Chemokine receptor CXCR2: physiology regulator and neuroinflammation controller?

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Abstract

The innate immune system is a crucial component of inflammatory reactions, while the central nervous system (CNS) is the most vulnerable site of the body to inflammatory tissue injury. Neuroinflammatory brain pathologies are disorders in which the CNS is threatened by its own immune system. Chemokine receptor CXCR2 and its ligands have been implicated in several neuroinflammatory brain pathologies, as well as in neutrophil recruitment and in the developmental positioning of neural cells.

This review focuses on the basics of CXCR2, its regulating role in bone marrow neutrophil recruitment, oligodendrocyte progenitor cell positioning and neural repair mechanisms, as well as its diverse roles in neuroinflammatory brain pathologies.

Keywords

CXCR2; chemokines; neutrophil; brain pathology; multiple sclerosis

1. Introduction

Chemokines and chemokine receptors are potential therapeutic targets for a variety of diseases. The suggested positive effect of chemokine signaling blockade range from drugs preventing cancer metastasis or ameliorating multiple sclerosis (MS) symptoms, to treatments inhibiting HIV or medications acting on roughly any other disease in which the chemokines and/or chemokine receptors have been implicated (Bielecki et al., 2008; Charo and Ransohoff, 2006; Garin and Proudfoot, 2011; Singh et al., 2010; Vasilescu et al., 2007). Research in academia and pharmaceutical industry has vastly expanded since the roles of chemokines and chemokine receptors in immunity unraveled. Moreover, the disclosure of their functions in homeostasis and under inflammatory conditions, make these molecules salient targets for the regulation of immunity (Charo and Ransohoff, 2006).

Currently, 19 different chemokine receptors have been discovered, separated into four different subfamilies: C, CC, CXC and CX_3C.. In humans, the CXC subfamily currently

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contains seven chemokine receptors and 15 ligands (Murphy et al., 2011). The CXC chemokine subfamily can be divided in a group of chemokines containing a glutamic acid-leucine-arginine (ELR) motif, and a group in which the ELR motif is absent (Hebert et al., 1993; Savarin-Vuaillat and Ransohoff, 2007). Seven of the 15 CXC ligands (CXCL1-3 and CXCL5-8) contain an ELR motif, all having high binding affinity for chemokine receptor CXCR2. Important roles in the innate immune system for CXCR2 were first described by Huber *et al.* shortly after CXCR2 was discovered as a neutrophil receptor (Huber et al., 1991; Murphy and Tiffany, 1991). Strong evidence for a role of CXCR2 in a neuroimmunological disease came a few years ago, when CXCR2 was shown to govern developmental positioning in the oligodendrocyte lineage (Robinson et al., 1998; Tsai et al., 2002). Extensive research in the past two decades has began to delineate the role CXCR2 plays in immunity, the central nervous system (CNS), and disease related contexts, all associated with the importance and the complexity of the functions of CXCR2.

Therefore, we will focus mainly on CXCR2, by addressing the importance of CXCR2 in neuroinflammatory pathologies, reviewing the basics of the receptor and its ligands, discussing expression of CXCR2 on neutrophils and the receptor's role in the recruitment of immune cells to the CNS, and going over the differential roles CXCR2 plays in the development and pathologies of the CNS.

2. Biological functioning of CXCL1-CXCR2

2.1. Chemokine receptor structure

CXCR2 was first described as IL-8 receptor IL-8RB by Murphy *et al.* (Murphy and Tiffany, 1991). Simultaneously another receptor was described by Holmes *et al.* as IL-8RA (Holmes et al., 1991), now known as CXCR1. CXCR1 and CXCR2 show 77% amino acid identity and are both mapped on human chromosome 2q34-q35 (Ahuja et al., 1992). CXCR2, and in some cases CXCR1, is highly conserved among vertebrates (Catusse et al., 2003; Hipkin et al., 2004; Huising et al., 2003a; Lee et al., 1992; Pighetti and Rambeaud, 2006; Prado et al., 1994). Genome comparison has shown about 70% sequence identity for human with rat (*Rattus norvegicus*) and murine (*Mus musculus*) CXCR1 and CXCR2 (Bozic et al., 1995; Dunstan et al., 1996; Fan et al., 2007). Although both receptors are fairly similar in structure and are well conserved, studies with CXCR2 germ line knock-outs have shown an extensive and non-redundant function for CXCR2 (Cacalano et al., 1994; Liu et al., 2010a; Liu et al., 2010b; Tsai et al., 2002), leaving the role of CXCR1, and interaction with its ligands, CXCL6 in rodents, and CXCL6 and CXCL8 in humans, unclear.

The DNA sequence of CXCR2 contains a single long open reading frame, encoding for protein domains which indicate membership in the G protein coupled receptors (GPCRs) of the rhodopsin superfamily (Holmes et al., 1991). CXCR2 is considered part of the rhodopsin Class A GPCR subfamily, which contains all chemokine receptors. The proposed structure of CXCR2 is mainly based on bovine rhodopsin (Palczewski et al., 2000), since studies investigating the structures of chemokine receptors have proven to be refractory so far (Lodowski and Palczewski, 2009). The receptor is comprised of seven transmembrane segments, three extracellular and three intracellular loops, an extracellular N-terminal domain, and a cytosolic C-terminal segment (Figure 1). The amino acid aspartate in the second extracellular loop and a LLKIL motif in the C-terminus are both required for rapid receptor internalization, while the second intracellular loop contains a DRY (Asp – Arg – Tyr) motif as the G protein docking site, giving the chemokine receptor the ability to signal upon ligand binding (Allen et al., 2007; Nasser et al., 2007).

