CXCL9-11 chemokines and CXCR3 receptor in teleost fish species

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A R T I C L E   I N F O

A B S T R A C T

The coordinated migration of immune cells from lymphoid organs to in or out of the bloodstream, and towards the site of infection or tissue damage is fundamental for an efficient innate and adaptive immune response. Interestingly, an essential part of this movement is mediated by chemoattractant cytokines called chemokines. Although the nature and function of chemokines and their receptors are well documented in mammals, much research is needed to accomplish a similar level of understanding of the role of chemokines in fish immunity. The first chemokine gene identified in teleosts (rainbow trout, *Oncorhynchus mykiss*) was CK1 in 1998. Since then, the identification of fish chemokine orthologue genes and characterization of their role has been more complex than expected, primarily because of the whole genome duplication processes occurring in fish, and because chemokines evolve faster than other immune genes. Some of the most studied chemokines are CXCL9, CXCL10, CXCL11, and the CXCR3 receptor, all involved in T cell migration and in the induction of the T helper 1 (Th1) immune response. Data from the zebrafish and rainbow trout CXCL9-11/CXCR3 axis suggest that these chemokines and the receptor arose early in evolution and must be present in most teleost fish. However, the pieces of knowledge also indicate that different numbers of gene copies can be present in different species, with distinct regulatory expression mechanisms and probably, also with different roles, as the differential expression in fish tissues suggest. Here, we revised the current knowledge of the CXCL9-11/CXCR3 axis in teleost fishes, identifying the gaps in knowledge, and raising some hypotheses for the role of CXCL9, CXCL10 CXCL11, and CXCR3 receptor axis in fish, which can encourage further studies in the field.

1. Introduction

Chemokines are a family of cytokines that coordinate the movement or migration of immune cells from lymphoid organs to the site of action, playing a fundamental role for an efficient innate and adaptive immune response [1]. Chemokines constitutively expressed also regulate homing, maturation, and even microenvironmental segregation within lymphoid organs of immune cells [2]. Beyond this function in immunity, chemokines play essential roles in normal development and growth, such as embryonic development, angiogenesis, and organogenesis [3–5], and tumor growth and metastasis [6,7]. This family of proteins is divided into subfamilies based on structural considerations of the two cysteines closest to the amine (N) terminus, allowing chemokines to be split into four subfamilies: CC, CXC, CX3C, and XC. Cysteines are directly juxtaposed in CC chemokines, while in CXC, chemokines have a variable amino acid between them. The CX3C chemokines have three amino acid residues between these two cysteines, while XC chemokines lack the first

and third cysteines of the motif [1]. Nowadays, 48 chemokines and 23 chemokine receptors have been identified in humans. Due to the promiscuity of this system, one chemokine can bind to one or several receptors, and one receptor can bind to several chemokines [8], resulting in a very intricate network of expression, regulation, and diverse roles.

The nature and function of chemokines and their receptors are well documented in mammals [9]. In teleost fish, less information has been accomplished for understanding the role of chemokines in immunity because they comprise a highly diverse group of approximately one-half of the known vertebrate species. The first chemokine gene identified in teleost (rainbow trout, *Oncorhynchus mykiss*) was CK1 in 1998 [10]. Since then, the identification of chemokine orthologue genes and characterization of their role has been more complex than expected. Primarily, because of the whole genome duplication processes occurring in fish, and because chemokines evolve faster than other immune genes [11].

From all the grand spectrum of chemokine families, this review aims

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to summarize the current knowledge on the CXCL9-11 chemokines and the CXCR3 receptor in teleost fish because of their potential roles in the induction of cellular immune response where Th1 cells play an essential role. Indeed, CXCL9-10/ CXCR3 axis is induced by interferon (IFN)-gamma [12] and CXCL10/CXCR3 interaction triggers the differentiation of naive T cells to T helper 1 (Th1) cells [13,14]. Interestingly, the functional characterization of the CXCL9-11/CXCR3 axis is an active focus of research even in human and other mammalian species because they are responsible for antiviral activity [15] or may also be involved in immunopathogenesis [16]. This is related to the fact that the Interestingly, as chemotactic proteins, the components of this axis are reported to be involved in T cell migration to the intestine and to the skin [17,18] Therefore, understanding the role of CXCL9-11/CXCR3 axis in teleost fishes can unveil relevant functions on T cell differentiation and recruitment into mucosal tissues and skin, which is one of the gaps of knowledge of the molecular and cellular mechanisms of adaptive immunity in fish. Understanding the role of CXCL9-11/CXCR3 axis in teleost fish such as the Atlantic salmon will contribute to the development of strategies for the control of infectious diseases specially in fish species of economic interest.

