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# Innate Immunity

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## Abstract

Research on the innate immunity has accelerated over the last decades. The main reason for this has been the discovery of receptors recognizing danger molecules from pathogens. This has been facilitated through genome and transcriptome sequencing of different fish species. Also, endogenous host molecules from sterile physiological insults may also bind to certain receptors and induce immunological processes. The magnitude and quality of adaptive immunity are known to be dependent on the instructions the innate response gives. This chapter gives an overview of selected innate immune organs/tissues, factors, and processes that have been suggested to possess important roles during innate immune response in fish.

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## Keywords

Innate immunity · Fish · TLR · Complement · Cytokines · Acute-phase proteins · Antimicrobial peptides · Chemokines · Epigenetics

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## Abbreviations

AMP	Antimicrobial peptide
APC	Antigen-presenting cell
APP	Acute-phase proteins. Resulting from acute inflammation
c1, c2, c3, c4, c5, c6, c7, c8, and c9	Complement components
c1q	A protein complex involved in the complement system
Cath11	Cathelicidin gene 1. Antimicrobial peptide
Cath2	Cathelicidin gene 2
Ccl19	Chemokine (C–C motif) ligand 19. A regulator of the induction of T-cell activation, immune tolerance, and inflammatory responses during continuous immune surveillance, homeostasis, and development
Cd4	Cluster of differentiation 4
Chemokine	A protein that can attract cells, toward a chemical gradient, having the specific receptor, and promote differentiation and multiplication of leukocytes, and cause tissue extravasation
CircRNA	Is a type of single-stranded RNA, which, unlike linear RNA, forms a covalently closed continuous loop. Can be protein coding and noncoding
CpG DNA	DNA that contains methylated nucleotides (CpG islands). Normally found in promoter regions, which modulate gene expression
CRP	C-reactive protein. An acute-phase protein
Cxcl13	Chemokine (C-X-C motif) ligand 13. Involved in chemotaxis of B lymphocytes
Cxcl9	Chemokine (C-X-C motif) ligand 9 is a small cytokine belonging to the CXC chemokine family. Plays role in chemotaxis
DAMPs	Damage-associated molecular patterns. Molecules released in a sterile inflammation or damage
DC	Dendritic cell
DEGs	Differentially expressed genes. From RNAseq data and bioinformatics
DEPs	Differential expressed proteins
dsRNA	Double-stranded RNA
Epigenetics	Is the study of heritable phenotype changes that do not involve alterations in the DNA sequence

Factor B (c3 convertase)	Protein that is activated by cleavage, yielding Bb and Ba fragments. Factor B is cleaved only when it is bound to c3b
Factor D	A serine protease present in blood and tissue in an active sequence but self-inhibited conformation. The only known natural substrate of factor D is factor B. Alternative pathway of complement activation
Factor H	The main cofactor of factor I
Factor I	Protein of the complement system (c3b/c4b inactivator). Alternative pathway of complement activation
Foxp3	Forkhead box P3. Regulator of the regulatory pathway in the development and function of regulatory T cells
Galectins	Proteins that bind specifically to $\beta$ -galactoside sugars
Hepcidin	An antimicrobial peptide
Histone modifications and DNA methylation	Both DNA methylation and histone modification are involved in establishing patterns of gene repression during development
HSP1	Heat shock protein I gene. Mitochondrial product
HSP90b	A chaperone protein that assists other proteins to fold properly, stabilizes proteins against heat stress, and aids in protein degradation
Ifn	Interferon (cytokine)
Ifnrel	Interferon-related
Ifng	Interferon-gamma
IL	Interleukin (cytokine)
IRF	Interferon regulatory factor
Isoforms (subtypes)	Alternatively spliced genes
KEGG	Kyoto Encyclopedia of Genes and Genomes
lncRNAs	A large and diverse class of transcribed RNA molecules with a length of more than 200 nucleotides that do not encode proteins
LPS	Bacterial lipopolysaccharide. A main constituent in gram-negative bacterial cell wall
MAP kinase 1	Mitogen-activated protein kinase I. Transcription factor
MAP	Mannan-binding lectin (MBL)-associated protein
MASPs	Serine proteases that function as a component of the lectin pathway of complement activation
MBL	Mannose-binding lectin (lectin pathway of complement activation)
MHC I and II	Major histocompatibility complex I and complex II. Function in antigen presentation

MicroRNAs	(miRNAs) are a family of small noncoding RNAs
MPO	Myeloperoxidase. An enzyme that catalyzes the formation of a number of reactive oxidant species
MyD88	Myeloid differentiation factor 88 (MyD88). A central component of the Toll-like receptor pathway
Mx	An interferon-induced GTP-binding protein
NOD-like receptors (NLRs) and C-type lectin receptors (CLRs)	Belongs to RIG-I and PPR family
PPRs	Pattern recognition receptors. Expressed on many types of cells, especially on antigen-presenting cells. Recognize repeating molecular patterns often found in pathogens
RNAseq	RNA sequencing
TLRs	Toll-like receptors (belongs to PPR family)
PAMPs	Pathogen-associated molecular patterns
Paraquat (PQ)	Toxic chemical that is widely used as an herbicide
Pentraxins	Family of acute-phase proteins produced during acute-phase response
Poly I:C	Polyinosinic:polycytidylic acid. Binds TLR3
QPCR	A real-time polymerase chain reaction, also known as quantitative polymerase chain reaction
RAG	Recombination-activating gene
RIG-I-like receptors (RLRs)	Retinoic acid-inducible gene I-like receptors. Belongs to RIG-I and PPR family
ROS	Reactive oxygen species
SAA	Serum amyloid A. A protein formed during acute-phase response
SETD3-OT	Has function on histidine methylation which belongs to epigenetic occurrence
ssRNA	Single-stranded RNA
STAT1	Signal transducer and activator of transcription 1. STAT1 can be activated by several ligands such as IFN- $\alpha$ , IFN- $\gamma$ , epidermal growth factor (EGF), platelet-derived growth factor (PDGF), IL-6, or IL-27
TGF- $\beta$	Transforming growth factor-beta
Th1 cells	Pro-inflammatory T cells that are responsible for cell-mediated immunity and phagocyte-dependent protective responses
Tnf	Tumor necrosis factor
Tnfr	Tumor necrosis factor receptor

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TRIF	TIR domain-containing adapter-inducing interferon- $\beta$ . An adapter in responding to activation of Toll-like receptors (TLRs)
Type I interferon	A role in antiviral responses
Type II interferon	A role in adaptive and innate immunity
Viperin	Virus-inhibitory protein, endoplasmic reticulum-associated, interferon-inducible. An IFN-inducible gene

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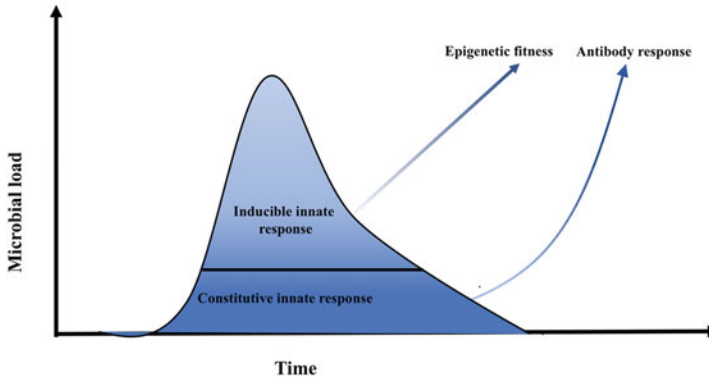
## 2.1 Introduction

### 2.1.1 Innate Immunity: The Concept

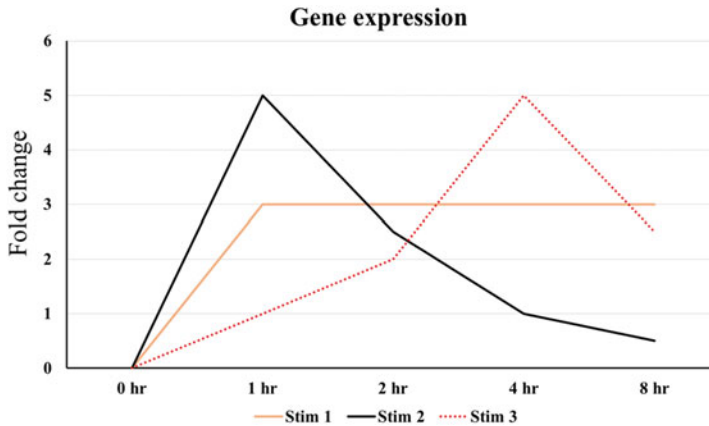
Innate immune defense is important for protecting the host from infection, not only in naïve fish but also in fish that have previously been infected. “Innate immunity has shed its older, disparaging title of ‘non-specific immunity’ and now stands as a proud partner with the adaptive immune system in protecting human hosts from infectious insults. For any who doubt the impressive protective capacity of the innate immune system, it is instructive to consider that only vertebrates boast the added benefits of an adaptive immune system, leaving most organisms on our planet to survive on innate immunity alone” (Turvey and Broide 2010). Indeed, this applies also to fish. The immune system of teleost fish is composed of two kinds of receptor types: The germline-encoded pattern recognition receptors (PRRs) and the antigen-specific receptors are made from gene arrangement after, e.g., pathogen infection. The latter consist of, e.g., antibodies, MHC I and MHC II, and T-cell receptors. In addition, numerous other receptors/molecules can take part in the innate immunity. The innate mechanisms can be divided into *constitutive* and *inducible*. The former represents rapid ongoing ligand binding to receptors and a quick response, while the inducible (e.g., many PPRs) acts slower—but with a higher magnitude (Paludan et al. 2020) (Figs. 2.1 and 2.2).

### 2.1.2 Innate Receptors

Innate and adaptive immunity can cooperate to clear the infections. Central receptors in the early innate responses are so-called Toll-like receptors (TLRs) and are vital for the communication between the innate and adaptive branches (Rivera et al. 2016). The germline-encoded pattern recognition receptors (PRRs) are central in the recognition of microbial components and for the activation of innate immunity, which may induce inflammatory response to eliminate pathogens. The PRRs, expressed in innate immune cells, include receptors such as Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), and C-type lectin receptors (CLRs). Upon recognition of microbial components known as pathogen-associated molecular patterns (PAMPs), PRRs



**Fig. 2.1** Simplified illustration shows how inducible innate immunity changes over time, whereas the constitutive is stable vs. time. To get complete sterilization and resolution, both inducible and constitutive innate immune responses plus antibodies are often needed. Epigenetic changes may contribute to better fitness/increased protection when fish are exposed to a second infection. Targeted gene expression surveys or transcriptomics has focused primarily on describing or identifying inducible genes (e.g., DEGs), while in contrast, factors contributing to the constitutive arm have been poorly described



**Fig. 2.2** Hypothetical time-course study of a gene (qPCR) expression. The orange line represents a gene with constitutive expression upon treatment with stimulant 1, and the black line demonstrates an early expression of the same gene after the fish were treated with stimulant 2, whereas the dotted red line represents a delayed expression of this gene after the fish were treated by stimulant 3. A specific gene may be induced by a certain stimulant and not by others, or there may be a stable, rapid, or delayed induction. The magnitude of induction may likely be dependent on number of specific receptors on cells or/and the number of cells that harbor specific receptors

induce intracellular signaling networks to activate transcription factors that regulate genes involved in inflammatory responses. Importantly, these innate immune signals also trigger dynamic chromatin changes. Such changes may in turn induce modulated gene-specific expression patterns resulting in even more pathogen elimination.

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## 2.2 Cells in the Innate Immune Response

The traditional view that the adaptive and innate immune defense is divided into two compartments is now more or less history. The fish innate immune cells comprise not only the “traditional” innate cells such as macrophages (Kordon et al. 2018; Rieger and Barreda 2011; Grayfer et al. 2014, 2018) and granulocytes (Pijanowski et al. 2013; Schmidt 1905) but also red blood cells (Puente-Marin et al. 2018, 2019b; Dahle et al. 2015; Wessel et al. 2015), thrombocytes (Stosik et al. 2019), B cells (Wu et al. 2020), and subtypes of T cells (Scapigliati et al. 2018).

### 2.2.1 Monocytes/Macrophages

Monocytes are large mononuclear circulating leukocytes, which become macrophages when they settle tissues and organs. The nucleus may display different oval-, kidney-, or bean-shaped conformations, while the cytoplasm is usually pale and agranular, with varying amounts of vesicles and lysosomes. Macrophages are one of the main immune cells performing phagocytosis, the other presumably being the neutrophilic granulocytes. Phagocytosis is a multistage process for removal and cellular ingestion and destruction by intracellular enzymes and other substances (Grayfer et al. 2014; Hodgkinson et al. 2015). In addition to being professional phagocytes, macrophages can also function as professional APCs by presenting antigen to T cells on MHC class II. Such functions have also been suggested from studies on fish monocytes/macrophages (Sugamata et al. 2009; Wittamer et al. 2011). It has been suggested that fish display at least three different phenotypes of macrophages, based on their activation processes: innately, classically, and alternatively activated macrophages (Wentzel et al. 2020; Hodgkinson et al. 2015). Innate activation occurs when a macrophage receives a stimulus from the recognition of a microbial substance (e.g., PAMP) through cell receptor(s) without any need for any co-stimulation. Classical activation, however, occurs with the combination of such a stimulus and the cytokine interferon-gamma (IFN-gamma). Both innate activation and classical activation typically lead to increased pro-inflammatory response as opposed to alternative activation. A presence of cytokines (interleukin 4 (IL4) and/or interleukin 13 (IL13)) induces a macrophage phenotype with a resolving function (wound healing and tissue repair). There is also a suggestion for a fourth type of macrophages, namely regulatory macrophage. Regulatory activation is associated with the cytokine interleukin 10 (IL10) and important for downregulation of the inflammatory process (Wiegertjes et al.

2016). However, macrophages are apparently able to change between different phenotypes, and there is still some uncertainty whether all these activation pathways perform the same way in fish, as in mammals (Forlenza et al. 2011). Please see Chap. 6 for a more thorough overview of macrophages in fish.

### 2.2.2 Dendritic Cells

Dendritic cells (DCs) are categorized as a professional APCs found within several different tissues and are very effective at initiating both innate and adaptive immune responses in mammals (Banchereau et al. 2000; Worbs et al. 2017). DCs are typically small cells, with several elongated, cytoplasmic processes (dendrites) that increase the cell surface area (Collin and Bigley 2018). Cell populations with DC-like morphology and functions have been reported from teleost fish (Shao et al. 2015; Bassity and Clark 2012; Haugland et al. 2012), but due to lack of specific markers it is currently unknown whether they are true homologs of the mammalian cell type.

