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CXC chemokine signaling in the liver: Impact on repair and regeneration

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Abstract

The process of liver repair and regeneration following hepatic injury is complex and relies on a temporally coordinated integration of several key signaling pathways. Pathways activated by members of the CXC family of chemokines play important roles in the mechanisms of liver repair and regeneration through their effects on hepatocytes. However, little is known about the signaling pathways utilized by CXC chemokine receptors in hepatocytes. Here we review our current understanding of the pathways involved in both CXC chemokine receptor signaling in other cell types, most notably neutrophils, and similar pathways operant during hepatocyte proliferation/ liver regeneration in order to formulate a basis for the function of CXC chemokine receptor signaling in hepatocytes.

Keywords

Liver injury; ischemia/reperfusion; hepatectomy; CXCR1; CXCR2

The phenomenon of liver regeneration has been promulgated for centuries. Greek mythology tells the stories of two different characters, Prometheus and Tityus, who were tortured by birds of prey, sent by the god Zeus to punish them, that ate from their livers every day, only to have them grow back to be eaten from the next day (1). Interestingly, the ancient Greeks held the liver in great regard, viewing it as the center of life, soul, and intelligence, therefore linking the regenerative capacity of the liver to the indestructibility of the soul (2). The wide breadth of liver pathology that effects populations today makes its regenerative capacity one of great interest. Despite the many advances in medicine over the years, and the ability of clinicians to provide support to failing organ systems such as the kidneys and lungs, liver failure is often lethal without transplantation. Similarly, traumatic injury to the liver as well as oncologic disease of the liver, both of which often necessitate resection, again bring the unique qualities of the liver into focus.

Liver regeneration involves numerous soluble mediators, growth factors, and metabolic factors that work to stimulate quiescent hepatocytes to enter into the cell cycle, replicate, and expand existing liver mass. A class of small proteins called chemokines play important roles in the processes of liver repair and regeneration. A better understanding of their various functions will have widespread implications, including the ability to minimize organ dysfunction and enhance graft survival rates after liver transplantation, allowance for

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utilization of livers from extended criteria donors, or performance of more aggressive surgical interventions for hepatic malignancies.

Basic Principles of Liver Regeneration

Hepatocytes possess the unique ability to proliferate upon appropriate stimulation, normally maintaining themselves in a stage of quiescence, known as the G_0 phase. The molecular basis of liver regeneration is composed of three different phases, including a priming phase, a proliferative phase, and a termination phase (3). These phases have also been qualified as cytokine, growth factor and metabolic pathways, respectively, as it pertains to the factors predominantly mediating a particular phase (4). It is important to note that while it is conceptually easier to denote the sequence of events into “phases,” there is in fact a highly coordinated, synchronous schema of interactions between growth factors, cytokines and other mediators that allow the process of liver regeneration to occur (5). Cytokines are a key factor in stimulating quiescent hepatocytes from the G_0 phase into the G_1 phase. TNFα and IL-6, along with the transcription factors, STAT3 and NF-κB, required for the initiation of liver regeneration (4, 6). Through activation of STAT3 and NF-κB, target genes are transcribed which are important to hepatocyte proliferation. Growth factors, specifically hepatocyte growth factor (HGF) and epidermal growth factor (EGF), then drive the cell from G_1 into the S phase of DNA replication (4). Arguably one of the most important mediators of liver regeneration is HGF, a 100kDa protein that was originally identified in 1984 (7, 8). A potent mitogen for hepatocyte growth, HGF is locally released and upregulated during the initiation of the regenerative process, cleaved from its inactive single-chain form into its active two-chain form by uPA (3).

