Developmental and Comparative Immunology 52 (2015) 37–47

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Comparative analysis of two types of CXCL8 from Japanese flounder (*Paralichthys olivaceus*)

Beibei Zhao, Takayuki Katagiri, Hidehiro Kondo, Ikuo Hirono *

Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato-ku, Tokyo 108-8477, Japan

ARTICLE INFO

Article history:
Received 25 February 2015
Revised 17 April 2015
Accepted 19 April 2015
Available online 23 April 2015

Keywords:
CXCL8_L1a
CXCL8_L1b
Japanese flounder
Immune response
Chemotaxis

ABSTRACT

A new type of CXCL8, named CXCL8_L1b, was identified in this research. Comparison of amino acid sequences of Japanese flounder CXCL8_L1b and CXCL8_L1a (BAB86884.1) showed only 41.2% identity. Transcripts of CXCL8_L1a were highly detected in spleen, kidney, gill and liver, while transcripts of CXCL8_L1b only were detected highly in spleen and kidney of apparently healthy fish. In fish challenged with *E. tarda*, transcripts of CXCL8_L1a were significantly increased at day 6, while no significant increase was detected in the mRNA level of CXCL8_L1b. On the other hand, fish infected by *S. iniae* significantly increased both transcripts of CXCL8_L1a and CXCL8_L1b at days 1 and 3. In VHSV-infected fish, only the transcripts of CXCL8_L1b were significantly induced at day 6. LPS and poly I:C stimulation of PBLs induced a high level of CXCL8_L1a transcripts, while CXCL8_L1b transcripts were significantly increased only post poly I:C treatment. To evaluate the chemotactic activity of CXCL8_L1a and CXCL8_L1b, Japanese flounder were intramuscularly injected with recombinant plasmids pCI-CXCL8_L1a and pCI-CXCL8_L1b. H & E staining showed that injections of both pCI-CXCL8_L1a and pCI-CXCL8_L1b caused strong immune responses in the form of intermuscular cell infiltration and capillary congestion. Injection of pCI-CXCL8_L1a and pCI-CXCL8_L1b significantly induced the expressions of genes related to inflammatory response such as IL-6 and CD8α on day 1 post-injection. The transcripts of IgM only significantly increased on day 7 post-injection of pCI-CXCL8_L1b.

1. Introduction

CXCL8, also known as Interleukin 8 (IL-8) or NAP-1 (neutrophil-activating peptide 1), is a secreted chemokine, which are chemotactic polypeptides of 8–15 kDa. By recruiting immune cells to injury sites, chemokines play an important role in both innate and adaptive immune responses (Esche et al., 2005). Most chemokines have four conserved cysteine residues which are important for their tertiary structure (Fernandez and Lolis, 2002; Joseph et al., 2010). In mammals, based on the position of the first two cysteine residues, chemokines are divided into CC, CXC and CX_3C subfamilies and a fourth subgroup of C chemokines lacking the first and the third cysteine residues. A CX subgroup lacking the second cysteine residue with five members was found in zebrafish (Nomiyama et al., 2008).

CXCL8, the first identified CXC chemokine, is an inflammatory chemokine (Zlotnik and Yoshie, 2012). It is minimally produced by non-stimulated cells in mammals although it can be secreted by a wide range of cells including macrophages, monocytes, and epithelial and endothelial cells (Bickel, 1993). Significant increases in CXCL8 transcripts have been detected after stimulation by bacteria (Eckmann et al., 1993; Hirao et al., 2000; Larsson et al., 1999), virus (Horne et al., 2004; Lane et al., 2001) and some cytokines such as IL-1 or tumour necrosis factor (TNF) (Qazi et al., 2011). In mammals, the presence of a Glu-Leu-Arg (ELR) motif in chemokines is required for receptor binding and signalling (Gerber et al., 2000). Chemokines with ELR motifs preferentially recruit neutrophils, while chemokines without an ELR motif mainly target lymphocytes and monocytes (Fernandez and Lolis, 2002). All currently identified CXCL8s in mammals are ELR positive, while CXCL8s in most teleost fish are ELR negative. However, for Japanese flounder CXCL8 (AB809049) the first 6–11 amino acids at the N-terminus are required for neutrophils migration, and not those corresponding in position to ELR motif in mammalian CXCL8 (Kurata et al., 2014).