### 2.2.3 Granulocytes

Granulocytes are leukocytes with cytoplasmic granules and often nucleus with varying shapes (lobes) (Flerova and Balabanova 2013). They are central pool of the innate immune cells (Lieschke and Trede 2009). The granulocytes have traditionally been grouped into neutrophilic granulocytes (neutrophils), eosinophilic granulocytes (eosinophils), and basophilic granulocytes (basophils), based on their staining characteristics with different dyes. However, this classification was originally developed for use in mammalian hematology and does not appear to always correlate well with characteristics of fish granulocytes (Kelenyi and Nemeth 1969; Drzewina 1909; Rombout et al. 2005).

#### 2.2.3.1 Neutrophils

Neutrophils typically possess nucleus with varying degrees of lobulation and contain granules that usually do not display marked affinity for staining with basic or acid dyes (such as hematoxylin and eosin). Neutrophils are generally most abundant between the granulocytes. In mammals, neutrophils are very mobile and are usually among the first cells to infiltrate tissue during onset and early phases of inflammation (Rosales 2018). Similar cellular recruitment speed has also been reported from teleosts (Lamas and Ellis 1994; Katzenback and Belosevic 2009; Havixbeck and Barreda 2015). Neutrophils are armed with a diverse arsenal of cellular weapons, making them effective combatants against invading pathogens (Havixbeck and Barreda 2015). Like macrophages, they are able to degrade ingested microbes and particles through production and release of reactive oxygen species (ROS) and proteases (Katzenback and Belosevic 2009; Rieger and Barreda 2011). They can also release different granules, upon degranulation, containing antimicrobial



proteins and enzymes such as myeloperoxidase (MPO) (Lieschke and Trede 2009). In addition, neutrophils are able to form extracellular traps, which contain antimicrobial factors (Palic et al. 2007; Pijanowski et al. 2013; Chi and Sun 2016; Zhao et al. 2017; Van et al. 2020).

Eosinophils, or acidophils, are described to contain cytoplasmic granules that stain bright red with the acidic dye (eosin). However, cellular identification based solely on cytochemical and/or histochemical staining characteristics may lead to misinterpretation as basophils, eosinophilic granule cells, mast cells, some neutrophils (also called heterophils), and rodlet cells also are capable to be dyed to various degrees. Consequently, it has been suggested that mammalian terminology should be used whenever possible for describing these cell types (Watanabe et al. 1997; Suljevic et al. 2017). In mammals, the eosinophils have immunological roles regarding both immune regulation, defense against parasitic infections, and allergic inflammatory reactions (Hogan et al. 2008). Teleost eosinophils have been reported to be phagocytic (Watson et al. 1963), and they increase in cell numbers and increase the degranulation activity as a response to infection (Balla et al. 2010).

### **2.2.3.2 Basophils**

Basophils are large granulocytes with staining of their cytoplasmic granules with a basic dye (hematoxylin). Basophils are rarely observed in teleost species (Tavares-Dias 2006). Their granules contain histamine, an inflammatory mediator, and basophils are associated with anaphylaxis, allergy, and hypersensitivity reactions (Chirumbolo 2012). As such, they are similar to the mast cells. Although not fully established, these granulocytes might also have other functions within the fish immune system (Odaka et al. 2018).

### **2.2.3.3 Eosinophilic Granule Cells**

Eosinophilic granule cells (EGCs) and rodlet cells have been observed in fish (Reite and Evensen 2006). Such cells resemble the classical mast cells (Reite 1998). Teleost EGCs have been identified in several species, as part of the host inflammatory response to injected vaccines, bacterial infection, parasite infestations, or other types of noxious stimuli (Rombout et al. 2011).

## **2.2.4 Thrombocytes**

Thrombocytes are oval-shaped, nucleated, and agranular cells located in fish. In some fish species, thrombocytes have been shown to be phagocytic and it has been discussed whether thrombocytes can function as APCs and/or is coupled to the innate immunity (Stosik et al. 2019; Passantino et al. 2005).

### 2.2.5 Red Blood Cells

From a RNAseq study on trout red blood cells exposed to either poly I:C, it was found that the cells expressed numerous transcripts of immune molecules—such as *ifna*, *tlr3*, *tlr9*, *mx*, and *ccl4* (Morera et al. 2011). Thus, the authors suggested that red blood cells indeed participate in innate immune response.

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## 2.3 Epigenetic Control of Innate Immunity

### 2.3.1 Epigenetics: The Concept

Epigenetics involves heritable factors that regulate spatiotemporal genome expression, which may induce different phenotypes. Two of the molecular mechanisms, histone modifications and DNA methylation, regulate gene expression at the chromatin level. In contrast, microRNAs are molecules that affect gene expression at the posttranslational level. Epigenetic histone modification involves acetylation/deacetylation, methylation/demethylation, and phosphorylation/dephosphorylation of specific histone amino acids. Pathogens have evolved a variety of strategies to modify host epigenetics. For example, they can (1) directly modify host proteins and chromatin, (2) attenuate PRR binding and signaling pathways, and (3) modulate the expression of activators and repressors of innate immunity. Hosts can abrogate pathogen-induced epigenetic changes to maintain their innate defense characters (Zhang and Cao 2019). Analysis of posttranslational processes on immunity has not generally been well studied in fish. However, in one study, the impact of histone modification after infectious necrosis virus infection (IPNV) and temperature control has been shown (Boltana et al. 2018). In this study, IPNV-infected fish that preferred a given temperature showed histone modification, which could explain modulated expression of *il1*, *il2*, *ifng*, and *ifnrg* receptor. The pattern of histone modification was different from IPNV-infected fish kept at constant temperature. In another study, spring viremia of carp virus (SVCV) infection induced histone modification in zebrafish (*Danio rerio*). The authors indicated that the *ifn*, *tlr*, and C-reactive protein promoters were methylated postinfection; thus, these genes were upregulated compared to controls (Medina-Gali et al. 2018). Since epigenetic modification of the genome is a heritable trait, epigenetic programming of brood stockfish, by, e.g., immunostimulants, may be a viable approach to produce offspring with higher innate disease resistance (Zhang et al. 2019).

### 2.3.2 Micro RNA

MicroRNAs (miRNAs) are a family of small noncoding RNAs that play vital roles in modulating host immune response. Accumulating evidence demonstrates that host

miRNAs are involved as mediators in regulating viral replication and host antiviral immunity in mammals. In a miiuy croaker macrophages, miR-3570 that was upregulated after rhabdovirus infection interfered and led to downregulation of type I interferon in the cells. In turn, this downregulation caused increased virus replication in cells (Xu et al. 2018a). Binding to Toll-like receptors (TLRs) and subsequent intracellular signaling may also bring about production of microRNA. This may result in a positive or negative feedback loop system regulating immune response. More on this complex issue is described in a review authored by Zhou et al. (2018).

### 2.3.3 Long Noncoding RNA

lncRNAs have been demonstrated to play pivotal roles in various biological processes, especially gene expression regulations, including transcriptional regulation, posttranscriptional control, and epigenetic processes. The functional significance of lncRNA lags far behind what is the status on mammals. However, a novel lncRNA (*SETD3-OT*) in turbot (*Scophthalmus maximus*) has been identified. From the annotation of neighboring adjacent genes, *SETD3-OT* might be involved in the regulation of cell apoptosis and cycle, the immune cell development, and the immune response against infection. The expression pattern of *SETD3-OT* was similar to the majority of the neighboring genes following *Aeromonas salmonicida* challenge. The *SETD3-OT* expression was high levels in mucosal surfaces in controls fish (intestine, gill, and skin), but was downregulated following *Vibrio anguillarum* infection (Yang et al. 2020). In another study, Nodavirus infected European sea bass (*Dicentrarchus labrax*) displayed many putative lncRNA, suggested to possibly be involved in immune responses (Pereiro et al. 2020). Other studies have also suggested lncRNA to be involved in the regulation of immune responses (Boltana et al. 2016; Valenzuela-Miranda and Gallardo-Escarate 2016; Paneru et al. 2016; Valenzuela-Munoz et al. 2018, 2019).

### 2.3.4 Small Interfering RNA and Circular RNA

In addition to microRNA and lncRNA, the methylation of mRNA, occurrence of small interfering RNAs, and circular RNAs may all contribute to epigenetic modulation of gene expression in vertebrates, including fish (Wang et al. 2018a). Olive flounder (*Paralichthys olivaceus*) experimentally infected with *Edwardsiella tarda* showed differentially expressed circRNA. The authors suggested that these belonged to the circRNA-miRNA-mRNA network, where KEGG analysis indicated that they were part of the *Herpes simplex* infection and intestinal immune network for IgA production (Xiu et al. 2019). Another study showed that circRNAs are involved in mammalian antiviral immunity (Wang et al. 2017). KEGG (Kyoto Encyclopedia of Genes and Genomes; [www.genome.jp](http://www.genome.jp)), a huge database integrating genomic, chemical, and systemic functional information, is often used

to find what cellular networks/pathways the DEGs belong to. It refers to what is described in and annotated from human/mice systems.

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## 2.4 Mucosal Innate Defense

### 2.4.1 Innate Immune Molecules of the Fish Skin

The skin of fishes protects fish from external pathogens. The outermost layer is mainly composed of epithelial cells, termed keratocytes. These cells cover scales and are highly phagocytic toward certain particles. They are also motile. The motility of fish keratocytes is studied in a number of fish species (Asbakk and Dalmo 1998; Tsuchida and Theriot 2013; Galbraith and Sheetz 1998; Jurado et al. 2005; Okimura et al. 2018; Ream et al. 2003). There has been limited research on the production of innate defense factors, but this topic deserves more attention. Whether the cells possess phagocytic receptors is not known, it seems that the cells are able to discriminate the uptake dependent on the kind of bacteria (Karlsen et al. 2012). The skin mucus contains an array of molecules enabling protection from pathogens. In a study on yellow catfish (*Pelteobagrus fulvidraco*), 133 differentially expressed proteins were found after bath infection with *Edwardsiella ictaluri*. A minority of these differentially expressed proteins were directly immune-related. Examples of the upregulated genes were complement component *c3*, *MAP kinase 1*, and *interferon-induced 35 kDa protein* (Xiong et al. 2020).

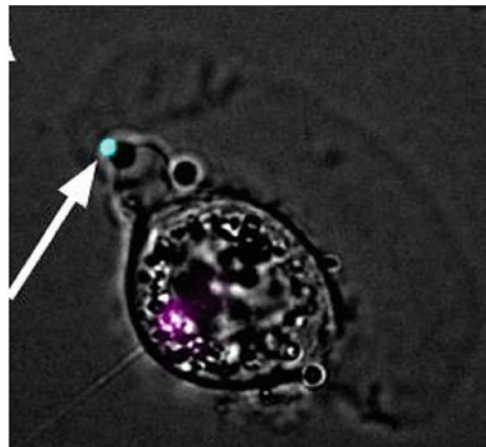
Among the antibacterial enzymes, the best studied in the fish skin is lysozyme. Lysozyme is a glycoside hydrolase that catalyzes the hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycan, which is the major component of gram-positive bacterial cell wall. However, it seems that lysozyme-like enzymes have activity also against gram-negatives, parasites, and virus, as reviewed by Dash et al. (2018). This review also contains detailed description of other skin-related innate immune factors (Dash et al. 2018).

How the mucus is obtained, for concomitant analysis of factors, will inevitably decide which substances will be found during a screening process. As an example: If the mucus sample contains cells or scales, it is clear that the samples also contain cellular factors and most probably also immune factors normally localized in deeper layers (e.g., connective tissue and muscle). The most gentle and sensible protocol is to adsorb the mucus using a tissue paper. While a wiping method using tissue paper also gives a good protein yield, this method comes with some degradations. If the research requires a high mucus yield together with substances from the epithelial layer, the wiping method is preferable (Faeste et al. 2020). It is quite difficult to discriminate between substances normally found in mucus compared to what is intracellularly or extracellularly localized in epidermis, subdermis, and

connective tissue. Thus, many reports describe the presence of substances not (only) found in the mucus itself but also found in the underlying tissue. As an example of the latter, the transcriptomic analysis of a skin sample ( $3 \times 1$  cm) from large yellow croaker (*Larimichthys crocea*) followed by *Cryptocaryon irritans* challenge revealed up to 1055 DEGs (differentially expressed genes) (96 h postinfection). Since many of the DEGs were clearly innate immune-related, it would have been interesting to see how many and which transcripts were from epithelial cells, connective tissue cells, blood cells, and muscle cells, respectively. Probably, a similar sampling protocol was followed by Liu et al. (2020a, b) where zebrafish were challenged with spring viremia of carp virus (SVCV)—causing skin lesions. This study revealed 320 DEPs (differentially expressed proteins) (48 h postinfection) and 181 DEPs (96 h postinfection). Sixteen of these were confirmed by means of QPCR analysis (Bai et al. 2020). DEPs often found are complement factors, and chemokines, heat shock proteins, MHC, cell adhesion molecules, TNF-induced protein, and many more were regulated (Liu et al. 2020b). In conclusion, analysis of skin innate defense mechanisms should discriminate between mucus itself, epidermis, subdermis, and connective tissue.

The epidermis consists of keratocytes, which are highly mobile cells and also possess (mostly overlooked) phagocytic activity (Asbakk and Dalmo 1998; Sveen et al. 2020). The immunological significance of their phagocytic ability is not yet fully understood. One theory is that they engulf as many particles they can before going into a cell death pathway and are sloughed off from the epidermis (Asbakk and Dalmo 1998). It is speculated that these cells possess some innate defense mechanisms (e.g., receptors) (Lindell et al. 2012). The epithelial layer of the fisheye cornea consists of cells that highly resemble skin keratocytes. These cells are not studied with respect to their innate defense abilities. We have preliminary results showing that these cells also engulf foreign particles (Fig. 2.3). For more details on mucosal immunity in fish, see Chap. 12.

**Fig. 2.3** Corneal epithelial cells of Atlantic salmon (*Salmo salar*) possess phagocytic ability, as illustrated by the intracellular presence of (cyan) microbeads. Lysosome is stained by pink color. Courtesy: Dalmo, Wolfson, Kjølstad, Svartaas (UiT)



### 2.4.2 Nasopharynx-Associated Lymphoid Tissue (NALT)

NALT has been discovered to harbor lymphocytes, but also genes central in induction of innate immunity. These include *mx1*, *tlr3*, *il1r*, *il8*, *tnfr*, *myD88*, *c3*, *c4*, *c7-1*, *cxcl9*, *cxcl9*, *cathl1*, *ccl19*, and *il6* (Tacchi et al. 2014; Yu et al. 2018). The significance of NALT-mediated innate response, compared to, e.g., skin or intestine, is not clear. Another assemblance of lymphoid cells can be found in the buccal cavity of rainbow trout (*Oncorhynchus mykiss*) that have been infected by *Flavobacterium columnare*. After infection, this buccal cavity lymphoid tissue was found to express innate factors such as *il8*, *il1b*, *chemokine like 19*, *cathl1* and *cathl2*, *rig1*, among other adaptive immune genes (Xu et al. 2020).

