Chapter

Macrophage: A Key Player of Teleost Immune System

Ragini Sinha

Abstract

Fish, the free-living organisms, residing in aquatic environment, are earliest vertebrates with fully developed innate and adaptive immunity. Immune organs homologous to those of mammalian immune system are found in fish. Macrophages are best known for their role in immunity, basic function of which being cytokine production and phagocytosis. Due to environmental adaptation and whole genome duplication, macrophages in teleost are differently modulated (pro-inflammatory, M1-type, and anti-inflammatory/regulatory, M2-type) and perform a variety of different functions as compared with those of mammals. Phagocytosis is a major mechanism for removing pathogens and/or foreign particles in immune system and therefore is a critical component of the innate and adaptive immune system. One of the most competent phagocytes in teleost is found to be macrophages/monocytes. Increasing experimental evidence demonstrates that teleost phagocytic cells can recognize and destroy antigens to elicit adaptive immune responses that involve multiple cytokines. A detail understanding of teleost macrophages and phagocytosis would not only help in understanding the immune mechanism but will also help in disease prevention in teleost.

Keywords: inflammatory response, cytokine production, macrophages, phagocytosis, teleost

1. Introduction

Fish, the first vertebrate group, appeared in evolution after adaptive radiation during the Devonian period, presenting the most successful and diverse group of vertebrates. Importantly, immune organs homologous to those of the mammalian system are found in fish. This population possesses complicated innate immune networks and are the earliest vertebrates that have fully developed both arms of the immune system, i.e., innate and adaptive immunity [1]. Macrophage lineage cells are integral to fish immune responses like any other vertebrate, and hence, recent fish immunology research focuses on fish macrophage biology. Macrophages are one of the most important immune cells that bridge the innate and adaptive immunity. It plays a crucial role in tight regulation of immune response by secreting different immune mediators [2, 3]. Macrophages are present in most animal tissues and play crucial roles in host protection and homeostasis. They are known by different names such as amebocytes, hemocytes, coelomocytes, granulocytes, monocytes, and macrophages,

but have similar morphology and comparable functions [4–6]. Due to whole genome duplication and environmental adaptation, teleost monocyte/macrophages possess a variety of different functions and modulation compared with those of mammals. The basic functions of macrophages are production of cytokines and phagocytosis in vertebrates. Monocytes give rise to macrophages during inflammatory conditions in both mammals and fish [7]. Macrophages play multiple roles in immune system. Macrophages are potent innate immune cells, which exert a crucial antimicrobial defense through phagocytosis and release of different antimicrobial mediators, including reactive oxygen and nitrogen species (ROS and RNS). Additionally, they also serve as professional antigen presenting cells (APCs) to activate the adaptive immune system (T and B cells) [8]. Macrophages pose the phagocytic activity, which is the initial step in the immune response in fish and is the major line of defense for all foreign material, including pathogenic agents [9]. Measurement of macrophage activation serves as a bio-indicator and reveals the impact of environmental stress as well as chemical contamination of the aquatic bodies.

2. Development of macrophages

Teleost blood cell development occurs within primitive waves of hematopoiesis [7]. In mammals, macrophages are predominantly derived from the hematopoietic precursors born in the yolk sac (YS) and the aorta-gonad-mesonephros (AGM) where embryonic and adult hematopoiesis occurs [10–14]. Likewise, macrophages originate from the rostral blood island (RBI) and ventral wall of dorsal aorta (VDA), the fish hematopoietic tissue equivalent to the mammalian YS and AGM for myelopoiesis, respectively [15–19]. During primitive hematopoiesis, embryonic mesoderm becomes committed to produce monopotent hematopoietic precursors in the rostral blood island that give rise to macrophages [20–22]. Following monopoiesis, first multilineage progenitor cells arise, known as erythromyloid progenitors (EMPs), which can develop into both erythroid and myeloid cells. Later, a population of hematopoietic stem cells (HSCs) arises in the AGM. The existence of renal marrow-derived HSCs has been documented in both zebrafish and ginbuna carp [23, 24]. The progenitor cells that are found in the kidney have been shown to be able to differentiate into erythrocytes, lymphocytes, thrombocytes, granulocytes, and monocytes. Monocytes mainly exist in the bone marrow, blood, and spleen. They can differentiate into inflammatory macrophages and dendritic cells during inflammation [25, 26]. Macrophages reside in a variety of tissues including lymphoid and non-lymphoid ones. Until recently, tissue macrophages were believed to arise from circulation monocyte precursors in response to different stimuli [27]. Recent evidence by fate-mapping blood cell lineages suggests that contribution of monocytes is limited in maintaining the population of tissue macrophages. Instead, tissue macrophages are “seeded” during primary haematopoiesis and self-maintain the resident population like that of the mammals [28–30]. There is a specific group of cytokines that act as hematopoietic group of cytokine, which can regulate the development of multiple cell lineages and can act individually or concurrently to stimulate a specific response. Hematopoietic cytokines are produced by a variety of cell types, which can act in paracrine, endocrine, juxtracrine, or autocrine manner on the target cells for their renewal and development [31, 32]. Cytokine sensitivity is determined by a complex regulatory network, a hematopoietic cytokine may induce different developmental changes in different circumstances. Specific cell lineage can be responsive to certain cytokines.

3. Role of transcription factor in macrophage development

The regulation of hematopoiesis is carried out in and orchestrated manner involving cell-cell and cell-extracellular matrix. Transcription factors play a critical role in determining the fate of development of macrophages. Transcription factors are DNA-binding proteins that recognize specific domains. Improper expression of transcription factors and activity results in serious consequences within the hematopoietic system including inhibition of proliferation [33, 34]. Synergistic interactions between transcription factors are generally required for the activation of specific genes. Apart from that, negative interaction between transcription factors is also necessary for the control of hematopoiesis [31].

3.1 Role of colony-stimulating factor-1

Macrophage colony-stimulating factor-1 (CSF-1) is an important growth and differentiation factor of both fish and mammalian macrophages [35]. The survival, proliferation, differentiation, and functionality of most of the macrophage lineage cells are governed by CSF-1 through binding to its cognate receptor (CSF-1R). CSF-1R is expressed exclusively on committed myeloid precursors and derivative macrophage populations [36–42]. CSF-1 has recently been identified in several fish species including trout [43], zebrafish [43], and goldfish [43, 44]. Recombinant trout CSF-1 was found to promote the proliferation of trout head kidney leucocytes [43]. Reports suggest that the recombinant goldfish CSF-1 (rg-CSF-1) induced chemotactic response and enhanced antimicrobial functions of macrophages. It plays a central role in regulation of goldfish pro-inflammatory macrophage responses [35]. Many teleost fish species have two distinct CSF-1 genes (CSF-1.1 and CSF-1.2) [43], which happen to work by upregulating pro-inflammatory components [45, 46]. A variety of cytokines can induce the production of CSF-1 by monocytes and macrophages, such as GM-CSF [47], TNF-α [48], IL-1 [49, 50], and INF-γ [51, 52]. The capacity of monocyte/macrophages to produce CSF-1 suggests that these cells can auto-regulate their own proliferation and functions [31]. CSF-1 also stimulates the production of several cytokines including G-CSF, GM-CSF, IL-1, IL-6, IL-8, and TNF-α and interferons [38, 53]. Cyprinid fish produce a soluble CSF-1 receptor (sCSF-1R) that downregulates their pro-inflammatory responses by reducing available soluble CSF-1. The sCSF-1R is produced by mature macrophages and not by monocytes and efficiently removes a variety of inflammatory events including macrophage chemotaxis, phagocytosis, and production of ROS intermediates and recruitment of leukocytes [54]. Circulating CSF-1 can effectively be cleared by the process of CSF-1 receptor-mediated internalization followed by intracellular destruction of the growth factor. Liver and splenic macrophages have been demonstrated to be capable of absorbing approximately 94% of the circulating CSF-1 [55, 56]. Adding CSF-1 to primary cultures has proven to increase the longevity of the cultures and can drive the culture from a heterogeneous population of progenitor monocyte and macrophage cells, toward a homogeneous population of macrophages [57].