The chemokine family is one of the most rapidly evolving gene clusters (Sequencing and Consortium, 2005). CXCL8, in the last decades, has been described in multiple vertebrates including mammals (Baggiolini et al., 1989; Seow et al., 1994), avians (Wu et al., 2008), amphibians (Cui et al., 2011), and several fish species

* Corresponding author. Laboratory of Genome Science, Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato-ku, Tokyo 108-8477, Japan. Tel.: +81 3 5463 0689; fax: +81 3 5463 0689.
E-mail address: hirono@kaiyodai.ac.jp (I. Hirono).

http://dx.doi.org/10.1016/j.dci.2015.04.011
0145-305X/

(van der Aa et al., 2010). Two lineages of CXCL8 were revealed recently in teleost fish (van der Aa et al., 2010). Both of them have different abilities to induce cytokine expression, but similar capability in migration of leukocytes (de Oliveira et al., 2013; van der Aa et al., 2012). Recently, a novel CXCL8_L3 group in teleost fish were identified by analysing 421 CXC chemokines (Chen et al., 2013). Gene expression analysis and chemotactic assay showed that the role of the teleost CXC chemokines are relatively well conserved (Chen et al., 2013). Studying their gene expression patterns and the target cells are important for understanding the complex chemokine pathway in fish. Japanese flounder is an economically important fish species in East Asia. Although five CXCL8 homologues were identified in Japanese flounder, their roles in immune response are still unclear.

Here, a new Japanese flounder CXCL8_L1b (AB778258) which showed low identity with the previous identified Japanese flounder CXCL8 (Supplementary data, Table 1) from an expressed sequence tag (EST) database was identified. To understand the roles of CXCL8 in Japanese flounder, CXCL8_L1a and CXCL8_L1b (Supplementary data) were studied. Gene expression analysis and chemotactic capabilities of both CXCL8_L1a and CXCL8_L1b were performed *in vitro* and *in vivo* in Japanese flounder.

2. Materials and methods

2.1. Cloning and sequence analysis of CXCL8_L1b in Japanese flounder

An EST of CXCL8_L1b was amplified from kidney cDNA of Japanese flounder infected by *Edwardsiella tarda* using three pairs of primers (Table 1) and the following PCR conditions: an initial denaturation step of 5 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and 1 min

Table 1
List of primers and their sequences used in experiments.