### 2.4.3 Gills in Innate Immunity

Gill-associated lymphoid tissue (GIALT) has been characterized in Atlantic salmon and different fish species (Resseguier et al. 2020; Haugarvoll et al. 2008). This tissue was, in Atlantic salmon, identified to express upregulated genes such as complement component *c3*, *il18*, *mx3*, *il20*, *ifn type II*, *viperin*, *rig1*, and *ifna* after ISAV challenge (Austbo et al. 2014; Valenzuela-Miranda et al. 2015). Pro-inflammatory (*il6*, *il17c1*) and anti-inflammatory (*il10*, *tgfb*) genes have been found, in rainbow trout gills, after Ich (*Ichthyophthirius multifiliis*) infection (Syahputra et al. 2019). Another study aimed at doing a transcriptomic survey of Atlantic salmon gills suffering from multifactorial pathologies. Genes that were differentially expressed were depicted to be involved in pathways such as cellular immune response (IL-17 signaling, IL-6 signaling, granzyme A signaling, crosstalk between dendritic cells and natural killer cells, granulocyte adhesion and diapedesis, and HMGB1 signaling), cytokine signaling (IL-17 signaling, IL-6 signaling, acute-phase response signaling, role of JAK family kinases in IL-6 type cytokine signaling, TNFR2 signaling, and HMGB1 signaling), and tissue damage and repair (Krol et al. 2020). Some of these genes possess central functions in innate immunity. More details on the gills' function in the immune response, please see Chap. 1.

### 2.4.4 Intestine in Innate Immunity

During the recent years, many excellent review articles describing the fish's intestinal immunity have been published (Dawood 2020; Nadal et al. 2020; Sitja-Bobadilla et al. 2016; Brugman 2016; Dezfuli et al. 2016; Scapigliati et al. 2018; Brinchmann et al. 2018). Recently, there have been many innovative approaches to better understand intestinal immunity. In one of these studies, proteomic and transcriptomic examination of *the intestinal mucus* in Tilapia infected with *Streptococcus agalactiae* showed that innate factors such as *c1r-like EGF domain*, *c1q-binding protein*, *hsp1*, *hsp90b*, *galectin*, and

*membrane attack complex component/perforin domain, conserved site, complement factor D, C-type lectin fold, il1, il1r, and foxp3* (Wu et al. 2016). Another study, in grass carp, did a transcriptomic and proteomic examination of the intestine after oral DNA vaccination (Li et al. 2020). The study revealed 250 and 50 immune-related DEGs and DEPs, respectively, after the oral vaccination. KEGG enrichment analysis showed genes and proteins participating in the Toll-like receptor signaling pathway, MAPK signaling pathway, NOD-like receptor signaling pathway, and the complement cascade were present both in the mucous and tissue homogenates. It is obvious that the intestinal innate mechanisms are quite diverse. More research using modern omic technologies will inevitably give us more information about the significance of the various intestinal innate factors that have on disease resistance.

Recently, a lymphoid structure in the cloacal region was discovered in Atlantic salmon (Loken et al. 2020). This may be the same as Inami et al. (2009) described in Atlantic cod—although the work on cod did not specify the anatomical localization properly (Inami et al. 2009). Whether this collection of lymphatic cells has any function in innate defense is not clear. However, through gene expression studies, genes encoding *il1b*, *il8*, *il10*, *hepcidin*, and *ccl19* were found. These genes likely play roles in the innate immunity.

Future studies of intestinal mucus must be carefully planned and executed to avoid contaminant cells and blood. This may give false assumptions with regard to the actual presence of innate factors.

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## 2.5 Innate Defense Mechanisms in Muscle Tissues

Skeletal muscles have been implicated in several atypical physiological processes including immune response, especially after pathogen challenge. When zebrafish were intramuscularly challenged by *Salmonella enterica*, pro-inflammatory *il1b* and *tnfa* were highly expressed in their skeletal muscle. Likewise, *hep* (hepcidin) and *il10* were also expressed (Chatterjee et al. 2016). The authors did not examine any presence of leukocytes in muscle tissue samples after the fish were challenged with *Salmonella*. Thus, it is likely that inflammatory leukocytes did contribute to the expression of the pro-inflammatory cytokines. The contribution of incoming leukocytes to the inflammatory event is discussed by Valenzuela et al. (2017) and Kaitetzidou et al. (2012). Similar to the induction of innate immune genes in skeletal muscle, Atlantic salmon infected with salmonid alphavirus and piscine reovirus showed altered gene expression in the heart tissue (Johansen et al. 2015). In this study, several innate genes were expressed in the heart muscle. The authors did not elaborate whether this contribution was caused by inflammatory cells or not.

## 2.6 Innate Defense Mechanisms in Kidney and Spleen

The kidney and spleen are hematopoietic organs capable of inducing and exerting innate immune responses (Uribe et al. 2011; Svingerud et al. 2012; Kumar et al. 2018). The head kidney is the principal immune organ responsible for phagocytosis, antigen processing, and formation of Igm and immune memory (Page et al. 2013; Stosik et al. 2018; Rauta et al. 2012; Rombout et al. 2005; Kim et al. 2017). Kidneys in fish are paired and have a Y shape along the body axis. The immune relevant part, the head kidney, is located anteriorly. The posterior is mostly the renal system. The form of the head kidney varies between species. In some species, there are two separate extensions in the most anterior part of the organ, while in salmonid species the kidney is present as a single organ (Press and Evensen 1999). It is acknowledged that the head kidneys' main function is hematopoiesis of lymphocytes, phagocytosis, antigen presentation, and maturation of lymphocytes. Its significance in innate immunity is not very well researched yet, although the head kidney leukocytes are armed with innate factors (Aballai et al. 2017; Gerdol et al. 2015; Cao et al. 2020; Hwang et al. 2017; Rozas-Serri et al. 2019; Zhou et al. 2019). It should be clear that cells in the posterior part of the kidney also have capability to express immune genes after pathogen challenge, as reported by Sudhagar et al. (2019).

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## 2.7 Innate Defense Mechanisms in the Spleen

It is acknowledged that the main functions of the spleen are in hematopoiesis of lymphocytes, antigen trapping, and destruction of red blood cells (Press and Evensen 1999). However, from RNAseq analysis it is evident that the spleen cells contain and express numerous innate immune genes such as those involved in chemokine signaling, Toll-like receptor signaling, RIG-1, and NOD-mediated signaling and complement cascade (Ali et al. 2014).

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## 2.8 Innate Defense Mechanisms in the Liver

The liver is acknowledged to produce acute-phase proteins, including complement components following infection or physiological insult. An array of innate defense factors has been found following a transcriptomic study of rainbow trout. This study revealed transcripts coding for genes important in acute-phase response, inflammatory response, genes coding for PAMP-binding receptors, and molecules central in chemotaxis (Martin et al. 2010). This finding suggests that the liver also has capacity to mount innate responses.



## 2.9 Receptors and Molecules of the Innate Immune Defense

Innate immunity is orchestrated by numerous molecules such as cytokines, complement factor, and receptors. Many molecules participate in both innate immunity and adaptive immunity. The following chapters describe the roles of selected innate molecules that have been ascribed to innate immunity—as central components.

### 2.9.1 Toll-Like Receptors (TLRs) as Pattern Recognition Receptors (PPRs)

The number of TLRs adds to other pattern recognition receptors (Tribouley et al. 1978) (including splice variants) such as different C-type lectin receptors, NOD-like (nucleotide-binding oligomerization domain-like) receptors (NLRs), RIG-1-like receptors, and scavenger receptors (Brubaker et al. 2015), and suggests that fish may very well be equipped with innate receptors that may likely be targets for innate immune training. TLRs are a family of pattern recognition receptors that bind pathogen-associated molecular patterns (PAMPs) (Pietretti & Wiegertjes, 2014). In addition, several TLRs are able to bind certain endogenous molecules called damage-associated molecular patterns (DAMPs) (following, e.g., trauma). TLRs are highly important since they represent a considerable diversity in their ligand-binding properties and thus facilitate responses against a wide array of pathogens. Genome duplication events in fishes during evolution have been attributed to the diversity of TLRs; therefore, differences with respect to the number of TLR loci exist between mammalian species and many fish species (Palti 2011). As an example: The genome of a mudskipper species (*Periophthalmodon schlosseri*) contains 11 copies of *tlr13* (You et al. 2014). Most vertebrate genomes are recognized to have at least one gene representing each of the seven major *tlr1*, *tlr2*, *tlr3*, *tlr4*, *tlr5*, *tlr7* and *tlr11* families (Roach et al. 2005). Within Osteichthyes, the large *tlr1* subfamily members include *tlr1*, *tlr2*, *tlr14*, *tlr18*, *tlr25*, *tlr27*, and *tlr28* (Nie et al. 2018). The *tlr3*, *tlr4*, and *tlr5* subfamilies recognize dsRNA, LPS, and bacterial flagellin. The *tlr7* subfamily ligands are nucleic acid motifs, whereas the *tlr11* family TLRs recognize an array of different molecules—from proteins to nucleic acids—reviewed by Nie et al. (2018). The ligand specificities for each TLR have not been very well studied in fish, though flagellin, synthetic triacetylated lipopeptide (Pam3CSK4), lipopeptides from gram-positive bacteria, and short double-stranded RNA (dsRNA) have been shown to interact with/bind to *tlr1/2*, *tlr5*, and *tlr22*, respectively (Nie et al. 2018). This means that fish immunologists assume that *tlr* localization and ligand specificities of fish *tlrs* are similar to mammalian counterparts. This is reviewed by Pietretti et al. (2014) and Kanwal et al. (2014). *Tlrs* are, in mice and humans, localized in the cell membrane and in the endoplasmic reticulum (ER), endosomes, and lysosomes (Fink et al. 2016). *Tlr* receptors 1, 2, 6, and 10 have, in human or mice models, been found to recognize a broad range of peptidoglycans and lipoproteins from, e.g., bacteria and parasites. These are located on the cell surface and, following engagement, there is intracellular signaling ending in NF- $\kappa$ B-dependent gene expression. NF- $\kappa$ B promotes expression of

pro-inflammatory cytokines. Viral recognition may be brought about by tlr3, tlr7, tlr8, and tlr9 where they potentially can bind dsRNA, single-stranded RNA (ssRNA), and CpG DNA. As found in zebrafish, the tlr22 may also bind dsRNA or poly I:C (a dsRNA mimic) (Li et al. 2017b) [Fitzgerald, 2020 #954]. These “antiviral receptors,” upon ligand binding, confer (via TRIF or/and MyD88) activation of interferon regulatory factors 3 and 7 (transcription factors), which in turn facilitates transcription of interferon type I expression.

Taken together, the common interpretation is that TLR activation results in the production of pro-inflammatory cytokines (e.g., *tnfa* and *IL-1 $\beta$* ) and/or in the expression and synthesis of transcription factors involved in protection against viruses, bacteria, and parasites (Sahoo 2020; Kanwal et al. 2014; Rauta et al. 2014; Zhang and Gui 2012; Palti 2011).

Tables 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 2.10, 2.11, 2.12, 2.13, 2.14, 2.15, 2.16, 2.17, 2.18, 2.19, 2.20, 2.21, and 2.22 give an updated overview of *tlrs 1–5, 7–9, 12–14, and 18–28* found in different fish species. This list is continuously growing as genome, and transcriptome sequences from new species are completed and analyzed. This will be done during the “Fish10K” project where the aim is to genome sequence 10,000 fish species during a ten-year period (Fan et al. 2020), and through the ongoing “Fish1K” project (Sun et al. 2016b) and Earth Biogenome Project (Lewin et al. 2018).

## 2.9.2 Interferon Type I

Interferons (IFNs) are a group of cytokines with important roles in defense against viral pathogens (cf. Chaps. 13 and 14). They are divided into two families, type I and type II, based on structural properties and functions. Both the type I and II IFN systems are essential to antiviral defense in innate and adaptive immunity (Zou and Secombes 2011) (Tables 2.23 and 2.24). In contrast to type I IFNs, which are more important in innate immunity, IFN- $\gamma$  (type II IFN) is exclusively produced in immune-related cells and is more important later in the immune response. In innate immune responses, IFN- $\gamma$  is produced by natural killer cells (Jung et al. 2012). During adaptive cell-mediated immune responses, IFN- $\gamma$  is produced by CD4-positive Th1 cells and CD8-positive cytotoxic T lymphocytes. IFNs induce the expression of a broad array of IFN-stimulated genes (isgs), which encode for proteins with direct antiviral activity, including inhibition of viral transcription, degradation of viral RNA, inhibition of translation, or modification of protein function. Several reviews of the interferon system of teleost fish have been presented over the years (Robertsen 2006; Workenhe et al. 2010; Zou and Secombes 2011; Secombes and Zou 2017). Chaves-Pozo and coworkers investigated the interferon response in the ovary of rainbow trout (*O. mykiss*). They found that the VHS virus strongly upregulated all the *ifn* genes studied, while the IPN virus either had no effect or strongly suppressed *ifn* gene expression (Chaves-Pozo et al. 2010). Valero and coworkers investigated *ifns* in the gonads of gilthead sea bream (*Sparus aurata*) and European sea bass (*D. labrax*). They evaluated the expression after infection with the disease viral nervous necrosis (VNN) in the brain

**Table 2.1** Updated list of *tlr1* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr3* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. Tlr1 can associate with *tlr2* and *tlr6* to expand their ligand-binding specificities. In general, *tlr1* may bind bacterial peptidoglycan and triacyl and diacyl peptides. *ND* not determined