Phospholipase Cγ1 (PLCγ1), phospholipase Cβ1 (PLCβ1), phospholipase D1 (PLD1), and phosphoinositide-3-kinase (PI3K) have been implicated in the mechanisms of hepatocyte proliferation immediately after HGF or EGF binding (9–12). PLCγ1 and PLCβ1 appear to play different roles in the regenerating liver, with PLCγ1 having more influence on the G2/M phase transition, and PLCβ1 seeming to trigger DNA replication (9). PLD1 may play a role in the activation of c-Jun/c-Fos transcription factors, further contributing to DNA synthesis (13). The HGF receptor, a c-met oncogene, has been shown to function through tyrosine kinase activity. However, Adachi, et al.(14), showed that pertussis-toxin sensitive G proteins were also involved in mitogen activated protein kinase (MAPK) activation and arachidonic acid release, specifically demonstrating that PLD activation was diminished to baseline levels in the presence of $G\alpha_i$ receptor complex inhibition. More recently, signaling through PI3K has been shown to be critical for the induction of cyclin D and DNA replication following HGF binding (12). Further downstream, MAPK-dependent production of arachidonic acid (AA) through PLA_2 results in production of prostaglandins, further stimulating DNA synthesis (11). Prostaglandins, most significantly PGE_2 and PGF_2, are known to promote growth in hepatocytes (15). Conversely, during conditions in which hepatocytes may be stressed, activation of PLA_2 and increased release of arachidonic acid may have a deleterious effect on hepatocytes (16). In the setting of hypoxic injury to hepatocytes, diminished ATP production leads to acidosis, therefore preventing activation of PLA_2 until the return to physiologic pH during reperfusion, resulting in AA release and increased cell death (16, 17). In vivo studies have revealed that COX-2-dependent conversion of arachidonic acid to prostaglandins is crucial to the induction of protective mechanisms within the liver, and that COX-2 inhibition contributed to greater hepatotoxicity in the setting of carbon tetrachloride (CCl_4) injury, perhaps indicating that the level of COX-2 following hepatic injury is important to recovery (18).

Experimental Models of Liver Injury, Repair, and Regeneration

Several different experimental models have been utilized to better understand the mechanisms by which liver regeneration occurs, with partial hepatectomy being the gold-standard. Although it is the most well studied model of liver regeneration to date, the hepatocytes which constitute the remnant liver after partial hepatectomy are not an accurate representation of the physiological scenario seen in many liver pathologies, in which hepatocytes are injured and/or stressed. Additional models of liver injury, such as acetaminophen toxicity, CCl_4 injury, and ischemia/reperfusion injury represent a means to assess the reparative and regenerative mechanisms in stressed and injured hepatocytes. While differences exist in the timing of molecular and cellular events between the different models, hepatocytes remain the nidus of the regenerative process, stimulated by various soluble mediators released after injury (19).

Chemokines and their Receptors

The term *chemokines* describes a family of chemotactic cytokines originally described as mediators of immune cell trafficking and function (20, 21). Chemokines are a group of small (8–10 kD), basic, heparin-binding proteins that are secreted by leukocytes as well as various tissue cells (20, 22). While mainly involved in leukocyte chemoattraction, chemokines have also been implicated in other cellular activities, including regulation of angiogenesis, fibrosis, proliferation, cytotoxicity and apoptosis (23–26). The nomenclature for chemokines is based on the configuration of a conserved amino-proximal cysteine-containing motif (27). There are currently four branches of the chemokine family, CXC, CC, CX_3C and C (where X is any amino acid). CC and CXC are the two major branches, whereas CX_3C and C each have only one representative, consisting of fractalkine (CX_3CL1) and lymphotactin (XCL1), respectively (28). The CC family is the largest, primarily involved in attracting mononuclear cells to sites of chronic inflammation, while members of the CXC family mediate the chemoattraction of neutrophils and monocytes to sites of acute inflammation (24). CXC chemokines can be further classified by the presence or absence of a Glu–Leu–Arg (ELR) amino acid motif in the amino terminus of the peptide. The ELR motif confers receptor-binding specificity (29, 30).

CXC chemokines exert their effects through the CXC chemokine receptors (CXCR) 1–6 (28). CXCR1 and CXCR2 bind specifically to CXC chemokines which contain the amino terminus sequence ELR (Glu-Leu-Arg) (Table 1) (23, 27). In addition to their leukocyte-chemoattractant properties, ELR^+ CXC chemokines have been shown to have important roles in angiogenesis and cellular proliferation (25, 31, 32). CXCR1 and CXCR2 are expressed by neutrophils, monocytes, CD8+ T cells, epithelial cells and endothelial cells, as well as in hepatocytes (33–35). CXC chemokine receptors are heptahelical transmembrane G protein-coupled receptors (GPCR), with the individual components that make up each receptor contributing to their unique interactions. The external interface contributes to ligand specificity, and the other domains, including the transmembrane sequences, cytoplasmic loops and C-terminal domain, allow for receptor signaling and internalization (23).

