Atypical chemokine receptors in renal inflammation

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Abstract: Chemokines are structurally related proteins which form a large family of chemotactic cytokines. They provide a general communication system for cells and regulate lymphocyte migration. These proteins orchestrate the formation of microenvironments in lymphoid tissue, promote lymphoid organogenesis and help foster vascular and lymphatic angiogenesis. In addition to the classical G protein-coupled chemokine receptors, many chemokines also bind to a family of nonsignaling proteins, now called interceptors (chemokine-internalizing proteins). Here we summarize recent data on the role of interceptors in chemokine biology with a focus on renal inflammation.

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Minireview
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Introduction

Chemokines are members of a family of chemotactic factors, defined by a conserved motif of cysteine residues [1, 2]. The position of the 2 N-terminal cysteines defines 4 chemokine subgroups, with the 2 large groups having their cysteines next to each other (CC) or being separated by a single amino acid (CXC). Chemokines function via 7 transmembrane-spanning G protein-coupled receptors which signal upon chemokine binding. Traditional chemokine receptors bind ligands of a single subgroup, and they are defined accordingly (CCRs, CXCRs, CX3CR1, XCR1). Activation of chemokine receptors typically triggers phospholipase C activation via the βγ-subunits (released from Gαi) resulting in calcium mobilization [2, 3].

As inflammatory cells migrate from the bloodstream, a chemokine signal is necessary for firm adhesion to the endothelium of venules. Therefore, chemokines need either to be expressed by endothelial cells, deposited on endothelial surfaces by platelets, or be transported through the endothelial layer, to be presented to passing leukocytes [4]. Having left the vessels, inflammatory cells travel through interstitial tissues most likely following haptotactic (matrix-bound) gradients towards the site of injury [3]. Defined structures of the extracellular matrix, particularly glycosaminoglycans and nonsignaling chemokine-binding proteins, are sites of chemokine presentation, and in the case of the interceptors, also transport [5]. These presenting structures change during inflammation [6]. Additionally, chemokine function is modified by the formation of dimers and multimers, occurring in the ligands and receptors [3].

An additional cascade of interactions between chemokines, soluble mediators, adhesion molecules and chemo-
Kine receptors are involved when cells exit the inflamed organ. Inflammatory cells (e.g. lymphocytes, dendritic cells) exit tissues through the lymphatic vessels. The role of the lymphatics, and particularly the lymphatic endothelial cells in this process, is a developing area of research. In the last 2 years, excellent reviews have summarized many aspects of the current literature on the role of chemokines in renal diseases [7–11]. In this brief review, we will discuss the family of nonsignaling receptors, called interceptors or atypical receptors, which have recently been shown to modify chemokine functions [12].

**Interceptors, Nonsignaling or Atypical Chemokine Receptors**

The atypical chemokine receptors are a family of 7 transmembrane-spanning proteins, which bind chemokines but do not signal via the typical G protein-mediated pathways [13–15]. This is thought to be due to the lack of the typical conserved sequence, the DRYLAIV motif, which is usually present on the second intracellular loop and normally enables G protein coupling [16]. Currently, members of this family include the Duffy antigen/receptor for chemokines (DARC), the protein D6 and the Chemocentryx chemokine receptor (CCX-CKR [12, 15]). In addition, the receptor CXCR7, which does not appear to signal via the classical pathways, has been provisionally associated with the interceptors.

As compared to chemokine receptors, the interceptors usually show a broader binding spectrum of chemokines, even binding ligands of different subgroups (table 1). Multiple functions for interceptors have been proposed which appear to depend in part on the particular microenvironment studied. The following functions have been suggested and/or documented under specific conditions.

- Interceptors are thought to compete with typical receptors by acting as scavengers (e.g. DARC expressed on red blood cells).
- Binding of chemokines at a site of high concentration and the release at lower concentrations might increase the half-life in the circulation (‘slow release substance’).
- Interceptors appear to bind and internalize chemokines leading to their degradation (as demonstrated for D6).
- Chemokines can be shuttled through cells and presented on the other side of the cells (as illustrated for DARC [17]). This decreases the concentration on one side of the cell, but increases it on the other side (e.g. the endothelial surface).
- Interceptors have been proposed to act as potential presentation structures on the endothelial surface.
- Interaction between typical and atypical receptors may help moderate the response of the typical receptor to the corresponding ligand (as demonstrated for CXCR7).

