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The two channel catfish intelectin genes exhibit highly differential patterns of tissue expression and regulation after infection with *Edwardsiella ictaluri*

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Summary
Intelectins (IntL) are Ca^{2+}-dependent secretory glycoproteins that play a role in the innate immune response. The mammalian IntL is also known as lactoferrin receptor (LfR) that is involved in iron metabolism. The objective of this study was to characterize the intelectin genes in both channel catfish and blue catfish, to determine their genomic organization and copy numbers, to determine their patterns of tissue expression, and to establish if they are involved in defense responses of catfish after bacterial infection. Two types of IntL genes have been identified from catfish, and IntL2 was completely sequenced. The genomic structure and organization of IntL2 were similar to those of the mammalian species and of zebrafish and grass carp, but orthologies cannot be established with mammalian IntL genes. The IntL genes are highly conserved through evolution. Sequence analysis also indicated the presence of the fibrinogen-related domain in the catfish IntL genes, suggesting their structural conservations. Phylogenetic analysis suggested the presence of at least two prototypes of IntL genes in teleosts, but only one in mammals. The catfish IntL genes exhibited drastically different patterns of expression as compared to those of the mammalian species, or even with the grass carp gene. The catfish IntL1 gene is widely expressed in various tissues, whereas the channel catfish IntL2 gene was mainly expressed in the liver. While the catfish IntL1 is constitutively expressed, the catfish IntL2 was drastically induced by intraperitoneal injection of *Edwardsiella ictaluri* and/or iron dextran. Such induction was most dramatic when the fish were treated with both the

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bacteria and iron dextran. While IntL1 was expressed in all leukocyte cell lines, no expression of IntL2 was detected in any of the leukocyte cell lines, suggesting that the up-regulated channel catfish IntL2 expression after bacterial infection may be a consequence of the initial immune response, and/or a downstream immune response rather than a part of the primary immune responses.

1. Introduction

Host defense starts with the recognition of molecular patterns from pathogens. Such molecular patterns are often made up of the cell wall components of bacteria harboring bacteria-specific carbohydrate chains that do not exist in the host organisms. Upon infection, the host recognizes such pathogen-specific carbohydrate chains by its lectins. Such lectins can function as phagocytosis receptors, or as soluble opsonins and agglutinins. In the case of phagocytosis receptors, mannose receptor binds to bacterial cell wall components containing terminal mannose residue and enhances their clearance by phagocytosis [1,2]. Soluble lectins are present in the plasma such as collectins and ficolins, and they function as opsonins or agglutinins for bacteria [3]. In addition, the soluble lectins form complexes with mannose-binding lectin-associated serine proteases in plasma. Binding of these complexes to targets activates complement system, which induces opsonization of the targets by phagocytes and the target killing by formation of the membrane attack complex, leading to the so-called lectin pathway that play important roles in innate immunity [4].

In addition to the mannose-specific lectins, animals also have lectins with affinity to galactose called galectins. Galectins have been reported to have functions in cell differentiation [5], apoptosis [6,7], recognition of tumor antigens [8], uptake of aged proteins [9], and binding of galactofuranose present in the carbohydrate chains of bacterial cell wall [4].

A recently identified member of the galectins, intelectin (IntL), was first identified in mouse as a homolog of *Xenopus laevis* oocyte lectin [10]. Mammalian IntL is a Ca^{2+}-dependent enteric lectin, which plays a role in pathogen recognition [4]. Recombinant human (*Homo sapiens*) IntL1, which exists as a 120 kDa homotrimeric structure unit, has specific affinities to D-pentoses and D-galactofuranosyl residues as well as recognizes *Nocardia rubra* arabinogalactan [4]. It was reported that mice with a genetic defect of IntL2 showed increased susceptibility to nematode *Trichinella spiralis* infection [11]. BALB/c mouse, which exhibited Th2 immune response polarization during intestinal infection of *Trichuris muris*, showed up-regulation of the IntL2 gene [12].

Mammalian IntL exists in the intestinal surface [12] and the major deposition of IntL was observed at the enterocyte brush border [13]. Additionally, mouse IntL transcript was observed in the Paneth cells located in the lower region of the intestinal crypts [10] and jejunal goblet cells [14]. The up-regulation of mouse IntL genes has also been reported in the lung after helminth parasite *Nippostrongylus brasiliensis* infection [15]. Moreover, mammalian IntL is thought to be involved in the stabilization of microvillar rafts. Microvillar rafts in the intestines and lungs are rich in glycoproteins and glycolipids, which provide anchorage for microbial adhesions [13]. Mammalian IntL shield against microbial infection by serving as decoy pathogen receptors or protecting exposed epithelial binding sites [13,16]. The intelectin is also named small intestine lactoferrin receptor (SI-LfR). The human SI-LfR cDNA transfected Caco-2 cell, a human small intestinal like cell line, showed higher SI-LfR binding with human Lf than mock-transfected cell [17]. Also, it was suggested that the SI-LfR binding with human milk lactoferrin (Lf) is important in maintaining the iron status for infants [18]. These previous reports on mammalian IntLs indicate that they have an important role in the innate immune response, mucosal stabilization, and iron metabolism.

The information about teleost fish IntLs is limited. To date, complete coding sequences of an IntL gene have been reported only from grass carp [19]. Several teleost fish ESTs or genomic sequences, showing similarity with mammalian IntLs, have been submitted to GenBank, but they have not been characterized. We previously identified ESTs similar to the mammalian intelectin genes from both channel catfish *Ictalurus punctatus*, and blue catfish *I. furcatus*, and identified them among the most highly up-regulated genes after bacterial infection with *Edwardsiella ictaluri* [20,21]. Similarly, previous studies in rainbow trout also identified intelectin as one of the acute phase response genes [22,23]. Given the strong up-regulation of intelectin genes in both catfish and trout livers following bacterial infection and the paucity of data on teleost intelectins, our objectives in this study were to isolate and sequence the complete cDNAs of intelectin genes of channel catfish and blue catfish, characterize the genomic structure of catfish intelectin genes, analyze their expression patterns in healthy catfish tissues, and study their expression after both bacterial infection and iron administration. Here we report that the channel catfish genome harbors two intelectin genes. They exhibit highly differential patterns of tissue expression, and are differentially regulated after bacterial infection.

2. Materials and methods

2.1. Identification and sequencing of catfish IntL cDNAs

BLAST searches were used to identify channel catfish and blue catfish ESTs encoding partial cDNAs for IntL genes. Bioinformatic analysis utilizing ClustalW [24] and VectorNTI (Invitrogen) identified two distinct IntL transcript types from each species, referred to here as IntL1 and IntL2. Complete cDNA sequences for IntL1 and IntL2 were obtained