2.2. Ligand biology

The characteristic ELR motif of the CXCR2 ligands, chemokines CXCL1-3 and CXCL5-8, assures binding with CXCR2 and is strongly associated with neutrophil attraction (Charo and Ransohoff, 2006). ELR+ CXC chemokines are located closely together in the human genome at chromosome 4q12-21, consistent with overlapping functions (Ransohoff, 2009).

An association between the CXC chemokines was already found in the late 1980's, when Richmond *et al.*, Sager *et al.* and Yoshimura *et al.* extracted and characterized the first ELR+ CXC chemokines (Anisowicz et al., 1987; Richmond et al., 1983; Richmond et al., 1985; Yoshimura et al., 1987). Rapidly, several other analogous chemokines were described, originally reported as $MGSA_{\alpha\beta\gamma}/GRO_{\alpha\beta\gamma}$, ENA-78, GCP-2 and NAP-1 and 2 in human, and as KC/MIP-2 and CINC in mice and rats respectively (Table 1). Until now a murine CXCL8 homolog has not been reported. However all other ELR+ CXC chemokines have been detected in mice and ELR+ CXC chemokine analogs have also been described in several other vertebrates. Some CXC chemokine and receptor analogs have been reported in the earliest vertebrates, suggesting a function advantageous for vertebrate biology (Huising et al., 2003b).

The importance of CXC chemokines for leukocyte recruitment in the innate immune system can be concluded from the extensive research showing that all ELR+ CXC chemokines have equal CXCR2 binding affinities and neutrophil chemoattractant properties (Addison et al., 2000; Ahuja et al., 1996). This leads to the suggestion that CXC chemokines are highly redundant, and the suggestion that a rapid chemokine expansion was necessary to cope with a continuous treat of viral infections (Huising et al., 2003b). Chemokines are also important for the induction of effector functions, raising intracellular Ca^{2+} levels, releasing granule contents and forming respiratory burst (Baggiolini et al., 1995). The early extraction of the MGSA/GRO chemokines (CXCL1-3) from tumor-derived human cells by Richmond *et al.,* showing its effect on melanoma cell growth and neural crest cells (Balentien et al., 1991; Bordoni et al., 1989), prefigured the importance of CXCL1-CXCR2 biology in cancer and CNS development (Horton et al., 2007; Tsai et al., 2002), while highly elevated CXCL1 levels during CXCR2 deficiency show the scavenging role of chemokine receptors, being one of their effector functions (Cardona et al., 2008).

2.3. Expression

The expression of CXCR2 on neutrophils and oligodendrocytes has been widely described for its roles in immunity, cancer growth and CNS development, but its expression has also been reported on human basophils, T-lymphocytes and endothelial cells (Krieger et al., 1992; Lippert et al., 2004; Raman et al., 2007; Ransohoff et al., 2007). Although this expression, as well as its murine expression on tissues and cells other than neutrophils and oligodendrocyte lineage cells, has been reported, characterization by immunocytochemistry has been shown to be of questionable specificity (Lindner et al., 2008; Liu et al., 2010a). Here we will mainly consider the functions CXCR2 exerts through its expression on neutrophils and in the oligodendrocyte lineage.

2.4. Activation, signaling and desensitization

Chemokine receptors are activated through signaling, based on the concept of a two-site interaction between the chemokine and its receptor (Rajagopalan and Rajarathnam, 2006). Although this interaction concept is based on ligand-receptor interaction studies of chemokine receptors CCR2 and CXCR4 (Crump et al., 1997; Monteclaro and Charo, 1996), more structures and binding modes of chemokine receptors and chemokines are currently solved (Wu et al., 2010; Salanga and Handel, 2011). For example, a recent study described similarities between the CXCR2 and CXCR4 binding modes (de Kruijf et al, 2011).

The first step in the ligand-receptor binding interaction is binding of the chemokine's N-terminal residues preceding the first cysteine, with the N-terminal domain of the receptor (site 1), followed by the binding of the ligand's ELR+ motif with the receptors 2nd and 3rd exoloops (site 2) (Figure 1). It appears that site 1 binding is essential for receptor selectivity and affinity (Rajagopalan and Rajarathnam, 2004), whereas the binding of the ELR+ motif of the chemokine with the 2nd binding site of the receptor stabilizes the binding and activates the signaling pathway (Kraemer et al., 2011).

Upon ligand-receptor binding, and subsequently receptor activation, the heterotrimeric G-protein, $G_{\alpha\beta\gamma}$, separates into the subunits G_{α} and $G_{\beta\gamma}$, through the exchange of GDP for GTP on the G_{α} subunit. The $G_{\beta\gamma}$ subunit then activates phospholipase C, which cleaves phosphatidylinositol (4,5)-biphosphate (PIP2) into secondary messengers inositol triphosphate (IP3) and diaglycerol. They in turn trigger signaling events such as the rise of intracellular calcium from intracellular stores, leading to responses like chemotaxis and degranulation (Murdoch and Finn, 2000). Next, depending on the cell type and chemokine receptor binding, activation of the classic mitogen-activated protein kinase (MAPK) signal transduction pathway takes place, leading to downstream transcription activation (Knall et al., 1996).

