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Review Article

Research Progress in Molecular Biology of Fish Immunoglobulin M (IgM)

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Abstract

Immunoglobulin (Ig) is a type of globulin produced by B lymphocytes during pathogenic infection of vertebrates. It has immune functions and can realize specific recognition and neutralization of corresponding antigens. As IgM is reported first in fish, IgM is the first antibody produced during immune responses and plays a vital role in systemic and mucosal immune tissues. IgM molecules have two forms: membrane-bound IgM (mIgM) and secreted IgM (sIgM). The latter is produced by plasmacytes and secreted into body fluid, existing as immunological effect molecules. The former embeds into B cytomembrane and exists as an antigen receptor. It binds with assistant molecules to form cell receptor compounds. This study reviews research progress on the structures and production processes of IgM genes in different fish species and the distribution characteristics of IgM on B cells, mediated signal pathways, and functions. It aims to enrich basic theoretical knowledge of fish immunology and provide some scientific references for disease control in fishes.

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Introduction

With the increasing consumption of aquatic products, aquaculture is developing rapidly. However, high-density aquaculture leads to the frequent occurrence of disease in aquatic animals. The prevalence of some diseases may even cause catastrophic impacts on aquaculture. Fish disease control is becoming increasingly urgent and a research hotspot for aquatic scientific researchers. Studying disease defense mechanisms based on the immune response of fishes is beneficial to understanding the evolutionary laws and characteristics of the immune system of vertebrates. It facilitates the research and development of vaccines for fish. Immunoglobulin is a vital molecule in the body-fluid immunity of fish. Studying immunoglobulin can bring about a more profound understanding of the immune mechanism of fishes and immunoglobulin evolutions and provide crucial references for disease control in fishes.

Three types of immunoglobulin (Ig) have been discovered in osteichthyan: IgM, IgT, and IgD (Dornburg et al., 2021). IgM is the first immunoglobulin to appear; IgT is the last. According to associated studies, IgT plays a vital role in the mucosal immune system of Osteichthyes, and its function is similar to IgA in mammals (Yu et al., 2022). The role of IgD in fish immunity has yet to be elaborated thoroughly. Wang Yimeng has demonstrated that IgD has a strong immune response in gills and might play a critical role in gills' mucosal-associated lymphoid tissue (Wang, 2022). IgM is an essential component of system immunity and plays a central role in mucosal immune tissues and mucus (Xia et al., 2014; Mirete-Bachiller and Gambon-Deza, 2023).

1. Structure of the IgM gene

Immunoglobulin M (IgM), discovered first in Osteichthyes, can generate effective specific humoral antibody reactions to various antigens. The immune system of animals develops gradually in the phylogenetic process. *Pheretima annulata* only has phagocytosis defense of phagocytes. IgM and cell immunity only developed in Osteichthyes and Chondrichthyes. IgG began to develop in amphibians. Mammals (e.g., rabbits) developed IgM, IgG, and IgA, and humans have five different Igs: IgM, IgG, IgA, IgD, and IgE (Xia et al., 2014). Therefore, fishes are the first species to develop immunoglobulin (Mirete-Bachiller and Gambon-Deza, 2023). IgM is the first immunoglobulin discovered in Osteichthyes, existing as tetramers. IgM in Chondrichthyes exists in the form of pentamers (Bilal et al., 2021). IgM lacks the J-chain in Osteichthyes, and its monomers mainly bond through covalent (disulfide) bonds. Currently, IgM is gained from cloning in many fish species, such as *Heterodontus francisci*, *Raja erinacea*, *Salmo salar*, *Oncorhynchus mykiss*, *Cyprinus carpio*, *Ctenopharyngodon idellus*, *Ictalurus punctatus*, *Epinephelus coioides*, *Siniperca chuatsi*, *Paralichthys olivaceus*, *Gadus morhua* (Bilal et al., 2021; Xia et al., 2014).

IgM has membrane-bound IgM (mIgM) and secreted IgM (sIgM). Its heavy-chain genes include three and four constant regions. The heavy-chain of sIgM genes in Osteichthyes have structural domains of (VH1)n—(D1)n—(JH1)n—CH1—CH2—CH3—CH4, and they mainly exist as tetramers in blood and pentamers in other vertebrates (Xiao et al., 2010). The mIgM has two additional transmembrane regions (TM): TM1 and TM2. The splicing site between the TM regions and constant regions is in CH3, which is different from other vertebrates and Chondrichthyes, in which the splicing site is in CH4 (Xia et al., 2014). Both mIgM and sIgM are formed by different selective alternative splicing by the same precursor mRNA (Xiao et al., 2010). sIgM in Osteichthyes and Chondrichthyes is formed by four constant regions. There are two types of sIgM in *Ginglymostoma cirratum* (Bakke et al., 2022). One type of sIgM has four constant regions, and the other has three constant regions, lacking the CH2 constant region (Bakke et al., 2022; Xia et al., 2014). Most sIgM in Osteichthyes exist as unique tetramers, but some IgMs exist as monomers. For Chondrichthyes, sIgM in *Lepidosiren* exist as pentamers, the same as those in tetrapods (Mirete-Bachiller and Gambón-Deza, 2022). mIgM in Chondrichthyes is similar to those in tetrapods, and they are formed by splicing between transmembrane exons and one splicing donor in CH4. The CH4 of most IgMs in Osteichthyes lack splicing sites; their mIgM is

