain development, constitutive and conditional loss of *Ackr3* in GABAergic neurons (Table 1) led to an abnormal distribution of interneurons in the cortex, suggesting a link between *Ackr3* expression and neuron migration (Sánchez-Alcañiz et al., 2011; Wang et al., 2011; Trousse et al., 2015). ACKR3 could have a cell intrinsic function as suggested by extracellular signal-regulated kinase (ERK)1/2 phosphorylation in cultured neurons, likely downstream of ACKR3 (Wang et al., 2011); however, it remains to be determined whether ACKR3-mediated ERK1/2 phosphorylation occurs in vivo and whether it is relevant during brain development. Alternatively, the role of ACKR3 in interneuron positioning could occur through cell-extrinsic effects as a scavenger receptor. *Ackr3* deficiency results in increased CXCL12 protein levels but unchanged *Cxcl12* mRNA levels in cortical homogenates (Sánchez-Alcañiz et al., 2011). Failure to maintain a CXCL12 gradient leads to accumulation of migrating interneurons in inappropriate locations in the cortex. Moreover, the abnormal distribution of interneurons was rescued when *Ackr3*^{-/-} interneurons were transplanted into *Ackr3*^{+/+} brain, suggesting that ACKR3 expression in other cells can rescue the phenotype (Sánchez-Alcañiz et al., 2011). Furthermore, a recent report has provided mechanistic insights into ACKR3-mediated CXCL12 endocytosis in interneurons by demonstrating that receptor phosphorylation was required for this process, whereas β -arrestins were dispensable (Saaber et al., 2019). Together, these findings in *Ackr3*-deficient mice support a role for ACKR3 as a scavenging receptor, particularly in the brain. Even though no obvious defects in the immune system were found in these mouse models, this aspect was not fully explored in the studies, implying that a knowledge gap might exist in this field (discussed in *Potential Role of ACKR3 within the Immune System at Steady State and during Inflammation*).

Microenvironment-Dependent Functions of ACKR3 and Nonchemokine Ligands. Differences observed in heart and brain tissues in *Ackr3*^{-/-} mice can, at least partially, be explained by the microenvironment having an impact on the biologic effects exerted by ACKR3. ACKR3 tissue-dependent functioning might be related to its capacity to interact with several ligands and therefore could be dependent on the surrounding cells producing or processing such ligands. First, within the chemokine system, CXCL12 displays six isoforms in humans and three in mice owing to alternative splicing. These isoforms have different C-terminal extensions and different expression patterns and functions [discussed in Janssens et al. (2018)]. Second, CXCL12 isoforms are processed post-translationally by the microenvironment, producing forms of CXCL12 with different binding and signaling properties on CXCR4 and ACKR3 (Peng et al.,

2012; Janssens et al., 2017, 2018; Szpakowska et al., 2018b). Additionally, the microenvironment changes during disease conditions. For example, CXCL11, which is a ligand for CXCR3, as well as for ACKR3, is normally not detectable in physiologic conditions but an inflammatory context, in which cytokines such as interferon are produced, can induce CXCL11 expression (Flier et al., 2001; Müller et al., 2010; Van Raemdonck et al., 2015; Singh et al., 2016). This implies that ACKR3 function may vary in pathologic conditions compared with steady state (Fig. 1), adding another layer of complexity. Lastly, ACKR3 tissue-dependent functions likely depend on the presence of other reported endogenous ligands of ACKR3 outside the chemokine system, including MIF (Alampour-Rajabi et al., 2015), intermediate opioid peptides (Ikeda et al., 2013), and possibly proteins in the adrenomedullin pathway (Klein et al., 2014).

MIF is an inflammatory cytokine that lacks the structural requirements to qualify as a chemokine; however, a previous study has suggested it should be considered as a pseudo-CXC chemokine (Bernhagen et al., 2007) as it binds with high affinity to CD74 ($K_d \approx 9$ nM), CXCR2 ($K_d \approx 1.4$ nM), CXCR4 ($K_d \approx 19.8$ nM), and to ACKR3 in the nanomolar range (K_d not determined) (Leng et al., 2003; Alampour-Rajabi et al., 2015; Bernhagen, 2018). MIF has a physiologic role as a chemoattractant (Bernhagen et al., 2007) and is involved in innate and adaptive immune responses by promoting macrophage activation and B-cell survival (Gore et al., 2008). Moreover, MIF is a mediator in several inflammatory conditions and cancers in an autocrine and paracrine manner by promoting tumor growth (Nobre et al., 2017) and inducing metastasis through CXCR4 (Dessein et al., 2010). Furthermore, MIF/ACKR3 signaling has been studied in platelets, where it prevents apoptosis (Chatterjee et al., 2014), providing evidence for a role of ACKR3 in the hematopoietic system.