TLR no	Species	Tissue expression	References
1	Common carp	Muscle, gills, skin, and others	Gong et al. (2017)
1	Common carp	Peripheral blood leukocytes, mid-kidney, spleen, and others	Fink et al. (2016)
1	Pufferfish	Heart, spleen, anterior kidney, and others	Oshiumi et al. (2003)
1	Pufferfish	Expressed in kidney	Oshiumi et al. (2003)
1	Channel catfish	Anterior kidney	Quiniou et al. (2013)
1	Channel catfish	Spleen, anterior kidney, kidney, gills, and others	Zhang et al. (2013a)
1	Rainbow trout	Spleen, anterior kidney	Palti et al. (2010b)
1	Atlantic salmon	ND	Salazar et al. (2016)
1	Brown trout	ND	Sudhagar et al. (2020)
1	Orange-spotted grouper	Anterior kidney, spleen, gills, skin, brain	Wei et al. (2011)
1	Large yellow croaker	Blood, spleen, heart, liver, kidney	Wang et al. (2013a)
1	Nile tilapia	Kidney, brain, spleen, intestine, muscle + other	Abouelmaatti et al. (2020)
1	Golden pompano	Skin, anterior kidney	Wu et al. (2018)
1	Sea perch	Anterior kidney, intestine, liver, spleen, heart	Li et al. (2018a)
1	Yellow catfish	Midgut, brain, foregut, head kidney, spleen, and other	Zhang et al. (2017b)
1	Blunt snout bream	ND	Lai et al. (2017b)
1	Miiuy croaker	Liver, head kidney, and other	Xu et al. (2016)
1	Grass carp	Spleen, mid-kidney, anterior kidney, and other	He et al. (2016)
1	Rock bream	Spleen, kidney, liver, and others	Elvitigala et al. (2015)
1.1	Zebrafish	Skin, liver, blood, spleen, testis, and others	Jault et al. (2004)
1	Tibetan fish (Przewalski's carp)	ND	Tong et al. (2015)
1	Yellow River scaleless carp	ND	Qi et al. (2017)
1-1	Spotted sea bass	Details not given	Fan et al. (2019)
1-2			
1	Maraena whitefish	Anterior kidney, kidney, and others	Altmann et al. (2016)

**Table 2.2** Updated list of *tlr2* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. *ND* not determined

TLR no	Species	Tissue expression	References
2-1	Common carp	Gills, spleen, and others	Gong et al. (2017)
2-2	Common carp	Spleen, kidney, and others	Gong et al. (2017)
2a	Common carp	Peripheral blood leukocytes, gut, anterior kidney, and other	Fink et al. (2016)
2b	Common carp	Heart and other	Fink et al. (2016)
2	Japanese flounder	Peripheral blood leukocytes	Hirono et al. (2004)
2	Pufferfish	Expressed in many tissues	Oshiumi et al. (2003)
2	Channel catfish	Anterior kidney, gills, spleen	Quiniou et al. (2013)
2	Channel catfish	Liver, brain, gills, trunk kidney, intestine, and others	Baoprasertkul et al. (2007a)
2	Channel catfish	Anterior kidney, spleen, heart, kidney, brain, and others	Baoprasertkul et al. (2007a)
2	Rainbow trout	Spleen, anterior kidney, thymus, macrophages, B cells, thrombocytes	Brietzke et al. (2016)
2	Orange-spotted grouper	Spleen, gills, anterior kidney	Wei et al. (2011)
2	Large yellow croaker	Blood, spleen, heart, anterior kidney, posterior kidney	Fan et al. (2015)
2b	Large yellow croaker	Intestine, blood, skin, liver	Ao et al. (2016)
2	Indian major carp, rohu	Spleen, intestine, gills, liver, blood	Samanta et al. (2012)
2	Darby's sturgeon	Blood, anterior kidney, brain, heart, muscle	Tang et al. (2020)
2	Golden pompano	Liver, intestine, blood, skin, anterior kidney	Wu et al. (2018)
2	Yellow catfish	Spleen, anterior kidney, foregut, gills, liver, and other	Zhang et al. (2017b)
2	Grass carp	Mid-kidney, anterior kidney, and other	He et al. (2016)
2	Zebrafish	Brain, liver, skin, spleen	Jault et al. 2004)
2	Gibel carp	Brain, gills, anterior kidney, and others	Fan et al. (2018)
2	Turbot	Kidney, liver, spleen, anterior kidney, and others	Zhang et al. (2016)
2	Miiuy croaker	Eye, gills, spleen, intestine, and others	Xu et al. (2013)
2	Indian major carp, mrigal	Gills, liver, kidney, intestine, blood	Basu et al. (2012b)
2	Catfish	Liver, brain, gills, trunk kidney, intestine, stomach, and others	Baoprasertkul et al. (2007a)

(continued)

**Table 2.2** (continued)

TLR no	Species	Tissue expression	References
2	Tibetan fish (Przewalski's carp)	Details not given	Tong et al. (2015)
2	Yellow River scaleless carp	ND	Qi et al. (2017)
2-1	Spotted sea bass	ND	(Fan et al. (2019)
2-2			
2	Maraena whitefish	Anterior kidney, kidney, and others	Altmann et al. (2016)
2	Goldfish	ND	Tu et al. (2016)

(Valero et al. 2015). The orange-spotted grouper, *Epinephelus coioides*, is a commercially important fish that is widely farmed in tropical waters, e.g., in Taiwan, Japan, Australia, and also Europe. Chen et al. characterized a type I *ifn* from this fish and determined the expression during nodavirus infection. Groupers infected with nodavirus had elevated levels of *ifn* and administration of recombinant IFN type I, which led to upregulated antiviral activity (Chen et al. 2014). In large yellow croaker (*L. crocea*), a type I group II interferon was identified by Ding and coworkers. The *ifn* was constitutively expressed in all examined tissues, spleen, liver, skin, head kidney, gills, blood, muscle, heart, brain, and intestine. The expression was rapidly upregulated in spleen and head kidney by poly I:C and *Aeromonas hydrophila* (Ding et al. 2019). A type I interferon gene was identified in Japanese eel (*Anguilla japonica*). The *ifn* was expressed constitutively in liver, spleen, intestine, gills, skin, kidney, heart, and muscle. After injection with LPS, poly I:C, and live *A. hydrophila*, expression levels increased in both liver, spleen, and kidney (Feng et al. 2017). A transgenic cell line for the detection of salmon interferons has been established. It is based on a CHSE-214 cell line containing a reporter construct expressing firefly luciferase under the control of a rainbow trout promoter for the IFN-induced *mx1* gene. The *mx* promoter was shown to respond to both salmon IFN type I and trout IFN type II in a dose-dependent manner, while there was no response to recombinant *tnfa* and *ilb* (Jorgensen et al. 2007). Three distinct members of type I interferons were identified in the mandarin fish (*Siniperca chuatsi*) by Laghari et al. Fish injected intraperitoneally with poly I:C resulted in an enhanced expression of all three genes in the head kidney. The disease infectious spleen and kidney necrosis virus (ISKNV) caused an increased but delayed response of *ifns* (Laghari et al. 2018). Liu and coworkers studied *ifn* subgroups of salmonid species like rainbow trout (*O. mykiss*), chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*Oncorhynchus kisutch*), Atlantis salmon (*Salmo salar*), and Arctic charr (*Salvelinus alpinus*) and compared them with other species. The analysis confirmed that salmonids have a complex (in terms of *ifn* subgroups present) and (large number of genes) type I *ifn* repertoire relative to other teleost fish (Liu et al. 2020a). Milne et al. studied three

**Table 2.3** Updated list of *tlr3* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr3* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. Tlr3 is able to bind double-stranded RNA, which is a molecular signature of certain viruses. ND not determined

TLR no	Species	Tissue expression	References
3	Pufferfish	Digestive tract	Oshiumi et al. (2003)
3	Silvery pomfret	Liver, kidney, intestine	Gao et al. (2020)
3	Common carp	Intestine, liver, kidney, spleen, and others	Yang and Su (2010)
3-1	Common carp	Spleen, skin, blood, and other	Gong et al. (2017)
3-2	Common carp	Gills, skin, blood, and others	
3	Yellow catfish	Liver, brain, swim bladder, and other	Zhang et al. (2017b)
3	Zebrafish	Skin, liver, blood, spleen, and other	Jault et al. (2004)
3	Gibel carp	Liver, gills, anterior kidney, brain, and others	Fan et al. (2018)
3	Sea perch	Spleen, liver, anterior kidney, and others	Wang et al. (2018b)
3	Spiny eel	Spleen, gills, and others	Han et al. (2017)
3	Snow trout	Kidney, liver, spleen, intestine, and others	Belwal et al. (2017)
3	Atlantic salmon	Spleen, anterior kidney, liver, heart, intestine	Vidal et al. (2015)
3	Orange-spotted grouper	Liver, anterior kidney, and others	Lin et al. (2013)
3	Rohu	Spleen, eye, intestine, blood, skin, and others	Samanta et al. (2013)
3	Japanese flounder	Anterior kidney, heart, gills, and others	Hwang et al. (2012)
3	Yellow croaker	Liver, intestine, heart, kidney, gills, and others	Huang et al. (2011)
3	Turbot	Anterior kidney, stomach, intestine, heart, and others	Hu et al. (2015a)
3	Channel catfish	Liver, muscle, spleen, and others	Baoprasertkul et al. (2006)
3	Channel catfish	Expressed in many tissues and organs, minor in blood	Zhang et al. (2013a)
3	Channel catfish	Liver, gills, spleen, stomach, and others	Bilodeau and Waldbieser (2005)
3	Rainbow trout	Liver, intestine, pyloric caeca, posterior and anterior kidneys, and others	Rodriguez et al. (2005)
3	Zebrafish	Gills, spleen, kidney, heart, brain, liver	Phelan et al. (2005)
3	Tibetan fish (Przewalski's carp)	Details not given	Tong et al. (2015)
3	Yellow River scaleless carp	ND	Qi et al. (2017)
3	Spotted sea bass	Details not given	Fan et al. (2019)

(continued)

**Table 2.3** (continued)

TLR no	Species	Tissue expression	References
3	Maraena whitefish	Liver, heart, kidney, skin, gills, and others	Altmann et al. (2016)
3	Atlantic cod	Details not given	Solbakken et al. (2016)
3	Grass carp	ND	Liao et al. (2017)
3	Goldfish	ND	Tu et al. (2016)
3	Lamprey	Skin, gills, peripheral blood leukocytes, kidney, and others	Kasamatsu et al. (2010)

distinct type I interferons in meagre (*Argyrosomus regius*), namely *ifnc*, *ifnd*, and *ifnh*. Constitutive expression was analyzed during larval development and in adult tissues (gills, midgut, head kidney, spleen). The spleen had high transcript levels of all three *ifns*. *Ifnd* and *ifnh* were also highly expressed in gills. The expression of each subgroup increased significantly across all four tissues following injection of poly I:C (Milne et al. 2018). In Atlantic salmon, Sun et al. identified an *ifn* multigene cluster encoding three *ifn* subtypes (*ifna*, *ifnb*, and *ifnc*). Each *ifn* subtype was constitutively expressed in head kidney. The three subtypes showed a striking difference in expression properties in response to stimulation with poly I:C. Both *ifna* and *ifnc* transcripts increased, while *ifnb* was only slightly induced by poly I:C (Sun et al. 2009). Type I interferon genes were cloned and characterized in the rock bream (*Oplegnathus fasciatus*). Their expression was upregulated in blood cells and head kidney by LPS, poly I:C, *E. tarda*, *Streptococcus iniae*, and iridovirus, and recombinant *ifn* I protein induced a rapid and transient expression of the *mx* gene in head kidney cells (Wan et al. 2012). An overview of type I *ifns* in different fish species with their activities is found in Table 2.23.

### 2.9.3 Interferon Type II

Interferon-gamma (*ifng*), the only type II interferon, is a pleiotropic pro-inflammatory and antiviral cytokine. In mammals, it is constitutively produced by NK cells, whereas T lymphocytes produce IFNG after activation or differentiation. IFNG is a key cytokine for innate and adaptive immunity against viral and intracellular bacterial infections and is involved in tumor control. An updated teleost interferon-gamma review has recently been published (Pereiro et al. 2019). Arts and coworkers made recombinant proteins of the carp (*Cyprinus carpio*) IFN- $\gamma$  sequences of both clusters (*ifng1* and *ifng2*) and tested their effects on expression of pro-inflammatory mediators (Arts et al. 2010). An interferon-responsive stable cell line RTG-3F7 has been developed for rainbow trout by modifying the RTG-2 cell line through transfection with a plasmid construct containing a promoter

**Table 2.4** A list of *tlr4* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr4* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. TLR4 binds the lipid moiety of bacterial lipopolysaccharide. *ND* not determined

TLR no	Species	Tissue expression	References
4all	Channel catfish	Gonads, gills	Quiniou et al. (2013)
4mb	Channel catfish	Gills, liver, spleen	
4	Channel catfish	Intestine, gills, and others	Zhang et al. (2013a)
4-1	Common carp	Intestine, gills, brain, and other	Gong et al. (2017)
4-2	Common carp	Intestine, spleen, gills, skin, blood, and others	
4.1	Grass carp	Heart, blood, liver, and others	Pei et al. (2015)
4.2	Grass carp	Skin, intestine, liver, spleen, and others	
4.3	Grass carp	Gills, skin, and others	
4.4	Grass carp	Heart, skin, and others	
4.1	Grass carp	Spleen, intestine, kidney, and others	Huang et al. (2012)
4-2	Grass carp	Spleen, intestine, kidney, and others	
4-3	Grass carp	Spleen, intestine, kidney, and others	
4-4	Grass carp	Spleen, kidney, anterior kidney, gills, and others	
4-1	Yellow catfish	Liver, anterior kidney, gills, brain, trunk kidney, heart, and other	Zhang et al. (2017b)
4.1	Zebrafish	Blood, skin, testis, digestive organ, brain, liver, heart, and other	Jault et al. (2004)
4.2	Zebrafish	Blood	
4	Rohu	Blood, spleen, gills, kidney, and others	Samanta et al. (2017)
4	Blunt snout	Kidney, muscle, heart, liver, and others	Tang et al. (2016)
4	Blunt snout	Blood, skin, heart, spleen, gills, and others	Lai et al. (2016)
4	Tibetan fish (Przewalski's carp)	Details not given	Tong et al. (2015)
4	Yellow River scaleless carp	ND	Qi et al. (2017)

element from the *IFN- $\gamma$*  responsive gene *TAP2* linked to a luciferase reporter gene and a hygromycin resistance gene. The results indicate that the stable cell line RTG-3F7 is an excellent tool for monitoring the presence of trout *ifng* in biological samples (Castro et al. 2010). The large yellow croaker (*L. crocea*) is an important mariculture fish species in China, and the bacterium *Vibrio harveyi* and the ciliate protozoan *C. irritans* are the two major pathogens of this species. The nucleotide sequence of *ifng* was obtained, and expression studies were performed. Fish were challenged with *V. harveyi* and *C. irritans*,



**Table 2.5** List of *tlr5* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr5* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. Bacterial flagellin is the ligand for TLR5. *ND* not determined, *S* soluble, *m* membrane

