Chapter

Immune System of Fish: An Evolutionary Perspective

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Abstract

Fishes are the most successful and diverse group of vertebrate animals, first appeared during Devonian period. Despite of certain differences, the immune system of fish is physiologically similar to that of higher vertebrates. The heterogenous group of fishes are the apparent link between innate immunity and the first appearance of the adaptive immune response. Importantly, fishes have immune organs homologous to that of mammalian immune system. In comparison to higher vertebrates, fishes live free in their environment from the early embryonic stage and during that time mostly they are dependent on non-specific immune system for their survival. In the fishes, non-specific immunity is the fundamental defense mechanism, therewith acquired immunity also plays key role in maintaining homeostasis by activation though a system of receptors proteins, which identify pathogen associated molecular pattern typical of pathogenic microorganism includes lipopolysaccharides, peptidoglycans, DNA, RNA and other molecules that are typically not present on the surface of multicellular organism. There are several external factors like environmental factors, biological factors, stress and internal factors like genetic makeup, age and sex, maternal effect etc. can affect immunological defense capabilities of the fishes.

Keywords: Fish immune system, innate immunity, adaptive immune response, defense mechanism, environmental factor

1. Introduction

Evolution has brought many genetical and physiological innovations in animal phyla including alteration in immune mechanism. Immune system of fish is a subject which provides unique insight towards evolution of defense system in vertebrate lineage. Fish as an earliest vertebrate in evolutionary history, has a distinct pattern of immune morphogenesis in comparison to other higher vertebrates. They are heterogeneous group of poikilothermic animals which include jawless fish (e.g., Lamprey) and jawed fish of class Chondrichthyes and Osteichthyes. Their physiology and immune system development vary among them and it is highly influenced by environmental parameters, unlike warm blooded vertebrates. External parameters like photoperiodism, temperature and oxygen concentration of water influence development and functioning of both innate (e.g., Complement, lysozyme activity) and adaptive immunity (e.g., IgM concentration) in fish [1]. Apart from

environmental influence some of the variations are inherited and evolved via genetic alterations. It appears mostly in the adaptive immune mechanism especially in form of genetic recombination process which is the key of diversification of repertoires of lymphocyte based antigen recognition receptors [2]. The role of various genes and organs involved in defense mechanism of jawed and jawless fishes are discussed here in order to provide complete information on progress or innovation in fish immune system.

2. Immunity of agnathans

Despite the diversification, many features of fish immunity i.e., immune gene expression, inflammation, wound healing, antigen pattern recognition receptors, signaling and trafficking of lymphocytes remains conserved across the vertebrate linage. These functions are mostly played by the cellular and humoral factors of the immunity. The agnathans lack hematopoietic organs i.e., spleen, thymus or kidney but they have unique strip of medullary tissue present throughout the length of trunk called Immune body [3]. The dedicated organs for immunity have not been so far detected but some of the area of lamprey typhlosole and renal folds carry hematopoietic stem cells and lymphoid like cells and differentiated cells including thrombocyte, granulocyte, monocyte, and lymphocyte like cells have also been detected [4]. The humeral factors like antimicrobial peptide coding genes i.e., cathelicidin genes has been detected in Atlantic hagfish (*Myxine glutinosa*) [5]. Other innate immunity related genes such as reactive oxygen species modulator I and Peroxiredoxin coding gene and NFκB inhibitor gene are being detected in immune body and other tissues which indicate for the presence of a well-developed innate defense mechanism [6]. The lamprey oral gland also found to secrete many defenses related functional proteins i.e., interferon-induced lethality protein-19 and disintegrins. The components involved in complement activation pathway have been detected in Lamprey [7]. The homologous components like C3, mannose-binding lectin (MBL), and MBL-associated serine proteases (MASP) of the lectin pathway and factor B of the alternative pathway have been identified from lamprey and/or hagfish but the cytolysis process in unique in terms of serum protein named "lamprey pore-forming protein" (LPFP).