Intermediate opioid peptides, such as BAM22, are produced in the adrenal cortex by subcapsular cell hyperplasia cells, and BAM22 has been shown to displace CXCL12 from ACKR3 ($IC_{50} = 32.2$ nM) (Szpakowska et al., 2018a). The BAM22/ACKR3 signaling axis has a critical role in the modulation of circulating glucocorticoids, which occurs through the increase of the amplitude of adrenocorticotrophic hormone-induced glucocorticoid diurnal oscillation in females (Ikeda et al., 2013). ACKR3 is highly expressed in the adrenal glucocorticoid-producing cells, especially in female mice compared with males, in support of the sex differences of the BAM22/ACKR3-dependent glucocorticoid oscillations.

Finally, adrenomedullin is a peptide hormone involved in angiogenesis and is implicated in cardiovascular diseases. A link between adrenomedullin and ACKR3 pathways may exist on the basis that haploinsufficiency of adrenomedullin partially rescued the lethal defects in *Ackr3*^{-/-} mice by normalizing the cardiac hyperproliferation (Klein et al., 2014). ACKR3 was suggested as an adrenomedullin scavenger, but a recent article showed that adrenomedullin does not displace CXCL12 from ACKR3 within the 6 pM to 1 μ M range (Szpakowska et al., 2018a); however, the presence of ACKR3 inhibited canonical adrenomedullin signaling (Klein et al., 2014), suggesting a crosstalk between adrenomedullin and ACKR3 pathways that remains to be fully explored.

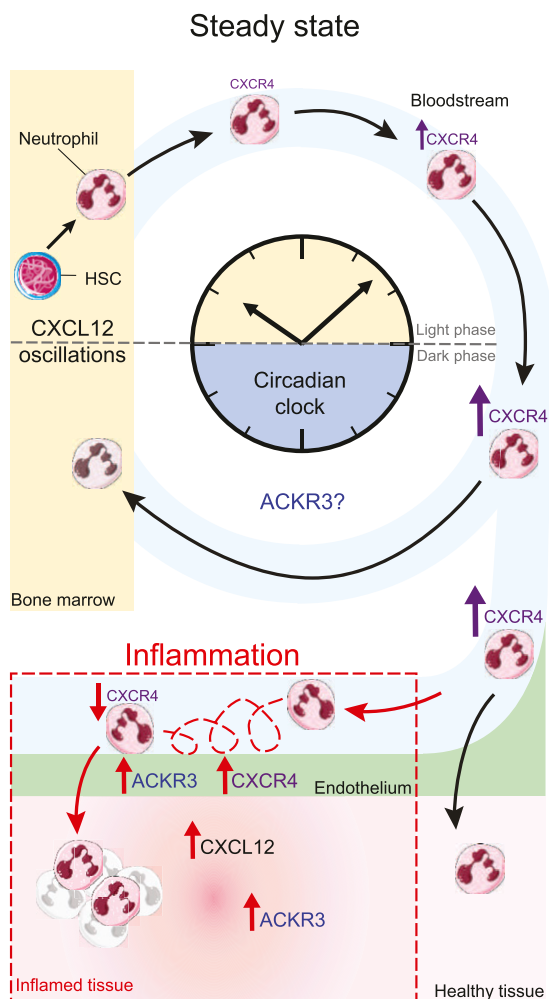


Fig. 1. Potential roles of ACKR3 in steady state and inflammation within the myeloid cell compartment. Neutrophils are produced in bone marrow (BM) from hematopoietic stem cells during granulopoiesis. They are released into the bloodstream after circadian oscillations and increase their surface CXCR4 expression while aging over time. After approximately 12 hours in circulation, at the end of the dark phase, aged CXCR4^{high} neutrophils migrate back to BM to be eliminated. As part of their patrolling function, they can migrate into healthy tissues. The role of ACKR3 is unknown at steady state, but it could potentially contribute to either the circadian oscillations of CXCL12 within BM via its scavenging activity or to the rhythmic release of neutrophils. When circulating neutrophils encounter inflammatory signals, they can adhere and roll on endothelium and extravasate from the bloodstream to infiltrate inflamed tissue, where they accumulate. Inflammation leads to an upregulation of CXCL12 within the tissue as a cue to attract immune cells. Furthermore, during inflammation, CXCR4 and ACKR3 are upregulated on endothelium as shown in lung inflammatory conditions. In addition, ACKR3 is reported to be upregulated in inflamed tissue (for example in lung alveolar epithelium upon lung inflammation), but its role in CXCL12-level regulation and subsequent immune cell recruitment is not completely understood.