Contribution of CXC Chemokines to Liver Repair and Regeneration

CXC chemokines are known to be important mediators of the inflammatory cascade following hepatic injury, and also appear to have a dichotomous role in hepatocytes that may be related to their level of expression (36). For example, induction of CXC chemokines at relatively low levels is associated with liver repair and regeneration, whereas high expression levels have been associated with hepatotoxicity (35, 37–39). The impact of CXC chemokines on the regenerative capacity of the liver was first examined using an in vivo model of partial hepatectomy (37). Partial hepatectomy represents a clinically relevant model of hepatic resection, a procedure often performed due to trauma or malignancy. ELR^+ CXC chemokines were found to be up-regulated after partial hepatectomy, and blockade of chemokines or CXCR2 resulted in diminished liver regeneration (37). Subsequent in vitro experiments demonstrated that hepatocytes treated with ELR^+ CXC chemokines proliferated to a degree similar to that induced by HGF. These studies (35, 37–39) provided evidence that ELR^+ CXC chemokines were important hepatocyte proliferative factors that functioned in vivo to promote liver regeneration after hepatectomy. However, as previously mentioned, the remnant liver after resection or hepatectomy, without Pringle maneuver, is comprised of unstressed hepatocytes. The role of CXC chemokines may be distinctly different in a setting in which hepatocytes are under significant stress.

This concept was first addressed in a murine model of hepatic ischemia/reperfusion (I/R) injury. Liver I/R represents a clinically relevant insult to the liver which produces significant inflammation and ultimately, parenchymal damage and organ dysfunction. There are two phases of liver injury following ischemia/reperfusion (39–41). The initial phase occurs during the first hours of reperfusion and is characterized by oxidant-induced hepatocellular injury and the induction of proinflammatory mediators (42–47). The later phase, which occurs many hours after reperfusion, is characterized by an intense inflammatory response culminating in the recruitment of activated neutrophils which directly injure hepatocytes via the release of oxidants and proteases (40, 48). The recruitment of neutrophils to the liver is CXC chemokine-dependent and thus these chemokines play a central role in the hepatic inflammatory response to I/R (49, 50).

Hepatocyte death following ischemia/reperfusion injury occurs through both apoptotic and necrotic mechanisms, with necrosis contributing most significantly after warm ischemia (51). Toxicity following ischemia/reperfusion injury is directly related to the generation and release of reactive oxygen species (ROS) by recruited neutrophils and resident Kupffer cells (52, 53). These ROS affect hepatocyte signal transduction and are directly cytotoxic. The c-Jun N terminal kinase (JNK) is a stress activated protein kinase member of the MAPK family which is activated by cytokines such as TNF-α and IL-1, as well as oxidants and other environmental stressors (54, 55). JNK has been shown to be activated following ischemia/reperfusion injury resulting in induction of both apoptosis and necrosis in hepatocytes (56, 57). Further study has specifically shown that with low levels of oxidative stress ERK1/2 and PKC/PKD based inhibition of JNK is protective in hepatocytes (58, 59). However, despite continued activation of ERK1/2 and PKC/PKD, high levels of oxidative stress overwhelm these protective mechanisms leading to sustained activation of JNK/AP-1 and resultant cell death (59, 60). Finally, Tsung, et al. (61) have demonstrated the importance of toll-like receptor 4 (TLR4) in recognizing endogenous damage associated molecular patterns (DAMPs) following ischemia/reperfusion injury, a process which appears to be of key importance in the overall pathogenesis of the inflammatory injury of warm hepatic I/R (61, 62). Interestingly, chimeric mice which lack non-parenchymal cell TLR4 were seen to be protected from I/R injury (61). These mice had diminished JNK and NF-κB activation, therefore implicating these intermediates in TLR4 signal transduction (61).

