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.

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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 teleosts, 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 IgM is expressed as a monomer in rainbow trout serum, and a tetramer in gut mucus (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-IgM 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-IgM, or anti-IgD antibodies to crosslink IgM and IgD on the surface of murine or human leukemia cell lines and these
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; Roess 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 (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 Cη 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 Cη 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 (Wilson et al., 1997). Overall, features of classifying the catfish cDNA as IgD included: (1) sequence similarities to mammalian IgD as demonstrated by phylogenetic analysis, (2) location of the catfish IgD 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 IgD from a long primary transcript containing a rearranged VDJ and Igμ and IgD genes (Wilson et al., 1997). This presence of the Cμ1 domain in all catfish membrane IgD transcripts indicates 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 (Igμ 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 IgD transcripts was hypothesized to be necessary since it would permit the covalent association of IgH 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 IgD transcripts is also observed in artiodactyls. For example, the nucleotide identities of the Cμ1 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 Cβ1 or Cμ1 (Zhao and Hammerstrom, 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 lamprey 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 (Ohda 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 (Ohda 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 holocephal may lack an IgD ortholog, since our searches of the existing database for elephant shark (Callorhinus milii) and a previous study of spotted ratfish (Hydrolagus coliei; 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., 2008; 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 IgG genes and the encoded IgG proteins are structurally highly conserved (Rogers et al., 2006; White et al., 1985; Zhao et al., 2007). For example, all mammalian IgG 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 (Cμ1) and exons 2 (H1) and 3 (H2) encode the extended hinge region that is characteristic of most mammalian IgG proteins. The second and third Ig domains (C62 and C63) are encoded by exons 4 and 5; exon 6 encodes the hydrophilic secreted tail (βSec) and exons 7 and 8 encode the membrane tail (βTM1 and βTM2). Mouse and rat IgG genes consist of six exons and encode for shorter IgG forms since these species lack the exons encoding H2 and C62. Also, while two hinge regions are present in the pig and giant panda IgG genes, these species only utilize their first hinge exon (H1) resulting in a less extended IgG hinge in these species (supplemental Figs. 1; Zhao et al., 2003, 2007).

In contrast, while the IgG genes of ectothermic vertebrates vary in their number of C6 exons, C6 exon duplications, and the number of IgG gene copies, the size of the expressed IgG in these species is relatively large. The number of unique C6 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 IgG transcripts that have blocks of repeated C6 domains and all teleost IgG molecules examined are chimeric since they include Cμ1. None of the cold-blooded vertebrate IgG 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 IgG in these species is compensated by the length of the IgG H chain that may allow for some flexibility.
IGHD1 is located directly 3′ of the functional Cμ gene and consists of 14 exons, with the exons encoding C62-C63-C64 repeated three times in tandem: C61-(C62-C63-C64)3-C65-C66-C67-TM. This gene encodes the chimeric membrane form of IgD and notably membrane IgD transcripts do not contain exon duplications. Similar IgD gene organizations with varying numbers of C62-C63-C64 block duplications also occur in Atlantic salmon, Atlantic halibut, grass carp, fugu and zebrafish (Hordvik, 2002; Hordvik et al., 1999; Saha et al., 2004; Xiao et al., 2010). The other two catfish IgD genes do not contain any exon duplications and are translated exons from the Cμ and membrane IgD genes (Aparicio et al., 2002). As in the catfish, the pufferfish membrane and secreted forms of IgD are encoded by separate genes; the gene encoding the putative secreted Ig form is linked to a separate array of D and JH genes found 5′ of the D and JH gene array linked to the Igμ and membrane IgD genes (Aparicio et al., 2002). Later, in a second study, which focused on membrane IgD, it was shown that the pufferfish membrane IgD gene lacks the tandem C62-C63-C64 duplication(s) found in ictalurids and salmonids. Instead the pufferfish gene has a longer tandem duplication of C61-C62-C63-C64-C65-C66 and the resulting cDNA transcript consisted of VD1-Cμ1-(C61-C62-C63-C64-C65-C66)2-C67-TM (Fig. 2; Saha et al., 2004). In the stickleback the IGH locus contains three arrays of VHD-JH-Cμ-Cδ-J4-Cμ-Cδ in tandem (Bao et al., 2010). The three IgD genes do not contain any exon duplications and are 90% identical at the amino acid level to each other. However, the Cδ5 exon in IGHD1 contains a nucleotide deletion. Thus, in stickleback, while there are three sets of IGH genes, there is no evidence of a functional difference between the IgD genes. In the salmonids, as a consequence of the tetraploid ancestry, there are two IGH loci on separate chromosomes. For example, in the Atlantic salmon each of the two IGH loci, have multiple duplications of the IgT gene, but only one set of the IgM and IgD genes (Yasuike et al., 2010). The corresponding Cδ exons of the two IgD genes in the IGHA and IGHB loci are very similar to each other and an alignment of Cδ translated exons from Igδ2 and Igδ1B gives an identity index of 97% (Hordvik, 2002). The main difference is in the number of C62-C63-C64 tandem duplications. In Igδ2 there are three C62-C63-C64 tandem duplications and in Igδ1B there are four (Yasuike et al., 2010). To date, the Atlantic cod IgD gene appears to be the one exception to the general teleost IgD structural pattern. It is much shorter and lacks C63, C64, C65, and C66 exons. Instead the Atlantic cod gene contains a tandem C61-C62 duplication separated by an intron containing a short exon termed δy. The second C61-C62 is followed by C67 and two TM exons and full-length transcripts consist of VDj-Cμ1-Cδ1-Cδ2-δy-C61-C62-C67-TM1-TM2 (Stenvik and Jorgensen, 2000). Here, it is
important to emphasize that when individual C6 domains from different teleost species are compared, it is clear that respective domains are homologous, i.e., D1 domains cluster together, D2 domains cluster together, etc. (supplemental Fig. 2). Also, previous phylogenetic analyses of different teleost C6 domains suggested that exons C65-C66-C57 arose from a gene duplication of exons C62-C63-C64 (Gambon-Deza et al., 2010; Hordvik et al., 1999) and teleost C61, C65 and C66 domains clearly are related to human C61, C62 and C63 domains, respectively (Hordvik et al., 1999; Wilson et al., 1997).