4. Activation of macrophages

Macrophage activation occurs under various intracellular as well as environmental influences. Based on the activation cue and the following effector functions,

macrophages have been broadly classified in two types: classically activated macrophages (M1) induced in a T helper 1 (T_H1) cytokine environment and alternatively activated macrophages (M2) induced in a T helper 2 (T_H2) cytokine environments [58]. In a different terminology, M1 macrophages have been termed to be "inflammatory," whereas M2 macrophages have been termed to be "healing" in nature. There have been studies indicating four different phenotypes of macrophages, which are innate activated, classically activated, and alternatively activated and regulatory macrophages. Classically activated macrophages present higher respiratory burst activity and iNOS expression as compared with innate activated macrophages [59]. Macrophages that are activated by microbial stimulus and innate danger signals without any influence of adaptive immune cells lead to the formation of the M1 population [5, 59]. M2 macrophages that form in the presence of T_H2 cytokines can again be classified into three groups: activated by IL-4/IL-13 or M2a macrophages [60], stimulated by Toll-like receptor (TLR) ligands in combination with second signal or M2b, developed in response to IL-10 or M2c [60].

4.1 M1 macrophage activation

Innate activation of M1 macrophages is induced by microbial stimulus, which can be detected by various receptors on the macrophage surface [61]. These microbial stimuli can activate macrophages through a large array of pattern recognition receptors (PRRs) [62]. Fish species poses a wide variety of PRRs both putative mammalian orthologues and fish-specific family members [63] and can be activated in the absence of exogenous cytokines. M1 macrophages are induced by pathogen associated molecular patterns (PAMPs) such as lipopolysaccharides (LPSs), a major component of outer membrane of Gram-negative bacteria [5, 6]. A number of publications show that in vitro stimulation of fish macrophages with LPS leads to increased respiratory burst activity and increased secretion of pro-inflammatory cytokines [64]. Classically activated macrophages require a microbial stimulus plus the presence of the cytokine INFγ (**Figure 1**) [65]. INFγ has been sequenced in fugu [66], rainbow trout [67], zebrafish [68], Atlantic salmon [69], catfish [70], common carp [71], goldfish [72], Atlantic cod [73], and flounder [74]. Certain fish species possess two distinct types of INFs. Both the isoforms, initially named INFγ1 and INFγ2, contain typical INFγ motifs and are now referred to as INFγ- related (INFγrel) and INFγ, respectively [75]. In carp and in grass carp, both isoforms are regulated by different stimuli [71, 76], *in vivo* bacterial infection in zebrafish embryo indicated that INFγ and INFγrel act partly redundantly, they have largely overlapping functions [77]. Goldfish INFγrel induced significantly higher phagocytosis and nitrite production in monocytes and macrophages, respectively, when compared with INFγ [72]. Research studies suggest that most probably INFγrel proteins are antiviral proteins without direct effects on M1/M2 polarization in fish [78]. It is particularly notable that certain teleosts possess two INFγ-receptor-binding chains (IFNGR1-1 and IFNGR1-2) in comparison to other vertebrates that have a single INFγ receptor 1 (INFGR1) [72, 79, 80]. These suggest that fish have adopted very unique strategies surrounding their M1 activation cytokine system. INFγ as a combination stimulus with LPS induces inflammatory M1 population. These macrophages show higher respiratory burst activity and nitric oxide synthase expression [62].

Classically activated macrophages are induced by a combination of INFγ and TNFα [81, 82]. Like its mammalian counterpart, teleost TNFα is one of the markers of M1 macrophages [83, 84]. Multiple isoforms of TNFα have been found in a

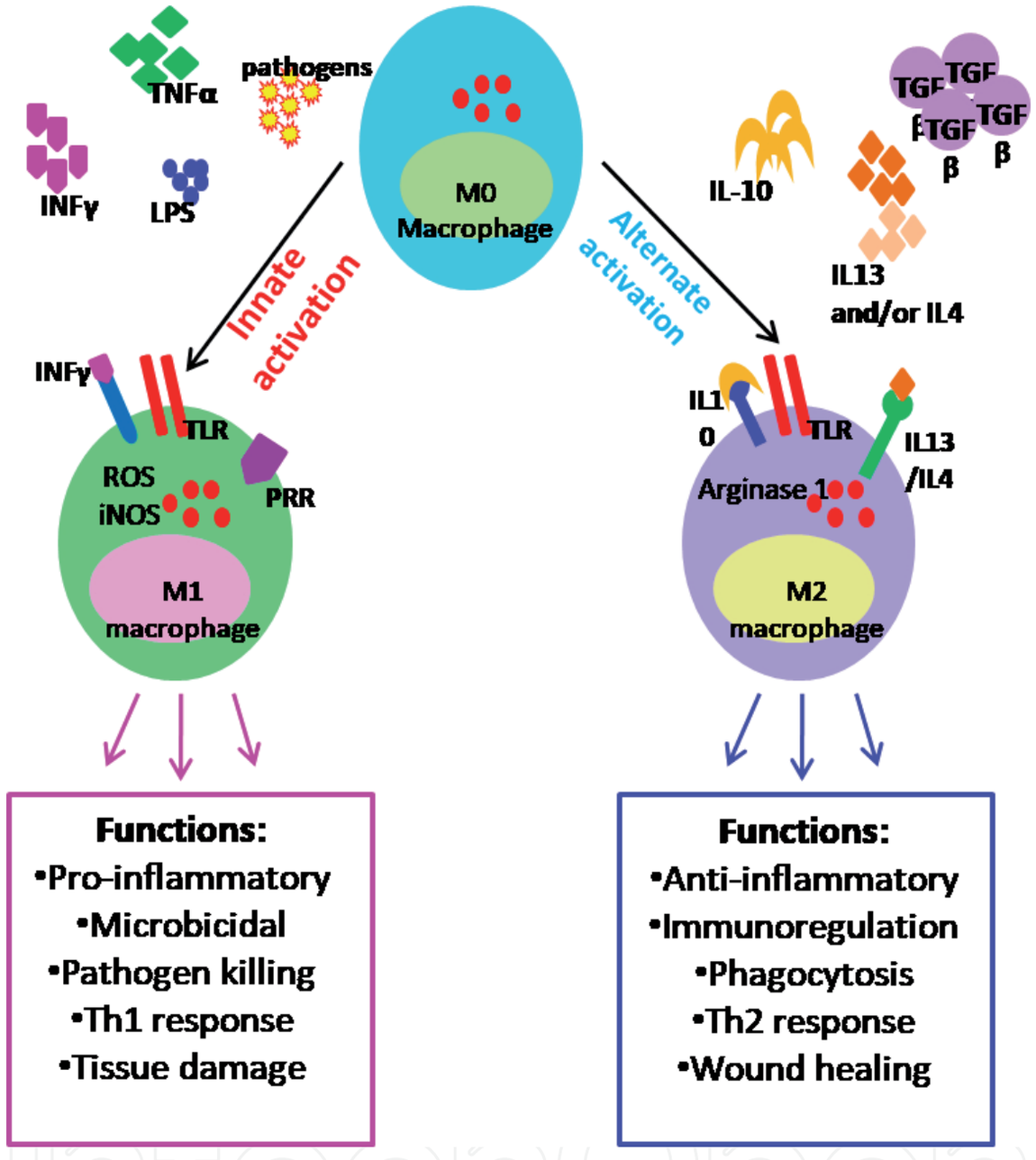

Figure 1.
M1 and M2 macrophage activation.