### Table 1. ‘Nonsignaling’ chemokine-binding proteins

<table>
<thead>
<tr>
<th>Interceptor</th>
<th>Ligands (homeostatic)</th>
<th>Ligands (inflammatory)</th>
<th>Site of expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>DARC</td>
<td>CCL2, CCL5, CCL7, CCL11, CCL13, CCL14, CCL17, CXCL5, CXCL6, CXCL8, CXCL11 [17]</td>
<td>red blood cells, blood endothelium (high endothelial venules, peritubular capillaries in the kidney), some lymphatic endothelial cells</td>
<td></td>
</tr>
<tr>
<td>D6</td>
<td>CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL13, CCL14,</td>
<td>lymphatic endothelial cells</td>
<td></td>
</tr>
<tr>
<td>CCX-CKR</td>
<td>CCL19, CCL21, CCL25</td>
<td>bronchial alveolar cells</td>
<td></td>
</tr>
<tr>
<td>CXCR7</td>
<td>CXCL12</td>
<td>CXCL11</td>
<td>blood and lymphatic endothelial cells, lymphocytes</td>
</tr>
</tbody>
</table>

**DARC in Renal Inflammation**

DARC binds 11 inflammatory chemokines of the CC and CXC groups (table 1) with a high affinity, but does not bind noninflammatory chemokines [18, 19]. This protein was initially identified as the Duffy red blood group antigen. As such it can be involved in transfusion reactions, and it mediates invasion of red blood cells by malaria parasites [4, 20, 21]. In addition to red blood cells, DARC is also expressed on a subset of endothelial cells, particularly the high endothelial venules in lymphoid tissue, venules of the skin, and peritubular capillaries in the kidney [22–27]. DARC has also been described on some regions of lymphatic vessels, the so-called pre-collectors [28]. In this regard, DARC appears to be expressed on blood endothelium at the sites of recirculation and extravasation of inflammatory cells [23–25, 27].
DARC has also been proposed to modulate the chemokine milieu at the lymphatic ‘exit site’ [28]. During renal inflammation, for example, in human crescentic glomerulonephritis as well as in all forms of allograft rejection (interstitial, vascular and humoral), the number of interstitial DARC-positive vessels have been shown to increase [23, 24]. We recently demonstrated that DARC on endothelial cells internalized chemokines on the basal side and mediated chemokine transcytosis [17]. This resulted in apical retention of intact chemokines and increased leukocyte migration across monolayers expressing DARC [17]. Mice overexpressing DARC on blood vessel endothelium displayed enhanced chemokine-induced leukocyte extravasation and contact hypersensitivity reaction [17]. The restricted expression of DARC in the tubulointerstitium, and the positive association with CCR5-positive T cells in the corresponding compartment, also suggested a role for transcytosis/presentation by DARC on specific endothelial cells in the human kidney [29].

_Darc_-deficient mice have been used in 2 studies to better characterize the potential function of DARC in renal inflammation. In neutrophil-mediated mouse models (lipopolysaccharide injection and ischemia-reperfusion injury), deficiency of DARC resulted in a significant reduction in leukocyte recruitment and improved morphology. In particular, the neutrophil recruitment to the post-ischemic kidney was significantly reduced in _Darc_-deficient mice [30]. We performed unilateral ureteral ligation and nephrotoxic serum nephritis studies in the _Darc_-deficient mice [31]. In both models, while an early increase in T cell recruitment was seen, at later time points, the chronic lesions and functional data were not different between the _Darc_-deficient and wild-type mice [31]. This suggests that while preserved recruitment of leukocytes is seen in chronic disease models in the absence of endothelial DARC, in acute neutrophil-dependent models, the injury process was ameliorated. Unfortunately, the overall interpretation is still difficult because of the complex functional consequences of DARC deficiency both on endothelial cells and erythrocytes. Depending on the microenvironment studied, the absence of DARC may have pro- or even anti-inflammatory effects. These questions will await experiments in which mice with selective cell-specific DARC deficiency can be used (deficiency on red blood cells versus endothelial cells).

**CXCR7 – A New Partner for Two Well-Known Chemokines**

For a long time, the CXCR4 receptor was thought to be the only binding site for the ligand CXCL12. The orphan receptor RDC1 was first cloned based on its homology with the conserved domain of G protein-coupled receptors. In addition, the gene was localized to chromosome 2, close to other CXC chemokine receptors [32, 33]. It was renamed CXCR7 recently, when it was found to bind the chemokines CXCL12 and CXCL11 with high affinity [34, 35]. Mice deficient in either CXCR4 or CXCL12 both show a similar embryonic lethal phenotype [36–38]. These include severe defects in lymphopoiesis, and neuronal and heart development [37, 38]. The binding of radiolabeled CXCL12/SDF-1 to embryonic liver cells of CXCR4-deficient mice led to the description of a second receptor, now called CXCR7 [34].

