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Preferential combination between the light and heavy chain isotypes of fish immunoglobulins

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ABSTRACT

Immunoglobulin light chain (IgL) is necessary for the assembly of an Ig molecule, which plays important roles in the immune response. IgL genes were identified in various teleost species, but the basic functions of different IgL isotypes and the preferential combination between IgL and IgH (Ig heavy chain) isotypes remain unclear. In the current study, by EST database searching and cDNA cloning in rainbow trout, 8 IgL sequences were obtained, which could be classified into the IgLκF, IgLκG, IgLσ and IgLλ isotypes, respectively. Trout IgL isotypes were highly expressed in the immune-related tissues, and participated in the immune responses in spleen and gut by stimulation with LPS and poly (I:C). The results of FACS and LC-MS/MS indicated that the IgLκG and IgLσ isotypes preferentially bonded with the heavy chains of IgM and IgT, respectively, in trout B cells and serum. In addition, the genomic organization of trout IgL isotypes and the utilization of recombination signal sequences were studied.

1. Introduction

As important molecules, immunoglobulins (Igs) play irreplaceable roles in the innate and adaptive immunities of jawed vertebrates and provide obligatory duties to eliminate extracellular and intracellular pathogens (Flajnik and Kasahara, 2010). A typical Ig molecule consists of two heavy (H) and two light (L) chains held together by disulfide bridges. IgH chains usually consist of one variable (V) domain and two to four constant (C) domains and IgL chains are composed of one V domain and one C domain. IgHV and IgLV are generated by random somatic rearrangement of the variable (V), diversity (D) (lacking in IgLV gene) and joining (J) segments in the corresponding gene loci, mediated by the recombination-activing gene products (RAG-1 and RAG-2), along with B cell developing (Jung et al., 2006; Parra et al., 2013). The specificity for an Ig molecule to recognize antigens is mainly determined through its antigen-binding site constituted by IgHV and IgLV (Danilova and Amemiya, 2009). The functional immunoglobulins are expressed on the surface of mature B lymphocyte as B cell receptors (BCRs) or secreted into body fluids, such as serum and gut mucus, as antibodies (Abs) to agglutinate pathogens (Parra et al., 2013).

An Ig isotype is defined according to its H chain isotype, especially the constant domains. In mammals, five Ig isotypes have been discovered: IgM, IgD, IgA, IgG, and IgE, and their H chain isotype is μ, δ, α, γ, and ε, respectively. While in teleost, three major Ig isotypes (IgM, IgD, and IgT/IgZ) have been identified, among which IgT/IgZ is the last one discovered (Danilova et al., 2005; Hansen et al., 2005) and is characterized as an antibody that specialized in mucosal immune responses in the gut and skin (Xu et al., 2013; Zhang et al., 2010, 2011). On the other side, two IgL isotypes, κ and λ, are expressed in mammals (Criscitiello and Flajnik, 2007). But in birds, only one IgL isotype is discovered, which is an evolutionary homolog to mammalian λ (Lundqvist

Abbreviations: Ab, antibody; BCR, B cell receptor; C domain, constant domain; CH, heavy chain constant domain; CL, light chain constant domain; DN, double negative; D segment, diversity segment; FACS, fluorescence-activated cell sorting; FBS, fetal bovine serum; J segment, joining segment; HKL, head kidney leukocyte; Ig, immunoglobulin; IgH, immunoglobulin heavy chain; IgL, immunoglobulin light chain; LC, liquid chromatography; MS, mass spectrometry; ORF, open reading frames; PBL, peripheral blood leukocyte; qPCR, quantitative real-time PCR; RAG, recombination-activing gene; RSS, recombination signal sequence; V domain, variable domain; VH, heavy chain variable domain; VL, light chain variable domain; V segment, variable segment.

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et al., 2006). The third IgL isotype, σ, seems to be expressed only in the ectothermic vertebrates, including cartilaginous fish, teleosts, and amphibians (Edholm et al., 2011; Schwager et al., 1991). While the recently identified IgL isotype, σ-cart, is unique to elasmobranches (Criscitiello and Flajnik, 2007). Therefore, all vertebrate IgLs are proposed to be grouped into four main ancestral branches: λ, κ, σ, and σ-cart (Criscitiello and Flajnik, 2007). Based on this nomenclature, the originally reported teleost IgL1 and IgL3 can be classified into IgLκG and IgLκF, respectively, and IgL2 into IgLσ. All of these three IgL isotypes have been identified in rainbow trout (*Oncorhynchus mykiss*) (Daggfeldt et al., 1993; Partula et al., 1996; Timmusk et al., 2000), zebrafish (*Danio rario*) (Haire et al., 2000; Zimmerman et al., 2008), common carp (*Cyprinus carpio* L.) (Ishikawa et al., 2004; Tomana et al., 1999, 2002), and channel catfish (*Ictalurus punctatus*) (Ghaffari and Lobb, 1993, 1997). Recently, another IgL isotype, phylogenetically homologous to mammalian λ, was found in channel catfish and Atlantic cod (*Gadus morhua*) (Edholm et al., 2009).