Liver recovery and repair after I/R injury in this model begins approximately 48 hours after reperfusion and is associated with increased expression of stathmin and marked hepatocyte proliferation (63). Liver repair and regeneration typically return the liver to its normal, homeostatic state within 5–7 days after reperfusion, depending on the severity of the injury. It is during this reparative/regenerative phase in which the function of CXC chemokines switches from a proinflammatory role to direct impingement on hepatocyte proliferation or death. Knockout of CXCR2, the primary receptor for ELR^+ CXC chemokines in rodents, resulted in accelerated liver recovery associated with increased activation of NF-κB and

STAT3 transcription factors resulting in increased hepatocyte proliferation (38). Antibody blockade of CXCR2 after induction of I/R injury had the same effect (38). These studies suggest that during the reparative/regenerative phase of I/R injury, ELR^+ CXC chemokines have harmful effects which delay liver recovery.

These apparent harmful effects of ELR^+ CXC chemokines is likely a result of specific signaling via CXCR2 in hepatocytes. While the presence and involvement of CXCR2 in murine models of hepatocyte injury and regeneration has been well characterized (37, 38), murine CXCR1 has only been recently identified (64, 65). Previous work has demonstrated that CXCR2 is constitutively expressed in hepatocytes (35), and may be up-regulated in the presence of certain cytokines (66). While CXCR2 and its ligands appear to play a key role in hepatocyte proliferation following partial hepatectomy, and hepatocyte toxicity following I/R injury or acetaminophen toxicity, the role of CXCR1 is less clear. CXCR1 is not constitutively expressed in the liver (64). This finding was recently confirmed, but CXCR1 was found to be induced in hepatocytes after I/R (34). Blockade and knockout of CXCR1 was found to result in delayed liver repair after I/R, although there were no observed changes in hepatocyte proliferation in vivo (34). While the effects of CXCR1 blockade or knockout on liver repair were not as striking as those observed with CXCR2, the findings suggest that CXCR1 has a divergent function in hepatocytes, compared to CXCR2.

While the stress level of the hepatocyte may alter its response to CXC chemokines, so may the concentration of available ligand. Following 70% partial hepatectomy, expression of ELR^+ CXC chemokines increase approximately 5-fold (37), whereas after I/R they increase hundreds- to thousands-fold (38). In vitro, stimulation of primary hepatocytes with CXC chemokines has hepatoprotective effects at low concentrations and progressively cytotoxic effects at increasingly greater concentrations, effects which are specific to CXCR2 (34, 38). Adenoviral-mediated liver overexpression (>100-fold) of the CXC chemokine, keratinocyte-derived chemokine, has been shown to result in massive hepatocellular necrosis within 48 hours (39). Collectively, these studies suggest that moderate increases in CXCR2 ligands, as occurs after partial hepatectomy may promote liver regeneration, whereas much larger increases in expression of CXCR2 ligands, as occurs after I/R injury, may be hepatotoxic and/or oppose hepatocyte proliferation and regeneration.

CXCR1 and CXCR2 Signal Transduction Pathways in Neutrophils

CXCR1 and CXCR2 signaling pathways have been well characterized in neutrophils. While the expression of these receptors on hepatocytes has been demonstrated, the signaling pathways utilized to alter hepatocyte function are unclear. Understanding the signaling pathways utilized by CXCR1 and CXCR2 in neutrophils may offer valuable insights into how these receptors function in hepatocytes.

CXCR1 and CXCR2 receptors are G-protein coupled and involve the $G\alpha_i$ family, specifically $G\alpha_{i2}$ and $G\alpha_{i3}$, as well as $G\alpha_{14}$ and $G\alpha_{16}$ (67–69). Following G protein activation, release of the βγ subunit activates two key pathways involved in generating neutrophil end responses, namely PLCβ and PI3K. PLC-β2/β3 catalyzes the conversion of PIP_2 into the second messengers DAG and IP_3. DAG activates PKC and PLA_2, and IP_3 stimulates Ca^{2+} mobilization. Activation of PKC leads to translocation of $p47^{phox}$ to the plasma membrane, assembly of NADPH oxidase, and generation of the respiratory burst (70). PI3K activation catalyzes the production of PIP_3. PI3K activity is central to several downstream pathways which influence neutrophil function, including Ras/Raf/MAPK and Akt, leading to neutrophil granule release, adhesion and chemotaxis, and respiratory burst (71, 72) (Figure 1). Additionally, expression and activation of the β_2 integrin CD11b, important to neutrophil adhesion, is modulated by PKC alongside of MAPK. MAPK and

PKC appear to function independently as discrete pathways, and both must be inhibited to completely abrogate integrin-dependent adhesion (73).

