Fish & Shellfish Immunology 42 (2015) 98–107

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Comprehensive and comparative transcription analyses of the complement pathway in rainbow trout

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ARTICLE INFO

Article history:
Received 13 June 2014
Received in revised form
23 October 2014
Accepted 28 October 2014
Available online 7 November 2014

Keywords:
BORN trout
CD93
CFD
CLEC4M
Complement system

ABSTRACT

The complement system is one of the most ancient and most essential innate immune cascades throughout the animal kingdom. Survival of aquatic animals, such as rainbow trout, depends on this early inducible, efficient immune cascade. Despite increasing research on genes coding for complement components in bony fish, some complement-related genes are still unknown in salmonid fish. In the present study, we characterize the genes encoding complement factor D (*CFD*), CD93 molecule (*CD93*), and C-type lectin domain family 4, member M (*CLEC4M*) from rainbow trout (*Oncorhynchus mykiss*). Subsequently, we performed comprehensive and comparative expression analyses of 36 complement genes including *CFD*, *CD93*, and *CLEC4M* and further putative complement-associated genes to obtain general information about the functional gene interaction within the complement pathway in fish. These quantification analyses were conducted in liver, spleen and gills of healthy fish of two rainbow trout strains, selected for survival (strain BORN) and growth (Import strain), respectively. The present expression study clearly confirms for rainbow trout that liver represents the primary site of complement expression. Spleen and gills also express most complement genes, although the mean transcript levels were generally lower than in liver. The transcription data suggest a contribution of spleen and gills to complement activity. The comparison of the two rainbow trout strains revealed a generally similar complement gene expression. However, a significantly lower expression of numerous genes especially in spleen seems characteristic for the BORN strain. This suggests a strain-specific complement pathway regulation under the selected rearing conditions.

1. Introduction

The complement system plays a central role in early pathogen defense. It consists of about 30 serum proteins in mammals, which constitute the classical, the lectin, and the alternative pathway (PW) (Fig. 1). Subsequently, three main processes are triggered: Opsonization of pathogens, release of anaphylatoxins, and formation of the membrane attack complex (MAC).

The complement cascade is presumably about one billion years old [1]. Some complement factors are present even in *Cnidaria* [2]. In invertebrates, exclusively the key complement component C3 and components of the lectin and the alternative pathway are present. The classical pathway, based essentially on antigen–antibody complexes arose first in jawed vertebrates (*Gnathostomata*) including cartilaginous and bony fish [3], where it evolved as bridge between innate and adaptive immunity [4]. The MAC-mediated cytolysis is also not present in invertebrates and probably evolved together with the classical PW [5].

Complement gene activation is mediated by several regulatory proteins providing a balanced complement concentration in proportion to the strength of the activation signal [6]. It has been suggested for fish that C-reactive proteins (CRPs) [7] and/or antibody–antigen complexes as well as surfaces of bacteria, viruses or fungi activate the

Abbreviations: aa, amino acid; AP, alternative pathway; bp, base pairs; cDNA, complementary DNA; CP, classical pathway; CR, complement receptor; CRP, C reactive protein; Ct, cycle threshold; kDa, kilo Dalton; LP, lectin pathway; mRNA, messenger RNA; pI, isoelectric point; PW, pathway; RCA, regulator of complement activation; qRT-PCR, real-time quantitative reverse transcriptase; PCR, polymerase chain reaction; TMD, transmembrane domain.

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http://dx.doi.org/10.1016/j.fsi.2014.10.032
1050-4648/