The signature molecules of adaptive immunity i.e., MHC genes, T cell receptors and B cell receptors are absent in primitive agnathans but in place there are lot of leucin rich repeats coding sequences indicating an alternative pathway of adaptive immunity [8]. Some of the research has found specific agglutinin-based memory for antigen recognition in Atlantic lamprey and agglutinin secreting cells in the intestine. The lamprey has unique lymphocytes expressing orthologous genes encoding B-cell signaling components i.e., PU.1/Spi-B. The classical VDJ gene recombination process which is required for creating diversifies repertoire of Ig based B cell receptors in higher vertebrates are absent in Agnathans. The Lymphoid like cells has found to express complex LRR carrying molecule called variable lymphocyte receptors (VLR) which under goes subsequent assembly through an entirely novel genomic mechanism in which large banks of LRR cassettes are used to build the 'diversity' region of the receptor molecules [8]. The basic composition of these VLR includes a conserved signal peptide, an N-terminal LRR (LRRNT), followed by nine variable and highly diverse LRRs, a connecting peptide, a C-terminal LRR (LRRCT), and a conserved C terminus (GPI)-anchor site and a hydrophobic tail. Upon antigen induction there is a marked proliferation of hematopoietic lymphoid cells and increased VLR protein receptors for variable antigen detection.

In adult lamprey the VLR gene expression has been detected in typhlosole, opistonephros, supra-neural body and blood. In contrast the pharyngeal regions of larvae or embryos are found to express VLR genes especially in oral tentacles and the gill filaments [9].

3. Immunity of osteichthyes

As per the cellular organization and physiologic requirement there are variations in pattern of immune system ontogeny in different group of fishes. There are many similarities between fish and human immune system but unlike human they have a resilient innate immunity which helps them to survive and adopt to the adverse condition inside water. Fishes do not have bone marrow and lymph nodes but head kidney plays a major role in hematopoiesis as well as direct antimicrobial activity through melanomacrophage centers (MMC). Apart from anterior and middle kidney, thymus and spleen are two important lymphoid organs present in fish [10]. The development pattern of fish lymphoid organs is variable according to the type of fish but we will discuss some of the well-known discoveries related to ontogeny of fish immune system.

The kidney (head and middle), thymus and spleen are the largest lymphoid organ in teleost fishes. The development sequence of lymphoid organ varies between freshwater and marine water fish species [11, 12]. In case of freshwater teleost e. g. carp, tilapia and trout, kidney is the first lymphoid organ to develop and spleen is the last organ. Lymphoid organs of marine fish develop differently in order of kidney, spleen and thymus respectively. In marine water teleost fishes, such as cobia (*Rachycentron canadum)*, Flounder (*Paralichthyus olivaceaus*), Sea bream (*Sparus aurata*), yellow tail (*Seriola deumerili*) and red sea bream (*Pagrus major*) the anterior kidney is the first lymphoid organ to appear followed by spleen and thymus [13, 14]. But in both cases thymus is the first organ to have lymphoid cells followed by kidney and spleen.

3.1 Kidney

In teleost fish, kidney functions similar to bone marrow in the vertebrates and is the largest site of hematopoiesis [11]. Immune cells are present over entire kidney whereas anterior or head kidney has the highest concentration of developing B-lymphoid cells [15]. The anterior kidney is aglomerular and has hematopoietic function [16] and unlike higher vertebrates, it is principal organ for phagocytosis, antigen processing, formation of IgM and immune memory through melanomacrophage centres [17]. In fish, the head kidney serves as an important endocrine organ, homologs to adrenal gland in mammals and release corticosteroids and other hormones [18]. Furthermore, anterior kidney is the major site for antibody production.

Anterior/head kidney is the initial common site for hematopoietic stem cells (HSC) development and differentiation. At early hatching condition rudimentary pronephric kidney use to carry undifferentiated precursor cells even in the absence of any blood islands which are believed to be the first site of pluripotent stem cell formation in mammalian yolk sac. Comparison with human immune system reveals that after migration of precursor cells from fetal liver and spleen, pro-myeloid cell formation occurs in bone marrow for life time and this is why anterior kidney of fish is similar in action to bone marrow of higher vertebrates [19].

In zebrafish a well-developed kidney can be found at 72 hours post fertilization (hpf) but hematopoietic cells appear at 96hpf [20] However this timeframe for appearance of hematopoietic cells may be different in different fishes (**Table 1**).

Species	Appearance of lymphoid organ	Appearance of hematopoietic cell	Appearance of lymphocytes	References
Zebra fish	72hpf	>96hpf	3wpf	[20]
Rainbow trout	<8dbh	5dbh	5dph	[21]
Seabream	<1dph	5dph	54dph	[22]
Channel catfish	NK	<3dph	<7dph	[23]
Common carp	1dph	NK	6dph	[24]

Hpf-hours post fertilization, wpf-week post fertilization, dbh- days before hatch, dph-days post hatch, NK-not known.