Potential Role of ACKR3 within the Immune System at Steady State and during Inflammation

The absence of obvious immune-hematopoietic defects in the available *Ackr3*^{-/-} mouse models does not exclude a role for ACKR3 in the immune system. Evidence suggests that the CXCL12/CXCR4 signaling axis can regulate the function of the immune system, notably by controlling immune cell subset migration and compartmentalization (Wei et al., 2006; Balabanian et al., 2012) or hematopoietic stem cell (HSC) homing, retention, and quiescence in bone marrow (BM)

(Sugiyama et al., 2006). ACKR3 mRNA and protein are also expressed in certain immune cell subsets, such as B cells and myeloid cells, as reported by several groups (Table 2). ACKR3 might also be involved in the circadian oscillation of CXCL12 expression levels, which regulate immune cell trafficking from and to BM (Fig. 1). In a similar manner to glucocorticoids, CXCL12 transcript and protein levels rhythmically oscillate in BM with light/dark cycles (Katayama et al., 2006; Méndez-Ferrer et al., 2008, 2010). This oscillation regulates retention in and mobilization from BM of CXCR4-expressing HSCs, which are released during sleep when CXCL12 levels are low and return to BM when CXCL12 levels have increased again (Méndez-Ferrer et al., 2010). CXCL12 is produced by osteoblasts in the bone fraction, endothelial cells around both endosteal and vascular niches, and perivascular mesenchymal stromal cells in the marrow fraction, with the latter representing a major contributor to the CXCL12 pool (Itkin et al., 2016). Depending on the production site, CXCL12 regulates either HSC maintenance or retention (Itkin et al., 2016; Asada et al., 2017). Considering that the ACKR3 scavenging function shapes CXCL12 gradients, the contribution of ACKR3 to the circadian oscillation of CXCL12 levels remains an open question (Fig. 1). Although most of this evidence relates to CXCL12/CXCR4 function, they indirectly point toward a role for ACKR3 in some processes within the immune system, both at steady state and in inflammatory settings. In the following sections, we explore this apparent knowledge gap focusing on myeloid cells.

Potential Role for ACKR3 in the Myeloid Compartment at Steady State. Myeloid cells, such as neutrophils, dendritic cells, or monocytes, are key players in innate immunity, and CXCL12/CXCR4 tightly regulates their homeostasis (De Filippo and Rankin, 2018), particularly their retention in BM and functioning in peripheral tissues (Chong et al., 2016; Evrard et al., 2018). CXCL12 promotes the extravasation of monocytes and their *in vitro* differentiation (Sánchez-Martín et al., 2011; Chatterjee et al., 2015), as well as the egress of plasmacytoid dendritic cells from BM (Chopin et al., 2016). Among myeloid cells, neutrophils are the most abundant type in peripheral blood. They are produced and released from BM after daily oscillations; consequently, neutrophil numbers in circulation vary during light/dark cycles (Ella et al., 2016). Neutrophils have a short lifespan in circulation (~12 hours) and when senescent, “aged” neutrophils express high levels of CXCR4, allowing them to migrate back to BM to be eliminated in a process called *clearance* (Fig. 1) (Casanova-Acebes et al., 2013). Their egress from BM might be partly due to changes in CXCL12 levels with the circadian rhythms (Méndez-Ferrer et al., 2008; Ella et al., 2016), as described for HSCs; however, other mechanisms may account for neutrophil release as it precedes CXCL12 oscillations (Casanova-Acebes et al., 2013). Recently, clock genes have been described as intrinsic aging regulators in neutrophils in combination with CXCR2 and CXCR4 (Adrover et al., 2019). Disruption of the aging process has consequences on immune cell trafficking at steady state and immune defense against infection. Although ACKR3 is detected in neutrophils, further studies are needed to determine its expression levels during neutrophil maturation and its possible contribution to this process. There is also a case for exploring whether cells of the microenvironment (e.g., cells in BM niches) express ACKR3 and how these factors could be related to neutrophil biology.