variety of fishes. These isoforms have been shown to enhance inflammatory gene expressions, macrophage chemotaxis, and phagocytosis [85–96]. Functional evaluation of fish TNFα has discovered some contradictory results. In some fish species, recombinant TNFα (rTNFα) was found to hardly activate macrophages [97–99], whereas the trout and goldfish TNFα1 and 2 are shown to be active in macrophages [87, 100]. Two different TNF receptors have been found in goldfish, namely TNF-R1 and TNF-R2, which bind the goldfish TNFα1 and TNFα2 in a homodimeric conformation unlike the trimeric conformations of mammalian TNF ligands and receptors [101]. The bacterial LPS readily induces the TNFα gene expression, which in turn plays a major role in polarizing the macrophages [99, 102]. From different studies it is clearly understood that fish possess a well-defined M1 polarization upon microbial stimuli.

4.2 M2 macrophage activation

M2 macrophages also known as alternatively activated macrophages can be generally characterized as having "anti-inflammatory" or "pro-healing" phenotypes (**Figure 1**) when developed in the presence of T_H2 cytokines IL-4 and/or IL-13 [103]. To date, at least two genes have been identified in fish that share homology with both the mammalian IL-4 and IL-13 cytokines (IL-4/13A and IL-4/13B) [104] even though variable number of copies of these genes are present in different fish due to genome duplication events [105]. Of the two may be IL-4/13A shows complete synteny with other genes in T_H2 cytokine complex [106]. There is a common homodimeric receptor subunit called IL-4Rα for both cytokines (IL-4 and IL-13) found in mammalian vertebrates [107], paralogues of which, IL-13Rα1 and IL-13Rα2, have also been identified in teleosts [108, 109]. Teleost recombinant IL-4/13A and IL-4/13B have anti-inflammatory roles including upregulation of immunosuppressive genes (TGF-β, IL-10, SAP1, and SOC3) and downregulation of pro-inflammatory cytokine gene expressions (TNFα, IL-1β, and INFγ) [110–112]. These M2 macrophages show increased arginase activity. In M1 macrophage, the iNOS enzyme converts L-arginine to L-cutrulin and NO. By contrast, in M2 macrophages, the enzyme arginase, a manganese metallo-enzyme, converts L-arginine to L-ornithine and urea [113, 114]. Mammals possess two arginase isoforms including arginase-1 located in cytosol and arginase-2 located in mitochondria [115]. Teleosts possess both the forms arginase-1 and arginase-2, which are found to be mitochondrial forms unlike that of their vertebrate counterparts. In carp, arginase-1 gene expression was found mainly in the mid kidney, whereas arginase-2 expression was found in all organs with the liver having the maximum expression [116]. Under stimulation of exogenous cAMP, carp head kidney-derived macrophages show upregulation of arginase-2 but not arginase-1 expression, suggesting that arginase-2 might be an excellent marker of M2 macrophages in fish.

M2 macrophages that are deactivated by glucocorticoids or by cytokines such as TGF-β or IL-10 are also referred to as regulatory macrophages. Glucocorticoids diffuse across plasma membrane and alter the expression of immune-related genes [60]. It has been shown to be a strong inhibitor of NO production in goldfish macrophages [117] and increases fish susceptibility to diseases due to its immunosuppressive nature [118–120]. Grass carp recombinant IL-10 and recombinant TGF-β1 have found to attenuate LPS-stimulated inflammatory gene expressions in monocyte/macrophages [121]. The goldfish TGFβ downregulates the nitric oxide response of TNFα-activated macrophages [122]. Mammalian IL-10 functions through IL-10R1 and IL-10R2 leading to activation of STAT3 [123]. Similar to mammalian IL-10, carp IL-10 acts through a signaling pathway involving phosphorylation of STAT3 and leading to upregulation of SOCS-3 expression [124]. An IL-10R1 has been found in zebrafish, goldfish, and grass carp [125, 126], whereas IL-10R2 has been found in rainbow trout [127]. These cytokines demonstrate an evolutionary conserved role in fish immunology.

5. Function of macrophages

Macrophages and monocytes serve as professional phagocytes in fish [128]. Phagocytosis is a specific type of endocytic process by which cell engulfs solid particulate targets. These solid particles (including microbial pathogens) are internalized to form phagolysosome followed by antigen degradation [129–132]. Phagocytosis plays

an essential role of linking the innate and adaptive immune response in vertebrates. It is well established that fish have both the innate and adaptive immune system in which macrophages happen to play a crucial role. The phagocytic mechanism depends on recognition of the foreign particle by cell surface receptors and killing by oxygen radicals [133, 134]. Phagocytosis plays a crucial role in the macrophage inflammatory immune response through hydrophobic interaction between the phagocytic membrane and the target particles. The multiple receptors present on the phagocyte can recognize their targets coated with opsonin molecules and form the phagosome by engulfing them [135]. Lysosome then fuses with the phagosome to form the phagolysosome, the vesicles in which the internalized microbes would be killed and degraded. Potent antimicrobial compounds including degradative enzymes (proteases, nucleases, phosphatases, lipases) and antimicrobial peptides (basic proteins and neutrophilic peptides) are generated by active phagocytes, which help in destruction of the phagocytosed pathogens [136–141]. Both M1 and M2-type macrophages form phagolysosomes. Reports suggest that M1 macrophages form a phagosome with relatively neutral pH as compared with M2 macrophages that form phagosomes with acidic pH [142]. Macrophages are known to be "professional" phagocytes along with polymorphonuclear cells (PMNs), monocytes, and dendritic cells in vertebrates. Apart from this, some "amateur" phagocytic cells (epithelial cells, fibroblasts, and B lymphocytes) show a lower degree phagocytic activity [129, 143]. Research suggests that succinate is critical in controlling phagocytosis in macrophages. Exogenous methyl-succinate was found to enhance phagocytosis, pro-inflammatory cytokine production, and expression of phagocytic genes [46].

The destruction of the internalized microorganism occurs by robust production of ROS (reactive oxygen species) by active macrophages. The multi-component enzyme NADPH assembles on the phagosome membrane during macrophage respiratory burst, which transfers electrons from NADPH to molecular oxygen-producing superoxide anion [144]. The functional sites of fish and mammalian NADPH oxidase are highly conserved. All of the components of NADPH oxidase have been found in teleosts, and fish ROS generation has been well documented following PAMP stimulation [145–148] and antimicrobial responses [149, 150].

