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Short communication

Identification, expression and immunological responses to bacterial challenge following vaccination of BLT1 gene from turbot, *Scophthalmus maximus*

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ABSTRACT

Leukotriene B4 (LTB4) is well known as a chemoattractant for leucocytes, recent studies also showed its involvement in adaptive immunity. The purpose of this work is to report the cloning, characterization and gene expression of leukotriene B4 receptor (BLT1) in turbot (*Scophthalmus maximus*), as well as the immunological response to challenge following vaccination with a live attenuated vaccine *Vibrio anguillarum* MVAV6203. The full cDNA sequence of turbot BLT1 was cloned. The open reading frame consists of 1119 bp nucleotides, which translate into 372 amino acid protein. A high conservation of amino acid sequence was found in the seven transmembrane (TM) domains and intracellular loops. The intracellular loop 3 consisting of a unique cluster of basic amino acid residues might be associated with signal transduction. High amino acid similarity and a phylogenetic tree confirmed it as a leukotriene B4 receptor member. The BLT1 gene is expressed in a wide range of tissues with the highest expression in kidney followed by spleen. The expression of turbot BLT1 was significantly up-regulated in spleen, gut and gill after vaccination and in kidney and skin after challenge. These results suggest a potential role of turbot BLT1 in protection against infection.

1. Introduction

Leukotriene B4 (LTB4), an oxidized fatty acid derived from arachidonic acid, is a well known chemoattractant for leucocytes (Samuelsson, 1983; Serhan et al., 1996). LTB4 is an inducer of the migration and adhesion of polymorphonuclear leucocytes to endothelial cells (Ford-Hutchinson et al., 1980; Gimbrone et al., 1984; Hoover et al., 1984). LTB4 also stimulates polymorphonuclear leucocytes to produce superoxide anions (Palmblad et al., 1984) and to secrete lysosomal enzymes (Rae and Smith, 1981). These biological responses induced by LTB4 are important in host defence mechanisms against foreign organisms. The biological properties of LTB4 are comparable to those of other chemotactic factors, such as C5a and bacterial formyl peptide, and are considered to be mediated through a G-protein-coupled receptor (GPCR) expressed on the cell surface of leucocytes (Andersson et al., 1986; Schepers et al., 1992; Schepers and McLeish, 1993). Mammals have at least two receptors for LTB4, high-affinity BLT1 (leukotriene B4 receptor 1) and low-affinity BLT2 (leukotriene B4 receptor 2), both of which are GRCR. Initially BLT1 was found to be expressed only in phagocytes (Samuelsson, 1983), and in fact no BLT1 expression occurs in naïve T cells. However, during the in vitro differentiation of naïve T cells into Th1 and Th2 ($CD4^+$) (Tager et al., 2003) and cytotoxic effector T cells ($CD8^+$) (Goodarzi et al., 2003; Ott et al., 2003) BLT1 expression is greatly up-regulated, suggesting the involvement of BLT1 in adaptive immune response. Additionally, LTB4–BLT1 pathway is involved in linking early immune system activation and early effector T cell recruitment (Tager et al., 2003). In Th17-differentiated T cells increased expression of BLT1 was also reported and BLT1-KO mice developed a milder symptom with low clinical scores and body weight loss than WT mice on mouse experimental allergic encephalomyelitis (EAE) model, suggesting the involvement of LTB4–BLT1 in Th17-induced multiple sclerosis (Kihara et al., 2010).

Previous studies have shown that fish mononuclear phagocytes, granulocytes and thrombocytes synthesize and release eicosanoid derivatives, such as prostaglandin E2 and leukotriene B4. These eicosanoids play a role in immune regulation in fish in a similar way to that reported in mammals (Rowley et al., 1995). Leukotriene B4 was found to induce enhanced migration of the eosinophilic G1 granulocyte in the dogfish (Hunt and Rowley, 1986). The proliferation of rainbow trout head kidney leukocytes in response to mitogen was stimulated by

Abbreviations: LTB4, leukotriene B4; BLT1, leukotriene B4 receptor; BLT2, leukotriene B4 receptor 2; TM, transmembrane; GPCR, G-protein-coupled receptor; VHSV, viral hemorrhagic septicemia virus; ORF, open reading frame; RACE, rapid-amplification of cDNA ends; ICL, intracellular loop.

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Table 1
Primers used for cloning, RACE, amplification and expression analysis.

