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Review

Research Progress on the Fish Complement C3 Gene

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Abstract

Complement (C) is a serum protein with immunity-related functions and enzymatic activities. It can induce inflammatory responses and antibody formation following activation by substances such as pathogens. Moreover, it is a crucial molecule in the congenital immune defense system. Complement manifests earlier than immunoglobulin in the evolutionary process of fish's immune system. Therefore, the complement system of fish is vital for congenital and acquired immunities. A complement system is a polymolecule system comprising more than 30 soluble proteins, membrane-bound proteins, and complement receptors. It can be activated through three pathways for immunoregulation, the clearance of the immune complex, etc. The liver primarily produces the fish complement C3, which is a significant component of the complement system of fishes. C3 is the hub of three activation pathways. Research works concerning the structure, generation process, expression, and functions of C3 in fishes are reviewed in this study.

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Introduction

Aquaculture is developing quickly in response to the annual escalation of market needs. The expansion of environment-friendly aquaculture has also emerged as a new trend in the industry. The increase in breeding density facilitates rapid pollution and, consequently, toxic substances in the water bodies, making it challenging to ensure the quality of aquatic products. Therefore, studying fish's immune systems and responses is necessary. The complement system of fish is an essential component of their immune systems. Investigating the biological functions of the complement system helps better understand the immune mechanism of fishes, thus enabling the prevention and control of diseases in them more effectively.

The complement system can be divided into a complete inherent component, a complete regulating component, and a complete receptor. C1~C9 belong to the complete inherent components. C3 is the complement component with the highest serum content, connecting specific and non-specific immunities (Li et al., 2016). C3, C4, and C5 are homologous and can produce anaphylatoxin following hydrolysis. All the C-terminal segments contain the structural domain of C345C (complement C3/4/5 C terminals), which facilitates combining C5b with C6 and C7 to create a membrane attack complex (Lv, 2020). C3 and C9 are crucial in complement activation, pathogen elimination, etc. Zhang Yanjie et al. demonstrated that the complement factor promotes phagocytosis, morphogenesis, and immune defense of fishes (Zhang et al., 2013)**Error! Reference source not found.**. C3 is the component with the highest content in the complement system and exhibits strong immune responses in various immune tissues.

1. Structure of the C3 gene

C3 is the molecule that occurs first in the evolutionary complement system. The system occurrence can be dated to the stage of coelenterate (Yu et al., 2012). Since the discovery of Ritg in 1912, C3 has been the most critical component of the complement system. Moreover, it plays an essential role in the classical pathway (CP), lectin pathway (LP), and alternative pathway (AP). It is the intersection of different pathways (Boshra et al., 2006). Several forms of C3 can be discovered in almost all Osteichthyes except *Gadus morhua*, *Hippoglossus hippoglossus,* and *Anarhichas minor Olafsen*, even though it is only encoded by one gene (Wang et al., 2008).

C3 comprises *α* chain and *β* chain. It belongs to the Thioester-containing protein (TEP) superfamily. Such molecules contain a thioester site where the highly conservative *α*2 macroglobulin (MG) combines with the protease region. The cleavage site between the *α* and *β* chains is four alkaline amino acids. They are connected according to disulfide and noncovalent bonds (Sfyroera et al., 2015). A complete C3 molecular is equipped with no bioactivity (Bavia et al., 2022). In many known C3 sequences, there is the conservative *β–α* processing sequence RXXR (most fishes are RRKR or RKRR. The typical sequence in mammals is RRRR. R is arginine, and K is lysine). The thioester domain (TED) of the *α* chain contains the thiolipid bond motif comprising five amino acids (GCGEQ, where G represents glycine, C represents hemimyosine, E represents glutamic acid, and Q represents glutamine). A unique intra-chain thioester bond between Cys and Gln hides in molecules. This site is important for the immune function of C3 (Løvoll et al., 2006).