Table 1.
Histogenesis of fish kidney.

By gradual differentiation immature precursor cells form cords, an aggregated form of more differentiated HSCs surrounded by blood vessels. These sinusoidal blood vessels are lined by fibroblastic reticular cells. Further development from pronephric to mesonephric kidney supports for the formation of erythroblast, myeloblast and lymphoblast.

3.2 Thymus

The lymphoid cells which are actually major immune blood cells initially are not differentiated in the head kidney. Thymus is the most important lymphoid organ which is found in all vertebrates including chondrichthyes and the osteichthyes but an exception in case of Lamprey and Hagfish which are known to be the primitive vertebrates. However, research for the presence of thymic analogue in lamprey has revealed Thy-1 reactivity which is mainly associated with thymus and Tcell development, has been captured in different tissues including typhlosole, opisthonephros, liver, external gill openings in larval lamprey [25]. Unlike mammals where thymus appears to carry and develop precursor cells migrated from bone marrow for T cells formation, in fish thymus is the first organ to be lymphoid. In fact, undifferentiated cells are found to be migrating from kidney to thymus through collagen fibers of pharyngeal septum during early developing stage of Turbot [13].

Thymus is present near gill arch and is closely associated with the pharyngeal epithelium internally facing towards head kidney. In zebrafish thymus appear as primordial outgrowth of pharyngeal epithelium at 54 hours post fertilization (hpf) (**Table 2**) and a developed thymus carry electro-lucent epithelial cells and mature lymphocytes [20]. The morphology of thymus varies in age dependent manner from species to species and within species. In carps, thymus alters from triangular to irregular shape and even the cortex as well as medulla changes their position. The distinct cortico-medullary junction is not present in all fish. The recombination activating genes (*rag*), which are responsible for rearrangement of immunoglobulin gene and T-cell receptor genes in immature B and T lymphocyte respectively are often used for histological localization of premature thymus. In zebra fish, the *rag1* gene expression at 92hpf distinguishes *rag1*+ cortex and *rag1*- medulla of thymus. Before this period *ikaros* gene which is responsible for lymphocyte differentiation is expressed in thymus at 72hpf [26].

Thymus of teleost is a bilobed homogenous organ placed in a dorsal projection in the epithelium of the operculum cavity and it is lined by mucus tissue of pharyngeal epithelium in structure that surrounds the lymphoid bark tissue is the characteristic

Species	Appearance of lymphoid organ	Appearance of hematopoietic cell	Appearance of lymphocytes	References
Zebrafish	54hpf	65hpf	3dpf	[20]
Rainbow trout	5dbh	1dh	3dph	[21]
Seabream	22-29dph	29-47dph	47dph	[22]
Channel catfish	NK	NK	5dph	[23]
Common carp	3dpf	NK	4-5dpf	[24]

Hpf-hours post fertilization, wpf-week post fertilization, dbh- days before hatch, dpf- days post fertilization, dph-days post hatch, NK-not known.

Table 2.
Histogenesis of fish Thymus:

of the fish thymus [27]. Thymus in the fishes has frequent record of variation in morphology due to the absence of cortico-medullary junction [28]. So, in many species it is not possible to differentiate between cortex and medulla that is found in higher vertebrates [29]. The involution of thymus in fish is more dependent on hormonal cycles and seasonal variations than on the age [18]. Teleost's thymus is much similar to mammalian in which erythrocytes, neutrophils and granulocytes are found in spleen whereas lymphocytes are major cell type found in thymus [18]. Thymus produces T lymphocytes involved in stimulation of phagocytosis, allograft rejection and antibody production by B cells [29].

3.3 Spleen

In teleost, spleen functions as major secondary immune organ, plays major role in the clearance of blood borne antigens and immune complexes in splenic ellipsoids and in the antigen presentation and initiation of adaptive immune response [30]. The size of spleen in fish is widely used as simple measurable immune parameter with potential role in immune response against parasite infections [31].

Spleen is the third important hematopoietic organ which originates in form of mesenchymal cell aggregate surrounded by blood capillaries. It is the third organ to be lymphoid but for a long time it carries erythroid cells only. The expression of Hox11 transcript factor which helps in survival of precursor splenic cells indicates splenic primordium appears during 5 dpf at left anterior gut portion of zebra fish [32], whereas it in rainbow trout it is found at 3dph (**Table 3**). The ellipsoids which are involved in plasma filtration and blood borne antigen trapping, appears at 3 months after hatching of zebrafish. These ellipsoids have narrow lumen which runs through reticular cells and macrophages.