2. CXCL9-11 chemokines and CXCR3 receptor axis in superior vertebrates

The first chemokine discovered was CXCL10 in 1985, initially identified in human U937 cells (histiocytic lymphoma cell line with monocytic characterization and origin), and from human placenta and spleen as an interferon (IFN)-gamma inducible product [19]; *mob-1* and *crg-2* are the rat and mouse homologs of human chemokine CXCL10, and they share 70 and 78% amino acid homology with these proteins, respectively [20]. CXCL10 is a small molecular weight protein (10 kDa) described as an inflammatory chemokine, and it is secreted from activated neutrophils, eosinophils [21], monocytes, epithelial cells, endothelial cells, stromal cells (fibroblasts), and keratinocytes in response to IFN-gamma [22]. CXCL10 is a chemoattractant for T cells, NK cells, and monocytes [23]. In humans, the CXCL10 gene is localized on chromosome 4, contains four exons and three introns, and encodes a 98 amino acid protein [19,24]. This chemokine is expressed in a wide range of human diseases and is involved in infectious, inflammatory diseases [25–27], autoimmune diseases [28] and cancer [29]. CXCL10 plays an essential role in the localization of leukocytes, exacerbates inflammation, and causes significant tissue damage [28].

CXCL9, at the beginning known as a monokine induced by IFN-gamma (MIG), was identified as a platelet factor 4-like protein selectively induced by IFN-gamma [30,31]. CXCL9 is secreted by T lymphocytes, NK cells, dendritic cells, macrophages, eosinophils, and by non-immune cells such as hepatic stellate cells, preadipocytes, thyrocytes, endothelial cells, tumor cells, and fibroblasts [32–35]. CXCL9 is also on chromosome 4 in a head-to-tail orientation regarding CXCL-10, separated by approximately 16 kb [36]. Studies revealed that CXCL9 and CXCL10 are chemokines that contain two conserved Cys-residues separated by one random residue in the NH2-terminal sequence and lack the conserved ELR (Glu-Leu-Arg) amino acid motif known to be involved in receptor binding for some chemokines [37]. They both act on CXCR3, the receptor for these two chemokines [38].

Another ELR negative, IFN-inducible CXC chemokine, was also identified in stimulated astrocytes and keratinocytes [32,35]. This third IFN-associated chemokine was named IFNγ-inducible protein-9 (IP-9) or IFN-inducible T cell α chemoattractant (I-TAC), and the gene is found on the human chromosome 4 [32,39]. The protein was related to CXCL9 and CXCL10 and showed a high affinity for CXCR3. Then, the new chemokine was called CXCL11. CXCL11 is secreted by human microvascular endothelial cells, keratinocytes, and fibroblasts and is also commonly secreted by peripheral blood mononuclear cells and astrocytes [34]. CXCR3 is the receptor for CXCL9, CXCL10, and CXCL11. It is a G-protein-coupled receptor (GPCR) that preferentially activates one of

several available cell-signaling pathways [40], generating a differential signaling and distinct biological functions in CD4+ T cells to regulate immunity. In mammals is essential for T-cell function and macrophage recruitment at sites of infection and injury. The chemokine ligands are involved in various infectious and pathological conditions, including tuberculosis [41]. In mammals, three variants have been described: CXCR3-A, CXCR3-B, and CXCR3-Alt, each with different size and composition characteristics and produced by alternative splicing. The difference in sequence between CXCR3A and CXCR3-B at the N terminus consists of 52 amino acids and exerts different cellular effects [42]. When CXCL9-11 chemokines bind, CXCR3-A induces chemotaxis and cell proliferation. In contrast, the binding of CXCR3-B with CXCL9-11 blocks cell migration and proliferation, thus triggering apoptosis [43].