formed by direct splicing of TM1 onto the terminal of exons of CH3, thus cutting the whole CH4. As a result, exons of mIgM only contain three constant regions. On the one hand, *Amia calva* and *Lepisosteidae,* the original *Actinopterygii*, have two splicing modes (Bradshaw and Sigel, 1972). On the other hand, *Amia calva* has a third splicing mode, another splicing donor site in the midstream of exons of CH3 that can form mIgM (Bradshaw and Sigel, 1972; Xiao et al., 2010). sIgM is the primary antibody type in serum; it distributes extensively in different tissues and plays a vital role in the humoral immune response, facilitating the phagocytosis effect and activating complement pathways. mIgM, known as B cell receptor (BCR), distributes on the surface of B cells. It is responsible for antigen recognition, mediating the endocytosis of antigens, and transferring extracellular signals (Yu et al., 2020).

IgM monomers of Osteichthyes are tetramers of two light chains and two heavy chains. There are intrachain disulfide bonds in the same chain, while different chains are connected by interchain disulfide bonds, forming 'Y-shaped' structures. IgM's light-chain and heavy-chain genetic structures in Osteichthyes cover the variable region (V) and the constant region (C). There are four relative conservative framework regions (FR) and three highly changed complementary determining regions (CDR) in the gene segment of V. The light chain of IgM is composed of two structural domains: the variable region (VL) and the constant region (CL). The heavy chain has one variable region (VH) and at least three constant regions (VH). Therefore, heavy-chain genes of IgM in Osteichthyes are aligned in the form of a 'translocon.' Gene segments of the variable region, diversified gene segments, gene segments of the linkage region, and gene segments of the constant region are connected successively. Such a structure plays a critical role in gene rearrangement and is the basis for the diversity of IgM (Xia et al., 2014).

2. Generation process of IgM

The immune response of fishes against pathological invasion is similar to that of mammals. There's no antibody when the antigen attacks the body for the first time. It may have antigen phagocytosis by macrophagocytes or delivery to the kidney and spleen through pinocytosis after entering the body. Next, antigen information is delivered to B lymphocytes, which proliferate and differentiate into antibody-producing cells. Later, antibodies are produced, and antigens are recognized and killed to protect the body, thus preventing pathogen invasion. IgM in mammals is the first antibody that appears in the development process of B cells. Pre-B cells in the marrow form IgM through the antigen-independent pathway and express it on the surface of B cells (Perdiguero et al., 2019). Heavy-chain molecules of IgM can be expressed as sIgM and mIgM through RNA variable splicing (Quiniou et al., 2011). The substitute cutting of mRNA transcript produces two types of mRNA. Exon sequences with TM1 and TM2 synthesize the mIgM, while TM1 and TM2 sequences are cut and synthesize the sIgM (Quiniou et al., 2011). The μ-chain mRNA and δ-chain mRNA formed by deleting the Cμ gene or Cδ gene during cutting are coded in the same cloned B cell, and they express the μ chain and δ chain with the same V region (Bunnoy et al., 2022; Hikima et al., 2011). The formed IgM molecules are embedded into the B cytomembrane.

3. Distribution characteristics of IgM on B cells

Understanding IgM mainly comes from studies of human and mice immune systems. However, more and more studies on the immunity of fishes are reporting the unique role of fishes in the evolutionary process, and the demand for aquaculture has increased in recent years.

Mature B cells in Osteichthyes are similar to B cells in mammals, both of which exist in the spleen (SPL). B cells are differentiated into plasmablasts and plasmocytes with a short life upon stimuli by antigens. Subsequently, some differentiated Ab-secreting cells (ASC) might be transferred to the head kidney and exist in this region as long-life plasmocytes (Bromage et al., 2004). Zwollo et al. found that B cells in the early development stage of rainbow trout can express early B cell factor (EBF) and recombination activating gene1 (RAG1). In contrast, in the late development stage, B cells cannot express these two

substances—although they can express the transcription factors Pax5 and mIgM (Zwollo, 2011). The terminally differentiated plasmacyte lacks Pax5 and mIgM and can secrete high-level sIgM (Zwollo, 2011). The three major B-cell masses of IgM^+/IgD^+, IgM^-/IgD^+, and IgT^+ have been identified from Osteichthyes (Yu et al., 2022). IgM^+/IgD^+ B cells have the highest content (Xu et al., 2020; Edholm et al., 2010). IgM^+/IgD^+ B cells distribute widely in peripheral blood leucocytes, head kidney, and spleen of Osteichthyes. Moreover, some studies have demonstrated that IgM secreted by B lymphocytes also exists in spawn, bile, body surface, and intestinal mucus (Hayman and Lobb, 1993; Lobb and Clem,1981; Salinas et al., 2021; Xu et al., 2013).