3.4 Appearance of Ig + cells

There is no clear-cut development pattern of Ig + cell in fish but mature B cells are found earlier in freshwater fish in comparison to marine fish. In Atlantic halibut (*Hippoglossus hippoglossus* L.) appearance of first Ig positive cell take time up to 66 dph in kidney (**Table 4**) [33]. Head kidney seems to be the major organ for B cell maturation and IgM production except in zebra fish where pancreas first gets Ig + detection [34]. At 10 dpf Ig transcripts can be located in pancreas of zebra fish and later on (19 dpf) in kidney. In rainbow trout cytoplasmic Ig (cIg) can be detected on 12 dbh followed by surface Ig on 8 dbh [36]. In contrast surface

Species	Appearance of lymphoid organ	Appearance of hematopoietic cell	Appearance of lymphocytes	References
Zebrafish	4dpf	30dpf	3 month	[20, 32]
Rainbow trout	3dph	NK	6dph	[21]
Seabream	12dph	NK	54dph	[22]
Channel catfish	NK	NK	5dph	[23]
Common carp	5dpf	NK	8dpf	[24]

hpf-hours post fertilization, wpf-week post fertilization, dph-days post hatch, days post fertilization, NK-not known.

Table 3.
Histogenesis of fish spleen:

Species	Appearance of Ig + cells	Organ	References
zebrafish	7 dpf	Whole fish	[34]
	10 dpf	pancreas	
Common carp	2 wpf	head kidney	[35]
Rainbow trout	cIg 12 d pre-hatching	head kidney	[36]
	sIg 8 d pre-hatching	head kidney	
Atlantic halibut	66 dph	kidney	[33]

hpf-hours post fertilization, wpf-week post fertilization, dpf- days post fertilization, dph-days post hatch.

Table 4.
Ontogenesis of Ig + cells.

Ig (sIg) is detected earlier (2 wpf) than cytoplasmic Ig + cells (4 wpf) in carp kidney. All investigations indicate that appearance of Ig + cells and immunocompetence development may show variation in time due to temperature and other external factor influence [35].

3.5 Other tissues

Apart from the major hematopoietic organ, there are additional lymphoid tissues in different organs of fish. Expression of *Ikaros,* which is a gene specific for lymphoid cell differentiation, is marked to be present in bilateral patches of brain at 24–96 hpf, heart, intestine and testes [37]. Fish do not have typical lymphocyte accumulation site which is so called Peyer's patches (PP) in mammals but few macrophage-like cells and leukocytes are found in gut. However, mucosa-associated lymphoid tissue (MALT) of fish can be found in different forms like gut associated lymphoid tissue (GALT), Gill associated lymphoid tissue (GIALT), Skin associated lymphoid tissue (SALT), nasal-associated lymphoid tissue (NALT), and the recently discovered buccal and pharyngeal MALTs. GALT is known to carry immunoglobulin expressing cells such as T and B cells in intraepithelial lymphocyte and lamina propria respectively. A maximum number of intraepithelial leukocytes are found in proximal and distal gut portion but their distribution and concentration vary according to species, diet, temperature and other external influence [38]. In teleost hind gut carries most of the Ig positive lymphocytes and the macrophages

associated with gut looks different comparison to kidney and spleen macrophage. These differential immune cells are found at 14 dph in *0reochromis.mossambicus* (Tilapia) and get fully matured during 7 weeks which is quite earlier in comparison to GALT maturation in *Burbus conchonius* (during 20 weeks). Such gut lymphoid cells can be seen during 8dpf in zebrafish whereas in rainbow trout are found in gut epithelial region during 13 dph. Occasionally at the age of 54dpf few lymphocytes like cells are found in gut and skin of sea bream which is a marine fish [35]. Unlike the mammals' fish like Rainbow trout secretes IgM, IgT/IgZ [37] and channel catfish secrets IgD in mucus [38]. These MALT associated Igs specific transcript expression can be detected at 4dpf in whole carp embryo but developed IgM and IgZ are found later during 4–6 weeks post-fertilization.