IgD in amphibians and reptiles is similar in length to most of the teleost IgD orthologs. Xenopus IgD transcripts consist of a rearranged VDJ, eight C6 domains and a TM; the Xenopus IgD gene contains eight C6 exons, followed by two TM exons (Ohta and Flajnik, 2006; Zhao et al., 2006). As for the reptiles, the IgD gene in the green anole lizard is single copy and consists of 11 C6 domains followed by two TM exons (Wei et al., 2009; and supplemental Fig. 1). Currently, the only transcripts sequenced for the green anole were obtained by nested PCR and they consist of VDJ-C61-C62-C63-C64-TM1-TM2. In contrast, the leopard gecko has two IgD genes that are quite different (Gambon-Deza and Espinel, 2008). The first IgD gene is directly 3′ of the IgM gene and contains 11 C6 exons and one TM exon and a membrane IgD transcript shows that the TM1 exon splices into a cryptic splice within the C67 domain. The second IgD gene, termed IgD2, is located ∼20 kb 3′ of the first IgD gene. It contains only seven Ig domains and is predicted to be the result of a partial duplication of the first IgD gene since the first four and seventh exons resemble C6 exons and exons 3 and 4 resemble IgA-like exons. Transcripts originating from this gene have not been published.

Interestingly, all cold-blooded vertebrates, except for elasmobranchs, lack a typical mammalian IgD secreted exon that is usually found 5′ of the TM exons (Fig. 2 and supplemental Fig. 1) and transcripts encoding a secreted IgD form are quite rare in these species. For teleosts, these transcripts arise by two different mechanisms: (1) the existence of a separate gene encoding a secreted IgD form as in the catfish and pufferfish or (2) alternative splicing of a membrane IgD gene as in the Atlantic salmon. As discussed above and in Section 3, catfish secreted IgD is encoded by the IGHD3 gene and pufferfish have a secreted IgD gene upstream of the IgM gene. Importantly, IgD can be detected in catfish serum by using the anti-IgDsc 2E5 monoclonal Ab (Bengten et al., 2002). As determined by western blot analyses two forms of secreted IgD of ∼130 and ∼180 kDa in size is routinely observed in sera from individual catfish and the difference in size may be due to either the inclusion or exclusion of the duplicated C62-C63-C64 domains (Fig. 3; Edholm et al., 2010). Based on densitometry of Coomassie blue-stained gels the concentration of secreted IgD in catfish serum is ∼0.04 mg/ml (Bengten et al., 2002). The evidence for an Atlantic salmon putative secreted IgD form comes from RT-PCR studies, which identified splice variants where the TM2 exon is spliced directly to C66. This splicing generates a transcript ending in a 14 amino acid tail (Hordvik, 2002). For reptiles, only the leopard gecko has been shown to have a secreted IgD form, which was identified as a cDNA transcript that ends with the C611 exon and contains 24 additional nucleotides that are spliced out of the membrane transcripts. This results in an IgD secreted form with an eight amino acid tail (Gambon-Deza and Espinel, 2008). In contrast, different secreted IgD (IgW or IgNARC) forms are more abundantly expressed in the elasmobranch species: nurse shark, horned shark, Heterodontus francisci, clearnose skate, bounded houndshark, Triakis scyllium (Anderson et al., 1999; Honda et al., 2010; Rumfelt et al., 2004). Likewise, short and long forms of IgD are found in the sarcopterygian dipnoi, as represented by the African lungfish, Protopterus annectens (Ota et al., 2003). The IgD genes in the elasmobranchs contain six C6 exons and alternative splicing produces both long and short forms of secreted and membrane IgD. Specifically, long secreted IgD transcripts contain six C6 exons and short IgD...
transcripts have only the first two C6 exons. The short membrane IgD transcripts also only contain the first two C6 exons, while the long membrane IgD transcripts contain the first four C6 domains. Furthermore, long IgD/IgW forms were identified in the nurse shark by using immunoprecipitation protocols (Greenberg et al., 1996).