Primers and their designated application in this study		
Primer name	Sequence (5′–3′)	Usage
CXCL8_L1bF1	TGGGAGGGAGATGAGAAAGG	Sequencing
CXCL8_L1bR1	AGTTACAACTGCCACGT	Sequencing
CXCL8_L1bF2	GATCACAGATCAGGGAAAGA	Sequencing
CXCL8_L1bR2	AAGAATGGTTTGTTCCCTGG	Sequencing
CXCL8_L1bF3	GCCAAGTTGGTTATGTGTAG	Sequencing
CXCL8_L1bR3	GTCCTGATGATGTGTGCCTT	Sequencing
CXCL8_L1b_STF	AGCAGCAGAGTCATCGTTGT	Quantitative PCR
CXCL8_L1b_STR	TTATCGCAGTGATAATTGGG	Quantitative PCR
CXCL8_L1a_STF	CTTTACAATTGTGGCACTCC	Quantitative PCR
CXCL8_L1a_STR	TCTTTGCAGTGAGAGCTGG	Quantitative PCR
CXCL8_L1b _qF	CAATTGTGGCACTCCTGGTTT	Quantitative PCR
CXCL8_L1b _qR	CCATTGCTCAGACTGGTTCCA	Quantitative PCR
CXCL8_L1a_qF	CATCGTTGTTGCTGTGATGGT	Quantitative PCR
CXCL8_L1a_qR	AGGCTCACCGCTTCACTGAT	Quantitative PCR
EF-1α_qF	CTCGGGCATAGACTCGTGGT	Quantitative PCR
EF-1α_qR	CATGGTCGTGACCTTCGCTC	Quantitative PCR
TNF-α_qF	CGAAGGCCTAGCATTCACTCA	Quantitative PCR
TNF-α_qR	TCGTGGGATGATGATGTGGTT	Quantitative PCR
IFN-γ_qF	TGTCAGGTCAGAGGATCACACAT	Quantitative PCR
IFN-γ_qR	GCAGGAGGTTCTGGATGGTTT	Quantitative PCR
IL-1β_qF	CAGCCACATCAGAGGCAACACAACA	Quantitative PCR
IL-1β_qR	TGGTAGCACCGGGCATTCT	Quantitative PCR
IL-6_qF	CAGCTGCTGCAAGAC	Quantitative PCR
IL-6_qR	GATGTTGTGCGCCGTCATC	Quantitative PCR
CXCL8_L1b_rF	GGGCATATGGGAGTGACTCCAAGAT	Recombinant plasmid
CXCL8_L1b_rR	CCCGAATTCTCATATCTTTCCCTGA	Recombinant plasmid
CXCL8_L1a_rF	GGGCATATGGTGAGCCTGAGAAGCCTA	Recombinant plasmid
CXCL8_L1a_rR	CCCGAATTCTCAGCGTCTTTTTGAA	Recombinant plasmid
CXCL8_L1b_T7_F	TTCACGCGTGGTACCTCTAGAG	Quantitative PCR
CXCL8_L1b_OR	CAGGAGTGCCACAATTGTAAAGAA	Quantitative PCR
CXCL8_L1a_T7_F	TCGAGAATTCACGCGTGGTA	Quantitative PCR
CXCL8_L1a_OR	CAACGATGACTCTGCTGCTCAT	Quantitative PCR
CXCL8_L1a_OF	ATTTCAAAAAGACGCTGAGCAGAT	Quantitative PCR
CXCL8_L1a_UTR_R	AGGGTCGTGTTGAGTTGTCTTTAAA	Quantitative PCR
CXCL8_L1b_OF	CAGAACAGTGTATCCCTGCAGAGT	Quantitative PCR
CXCL8_L1b_UTR_R	TCTTTCCAAGAGGTGGCAGTGT	Quantitative PCR
CD4-1_qF	CCAGTGGTCCCCACCTAAAA	Quantitative PCR
CD4-1_qR	CACTTCTGGGACGGTGAGATG	Quantitative PCR
CD4-2_qF	CACAGCGAGGACGTCAGAAA	Quantitative PCR
CD4-2_qR	TCTCTCCCATCACTCCTTTAGCA	Quantitative PCR
CD8α_qF	CCTCTCCCCATACATTGATTCC	Quantitative PCR
CD8α_qR	CCGAGCTTTGCTGAAGGACTT	Quantitative PCR
CD8β_qF	GATGACACTCAAACCTCCAGTCAA	Quantitative PCR
CD8β_qR	GCCATCCTGTGCAAAATTCTTC	Quantitative PCR
IgM_qF	ATGGATCCTACTCGGCTTATGG	Quantitative PCR
IgM_qR	TGGTAAACAACGCAGCTGT	Quantitative PCR
IgD_qF	AACACTAGCAAGCCCCAACAA	Quantitative PCR
IgD_qR	CTCCTGGTAACCAGTTGCCTTT	Quantitative PCR
MHCIIα_qF	GCCAGACTGAAATTCATCGCT	Quantitative PCR
MHCIIα_qR	CCAGATCTTGGTCAGTGATTGG	Quantitative PCR
MHCIIβ_qF	CCTGGGTCTGACCTTATCTCTG	Quantitative PCR
MHCIIβ_qR	CCAAGTCCAGGACCAGACTGAC	Quantitative PCR