4. Fish innate immunity

Non-specific immunity found in all living organisms and is the first line of defense against all pathogens, also plays an important role in the activation of adaptive immune response. The cells of the innate system recognize and respond to pathogens in a generic way. It also possesses memory as the host evolves its innate immune components based on evolutionary experience of its ancestors encountering similar pathogens [39]. Innate immunity is commonly divided into three compartments: surface barrier, humoral factors and cellular factors. As the first line of defense, it is not surprising that the majority of the broad-spectrum parameters of innate immunity are highly conserved across species and taxa. In all jawed vertebrates, the innate immune system features a rapid defensive response towards invading pathogens and tissue damage. However, it cannot provide well-directed, specific protection from individual pathogens or long-term immunological memory.

4.1 Surface barrier

Mucus, skin, gills and gastrointestinal (GI) tract acts as first line of barrier to any infection. Layer of mucus present in skin, gills and GI tract entraps microorganisms by continuously sloughing and inhibits colonization. Mucus of fish is toxic to certain microorganism due to presence of some humoral factors. The rate of mucus production increases in response to infection or by physical or chemical irritants [40].

The epidermis of fish skin is composed of non-keratinized living cells and the integrity of these cells plays vital role in maintaining osmotic balance and excluding microorganisms. Rapid healing is also observed in epidermis of fishes [41].

Large surface area of delicate gill epithelium considered as important route of pathogen entry. The gills are protected by mucus production and highly responsive epithelium resulting in hyperplasia, frequently seen in various gill infections. Phagocytic cells line the branchial capillaries, lymphoid cells on the caudal edge of the intrabranchial septum.

GI tract is lined by mucus membrane and also the digestive enzymes, bile and low pH of stomach provides an extremely hostile environment for pathogens.

4.2 Humoral factors

There is array of soluble substances which have protective function which inhibits the growth of microorganisms and neutralizes the enzymes on which pathogen depends. The classification of humoral parameters is commonly based on their pattern recognition specificities or effector functions.

4.2.1 Growth inhibitors

Growth inhibitors acts either by depriving microorganism of essential nutrients or by interfering with their metabolism. Transferrin occurs in serum, exerts a bacteriostatic and fungistatic effect. Transferrin is a protein with high Iron (Fe) binding capacity, which is an essential element for growth of microorganism and deprives them of iron [42]. Pathogenic bacteria may produce their own chelating agents like siderophores to overcome this defense mechanism and hyperferremic activity acting as a counter response has been demonstrated in some fish species. Transferrin is also an acute phase protein invoked during an inflammatory response to remove iron from damaged tissue [42] and an activator of fish macrophages [43]. Interferons are another virus inducible cytokine which induces the expression of Mx and other antiviral proteins [44]. Grinde (1989) studied the antibacterial effect of two lysozyme variants (Types I and II), purified from the head kidney of rainbow trout, on seven Gram-negative bacterial fish pathogens [45]. INFα and β are cytokines with a nonspecific antiviral function that is based on the inhibition of nucleic acid replication within infected cells. Interferons are potent activator of downstream antiviral defenses and the type I Interferons (IFN-α and β) induces expression of wide range of Interferon stimulated genes (ISG) inducing Mx, Viperin, ISG 15, PKR leading to enhanced antiviral state. Type II interferons (IFN- γ) promotes Th 1 cell responses produced primarily by CD4 + Th 1 cells and NK cells. Th 1 cell provide defense against intracellular pathogens such as viruses and bacteria by inducing apoptosis restricting cell proliferation during viral infection. Fish IFN also modulates cytokines and chemokines expression and is potent inducer of proinflammatory cytokines such as IL-1, IL-6, IL-12 and tumor necrosis factor (TNF).

4.2.2 Enzyme inhibitors

Pathogens produce enzymes in order to penetrate and obtain nutrients from their hosts. Tissue fluids and serum of vertebrates contains many enzyme inhibitors which are thought to defend body against autodigestion and also plays an important role in neutralizing enzymes produced by pathogens. Fish plasma contains a number of protease inhibitors, principally α1-antiproteinase and α2-macroglobulin (α2M). Many bacteria produce proteolytic toxins which digest host tissue proteins as a source of amino acids. An important protease produced by *A. salmonicida* is resistant to rainbow trout α1-antiproteinase but is inhibited by α2M [46]. The difference in α2M activity between two different trout species (rainbow trout and brook trout) has been found to correlate with their resistance to *A. salmonicida* infection [46] suggesting that α2M may play a role in defense against furunculosis.

