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Review

Recent findings on the structure and function of teleost IgT

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ABSTRACT

As key effector molecules of jawed vertebrate's adaptive immune system, immunoglobulins are produced by B lymphocytes, either as a secretory form (antibody) or as a membrane form (B cell receptor). Until recently, teleost fish B cells were thought to express only two classes of immunoglobulins, IgM and IgD. In addition, IgM in these species was thought to be the only immunoglobulin isotype responding to pathogens both in systemic or mucosal compartments. However, the unexpected discovery of IgT, a new teleost immunoglobulin unearthed in 2005, has provided for new opportunities to analyze further roles of teleost immunoglobulins in these two physiologically distinct compartments. The smoke about the potential function of IgT has cleared recently with the finding that this immunoglobulin appears to be specialized in gut mucosal immunity. Significantly, the new capability of measuring not only IgM but also IgT responses will greatly facilitate the evaluation and understanding of fish immune responses as well as the protective effects of fish vaccines. The purpose of this review is to summarize the molecular characterization of new IgT orthologs and subtypes in teleosts, as well as to describe the new findings concerning the protein structure of IgT, the B cells producing it, and its role in mucosal immunity.

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1. Introduction

The adaptive immune system (AIS) appears to have emerged in the common ancestor of all vertebrates, more than 500 million years ago [1,2], since a parallel version of jawed vertebrate AIS was discovered recently in jawless vertebrates [3–5]. Antigen recognition in jawless vertebrates is mediated by variable lymphocyte receptors (VLRs) [6] whereas in jawed vertebrates the key molecules involved in antigen recognition are the B and T cell receptors (BCR and TCR respectively).

A typical immunoglobulin (Ig) molecule consists of two heavy (H) and two light (L) chains (H_2L_2 unit), each of which containing one amino-terminal variable (V) immunoglobulin superfamily (IgSF) domain and one (in the L chain) or more (in the H chain) carboxyl-terminal constant (C) IgSF domain. Generally, the paired V domains provided by each of the H and L chains, are associated non-covalently and confer antigenic specificity, while the H chain C domains define effector functions of the immunoglobulins through binding to their receptors on effector cells or activating other immune mechanisms, such as complement [7]. Immunoglobulins can be classified into different isotypes (classes) and subtypes (subclasses) based on the nature of the C domains of their H chains. In mammals, five Ig isotypes, IgM, IgD, IgG, IgE, and IgA, have been identified, which possess specific effector functions in humoral and cellular immune responses (see reference [2] for a recent review on structural, functional and evolutionary aspects of vertebrate immunoglobulins). Among the antibody isotypes, IgM is the most ancient and the only isotype functionally conserved in all gnathostomes (jawed vertebrates) [8]. IgD has been found in all gnathostomes, except birds, indicating that it is also a primordial antibody class despite its highly plastic structure and unclear function in evolution (see reference [2]).

Immunoglobulin isotypes have evolved to play specialized roles either within mucosal or systemic compartments. In mammals and birds, IgM, IgG and IgY isotypes have major roles in systemic responses, while IgA is the main player in mucosal areas. In amphibians, IgM and IgY play a prevalent role in systemic immunity whereas IgX is an isotype chiefly expressed in the gut [9]. Until recently, teleost fish were thought to contain only two classes of immunoglobulins, IgM and IgD. It was also generally accepted that IgM was the only immunoglobulin class responding to antigenic challenge both in systemic and mucosal compartments, and thus, teleost were believed to be devoid of an immunoglobulin specialized in mucosal surfaces. A new teleost Ig H chain gene, named *ighτ* in rainbow trout [10] or *ighζ* in zebrafish [11], was reported in 2005. The assembled immunoglobulin containing the *ighτ* product was named IgT in trout (or IgZ in zebrafish), and it was suggested to