3. CXCL9-11 chemokines and CXCR3 receptor axis in teleost fish

3.1. Chemokines in teleost fish

The study of chemokines and the immune system in teleost is of interest because fish are considered an evolutive intermediate step between organisms with only mechanisms of innate immunity (invertebrates) and organisms with well-developed mechanisms of adaptive immunity (mammals). Therefore, the functionality of their different immune cell types and molecules might also be transitional between innate and acquired responses. Teleost fishes comprise a diverse group of fish species that make up about half of the known vertebrate species; thus, the complexity and diversity of fish chemokines are expected to be huge in this context. Accordingly, many more chemokine sequences have been identified in fish than in mammals, probably due to the extensive intrachromosomal gene duplications and the exposure to different infectious experiences [11]. However, homologies with mammalian chemokines have only been established in the case of fish chemokines with well-conserved homeostatic roles [44]. The genes encoding the CXC chemokines are expressed in various organs of different fish species (Table 1). They have different functions (Table 2), including chemotactic functions for monocytes and lymphocytes in several fish species. Twenty-five CXC chemokines have been found in zebrafish (*Danio rerio*), while more than ten have been identified in catfish and rainbow trout, as well as Atlantic salmon (*Salmo salar*) and yellow croaker (*Larimichthys crocea*) [44,45]. Phylogenetic analysis of teleost sequences has identified six different clades for CXC chemokines: CXCa, CXCb, CXCc, CXCd, CXCL12, and CXCL14 [46]. However, fish species showed significant differences, and thus, there are no chemokines for each clade in all fish species. Studies report four groups of CXC chemokines in rainbow trout, namely CXCL8_L1 (CXCa), CXCL10 (CXCb), CXCL_F1a (CXCd1), and CXCL_F1b (CXCd2) [47,48].

3.2. CXCL9-11 chemokines in teleost fish

As in mammals, fish CXCL9, CXCL10, and CXCL11 were first classified based on the presence of the two conserved cysteine residues. Additionally, they have been clustered in no-ELR chemokine near their N-terminus [47,49]. The CXCL9, CXCL10, and CXCL11 ligands of CXCR3 in mammals are likely to have originated from a relatively recent common ancestor [50], but the situation in fish is variegated. As for other chemokines, the availability of genome sequences of fish species and phylogenetic analysis revealed a greater number of genes and complexity in this family of CXCL9-11 chemokines, such as the phylogenetic relationship of fish CXCL9-11 genes is still uncertain [47]. This latter also impacts the nomenclature of these (and others) fish chemokines, which differ depending on the fish species studied. For example, in zebrafish, a cluster of seven putative CXCL11 genes was designated CXCL11aa, ac, ad, ae, af, and ag genes [45]. This group of genes was proposed to be called CXCL11_L2 according to a more recent phylogenetic analysis done by Chen et al. [47]. In this same report, a previously CXCL-10 gene identified in rainbow trout and other cyprinid molecules

Table 1
Expression of the CXCL9-11/CXCR3 in teleost fish.