Similar to the binding of a chemokine with its receptor during activation, the induction of internalization of CXCR2, depends on the interactions between the N-terminal of the chemokine and the N-domain of the chemokine receptor (Prado et al., 2007). The efficiency of receptor internalization, as well as the choice for the intracellular trafficking pathway of CXCR2, depends on motifs on the C-terminus of the receptor (Baugher and Richmond, 2008). *In vitro* studies in HL60 and HEK293 cell lines show that binding of adaptor proteins and phosphorylation of serine residues in the receptor's C-terminus facilitate intracellular movement of CXCR2 (Raman et al., 2009). Internalization of CXCR2 through clathrin-coated pits, and association with different cellular trafficking regulators, as Rab GTPases like Rab5, results in either the recycling or lysosomal sorting pathway of CXCR2 (Neel et al., 2005). In case of the recycling pathway, CXCR2 returns back to the cell surface after dephosphorylation, while, in case of the lysosomal sorting pathway, the receptor will be degraded by proteolytic enzymes (Fan et al., 2003).

3. Immunobiology of CXCR2

3.1. Bone marrow to lesion

The regulation of neutrophil recruitment from the bone marrow to the site of inflammation is incompletely known, but research is pointing toward major roles for CXCR2 and CXCR4 (Borregaard, 2010). Upon acute injury, two different reactions take place simultaneously (Figure 2).

First, pro-inflammatory cytokine interleukin-1β (IL-1β) is released within damaged tissue as a reaction to injury. IL-1β is processed from the inactive proIL-1β into the bioactive IL-1β by either inflammasome caspase-1 in monocytes and macrophages, or proteinase 3 in neutrophils (van de Veerdonk et al., 2011). Since macrophage are situated in a specialized environment with constant exposure to microbial stimuli, the macrophage inflammasome needs to be activated by two signals, Toll like receptor (TLR) stimulation and a secondary adenosine triphosphate "danger" signal. The monocyte inflammasome is activated through TLR stimulation only, because monocytes normally remain in a pathogen free environment (Netea et al., 2008). The release of IL-1β at the site of injury leads to activation of intracellular adhesion molecule-1, while the concurrent release of CXCR2 ligands induces a sequential assembly of tumor necrosis factor-α (TNF-α) and leukotriene B4 (LTB4), all leading to activation of adherence factors on endothelial cells at the site of the lesion,

facilitating the migration of neutrophils to the site of injury (Coelho et al., 2008; Hu et al., 2010a; Hu et al., 2010b; McDonald et al., 2010; Ramos et al., 2006). Simultaneously, CXCR2 ligands are produced by macrophages and endothelial cells in the periphery or astrocytes in the CNS, in order to form a chemotactic gradient to guide neutrophils through the bloodstream to the injured site (McDonald et al., 2010; Oh et al., 1999; Vieira et al. 2009).

Secondly, G-CSF is produced quickly by the release of IL-17A from activated γδ T cells and natural killer T cells immediately after injury (Kerns et al., 2009; Ley et al., 2006). The release of G-CSF weakens the retention signal of CXCR4 with CXCL12 in the bone marrow, by decreasing CXCR4 and CXCL12 expression, concurrently inducing thrombopoietin (TPO) production by bone marrow stroma, and triggering the release of CXCR2 ligands by megakaryocytes, preparing the release of neutrophils from the bone marrow (Eash et al., 2010; Greenbaum and Link, 2011; Köhler et al., 2011; Petit et al., 2002; Wenger et al., 2008; Wu et al., 2001). Neutropils are then mobilized into the circulation, and replace the neutrophils recruited to the site of injury (Furze and Rankin, 2008).

3.2. Protection and destruction

The complexity of the innate immune system evidently shows that many factors work together in order to provide protection against pathogens and disease. The non-redundant role of CXCR2 in the innate immune system is indicated by its significance in the regulation of neutrophil recruitment and the activation of neutrophil effector functions. Evidence for its non-redundancy was shown *in vivo*, when Cacalano *et al.* generated the first germline CXCR2 knockout mice (CXCR2$^{-/-}$) in 1994 (Cacalano et al., 1994). Characteristics of these mice include neutrophilia and impairment in the recruitment of neutrophils during acute inflammatory conditions (Cacalano et al., 1994). Our laboratory recently described a similar high neutrophil count in the blood of CXCR2$^{-/-}$ mice. However, the CXCR2$^{-/-}$ mice did show a neutrophil response into the brain after 5 days of inflammatory cuprizone-induced demyelination similar to that of wild-type mice (Liu et al., 2010a). Flow cytometric staining of infiltrated cells with Ly6G and CD45, as well as peripheral co-staining of Ly6G and CXCR2, suggested that CXCR2-positive neutrophils were required for extensive demyelination in the corpus callosum of wild-type mice, while the CXCR2$^{-/-}$ mice showed resistance to demyelination. This observation suggests that CXCR2 is necessary for the promotion of selective neutrophil effector functions in the CNS, whereas on the contrary, the CXCR2$^{-/-}$ mice in the study of Cacalano *et al.* showed no impairment in functions required for peripheral bacterial clearance (Cacalano et al., 1994; Liu et al., 2010a). These observations lead to the hypothesis that CXCR2 exerts varying effector funtions in case of acute versus chronic inflammatory reactions, as well as in peripheral versus CNS environments.

