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Insights into the function of IgD

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ABSTRACT

IgD, previously thought to be a recent addition to the immunoglobulin classes, has long been considered an enigmatic molecule. For example, it was debated if IgD had a specific function other than as an antigen receptor co-expressed with IgM on naive B cells and if it had an important role in mammalian immunity. However, during the past decade extensive sequencing of vertebrate genomes has shown that IgD homologs are present in all vertebrate taxa, except for birds. Moreover, recent functional studies indicate that IgD likely performs a unique role in vertebrate immune responses. The goal of this review is to summarize the IgD gene organization and structural data, which demonstrate that IgD has an ancient origin, and discuss the findings in catfish and humans that provide insight into the possible function of this elusive immunoglobulin isotype.

1. Introduction

It is well known that teleost B cells share many similarities with mammalian B cells, including immunoglobulin (Ig) gene rearrangements, allelic exclusion, production of membrane Ig and secreted Ig forms (reviewed in Bengten et al., 2000, 2006a; Flajnik and Kasahara, 2010; Miller et al., 1994; Solem and Stenvik, 2006; Warr, 1995) and the use of B cell receptor (BCR) accessory proteins CD79a and CD79b for Ig signal transduction (Edholm et al., 2010; Sahoo et al., 2008). Also, it is established that IgM is the predominant Ig isotype found in teleost serum, while the other Ig isotypes, for example, IgD in channel catfish, *Ictalurus punctatus* (Bengten et al., 2002; Edholm et al., 2010) and IgT in rainbow trout, *Oncorhynchus mykiss* (Zhang et al., 2010), are found in lesser amounts. In most teleost, serum IgM is expressed as a tetramer, although IgM monomers have been described in giant grouper, *Epinephelus itaira* (Clem, 1971) and sheepshead, *Archosargus probatocephalus* (Lobb and Clem, 1981). In contrast, serum IgT is expressed as a monomer in rainbow trout serum, and a tetramer in gut mucous (Zhang et al., 2010). At present serum IgD has only been described in catfish by using Western blot analyses and whether it exists as a monomer has yet to be determined. Currently, IgT has been identified at the cDNA/DNA level in zebrafish *Danio rerio* (IgZ; Danilova et al., 2005), common carp, *Cyprinus carpio* (Savan et al., 2005a), rainbow trout (Hansen et al., 2005), Japanese pufferfish, *Takifugu rubripes* (Savan et al., 2005b), grass carp, *Ctenopharyngodon idella* (Xiao et al., 2010), three-spined stickleback, *Gasterosteus aculeatus* (Gambon-Deza et al., 2010), and Atlantic salmon, *Salmo salar* (Tadiso et al., 2010). However, PCR and genomic sequencing studies have failed to isolate a catfish IgT gene homolog. Also, since IgT transcripts were not found among the ~500,000 catfish expressed sequence tags in the NCBI database it is likely that the issue of catfish IgT will only be resolved once the sequencing and annotation of the catfish IgH locus (IGH) is completed. Currently, the only immunoglobulins described in catfish are IgM and IgD and catfish IgM, like teleost IgM in general, has been well studied and shown to be a structural and functional homolog of mammalian IgM (Bengten et al., 2000, 2006b; Warr, 1995). In contrast, much less is known about teleost IgD function. Hence, this review will focus on IgD and since IgD has often been described as the most enigmatic vertebrate Ig, the structure and function of teleost IgD is discussed in the context of what we know concerning mammalian (and other vertebrate) IgD structure and function.

IgD was first discovered in human serum as a myeloma protein in 1965 and then in a companion study was shown to be present in normal serum (Rowe and Fahey, 1965a,b). Later it was identified on the surface of B cells (Van Boxel et al., 1972). Yet a unique function, in addition to initiating BCR signal transduction in mature naive B cells, was not identified until recently (Chen et al., 2009), and the significance or function of IgD has been a subject of debate (reviewed in Geisberger et al., 2006). For example, it was hypothesized that since anti-Igμ treatment of mouse immature B cells resulted in clonal anergy or clonal deletion, the signaling through IgD in mature B cells would result in a qualitative different signal from that of IgM (Nossal et al., 1979; Pike et al., 1982). Support for this notion came from studies which used anti-Igμ or anti-Igδ antibodies to crosslink IgM and IgD on the surface of murine or human leukemia cell lines and these