CXCR1 and CXCR2 have intertwined yet diverse roles in neutrophil activation, and receptor-specific pathways have been identified for both receptors. Using receptor-specific antibodies, it was determined that CXCR1 was uniquely linked to activation of respiratory burst through the action of phospholipase D, known to catalyze the hydrolysis of phosphatidylcholine to phosphatidic acid and choline, thus allowing phosphatidic acid (along with DAG) to activate NADPH oxidase (74). Matrix metalloproteinase-9 (MMP-9) is present in the tertiary granules of neutrophils and can be released through ERK1/2 and PKC dependent pathways as a result of IL-8 stimulation. Blockade of CXCR2, but not CXCR1, diminished the release of MMP-9 in IL-8 treated neutrophils, suggesting a CXCR2 dependent system (75).

The differences between CXCR1 and CXCR2 may be further explored by examining the unique effect of ligand concentration, as well as their individual patterns of receptor internalization and desensitization. Previous work has demonstrated that IL-8 induces a time- and dose-dependent internalization of CXCR1 and CXCR2, with CXCR2 internalized more quickly than CXCR1, but CXCR1 recovering more quickly (76). Ligand concentration may play a role in receptor internalization, as it has recently been proposed that high ligand concentration may allow for receptor endocytosis via clarthrin-coated pits and subsequently act as a stop signal. This is in contrast to the generation of signaling for chemotaxis, which only requires low ligand concentrations, and does not result in receptor endocytosis (77). The concentration of chemoattractant needed to bring about chemotaxis versus granule enzyme release and respiratory burst also differ, with higher concentrations being necessary for the induction of granule release and respiratory burst (78). Neutrophils are known to exhibit a cross-desensitization effect in response to IL-8, and generation of these cross-desensitization signals may be related to the extent and length of activation of the receptor. Rapid phosphorylation of the carboxyl terminus of CXCR2 leads to receptor internalization and may prevent its ability to generate a cross-desensitization signal, indicating that CXCR1 may be responsible for this action (79). Collectively, this research indicates that the ability of CXCR1 and CXCR2 to generate certain signals is dependent upon ligand concentration and the length of receptor activation. While the CXCR1 and CXCR2 receptors in neutrophils certainly have commonalities, it is obvious that they have very distinct roles as well. Based on what is known about each receptor individually, it is possible that CXCR1 may have a more regulatory function, including a role in regulation of CXCR2 (33).

Proposed Signaling Pathways of CXCR1 and CXCR2 in Hepatocytes

Based on the current understanding of various signaling pathways operant in hepatocytes, and the known signal transduction pathways utilized by CXCR1 and CXCR2 in neutrophils, we propose a working hypothesis of signal transduction via CXCR1 and CXCR2 in hepatocytes. It is likely that hepatocyte CXCR1/2 are G-protein-coupled receptors (14, 67, 80). In addition, PI3K, PLC and PLD again all have significant roles in both neutrophil CXCR1/2 signaling as well as in hepatocyte proliferation, therefore it is probable that these elements are likewise a part of hepatocyte CXCR1/2 signaling (9–12, 72, 81). Downstream effectors in murine hepatocyte signal transduction including PKC, the MAPK ERK1/2, and PLA_2 have been linked to both proliferative and cytotoxic effects, suggesting a potential role for these in hepatocyte CXC chemokine signaling. These mechanisms may involve significant cross-talk between receptor pathways, with CXCR2 signaling dominant early, and CXCR1 signaling playing a role later, after it the receptor is upregulated (33, 34).

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We propose a system where signaling via CXCR2 induces a proliferative effect when low concentrations of ligand are present with little or no activity from CXCR1 signaling (Figure 2). As observed by Czaja, et al. (58–60), under conditions of a low stress environment, signaling through ERK1/2 and PKC is able to suppress JNK/AP-1 activation, therefore minimizing cytotoxic effects. Specifically, proliferative signals are likely mediated through MAPK/ERK1/2 activation, and PLC induced PLA_2 stimulation of prostaglandin production, as has been seen during healthy hepatocyte proliferation following partial hepatectomy (11, 15). Conversely, when high ligand concentrations are present, we propose that CXCR2 predominantly activates toxicity pathways. In this scenario, it is probable that the high stress environment induced by an injury such as ischemia/reperfusion overwhelms the protective inhibition of ERK1/2 and PKC/PKD, leading to JNK/AP-1 activation and hepatocyte toxicity (58, 59). Additionally, in conditions of high chemokine concentrations and stressed/injured hepatocytes, PLA_2 activation may lead to excessive production of arachidonic acid leading to further toxicity, and perhaps overwhelming any proliferative effect of prostaglandins (16). CXCR1 is also activated, providing some protective effects, and also exerting a regulatory effect on CXCR2 (34, 38).