Classically activated M1 macrophages abundantly express high levels of inducible nitric oxide synthase enzyme iNOS, which catalyze the conversion of L-arginine to L-citruline, resulting in the production of nitric oxide (NO) [151]. iNOS serves as a marker of M1 macrophage and is upregulated in response to INFγ, TNFα, and microbial compounds [82]. The fish iNOS has been characterized with marked similarity to the mammalian enzyme counterpart. The fish iNOS gene expression is induced by antimicrobial and inflammatory stimuli including cleaved transferring products [152, 153]. iNOS plays an important role in protection of fish from a variety of pathogens.

Another hallmark of M1 macrophages is upregulation of the expression of indoleamine2,3-dioxygenase (IDO) enzyme that depletes local tryptophan levels [154]. Tryptophan degradation produces certain metabolites that may inhibit T cell proliferation. Teleost IDO is less effective in tryptophan degradation as compared with their mammalian counterparts [155].

6. Conclusion

Teleosts are found throughout the world and are highly susceptible to variations caused by natural as well as man-made external changes, which affect their immune

system. Macrophages are one of the basic immune cells found in teleosts like their mammalian counterparts, which play a crucial role in bridging the innate and adaptive immunity in fish. Macrophages of teleost fish exhibit many functions from that of homeostasis to host immune defense. They possess the phagocytic activity, which is initial step of defense in fish immunity. Measurement of macrophage activation serves as a bioindicator of fish health. Teleosts have shown to have different macrophage polarizations (M1 and M2) pathways under different stimuli, which provides a great support in understanding the evolutionary development of fish immune system. Despite having multiple isoforms of key macrophage cytokines in fish, functional studies of these have been limited. Whole-genome duplication events are responsible for the availability of multiple isoforms of immune mediators in different fish [156]. A greater understanding of teleost macrophages and their function with growing genetic resources would help widely in deciphering the minutes of fish immune system and its evolutionary linkage with that of their mammalian counterparts.

Acknowledgements

RS is thankful to the Council of Scientific and Industrial Research (CSIR), Human Resource Development Group, Govt. of India for her NET-SRF fellowship (Grant No. 09/202(0078)/2018-EMR-I). The author is thankful to Prof. Dipak Kumar Mandal for his guidance and support and to Monisha Das and Nibedita Sharma for their constant encouragement.

Conflict of interest

The author declares no conflict of interest.

Note/thanks/other declarations

The author thanks the Head of the Department of Zoology, for providing the assistance in the research work.

Author details

Ragini Sinha
Department of Zoology, Visva-Bharati University, Santiniketan, West Bengal, India

*Address all correspondence to: raginniissinha5@gmail.com

References

[1] Zhu L, Nie L, Zhu G, Xiang L, Shao J. Advances in research of fish immune-relevant genes: A comparative overview of innate and adaptive immunity in teleosts. Developmental and Comparative Immunology. 2013;**39**:39-62. DOI: 10.1016/j.dci.2012.04.001

[2] Rajaram MV, Ni B, Dodd CE, Schlesinger LS. Macrophage immunoregulatory pathways in tuberculosis. Seminars in Immunology. 2014;**26**:471-485. DOI: 10.1016/j.smim.2014.09.010

[3] Oishi Y, Manabe I. Macrophages in inflammation, repair and regeneration. International Immunology. 2018;**30**:511-528. DOI: 10.1093/intimm/dxy054

[4] Bilej M, De Baetselier P, Beschin A. Antimicrobial defense of the earthworm. Folia Microbiologica. 2000;**45**(4):283-300. DOI: 10.1007/BF02817549

[5] Wiegertjes GF, Wentzel AS, Spaink HP, Elks PM, Fink IR. Polarization of immune responses in fish: The 'macrophages first' point of view. Molecular Immunology. 2016;**69**(3):146-156. DOI: 10.1016/j.molimm.2015.09.026

[6] Lu XJ, Chen J. Specific function and modulation of teleost monocytes/macrophages: Polarization and phagocytosis. Zoological Research. 2019;**40**(3):146-150

[7] Hodgkinson JW, Grayfer L, Belosevic M. Biology of bony fish macrophages. Biology. 2015;**4**(4):881-906. DOI: 10.3390/biology4040881

[8] Joerink M, Ribeiro CMS, Stet RJM, Hermsen T, Savelkoul HFJ, Wiegertjes GF. Head kidney-derived macrophages of common carp (*Cyprinus carpio* L.) show plasticity and functional polarization upon differential stimulation. The Journal of Immunology. 2006;**177**(1):61-69. DOI: 10.4049/jimmunol.177.1.61

[9] Bennani N, Alliana AS, Lafaurie M. Evaluation of phagocytic activity in a teleost fish, *Dicentrachus labrax*. Fish and Shellfish Immunology. 1995;**5**:237-246. DOI: 10.1016/S1050-4648(05)80017-8

[10] Boisset JC, van Cappellen W, Andrieu-Soler C, Galjart N, Dzierzak E, Robin C. In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium. Nature. 2010;**464**(7285):116-120. DOI: 10.1038/nature08764

[11] de Bruijn MF, Speck NA, Peeters MC, Dzierzak E. Definitive hematopoietic stem cells first develop within the major arterial regions of the mouse embryo. The EMBO Journal. 2000;**19**(11):2465-2474. DOI: 10.1093/emboj/19.11.2465

[12] Medvinsky A, Dzierzak E. Definitive hematopoiesis is autonomously initiated by the AGM region. Cell. 1996;**86**(6):897-906. DOI: 10.1016/s0092-8674(00)80165-8

[13] Bertrand JY, Jalil A, Klaine M, Jung S, Cumano A, Godin I. Three pathways to mature macrophages in the early mouse yolk sac. Blood. 2005;**106**(9):3004-3011. DOI: 10.1182/blood-2005-02-0461

[14] Palis J, Chan RJ, Koniski A, Patel R, Starr M, Yoder MC. Spatial and temporal emergence of high proliferative potential hematopoietic precursors during murine embryogenesis. Proceedings of the National Academy of Sciences of the United States of America.

2001;**98**(8):4528-4533. DOI: 10.1073/pnas.071002398

[15] Herbomel P, Thisse B, Thisse C. Ontogeny and behaviour of early macrophages in the zebrafish embryo. Development. 1999;**126**(17):3735-3745

[16] Herbomel P, Thisse B, Thisse C. Zebrafish early macrophages colonize cephalic mesenchyme and developing brain, retina, and epidermis through a M-CSF receptor-dependent invasive process. Developmental Biology. 2001;**238**(2):274-288. DOI: 10.1006/dbio.2001.0393

[17] Jin H, Sood R, Xu J, et al. Definitive hematopoietic stem/progenitor cells manifest distinct differentiation output in the zebrafish VDA and PBI. Development. 2009;**136**(4):647-654. DOI: 10.1242/dev.029637

[18] Xu J, Du L, Wen Z. Myelopoiesis during zebrafish early development. The Journal of Genetics and Genomics. 2012;**39**(9):435-442. DOI: 10.1016/j.jgg.2012.06.005

[19] Yu T, Guo W, Tian Y, Xu J, Chen J, Li L, et al. Distinct regulatory networks control the development of macrophages of different origins in zebrafish. Blood. 2017;**129**(4):509-519. DOI: 10.1182/blood-2016-07-727651