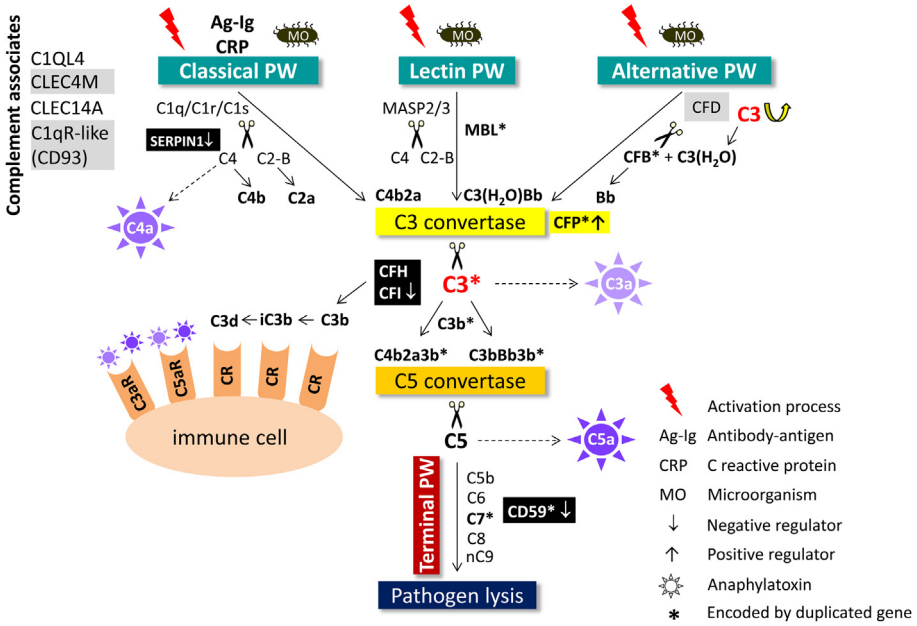

Fig. 1. Schematic representation of the predicted complement activation pathways and subsequent processes in rainbow trout. The complement system comprises the classical, the lectin and the alternative pathway (PW, highlighted in cyan) induced by specific activators as indicated by red arrows. Asterisks indicate components encoded by duplicated genes. The legend provides a description for further symbols. Black boxes mark regulatory factors. Gray boxes highlight the coding sequences of three complement and associated genes isolated in rainbow trout in the present study.

classical pathway [8] (Fig. 1). The lectin and the alternative PW are induced by microbial surfaces [9]. The binding of activated C3 and C4 proteins to those pathogenic surfaces enhances the phagocytosis of coated microbes. This process is referred to as opsonization. C3 plays a major role during this process as it is part of the so-called C3 convertase, which promotes increased levels of activated C3 *via* an amplification loop [10]. The parallel release of the anaphylatoxins C3a and C5a as cleavage products of C3 and C5, respectively, mediate leucocyte migration and induce respiratory burst. However, C5a is the more important anaphylatoxin in mammals [11]. On the other hand, three C3 isotypes are present in rainbow trout providing three C3a anaphylatoxins with different impacts on effector functions [12]. Furthermore, the three C3b cleavage products differ in their binding specificity [13]. Initial studies validated the functionality of the anaphylatoxin receptors C5aR [14–16] and C3aR [17] on rainbow trout B lymphocytes. The high gene sequence similarity of *C5aR* and *C3aR* suggests that both genes share a common ancestor [17]. A further anaphylatoxin, C4a, acts similar as C3a and C5a, but significantly weaker and seems to be less relevant in mammals [18].

The activated classical, lectin and/or alternative pathways lead to formation of the C5 convertases C4b2a3b and C3Bb3b, respectively, which cleave C5, an initial component of the MAC. C5b binds to the pathogenic surface as a starting point for the accumulation of the pore-building components C6, C7, C8 and numerous C9 [19], which allow for an osmotic influx of fluid into the cell and finally for cytolysis [20].

To prevent pathologic consequences [21], regulatory molecules monitor the most important activation sites (Fig. 1, black boxes). SERPIN1, also known as C1 inhibitor, is interacting with the C1-associated serine proteases C1r and C1s [22] as well as MASP1 and MASP2 [23] to inhibit the autoactivation of the classical and lectin pathway [24]. The complement factor I (CFI) is responsible for the fragmentation of free C4b and C3b [25]. Co-factors for CFI are complement factor H (CFH) and C4BP [26]. CD59 is a negative regulator of the MAC pathway, encoded by a duplicated gene in rainbow trout [27]. CD59 controls the accumulation of C9 molecules to the C5b-C8 complex preventing the formation of the pore [28]. Properdin (CFP) is a positive regulator of the complement system. The factor is encoded by the genes *CFP1*, *CFP2*, and *CFP3* in rainbow trout [29] stabilizing the C3 convertase of the alternative PW [30].

All mammalian complement components are present as orthologs in fish except for the crucial feature complement factor D (CFD), acting as serine protease of the alternative pathway. At the beginning of our investigations, a *CFD* ortholog was unknown in bony fish. Since recent microarray studies identified *CFD* as differentially expressed in naïve and infected rainbow trout of two strains [31,32], we aimed at the characterization of the rainbow trout *CFD* cDNA sequence. However, assembling the salmon genome identified meanwhile a further piscine *CFD* gene (GenBank BT049507). In parallel, we analyzed structure and function of the genes coding for CD93 molecule (*CD93*) and C-type lectin domain family 4, member M (*CLEC4M*) in rainbow trout, representing complement related molecules.

Despite a wide range of available sequence information and reports about the function of individual teleost complement factors in recent two decades, comparative expression profiles of a comprehensive set comprising all known complement genes of one single fish species have not been recorded until now. The present study compares transcript levels of complement-related genes in liver, spleen, and gills of healthy adult rainbow trout grown under identical rearing conditions. In mammals, the liver is deemed to be the main complement expression site, despite a not negligible extrahepatic expression of several complement genes [33]. Since extrahepatic complement expression is contributing considerably to systemic host defense mechanisms in rainbow trout, we included spleen and gills into our analyses. Additionally, our investigations of complement expression patterns focus on two rainbow trout strains with significantly different levels of robustness [31–35]. Therefore, they are expected to accommodate different expression levels of immune molecules such as complement components. The strain BORN is bred since 1975 in brackish water of the Baltic Sea (Born, Germany) and selected for survival whereas the other rainbow trout