Chemokine	Alternative name	Species	Tissue or cells	Expression (constitutive, induced by pathogen or PAMPs)	Reference
CXCL10	γIP-10	*Oncorhynchus mykiss*	RTS11	Key marker gene for rIFN-γ biological activity. Up-regulated by LPS and poly (I:C) stimulation.	[52]
CXCL10	IP10	*Cyprinus carpio*	Head kidney	Increases its expression after induction with LPS	[59]
CXCL10	N.A.	*Ictalurus punctatus and I. furcatus*	Head kidney, spleen, liver, gill, skin, stomach, and intestine	Up-regulation of CXC10 after bacterial infection (*Edwardsiella ictaluri*)	[55]
CXCL10	γIP	*Oncorhynchus mykiss*	Gonad	Up-regulation after intraperitoneally injected with IPNV	[53]
CXCL10	γIP	*Oncorhynchus mykiss*	Head kidney and spleen	Up-regulation after intraperitoneally injected with IPNV	[54]
CXCL10	γIP	*Oncorhynchus mykiss*	Head kidney and spleen	Up-regulation after LPS injection	[54]
CXCL10	γIP-10	*Oncorhynchus mykiss*	Gill, spleen, head kidney, and liver	Constitutive expression	[52]
CXCL10	N.A.	*Oreochromis niloticus*	Liver, spleen, and head kidney	Transcriptional up-regulation after stimulations with LPS, *Streptococcus agalactiae*, and Poly I: C	[58]
CXCL10	N.A.	*Scophthalmus maximus*	Spleen and head kidney	High expression levels in tested tissues after bacterial infection (*Vibrio anguillarum*)	[60]
CXCL10	N.A.	*Miichthys miiuy*	Eye, fin, gill, heart, kidney, liver, muscle, and spleen.	Constitutively expressed in all tissues and high expression levels in the kidney and spleen after bacterial infection (*V. anguillarum*)	[61]
CXCL10	N.A.	*Salmo salar*	Spleen	Up-regulated after the administration of *Aerominas salmonicida* vaccine	[66]
CXCL10	CXCL11_L1	*Oncorhynchus mykiss*	Muscle	Up-regulated in vaccinated fish with DNA vaccine of viral haemorrhagic septicaemia virus (VHSV)	[84]
CXCL11	N.A.	*Oncorhynchus mykiss*	Spleen	Up-regulation in fish infected with *Y. ruckeri*	[64]
CXCL11	N.A.	*Oncorhynchus mykiss*	Gill	Up-regulation in fish infected with *Ichthyophthirius multifiliis*	[65]
CXCL11	N.A.	*Sebastes schlegelii*	Head kidney, liver, gill, blood, intestine, spleen, brain, and skin	Up-regulation in head kidney, spleen, liver, and gill after bacterial infection (*Aeromonas salmonicida*)	[85]
CXCL11	CXCL11_L3	*Monopterus albus*	Spleen, kidney, muscle, liver, blood	High expression levels in spleen after bacterial infection (*Aeromonas veronii*)	[86]
CXCR3	N.A.	*Oncorhynchus mykiss*	Blood, head and caudal kidney, spleen, thymus, gills, intestine, adipose tissue, brain, heart, muscle, ovary, liver, scales, skin, adipose and tail fins	Constitutively expressed in all tissues	[71]
CXCR3	N.A.	*Ctenopharyngodon idella*	Brain, intestine, spleen, head kidney, trunk kidney, gill, muscle, liver and blood	Constitutively expressed in the central nervous system	[87]
CXCR3	N.A.	*Oryzias latipes*	Mononuclear phagocytic cells	Marker of innate immune cells	[88]
CXCR3	N.A.	*Sebastes schlegelii*	Head kidney, liver, gill, blood, intestine, spleen, braind and skin	High expression levels in tissues after bacterial infection (*Aeromonas salmonicida*)	[85]
CXCR3	N.A.	*Scophthalmus maximus*	Spleen, head kidney, liver and blood	Up-regulation after LPS injection	[48]
CXCR3	N.A.	*Oncorhynchus mykiss*	Muscle	Up-regulated in vaccinated fish with DNA vaccine of viral haemorrhagic septicaemia virus (VHSV)	[84]
CXCR3.1	N.A.	*Plecoglossus altivelis*	Monocytes/macrophages, kidney, spleen, gill, and liver	Constitutively expressed in macrophages	[79]
CXCR3.2	N.A.	*Plecoglossus altivelis*	Monocytes/macrophages, kidney, spleen, gill, and liver	Induced after *E. coli* infection by a ROS-dependent mechanism	[79]

N.A. No alternative name.

previously called CXCb were clustered together and were proposed to be called CXCL11_L1 (41). It appears that the final classification of these genes must be approached not only from the structural studies but also from the experimental perspective [49]. Thus, to define true chemokine orthologs between fish and mammals, functionality and regulation should be evaluated for each gene [49]. In this case, the expression of CXCL9, CXCL10, and CXCL11 shall be induced by IFN-gamma; they should be involved in T cell or NK-type cell recruitment and act through the CXCR3 receptor.

3.2.1. CXCL10

A chemokine homologous to CXCL10 from mammals was reported ten years ago in rainbow trout [51]. Its expression showed up-regulation in response to IFN-gamma, just as its mammalian counterpart [52]. Moreover, poly I:C but not LPS [51] induces CXCL10 expression, suggesting its role in viral defense [53,54]. Importantly, CXCL10 is induced by the viral hemorrhagic septicemia virus (VHSV), but not by Infectious pancreatic necrosis virus (IPNV), indicating a pathogen-specific regulation [53]. Based on phylogenetic analysis for CXC chemokines in fish, Chen et al. proposed that CXCL10 should be designated as CXCL11_L1 [47]. The use of recombinant IL-1beta, IFN type I, and IFN-gamma increased the levels of CXCL10 mRNA in the rainbow trout Gonad-*2* (RTG2) and Rainbow Trout Spleen-11 (RTS11) cell lines, as well as in primary cell culture of head kidney leukocytes. This indicates that

Table 2
Functional studies of CXCL9-11 and CXCR3 of teleost fish.