Varying functions of CXCR2 are also suggested in other conditions. For example, CXCR2 is considered to create favorable conditions for wound healing in the periphery through the promotion of neutrophil recruitment (Zaja-Milatovic and Richmond, 2008). But on the other hand, depletion of CXCR2-positive neutrophils in subarachnoid hemorrhage is associated with the prevention of delayed cerebral vasospasm (Provencio et al., 2011). Furthermore, CXCR2 and its ligands are assumed to increase cellular senescence in early tumorigenesis, inducing growth arrest in cells. In the case of late stage tumorigenesis, mutations in tumorous cells create circumstances in which ELR+ chemokines are unable to induce cellular senescence, and instead are implicated to create circumstances favoring metastasis formation (Lazanecc and Richmond, 2010). This versatility sets the effector functions of CXCR2, depending on the circumstances, constantly on the border of protection and destruction.

3.3 Cancer regulation

Involvement of CXCR2 ligands in cancer was described, shortly after the first CXCR2 ligands were defined as molecules important in leukocyte recruitment and functioning. The extraction of CXCL1-3 from tumor-derived human melanoma cells and its involvement in melanoma cell growth, suggested roles for CXCR2 and its ligands in tumor regulation as well (Balentien et al., 1991). Now, CXCR2 and all CXCR2 ligands have been implicated in tumor progression including tumor growth, vessel formation and cancer cell proliferation, as well as in neutrophil recruitment to the tumor microenvironment, in which the neutrophils are also associated with tumor development and growth (Horton et al., 2007; Raman et al., 2011).

Robinson *et al.* described involvement of CXCR2 in oligodendroglioma proliferation and reported upregulation of CXCL1 in primary brain tumors, showing a CXCR2 signaling role in the induction of tumor growth in the brain (Robinson et al., 2001). CXCL8, along with CXCL1 and possibly other CXC chemokines, are produced in the tumor microenvironment through, among others, nuclear factor-kappa B (NF-κB) activation (Amiri et al., 2006; Martin et al., 2009). And although chemotherapy and radiation are used to conquer cancer, they also produce reactive oxygen species in the tumor microenvironment, which in turn activates NF-κB and thus leads to the production of factors like CXC chemokines involved in tumor progession (Reuter et al., 2010).

This is a double-edged sword situation, in which a risk has to be taken in order to defeat the pathology. Even though many reports describe how CXCR2 and its ligands promote tumorigenesis, it has also been shown that they provide protection in the early stages of tumorigenesis (Lazanecc and Richmond, 2010).

4. Differential roles of CXCR2 in the brain

4.1. Oligodendrocyte progenitor cell proliferation and protection

The expression of CXCR2 on oligodendrocyte progenitor cells (OPCs) in the brain and spinal cord was described, after the discovery of CXCL1 production by spinal cord astrocytes (Glabinski et al., 1997; Robinson et al., 1998; Tsai et al., 2002). Chemokine CXCL1, in combination with platelet derived growth factor (PDGF), stimulated the proliferation of OPCs in the spinal cord *in vitro* (Robinson et al., 1998). CXCR2 on OPCs *in vivo* interacted with CXCL1 to arrest the migration of OPCs through the rapid and reversible inhibition of PDGF chemokinesis in a transwell assay. Using slice culture, it could be shown that CXCL1 also increased OPC interaction with extracellular matrix substrates, possibly accounting for migration arrest. In postnatal mice, the absence of CXCR2 led to a reduction in myelin thickness, with an increased G-ratio of radial myelin thickness to axonal perimeter, during spinal cord development (Padovani-Claudio et al., 2006; Tsai et al., 2002). Moreover, Tirotta *et al.* described that CXCR2 protects striatum OPCs, differentiated in culture from mice at postnatal day 1, from apoptosis after 6 days of pro-apoptotic IFN-γ and CXCL10 treatment (Tirotta et al., 2011). Altogether, this indicates that in the CNS, interaction of CXCL1-CXCR2 possibly prevents OPCs from apoptosis, and controls the positioning and proliferation of OPCs in the developing spinal cord through a regulating action on PDGF.

4.2. Neuroinflammation

Neuroinflammatory reactions in neuropathological diseases such as MS, traumatic brain injury (TBI) and Alzheimer's disease (AD) are associated with the presence of chemokines and cells bearing chemokine receptors. Evidence for the role of chemokines and its receptors in MS is well described (Holman et al., 2011), but evidence for these factors in TBI and AD

is found more recently. Studies in AD and TBI indicated that chemokine receptors may play essential roles in the extent of cortical damage and β-amyloid (Aβ) plaque deposition (Cho et al., 2011; Lee et al., 2010; Semple et al., 2010).