TLR no	Species	Tissue expression	References
5 s	Pufferfish	Reproductive organ, heart, gills, brain, skin, eye, liver, spleen	Oshiumi et al. (2003)
5		Digestive tract, reproductive organ, liver,	
5all	Channel catfish	Anterior kidney, liver, gonads, posterior kidney	Quiniou et al. (2013)
5 mb		Anterior kidney, liver, gonads, posterior kidney	
5-1 and 5-2	Channel catfish	Expressed at low levels in many organs and tissues	Zhang et al. (2013a)
5 s		Liver, anterior kidney, kidney, and others	
5	Channel catfish	Ovary, liver, anterior kidney, skin, and others	Baoprasertkul et al. (2007b)
5	Channel catfish	Liver, kidney, and others	Bilodeau and Waldbieser (2005)
5	Golden pompano	Intestine, liver, kidney, blood	Zhu et al. (2020)
5 s		Blood, kidney, spleen, skin, intestine	
5	Common carp	Muscle, spleen, and other	Gong et al. (2017)
5	Yellow catfish	Liver, gills, blood, trunk kidney, and other	Zhang et al. (2017b)
5a	Zebrafish	Low expression in digestive organ, negligible in others	Jault et al. (2004)
5	Spiny eel	Liver, eye, gills, muscle, fins, and others	Han et al. (2017)
5a	Yellow River scaleless carp	ND	Qi et al. (2017)
5 m	Tibetan fish (Przewalski's carp)	Details not given	Tong et al. (2015)
5.1	Ya-fish	Liver, spleen, anterior kidney, and others	Du et al. (2019a)
5.2		Liver, spleen, and others	
5	Spotted sea bass	Details not given	Fan et al. (2019)
5	Silver pomfret	Liver, kidney, spleen, intestine, and others	Gao et al. (2018)
5 s	Miiuy croaker	Liver, kidney, skin, and others	Huo et al. (2018)
5	Dark barbel fish	Anterior kidney, spleen, and others	Qin et al. (2018)
5 s		Anterior kidney, spleen, and others	

(continued)

**Table 2.5** (continued)

TLR no	Species	Tissue expression	References
5	Pacific red snapper	Intestine, liver, anterior kidney, brain, muscle, and others	Reyes-Becerril et al. (2017)
5	Triploid crucian carp	Kidney, liver, anterior kidney, heart, and others	Zhang et al. (2017a)
5 s	Striped catfish	Anterior kidney, kidney, liver, spleen, and others	Jayaramu et al. (2017)
5	Orange-spotted grouper	Skin, anterior kidney, spleen, kidney, and others	Bai et al. (2017)
5 s	Orange-spotted grouper	Liver, spleen, and others	
5	Maraena whitefish	Liver, fins, heart, skin, and others	Altmann et al. (2016)
5 s		Liver and others	
5a	Grass carp	Anterior kidney, kidney, muscle, and others	Jiang et al. (2015)
5b		Anterior kidney, kidney, heart, gills, and others	
5 s	Gilthead sea bream	Liver, peritoneal exudate, blood, spleen, thymus	Munoz et al. (2013)
5	Indian major carp, mrigal	Liver, skin, muscle, and others	Basu et al. (2012a)
5	Japanese flounder	Kidney, liver, heart, gills, and others	Hwang et al. (2010)
5a	Blunt snout bream	Liver, kidney, and others	Zhan et al. (2019)
5b		Spleen, kidney, heart, and others	
5	Atlantic salmon	ND	Salazar et al. (2016)
5 s			
5	Lamprey	Peripheral blood leukocytes, skin, gills, and others	Kasamatsu et al. (2010)

respectively. One day after injection with *V. harvey*, all 10 tissues investigated had a higher expression of *ifng*, while only spleen, muscle, intestine, heart, and skin had higher expression after infection with *C. irritans* (Chen et al. 2015). Jung and coworkers produced a recombinant *ifng* (*rifng*) from the olive flounder (*P. olivaceus*). Stimulation of kidney leukocytes in vitro with *rifng* induced the gene expression of *il1b*, *signal transducer and activator of transcription 1 (stat1)*, *CXCL-13*-like chemokine (*cxcl13*), and *ifng*. Intraperitoneal injection of a mixture of *rifng* and *E. tarda* into olive flounder resulted in a survival rate of 60% compared to 0% in the group treated with *E. tarda* only (Jung et al. 2012).

The extensive use of paraquat (PQ) in agricultural practice throughout the world may compromise the integrity of biological systems in fish. PQ toxicity has been found to be mediated by the production of free radicals, which cause oxidative damage to cells. In a

**Table 2.6** Updated list of *tlr7* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr7* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. TLR7 recognizes internalized single-stranded RNA found in viruses. *ND* not determined

TLR no	Species	Tissue expression	References
7	Pufferfish	Kidney, gills, reproductive organ	Oshiumi et al. (2003)
7	Channel catfish	Spleen, anterior kidney, posterior kidney	Quiniou et al. (2013)
7	Channel catfish	Anterior kidney, kidney, brain, spleen, heart, gills, intestine, skin, and others	Zhang et al. (2013a)
7-1	Common carp	Brain, gills, skin, blood, and others	Gong et al. (2017)
7-2		Gills, heart, skin	
7	Common carp	Spleen, liver, heart, kidney, and others	Tanekhy et al. (2010)
7	Yellow catfish	Brain, spleen, swim bladder, anterior kidney, and other	Zhang et al. (2017b)
7	Zebrafish	Heart, skin, blood, brain, and others	Jault et al. (2004)
7	Tibetan fish (Przewalski's carp)	Details not given	Tong et al. (2015)
7	Yellow River scaleless carp	ND	Qi et al. (2017)
7	Spotted sea bass	Details not given	Fan et al. (2019)
7	Maraena whitefish	Anterior kidney and others	Altmann et al. (2016)
7a-c	Atlantic cod	No details given	Solbakken et al. (2016)
7	Zig-zag eel	Gills, muscle, spleen, intestine, and others	Han et al. (2019)
7	Barbel chub	Spleen, brain, heart, and others	Jin et al. (2018)
7	Golden pompano	Spleen, kidney, muscle, gills, skin, and others	Wei et al. (2017)
7	Tongue sole	Kidney, spleen, liver, heart, and others	Li and Sun (2015)
7	Large yellow croaker	Spleen, kidney, muscle, gills, skin, and others	Qian et al. (2013)
7	Grass carp	Spleen, skin, heart, intestine, gills, and others	Yang et al. (2012)
7	Rainbow trout	Spleen, anterior kidney, and others	Palti et al. (2010a)
7	Goldfish	ND	Tu et al. (2016)
7/8a	Lamprey	Peripheral blood leukocytes, skin, and others	Kasamatsu et al. (2010)
7/8b		Peripheral blood leukocytes, heart, and others	

**Table 2.7** List of *tlr8* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr8* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. TLR8 may recognize guanosine and uridine-rich single-stranded RNA found in viruses. Tlr8 associates with *tlr7*. *ND* not determined

TLR no	Species	Tissue expression	References
8	Pufferfish	Reproductive organ	Oshiumi et al. (2003)
8	Channel catfish	Spleen, anterior kidney, posterior kidney	Quiniou et al. (2013)
8a-1	Channel catfish	Anterior kidney, kidney, spleen, and others	Zhang et al. (2013a)
8a-2		Spleen, anterior kidney, kidney, heart, brain, and others	
8-1	Common carp	Gills and other	Gong et al. (2017)
8-2		Gills, intestine, skin, heart, and other	
8-3		Intestine, kidney, spleen, and other	
8	Common carp	Spleen, skin, brain, gonads, and others	Shan et al. (2018b)
8-2	Yellow catfish	Spleen, brain, liver, foregut, and other	Zhang et al. (2017b)
8.1	Zebrafish	Heart, blood, liver, ovaries, digestive organ, and others	Jault et al. (2004)
8.2		Skin, blood, heart, and others	
8	Tibetan fish (Przewalski's carp)	ND	Tong et al. (2015)
8a	Yellow River scaleless carp	ND	Qi et al. (2017)
8b			
8	Spotted sea bass	ND	Fan et al. (2019)
8.1	Maraena whitefish	Anterior kidney, kidney, and others	Altmann et al. (2016)
8.2		Anterior kidney, kidney, heart, and others	
8a-1	Atlantic cod	ND	Solbakken et al. (2016)
8	Zig-zag eel	Spleen, gills, and others	Han et al. (2019)
8	Barbel chub	Spleen, brain, skin, kidney, heart, muscle, and others	Jin et al. (2018)
8	Golden pompano	Spleen, gills, muscle, skin, and others	Wei et al. (2017)
8	Large yellow croaker	Spleen, gills, muscle, skin, and others	(Qian et al. 2013)
8	Grass carp	Swim bladder, spleen, brain, posterior intestine, anterior kidney, skin, and others	Chen et al. (2013)
8a	Grass carp	ND	Liao et al. (2017)
8b			
8a1	Rainbow trout	Spleen, anterior kidney, kidney, and others	Palti et al. (2010a)
8a2		Spleen, anterior kidney, kidney, and others	
8	Turbot	Blood, spleen, anterior kidney, and others	Dong et al. (2016)

**Table 2.8** A list of *tlr9* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr9* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. TLR9 binds unmethylated CpG sequences in DNA—often found in prokaryotes. *ND* not determined

TLR no	Species	Tissue expression	References
9	Pufferfish	Kidney, digestive organ, skin, heart	Oshiumi et al. (2003)
9	Channel catfish	Anterior kidney, spleen	Quiniou et al. (2013)
9	Channel catfish	Heart, anterior kidney, kidney, and others	Zhang et al. (2013a)
9	Common carp	Kidney, gills, brain, heart, and others	Gong et al. (2017)
9	Common carp	Kidney, gills, and others	Gong et al. (2017)
9	Yellow catfish	Spleen, anterior kidney, kidney, hindgut, and other	Zhang et al. (2017b)
9	Yellow catfish	Spleen, anterior kidney, gills, intestine, adipose tissue, and others	Zhang et al. (2017b)
9	Zebrafish	Blood, heart, testis, and others	Jault et al. (2004)
9	Tibetan fish (Przewalski's carp)	Details not given	Tong et al. (2015)
9	Yellow River scaleless carp	ND	Qi et al. (2017)
9	Spotted sea bass	Details not given	Fan et al. (2019)
9	Silver pomfret	Spleen, kidney, liver, intestine, gills, and others	Gao et al. (2018)
9	Maraena whitefish	Anterior kidney, gills, and others	Altmann et al. (2016)
9a-e	Atlantic cod	ND	Solbakken et al. (2016)
9	Zig-zag eel	Spleen, muscle, fin, gills, and others	Han et al. (2019)
9	Golden pompano	Spleen, kidney, skin, and others	Wei et al. (2017)
9	Turbot	Brain, anterior kidney, blood, intestine, gills, and others	Dong et al. (2016)
9	Blunt snout bream	Kidney, heart, spleen, gills, liver, and others	Zhan et al. (2019)
9	Grass carp	ND	Liao et al. (2017)
9a	Orange-spotted grouper	Spleen, anterior kidney, liver, kidney	Lee et al. (2015)
9b		Spleen, anterior kidney, liver, kidney	
9	Pacific red snapper	Intestine, leukocytes, liver, skin, anterior kidney	Reyes-Becerril et al. (2015)
9	Rainbow trout	Spleen, anterior kidney, peripheral blood leukocytes, gut	Ortega-Villaizan et al. (2009)
9A		Spleen, skin, kidney, brain, and others	Yao et al. (2008)

(continued)

**Table 2.8** (continued)

TLR no	Species	Tissue expression	References
9B	Large yellow croaker	Spleen, skin, liver, and others	
9	Gilthead sea bream	Peripheral blood leukocytes, peritoneal exudate, gills, spleen, anterior kidney, and others	Cuesta et al. (2008)
9	Gilthead sea bream	Gills, spleen, gut, anterior kidney, muscle, and others	Franch et al. (2006)
9	Japanese flounder	Spleen, kidney, intestine, gills, blood, and others	Takano et al. (2007)
9	Goldfish	ND	Tu et al. (2016)

**Table 2.9** Tlr12 found in Yellow River scaleless carp (common name). See citation for corresponding Latin names. TLR12 recognizes profilin found in certain parasites. *ND* not determined

TLR no	Species	Tissue expression	References
12	Yellow River scaleless carp	ND	Qi et al. (2017)

study by Ma et al. (2014), the acute toxicity of PQ in common carp (*C. carpio*) was determined. The results suggest that PQ exposure may result in suppression or excessive activation of the immune system that leads to immune dysfunction and reduced immunity (Ma et al. 2014). In the report of Pereiro et al. (2016), an antiviral turbot (*S. maximus*) interferon-gamma gene was characterized, and its expression pattern under basal conditions, after type I ifn administration and viral and bacterial infections, was evaluated (Pereiro et al. 2016). The intramuscular injection of an expression plasmid encoding turbot *ifn* gene was not able to affect the transcription of numerous immune genes directly related to the activity of ifng. It was neither able to reduce the mortality caused by a VHSV nor *A. salmonicida* challenge. Shibasaki and coworkers cloned and characterized two ginbuna crucian carp (*Carassius auratus langsdorfii*)-specific isoforms of *ifng* called *ifng rel1* and *ifng rel2*. Recombinant ifng rel1 and ifng rel2 showed high antiviral activities against the lethal crucian carp hematopoietic necrosis virus (Shibasaki et al. 2014).