Chemokine	Alternative name	Species	Tissue or cells	Function	Reference
CXCL9	N.A.	*Salmo salar*	Gill	Resistant fish to amoebic gill disease had a significant reduction in the CXCL9 transcriptional level	[68]
CXCL10	CXCL11_L1, γIP	*Salmo trutta*	Gills, thymus, mid-gut, spleen, liver, and kidney	Attracting activated and memory T cells	[89]
CXCL10	CXCL11_L1, γIP	*Oncorhynchus mykiss*	Head kidney leucocytes	Involved in regulation of inflammation during the early stages of infection	[72]
CXCL10	N.A.	*Salmo salar*	Muscle	Attraction of T-cells, B-cells, and APCs	[90]
CXCL10	CXCL11_L1, γIP	*Oncorhynchus mykiss*	Head kidney	Regulation of CD4+ cells and macrophages migration	[72]
CXCL11	CXCL11_L3	*Monopterus albus*	Spleen, kidney, liver, and intestine	Attraction of immune cells to protect the skin of the Asian swamp eel in absence of scales	[86]
CXCL11	N.A.	*Ictalurus punctatus*	Gills	High expression in resistant fish to bacterial infection (*Edwardsiella ictaluri*) and fish exposed biotic and abiotic stresses	[63]
CXCL11	N.A.	*Danio rerio*	Embryos	Inducible by bacterial infection (*Mycobacterium marinum*) as the functional ligands of Cxcr3.2	[80]
CXCR3	N.A.	*Ctenopharyngodon della*	Monocytes/macrophages, liver, spleen, gill, and kidney	Regulation of induction of macrophage polarization	[79]
CXCR3	N.A.	*Cyprinus carpio*	Gill, gut, head kidney, spleen	Macrophage-mediated responses	[91]
CXCR3.2	N.A.	*Danio rerio*	Embryos	Required for macrophage motility and recruitment to sites of mycobacterial infection.	[70]
CXCR3.2	N.A.	*Danio rerio*	Embryos	Induces up-regulation of genes of lysosomal function and vesicular traffic and increased microbicidal activity of macrophages.	[70]

N.A. No alternative name.

pro-inflammatory and antiviral cytokines can control the expression of CXCL10 trout chemokine. Some functional studies have also reported that CXCL10 from rainbow trout has chemotactic activity towards head kidney leukocytes. Cells that migrated have a higher abundance of CD4 transcripts than populations that did not migrate, suggesting that CD4+ T cells are one of the main attracted cell types [47]. In channel catfish (*Ictalurus punctatus*) and blue catfish (*I. furcatus*) catfish, the first chemokine identified was a CXC chemokine, most likely CXCL10 [55]. This chemokine, which is constitutively expressed in most tissues in channel catfish and blue catfish, shows different regulation patterns upon infection with *Edwardsiella ictaluri*. Under natural and experimental challenge conditions, channel catfish are highly susceptible to *E. ictaluri*, whereas blue catfish are generally resistant. In this context, CXCL10 was strongly induced in channel catfish in response to the infection but only slightly induced in blue catfish, suggesting that this chemokine is not involved in resistance mechanisms against this pathogen [55]. In Atlantic salmon, a CXCL10-like chemokine mRNA (accession number EF619047.1) has been reported. In cell lines derived from salmon head kidney leucocytes (SHK-1 and TO), CXCL10 is not expressed but its mRNA level increased after infection with Salmon Alpha Virus [56]. This agrees with reports about the activity of its orthologous gene in mammalian, known to be implicated primarily in the immune response to viral or intracellular bacterial infections [57]. The chemokine CXCL10 has also been identified and characterized in Nile tilapia (*Oreochromis niloticus*). The amino acid sequence contains the four conserved cysteine residues and was found to be homologous to the CXCL10 of other species. Expression analysis revealed that CXCL10 was highly expressed in the spleen and widely present in other tissues, including the liver, intestine, and head kidney. Nile tilapia CXCL10 was transcriptional up-regulated after *in vivo* stimulation with polyinosinic: polycytidylic acid (poly I:C) and lipopolysaccharide (LPS), and after bacterial infection (*Streptococcus agalactiae*) [58]. Similarly, in common carp (*Cyprinus carpio L.*) a CXCL10 putative sequence homologous to the human gene was identified. The sequence encoding the four conserved cysteines residues, with first two cysteines separated with phenylalanine, was transcribed in head kidney, spleen, liver, and gills [59]. A sequence with homology to the CXCL10 chemokine gene is also identified in turbot (*Scophthalmus maximus*). Analysis showed induced expression of the chemokine in the liver, spleen, and head kidney after bacterial challenge (*V. anguillarum*), which was kept at high levels for 24 h. The chemokine expression was only detected in tissues of turbot