A developmental role of CXCR7 has been demonstrated [39–43]. In mice with a CXCR7 reporter knockin, CXCR7 was localized to cardiomyocytes, vascular endothelial cells of lung and heart, the cerebral cortex and in osteocytes [44]. The majority of CXCR7-deficient mice died within the first week after birth due to cardiovascu-
lar malformations (ventricular septal defects and semilunar heart valve malformation) with myocardial degeneration and fibrosis [44, 45]. In a conditional knockout where CXCR7 was deleted on endothelial cells, this phenotype was recapitulated [45]. The migration of CXCR4-positive primordial germ cells was dependent on the expression of CXCR7-positive somatic cells in zebrafish [41]. The knockdown of CXCR7 resulted in misguidance of the primordial germ cells [41, 42]. This has an important implication, as the scavenging of chemokines by CXCR7-positive cells was necessary to localize CXCR4 to particular microenvironments. For this process to occur, CXCR7 and CXCR4 apparently need to be expressed on different cells, for example, CXCR7 expression on endothelial cells.

CXCR7 is an ‘atypical’ chemokine receptor, as binding of chemokines does not result in mobilization of intracellular calcium, nor does it provoke direct chemotaxis of CXCR7-positive cells [35, 46]. On the other hand, CXCR7 does appear to be involved in a variety of physiological and pathological processes including the recruitment of stem cells [47], modulation of CXCL12-induced migration [35], internalization of the receptor after chemokine exposure [35], and tumor formation [34, 48]. A role for CXCR7 in the transendothelial migration of tumor cells was recently defined [49]. Ligand binding to CXCR7 led to the recruitment of β-arrestin2. Either CXCL11 or the small molecule CXCR7 ligand was found to block the transendothelial migration of tumor cells (which were CXCR4 and CXCR7 double positive). Inhibition of CXCL12-induced migration by the small molecule CXCR7 ligand was even more pronounced than that seen with a CXCR4 antagonist. Renal stem cells have also been shown to be CXCR4 and CXCR7 positive [47]. Blocking either CXCR4 or CXCR7 was found to reduce their engraftment in acute renal failure [47]. Transendothelial migration of these stem cells also required the presence of both receptors. CXCR7 was found to be essential for cell adhesion to endothelial cells [47].

Interestingly, heterodimers form between CXCR7 and CXCR4, which can impair CXCL12-mediated responses in primary lymphocytes [50]. This is most likely due to stabilization of the conformational state.

The induction of the chemokine CXCL8/IL-8 has been demonstrated in prostate carcinoma cells via CXCR7 [51]. If this can be confirmed in other cell types, it may represent an important mechanism how CXCR7 expressed on endothelial cells can help promote the activation of a proinflammatory phenotype. The proposed functions of CXCR7 are summarized in table 1.

The expression of CXCR7 on inflammatory cells like monocytes, B cells, and dendritic cells is still somewhat controversial [52, 53]. The chemotaxis of T cells towards CXCL12 can be blocked by an anti-CXCR7 antibody [34, 35]. In gene chip analysis of human immune cells, the expression of CXCR7 was demonstrated on B cells, some T cell subsets and NK cells [45]. CXCR7 deficiency has only a marginal effect on B cell development in mice [45]. Because even relatively low levels of expression of CXCR7 on lymphocytes could have a major impact on the local inflammatory milieu, the controversy may be due in part to the specific model systems used and the sensitivity of the antibodies used.

Relatively little is known about the expression of CXCR7 in human tissues, particularly under pathological conditions. CXCR7 was described on blood endothelial cells and was found to be upregulated on human umbilical vein endothelial cells after stimulation with proinflammatory cytokines [34]. The most prominent cell adhesion was found when CXCR4 and CXCR7 were both expressed on the adhering leukocyte, and CXCR7 was expressed by the endothelial cells [34]. The induction of CXCR7 was described on endothelial cells after they had been transformed by Kaposi’s sarcoma-associated herpesvirus [54]. The proliferation of these cells could be prevented via blockade of CXCR7 by antisense molecules [55]. Malignant brain tumors were also found to contain CXCR7-positive vessels [56]. While an important role has been demonstrated for CXCL12 and CXCR4 in renal vasculature biology, a role for CXCR7 has not yet been described [57, 58]. While the effects can be demonstrated in animal models, the molecular role of endothelial CXCR7 expression is still unclear.