The antiviral activity of ifn gamma against IPNV and salmonid alphavirus (SAV) was studied by Sun et al. (2011). The studies were performed in Atlantic salmon TO cells and Chinook salmon embryo cells (CHSE-214). Ifn- $\gamma$  induced antiviral activity against both IPNV and SAV3 in salmon cells (Sun et al. 2011). The marine flatfish Atlantic halibut (*Hippoglossus hippoglossus*) is of great commercial interest. However, due to poorly developed larva at hatching and a long live-feed stage, aquacultural use of this species is limited. Øvergård and coworkers cloned and characterized the gene encoding the *ifng*. A constitutive expression was found in both lymphoid and non-lymphoid organs with

**Table 2.10** Updated list of *tlr13* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr13* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. It has been suggested that *tlr12* binds 23S ribosomal RNA (rRNA) found in bacteria. *ND* not determined

TLR no	Species	Tissue expression	References
13	Darby's sturgeon	Anterior kidney, heart, and others	Tang et al. (2020)
13	Orange-spotted grouper	Spleen, anterior kidney, liver, and others	Liang et al. (2018)
13	Tibetan fish (Przewalski's carp)	Details not given	Tong et al. (2015)
13	Yellow River scaleless carp	ND	Qi et al. (2017)
13-1	Spotted sea bass	ND	Fan et al. (2019)
13-2			
13-3			
13	Miiuy croaker	Liver, spleen, anterior kidney, and others	Wang et al. (2016a)
13	Soiny mullet	Anterior kidney, skin, and others	Qi et al. (2020)
13-like genes	Brown trout	ND	Sudhagar et al. (2020)

**Table 2.11** A list of *tlr14* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr14* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. The natural ligand for TLR14 is not known. Since it is a member of *tlr1* subfamily one can speculate that this receptor may bind similar ligands as TLR1/2/6—which are ligands from bacteria. *ND* not determined

TLR no	Species	Tissue expression	References
14	Golden pompano	Skin, intestine, kidney, blood	Wu et al. (2019)
	Ya-fish ( <i>Schizothorax prenanti</i> )	Peripheral blood leukocytes, brain, anterior kidney, spleen, eye	Li et al. (2018c)
14a	Lamprey	Gills	Ishii et al. (2007)
14b	Lamprey	Gills, gut, egg, skin, and others	
14	Spotted sea bass	ND	Fan et al. (2019)
14	Atlantic cod	ND	Solbakken et al. (2016)
14	Japanese flounder	Kidney, spleen, liver, gills, and others	Hwang et al. (2011)
14a	Lamprey	Gills	Kasamatsu et al. (2010)
14b		Gills, peripheral blood leukocytes, intestine, and others	
14c		Peripheral blood leukocytes, heart, gills, brain, eye, and others	
14d		Liver, kidney, intestine, and others	

**Table 2.12** List of *tlr18* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr18* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. TLR18 is also a member of the TLR1 subfamily and may bind molecules from bacteria. *ND* not determined

TLR no	Species	Tissue expression	References
18	Channel catfish	Gills, gonads, posterior kidney	Quiniou et al. (2013)
18	Channel catfish	Gills, kidney, brain	Zhang et al. (2013a)
18-1	Common carp	Kidney, intestine, heart, and other	Gong et al. (2017)
18-2		Kidney, intestine, gills, heart, and other	
18	Common carp	Skin, spleen, liver, hindgut, and others	Shan et al. (2018a)
18	Atlantic salmon	Muscle, liver, spleen, gills, and anterior kidney	Lee et al. (2014)
18	Tibetan fish (Przewalski's carp)	Details not given	Tong et al. (2015)
18	Yellow River scaleless carp	ND	Qi et al. (2017)
18	Grass carp	ND	Huang et al. (2012)
18	Grass carp	Spleen, gills, heart, and others	Huang et al. (2015)

**Table 2.13** Updated list of *tlr19* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr19* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. Teleost-specific TLR19 is localized in the endosomes and recognizes dsRNA analogs. *ND* not determined

TLR no	Species	Tissue expression	References
19	Channel catfish	Anterior kidney, spleen, posterior kidney	Quiniou et al. (2013)
19	Channel catfish	Heart, anterior kidney, kidney, and others	Zhang et al. (2013a)
19	Common carp	Brain, heart, skin, and other	Gong et al. (2017)
19	Tibetan fish (Przewalski's carp)	Details not given	Tong et al. (2015)
19	Yellow River scaleless carp	ND	Qi et al. (2017)
19	Grass carp	ND	Huang et al. (2012)
19	Atlantic salmon	Spleen, anterior kidney, gills, muscle, liver	Lee et al. (2014)
19	Brown trout	ND	Sudhagar et al. (2020)



**Table 2.14** A list of *tlr20* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr20* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. TLR20 is proposed to play a role in the immune response of carp to protozoan parasites. *ND* not determined

TLR no	Species	Tissue expression	References
20	Channel catfish	Anterior kidney, spleen	Quiniou et al. (2013)
20-1	Channel catfish	Anterior kidney, kidney, spleen, heart, gills, and others	Zhang et al. (2013a)
20	Channel catfish	Stomach, anterior kidney, liver, spleen, intestine, gills, and others	Baoprasertkul et al. (2007b)
20a	Atlantic salmon	Spleen, anterior kidney, gills, muscle, liver	Lee et al. (2014)
20b		Spleen, anterior kidney, muscle, gills	
20c		Spleen, anterior kidney, gills, muscle, liver	
20d		Spleen, gills, anterior kidney, liver, muscle	
20	Tibetan fish (Przewalski's carp)	ND	Tong et al. (2015)
20	Yellow River scaleless carp	ND	Qi et al. (2017)
20.2	Grass carp	Spleen, anterior kidney, liver, brain, and others	Huang et al. (2016)
20a	Grass carp	ND	Huang et al. (2012)
20b			
20a	Atlantic salmon	Spleen, anterior kidney, gills, and others	Lee et al. (2014)
20b		Spleen, anterior kidney, muscle, and others	
20c		Spleen, anterior kidney, gills, muscle, liver	
20d		Spleen, gills, anterior kidney, liver, muscle	
20a-d	Zebrafish	ND	Pietretti et al. (2014)
20	Goldfish	ND	Tu et al. (2016)

relatively high expression in the thymus and gills (Overgard et al. 2012). An overview of type II ifng in different fish species is presented in Table 2.24.

## 2.9.4 Tnfa

Tumor necrosis factor-alpha (*tnfa*) is a cytokine involved in systemic inflammation, apoptosis, cell proliferation, and regulation of immune cells (Wiens and Glenney 2011). It is produced mainly by activated macrophages as a membrane or secreted form. The main pro-inflammatory effects are mediated through the activation of endothelial cells (Roca et al. 2008). In bony fish, *tnfa* was first discovered in Japanese Flounder (Hirono et al.

**Table 2.15** Updated list of *tlr21* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr21* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. It has been suggested, similar to TLR9, that TLR21 may bind unmethylated CpG sequences in DNA. *ND* not determined

TLR no	Species	Tissue expression	References
21	Pufferfish	Gills, heart, reproductive organ	Oshiumi et al. (2003)
21	Channel catfish	Spleen	Quiniou et al. (2013)
21	Channel catfish	Kidney, spleen, heart, gills, anterior kidney, and others	Quiniou et al. (2013)
21	Channel catfish	Stomach, liver, spleen, intestine, kidney, ovary, gills, and others	Baoprasertkul et al. (2007b)
21-1	Common carp	Gills, skin, heart, brain, and other	Baoprasertkul et al. (2007b)
21-2		Spleen, heart, skin, and other	
21	Common carp	Spleen, anterior kidney, gills, and others	Li et al. (2018b)
21	Atlantic salmon	Spleen, gills, anterior kidney, muscle, liver	Lee et al. (2014)
21	Tibetan fish (Przewalski's carp)	Details not given	Tong et al. (2015)
21	Yellow River scaleless carp	ND	Qi et al. (2017)
21	Spotted sea bass	ND	Fan et al. (2019)
21	Maraena whitefish	Gills, heart, kidney, anterior kidney, skin, and others	Altmann et al. (2016)
21	Atlantic cod	ND	Solbakken et al. (2016)
21	Atlantic cod	Kidney, gills, testis, spleen, and others	Sundaram et al. (2012b)
21	Blunt snout bream	Gills, kidney, muscle, liver, and others	Zhan et al. (2019)
21	Grass carp	ND	Huang et al. (2012)
21	Grass carp	Skin, spleen, intestine, anterior kidney, and others	Wang et al. (2013b)
21	Large yellow croaker	Spleen, gills, anterior kidney, intestine, and others	Sun et al. (2016a)
21	Large yellow croaker	Spleen, anterior kidney, stomach, liver, and others	Sun et al. (2018)
21	Olive flounder	Spleen, gills, heart, muscle, and others	Gao et al. (2013)
21	Orange-spotted grouper	Kidney, anterior kidney, heart, spleen, and others	Li et al. (2012)
21	Mudskipper	Gills, reproductive organ, kidney, brain, and others	Qiu et al. (2019)
21	Atlantic salmon	Spleen, gills, anterior kidney, muscle, liver	Lee et al. (2014)

(continued)

**Table 2.15** (continued)

TLR no	Species	Tissue expression	References
21	Zebrafish	ND	Pietretti et al. (2014)
21	Zebrafish	Spleen, gills	Sundaram et al. (2012a)
21	Darby's sturgeon	Gills, intestine, anterior kidney, and others	Qi et al. (2018b)
21	Nile tilapia	Brain, gills, heart, muscle, stomach, intestine, skin, and others	Pang et al. (2017)
21	Yellowtail	Spleen, anterior kidney, intestine, liver, gills	Reyes-Becerril et al. (2016)
21a	Lamprey	Peripheral blood leukocytes, gills, and others	Kasamatsu et al. (2010)
21b	Lamprey	Gills and others	Kasamatsu et al. (2010)
21c	Lamprey	Gills, heart, muscle, kidney, liver, eye, and others	Kasamatsu et al. (2010)

2000) rainbow trout (Laing et al. 2001) and has since been characterized in a number of species. Fish have 14 tumor necrosis family genes. Their genomic existence and location have been investigated in the Japanese pufferfish (fugu) (*Takifugu rubripes*) (Biswas et al. 2015). Fugu was found to possess nine *tnf* superfamily genes including seven newly identified and two that had been previously reported. Poly I:C caused an elevated expression of three fugu *tnf* superfamily 10 genes in head kidney cells. *Tnfa* is an important factor for bacterial pathogen killing. *A. salmonicida* subsp. *salmonicida* is highly pathogenic for turbot, an economically important cultured flatfish in Europe, China, and Chile. In *A. salmonicida*-infected fish, the number of *tnfa* immunopositive cells was significantly increased in the kidney and spleen (Coscelli et al. 2016). Immunoreactive cells were also present in the digestive tract, liver, heart, gills, and skin (Ronza et al. 2015).

The striped trumpeter (*Latris lineata* Forster) is a new species in Tasmanian waters. The *tnfa* was cloned, and the expression was analyzed in response to an ectoparasite *Chondracanthus goldsmidi*. A significant upregulation was found in the gills, which are the site of parasite attachment. Head kidney cells showed a significant upregulation of *tnfa*, but spleen cells did not (Covello et al. 2009). The European sea bass (*D. labrax*) is intensely aquacultured in the Mediterranean area. The bacterial pathogen *V. anguillarum* provokes the highest mortality among several pathogens of this species. Available vaccines do not achieve the desired protection. In a recent study, recombinant *tnfa* was used as adjuvant in a commercial sea bass oral vaccine against *V. anguillarum*. *Tnfa* significantly enhanced disease resistance and induced recruitment of gut intraepithelial lymphocytes (Galindo-Villegas et al. 2013). In rainbow trout, two *tnfa* genes have been described. Recently, a third *tnfa* (*tnfa3*) that has low identities to known trout molecules was reported.

**Table 2.16** List of *tlr22* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr22* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. TLR22 is a typical fish-specific TLR and is suggested to bind double-stranded RNA. *ND* not determined

TLR no	Species	Tissue expression	References
22	Pufferfish	Kidney	Oshiumi et al. (2003)
22	Channel catfish	Anterior kidney, gills, spleen, posterior kidney	Quiniou et al. (2013)
22	Channel catfish	Anterior kidney, kidney, intestine, liver, gills, heart, and others	Zhang et al. (2013a)
22-1	Common carp	Kidney, spleen, gills, heart, and other	Gong et al. (2017)
22-2		Spleen, heart, gills, skin, blood, and other	
22-3		Heart, skin, and others	
22	Common carp	Gills, anterior kidney, brain, hindgut, foregut, and others	Li et al. (2017a)
22	Rohu	Kidney, muscle, spleen, and others	Li et al. (2017a)
22	Rohu	Liver, kidney, gills, heart, eye, and others	Samanta et al. (2014)
22	Catla	Kidney, intestine, brain, and others	Samanta et al. (2014)
22	Yellow catfish	Spleen, anterior kidney, gills, heart	Zhang et al. (2017b)
22	Turbot	Anterior kidney, kidney, spleen, heart, intestine, stomach, and others	Hu et al. (2015b)
22	Rainbow trout	Spleen, anterior kidney, trunk kidney, and others	Rebl et al. (2007)
22L		Spleen, anterior kidney, trunk kidney, gills, and others	
22	Tibetan fish (Przewalski's carp)	ND	Tong et al. (2015)
22	Yellow River scaleless carp	Liver, muscle, and others	Qi et al. (2019)
22a	Yellow River scaleless carp	ND	Qi et al. (2017)
22b			
22	Spotted sea bass	ND	Fan et al. (2019)
22	Triploid crucian carp	Anterior kidney, kidney, muscle, liver, and others	Zhang et al. (2017a)
22a	Maraena whitefish	Anterior kidney, liver, heart, kidney, and others	Altmann et al. (2016)
22b		Anterior kidney, kidney, and others	

(continued)

**Table 2.16** (continued)

TLR no	Species	Tissue expression	References
22	Gilthead sea bream	Spleen, thymus, gills, and others	Munoz et al. (2014)
22a	Grass carp	ND	Huang et al. (2012)
22b		ND	
22	Grass carp	Anterior kidney, spleen, kidney, gills, and others	Lv et al. (2012)
22	Grass carp	Gills, anterior kidney, kidney, midgut, and others	Lv et al. (2012)
22a	Mudskipper	Spleen, gills, kidney, intestine, and others	Qiu et al. (2019)
22b		Spleen and others	
22c		Spleen, kidney, and others	
22d		Kidney, brain, spleen, and others	
22	Soiny mullet	Liver, spleen, and others	Qi et al. (2020)
22	Zebrafish	Spleen, gonads, and others	Sundaram et al. (2012a)
22	Darby's sturgeon	Heart, gills, kidney, anterior kidney, and others	Sundaram et al. (2012a)
22	Nile tilapia	Spleen, gills, and others	Pang et al. (2017)
22a	Atlantic cod	Kidney, liver, gills, testis, and others	Sundaram et al. (2012b)
22b		Kidney, liver, gills, and others	
22c		Kidney, gills, testis, liver, spleen, and others	
22d		Gills, kidney, spleen, liver, and others	
22e, f, l		Low in all organs and tissues	
22g		Gills and low in other organs and tissues	
22h		Kidney, gills, liver, spleen, anterior kidney, blood, and others	
22i		Gills, liver, spleen, kidney, and others	
22j		Kidney, gills, liver, spleen, anterior kidney, and others	
22 k		Anterior kidney, kidney, spleen, liver, gills, and others	
22a-1		Atlantic cod	
22-1	Ya-fish	Gills, liver, kidney, and others	Du et al. (2019b)
22-2		Spleen, brain, heart, and others	
22-3		Spleen, kidney, liver, anterior kidney, brain, heart, and others	
22	Atlantic salmon	ND	Salazar et al. (2016)

(continued)

**Table 2.16** (continued)

TLR no	Species	Tissue expression	References
22	Wuchang bream	Similar expression levels in many organs and tissues	Lai et al. (2017a)
22	Orange-spotted grouper	Kidney, anterior kidney, peripheral leukocytes, spleen, heart, and others	Ding et al. (2012)
22	Large yellow croaker	Anterior kidney, heart, spleen, blood, and others	Xiao et al. (2011)
22	Lamprey	Eye, low in other organs	Kasamatsu et al. (2010)

