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Molecular characterisation of four class 2 cytokine receptor family members in rainbow trout, *Oncorhynchus mykiss*

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ABSTRACT

The interleukin (IL)-10 cytokine family includes IL-10, IL-19, IL-20, IL-22, IL-24, IL-26 and the lambda/type III interferons. They are highly pleiotropic and mediate a variety of activities, including immune suppression and antibacterial immunity. To exert their functions they signal through a heterodimeric receptor composed of a subunit with a long intracellular domain (R1 type receptors; IL-10R1, IL-20R1 or IL-22R1) and a subunit with a short intracellular domain (R2 type receptors; IL-10R2 or IL-20R2). In this study we report the identification of three R1 type receptors (named IL-10R1/CRFB7, IL-20R1a/CRFB8a and IL-20R1b/CRFB8b) and one R2 type receptor (named IL-10R2/CRFB4) in rainbow trout. The nomenclature of the receptors was supported by homology analysis, conserved motifs and phylogenetic tree analysis, confirming they belong to the piscine class 2 cytokine receptor family. For instance, they all displayed the presence of characteristic features, such as conserved fibronectin type-III domains. Expression analysis in tissues collected from healthy fish revealed different patterns of expression for each receptor, suggesting their potential involvement in different types of immune responses. When studying the modulation of the genes in cell lines and primary cultures, a greater effect was observed in the cell lines, where the expression of most receptors was affected by incubation with microbial mimics (LPS and PolyI:C) or the pro-inflammatory cytokine rIFN-γ. In addition, expression of the four receptors was modulated by viral infection, suggesting a potential involvement of such receptors and their ligands in antiviral defence.

1. Introduction

Helical cytokines exert their functions after binding to cell surface receptors (also known as helical cytokine receptors), which activates signal transduction pathways leading to a cascade of intracellular events in the target cell. Helical cytokine receptors that require an association of the intracellular domain of the receptor chain with tyrosine kinase enzymes are classified into class 1 or 2 cytokine receptors. This classification is based on structural differences and more specifically on the existence of conserved motifs in the extracellular domains (Krause and Pestka, 2005; Langer et al., 2004). The class 2 cytokine receptor family (CRF2), includes six different receptor chains for the nine to ten ligands of the interleukin (IL)-10 cytokine family known in mammals (Booth and George, 2013; Kotenko et al., 2001; Trivella et al., 2010; Xie et al., 2000). These receptors are grouped into the R1 and R2 receptor subunits. The R1 type receptors have a long intracellular domain and include IL-10R1, IL-20R1, IL-22R1 and IL-28R in mammals, usually sharing high affinity towards their ligands. The R2 type subunits comprise IL-10R2 and IL-20R2, and contain a short intracellular domain. Dimerisation of both receptor chains upon binding to their respective ligand triggers intracellular signalling pathways (Kotenko and Langer, 2004; Trivella et al., 2010). In addition to the transmembrane chains, there is also a receptor for IL-22 that consists only of an extracellular domain, named the IL-22 binding protein (IL-22BP) or IL-22R2 (Xu et al., 2001). Structurally, CRF2 members are characterised by the presence of three characteristic regions: an extracellular domain (organised into two fibronectin type-III (FNIII) domains), a transmembrane region, and the intracellular domain which ranges between 60 to 300 aa depending on whether a short or long cytoplasmic chain is present (Bazan, 1990; Trivella et al., 2010). The two FNIII domains in the extracellular regions are referred to as D1 and D2 according to their distal or proximal distance to the membrane, respectively (Langer et al., 2004).

IL-10 signals through the receptor complex composed of a high affinity IL-10R1 long chain and IL-10R2 short chain (Josephson et al., 2001; Kotenko et al., 1997). The IL-10R1 was first isolated in mice (Ho et al., 1993) and later in humans (Liu et al., 1994). The mouse IL-10R1 contains 575 aa whereas the human protein contains 578 aa, and they share an aa identity of 60%. In terms of tissue

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distribution, human IL-10R1 has a low expression in lymphoid organs such as thymus, spleen and peripheral blood leucocytes (Ho et al., 1993; Liu et al., 1994). The IL-10R2 gene was first described as the ubiquitously expressed human CRFB4 gene in 1993 and identified later as IL-10R2 in 1994 (Gibbs and Pennica, 1997; Kotenko and Langer, 2004; Kotenko et al., 1997; Lutfalla et al., 1993). In addition to acting as a second chain subunit for the IL-10 receptor complex, it also serves as an accessory receptor chain for IL-20, IL-22, IL-26 and the type III interferons (Kotenko et al., 1997; Trivella et al., 2010).

IL-19, IL-20 and IL-24 signal through a heterodimeric receptor complex composed of the two subunit chains IL-20R1 and IL-20R2 (Blumberg et al., 2001; Dumoutier et al., 2001; Wang et al., 2002). Human IL-20R1 contains 553 aa whereas the IL-20R2 only contains 311 aa. Both receptors have differences in their tissue and cell distribution. For instance, IL-20R2 has a more limited expression pattern in tissues and cell types relative to IL-20R1, indicating that the latter could function independently of IL-20R2 (Blumberg et al., 2001; Sheikh et al., 2004; Wolk et al., 2002). Both receptors are highly expressed in human skin, and are up-regulated in human psoriatic skin, suggesting their involvement in skin diseases, such as psoriasis (Blumberg et al., 2001).

IL-22 signals through a heterodimeric receptor complex composed of two transmembrane chains, IL-10R2 and IL-22R (Kotenko et al., 2001; Xie et al., 2000). Findings in mammals suggest that IL-22 has a higher affinity to the IL-22R binding chain. Following binding to this receptor, an alteration of the cytokine conformation occurs allowing the interaction with another receptor, the accessory chain IL-10R2. Since the latter receptor is constitutively expressed in most cell types, it is the restricted expression of IL-22R in tissues such as skin, digestive and respiratory tracts that dictates the sites of action of IL-22 (Trivella et al., 2010; Wolk et al., 2004). IL-22R can also be used for the signalling of IL-20 and IL-24 combined with IL-20R2 (Langer et al., 2004).