Interestingly, some of the concentration-dependent effects seen in neutrophils parallel what has been observed in vivo in the liver (38, 50). The suggested cross-regulatory effect of CXCR1 on the expression of CXCR2 in neutrophils (33) may also be seen between CXCR1 and CXCR2 in hepatocytes as well, and further experimentation will be required in order to elucidate how these two receptors act in concert. Since we know that CXCR1 is inducible in the setting of ischemia/reperfusion, and that high concentrations of CXC chemokines are present in ischemia/reperfusion, it is conceivable that this receptor may have some kind of an impact on the induction of hepatocyte proliferation versus hepatocyte toxicity. Since the signaling mechanisms of hepatocyte CXCR1 and CXCR2 remain to be elucidated, there are likely other relevant pathways yet to be discovered that may prove to be significant in the regulation of liver pathology.

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List of Abbreviations

TNF-α	tumor necrosis factor α
IL-6	interleukin-6
STAT3	signal transducer and activator of transcription 3
NF-κB	nuclear factor-κB
HGF	hepatocyte growth factor
EGF	epidermal growth factor
PLCγ1	phospholipase Cγ1
PLCβ1	phospholipase Cβ1
PLD	phospholipase D
PI3K	phosphoinositide-3-kinase
MAPK	mitogen-activated protein kinase
PLA_2	phospholipase A_2

PGE_2	prostaglandin E_2
PGF_2	prostaglandin F_2
ATP	adenosine triphosphate
AA	arachadonic acid
COX-2	cyclo-oxygenase-2
CCl_4	carbon tetrachloride
ELR	Glutamine-Leucine-Arginine
GPCR	G protein coupled receptor
I/R	ischemia/reperfusion
ROS	reactive oxygen species
JNK	c-Jun N-terminal kinase
IL-1	interleukin-1
ERK	extracellular signal related kinase
PKC	protein kinase C
PKD	protein kinase D
AP-1	activator protein-1
TLR4	toll-like receptor 4
DAMP	damage associated molecular patterns
PIP_2	phosphatidylinositol diphosphate
DAG	diacylglycerol
IP_3	inositol triphosphate
NADPH	nicotinamide adenine dinucleotide phosphate
PIP_3	phosphatidylinositol triphosphate
MMP-9	matrix metalloproteinase-9
IL-8	interleukin-8