In the African lungfish membrane IgD forms have yet to be identified, but sequencing showed that secreted IgD transcripts either contain seven C6 exons or two C6 exons. Finally, recent annotation of the monocrotone duck-billed platypus genome demonstrated that the platypus IgD gene is located between the IgM and IgG1 genes (Gambon-Deza et al., 2009; Zhao et al., 2009). It consists of 10 C6 exons followed by two TM exons and to date a secreted exon has not been found. Besides resembling the IgD expressed by teleosts, amphibians and leopard gecko in its length, phylogenetic analyses and amino acid comparisons show that all of the platypus C6 domains have a corresponding domain in reptile IgD. Also a domain by domain comparison demonstrated that platypus C61, C66 and C67 domains are homologous to the mammals C61, C62 and C63 (Zhao et al., 2009).

Taken together the available IgD sequencing data indicate, in marked contrast to IgM, that IgD genes have undergone numerous alterations involving both exon duplications and deletions leading to different IgD structural forms in different species (Hordvik et al., 1999; Ohta and Flajnik, 2006; Rogers et al., 2006; Stenvik and Jorgensen, 2000; Tucker et al., 1980; Wagner et al., 2004; White et al., 1985; Wilson et al., 1997; Zhao and Hammarstrom, 2003; Zhao et al., 2002).

3. IgD-expressing B cells in humans and catfish

Most of what we know about IgD expression and function comes from studies performed using human and mouse tissues and cell lines, and knockout mice. Also, it was only after the initial sequencing of IgD in catfish and in other teleosts, followed by the widespread sequencing of different genomes, that IgD was found in vertebrates other than primates and rodents. However, despite the many publications demonstrating that IgD has a more ancient origin than originally thought (see above), very few reports have focused on the function and cellular expression of this isotype in vertebrates, except for the studies performed in humans and mice. Consequently, this section summarizes our recent characterization studies of catfish IgD-bearing cell populations and how these relate to human IgD-bearing cells.