Boshra et al. pointed out that IgM^+B cells in peripheral blood leucocytes of Osteichthyes account for about 30–60% (Boshra et al., 2006). Wu Xia investigated percentages of $mIgM^+$ B cells in the peripheral blood, head kidney, abdominal muscles, and spleen of *Myxocyprinus asiaticus*, valuing them at 15.9%, 10.7%, 4.32%, and 4.16%, respectively (Wu, 2019). Wu and co-workers found that distributions of IgM^+ B cells vary among tissues, accounting for the highest percentage (37.6%) in peripheral blood, followed by the spleen (33.7%), head kidney (23.9%), and metanephros (20%) (Wu et al., 2018).

4. IgM-mediated signal pathways

Intracellular parts of mIgM and mIgD are the same, with only three kinds of amino acids. Both mIgM and mIgD have the same process of downstream activation of PTK (protein tyrosine kinase) and Ca^{2+} releasing, but the responses after B cell activation differ (Bromage et al., 2004). Løset et al. found that the co-expression of mIgM and mIgD on B cells can make different concentrations of antigens choose the optimal binding sites (Løset et al., 2004). IgM contains five Fc segments, and it is easier to activate complement in IgG, in which CH3 is the complement activation site. IgM of mammals has to be transported under the action of the J-chain. IgM^+ B cells of rainbow trout can take in particles. B cells with endocytosis activate the formation of intracellular phagocytic lysosome and a series of degradation pathways downstream, showing a relatively robust immune response (Xu et al., 2020).

5. Functions of IgM

IgM is an immunoglobulin that exists extensively in vertebrates and plays an essential role in the immune system of mammals (Xia et al., 2014). IgM in serum has the strongest ability to bind and activate complement. Its opsonization and agglutination are stronger than IgG and crucial for preventing bacteremia and septicemia.

Due to increasing attention to the immunity of fishes, there are many studies on heavy-chain gene cloning, sequence analysis, composition form, the rearrangement mechanism, and transcription regulation of IgM in fishes. The specific IgM antibody in Osteichthyes not only activates complement to act on red blood cells marked by artificial epitope and specific antigen reaction but also kills or neutralizes bacterial pathogens such as *Aeromonas salmonicida*, *Cryptobia salmositica*, infectious hematopoietic organ necrosis virus (IHNV) and viral hemorrhagic sepsis virus (VHSV). It has been proved that the IgM antibody in Osteichthyes mediates the antigen specificity opsonisation and phagocytosis of bacterial pathogens, such as the phagocytosis of *Francisella* and *photobacterium damsela* (Nawaz et al., 2022).

By RT-PCR, Liu Yue et al. detected IgM expressions in the perch's tissues and organs and found the highest expression in the head kidney, followed by the spleen (Liu et al., 2013). There was hardly any expression of IgM in the heart, muscle, or brain. The expression of the IgM gene in the skin of perch was lower than in *Pelteobagrus fulvidraco*, which might be because there was no scale on the surface, and the mucosal immunity of the skin was more important (Xu et al., 2019). After being injected with *Aeromonas hydrophilia*, the transcription expression of IgM in the hemocyte, spleen, and head kidney of *Mystus macropterous* increased significantly in 1-10 d and then tended to be expected after 15d (Li et al., 2011). After being infected with *Edwardsiella tarda* by intraperitoneal injection, IgM expression in the hemocyte, spleen, and head kidney of *Ictalurus punctatus* increased significantly in 1-13d (Zilberg and Klesius, 1997). These results showed that in

three weeks of antigen stimuli, IgM expressions in the spleen and head kidney of fishes increased significantly, and IgM molecules in fishes produced solid immune responses three weeks after antigen stimuli (Xia et al., 2014; Liu et al., 2013; Xu et al., 2019; Li et al., 2011).

6. Conclusions and prospects

Most IgM in Osteichthyes exist as tetramers, and IgM monomers comprise two light chains (L chain) and two heavy chains (H chain). The H-chain constant regions have many amino acid sequences, more complicated structures, and a lot of binding sites with antigens. By contrast, the L-chain is more straightforward and has fewer binding sites. There are many fish species, and IgM-mediated immune signal pathways in different fishes might differ. After being infected by different pathogens like viruses, bacteria, and parasites, fish's immune response laws might differ. Hence, the acting mechanism of IgM in fish's immune system has to be further investigated.

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