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experiments demonstrated that anti-IgM treatment resulted in growth inhibition as indicated by decreased DNA synthesis, while anti-IgD antibodies did not affect proliferation (Ales-Martinez et al., 1988; Mongini et al., 1989). However, in the 1990s, studies using transgenic (knockout) mice that were germline deficient for either IgD or IgM demonstrated that IgM or IgD, each by itself was capable of functioning as a BCR and inducing normal B cell immune responses (Lutz et al., 1998; Nitschke et al., 1993; Roes and Rajewsky, 1993). Briefly, transgenic mice lacking IgM, expressed only IgD on pre- and immature B cells, which not only matured normally, but also populated peripheral tissues in a manner similar to that observed in normal control mice that expressed both IgM and IgD. These IgM knockout mice also mounted a fairly normal antibody response even though antibody affinity maturation was delayed. Similar results were also found in the studies using IgD knockout mice. Thus, it was concluded that while IgD appears not to be required for a normal B cell response, clearly IgD could substitute for IgM in regards to both the B cell maturation process and immune function, at least in mice. More recently, studies in human and catfish have provided evidence indicating that IgD, in addition to functioning as an Ag-binding receptor, is involved in immune responses to certain pathogens and plays a role as a mediator of innate immunity (Chen et al., 2009).

2. IgD is found in most major taxa of jawed vertebrates and displays remarkable structural plasticity between species

Before the identification of an IgD homolog in catfish, IgD was believed to represent a recently evolved Ig exclusive to primates and rodents (Wilson et al., 1997). In fact it was only recently appreciated that IgD is also found in a wide variety of other vertebrates, including both mammals and ectotherms. Thus, it appears that IgD and its orthologs represent an ancient Ig class, most likely as old as IgM. The catfish IgD homolog was originally identified as a cDNA, which consisted of a rearranged VH region (VDJ) spliced to a Cμ1 domain, followed by seven novel Cδ domains, a transmembrane (TM) region, and a short cytoplasmic tail (CYT) consisting of five amino acids, K-V-K-I-A (Wilson et al., 1997). The finding of such a long IgD-like transcript was surprising since human and mouse IgD have three and two Cδ domains, respectively. A second catfish IgD cDNA was also identified in the same cDNA library screen. This cDNA was a partial transcript and it lacked the TM region and ended in a predicted secreted tail (Bengten et al., 2002; Wilson et al., 1997). Overall, the features supporting the classification of these novel cDNAs as IgD included: (1) sequence similarities to mammalian Igδ as demonstrated by phylogenetic analysis, (2) location of the catfish Igδ gene immediately 3′ of the Igμ gene in the IGH chain locus, (3) co-expression with Igμ in the catfish clonal 3B11 B cell line and (4) the apparent expression of Igδ from a long primary transcript containing a rearranged VDJ and Igμ and Igδ genes (Wilson et al., 1997). This presence of the Cμ1 domain in all catfish membrane Igδ transcripts indicated that catfish IgD, like mammalian IgD, is produced by alternative splicing of mRNA and not by mechanisms involving classical isotype switching by chromosomal recombination. Moreover, the finding that Ig exons of different isotypes (IgM and IgD), can combine was novel and later it was observed to occur in all teleost IgD cDNAs sequenced to date (Fig. 1; Gambon-Deza et al., 2010; Hirono et al., 2003; Hordvik, 2002; Hordvik et al., 1999; Saha et al., 2004; Stenvik and Jorgensen, 2000). This inclusion of Cμ1 in catfish Igδ transcripts was hypothesized to be necessary since it would permit the covalent association of Igδ H chains with catfish IgL chains, i.e., the Cμ1 domain provides the appropriately located cysteine required for IgH–IgL chain association. The inclusion of Cμ1 or a Cμ1-like sequence in Igδ transcripts is also observed in artiodactyls. For example, the nucleotide identities of the Cδ1 exon in cow, *Bos taurus*; sheep, *Ovis aries*; and pig, *Sus scrofa*, range from 95.4% to 98.7% when compared to their respective Cμ1 exon of each species and in the pig the first CH exon in an IgD transcript can either be a Cδ1 or Cμ1 (Zhao and Hammarstrom, 2003; Zhao et al., 2002). Also, IgD has been identified in other mammalian species such as, dogs (Rogers et al., 2006), horses (Wagner, 2006) and giant pandas (Zhao et al., 2007). As for the non-mammalian taxa, IgD genes and cDNAs have been described in the leopard gecko (*Eublepharis macularius*; Gambon-Deza and Espinel, 2008), green anole lizard (*Anolis carolinensis*; Wei et al., 2009), Chinese soft-shelled turtle (*Pelodiscus sinensis*; Xu et al., 2009), *Xenopus tropicalis* (Ohta and Flajnik, 2006) and most recently in the platypus (*Ornithorhynchus anatinus*; Zhao et al., 2009). Notably, phylogenetic analyses demonstrate that shark IgW is an ortholog of IgD, which further illustrates the ancient origin of this isotype (Ohta and Flajnik, 2006). IgW was originally identified as IgNARC in the nurse shark (*Ginglymostoma cirratum*; Greenberg et al., 1996), IgW in the sandbar shark (*Carcharhinus plumbeus*; Berstein et al., 1996), and IgX in the clearnose skate (*Raja eglanteria*; Anderson et al., 1999), reviewed by Flajnik and Rumfelt (2000). However, it appears, at least from the present available data, that cartilaginous fish belonging to the subclass of holocephali may lack an IgD ortholog, since our searches of the existing database for elephant shark (*Callorhinchus milii*) and a previous study of spotted ratfish (*Hydrolagus colliei*; Rast et al., 1998) immunoglobulins failed to identify any IgD gene or cDNA sequences. In addition, IgD is not present in the galliforms (chicken, *Gallus gallus*; Zhao et al., 2000), anseriforms (duck, *Anas platyrhynchos*; Lundqvist et al., 2001) and in some mammals such as the rabbit and opossum (*Oryctolagus cuniculus*; Lanning et al., 2003; *Monodelphis domestica*; Wang et al., 2009; reviewed in Sun et al., 2010).