[20] Bertrand JY, Kim AD, Violette EP, Stachura DL, Cisson JL, Traver D. Definitive hematopoiesis initiates through a committed erythromyeloid progenitor in the zebrafish embryo. Development. 2007;**134**:4147-4156. DOI: 10.1242/dev.012385

[21] Lieschke GJ, Oates AC, Paw BH, Thompson MA, Hall NE, Ward AC, et al. Zebrafish SPI-1 (PU.1) marks a site of myeloid development independent of primitive erythropoiesis: Implications for axial patterning. Development Biology. 2002;**246**:274-295. DOI: 10.1006/dbio.2002.0657

[22] Willett CE, Cortes A, Zuasti A, Zapata AG. Early hematopoiesis and developing lymphoid organs in the zebrafish. Developmental Dynamics. 1999;**214**:323-336

[23] Kobayashi I, Kuniyoshi S, Saito K, Moritomo T, Takahashi T, Nakanishi T. Long-term hematopoietic reconstitution by transplantation of kidney hematopoietic stem cells in lethally irradiated clonal ginbuna crucian carp (*Carassius auratus* langsdorfii). Developmental and Comparative Immunology. 2008;**32**:957-965. DOI: 10.1016/j.dci.2008.01.006

[24] Traver D, Winzeler A, Stern HM, Mayhall EA, Langenau DM, Kutok JL, et al. Effects of lethal irradiation in zebrafish and rescue by hematopoietic cell transplantation. Blood. 2004;**104**:1298-1305. DOI: 10.1182/blood-2004-01-0100

[25] Ray R, Rai V. Lysophosphatidic acid converts monocytes into macrophages in both mice and humans. Blood. 2017;**129**(9):1177-1183. DOI: 10.1182/blood-2016-10-743757

[26] Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. Nature Reviews Immunology. 2011;**11**(11):762-774. DOI: 10.1038/nri3070

[27] Van Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. Mononuclear phagocytic system: New classification of macrophages, monocytes and of their cell line. Bulletin of World Health Organization. 1972;**47**:651-658

[28] Ginhoux F, Jung S. Monocytes and macrophages: Developmental pathways and tissue homeostasis. Nature Reviews Immunology. 2014;**14**:392-404. DOI: 10.1038/nri3671

[29] Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, et al. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. Immunity. 2013;**38**:792-804. DOI: 10.1016/j.immuni.2013.04.004

[30] Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. Immunity. 2013;**38**:79-91. DOI: 10.1016/j.immuni.2012.12.001

[31] Hanington PC, Tam J, Katzenback BA, Hitchen SJ, Barreda DR, Belosevic M. Development of macrophages of cyprinid fish. Developmental and Comparative Immunology. 2009;**33**(4):411-429. DOI: 10.1016/j.dci.2008.11.004

[32] Ihle JN. Pathways in cytokine regulation of hematopoiesis. Annals of the New York Academy of Sciences. 2001;**938**:129-130. DOI: 10.1111/j.1749-6632.2001.tbo3581.x

[33] Gewirtz AM, Calabretta B. A c-myb antisense oligodeoxynucleotide inhibits normal human hematopoiesis in vitro. Science. 1988;**242**(4883):1303-1306. DOI: 10.1126/science.2461588

[34] Anfossi G, Gewirtz AM, Calabretta B. An oligomer complementary to c-myb-encoded mRNA inhibits proliferation of human myeloid leukemia cell lines. Proceedings of the National Academy of Sciences of the United States of America. 1989;**86**(9):3379-3383. DOI: 10.1073/pnas.86.9.3379

[35] Grayfer L, Hanington PC, Belosevic M. Macrophage colony-stimulating factor (CSF-1) induces pro-inflammatory gene expression and enhances antimicrobial responses of goldfish (*Carassius auratus* L.) macrophages. Fish and Shellfish Immunology. 2009;**26**(3):406-413. DOI: 10.1016/j.fsi.2008.12.001

[36] Garceau V, Smith J, Paton IR, Davey M, Fares MA, Sester DP, et al. Pivotal advance: Avian colony-stimulating factor 1 (CSF-1), interleukin-34 (IL-34), and CSF-1 receptor genes and gene products. The Journal of Leukocyte Biology. 2010;**87**:753-764. DOI: 10.1189/jlb.0909624

[37] Hanington PC, Wang T, Secombes CJ, Belosevic M. Growth factors of lower vertebrates: Characterization of goldfish (*Carassius auratus* L.) macrophage colony-stimulating factor-1. Journal of Biological Chemistry. 2007;**282**:31865-31872. DOI: 10.1074/jbc.M706278200

[38] Pixley FJ, Stanley ER. CSF-1 regulation of the wandering macrophage: Complexity in action. Trends in Cell Biology. 2004;**14**:628-638. DOI: 10.1016/j.tcb.2004.09.016

[39] Petit J, Embregts CWE, Forlenza M, Wiegertjes GF. Evidence of trained immunity in a fish: Conserved features in carp macrophages. The Journal of Immunology. 2019;**203**(1):216-224. DOI: 10.4049/jimmunol.1900137

[40] Dai XM, Ryan GR, Hapel AJ, Dominguez MG, Russell RG, Kapp S, et al. Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. Blood. 2002;**99**:111-120. DOI: 10.1182/blood.V99.1.111

[41] Guilbert LJ, Stanley ER. Specific interaction of murine colony-stimulating factor with mononuclear phagocytic cells. Journal of Cell Biology. 1980;**85**:153-159. DOI: 10.1083/jcb.85.1.153

[42] Lichanska AM, Browne CM, Henkel GW, Murphy KM, Ostrowski MC, McKercher SR, et al. Differentiation of the mononuclear phagocyte system during mouse embryogenesis: The role of transcription factor PU.1. Blood. 1999;**94**:127-138. DOI: 10.1182/blood.V94.1.127.413k07_127_138

[43] Wang T, Hanington PC, Belosevic M, Secombes CJ. Two macrophage colony-stimulating factor genes exist in fish that differ in gene organization and are differentially expressed. The Journal of Immunology. 2008;**181**(5):3310-3322. DOI: 10.4049/jimmunol.181.5.3310

[44] Neumann NF, Barreda DR, Belosevic M. Generation and functional analysis of distinct macrophage sub-populations from goldfish (*Carassius auratus* L.) kidney leukocyte cultures. Fish and Shellfish Immunology. 2000;**10**:1-20. DOI: 10.1006/fsim.1999.0221

[45] Grayfer L, Kerimoglu B, Yaparla A, Hodgkinson JW, Xie J, Belosevic M. Mechanisms of fish macrophage antimicrobial immunity. Frontiers in Immunology. 2018;**9**:1105. DOI: 10.3389/fimmu.2018.01105

[46] Yang DX, Yang H, Cao YC, Jiang M, Zheng J, Peng B. Succinate promotes phagocytosis of monocytes/macrophages in teleost fish. Frontiers in Molecular Biosciences. 2021;**8**:644957. DOI: 10.3389/fmolb.2021.644957

[47] Horiguchi J, Warren MK, Kufe D. Expression of the macrophage-specific colony-stimulating factor in human monocytes treated with granulocyte macrophage colony-stimulating factor. Blood. 1987;**69**:1259-1261

[48] Oster W, Lindemann A, Horn S, Mertelsmann R, Herrmann F. Tumor necrosis factor (TNF)-alpha but not TNF-beta induces secretion of colony stimulating factor for macrophages (CSF-1) by human monocytes. Blood. 1987;**70**:1700-1703