In humans (and mice), IgM and IgD are expressed on the surface of naive mature B cells by alternative splicing of a long primary RNA transcript. These double positive B cells (IgM+/IgD+) make up the majority of the peripheral B cells and upon Ag binding, they down-regulate their IgD expression. In contrast, the majority of long-lived memory B cells have class switched, undergone somatic hypermutation and express either high affinity IgG, IgE, or IgA (McHeyer-Williams, 2003). Interestingly, class switching is also involved in generating a unique IgM- /IgD+ (IgD-only) B cell population in humans, i.e., the cells use a cryptic switch region found between the Cµ and C6 genes (Chen et al., 2009). Human IgD-only B cells while rare in peripheral blood (~3%), have been shown to make up a substantial population (20–25%) of the human upper respiratory tract mucosal B cells and in the past 12 years, several laboratories have been focusing on this unique IgD-only B cell population (Chen et al., 2009; Kluin et al., 1995; Owens et al., 1991; White et al., 1990; Yasui et al., 1989). Most IgD-only B cells are found associated with tonsillar tissues. The IgD-only B cells are larger, have more cytoplasm than conventional naive IgM+/IgD+ B cells, and exhibit a centroblast morphology (Billian et al., 1996; Liu et al., 1996). Notably, ~90% of these IgD-only B cells utilize IgL α chains (Arpin et al., 1997) and sequence analyses showed that these cells express highly mutated VH and VL regions. Also, since the mutations are found scattered throughout the CDR and FR regions it was proposed that the different V regions would probably be unable to bind Ag (Liu et al., 1996; Seifert et al., 2009; Wilson et al., 1998; Zheng et al., 2005). Moreover, studies performed by Chen et al. (2009) demonstrated that secreted IgD made by the IgD-only B cells is bound to the surface of basophils (and mast cells) by a calcium-mobilizing receptor. First, immunofluorescence staining showed that IgD is found on the surface and in the cytoplasm of mucosal and circulating basophils, which indicates IgD-bound immune complexes are internalized. Second, cross-linking of basophil-bound IgD using an anti-IgD mAb triggered an intracellular calcium flux and induced secretion of B cell activation factors and cytokines, including BAFF (B cell-activating factor of TNF family), APRIL (a proliferation-inducing TNF ligand), IL-3, and IL-4. Third, IgD cross-linking induced basophils to secrete the antimicrobial factors cathelicidin, pentraxin-3, and β-defensin. In addition, the authors showed that supernatants collected from IgD cross-linked basophils inhibited the growth of the respiratory pathogens Moraxella catarrhalis and Haemophilus influenzae. However, even though the putative IgD-binding receptor (FcR) was not isolated, the authors’ studies suggest that it is a high affinity receptor since adding exogenous labeled IgD to cultured basophils did not lead to an increase in surface IgD staining, indicating saturation of the binding sites. Exogenous IgD could only be bound by the basophils after they were stripped of their IgD and IgD binding by pre-treated basophils was abolished if the “added” IgD was denatured or if stripped basophils were treated with trypsin. Interestingly, in this same study, patients with autoinflammatory syndromes were shown to have more circulating and mucosal IgD-only B cells and more mucosal “IgD-armed” basophils than healthy subjects, which suggests that inflammation promotes the switch to IgD. Also, cross-linking of IgD-armed basophils (from these patients) resulted in the increased secretion of both IL-1 and TNF, which suggests that IgD bound to the surface of basophils in the respiratory mucosa is able to trigger an innate immune response (Chen et al., 2009).

As for catfish IgD, in the same study it was shown by using flow cytometry that catfish express two types of IgD+ B cell
Fig. 4. Catfish express three types of IgD−-bearing B cells. The single IgM+/IgD− B cells, double positive IgM+/IgD+, and IgD−-only (IgM−/IgD+) B cells are depicted with their membrane immunoglobulins associated with CD79 accessory molecules. IgM+ B cells produce secreted IgM tetramers and the IgD−-only B-cells are the main producers of "V-less" secreted IgD (ref). Immunoglobulin domains are represented as in Fig. 2: C, black; C, dark grey, CL, light grey; V, white. The CD79a and CD79b Ig domains are stripped and Y marks the location of the two tyrosine residues in the immunoreceptor tyrosine-based activation motifs.