The increased expression of both CXCR7 ligands have been described in animal models of renal allograft rejection [59–63]. We investigated the expression of CXCR7 in human renal allograft injury, CXCR7 and its corresponding ligands were quantified at the mRNA level using quantitative PCR and the protein localized in renal allograft biopsies [64]. CXCL12 and CXCL11 mRNA were significantly induced in biopsies with borderline lesions and with acute allograft rejection [64]. Expression of CXCR7 mRNA was also documented.

CXCR7 protein was localized in 64 renal biopsies including samples with acute rejection, chronic allograft injury, normal pretransplant biopsies as well as in a series of protocol biopsies [64]. CXCR7 was found to be expressed on rare peritubular vessels, but not on glomerular endothelial cells in pretransplant controls. The number of CXCR7-positive peritubular vessels increased signifi-
cantly in biopsies with acute interstitial and acute vascular rejection. Double staining with the lymphatic marker podoplanin demonstrated that a subset of CXCR7-positive vessels was identified as lymphatic vessels [64]. CXCR7-positive blood and lymphatic vessels increased during allograft rejection.

Thus, in human renal allografts, CXCR7 is situated at 2 strategic sites (peritubular capillaries and lymphatic vessel) at which the inflammatory cell influx and the efflux from the allograft is regulated. Further studies are necessary to define the functional role of CXCR7 on endothelial cells. Unfortunately, CXCR7-deficient mice die shortly after birth due to heart defects, therefore, inducible knockout animals and antagonist studies will have to be used to define the functional role of CXCR7 in renal allograft injury [44, 45].

**D6 Is a Chemokine Scavenger**

D6 binds multiple proinflammatory CC chemokines (table 1) [65]. D6 is localized to endothelial cells of lymphatic vessels in the skin [66]. In contrast to DARC, which appears to shuttle and present chemokines, D6 is thought to primarily act as a chemokine scavenger [17, 67]. The chemokines that bind D6 are rapidly internalized and degraded, while D6 is recycled to the cell surface [68]. D6-mediated chemokine internalization occurs without significant surface reduction of D6 due to internalization in recycling endosomes [68]. D6-deficient mice demonstrate exacerbated responses in skin inflammation and increased cell numbers in draining lymph nodes [69]. Recently, interest has focused on the potential role of D6 on leukocytes where it may help balance the inflammatory response which is necessary for repair following inflammatory tissue injury [67].

**CCX-CKR – A New Kid on the Block**

In contrast to D6 and DARC, CCX-CKR binds homeostatic chemokines including CCL19, CCL21, and CCL25 [70]. CCL21 is thought to play a role in renal inflammation [71, 72]. CCX-CKR sufficiently scavenges CCL19, which leads to intracellular degradation [73]. Expression of CCX-CKR was found predominantly in heart and lungs and was localized to bronchial epithelial cells in the lung [74]. To date, CCX-CKR has not been studied in the kidney.

**General Functions of ‘Nonsignaling’ Receptors**

The atypical chemokine receptors add another level of complexity to the chemokine system. Many studies of the biologic roles of these proteins using classical knockout animals have not shown a phenotype which suggests a redundant mode of action. Importantly, nature has evolved a red blood cell-specific human ‘knockout’ of DARC in areas of endemic malaria. This suggests an evolutionary pressure to conserve the endothelial DARC expression. Generally, both classical as well as nonsignaling chemokine receptors can bind and internalize chemokines. The net result for the inflammatory process appears to depend on the local microenvironment. A new concept is highlighted by the role of CXCR7 during development. In this context, the scavenging action of the receptor is thought to help focus chemokine gradients necessitating its localization in specific microenvironments. If the same mechanism would be true for mammalian cells, the expression of atypical receptors on endothelial cells might be involved in the recruitment of inflammatory cells via the formation of defined chemokine gradients.

**Conclusions**

The family of nonsignaling chemokine receptors is emerging as major players in chemokine biology. We are just starting to understand the role of DARC in renal inflammation, and data on the other atypical chemokine receptors is slowly accumulating. Further studies on the functional role of these proteins might define them as therapeutic targets in renal inflammation.

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