[49] Harrington M, Konicek BW, Xia XL, Song A. Transcriptional regulation of the mouse CSF-1 gene. Molecular Reproduction and Development. 1997;**46**:39-44 [discussion 5]. DOI: 10.1002/(SICI)1098-2795(199701)46:1<39::AID-MRD7>3.0.CO;2-S

[50] Gruber MF, Williams CC, Gerrard TL. Macrophage-colony-stimulating factor expression by anti-CD45 stimulated human monocytes is transcriptionally up-regulated by IL-1 beta and inhibited by IL-4 and IL-10. The Journal of Immunology. 1994;**152**:1354-1361

[51] Horiguchi J, Warren MK, Ralph P, Kufe D. Expression of the macrophage specific colony-stimulating factor (CSF-1) during human monocytic differentiation. Biochemical and Biophysics Research Communication. 1986;**141**:924-930. DOI: 10.1016/s0006-291x(86)80131-0

[52] Oster W, Lindemann A, Mertelsmann R, Herrmann F. Regulation of gene expression of M-, G-, GM-, and multi-CSF in normal and malignant hematopoietic cells. Blood Cells. 1988;**14**:443-462

[53] Chitu V, Stanley ER. Colony-stimulating factor-1 in immunity and inflammation. Current Opinion in Immunology. 2006;**18**:39-48. DOI: 10.1016/j.coi.2005.11.006

[54] Rieger AM, Hanington PC, Belosevic M, Barreda DR. Control of CSF-1 induced inflammation in teleost fish by a soluble form of the CSF-1 receptor. Fish and Shellfish Immunology. 2014;**41**:45-51. DOI: 10.1016/j.fsi.2014.03.035

[55] Bartocci A, Mastrogiannis DS, Migliorati G, Stockert RJ, Wolkoff AW, Stanley ER. Macrophages specifically regulate the concentration of their own growth factor in the circulation. Proceedings of the National Academy of Sciences. 1987;**84**:6179-6183

[56] Tushinski RJ, Oliver IT, Guilbert LJ, Tynan PW, Warner JR, Stanley ER. Survival of mononuclear phagocytes depends on a lineage-specific growth factor that the differentiated cells selectively destroy. Cell. 1982;**28**:71-81. DOI: 10.1016/0092-8674(82)90376-2

[57] Hanington PC, Hitchen SJ, Beamish LA, Belosevic M. Macrophage colony stimulating factor (CSF-1) is a central growth factor of goldfish macrophages. Fish and Shellfish Immunology. 2009;**26**(3):406-413. DOI: 10.1016/j.fsi.2008.12.001

[58] Forlenza M, Fink IR, Raes G, Wiegertjes GF. Heterogeneity of macrophage activation in fish. Developmental and Comparative Immunology. 2011;**35**:1246-1255. DOI: 10.1016/j.dci.2011.03.008

[59] Gordon S. Alternative activation of macrophages. Nature Reviews Immunology. 2003;**3**:23-35. DOI: 10.1038/nri978

[60] Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends in Immunology. 2004;**25**(12):677-686. DOI: 10.1016/j.it.2004.09.015

[61] Janeway CA Jr, Medzhitov R. Innate immune recognition. Annual Review of Immunology. 2002;**20**:197-216. DOI: 10.1146/annurev.immunol.20.083001.084359

[62] Taylor PR, Martinez-Pomares L, Stacey M, Lin HH, Brown GD, Gordon S. Macrophage receptors and immune recognition. Annual Reviews of Immunology. 2004;**23**:901-944. DOI: 10.1146/annurev.immunol.23.021704.115816

[63] van der Vaart M, Spaink HP, Meijer AH. Pathogen recognition and activation of the innate immune response in zebrafish. Advances in Hematology. 2012;**2012**:159807. DOI: 10.1155/2012/159807

[64] Rieger AM, Barreda DR. Antimicrobial mechanisms of fish leukocytes. Developmental and Comparative Immunology. 2011;**35**:1238-1245. DOI: 10.1016/j.dci.2011.03.009

[65] Dalton DK, Pitts-Meek S, Keshav S, Figari IS, Bradley A, Stewart TA. Multiple defects of immune cell function in mice with disrupted interferon gamma genes. Science. 1993;**259**(5102):1739-1742. DOI: 10.1126/science.8456300

[66] Zou J, Yoshiura Y, Dijkstra JM, Sakai M, Ototake M, Secombes C. Identification of an interferon gamma homologue in Fugu, *Takifugu rubripes*. Fish and Shellfish Immunology. 2004;**17**:403-409. DOI: 10.1016/j.fsi.2004.04.015

[67] Zou J, Carrington A, Collet B, Dijkstra JM, Yoshiura Y, Bols N, et al. Identification and bioactivities of IFN-γ in rainbow trout *Oncorhynchus mykiss*: The first Th1- type cytokine characterized functionally in fish. The Journal of Immunology. 2005;**175**:2484-2494. DOI: 10.4049/jimmunol.175.4.2484

[68] Igawa D, Sakai M, Savan R. An unexpected discovery of two interferon gamma-like genes along with interleukin (IL)-22 and -26 from teleost: IL-22 and -26 genes have been described for the first time outside mammals. Molecular Immunology. 2006;**43**:999-1009. DOI: 10.1016/j.molimm.2005.05.009

[69] Robertsen B. The interferon system of teleost fish. Fish and Shellfish Immunology. 2006;**20**:172-191. DOI: 10.1016/j.fsi.2005.01.010

[70] Milev-Milovanovic I, Long S, Wilson M, Bengten E, Miller NW, Chinchar VG. Identification and expression analysis of interferon gamma genes in channel catfish. Immunogenetics. 2006;**58**:70-80. DOI: 10.1007/s00251-006-0081-x

[71] Stolte EH, Savelkoul HFJ, Wiegertjes G, Flik G, van Kemenade BM. Differential expression of two interferon-γ genes in common carp (*Cyprinus carpio* L.). Developmental and Comparative Immunology. 2008;**32**:1467-1481. DOI: 10.1016/j.dci.2008.06.012

[72] Grayfer L, Garcia EG, Belosevic M. Comparison of macrophage antimicrobial responses induced by type II interferons of the goldfish (*Carassius auratus* L.). Journal of Biological Chemistry. 2010;**285**:23537-23547

[73] Furnes C, Seppola M, Robertsen B. Molecular characterisation and expression analysis of interferon gamma in Atlantic cod (*Gadus morhua*). Fish and Shellfish Immunology. 2009;**26**:285-292. DOI: 10.1016/j.fsi.2008.12.002

[74] Jung CY, Hikima J, Ohtani M, Jang HB, del Castillo CS, Nho SW, et al. Recombinant interferon-γ activates immune responses against *Edwardsiella tarda* infection in the olive flounder, *Paralichthys olivaceus*. Fish and Shellfish Immunology. 2012;**33**:197-203. DOI: 10.1016/j.fsi.2012.04.015

[75] Savan R, Ravichandran S, Collins JR, Sakai M, Young HA. Structural conservation of interferon gamma among vertebrates. Cytokine & Growth Factor Reviews. 2009;**20**(2):115-124. DOI: 10.1016/j.cytogfr.2009.02.006