The role of CXCR2 during neuroinflammation specifically, has been studied and well described before in the experimental autoimmune encephalomyelitis (EAE) mouse model for MS (Cardona et al., 2008; Carlson et al., 2009; Ransohoff RM, 2009; Ransohoff RM et al., 2007). More recently, we described the function of CXCR2 in an animal model for MS: the cuprizone-induced demyelination model. In this model, the role of CXCR2 on myeloid and non-myeloid cells in relation to the destruction and repair of myelin was described (Liu et al., 2010a; Liu et al., 2010b).

4.2.1. Multiple Sclerosis—MS is a chronic inflammatory demyelinating disease of the CNS, characterized by infiltrating inflammatory components into the CNS and ultimately failing remyelination. The infiltrating inflammatory cells are mostly considered to be T-cells, B-cells and macrophages from the adaptive immune system. But recently the role of the innate immune system is also considered for the involvement in the onset or progression of MS (Carlson et al., 2008; Chabas et al., 2011; McColl et al., 1998; Weiner, 2009). CXCR2 chemokine CXCL1 has been implicated in EAE, CXCL1 expression by reactive astrocytes has been reported at lesion edges, and measurements of serum levels in MS patients showed upregulation of CXCL8, suggesting a role of the innate immune system, and more specifically CXCR2 in MS (Glabinski et al., 1998; Lund et al., 2004; Omari et al., 2006).

The effect of CXCL1 on neutrophil recruitment into the CNS was shown in a transgenic MBP-CXCL1 mouse model, in which expression of CXCL1 by oligodendrocytes led to neutrophilia in the CNS, behavioral impairment and blood-brain-barrier breakdown (Tani et al., 1996). The inoculation of a neurotropic virus into the CNS, simulating immune-mediated demyelination as in MS, also elevated the expression of CXCR2 ligands, suggesting the recruitment of CXCR2-positive neutrophils into the CNS for virus clearance. Deficiency of CXCR2 led to lessened neutrophil infiltration, but had no effect on virus specific T cell viral clearance (Hosking et al., 2009). Another demyelinating animal model for MS recently used by our laboratory, cuprizone-induced demyelination, showed CXCR2-positive neutrophils in the CNS after 5 days of cuprizone feeding (Liu et al., 2010a). After 3 to 6 weeks of cuprizone feeding, $CXCR2^{+/+}$ mice showed extensive demyelination, while $CXCR2^{-/-}$ mice were relatively resistant to cuprizone-induced demyelination. Moreover, bone marrow chimeric mice, $CXCR2^{+/-} \rightarrow CXCR2^{+/+}$ and $CXCR2^{+/-} \rightarrow CXCR2^{-/-}$, revealed equal susceptibility to demyelination, but $CXCR2^{-/-} \rightarrow CXCR2^{+/+}$ showed resistance to demyelination (Liu et al., 2010a). To control for the impact of radiation with the bone marrow chimeric mice, as it may influence the migration of myeloid cells into the CNS, also $CXCR2^{+/+} \rightarrow CXCR2^{-/-}$ were tested on cuprizone-induced demyelination. These mice showed demyelination as extensively as the $CXCR2^{+/+}$ mice, indicating that in cuprizone-induced demyelination, CXCR2-positive myeloid cells are essential in the demyelination process.

CXCR2 has also been implicated as an essential element in EAE. Induction of CXCL1 and CXCL2 transcription by CD4-positive T cells was shown to be essential in EAE, and CXCR2-positive neutrophils were able to restore susceptibility for EAE in $CXCR2^{-/-}$ mice (Carlson et al., 2008). The use of a CXCR2 antagonist led to a reduction in EAE score, and the overexpression of CXCL1 by astrocytes in double transgenic mice led to a reduction in inflammation and demyelination in EAE, possibly due to CXCR2 receptor downregulation (Kerstetter et al., 2009; Omari et al., 2009). Moreover, Liu *et al.* showed similar

susceptibility for EAE and cuprizone-induced demyelination in $CXCR2^{+/-} \rightarrow CXCR2^{+/+}$ and $CXCR2^{+/-} \rightarrow CXCR2^{-/-}$ bone marrow chimeric mice (Liu et al., 2010b).

Remyelination was improved in the $CXCR2^{+/-} \rightarrow CXCR2^{-/-}$ bone marrow chimeras during the recovery phase (Liu et al., 2010b), while also mice treated with a CXCR2 antagonist showed better remyelination in the spinal cord after EAE (Kerstetter et al., 2009), leading to the suggestion that CXCR2 expression on myeloid cells is essential in the damage of myelin during chronic CNS inflammation in animal models for MS, while CXCR2 on non-hematopoietic cells is responsible for the inhibition of myelin repair, which may be in line with CXCR2's arresting role on OPC proliferation in the developing spinal cord (Liu et al., 2010a; Tsai et al., 2002).

4.2.2. Traumatic brain injury—Several clinical studies reported highly elevated levels of CXCL8 in the cerebrospinal fluid (CSF) following severe TBI (Hayakata et al., 2004; Kossmann et al., 1997; Maier et al., 2001; Whalen et al., 2000), indicating that a neuroinflammatory response might be following injury via CXCR2 leukocyte recruitment. But recently, a more apparent link between CXCL8 and CXCR2 was established in a clinical TBI study with a small subset of patients, where poor patient survival of patients with enlarged contusions after TBI, was correlated with elevated CXCL8 serum concentrations (Rhodes et al., 2009).