IgL gene configuration is classified as either "translocon" or "cluster" in genome and assembled by RAG-1 and RAG-2 through recognizing the recombination signal sequences (RSS) at the border of V and J segments (Criscitiello and Flajnik, 2007; Krangel, 2003; Mcblane et al., 1995). Mammalian IgL gene is organized as "translocon" arrangement, with multiple V and J segments arrayed upstream of C gene segments as (V_n-J_n-C_n) (Hieter et al., 1980; Hofker et al., 1989; Vasicek and Leder, 1990). Teleost and cartilaginous fish IgL genes exhibit a cluster-type organization as $(V\text{-}J\text{-}C)_n$ (Cannon et al., 2004; Criscitiello and Flajnik, 2007). Beyond this, more than one copy of IgLκ loci are found in different chromosomes of some teleost species (Edholm et al., 2011; Hikima et al., 2011; Yasuike et al., 2010), which probably arose because of the teleost-specific whole-genome duplication.

The ratio of expressed Igκ and Igλ is various in different mammalian species from 1:9 to 9:1 (Gorman and Alt, 1998). In human, Igκ-expressed B cells had less Igλ rearrangement but Igλ-expressed B cells all contained Igκ rearrangement during early B cell development (Brauninger et al., 2001). In teleost, rainbow trout peripheral blood leukocytes (PBLs) expressed IgLκG to IgLσ at a ratio of approximately 85:15 (Timmusk et al., 2000), while catfish serum IgM associated IgLκF and IgLκG were at a ratio of 60:40 (Lobb, 1986). Recently, catfish IgM^-/IgD^+ cells were found only express Igσ (Edholm et al., 2009, 2010). However, the investigation that human without λ or κ exhibited no striking immune deficiency (Gorman and Alt, 1998) together with the discovery that the certain classes of antibodies without L chain in camel (Nguyen et al., 2000), bovine (Hamerscasterman et al., 1993) and shark (Greenberg et al., 1995) had a normal function indicated that IgL might be less important in binding antigen. Thus the functions of IgL and its isotypes are still a mystery (Edholm et al., 2011; Hikima et al., 2011; Pilstrom, 2002). In teleost, with the newly uncovered IgLλ, the relationship between IgL and IgH as well as the functions of the IgL isotypes are eager to be explored. Here, we characterize all four IgL isotypes in rainbow trout, and describe their genomic organization, expression pattern and phylogenetic relationship. Moreover, the preferential usage of trout IgL isotypes by IgM and IgT is also quantified.

2. Materials and methods

2.1. Experimental fish

Rainbow trout (weighing 70–100 g) were obtained from Zhanghe Reservoir Rainbow Trout Farm (Jingmen, China) and maintained in aquarium tanks using a water recirculation system involving extensive filtration and thermostatic temperature control. Water temperature was maintained continuously at 15–17 °C. The fish were acclimated to the aquarium tanks for 2 weeks before being used in experiments.

Fifty trouts were divided into two groups, and intraperitoneally (i.p.) injected with LPS (from *Escherichia coli* 0111:B4; Sigma-Aldrich) and poly (I:C) (Sigma-Aldrich), separately, at a dose of 100 μg per 50 g fish body weight. At 0, 1, 3, 7, 14, 21 and 28 d after injection, three fish from each group were euthanized with tricaine (MS-222, Sigma-Aldrich), and the spleen and gut were collected. The samples were flash frozen in liquid nitrogen and then transferred to −80 °C freezer for RNA extraction. All animal experiments were approved by the Committee on the Ethics of Animal Experiments of the Chinese Academy of Sciences.

2.2. Total RNA isolation and cDNA synthesis

Total RNA was extracted from the tissues sampled from the healthy and challenged fish using the Trizol reagent (Invitrogen), as well as from the sorted IgM^+, IgT^+, and double negative (DN) cells using the RNeasy mini kit (Qiagen). All extracted RNA was digested with an RNase-free DNase (Qiagen). In order to normalize the gene expression levels for each sample, same amounts (500 ng) of the total RNA were used for cDNA synthesis in a 25 μl reaction volume with M-MLV reverse transcriptase (Promega). The synthesized cDNA was diluted with 75 μl of RNase-free water and used as template for regular RT-PCR and quantitative real-time PCR (qPCR).

2.3. Cloning of trout IgL isotypes

The constant regions of trout IgL1 (GenBank accession No. X65260), IgL2 (U69987), and IgL3 (GQ217535) were used as queries to search trout EST database at NCBI (http://www.ncbi.nlm.nih.gov). All of the identified EST sequences were assembled into 8 IgL subisotypes, based mainly on the amino acid identities of the constant regions. Within each isotype when the identities were less than 80% they would be defined to different subisotypes. Specific primers (Supplemental Table 1) were designed to amplify the cDNAs containing the open reading frames (ORFs) of each IgL subisotype. Ex Taq HS (Takara) was used in all PCR, and the PCR products were cloned into the pMD19-T vector (Takara) for sequencing.

2.4. Sequence and phylogenetic analyses

Obtained nucleotide sequences were translated to amino acid sequences using the ExPASy online tools (http://web.expasy.org/). Signal peptides were predicted using the SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/). Multiple sequence alignments were performed using the CLUSTAL X version 2.1 with default parameters according to the IMGI (the International ImMunoGeneTics information system) scientific chart (Lefranc et al., 2005) and then manually adjusted. Phylogenetic tree was constructed with the MEGA 4.0 package, with a neighbor-joining algorithm. Bootstrap values were derived from 1000 replications. Trout β_2-microglobulin was used as outgroup.

2.5. Genomic analysis of trout IgLs

Trout genome data (Berthelot et al., 2014) were downloaded from European Nucleotide Archive under the accession code CCAF000000000 and then, local BLAST was performed. Trout IgL gene loci and the transcriptional orientations of the V, J and C segments were determined based on the results of local BLAST. The copy numbers of each IgL subisotype were counted. The conserved RSS motif was scanned manually upstream and downstream of the