ACKR3 and the Myeloid Compartment in Inflammatory Settings. ACKR3 expression is usually faint or undetectable at steady state in the endothelium and in myeloid cells, but it can be upregulated during inflammation, for instance, by proinflammatory cytokines, such as interleukin-8 (Singh and Lokeshwar, 2011) or IL-1 β in vitro (Watanabe et al., 2010) and by environmental cues, such as lipopolysaccharide (Cao et al., 2016; Konrad et al., 2017; Ngamsri et al., 2017) or during infection by oncoviruses [reviewed in Freitas et al. (2014)]. Along this line, ACKR3 is highly upregulated during monocyte-to-macrophage differentiation in vitro, switching to a more pro-inflammatory cell phenotype (Ma et al., 2013; Chatterjee et al., 2015). Another example can be found during central nervous system inflammation, where ACKR3 is upregulated in endothelial cells of the blood-brain barrier (Cruz-Orengo et al., 2011). Antagonizing the scavenging activity of ACKR3 using small-molecule CCX771 blocked leukocyte infiltration in the parenchyma, including that of CD11b⁺ myeloid cells, preventing chronic inflammation and therefore improving disease recovery (Cruz-Orengo et al., 2011). This could be associated with a restoration of the CXCL12 polarity along the blood-brain barrier, which is essential for its integrity and for preventing infiltration of CXCR4⁺ cells (McCandless et al., 2008).

Furthermore, the role of ACKR3 has been explored in pulmonary inflammation regarding the lung epithelial barrier function and the recruitment of myeloid cells (Fig. 1). In an acute inflammation mouse model induced by lipopolysaccharide inhalation, ACKR3 protein is upregulated in the lung tissue, both in epithelial and endothelial cells (Ngamsri et al., 2017); however, in chronic lung injury, mouse models induced upon repeated bleomycin injection or hydrochloric acid inhalation, ACKR3 mRNA and protein levels are decreased in endothelial cells (Cao et al., 2016). These findings indicate that ACKR3 may play a role in the early stages of inflammation. Interestingly, in acute inflammation, CXCL12 mRNA and total protein levels in lung homogenates were increased (Cao et al., 2016; Konrad et al., 2017; Ngamsri et al., 2017), and at least mRNA levels remained high in chronic inflammatory settings (Cao et al., 2016). Regarding immune cell recruitment, neutrophils were recruited to the lung tissue in acute inflammation (Konrad et al., 2017; Ngamsri et al., 2017), whereas macrophages were recruited in chronic inflammation (Cao et al., 2016); however, these studies did not explore neutrophil and macrophage recruitment in both short- and long-term inflammatory processes. Pharmacologic modulation of ACKR3 with either CCX771 (Ngamsri et al., 2017) or TC14012 (Cao et al., 2016) prevented microvascular permeability and further alveolar epithelial damage. Although both molecules induce β -arrestin recruitment to ACKR3, to our knowledge, the downstream signaling pathways have not been assessed (Zabel et al., 2009; Montpas et al., 2015). These molecules can be considered functional antagonists because of their capacity to displace CXCL12 from ACKR3, thus inhibiting the decoy activity of the receptor. Whereas the potential therapeutic benefit of targeting ACKR3 is promising, it cannot be claimed so far which function of ACKR3 contributes to disease improvement (i.e., as a signaling or scavenging receptor). It will be essential to determine where ACKR3 is being expressed using reporter mouse models to decipher its potential protective or proinflammatory role in central nervous system and pulmonary inflammatory diseases.

Lastly, chronic inflammation can promote the progression of cancer (Hanahan and Weinberg, 2011) and is often initiated and maintained by infiltrating immune cells that secrete cytokines and chemokines in the tumor microenvironment (Nagarsheth et al., 2017). For example, in breast cancer, the microenvironment likely induces myeloid-derived suppressor cells that contribute to immune evasion and consequently sustain tumor growth [reviewed in Markowitz et al. (2013)]. CXCL12 also plays an important role in breast cancer. Indeed, CXCL12 production by cancer-associated fibroblasts enhances proliferation and survival of cancer cells, as well as tumor growth and angiogenesis (Orimo et al., 2005). Additionally, CXCL12 facilitates tumor cell intravasation by affecting vasculature integrity (Ahirwar et al., 2018) and contributes to immune evasion by recruitment of T cells, which differentiate into immunosuppressive regulatory T cells in the tumor (Su et al., 2017; Costa et al., 2018). Furthermore, sites in the body that constitutively display high concentrations of CXCL12, such as lung or BM, are common metastatic destinations for breast cancer cells (Müller et al., 2001). Interestingly, blockade of CXCR4 with antagonist AMD3100 in breast cancer mouse models reduced the number of immunosuppressive regulatory T cells and neutrophils in the tumor, improving the immunosuppressive microenvironment (Chen et al., 2019). CXCR4 blockade further reduced activated cancer-associated fibroblasts numbers and increased the transcription of genes associated with antitumor immunity, such as *Ifng* and *Gzma* in the tumor mass.