Although several clinical studies have indicated CXCL8 as a marker following TBI, little research is performed in TBI animal models. A study in 2006 showed rapid upregulation of CXCR2 ligands in rats, as well as neuronal immunoreactivity of CXCR2 following TBI (Vallès et al., 2006). Unfortunately, immunolocalization of CXCR2 has subsequently been found to be nonspecific (Lindner et al., 2008;2009). Semple *et al.* performed a TBI simulating experiment in mice recently, resulting in upregulation of G-CSF, CXCL1 and CXCL2 in the CNS at 12–24 hours after brain injury (Semple et al., 2010). Mice deficient for CXCR2 showed elevated levels of CXCL1 and reduced neutrophil infiltration after brain injury. Moreover, a correlating attenuation of neuronal loss and tissue damage was described in $CXCR2^{-/-}$ mice (Semple et al., 2010). This suggests a potentially significant role of CXCR2 following TBI, but further research should reveal the specific role of CXCR2 in TBI, to seriously consider it as a possible therapeutic target in the future.

4.2.3. Alzheimer's disease—AD is a major cause of dementia and is the most common neurodegenerative disease in the elderly. The neuropathology of AD is associated with extracellular Aβ plaques and intracellular neurofibrillary tangles along with marked neuroinflammation, including microgliosis (Reitz et al., 2011). Recent evidence has implicated pro-inflammatory molecules within the AD brain, and, more specifically, chemokine receptors have been associated with a role in Aβ deposition and tau pathology (Bhaskar et al., 2010; Cho et al., 2011; Fuhrmann et al., 2010; Lee et al., 2010). Despite that, clinical interventions based on these discoveries are not elucidated yet, since several clinical trials have turned out to be controversial (de Jong et al., 2007; Schwartz and Shechter, 2010).

The involvement of CXCR2 in AD remains to be clarified too, since only a few studies on the role of CXCR2 in AD have been performed so far (Bakshi et al., 2011; Xia and Hyman, 2002). The studies imply that CXCR2 may enhance AD pathology and that deficiency in CXCR2 reduces Aβ deposition (Bakshi et al., 2011; Xia and Hyman, 2002).

Another study with mice expressing transgenes encoding mutated human presenilin 1 (PS1), the polypeptide linked to familial forms of AD, showed involvement of CXCR2 ligand CXCL1. Resting microglia showed upregulation of CXCL1, and downregulation of neural

progenitor cell proliferation, in mice carrying a PS1 mutation, linking neurogenesis impairment and CXCL1 expression in an AD mouse model (Choi et al., 2008). Moreover, a recent clinical study revealed that CSF CXCL1 was lower in cognitively normal as compared with cognitively impaired individuals (Craig-Shapiro et al., 2011), while it has also been shown that CXCL8 CSF levels are significantly increased in AD patients (Zhang et al., 2008).

Although these data point to a possible harmful role of CXCR2 in AD pathology, too little is known to provide specific conclusions at present. Additional research needs to be performed to decipher the exact role of CXCR2 in this neurodegenerative disease.

5. Conclusion and future perspectives

The significance of CXCR2 and its ligands in multiple diseases and disease models has been described. CXCR2 is a promising potential therapeutic target, since brain penetrant inhibitors and CXCR2 antagonist providing promising results in various clinical trails for Alzheimer's disease and COPD, respectively (Chapman et al., 2009; Frisardi et al., 2010). The mechanisms of CXCR2 in different disease models and environments have not been totally defined yet, so vigilance should be exercised because of the complex biological role CXCR2 carries out.

The role of CXCR2 in the environment of the periphery and the CNS, and during acute and chronic inflammatory reactions, can be very different. Although CXCR2 exerts this versatile role, development of CXCR2 antagonists is logically based on the harmful role CXCR2 seems to play in the different pathologies described here. But it should be noted that CXCR2 is also very important in the physiology of the body, exemplified by the striking phenotype of CXCR2 germline knockout mice (Cacalano et al., 1994). Moreover, some studies have shown that depletion of CXCR2 or neutrophils after initiation of diseases worsens the outcome (Bai et al, 2010; Hosking et al, 2009). This promiscuity in the functioning of CXCR2 may represent the chemokine receptor as a phenomenon, for which it is hard to create standard inhibitors or blockers.

Despite CXCR2's versatility, also several similarities in the functioning of CXCR2 can be extracted from the studies performed on CXCR2. For example the observation that CXCR2 and its ligands induce cellular senescence during early tumorigenesis (Lazannec and Richmond, 2010), can also be considered a role of CXCR2 under normal circumstances in the body's physiology. After all, the function of CXCR2 in the innate immune system is to guard the body from infections and disease. In case of an auto-immune disoder as MS, or in case of late tumorigenesis, CXCR2 possibly exerts an autocrine and paracrine ligand signaling, responsible for the maintenance of high ligand levels and the induction of damage, instead of providing protection. Depletion of neutrophils in this case might worsen the outcome, since eliminating the initial immune response and allowing escape of initial clearance.

Regarding the current knowledge about CXCR2, it is important to keep investing effort into the specific role of CXCR2 in different diseases. Since chronic CXCR2 inhibition could be difficult, especially in the CNS, acute inhibition of CXCR2 in TBI or subarachnoid hemorrhage should be considered. Additional information should provide more evidence, to better mark a therapeutic target in the CXCR2 biology for its future application in medicine.