[76] Chen WQ, Xu QQ, Chang MX, Zou J, Secombes CJ, Peng KM, et al. Molecular characterization and expression analysis of the IFN-gamma related gene (IFN-gammarel) in grass carp *Ctenopharyngodon idella*. Veterinary Immunology and Immunopathology. 2010;**134**(3-4):199-207. DOI: 10.1016/j.vetimm.2009.09.007

[77] Sieger D, Stein C, Neifer D, van der Sar AM, Leptin M. The role of gamma interferon in innate immunity in the zebrafish embryo. Disease Model and Mechanism. 2009;**2**(11-12):571-581. DOI: 10.1242/dmm.003509

[78] Shibasaki Y, Yabu T, Araki K, Mano N, Shiba H, Moritomo T, et al. Peculiar monomeric interferon gammas, IFN _rel 1 and IFN _rel 2, inginbuna crucian carp. FEBS Journal. 2014;**281**:1046-1056. DOI: 10.1111/febs.12666

[79] Aggad D, Stein C, Sieger D, Mazel M, Boudinot P, Herbomel P, et al. In vivo analysis of IFN-γ1 and IFN-γ2 signaling in zebrafish. The Journal of Immunology. 2010;**185**:6774-6782. DOI: 10.4049/jimmunol.1000549

[80] Yabu T, Toda H, Shibasaki Y, Araki K, Yamashita M, Anzai H, et al. Antiviral protection mechanisms mediated by ginbuna crucian carp interferon gamma isoforms 1 and 2 through two distinct interferon γ-receptors. Journal of Biochemistry. 2011;**150**:635-648. DOI: 10.1093/jb/mvr108

[81] Mosser DM. The many faces of macrophage activation. The Journal of Leukocyte Biology. 2003;**73**(2):209-212. DOI: 10.1189/jlb.0602325

[82] Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nature Reviews Immunology. 2008;**8**(12):958-969. DOI: 10.1038/nri2448

[83] Nguyen-Chi M, Laplace-Builhe B, Travnickova J, Luz-Crawford P, Tejedor G, Phan QT, et al. Identification of polarized macrophage subsets in zebrafish. Elife. 2015;**4**:e07288. DOI: 10.7554/eLife.07288

[84] Ronza P, Losada AP, Villamarin A, Bermudez R, Quiroga MI. Immunolocalization of tumor necrosis factor alpha in turbot (*Scophthalmus maximus*, L.) tissues. Fish and Shellfish Immunology. 2015;**45**:470-476. DOI: 10.1016/j. fsi. 2015.04.032

[85] García-Castillo J, Pelegrín P, Mulero V, Meseguer J. Molecular cloning and expression analysis of tumor necrosis factor α from a marine fish reveal its constitutive expression and ubiquitous nature. Immunogenetics. 2002;**54**:200-207. DOI: 10.1007/s00251-002-0451-y

[86] Hirono I, Nam BH, Kurobe T, Aoki T. Molecular cloning, characterization, and expression of TNF cDNA and gene from Japanese flounder *Paralychthys olivaceus*. The Journal of Immunology. 2000;**165**:4423-4427. DOI: 10.4049/jimmunol.165.8.4423

[87] Grayfer L, Walsh JG, Belosevic M. Characterization and functional analysis of goldfish (*Carassius auratus* L.) tumor necrosis factor-alpha. Developmental and Comparative Immunology. 2008;**32**:532-543. DOI: 10.1016/j.dci.2007.09.009

[88] Kadowaki T, Harada H, Sawada Y, Kohchi C, Soma GI, Takahashi Y, et al. Two types of tumor necrosis factor-α in bluefin tuna (*Thunnus orientalis*) genes: Molecular cloning and expression profile in response to several immunological stimulants. Fish and Shellfish Immunology. 2009;**27**:585-594. DOI: 10.1016/j.fsi.2008.12.006

[89] Laing KJ, Wang T, Zou J, Holland J, Hong S, Bols N, et al. Cloning and expression analysis of rainbow trout *Oncorhynchus mykiss* tumour necrosis factor-α. European Journal of Biochemistry. 2001;**268**:1315-1322. DOI: 10.1046/j.1432-1327.2001.01996.x

[90] Lam FWS, Wu SY, Lin SJ, Lin CC, Chen YM, Wang HC, et al. The expression of two novel orange-spotted grouper (*Epinephelus coioides*) TNF genes in peripheral blood leukocytes, various organs, and fish larvae. Fish and Shellfish Immunology. 2011;**30**:618-629. DOI: 10.1016/j.fsi.2010.12.011

[91] Nascimento DS, Pereira PJB, Reis MIR, do Vale A, Zou J, Silva MT, et al. Molecular cloning and expression analysis of sea bass (*Dicentrarchus labrax* L.) tumor necrosis factor-α (TNF-α). Fish and Shellfish Immunology. 2007;**23**:701-710. DOI: 10.1016/j.fsi.2010.12.011

[92] Ordás MC, Costa MM, Roca FJ, López-Castejón G, Mulero V, Meseguer J, et al. Turbot TNFα gene: Molecular characterization and biological activity of the recombinant protein. Molecular Immunology. 2007;**44**:389-400. DOI: 10.1016/j.molimm.2006.02.028

[93] Saeij JP, Stet RJ, Groeneveld A, van Kemenade LB, van Muiswinkel WB, Wiegertjes GF. Molecular and functional characterization of a fish inducible-type nitric oxide synthase. Immunogenetics.

2000;**51**:339-346. DOI: 10.1007/s002510050628

[94] Savan R, Kono T, Igawa D, Sakai M. A novel tumor necrosis factor (TNF) gene present in tandem with the TNF-α gene on the same chromosome in teleosts. Immunogenetics. 2005;**57**:140-150. DOI: 10.1007/s00251-005-0768-4

[95] Zhang A, Chen D, Wei H, Du L, Zhao T, Wang X, et al. Functional characterization of TNF-α in grass carp head kidney leukocytes: Induction and involvement in the regulation of NF-κB signaling. Fish and Shellfish Immunology. 2012;**33**:1123-1132. DOI: 10.1016/j.fsi.2012.08.029

[96] Hong S, Li R, Xu Q, Secombes CJ, Wang T. Two types of TNF-α exist in teleost fish: Phylogeny, expression, and bioactivity analysis of type-II TNF-α3 in rainbow trout *Oncorhynchus mykiss*. The Journal of Immunology. 2013;**191**:5959-5972. DOI: 10.4049/jimmunol.1301584

[97] Wang T, Secombes CJ. The cytokine networks of adaptive immunity in fish. Fish and Shellfish Immunology. 2013;**35**(6):1703-1718. DOI: 10.1016/j.fsi.2013.08.030

[98] Roca FJ, Mulero I, López-Muñoz A, Sepulcre MP, Renshaw SA, Meseguer J, et al. Evolution of the inflammatory response in vertebrates: Fish TNF-alpha is a powerful activator of endothelial cells but hardly activates phagocytes. The Journal of Immunology. 2008;**181**:5071e81. DOI: 10.4049/jimmunol.181.7.5071

[99] Forlenza M, Magez S, Scharsack JP, Westphal A, Savelkoul HF, Wiegertjes GF. Receptor-mediated and lectin-like activities of carp (*Cyprinus carpio*) TNFalpha. The Journal of Immunology. 2009;**183**:5319e32. DOI: 10.4049/jimmunol.0901780