**Table 2.17** Updated list of *tlr23* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr23* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. The ligand for TLR23 is unknown, *ND* not determined

TLR no	Species	Tissue expression	References
23	Spotted sea bass	Details not given	Fan et al. (2019)
23	Atlantic cod	ND	Solbakken et al. (2016)
23a	Atlantic cod	Kidney, gills, liver, and others	Sundaram et al. (2012b)
23b		Low in all tissues and organs	
23a	Mudskipper	Kidney, spleen, gills, and others	Qiu et al. (2019)
23b-e		Kidney, spleen, and others	
23f		Kidney and others	
23 g		Spleen, kidney, and others	

**Table 2.18** List of *tlr24* found in different fish species (common names). See citation for corresponding Latin name. The main tissue distribution of *tlr24* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. The ligand for TLR23 is unknown

TLR no	Species	Tissue expression	Reference
24a	Lamprey	Peripheral blood leukocytes, liver	Kasamatsu et al. (2010)
24b		Peripheral blood leukocytes, heart, and others	

The constitutive expression of *tnfa3* was generally lower than the other two genes in tissues and cell lines. Expression of all three *tnfa* isoforms could be modulated by crude LPS, peptidoglycan, poly I:C and recombinant *I*fng in cell lines and primary macrophages, and bacterial and viral infections (Hong et al. 2013). The genomic location of the two *tnfa* genes in zebrafish (*D. rerio*) and medaka (*Oryzias latipes*) was recently determined. Zebrafish *tnfa1* and *tnfa2* were found on chromosomes 19 and 15, and medaka *tnfa1* and *tnfa2* on chromosomes 11 and 16, respectively. There was a constitutive expression of the genes in

**Table 2.19** Updated list of *tlr25* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr25* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. The ligand for TLR25 is unknown, *ND* not determined

TLR no	Species	Tissue expression	References
25	Channel catfish	Gills, kidney, anterior kidney	Quiniou et al. (2013)
25	Channel catfish	Gills, anterior kidney, kidney, and others	Lee et al. (2020)
25	Nile tilapia	Spleen, anterior kidney, skin, muscle	Lee et al. (2020)
25-1	Common carp	Muscle, gills, heart, blood, and other	Gong et al. (2017)
25-2		Muscle, heart, skin, blood, and other	
25a-g	Atlantic cod	ND	Solbakken et al. (2016)
25	Grass carp	ND	Huang et al. (2012)
25	Grass carp	ND	Liao et al. (2017)
25	Darby's sturgeon	Kidney, gills, skin, heart, and others	Liao et al. (2017)

**Table 2.20** Updated list of *tlr26* found in different fish species (common names). See citations for corresponding Latin names. The main tissue distribution of *tlr26* mRNA is also given, where the highest expression is found in the first organ/tissue listed, and so on. *TLR26* is not yet found in any other fish species. The ligand is unknown

TLR no	Species	Tissue expression	References
26	Channel catfish	Anterior kidney, kidney, spleen	Quiniou et al. (2013)
26	Channel catfish	Anterior kidney, kidney, brain, gills, and others	Zhang et al. (2013a)
26	Yellow catfish	Anterior kidney, blood, spleen, heart, and others	Liu et al. (2019)

**Table 2.21** *Tlr27* is found in spotted gar, coelacanth, and elephant shark. See citation for corresponding Latin name. *ND* Not determined. Its ligand is unknown

TLR no	Species	Tissue expression	Reference
27	Spotted gar, coelacanth, elephant shark	ND	Wang et al. (2015)

**Table 2.22** *Tlr28* is found in miiuy croaker. See citation for corresponding Latin name. Like *tlr27*, the *tlr28* belongs to the *tlr1* subfamily. Its ligand is unknown

TLR no	Species	Tissue expression	Reference
28	Miiuy croaker	Liver, eye, skin, gills, heart	Wang et al. (2016b)

**Table 2.23** Innate immunity: interferon type I genes in fish

Fish Species	Subject	References
Rainbow trout	Response in ovary against virus	Chaves-Pozo et al. (2010)
Orange-spotted grouper	Functional analysis in response to virus infection	Chen et al. (2014)
Large yellow croaker	Characterization, function	Ding et al. (2019)
Japanese eel	Identification, expression	Feng et al. (2017)
CHSE-214 cell line	Mx1 promoter–reporter system	Jorgensen et al. (2007)
Mandarin fish	Functional, signaling, and transcriptional differences	Laghari et al. (2018)
Salmonids	Evolution of subgroups	Liu et al. (2020a)
Meagre	Discovery, expression	Milne et al. (2018)
Review		Robertsen (2006)
Review	Evolution of interferons and interferon receptors	Secombes and Zou (2017)
Atlantic salmon	Multigene cluster, identification, expression	Sun et al. (2009)
Gilthead sea bream, European sea bass	Characterization, expression	Valero et al. (2015)
Zebrafish	Interferon-induced proteins (IFITs)	Varela et al. (2014)
Rock bream	Cloning, functional characterization	Wan et al. (2012)
Review		Workenhe et al. (2010)
Review		Zou and Secombes (2011)

**Table 2.24** Innate immunity: Presence of interferon type II (*ifng*) genes in fish

Fish Species	Subject	References
Common carp	Functional analysis	Arts et al. (2010)
Cell line (Rainbow trout)		Castro et al. (2010)
Large yellow croaker	Characterization, expression	Chen et al. (2015)
Olive flounder	Response against <i>Edwardsiella tarda</i> infection	Jung et al. (2012)
Turbot	Characterization, expression	Pereiro et al. (2016)
Review		Pereiro et al. (2019)
Ginbuna crucian carp	Isoforms	Shibasaki et al. (2014)
Atlantic salmon TO cells, CHSE-214 cells	Antiviral activity	Sun et al. (2011)
Atlantic halibut	Characterization, expression	Overgard et al. (2012)



different tissues. An increased expression of both was induced in head kidney cells by LPS in vitro (Kinoshita et al. 2014). Li and Zhang studied a *tnfa* homologue from the Tongue sole (*Cynoglossus semilaevis*) named *CsTNF1*. Expression of *CsTNF1* was detected in liver, spleen, kidney, blood, gill, brain, muscle, heart, and intestine, and was upregulated by experimental challenge with bacterial and viral pathogens (Li and Zhang 2016).

Meagre (*Argyrosomus regius*) is an emerging aquaculture species found in the Mediterranean area and Black Sea due to its large size, fast growth, low feed conversion ratio, and high processing yield. Two types of *tnfa* were expressed in meagre (type 1 and type 2). *Tnfa1* was more highly expressed in head kidney and gills. Both isoforms increased in expression in head kidney following injection with LPS (Milne et al. 2017). Atlantic bluefin tuna (*Thunnus thynnus*) was introduced into Mediterranean aquaculture in the early nineties, and has become the most valuable finfish aquaculture, representing more than half of the world's total production (Pleic et al. 2014). Tuna aquaculture is a capture-based activity, where wild-caught tuna is cultured in marine cages for a period of time in order to increase their protein and fat content. The full-length cDNA and gene sequences of Bluefin tuna *tnfa1* and *tnfa2* were determined, and expression studies showed that they were constitutively expressed in liver and head kidney at similar levels. Expression of both cytokines was examined in acute and chronic natural infection of the parasites *Pseudocycnus appendiculatus* and *Didymosulcus katsuwonocola* (Pleic et al. 2015). *D. katsuwonocola*-infected gills showed significantly higher expression of *tnfa2*, while *tnfa1* showed no difference in expression with either *Pseudocycnus appendiculatus*- or *Didymosulcus katsuwonocola*-infected gills.

Rainbow trout red blood cells (RBCs) are able to endocytose nanostructured *tnfa* in vitro despite not being phagocytic cells, and in response to nanostructured *tnfa*, the expression of different immune genes could be modulated (Puenta-Marin et al. 2019a).

*Tnfa* was cloned in large yellow croaker (*Pseudosciaena crocea*), mainly distributed in coastal regions of East Asia, and is one of the most important cultured marine fish in China. *Vibrio parahemolyticus* challenge demonstrated enhanced expression of *tnfa* in head kidney and blood (Xie et al. 2008). *Tnfa* was also identified in grass carp (*Ctenopharyngodon idella*), and its role in signaling was defined (Zhang et al. 2012). Additionally, *tnfa* is involved in the control of ovulation (Crespo et al. 2010, 2015) (Table 2.25). For more details, please see Chap. 10 (“cytokines”) by Dr. C. Secombes.

### 2.9.5 The Complement System

The mammalian complement system is composed of about 35 plasma and membrane-associated proteins. The main functions of the complement system are opsonization, inflammation, and formation of the cytolytic membrane attack complex. The proteins are mostly produced by liver hepatocytes and secreted to the blood, except for some like factor D and c1q. Several components of the teleost complement contain isoforms like c3, c4, c5, c7, factor B, factor I, and MBL. Most homologs of mammalian complement

**Table 2.25** Innate immunity: tnfa genes in different fish species

Species	Subject	References
Japanese pufferfish	Genomic identification, expression	Biswas et al. (2015)
Turbot	<i>Aeromonas salmonicida</i> infection, immunohistochemical study	Coscelli et al. (2016)
Three striped trumpeter	Cloning, sequencing, expression in response to ectoparasite <i>Chondracanthus goldsmidi</i>	Covello et al. (2009)
Brown trout	Ovulatory mechanism	Crespo et al. (2010)
Brown trout	Ovulation	Crespo et al. (2015)
European sea bass	Oral vaccine adjuvant	Galindo-Villegas et al. (2013)
Rainbow trout	Phylogeny, expression, bioactivity	Hong et al. (2013)
Zebrafish, medaka	Genomic location, expression	Kinoshita et al. (2014)
Tongue sole	Expression, antibacterial and antiviral defense	Li and Zhang (2016)
Review	Regulator in adipose tissue	Liu et al. (2015)
Meagre	Discovery, distribution, expression	Milne et al. (2017)
Atlantic bluefin tuna	Characterization, expression	Pleic et al. (2014)
Atlantic bluefin tuna	Expression, role in acute and chronic parasitic infection	Pleic et al. (2015)
Rainbow trout	Expression of immune genes post-endocytosis of nanostructured tnfa	Puente-Marin et al. (2019a)
Gilthead sea bream, zebrafish	Activator of endothelial cells	Roca et al. (2008)
Turbot	Immunohistochemical distribution	Ronza et al. (2015)
Turbot	Immune response to <i>Enteromyxum scophthalmi</i> (Myxozoan)	Ronza et al. (2015)
Review	TNF and TNF superfamilies	Wiens and Glenney (2011)
Large yellow croaker	Characterization, response to bacterial infection	Xie et al. (2008)
Grass carp	Characterization, immune challenge in vitro and in vivo	Zhang et al. (2012)

components are present in teleosts and have been shown to be expressed in a variety of tissues like the kidney, skin, and intestine. Several reviews on the complement system of teleosts are available (Zhang et al. 2013b; Nakao et al. 2011; Uribe et al. 2011; Boshra et al. 2004, 2006). Additional information of the complement system in fish is given in Chap. 9.

### 2.9.5.1 c3

c3 is the central complement component and has been isolated, purified, and characterized in many teleost species. Recently, four c3 isoforms were purified from the Nile tilapia (*Oreochromis niloticus*) serum and were shown to possess an intrachain thioester bond. All named c3-1, c3-2, c3-3, and c3-4 showed the two-chain polypeptide structure typical of c3 (Abdel-Salam et al. 2014). Forn-Cuni et al. confirmed the presence of three c3 genes and in addition identified five more c3 genes in the zebrafish (*D. rerio*) genome (Forn-Cuni et al. 2014). Maternal immunization of female zebrafish with formalin-killed *A. hydrophila* caused a significant increase in c3 and factor b contents in the mother, a corresponding rise in the offspring, and induced a remarkable increase in the hemolytic activities in both the mother and offspring (Wang et al. 2009). The dojo loach (*Misgurnus anguillicaudatus*) is one of the most commercially cultured fish species in Eastern Asian countries including China, Japan, and Korea. Three isoforms of c3 were discovered in *M. anguillicaudatus* named c3-1, c3-2, and c3-3, respectively. The expression of c3-1 and c3-3 was mainly detected in liver followed by spleen and gonad. The mRNA levels were upregulated in the gill, skin, liver, and spleen after bath infection with *A. hydrophila* (Xu et al. 2018b). Furthermore, the complete nucleotide sequence of c3 from two Antarctic teleosts *Trematomus bernacchii* (two isoforms) and *Chionodraco hamatus* (a single isoform) was determined (Melillo et al. 2015).

Rainbow trout c3a and c5a receptors were cloned and functionally characterized. Both anaphylatoxin receptors were expressed at considerable levels by B cells. Treatment by lipopolysaccharide led to a significant upregulation of both receptors, suggesting that B cells play a role in the development of an inflammatory response (Li et al. 2007).

### 2.9.5.2 Classical Pathway

The classical complement pathway involves c1, c2, and c4. The first complement component (c1) is activated by recognition of antigen-bound immunoglobulins, and proteolytically activates c4 and c2 into c4b and c2a, respectively. Rock bream (*Oplegnathus fasciatus*) is one of the most economically important marine fish species in South Korea, which geographically distribute in the coastal water, especially in coral beds of the Pacific and Indian Ocean. Rock bream complement components c1r and c1s were characterized, and homology analysis showed 73.4% and 58% amino acid identity with orthologs of *Pundamilia nyererei* of Lake Victoria and the Japanese rice fish, *Oryzias latipes*, respectively. c1r was highest expressed in blood and c1s in the liver. The transcription of both components was found to be upregulated in response to pathogenic bacteria *E. tarda* and *S. iniae* and virus (rock bream iridovirus) (Godaheewa et al. 2015).

Grass carp (*C. idella*) is susceptible to *A. hydrophila* infections. In the study of Dang et al., grass carps were given intraperitoneal injections of live *A. hydrophila* and 4, 8, 12, 24, 48, and 72 h after RNA sequencing of spleen tissue was performed. Four to 72 h after infection, the complement system, represented by c2, c3, c4, c5, c8a, c1q, and mbl, was upregulated with a transitory downregulation at 12 h (Dang et al. 2016).

Tissue of *C. idella* was infected with *A. hydrophila*. Two cDNA sequences of *c4* from the common carp (*C. carpio*) were isolated sharing only 32% identity of amino acid level and having distinct binding specificities (Mutsuro et al. 2005).