With the availability of genomes for zebrafish (*Danio rerio*) and two pufferfish species (*Takifugu rubripes* and *Tetraodon nigroviridis*), putative homologues of the mammalian CRF2 family have been identified and characterised in lower vertebrates, but the orthology between mammalian and piscine species molecules is not straightforward (Krause and Pestka, 2005; Stein et al., 2007). According to the mammalian nomenclature, the fish molecules have been named cytokine receptor family B (CRFB) 1 to 17 members (Aggad et al., 2010; Krause and Pestka, 2005; Levraud et al., 2007; Lutfalla et al., 2003; Stein et al., 2007). An IL-10R1 molecule has also been identified in goldfish *Carassius auratus L.* and grass carp *Ctenopharyngodon idellus*, with functional evidence for IL-10 signalling (Grayfer and Belosevic, 2012; Wei et al., 2013). Most recently CRFB4 and CRFB5 have also been characterised in grass carp (Wei et al., 2014). The present work describes the sequencing and characterisation of four receptor chains with homology to IL-10R1/CRFB7, IL-10R2/CRFB4 and IL-20R1/CRFB8 molecules in rainbow trout *Oncorhynchus mykiss*.

2. Material and methods

2.1. Maintenance of rainbow trout

Rainbow trout, weighing approximately 100 g, were purchased from the Mill of Elrich Trout Fishery (Aberdeenshire, UK) and maintained in 1-m-diameter aerated fibreglass tanks supplied with a continuous flow of recirculating freshwater at 15 ± 1 °C. Fish were fed twice daily on standard commercial pellets (EWOS, Scotland), and were given a 2-week acclimatisation period prior to treatment.

2.2. Cloning and sequencing of four receptor genes

Blast search of homologous sequences in databases and cloning of cDNA sequences were carried out as described previously (Wang and Secombes, 2003; Wang et al., 2011). IL-10R1/CRFB7 was cloned by 3′-RACE using primers F1 and F2 designed based on the 5′UTR sequence of an EST (accession number: CA388246). IL-10R2/CRFB4 was obtained by 5′-RACE using primers R1 and R2 designed based on the sequence of an EST (accession number: CA354684) and 3′-RACE with primers F2 and F3 designed based on the 5′UTR sequence of the 5′-RACE product. IL-20R1a/CRFB8a was a random sequenced cDNA clone from a full-length cDNA library as described previously (Wang et al., 2005). IL-20R1b/CRFB8b was cloned by 5′ and 3′-RACE using primers designed based on the sequence of another EST (accession number: BX866651). The nucleotide sequences of the receptors are deposited in the EMBL/DDBJ/GenBank nucleotide sequence database under the following accession numbers: IL-10R1/CRFB7: FN824524, IL-10R2/CRFB4: FN824526, IL-20R1a/CRFB8a: AJ555870 and IL-20R1b/CRFB8b: FN824525 and are detailed in Appendix: Supplementary Figs. S1–S4. Primer sequences are listed in Table 1.

Table 1
Oligonucleotide primers used for cloning and expression analysis.

Primer name (forward and reverse)	Primer sequence (5′ → 3′)	Primers used
IL-10R1/CRFB7 F1	CCAACTGAGCCTTCCCGTCTC	Cloning of IL-10R1/CRFB7
IL-10R1/CRFB7 F2	GAAGGCTGAGCTGATGAGGGAC	(3′-RACE)
IL-10R2/CRFB4 F2	CATTTCAGTGTCGCTGGACTACA	Cloning of IL-10R2/CRFB4
IL-10R2/CRFB4 F3	CACAGAACATCTCTCGGGCAAC	(3′-RACE)
IL-10R2/CRFB4 R1	TTGGCCTTAGCGGTGCTCTC	Cloning of IL-10R2/CRFB4
IL-10R2/CRFB4 R2	CCATTGCTCCAGCTCCAACAC	(5′-RACE)
IL-20R1b/CRFB8b F1	CTGGTCAACATTGCCAAGCC	Cloning of IL-20R1b/CRFB8b
IL-20R1b/CRFB8b F2	CAACATCCCTGCCCTGATCC	(3′-RACE)
IL-20R1b/CRFB8b R1	ACACATCCCAGTCAAAACATTCTCTAC	Cloning of IL-20R1b/CRFB8b
IL-20R1b/CRFB8b R2	CTCAATGTCAGATGCCTCACTTTC	(5′-RACE)
EF-1 α F	CAAGGATATCCGTCGTGGCA	Real-time PCR
EF-1α R	ACAGCGAAACGACCAAGAGG	
IL-10R1/CRFB7 F	CATCTCAGACTGTGTGTCAGGTGAAG	Real-time PCR
IL-10R1/CRFB7 R	GGTCCAATTAATATTGCCATACCTGG	
IL-10R2/CRFB4 F	GGCCAGGAGAAGAAGGTCACAC	Real-time PCR
IL-10R2/CRFB4 R	TTGGCCTTAGCGGTGCTCTC	
IL-20R1a/CRFB8a F	AAGCCCCCCTTTCCTGGAAATATC	Real-time PCR
IL-20R1a/CRFB8a R	TCCCATGATCCCTCCTGACAATC	
IL-20R1b/CRFB8b F	GAAAAGTCCCCCTTTCCTGGAAATATT	Real-time PCR
IL-20R1b/CRFB8b R	TCGCATGATCCCTCCTTTGG	