4.2.3 Lysins

Various lytic enzymes either in single or in combination may cause lysis of pathogenic cells. Lysins in fishes include complement, lysozyme and antimicrobial peptides. Lysozyme is the most studied innate response in fish which act on the peptidoglycan layer of bacterial cell walls resulting in the lysis of bacteria [47]. Lysozymes synthesized both in liver and extra hepatic sites and are present in mucus, lymphoid tissue, plasma as well as in other fluids and is also expressed in a wide variety of tissues [48] and involved in a comprehensive defense mechanism, such as bacteriolysis, opsonization, as well as restricted antiviral and antineoplastic activity, as found in higher vertebrates [49].

Studies of the integument and integument secretions of fish [50] have demonstrated an important role of antimicrobial peptides in host defense against viruses and bacteria [51]. These peptides are found in mucus, gills and liver tissue of teleost fishes [52] and include liver expressed antimicrobial peptides (LEAP), Defensins, Piscidins, and Cathelicidin.

Complement system is the biochemical cascade that helps or complements the ability of antibiotics to clear pathogens from the host. Complement system plays major role in the link between both innate and adaptive immune responses that allows an integrated host defense to pathogenic challenges [53]. Complement system plays multiple functions like mediating inflammatory vasodilation, lysis of bacterial cells and infected cells, opsonization to foreign particles to enhance phagocytosis, clearance of apoptotic cells and also in alternation of molecular structure of viruses. The bactericidal activity of complement has been reported in many fishes [54]. Complement system gets activated by three pathways- the classical pathway, which is triggered by antibody binding to the cell surface [55], the alternative pathway, which is independent of antibodies and is activated directly by foreign microorganisms, and the lectin pathway, which is activated by the binding of a protein complex consisting of mannose/mannan-binding lectin in bacterial cells [56].

4.2.4 Agglutinins and precipitins

Mucosal or serum agglutinins and precipitins are lectins like C-type lectins and pentraxins. The C-type lectins have binding capacity for different carbohydrates like mannose, N-acetyl glucosamine or fucose in the presence of Ca ions, and the interaction between carbohydrate binding protein and carbohydrate leads to opsonization, phagocytosis and activation of the complement system [57]. Mannose binding lections (MBL) are the most studied lections which show specificity for mannose, N-acetyl glucosamine, fructose and glucose. Lections, with various carbohydrate specificities, have been isolated from the serum of several fish species [58]. Pentraxins (C-reactive protein, CRP and serum amyloid protein, SAP) are lectins, which are present in the body fluids of both invertebrates and vertebrates and are commonly associated with the acute phase response [59]. Pentraxins are pattern recognition proteins that are important component of acute phase response to infection or injury. Some best known pentraxins are C-reactive protein (CRP) which is known to bind with phosphoryl choline present on many microbial cell wall and Serum amyloid protein (SAP) binds to phosphoethanolamine, glycans and also known to bind LPS of Gram-negative bacteria [60].

4.3 Cellular factors

The cellular components of the fish's innate immune system consist of many different types of cells such as monocytes/macrophages, granulocytes as mast cells/eosinophilic granule cells, and neutrophils, dendritic cells, and natural killer cells (NK cells). When an innate immune cell encounters and recognizes a pathogen through its pathogen-associated molecular pattern (PAMP), the immune cells get activated and can participate in several responses depending on their cell subtype, including phagocytosis and subsequent destruction of pathogens [61].

4.3.1 Macrophages/monocytes

Macrophages are the first cells to arrive and respond to the site of infection. Macrophages are derived from hematopoietic progenitor cells (immature cells), which differentiate through circulating monocytes or via tissue-resident

macrophages namely kuffer cells in liver, glial cells in brain, etc. [62]. Macrophage differentiation is controlled by engagement of the colony-stimulating factor 1 receptor (CSF1R) [63] first identified in the elephant shark (*Callorhinchus milii*) genome [64]. Macrophages in teleost play a role in both the innate and adaptive immune systems and are vital players during inflammation and pathogen infection. In the innate immune system, macrophages destroy pathogens through phagocytosis, reactive oxygen species (ROS) and nitric oxide (NO) production, and the release of several inflammatory cytokines and chemokines, similar to mammalian macrophages [65]. Similar to mammals, teleost fish also have functionally distinct macrophages [66]. In teleost fish species, M1 (classically activated macrophages) are characterized by the production of pro-inflammatory cytokines such as TNFa and IL-1b and production of ROS and NO [67], and these cells may rapidly kill pathogens by engulfment and production of toxic reactive intermediates, phagolysosomal acidification, and restriction of nutrient availability [66]. Whereas M2 are alternatively activated macrophages and are mainly associated with immunosuppression, trauma, and anti-inflammatory cytokines such as interleukin (IL)-10 [68].