Acknowledgments

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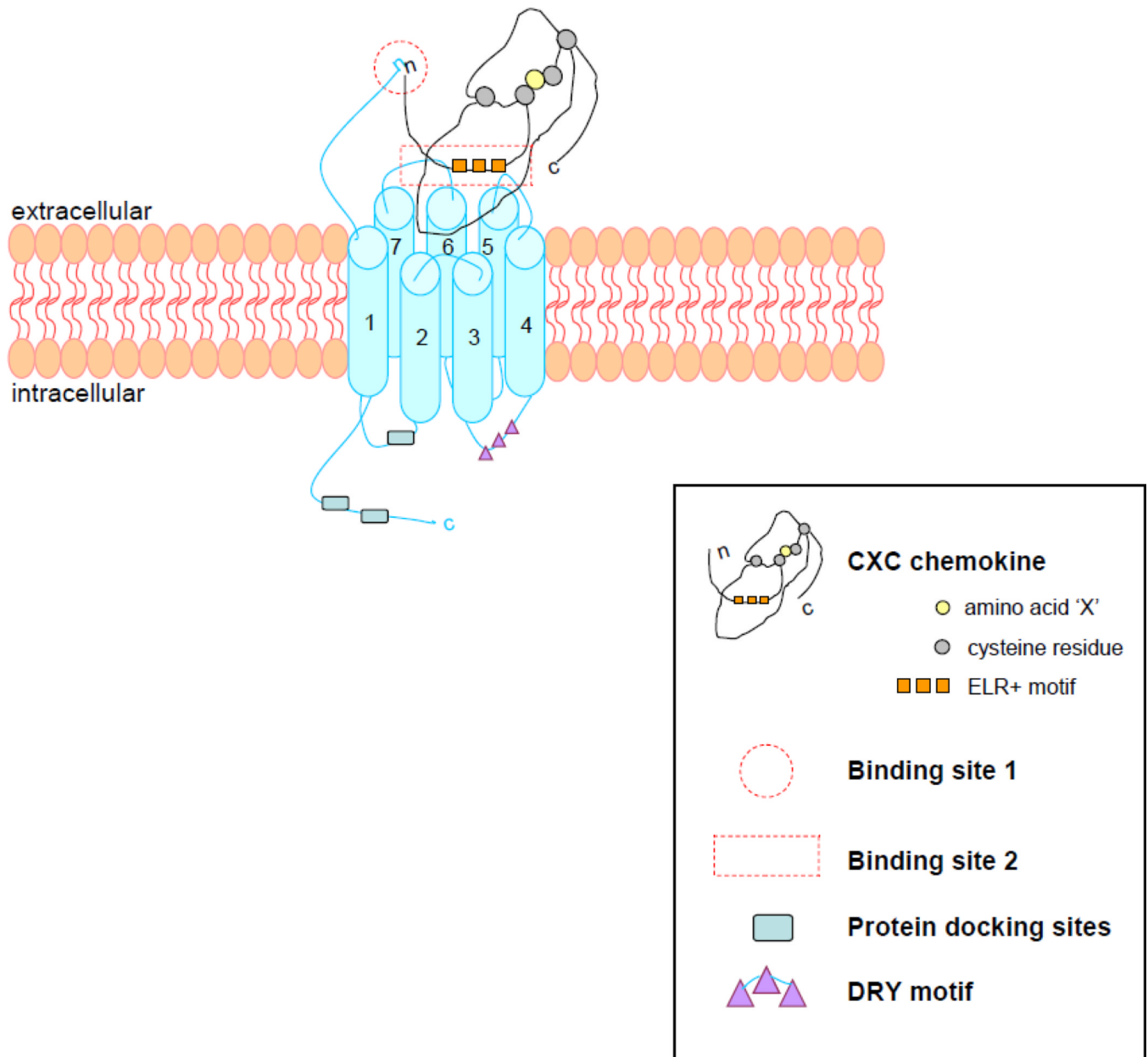
Figure 1. CXCR2 structure and ligand binding concept

The CXCR2 chemokine receptor is compromised of seven transmembrane segments, three extracellular and three intracellular loops, an extracellular N-terminal domain, and an intracellular C-terminal domain. It contains a DRY motif on the second intracellular loop, functioning as G-protein docking site, and other protein docking sites as the LLKIL motif on the C-terminus.