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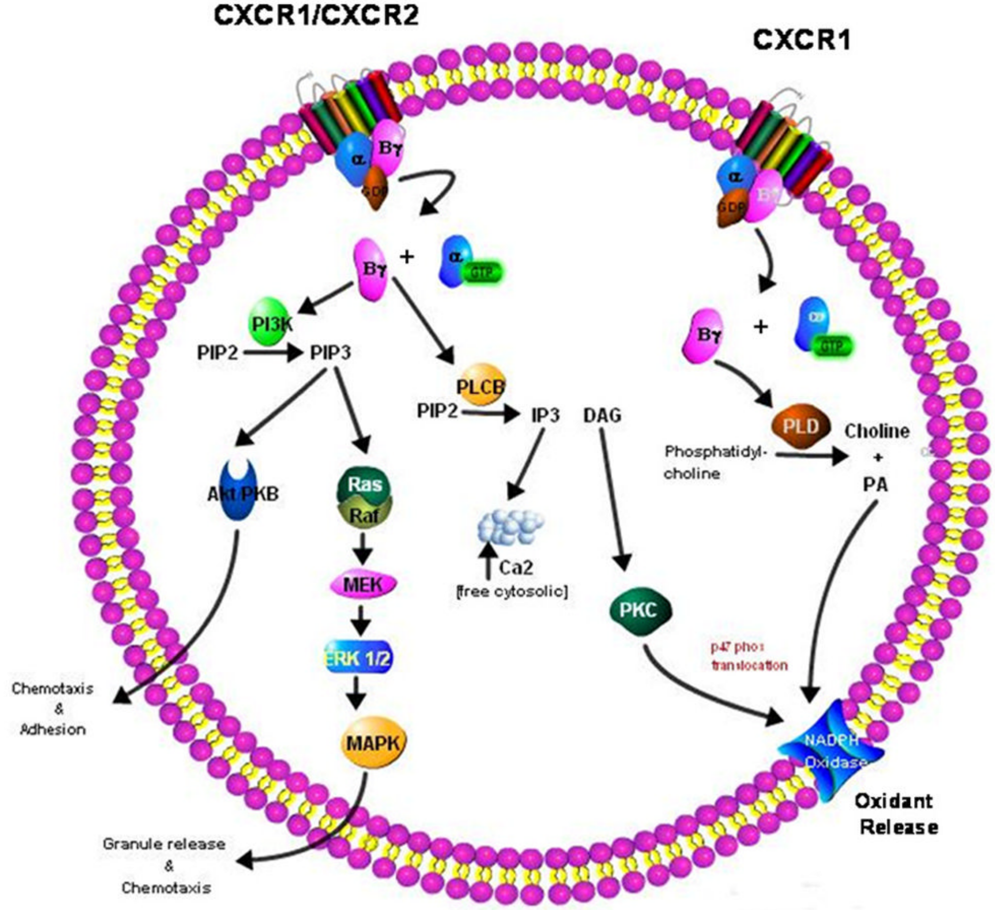

Figure 1.

Signaling pathways of CXCR1 and CXCR2 in neutrophils.

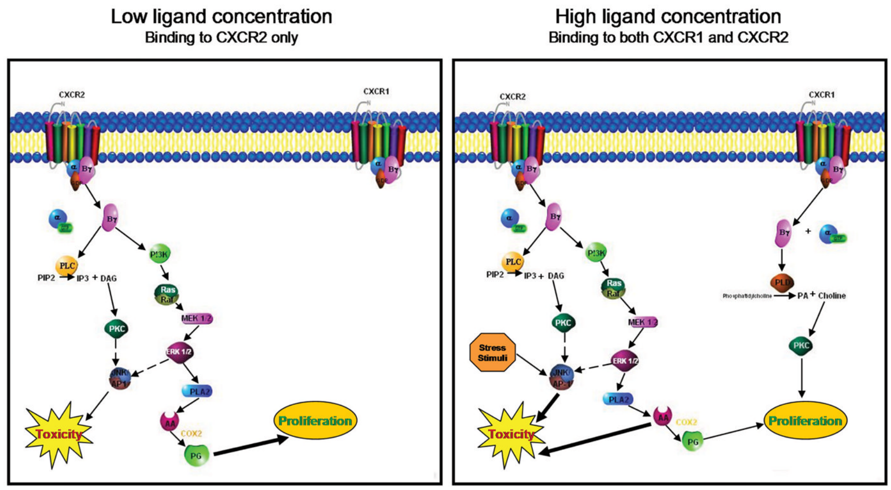

Figure 2.
Proposed signaling pathways utilized by CXCR1 and CXCR2 in hepatocytes under conditions of low and high ligand availability.

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Table 1

CXC chemokines and their receptors.

ELR+ CXC Chemokines Ligands for CXCR1 and CXCR2			ELR− CXC Chemokines Ligands for CXCR3-6		
	Old Nomenclature			Old Nomenclature	
Chemokine	**Human**	**Mouse**	**Chemokine**	**Human**	**Mouse**
CXCL1	Gro-α	KC	CXCL4	PF4	PF4
CXCL2	Gro-β	MIP-2	CXCL9	MIG	MIG
CXCL3	Gro-γ	DCIP-1	CXCL10	IP-10	CRG-2
CXCL5	ENA-78	LIX	CXCL11	I-TAC	I-TAC
CXCL6	GCP-2	GCP-2	CXCL12	SDF-1	SDF-1
CXCL7	NAP-2	TCK-1	CXCL13	BCA-1	BLC
CXCL8	IL-8	none	CXCL14	BRAK	BRAK
			CXCL15	Lungkine	Lungkine
			CXCL16	none	none