4.3.2 Phagocytic B cells

Phagocytosis mediates the primary action of the teleost immune system, is the central effector mechanism of innate immunity, and also plays an essential role in linking the innate and adaptive immune responses in vertebrates. Phagocytosis is an endocytic process of phagocytes by which other cells or particles, including microbial pathogens, are ingested or engulfed to form phagosomes and phagolysosomes, followed by the destruction of the invader or the continued processing of antigenic information, eventually initiating adaptive immunity in vertebrates [69]. Classical phagocytosis is mainly versed by "professional" phagocytes, like macrophages/monocytes, neutrophils, and dendritic cells. Moreover, some "amateur" phagocytes such as epithelial cells and fibroblasts can also internalize antigens particulate to a much lower degree compared to professional phagocytic cells [70]. It is very well known that B cells in all vertebrates are functional antibody-secreting cells (ASCs) for producing specific antibodies in response to certain invading foreign antigens and those them play vital roles in adaptive immunity [71]. It was a long-held paradigm that B cells are non-phagocytic cells, even though evidence has been reported that CD5+ B-cell lymphoma could differentiate to macrophage-like cells [72]. In 2006, for the first time, it was reported that B cells derived from teleost fish and frog are competent of phagocytic and bactericidal activity through the formation of phagolysosome, which was previously only identified in professional phagocytes [73]. Moreover, teleost fish, this novel phagocytic capability of B cells has also been notified into other vertebrates like reptiles [74], mice, and humans [75]. IgM+ B cell is the most abundant immunoglobulin present in the serum of teleost fish and was first reported in rainbow trout (*Oncorhynchus mykiss*) and catfish (*Ictalurus punctatus*) for their characteristic phagocytic and bacteria-killing abilities [73]. In the subsequent study, in rainbow trout the IgM−/IgT+ B-cell subset, which uniquely secretes IgT, gets identified, capable of phagocytic and microbicidal activity [76]. In recent years, the phagocytic B cells of teleost fish have been identified from about ten teleost fishes but were only focused on IgM+ B-cell subsets due to the deficiency of specific mAbs against IgT or IgD in these fish species [69]. The phagocytic activity of IgM+ and IgT+ B cells could be significantly increased after incubation with antiserum or complement-opsonized target particles [77]. The regulatory mechanisms of interleukin IL-6 and IL- 10

are recognized in the phagocytic activity of teleost IgM+ B cells [78], where IL-10 could enhance the phagocytosis of IgM+ B cells in flounder [79]. A number of B Cell receptor (BCR) like mIgM, CD79a, CD79b [80], and other cell receptors, such as Toll-like receptors (TLRs), Retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) and NOD-like receptors (NLRs), which are common pattern recognition receptors (PRRs) of professional phagocytic cells, may also be involved in B-cell phagocytosis [81]. The concurrence of complement and phagocytic B cells indicates the essential importance of B cells in the linkage of innate and adaptive immunity. The highly variable phagocytic abilities for the IgM+ B cells to ingest different microbial particles were also reported in zebrafish (*Danio rerio*), lumpfish (*Cyclopterus lumpus* L.), half-smooth tongue sole (*Cynoglossus semilaevis*), large yellow croaker (*Larimichthys crocea*), and Japanese flounder (*Paralichthys olivaceus*) [82]. Teleost phagocytic B cells study is still at an early stage, and more efforts are required for further detailed investigation of immune functions in teleosts.

4.3.3 NK cells

Non-specific cytotoxic (NCC) cells are akin to mammalian natural killer (NK) cells, but they do not contain cytoplasmic granules like NK cells and having pleomorphic clefted nucleus with little cytoplasm with different killing mechanism [83]. They share several similarities, mainly the competent lytic cycle, the target cells for lysis, recognition of target cell, and the effecters to lyse the infectious microorganisms [84]. In almost all fish species, NK cells or NK-like functional activities have been described [85]. Cells with NCC activity are primarily present in the blood, lymphoid tissues, and the gut. NCC needs to physically contact target cells without membrane fusions or fragmentation [86]. The smallest leucocyte NCC targets various cells, including tumor cells, transformed cells, virus-transformed cells, and protozoa parasites [87]. The killing is spontaneous, non-specific, and does not require any apparent induction period. NCCs are reported to be most active in the head kidney of teleosts, but spleen and peripheral blood leukocytes (PBL) also demonstrate cytolytic abilities [88]. The NCC activities are influenced by age, strain, temperature, stress, and activity are more pronounced when specific responses are less active.