### 2.9.5.3 Alternative Pathway

Factor B and factor D are both components of the alternative pathway of complement. Following activation of the alternative pathway, factor B is cleaved into Ba and Bb fragments. In rainbow trout, factor B is known to act as a C3 convertase, but the function of the Ba fragment is unknown. The expression patterns of tongue sole (*Cynoglossus semilaevis*) factor B and the biological activity of the Ba fragment were studied by Li and Sun (2017). Expression of *factor B* was high in liver, muscle, and heart and low in intestine, blood, and kidney. Bacterial infection (*E. tarda*, *Pseudomonas fluorescens*, and *V. harveyi*) induced an expression in kidney, spleen, and liver in a time-dependent manner. For the first time, it was found that overexpression of *Ba* significantly reduced bacterial dissemination in fish tissues, indicating that *Ba* possesses antimicrobial activity and may inhibit bacterial infection in fish (Li and Sun 2017).

Rock bream (*Oplegnathus fasciatus*) complement factor D (Cfd) was characterized and expression was analyzed. Factor D encodes 277 amino acids for a 30 kDa polypeptide and was most highly expressed in the liver and spleen. Transcription of *factor D* was upregulated in the spleen by lipopolysaccharide, *S. iniae*, rock bream iridovirus, and poly I:C (Godahewa et al. 2016). Rainbow trout liver seems not to be an important transcription site of the genes *c1q*, *factor B (cfb)*, and *c7-2*. The novel characterized factor D of rainbow trout had 253 amino acids with a molecular weight of 27.2 kDa and shared a sequence identity with its human ortholog of 45% (Kobis et al. 2015).

### 2.9.5.4 Lectin Pathway

The central components of the lectin pathway are MBL and MASPs. Teleost fish often possess several genes encoding different subtypes. Kania et al. (2010) characterized three homologs of mannan-binding lectin (named *MBL H-1*, *MBL H-2*, *MBL H-3*) (Gene: *mb1 and variants*) in the rainbow trout. They were expressed in the spleen, anterior intestine, and liver. *MBL H-1* and *H-3* were also found in the vascular system. *MBL H-1* had the highest expression level in the anterior intestine followed by gill, thymus, and skin, while the highest expression level of *MBL H-2* and *MBL H-3* occurred in the anterior intestine (Kania et al. 2010).

*C. semilaevis* mannan-binding lectin (Mbl)-associated protein 34 (MAP34) and Mbl-associated serine protease 1 (MASP1) are key factors involved in complement activation through MAPs' ability to bind to M1 and MBL. Remarkably, in contrast to a negative regulatory role of mammalian MAP, the teleost *C. semilaevis* Map 34 exerts a positive effect on the activation of the lectin pathway (Li et al. 2016).

### 2.9.5.5 Terminal Pathway

c5, c6, c7, c8, and c9 are the components engaged in building the membrane attack complex. Native c5a of rainbow trout and recombinant infectious hematopoietic necrosis virus glycoprotein (G) fusion protein was constructed to test the adjuvant activity of rainbow trout c5a. At four to sixteen weeks postinjection, the serum Igm antibody levels were higher than those injected with G-protein alone, suggesting that c5a acts as molecular adjuvant in rainbow trout (Wu et al. 2014).

Grass carp (*C. idella*) is an economically important species, and its global production is more than 4.5 million tons per year making it the most highly consumed freshwater fish species in the world. *A. hydrophila* is the causative pathogen of intestinal hemorrhage, which has caused great economic loss in grass carp aquaculture. Fish were intraperitoneally injected with *A. hydrophila*, and the transcriptomic response was tested in the spleen. A total of 1591 genes were upregulated, and 530 were downregulated. *c1*, *c7*, and *c8b* were upregulated indicating activation of the classical pathway (Yang et al. 2016). *c7* was isolated and characterized from grass carp. The predicted amino acid sequence of *c7* cDNA exhibited 55.4% and 48.3% homology with rainbow trout *c7-1* and zebrafish *c7*, respectively. *c7* gene expression was detected in trunk kidney, liver, head kidney, skin, spleen, heart, and intestine. Significant changes in *c7* transcript expression were detected following *A. hydrophila* infection, especially in head kidney and spleen (Shen et al. 2012).

Full-length *c8a* and *c8b* sequences from a cDNA library of rock bream (*Oplegnathus fasciatus*) and their genomic sequences were obtained. Quantitative real-time PCR analysis showed that both components were expressed in all examined tissues, with highest expression in the liver. Pathogen challenge, including *E. tarda*, *S. iniae*, and rock bream iridovirus, led to upregulation of both (Wickramaarachchi et al. 2013).

Complement component c9 is the last component that is involved in the formation of the membrane attack complex on the surface of target cells. The full-length *c9* cDNA sequence was found in the southern catfish (*Silurus meridionalis*) and showed similarity with other teleost fish. The mRNA expression was highest in the liver and observed also in the spleen, head kidney, stomach, and intestine. Intraperitoneal injection of *A. hydrophila* gave upregulation of *c9* in the liver, spleen, and intestine (Fu et al. 2019).

The large yellow croaker *Larimichthys crocea* is one of the most important marine fish in China and East Asian countries. Complement components *c7* and *c9* were characterized by Guo et al. (2016). *c7* and *c9* were mainly expressed in liver, but low levels were also constitutively expressed in most tissues. Fish challenged with *Vibrio alginolyticus* showed a rapidly upregulated response in the liver and head kidney (Guo et al. 2016). Miiuy croaker, *Miichthys miiuy*, belongs to the family Sciaenidae of the order Perciformes and mainly distributes from the western Japan Sea to the East China Sea. In China, it has been widely cultured since the late 1990s for its good taste and high nutritive and medicinal value. A truncated *c9* cDNA sequence encoding 461 amino acids was cloned and characterized in the miiuy croaker (*M. miiuy*). The *c9* of miiuy croaker shows the highest amino acid identity score with fugu *c9* (61%) and the lowest with zebrafish *c9* (36%). The highest levels of transcripts were detected in liver of both healthy and *V. anguillarum*-

infected fish (Meng et al. 2012). Full-length *c9* sequence was identified from a cDNA library of rock bream (*O. fasciatus*), and its genomic sequence was obtained. Quantitative real-time RT-PCR analysis confirmed that *c9* was constitutively expressed in all the examined tissues, with highest expression occurring in the liver. Pathogen challenge including *E. tarda*, *S. iniae*, lipopolysaccharide endotoxin, and rock bream iridovirus led to upregulation of *c9* in liver but resulted in no change in the peripheral blood cells (Wickramaarachchi et al. 2012). The transcriptional expression of central complement components during the ontogeny of the common sole (*Solea solea*) was studied by Ferraresso et al. (2016). The *c2*, *c3*, and *fb* showed a gradual increase in expression between 4 and 33 days post-hatch (dph). *c4* and *masp1* showed no differences in expression during the development, while *c1qb* showed a very high level of expression. Terminal components, *c5*, *c6*, *c7*, *c8*, and *c9*, showed an increase in expression until the onset of metamorphosis and a second increase after metamorphosis (Ferraresso et al. 2016).

#### 2.9.5.6 Complement Regulation

Complement activation is controlled by both fluid phase and membrane inhibitors. Factor I regulates complement by proteolytic cleavage of components *c3b* and *c4b*. Factor H, the main cofactor of factor I, regulates the alternative pathway by acting in the breakdown of *c3b* to *ic3b*. Factor I (*cfi*) and factor H (*cfh*) of rainbow trout were cloned and characterized. The deduced amino acid sequences of factor I and factor H exhibited 42% and 32% identity with human orthologs, respectively (Anastasiou et al. 2011). The deduced amino acid sequence of factor H from large yellow croaker (*Larimichthys crocea*) showed 28% and 34% identity with human and rainbow trout orthologs, respectively. The highest expression levels were found in liver, kidney, and spleen. After injection with *V. alginolyticus*, the expression levels were upregulated in all three tested tissues (Qi et al. 2018a). Black rockfish (*Sebastes schlegelii*) is an important aquaculture species in the Republic of Korea. A *c1 inhibitor gene* from black rockfish was cloned and characterized by Nilojan et al. The *c1 inhibitor* was most highly expressed in the liver followed by the gills (Nilojan et al. 2018) (Table 2.26).

#### 2.9.6 Acute-phase Component

During infection, stimulation with strong danger signals or stress, the fish may respond to produce acute-phase proteins (APPs). Especially, IL-1, IL-6, and *tfa* are able to induce acute-phase response, as observed in higher vertebrates. The most common APPs are pentraxins such as serum amyloid A (SAA) and C-reactive protein (CRP). Dissimilar to many mammalian species, the fish show a modest acute-phase response when it comes to concentration of pentraxins in serum. CRP may be able to bind to (opsonize) certain bacteria, fungi, and parasites, activate the complement system, agglutinating particles, and may infer production of cytokines. There are two forms of SAA, one of them being acute-phase SAA. SAA may neutralize pathogen activity, reduce tissue damage, and

**Table 2.26** Table of complement components found in the listed fish species

Component	Teleost species	References
c1, c1r, c1s	Rock bream Grass carp	Godahewa et al. (2015) Yang et al. (2016)
c1 inhibitor	Black rockfish	Nilojan et al. (2018)
c3	Nile tilapia Common sole Zebrafish Zebrafish Dojo loach <i>Trematomus bernacchii</i> , <i>Chionodraco hamatus</i> Grass carp	Abdel-Salam et al. (2014) Ferraresso et al. (2016) Forn-Cuni et al. (2014) Wang et al. (2009) Xu et al. (2018b) Melillo et al. (2015) Dang et al. (2016)
c3a receptor	Rainbow trout	Li et al. (2007)
c4	Common carp	Mutsuro et al. (2005)
c5	Rainbow trout	Wu et al. (2014)
c5a receptor	Rainbow trout	Li et al. (2007)
c7	Large yellow croaker Grass carp	Guo et al. (2016) Shen et al. (2012)
c8	Rock bream	Wickramaarachchi et al. (2013)
c9	Sothern catfish Miiuy croaker Large yellow croaker Rock bream	Fu et al. (2019) Meng et al. (2012) Guo et al. (2016) Wickramaarachchi et al. (2012)
Factor B	Rock bream Tongue sole	Godahewa et al. (2016) Li and Sun (2017)
Factor D	Rainbow trout Rock bream	Kobis et al. (2015) Godahewa et al. (2016)
Factor H	Rainbow trout Large yellow croaker	Anastasiou et al. (2011) Qi et al. (2018a)
Factor I	Rainbow trout	Anastasiou et al. (2011)
MBL	Rainbow trout	Kania et al. (2010)
MAP/MASP	Tongue sole	Li et al. (2016)
Ontogeny	Common sole	Ferraresso et al. (2016)
Bacterial infection	Grass carp	Dang et al. (2016)

restore homeostasis. Transferrin, haptoglobin, ceruloplasmin, alpha-2-macroglobulin, lectins, and complement component c3 are all considered to be AAPs. Most of these have regulatory activities limiting infection and restoring the physiological balance. Several reviews covering this topic are recommended (Roy et al. 2017; Bayne and Gerwick 2001; de Magalhaes et al. 2020; Magnadottir 2014; Nakao et al. 2011).

## 2.9.7 Chemokines and Their Receptors

The major function of chemokines is to guide the migration of cells. An example is chemokine-guided migration of leukocytes to inflammatory foci. Other functions involve immune surveillance where chemokines direct homing of leukocytes to lymphatic tissues. Some chemokines have function in growth of new blood vessels and wound healing. Chemokines are classified into four main subfamilies (CXC, CC, CX3C, and XC) dependent on the amino acid sequences (first two cysteine amino acid residues). Most of the chemokines bind to specific chemokine receptors on cells. Fish display numerous genes for different chemokines and chemokine receptors, suggested due to gene duplication events. Functional and significance studies of chemokine expression are generally not very well examined in fish. However, exceptions exist. We list the most recent findings within functional chemokine research. It has been shown that grass carp cxcl20b possessed antibacterial activity by attaching to the bacterial membrane (Xiao et al. 2020). In another study, it was shown that common carp Cxcl1 stimulated neutrophil extracellular trap formation—which was suggested to be an antipathogenic event (Pijanowski et al. 2020). Moreover, an ayu (*Plecoglossus altivelis*) CC-like chemokine was found to possess chemotactic activity against monocytes and neutrophils in vivo and in vitro (Yu et al. 2019). Chemotactic activity of Ccl4 has been shown in the golden pompano (*Trachinotus blochii*). This recombinant chemokine had also antimicrobial activity against *E. tarda* and *Escherichia coli* (Sun et al. 2019). Furthermore, a rainbow trout CC chemokine (Ck11) also displayed antimicrobial activity against different gram-positive and gram-negative bacteria by attaching to and disrupting their cell membranes (Munoz-Atienza et al. 2019).

## 2.9.8 Antibacterial Peptides (AMPs)

AMPs are a diverse class of highly conserved molecules that are produced as a first line of defense in all multicellular organisms, including fish. These small peptides (12–50 amino acids) are essential components of innate immunity capable of antimicrobial activity against a broad range of microbial pathogens (Semple and Dixon 2020; Zhang and Gallo 2016). Functionally, they can be described as either membrane disruptive AMPs, which induce membrane permeabilization, or they can be non-membrane disruptive where they can be internalized in cells and act on intracellular targets (Semple and Dixon 2020). In general, fish AMPs may be categorized into five different classes based on their structure:  $\beta$ -defensins, cathelicidins, hepcidins, histone-derived peptides, and piscidins (Brunner et al. 2020). As for chemokines and TLRs, fish possess numerous genes for antimicrobial peptides. Recently, reviews on the significance of AMPs are published (Brunner et al. 2020; Chaturvedi et al. 2020; Valero et al. 2020; Chen et al. 2020; Shabir et al. 2018).



## 2.10 Conclusion and Future Research

It is clear that fish are indeed equipped with an arsenal of defense mechanisms to prevent infection. An earlier report has used Rag knockout mutants (*rag1*<sup>-/-</sup> zebrafish), which possess no serum Igm, to assess the significance of the innate immune system in comparison with control fish with a *rag1*<sup>+/-</sup> genotype. An experimental challenge experiment revealed that the *rag*<sup>-/-</sup> zebrafish displayed similar protection as the controls (Tokunaga et al. 2017). This underscores the notion that the innate immune system alone may likely be as effective as a fully immune-equipped fish. However, immunized fish will normally acquire higher disease resistance than naïve fish. The concept of trained innate memory should be addressed as a trained innate immune system likely would add higher protection level during infection. Trained innate immunity involves activation of innate defense factors that in turn confer increased disease resistance to infection by homologous or heterologous pathogens. Trained immunity can be transferred to offspring as training induces heritable epigenetic changes.

The future will bring a vast more knowledge of innate immune factors through fish genomic and transcriptomic studies, and it is likely that many more innate immune factors will be revealed. To find their significance in the innate immune defense, these must be functionally examined.

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