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represent the last immunoglobulin class to be found in a vertebrate species [12]. During the last five years *igh*τ/*igh*ζ has been cloned and characterized at the gene level in a number of teleosts species [13–18]. However, until very recently its protein structure, production, and potential role in immunity were not reported. In that regard, a 2010 study revealed that rainbow trout IgT is an immunoglobulin specialized in gut mucosal immune responses, while IgM appears to be specialized in systemic immunity [19]. These findings have challenged the paradigm that specialization of immunoglobulin isotypes into mucosal and systemic areas arose during tetrapod evolution. The new capability of measuring not only IgM but also IgT responses will greatly facilitate the evaluation and understanding of teleost immune responses as well as the protective effects of fish vaccines. In this review, we summarize the molecular characterization of newly discovered *igh*τ/*igh*ζ genes in teleosts, as well as recently reported aspects of the protein structure of IgT, its production by a novel lineage of B cells and its role in immunity.

2. Characterization of the genes encoding IgT

2.1. Organization and rearrangement of the igh locus in teleost fish

The immunoglobulin heavy (IgH) and light (IgL) chains are encoded by separate genomic loci, *igh* and *igl*, respectively, and their individual V and C domains are each encoded by independent elements: the variable (*V*), diversity (*D*, only for H chains), and joining (*J*) gene segments for the V domain, and individual constant (*C*) gene segments for the C domains. The V domain of the IgH and IgL chains is functionally divided into three hypervariable sequences termed complementarity-determining regions (CDR) that are located between four relatively stable sequences named framework regions (FR). The diversity of the V domain is provided mostly by the three CDR. CDR1 and CDR2 are encoded by the *V* gene alone, while CDR3 is encoded by the *V-J* or *V-D-J* rearrangement junction and thus represents the most diverse CDR [7].

To date, two major organization types of *igh* and *igl* loci have been reported: translocons or clusters [2]. In humans, the *igh* locus is in 'translocon' configuration: *V-D-J-Cμ-Cδ-Cγ3-Cγ1-Cγ2b-Cγ2a-Cε-Cα*. The messenger RNAs for the H chains of IgM (μ) and IgD (δ) are generated by alternatively splicing the recombined *VDJ* to either *Cμ* or *Cδ* in their *igh* loci, whereas the H chains of IgG (γ), IgE (ε), and IgA (α) are expressed through a process known as class-switch recombination. In cartilaginous fish, the *igh* genes are in the 'cluster' configuration with many sets of $V(D)_{2-3}JC$ clusters [1,2].

Until five years ago, the *igh* loci in bony fish were thought to be organized only in 'translocon' configuration, where multiple *V* gene segments are upstream of multiple *D* and *J* segments, followed by a *Cμ* and *Cδ* genes, encoding and generating the μ and δ chains by alternative splicing [20,21]. However, the detailed analyses of the complete *igh* loci from rainbow trout (*Oncorhynchus mykiss*) [10] and zebrafish (*Danio rerio*) [11] revealed a novel genomic architecture that had never been observed in any *igh* loci: upstream of the known (V_H)-*DJCμCδ* elements for μ and δ IgH chains, another set of V_H-*DJC* elements were found, which encoded for the H chain (τ, for teleost fish) of a previously unknown Ig isotype, IgT, named by Hansen and colleagues [10]. In zebrafish this new immunogobulin was termed IgZ [11]. To avoid a mixed terminology, throughout this review we will mostly use the term 'IgT' to refer to IgT/IgZ, and we will use '*igh*τ' for the gene encoding its H chain. The organization of the above mentioned *igh*τ-*igh*μ locus is strikingly similar to that of the mouse *Tcrd-Tcra* locus encoding T cell receptor δ (TCRδ) and TCRα. In both loci, upstream *V* segments rearrange either to *DJC*τ (or *DJCδ*) to encode τ (or TCRδ) or to *DJCμ* (or *DJCα*) to encode μ (or TCRα) [1,11,12]. Based on the aforementioned architecture of the *igh*τ-*igh*μ locus, and despite of the fact that class switching mechanisms are absent in fish [22,23], B cells of these species were predicted to express either IgT or IgM, which was demonstrated to be the case in rainbow trout at protein level [19] and in zebrafish at gene level [24].