exposed to the pathogen [60]. In miiuy croaker (*Miichthys miiuy*) a sequence with 65.6% identity with human CXCL10 was identified. This gene was constitutively expressed in all tissues tested; the higher mRNA levels were detected in the intestine and eye, and the lower levels in the bladder, fin, gill, heart, kidney, liver, muscle, and spleen. Upon bacterial infection (*V. anguillarum*), gene expression was up-regulated in the kidney and spleen; however, it was down-regulated in the liver [61].

3.2.2. CXCL11

The status with CXCL11 in fish is variegated and specific expansions have taken place in some cases. In zebrafish, a cluster of seven putative CXCL11 genes, grouped together in a single locus on chromosome 5, share homology and synteny with human CXCL11 [62]. An association between the putative isoforms of CXCL11 ligands and CXCR3 receptors has not been described, and the *in vivo* relevance of this signaling axis has not been addressed in this fish species.

In the genome of catfish, three chemokine genes were identified, *i.e.*, CXCL11.1, CXCL11.2, CXCL11.3. In addition, the expression was evaluated after a bacterial infection (*Edwardsiella ictaluri*). Interestingly, CXCL11.3 was significantly higher in resistant than in susceptible fish. A similar pattern of expression for CXCL11 chemokine was observed between resistant and susceptible fish with biotic and abiotic stresses [63]. Thus, the levels of expression of the CXC chemokines can be a useful indicator of disease or stress resistance. In rainbow trout, the gene sequence of the chemokine CXCL11 was identified by transcriptomic analysis in fish challenged with *Yersinia ruckeri* [64] and *Ichthyophthirius multifiliis* [65], as up-regulation during infectious processes occurred. In Atlantic salmon, a transcriptomic analysis revealed that the chemokine CXCL11 is differentially expressed in fish vaccinated against *Aeromonas salmonicida* [66]. As far as we know, to date no functional analyses of this chemokine have been performed in salmonid species.

3.2.3. CXCL9

The least studied chemokine of the axis in teleost fish is CXCL9. Like CXCL11, sequences corresponding to CXCL9 orthologous has been reported in several species of teleost fish through transcriptomic analysis, but functional information is scarce. One study conducted on carp showed that stress by confinement downregulated the gene expression of CXCL9 in the nucleus preopticus, pituitary gland, and head kidney, all organs of the stress axis [67]. Transcriptomic analysis of amoebic gill disease resistance in Atlantic salmon showed that expression of CXCL9

had significant reduction in the transcription following infection compared to the naïve animals [68].

3.3. CXCR3 receptor in teleost fish

Regarding the CXCR3 receptor, one copy of this chemokine receptor is present in the mammalian genome, while in amphibians, reptiles, and lobe-finned fish, including rainbow trout, two genes related to CXCR3 (CXCR3.1 and CXCR3.2) have been found [69]. In zebrafish, the CXCR3 gene is triplicated: CXCR3.1, CXCR3.2, and CXCR3.3 [70]. The sequences of the rainbow trout CXCR3 receptors share only 36.9% identity at the amino acid level [71], suggesting that they can have different ligands and different functions. CXCR3.1 and CXCR3.2 are constitutively expressed in different rainbow trout tissues, and the expression of CXCR3.1 is significantly higher than CXCR3.2 in the thymus, adipose fin, caudal kidney, cephalic kidney, gonad, and spleen. On the contrary, the expression of CXCR3.2 is significantly higher than CXCR3.1 in caudal fins, liver, and blood [71]. The differential expression in tissues for CXCR3.1 and CXCR3.2 receptors also indicates that they fulfill different roles in fish immunity. Only CXCL10 has been identified as a potential ligand for CXCR3 in rainbow trout [51,72], and it remains to be determined whether CXCR3.1 and CXCR3.2 bind the same ligands or not [47]. Both CXCL10 and CXCR3.1 are up-regulated by poly I:C, IL-1beta, and TNF-alpha. On the other hand, the expression of CXCR3.2 was negatively regulated by poly I:C and peptidoglycan (PGN) [14,71].