4.3.4 Stromal cells

Stromal cells are connective tissue cells of organs that act in a supportive capacity to the parenchymal cells performing specific organ functions. During the last decade, when the complexity and function of stromal cells were revealed in immune functions, the stromal cells were considered "non-hematopoietic immune cells" before that it was merely known for providing a structural framework upon which hematopoietic immune cells could function [89]. The growing evidence suggests that non-hematopoietic stromal cells exhibit a capacity for diverse cell intrinsic and extrinsic immune function in many non-lymphoid tissues, including the intestine, where it plays multiple immune responses inflammation at this mucosal site [90]. Intestinal stromal cells are non-professional immune cells that recognize bacteria and other cells via TLR or NLR and modulate T-cell function [91]. Stromal cells have various mechanisms to directly sense bacterial contact, respond rapidly on contact with pathogen proving protective immune response, and respond to cytokine signals from the epithelium and thus amplify both protective and potential deleterious immune responses [92].

4.3.5 Red blood cells

Unlike mammalian cells, fish red blood cells are nucleated and contain organelles in their cytoplasm [93]. The nucleated fish red blood cells are well known for gaseous exchange but recently their new biological role in immune response has been reported [94]. Nucleated red blood cells (RBCs) of fish contain the transcriptional and translational machinery necessary to produce characteristic molecules of the immune system to respond against various infectious agents and play an active role in maintaining homeostasis of the fish immune system [95]. The nucleated RBC are reportedly involved in both innate and adaptive immune responses in fish [96]. Nucleated RBCs are able to phagocytose, acts as antigen-presenting cells [97, 98], recognizes pathogen associated molecular pattern (PAMPs) by specific pathogen recognition receptors (PRRs), modulate leukocyte activity, release cytokine-like factors [99, 100] and also induces interferon in fish [101]. The expression of immune-relevant genes in RBC had shown a wide repertoire of TLRs in *Salmo salar* and *Oncorhynchus mykiss*, which allow them to respond to both bacterial and viral infections [95]. However, to know more about the involvement of RBC in immune response, more studies are required and several researchers are working on it.

4.3.6 Intestinal cells

The gastrointestinal tract cells function in digestion and maintain immune homeostasis to protect the body from potentially harmful microbes and induce a tolerogenic response to innocuous food, commensals, and self-antigens. Fish have local mucosal defense in the gut to sample antigens and produce local immunoglobulin responses [102]. Leucocytes are abundantly present in the fish gut's lamina propria and intestinal epithelium [103]. The indication of specific antibody secretion in the fish intestine comes after intestinal or immersion immunization of various fish species, which were rarely detectable after systemic immunization [104]. Immunoglobulins (Ig) produced in the intestine are a result of local synthesis was get confirmed after intravenous administration of radiolabeled Ig, which never reached the mucosal secretions. Ig isotype (IgT) is specialized for mucosal immunity, and in trout fish, the IgT response to a gut parasite is restricted to the intestine [102]. The Polymeric immunoglobulin receptor (pIgR), an essential component of mammalian mucosal immunity, has also been described in few fish species [105]. Ig + B cells and Ig-T cells are abundantly present in fish's gut, but limited data is available regarding their functional relevance [106].

The fish intestine, especially the posterior segment, is immunologically active and armored with various immune cell types, including B cells, macrophages, granulocytes, and T cells.

4.3.7 Fish gill

Diseases associated with gill damage, cause substantial losses in the aquaculture industry not only through an increased mortality rate among fish but also through impaired growth and also by increased treatment and sanitation cost. Damage to gill tissues is specially characterized by inflammation and increased epithelial cells hyperplasia or hypertrophy. A gill epithelium of salmonids has higher number of MHC class II positive cells [107] whereas low number of macrophages like cells has been detected in gill epithelium of presumably healthy salmonid fish [108].

5. Conclusions

Fish immunity although similar to other higher organisms, there is differences owing to their natural habitat. Fish are a heterogeneous group of poikilothermic animals consist of jawless fish and jawed fish of class Chondrichthyes and Osteichthyes. Their physiology and immune system development vary among them and is highly influenced by environmental parameters, unlike warm blooded vertebrates. Here we highlighted the development of immune system in different class of fish along with components of immune system.

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Conflict of interest

The authors declare no conflict of interest.

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