2. Generation process of C3

Complements are proteins with enzymatic activity. They are present in serum and interstitial fluid in humans and vertebrates. Complements are synthesized in several histocytes. Hepatocytes constitute a significant source of most plasma complement proteins, especially C3. According to the individual development and extrahepatic synthetic spectra of *O. mykiss*, there is an extensive distribution of multiple complement components (Løvoll et al., 2006). The comparative study of *H. hippoglossus* and *G. morhua* revealed that the mRNA and proteins of the C3 gene had been detected in several tissues following incubation (Lange et al., 2006). Complement is synthesized for a small content in other sites, such as endothelial cells, lymphocytes, monocytes/macrophages and neurogliocytes,

and so on (Fabry et al., 1994). Among them, monocytes/macrophages can produce all the complement components, whereas other cells or tissues can produce some complement components (Fabry et al., 1994).

The upstream recognition molecules of the serum complement (C1q), mannose-binding lectin (MBL), and ficolin complement participate in the classical pathway (CP) and lectin pathway to finally activate C3. C3a and C3b molecules assume their roles after C3 is split into C3a and C3b. Sulfur lipid bonds in C3b allow C3 molecules to connect with receptor molecules on the cell surface by forming ester groups or amido bonds. Generally, the sulfur lipid bonds in C3 are hidden in the hydrophobic region and can only be exposed after C3 is split into C3b segments by the C3 invertase (Law et al., 1997). After C3 is divided into C3b, the sulfur lipid bonds in C3b are exposed and participate in the trans-acylation between the nucleophilic group on the cell surface and complex carbohydrate or immune complex, thus strengthening the humoral immune response of antigen (Sahu and Pangburn, 1996). Furthermore, C3b can bind with the H factor. Under the participation of CD46 and CR1, C3B is also split into iC3b and C3f by complement factor I (CFI). C3b is finally divided into C3c, C3dg, and C3d (Nishida et al., 2006).

C3 is the most crucial component in the complement system. It is the activation and effect center. Three activation pathways of the complement system aggregate at C3 and then produce the corresponding effect. C3 is also related to the initiation of activation of AP, which also belongs to a spontaneous activation, a "tickover" mechanism called C3 (Bavia et al., 2022). C3, accounting for about 1% of total complements, triggers "tickover" every hour. C3 with structural changes is known as C3(H_2O), which can combine with factor B. Factor B can be split by the serum protease factor D, which is activated because of conformation changes, thereby causing a series of effects to trigger the splitting activation of C3.

3. Expression of C3

Previous studies primarily concentrated on the expressions of C3 in humans, but there are few studies on the C3 expression in fishes and other species. Because C3 molecules are present at the hub of complements and are the core molecules of complement activation pathways, they have been widely researched in recent years. The C3 gene in several species, including protein expression, gene and protein sequence analysis, structure and functional analysis, and so on, have been examined and identified over time (Nishida et al., 2006).

Because the tissues in invertebrates have not been differentiated yet in the early stage, the complement components can only be produced in the body cavity equivalent to invertebrates or the original cavity of the blood vessels of vertebrates (Clow et al., 2004). Bilateral animals have three germ layers, and complement components hide in the cavity enclosed by the middle germ layer. The two-layer structure of coelenterate has only one coelenteron wrapped by the endoderm, and the C3 gene of coelenterate has expressions of the antenna, pharynx, and mesenteric endoderm in the three tissues of endoderm of adults (Wang et al., 2015). In mammals, the C3 gene is primarily expressed in the liver and in other tissues, such as the central nervous system, gastrointestinal tract, reproduction, and lymph (Gasque et al., 2000).