4. Functional studies

In mammals, functional studies done *in vitro* have shown that CXCL9 and CXCL10 bias the polarization of T cells in Th1 and Th17 effector cells [14]. CXCR3 drives Th1 polarization through phosphorylation of STAT1, STAT4, and STAT5. On the contrary, CXCL11/CXCR3 interaction induces an immunotolerant state characterized by IL-10 (Type 1 regulatory cells (TR1)) and IL-4 (T helper 2 (Th2)) cells. Cell signaling involves the p70 kinase/mTOR in STAT3- and STAT6-dependent pathways [73]. CXCL11 binds to the CXCR3 receptor with a higher affinity than the CXCL10 ligand, suggesting that CXCL11 has the potential to mediate and restrict inflammatory responses [73]. Therefore, despite being chemokines of the same subfamily, it seems that each one plays different roles in immunity.

In fish, CD4+T cells have been identified and characterized in several fish species [74–78] but no such studies of the role of CXCL9-11/CXCR3 on T cell polarization exist for teleost fishes. Instead, the role of the CXCL9-11/CXCR3 axis on macrophage functions has been reported. For example, in ayu (*Plecoglossus altivelis*), grass carp (*Ctenopharyngodon idella),* and green spotted pufferfish (*Tetraodon nigroviridis*), the isoforms CXCR3.1 and CXCR3.2 are also expressed in macrophages and contribute differentially to macrophage polarization. CXCR3.1 gene in ayu is constitutively expressed in macrophages, whereas the CXCR3.2 gene is induced post-infection with *Escherichia coli*. Upon *E. coli* infection, CXCR3.1+ and CXCR3.2+ macrophages showed an M1 and an M2 phenotype, respectively. CXCL9-11-like proteins mediated M1 and M2 polarization by interacting with the CXCR3.1 and CXCR3.2 proteins on macrophages, respectively [79]. Another study using the optically accessible zebrafish embryo model explored the function of the CXCR3-CXCL11 in macrophage recruitment. For this, an axis interruption was performed and infection with *Mycobacterium marinum* was used to study the effects of mutations on a fish infectious disease model. During infection of zebrafish embryos, CXCR3.2 deficiency limited the macrophage-mediated dissemination of mycobacteria. Furthermore, the loss of the CXCR3.2 function attenuated the formation of granulomatous lesions and led to a reduction in the total bacterial burden [80]. Thus, the disruption of CXCR3.2 increases the resistance to mycobacterial infection [80,81]. Furthermore, the transcriptomic analysis showed that CXCL11aa was the most highly induced gene in infected macrophages with *M. marinum*, suggesting that this chemokine can be the functional

ligands of CXCR3.2. In this line, other studies show that CXCR3.2, and CCR2 both mediate the recruitment of macrophages to injury [41,70,82, 83]. Interestingly, mutation of CXCR3.2 induces up-regulation of genes of lysosomal function and vesicular traffic and increased microbicidal activity of macrophages [70].

5. Concluding remarks

In the axis CXCL9-11/CXCR3, the chemokines CXCL9, CXCL10, and CXCL11 have been identified in some teleost fish species. The availability of genome sequences of fish species and phylogenetic analysis revealed a great number of genes and complexity in the family of CXCL9-11 chemokines; thus, the phylogenetic relationship of fish CXCL9-11 genes is still uncertain. This latter also impacts the nomenclature of these (and others) fish chemokines, which differ depending on the fish species studied.

Functional characterization is scarce, particularly for fish species of interest in aquaculture (Table 1). No clear inferences about the functions of the chemokines CXCL9, CXCL10, and CXCL11 can be made based on their structural similarities to their mammalian counterparts.

The presence of multiple genes in some fish species makes it extremely important to use biochemical or genetic strategies to define functions in wet-lab experiments. Furthermore, functional studies are essential to understand the role of the CXCL9-11/CXCR3 axis in fish immune response and protection against infectious diseases, particularly in fish species of interest in the aquaculture industry.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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