Here, it is also important to emphasize that structural variations of IgD occur between different vertebrates. In placental mammals, the Igδ genes and the encoded IgD proteins are structurally highly conserved (Rogers et al., 2006; White et al., 1985; Zhao et al., 2007). For example, all mammalian IgD genes currently sequenced are located directly 3′ of the Igμ gene and with the exception of rodents all consists of eight exons. Exon 1 encodes the first Ig domain (Cδ1) and exons 2 (H1) and 3 (H2) encode the extended hinge region that is characteristic of most mammalian IgD proteins. The second and third Ig domains (Cδ2 and Cδ3) are encoded by exons 4 and 5; exon 6 encodes the hydrophilic secreted tail (δSec) and exons 7 and 8 encode the membrane tail (δTM1 and δTM2). Mouse and rat IgD genes consist of six exons and encode for shorter IgD forms since these species lack the exons encoding H2 and Cδ2. Also, while two hinge regions are present in the pig and giant panda IgD genes, these species only utilize their first hinge exon (H1) resulting in a less extended IgD hinge in these species (supplemental Fig. 1; Zhao et al., 2003, 2007).

In contrast, while the IgD genes of ectothermic vertebrates vary in their number of Cδ exons, Cδ exon duplications, and the number of IgD gene copies, the size of the expressed IgD in these species is relatively large. The number of unique Cδ domains in these different species ranges from 6 to 11, and some species have two exons that encode their TM and others have only a single TM exon. Also, some teleost express IgD transcripts that have blocks of repeated Cδ domains and all teleost IgD molecules examined are chimeric since they include Cμ1. None of the cold-blooded vertebrate IgD genes sequenced to date have individual exons that would encode a hinge, i.e., the C regions are composed of Ig domains. Perhaps, the lack of a hinge in the IgD in these species is compensated by the length of the IgD H chain that may allow for some flexibility.

Briefly and beginning with the teleosts, in the catfish the IGH locus spans ~1 Mbp and contains multiple internal duplications and transpositions. It contains three IgD genes termed IGHD1, IGHD2 and IGHD3 and each is individually linked to either a functional Igμ gene or an Igμ pseudogene (Bengten et al., 2006a,b, 2002).