populations (see below) and a population of circulating granular cells that are armed with exogenous IgD via a putative IgD-binding receptor. Currently, the origin of these granular cells is unknown, however at the message level these cells did not express message for either membrane or secreted IgD, IgM, or TCR and they did not react with an anti-catfish neutrophil mAb. However, these IgD-granulocytes could not be isolated via direct selection techniques as this invariably resulted in degranulation and granulocyte destruction. Recently, a more detailed characterization of catfish IgD-only B cells was published by Edholm et al. (2010). Basically, catfish express three different types of B cells: single positive IgM+/IgD− B cells, double positive IgM+/IgD+ B cells and IgD−-only B cells (Fig. 4). The IgM+/IgD− and IgM+/IgD+ B cells are small agranular lymphocytes and are morphologically indistinguishable from each other, while the IgD-only B cells resemble human IgD-only B cells. They are large, exhibit a plasmablast morphology and have a higher cytoplasm to nucleus ratio as compared to catfish IgM+ B cells and in contrast to mammalian IgD-only B cells. These catfish IgD-only B cells can represent as much as 60–80% of the total peripheral blood B cells, depending on the individual fish. Notably, as shown by cell sorting experiments, co-immunoprecipitations, and RT-PCR, catfish IgD-only B cells exclusively utilize IgL σ chains, an IgL isotype restricted to ectothermic vertebrates. Also, even though the catfish IgM+/IgD− and IgM+/IgD+ B cell populations have the potential to utilize any of the four different IgL chain isotypes (IgL F, IgL G, IgL λ and IgL σ) most IgM+ B cells utilize the IgL κ orthologs, F and G, i.e., only 2–5% of IgM+ B cells isolated from peripheral blood express IgL σ or IgL λ chains, isotypes known to have very limited repertoires in catfish (Edholm et al., 2009). Currently, while the different roles of catfish IgM+/IgD+ and IgD-only B cells have not been defined, we hypothesize that catfish membrane IgD functions as a typical Ag binding receptor. Both cell types express message for membrane IgD with functionally rearranged VDJ regions and the B cell accessory signaling molecules CD79a and CD79b. Furthermore, co-immunoprecipitation experiments demonstrate that membrane IgD associates with IgL chains, and requires association of CD79 molecules for B cell surface expression. Furthermore, no bias in the V, D or J family usage of membrane IgD transcripts was observed in the different IgD-expressing B cell types. In addition, anti-IgD cross-linking in IgM+/IgD+ B cells induces a calcium flux, albeit it is slower than the response induced by IgM cross-linking of IgM+/IgD− and IgM+/IgD+ B cells (Edholm unpublished). Whether this is due to lower levels of IgD expression on the surface of IgD+ B cells and/or the nature of the anti-IgD mAb has not been determined.
Probably the most surprising observation from the catfish IgD⁺ B cell studies was the finding that secreted IgD transcripts isolated from sorted IgD-only B cells lacked V regions, instead the Cδ1 was directly spliced to a functional leader sequence located ~8 kb 5′ of the Cδ1 in the “IgD secreted form” IGHδ1 gene (Edholm et al., 2010). The isolation of catfish V-less secreted IgD transcripts is intriguing specifically when considering Chen et al. (2009) showed that both M. catarrhalis and H. influenzae bound IgD secreted by stimulated human IgD-only B cells, regardless of the IgD’s Ag specificity. Also, as previously demonstrated (Riesbeck and Nordstrom, 2006), this secreted IgD bound M. catarrhalis IgD-binding protein (MID), which is also expressed by H. influenzae. MID is an ~200 kDa adhesin with two functional domains, one mediates adhesion to mucosal epithelial cells and the other binds to the Cδ1 domain of IgD and studies show that the purified protein activates IgD⁺ B cells. Therefore, our working hypothesis for catfish IgD function is that IgD-only B cells, and possibly a subset of IgM⁺/IgD⁺ B cells, becomes activated by a certain “pathogen(s)” that can bind IgD. Once activated, these cells secrete V-less IgD, which may function as a pattern recognition molecule when bound to circulating granulocytes. This may also explain the low and varying levels of secreted IgD in catfish serum. In turn, the granulocytes upon binding the “target pathogen” through the IgD Fc-region would become activated. Here, it should be noted that the frequency of IgD-only B cells and IgD-bearing granulocytes varies among individual fish and the presence of these two populations do not always correlate (Edholm et al., 2010 and unpublished). Consequently, our studies are focused on identifying possible “target pathogens” or IgD-only B cell activation triggering events and isolating and determining the nature of the IgD-bearing granulocytes. Additionally, another important question that needs to be addressed is the relationship or lineage of the different IgD⁺ B cell populations. It may be that the catfish IgM⁺/IgD⁺ B cells represent traditional naive B lymphocytes, which upon Ag activation differentiate into IgM producing plasma cells or memory B cells, while the IgD-only B cells constitute a separate B cell lineage that expands in response to a yet unknown stimulus. Alternatively, it may be that the small population of IgM⁺/IgD⁺/Igσ⁺ B cells upon activation differentiate into IgD-only B cells.

4. Conclusion

In conclusion and as noted by others (Flajnik and Kasahara, 2010; Ohta and Flajnik, 2006; Zhao et al., 2009) IgD represents an ancient Ig isotype that is found in all vertebrate taxa, except for birds. Moreover, IgD has been shown to display remarkable plasticity as illustrated by the occurrence of multiple structural variants and splice forms in the different vertebrates. Also the changes in the IgD genes that result in long chimeric molecules in teleost as compared to shorter hinge containing molecules in placental mammals indicates that IgD has been subjected to species-specific adaptations. For example, the finding of IgD-only B cells and the fact that secreted IgD can be bound by granulocytes via an IgD-binding receptor suggests that IgD plays a direct role in immune defense at least in these two species.

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Appendix A. Supplementary data


Pelodiscus sinensis