Designation	Sequence (5′–3′)	Function
3′-R	CGTCCAAATGTCACTGCCTTCGCCTTCTTC	3′ RACE
5′-R	GCTGAGCCAAACTCCCAGCCCCGGC	5′ RACE
Full-F	AAGCAGTGGTATCAACGCAGAGTAC	Amplification of complete ORF
Full-R	CACCACAGGCACCTCCTCCT	Amplification of complete ORF
qBLT1-F	CACCTCTATTCCTGCGGTACCT	Real-time PCR analysis
qBLT1-R	GTGTGCTTGGTCCTCATCCTCT	Real-time PCR analysis
β-Actin-F	GATGGTGGGTATGGGCCAGAAG	Reference gene for Real-time PCR
β-Actin-R	ATGTCACGCACGATTTCCCTCTC	Reference gene for Real-time PCR

leukotriene B4 (Secombes et al., 1994). In turbot (*Scophthalmus maximus*) the levels of LTB4 were significantly increased in the supernatants after VHSV (viral hemorrhagic septicemia virus) infection and exogenous LTB4 significantly inhibited VHSV replication in RTG-2 cells, suggesting that LTB4 may play a significant role in VHSV replication (Tafalla et al., 2002). In the present study we cloned the cDNA sequence in turbot and characterized it as a BLT1 sequence firstly in fish. BLT1 of turbot was isolated to understand the amino acids conserved between various species, the primary structure and tissue distribution. Additionally the expression profile of turbot BLT1 after bath-vaccination with a live attenuated vaccine *Vibrio anguillarum* MVAV6203 and challenge by its virulent strain were investigated.

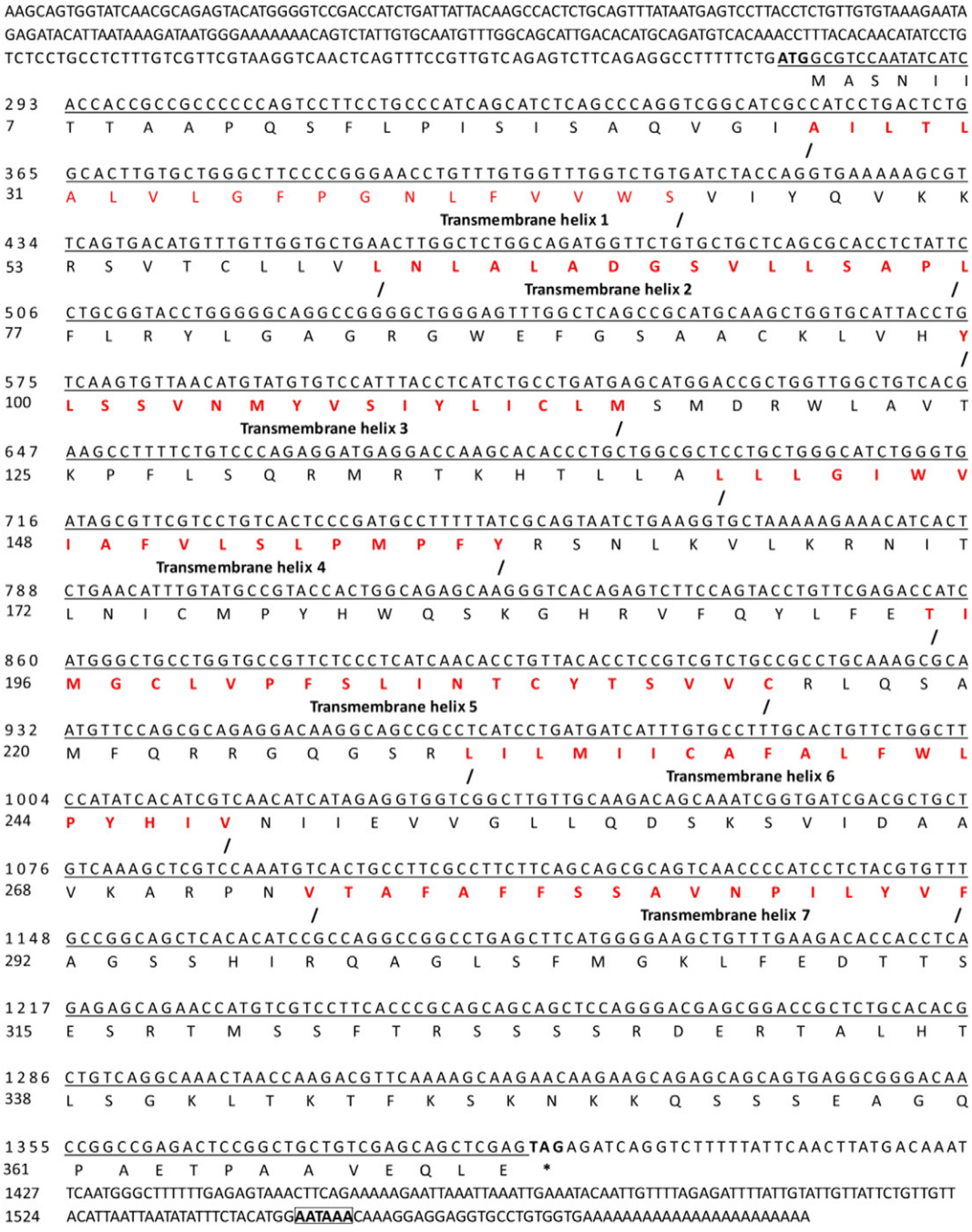

Fig. 1. The BLT1 cDNA sequences and deduced amino acid sequences of turbot. Boxes and asterisks indicate the polyadenylation signal and stop codon respectively. Red letters indicate the amino acid sequences which locate in the transmembrane region.