In fishes such as *Pseudosciaena crocea* and *Labeo rohit*, the liver is viewed as the principal organ for the synthesis of C3 (Wang et al., 2015; Pushpa et al., 2014). Except for a high expression in the liver, the extrahepatic expression of C3 has been reported in many species. For example, C3 has expressions in the gills, skin, skeletal muscles, and heart of *O. mykiss*, *Epinephelus coioides*, carp, *Dicentrarchus labrax*, *sea bass,* and *Atlantic salmon*, among others. However, expression levels differed (Qi et al., 2011; Sigh et al., 2004; Saeij et al., 2003; Mauri et al., 2011). After incubation, the expression distributions of C3 in 5~99d *H. hippoglossus* were detected using an immunohistochemical technique. This approach revealed the C3 expression in the yolk sac, muscle, liver, brain, cartilage, eyes, heart, stomach, intestines, esophagus, throat, and kidney in different stages (Lange et al., 2004). The expression distributions of C3 in Atlantic cod aged from 1d to 57d were detected using the same approach. The results demonstrated that C3 existed in the yolk sac at 1d

and in the liver, brain, kidney, and muscle at 2d. Moreover, the C3 protein was expressed in other organs in different development stages, such as the eyes, notochord, stomach, intestines, spleen, heart, and gills (Lange et al., 2004). Although the size of C3 in different species and expression levels in different tissues and organs differ, it has stable functions because some acting sites in the conservative functional areas are highly conservative. Due to the various functions of each protein, its spatial and temporal expressions might vary to different extents. With system evolution, the complement components are mainly produced by the liver of vertebrates or hepatopancreas and hemocytes of invertebrates.

4. *Functions of C3*

4.1 Bioactivity of C3

(1) Cytotoxicity, sterilization, and dissolution effect: when the membrane attack complex (MAC) is generated, tubular structures are formed on the target cell surface to create pores and cause the breakage of target cells. MAC is the immune measure for the body to resist microbial infection (e.g., bacteria). The excessive activation of MAC may trigger the body's autoimmunity and cause damage to local tissues (Aleshin et al., 2012). (2) Opsonisation: C3 in Osteichthyes can mediate and eliminate pathogens through target marking and change the conformation of phagocytes and pathogen cell surface molecules. (3) Removal of immune conjugates: fragment crystallizable (Fc) is affected by absorption between C3b and antibody, thus promoting the cleavage reaction of the immune complex (IC). (4) Inflammation mediating effect: The C3a anaphylatoxin of *O. mykiss* can combine with head–kidney cells, thus causing inflammatory reactions, such as respiratory bursts (Kania et al., 2010). The C3 molecules of mammals are split into C3b and then combined with the antigen-antibody complex and transfer complex into the liver for removal via blood circulation (Mihlan et al., 2011). Moreover, C3d is the smallest fragment of the complement molecule that cannot be split by protease. As a new adjuvant, C3d is integrated with target protein to prepare a vaccine, which enjoys promising application prospects. It shares a covalent linkage with antigens, facilitating cell activation and antibody affinity.

4.2 Complement C3-related diseases

C3 can be activated by the human immunodeficiency virus (HIV) in the early infection stage. By differently targeting the HIV infection, C3 activation can only produce some MAC to kill and dissolve the virus. It emphasizes opsonization to make HIV enter into cells or infect other cells out of cells (Webster et al., 2012). In rats with complement factor H deficiency, the deposition of C3 in the basal membrane of capillary loops requires the participation of i C3b metabolites in the complement regulatory protein (Thurman 2012). Therefore, the deficiency of C3 can hinder the metabolism of i C3b and the deposition of C3 in the basal membrane of glomeruli, thus avoiding dense deposit disease (DDD) of glomeruli (Thurman 2012).

5. Conclusions and prospects

To summarise, C3 is a core component of the complement system and develops a critical role. Therefore, profound investigations on the evolution and function of C3 are vital to studies on the evolution and functions of the whole complement system. Recent research works on C3 emphasize elaborating the action mechanism of the immune function. Are there more non-complement functions of C3 and any other essential tasks in the complement system of fishes except for the above four functions? What is the role of C3 in the complement system under bacterial, virus, and parasitic infections? These scientific problems must be further explored in the future.

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