Subsequent reports have showed that IgT orthologs exist in almost all studied species belonging to the main orders of teleost fish (see Table 1), such as fugu (*Takifugu rubripes*) [13], common carp (*Cyprinus carpio*) [14,15], zebrafish [25], stickleback fish (*Gasterosteus aculeatus*) [16,17], grass carp (*Ctenopharyngodon della*) [18], Atlantic salmon (*Salmo salar*) [26,27], Chinese perch (*Siniperca chuatsi*), and orange-spotted grouper (*Epinephelus coioides*). The only exception where IgT has not been found thus far is in the channel catfish (*Ictalurus punctatus*) [28]. As the genome sequence of the channel catfish had not been finished at present time, it is possible that IgT may be found once the genome of this species is completed.

It is important to highlight that most of the above species possess more than one subclass of IgT, which may be encoded by duplicated *igh*τ genes in the same locus, as for example in stickleback fish [16,17]. Alternatively, these additional IgT subclasses are found in different *igh* loci. For instance, in Atlantic salmon, two *igh* loci were discovered (see Table 1), in each of which, 3 or 5 $V_HD\tau J\tau C\tau$ clusters are upstream of one copy of $V_HD\mu J\mu C\mu C\delta$, encoding for three putatively functional IgT subclasses [26,27]. Similarly, two *igh*

Table 1
Organization of teleost *igh* loci and the constant regions encoded by *igh*τ.

Order	Species	Potentially functional IgT Subtypes	Organization of *igh* locus	Constant region	Reference[a]
Salmoniforms	*Oncorhynchus mykiss*	IgT1	$V_HD\tau J\tau C\tau$-$V_HD\mu J\mu C\mu C\delta$	Cτ1-Cτ2-Cτ3-Cτ4	[10]
		IgT2	Data not available		[11]
	Salmo salar	IgT4, IgT5	locus A: $(V_HD\tau J\tau C\tau)_5$-$V_HD\mu J\mu C\mu C\delta$	Cτ1-Cτ2-Cτ3-Cτ4	[26,27]
		IgT2	locus B: $(V_HD\tau J\tau C\tau)_3$-$V_HD\mu J\mu C\mu C\delta$		
Cypriniformes	*Danio rerio*	IgT1	$V_HD\tau J\tau C\tau$-$D\mu J\mu C\mu C\delta$	Cτ1-Cτ2-Cτ3-Cτ4	[11]
		IgT2	Data not available		[25]
	Ctenopharyngodon idella	IgT1	$V_HD\tau J\tau C\tau$-$D\mu J\mu C\mu C\delta$	Cτ1-Cτ2-Cτ3-Cτ4	[18]
		IgT2	Data not available		DQ478943
	Cyprinus carpio	IgT1	Data not available	Cτ1-Cτ2-Cτ3-Cτ4	[15]
		IgT2 (chimeric IgM-IgT)		Cμ1–––Cτ4	[14,15]
Tetraodontiformes	*Takifugu rubripes*	IgT	$V_HD\tau J\tau C\tau$-$D\mu J\mu C\mu C\delta$	Cτ1–H[b]––Cτ4	[13]
Gasterosteiformes	*Gasterosteus aculeatus*	IgT1, IgT2, IgT3, IgT4	$(V_HD\tau J\tau C\tau$-$D\mu J\mu C\mu C\delta)_3$-$V_HD\tau J\tau C\tau$	Cτ1—Cτ3-Cτ4	[16]
					[17]
Perciformes	*Siniperca chuatsi*	IgT	Data not available	Cτ1-Cτ2-Cτ3-Cτ4	DQ016660
	Epinephelus coioides	IgT	Data not available	Cτ1-Cτ2-Cτ3-Cτ4	GU182366

[a] When no literature is available the GenBank accession number is showed.
[b] H, the putative hinge region: $GPV(KPTV)_5$.