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Channel catfish immunoglobulins: Repertoire and expression

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Abstract

The channel catfish, *Ictalurus punctatus*, is widely recognized as an important model for studying immune responses in ectothermic vertebrates. It is one of the few fish species for which defined viable in vitro culture systems have been established and is currently the only fish species from which a variety of functionally distinct clonal leukocyte lines are available. Moreover, there is a large basis of biochemical and molecular information on the structure and function of catfish immunoglobulins (Igs). Catfish, as other teleosts, have a tetrameric homolog of IgM as their predominant serum Ig plus a homolog of IgD. They also have genetic elements basically similar to those of mammals, which encode and regulate their expression. The catfish Ig heavy (H) chain locus is a translocon-type locus with three Igδ genes linked to an Igμ gene or pseudogene. The catfish IgH locus is estimated to contain approximately 200 variable (V) region genes representing 13 families as well as at least three diversity (D) and 11 joining (JH) genes. The catfish has two light (L) chain isotypes, F and G, both encoded by loci organized in multiple cassettes of VL-JL-CL with the VL in the opposite transcriptional orientation. Hence, all requisite components for encoding antibodies are present in the catfish, albeit with certain variations. In the future, whether or not additional unique features of Ig function and expression will be found remains to be determined.

Keywords: Catfish; Antibodies; Immunoglobulins; Gene expression; Repertoire; Diversity; IgH chain enhancer

1. Introduction

Channel catfish (*Ictalurus punctatus*) immunogenetics is of interest not only because of the economic significance of this aquacultured species, but also because the catfish is currently one of the best-understood models of teleost fish immune functions. The advantages of catfish as an immunologic model include: (1) a large base of biochemical information on the structure and function of catfish immunoglobulins (Igs) and antibodies [1–7]; (2) well-established and defined in vitro cell culture systems permitting studies on antigen presentation, cell/cell cooperation and temperature effects on immune responses [8–19]; (3) the availability of cloned leukocyte lines of defined phenotype (including B

Abbreviations Ig, immunoglobulin; H, heavy; L, light; C, constant; TM, transmembrane; s, secreted; V, variable; D, diversity; J, joining; AID, activation-induced cytidine deaminase; MLC, mixed leukocyte culture; PBL, peripheral blood leukocytes.

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cells, macrophage/monocytes, cytotoxic and noncytotoxic T cells, antibody-dependent cytotoxic cells, and NK-like cells) [20–30]; (4) panels of monoclonal antibody reagents directed against both immune molecules and surface markers of immunocytes [31–41]; and (5) a significant number of cloned immune function molecules [41–64].

The immunoglobulin studies in many cases have gone well beyond the initial structural description of IgM-like tetrameric molecules consisting of eight each heavy (H) and light (L) chains to contribute greatly to our understanding of Ig gene expression in teleosts. Such findings made initially in catfish include the delineation of an unusual pathway of alternative RNA processing by which the expression of membrane receptor and secreted forms of teleost IgM is achieved [43,48,65–68], the discovery that the Ig heavy (IgH) transcriptional enhancer is unique among vertebrates in its location, structure and mode of function [60,69] and the discovery that teleosts produce (in addition to IgM) a second class of Ig homologous to IgD. IgD was previously considered to be recently evolved having been described initially only in primates and rodents [52,70–73], but more recently after the finding in fish, it has also been identified in artiodactyls [74,75].

1.1. The IgH germline repertoire

It was initially thought that the catfish IgH locus was a simplified version of the mammalian translocon IgH locus [76–78] and should therefore, at least in principle, be easily understood. However, as knowledge of catfish antibodies and IgH genes has increased, the picture has become more complicated. This complexity may best be appreciated in historical context. The catfish was the first teleost in which the IgM heavy (μ) chain sequence was described at both the cDNA [42,79] and genomic [43] levels. In most respects the catfish μ gene seemed typical of all other vertebrate μ genes, i.e. it possessed four constant (C) domain-encoding exons and two transmembrane (TM) exons, through which it encoded both secreted (μs) and membrane receptor (μm) polypeptide forms [43]. However, it was shown to have a major peculiarity. In other vertebrate classes (elasmobranchs, amphibia, reptiles, mammals) the μm form of the μ chain message results from the splicing of the two TM exons into a cryptic splice site within the Cμ4 exon [80]. In contrast the catfish TM exons are spliced to the regular splice donor site of the Cμ3 exon, thereby completely eliminating the Cμ4-region domain from the μm [43]. This unusual structure does not appear to interfere with the ability of the μm to function as an antigen receptor [19,33,81]. Subsequently this scenario has been found in all species of teleosts examined [65–68] and this unusual pathway of RNA processing appears to be evolutionarily ancient, i.e. it is present in the more ancient holostean fishes, the gar, *Lepisosteus osseus*, and bowfin, *Amia calva* [82].

Although some antigenic evidence had been presented to argue for the existence of IgM subclasses in catfish [83,84], the application of molecular biology to the resolution of the question of IgM complexity initially indicated catfish possessed only one gene encoding a functional μ H chain [42,43]. Likewise, in all species of teleosts examined, the only IgH chain constant region gene described at both the cDNA or genomic levels was μ. Hence it was widely accepted that IgM was the only Ig class expressed by teleosts [76]. The realization that this picture was incomplete followed the identification of the IgH chain of catfish IgD (δ; [52]). The reasons for naming this second IgH gene δ were (1) sequence similarities to mammalian δ; (2) a position in the IgH locus immediately downstream of μ; (3) co-expression with μ in some catfish B cells and; (4) expression of δ from a long primary transcript containing a rearranged variable region (V) consisting of VH, diversity (D), joining (JH) segments and μ and δ genes [52]. The mature δ mRNA is produced from this primary transcript by alternative pathways of RNA processing. Thus expression of IgD is accomplished, in both catfish and mammals, by mechanisms that do not involve isotype (class) switching by chromosomal recombination. Briefly, the structure of catfish δ, as revealed at the cDNA level [52], showed two unique features. First, it contained seven δ C region domains, followed by either a typical membrane anchoring or secretory tail. Second, it included, by an unusual pathway of RNA processing, the first domain of μ (Cμ1) between the rearranged VHDJ and Cδ1 domain. This inclusion of Cμ1 was postulated to permit the covalent association of the δ polypeptide chain with catfish IgL chain, since the Cμ1 domain