The CXCR2 ligand N-terminus binds with the N-terminal domain of the receptor (site 1), after which the ELR+ motif of the chemokine binds with the second and third extracellular loop of the receptor (site 2), to control the binding and to activate receptors signaling pathways.
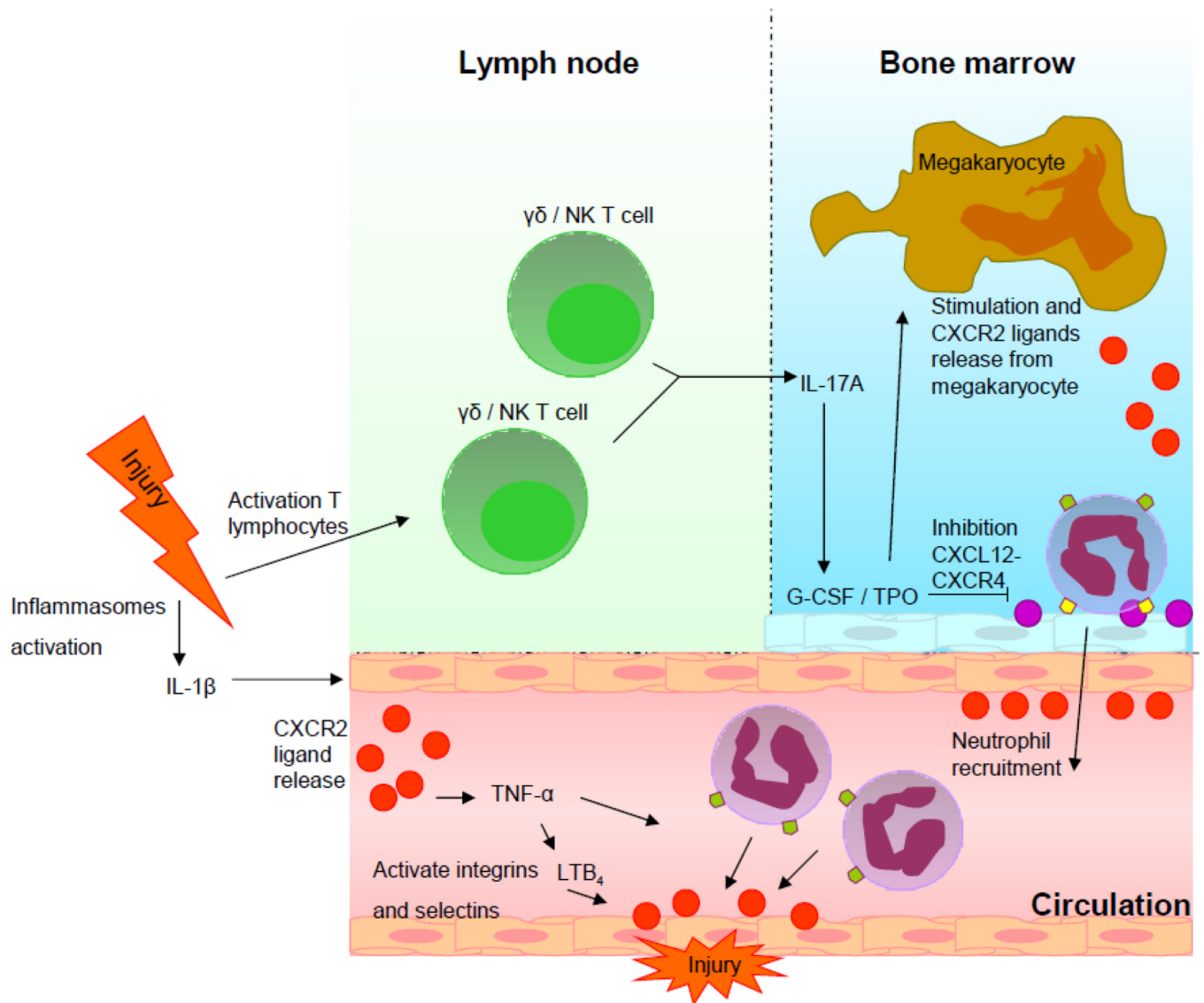
Figure 2. Hypothetical model of CXCR2's regulating role in two separate recruiting events
Upon acute injury, two different reactions take place simultaneously: release of neutrophils from the bone marrow (i) and local neutrophil recruitment (ii).

a. Injury activates $\gamma\delta$ and natural killer T cells, which produces IL-17A. This induces transcription of G-CSF in the bone marrow, which activates TPO. Stimulation by TPO inhibits CXCL12-CXCR4 binding and activates the release of CXCR2 ligands from megakaryocytes. The release of the CXCR2 ligands and weakening of the CXCR2 binding, leads to mobilization of neutrophils from the bone marrow.

b. Activation of inflammasomes leads to IL-1β release and stimulation of local CXCR2 ligand production, inducing a subsequential production of TNF-α and LTB$_4$, therewith activating integrins and selectins on the endothelial cells close the site of the lesion. CXCR2 ligands are presented at the site of the lesion, creating a chemotactic gradient for the recruitment of neutrophils through the interaction of CXCR2 and CXCR2 ligands.

Table 1

The CXCR2 ligands and their secondary names

Murine (Mus musculus) CXC chemokines and rat (Rattus norvegicus) CXC chemokine.

Ligands	Secondary names	Characterization
CXCL1	Gro-1, Gro-α, MGSA-α, NAP-3, *KC*	Anisowciz *et al.*, 1987 Richmond *et al.*, 1988
CXCL2	Gro-2, Gro-β, MGSA-β, MIP-2α, *MIP-2*	Haskill *et al.,* 1990
CXCL3	Gro-3, Gro-γ, MGSA-γ, MIP-2β	Haskill *et al.,* 1990
CXCL5	ENA-78	Walz *et al.,* 1991
CXCL6	GCP-2	Proost *et al.*, 1993
CXCL7	NAP-2, CTAP-III	Moser *et al.*, 1991
CXCL8	IL-8, *CINC*	Baggiolini *et al.*, 1989