ACKR3 is upregulated in several types of cancer, including breast cancer, frequently in tumor-associated vasculature, as well as in the primary tumors (Freitas et al., 2014). The clinical relevance of ACKR3 in breast cancer has been discussed elsewhere as a part of the mini-review series "From Insight to Modulation of CXCR4 and ACKR3 (CXCR7) Function" (Neves et al., 2019). Studies using mouse orthotopic xenografts suggest that ACKR3 might play a role in maintaining proliferation in the primary tumor while decreasing intravasation of tumor cells and thus reducing metastasis (Hernandez et al., 2011). Furthermore, ACKR3 endothelial expression is likely involved in preventing breast cancer metastasis (Stacer et al., 2016). Collectively, these findings highlight the dual role of ACKR3 either expressed in the primary tumor or in the tumor-associated vasculature. Further research is warranted to understand the underlying mechanisms of ACKR3 that impact the different stages of breast cancer progression and whether this occurs through ACKR3 itself or via modulation of the CXCL12/CXCR4 axis. ACKR3 could shape CXCL12 (and likely CXCL11) availability within the tumor owing to its scavenging activity, which could decrease tumor cell intravasation and metastasis as reported (Hernandez et al., 2011; Stacer et al., 2016; Ahirwar et al., 2018) and potentially immune cell infiltration. In essence, decreasing CXCL12 availability could inhibit CXCR4 responses and thus reverse the immunosuppressive microenvironment in breast cancer, including reduced Treg and increased cytotoxic T-cell numbers.

Discussion

After being orphaned in 2005, CXCR7 was proposed to act as an atypical chemokine receptor, ACKR3, with its primary role as a decoy receptor for CXCL12 and CXCL11, whose

function was to merely internalize the ligands. Over the years, accumulating evidence has supported the concept of ACKR3 being a major regulator of the CXCL12/CXCR4 axis and possibly CXCL11/CXCR3. Furthermore, in some cell lines ACKR3 displays β -arrestin-biased signaling capacity in vitro in response to its chemokine and non-chemokine ligands, an observation mainly supported by β -arrestin recruitment and ERK1/2 phosphorylation studies (Zabel et al., 2009; Rajagopal et al., 2010; Wang et al., 2011; Alampour-Rajabi et al., 2015); however, ERK1/2 phosphorylation has not been formally demonstrated to be β -arrestin-mediated for ACKR3 and particularly in in vivo settings. Moreover, this concept has recently been challenged, as a panel of known β -arrestin-biased receptors unexpectedly required functional G proteins to elicit ERK1/2 phosphorylation activity, whereas β -arrestins were not essential (Grundmann et al., 2018). This result can be highly relevant to ACKR3, which is already known to engage with, but not activate, G proteins (Levoye et al., 2009). In addition, studies on CRISPR-Cas9-mediated β -arrestin knockout cell lines have shown that β -arrestins might not be necessary for ERK1/2 phosphorylation (Luttrell et al., 2018). Instead, they may act as regulators that fine-tune ERK1/2 phosphorylation, depending on the cell type and its strength in potentiating G-protein or β -arrestin-mediated signaling (Luttrell et al., 2018). Together, these recent findings raise questions about the molecular mechanisms by which ACKR3 is exerting its still underappreciated functions in homeostatic processes (e.g., in hormonal and neuronal systems and potentially in the hematopoietic system).

To conclude, ACKR3 expression and function in immune cells remain poorly understood, and future research should focus on: 1) unambiguously characterizing the ACKR3 expression patterns in physiologic and pathologic contexts; 2) clarifying the mechanisms by which ACKR3 acts as a signaling or a scavenging receptor; and 3) understanding its function in homeostatic processes, such as the circadian oscillation of CXCL12 levels or neutrophil trafficking, as well as in pathologic conditions (Fig. 1). Importantly, inflammation-related pathologic conditions can highly dysregulate ACKR3 expression. Thus, mechanistically deciphering the precise contribution of ACKR3 to immune cell recruitment in inflammatory context should identify ACKR3 as a novel therapeutic target in various diseases and cancer.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Koenen, Bachelierie, Balabanian, Schlecht-Louf, Gallego.

Note Added in Proof—The current affiliation of the third author was changed from that used for the Fast Forward version published April 30, 2019.

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