[100] Zou J, Peddie S, Scapigliati G, Zhang Y, Bols NC, Ellis AE, et al. Functional characterisation of the recombinant tumor necrosis factors in rainbow trout, *Oncorhynchus mykiss*. Developmental and Comparative Immunology. 2003;**27**:813e22. DOI: 10.1016/s0145-305x(03)00077-6

[101] Grayfer L, Belosevic M. Molecular characterization of tumor necrosis factor receptors 1 and 2 of the goldfish (*Carassius aurutus* L.). Molecular Immunology. 2009;**46**:2190-21999. DOI: 10.1016/j.molimm.2009.04.016

[102] Roca FJ, Mulero I, Lopez-Munoz A, Sepulcre MP, Renshaw SA, Meseguer J, et al. Evolution of the inflammatory response in vertebrates: Fish TNF-alpha is a powerful activator of endothelial cells but hardly activates phagocytes. The Journal of Immunology. 2008;**181**(7):5071-5081. DOI: 10.4049/jimmunol.181.7.5071

[103] Gordon S, Martinez FO. Alternative activation of macrophages: Mechanism and functions. Immunity. 2010;**32**(5):593-604. DOI: 10.1016/j.immuni.2010.05.007

[104] Ohtani M, Hayashi N, Hashimoto K, Nakanishi T, Dijkstra JM. Comprehensive clarification of two paralogous interleukin 4/13 loci in teleost fish. Immunogenetics. 2008;**60**:383-397. DOI: 10.1007/s00251-008-0299-x

[105] Wang T, Secombes CJ. The evolution of IL-4 and IL-13 and their receptor subunits. Cytokine. 2015;**75**:8-13. DOI: 10.1016/j.cyto.2015.04.012

[106] Yamaguchi T, Takizawa F, Fischer U, Dijkstra JM. Along the axis between Type 1 and Type 2 immunity: Principles conserved in evolution from fish to mammals. Biology (Basel).

2015;**4**(4):814-859. DOI: 10.3390/biology4040814

[107] Mueller TD, Zhang JL, Sebald W, Duschl A. Structure, binding, and antagonists in the IL-4/IL-13 receptor system. Biochimica et Biophysica Acta. 2002;**1592**(3):237-250. DOI: 10.1016/s0167-4889(02)00318-x

[108] Wang T, Huang W, Costa MM, Martin SAM, Secombes CJ. Two copies of the genes encoding the subunits of putative interleukin (IL)-4/IL-13 receptors, IL-4Rα, IL-13Rα1 and IL-13Rα2, have been identified in rainbow trout (*Oncorhynchus mykiss*) and have complex patterns of expression and modulation. Immunogenetics. 2011;**63**:235-253. DOI: 10.1007/s00251-010-0508-2

[109] Zhu L, Pan P, Fang W, Shao J, Xiang L. Essential role of IL-4 and IL-4Rα interaction in adaptive immunity of zebrafish: Insight into the origin of Th2-like regulatory mechanism in ancient vertebrates. The Journal of Immunology. 2012;**188**:5571-5584. DOI: 10.4049/jimmunol.1102259

[110] Wang T, Johansson P, Abos B, Holt A, Tafalla C, Jiang Y, et al. First in-depth analysis of the novel Th2-type cytokines in salmonid fish reveals distinct pat-terns of expression and modulation but overlapping bioactivities. Oncotarget. 2016;**7**:10917-10946

[111] Yang ZJ, Li CH, Chen J, Zhang H, Li MY, Chen J. Molecular characterization of an interleukin-4/13B homolog in grass carp (*Ctenopharyngodon idella*) and its role in fish against Aeromonas hydrophila infection. Fish and Shellfish Immunology. 2016;**57**:136-147. DOI: 10.1016/j.fsi.2016.08.022

[112] Stocchi V, Wang T, Randelli E, Mazzini M, Gerdol M, Pallavicini A, et al. Evolution of Th2 responses: Characterization of IL-4/13 in sea bass (*Dicentrarchus labrax* L.) and studies of expression and biological activity. Scientific Reports. 2017;**7**:2240

[113] Green SJ, Crawford RM, Hockmeyer JT, Meltzer MS, Nacy CA. Leishmania major amastigotes initiate the L-arginine-dependent killing mechanism in IFN-gamma-stimulated macrophages by induction of tumor necrosis factor-alpha. The Journal of Immunology. 1990;**145**:4290-4297

[114] Barksdale AR, Bernard AC, Maley ME, Gellin GL, Kearney PA, Boulanger BR, et al. Regulation of arginase expression by T-helper II cytokines and iso-proterenol. Surgery. 2004;**135**:527-535. DOI: 10.1016/j.surg.2003.10.007

[115] Jenkinson CP, Grody WW, Cederbaum SD. Comparative properties of arginases. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 1996;**114**:107-132. DOI: 10.1016/0305-0491(95)02138-8

[116] Joerink M, Savelkoul HFJ, Wiegertjes GF. Evolutionary conservation of alternative activation of macrophages: Structural and functional characterization of arginase 1 and 2 in carp (*Cyprinus carpio* L.). Molecular Immunology. 2006;**43**:1116-1128. DOI: 10.1016/j.molimm.2005.07.022

[117] Wang R, Belosevic M. The in vitro effects of estradiol and cortisol on the function of a long-term goldfish macrophage cell line. Developmental and Comparative Immunology. 1995;**19**:327-336. DOI: 10.1016/0145-305x(95)00018-o

[118] Maule AG, Tripp RA, Kaattari SL, Schreck CB. Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus*

tshawytscha). The Journal of Endocrinology. 1989;**120**:135-142. DOI: 10.1677/joe.0.1200135

[119] Pickering AD, Pottinger TG. Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without reducing the white blood cell count. The Journal of Fish Biology. 1985;**27**:611-619. DOI: 10.1111/j.1095-8649.1985.tb03206.x

[120] Maciuszek M, Rydz L, Świtakowska I, Verburg-van Kemenade BML, Chadzińska M. Effects of stress and cortisol on the polarization of carp macrophages. Fish and Shellfish Immunology. 2019;**94**:27-37. DOI: 10.1016/j.fsi.2019.08.064

[121] Wei H, Yin L, Feng S, Wang X, Yang K, Zhang A, et al. Dual-parallelinhibition of IL-10 and TGF-beta1 controls LPS-induced inflammatory responsevia NF-kappaB signaling in grass carp monocytes/macrophages. Fish and Shellfish Immunology. 2015;**44**:445-452. DOI: 10.1016/j.fsi.2015.03.023

[122] Haddad G, Hanington PC, Wilson EC, Grayfer L, Belosevic M. Molecular and functional characterization of goldfish (*Carassius auratus* L.) transforming growth factor beta. Developmental and Comparative Immunology. 2008;**32**:654e63. DOI: 10.1016/j.dci.2007.10.003

[123] Ferrante CJ, Leibovich SJ. Regulation of macrophage polarization and wound healing. Advances in Wound Care. 2012;**1**:10-16

[124] Piazzon MC, Savelkoul HS, Pietretti D, Wiegertjes GF, Forlenza M. CarpIl10 has anti-inflammatory activities on phagocytes, promotes proliferation ofmemory T cells, and regulates B cell differentiation and antibody secretion. The Journal of Immunology. 2015;**194**:187-199. DOI: 10.4049/jimmunol.1402093

[125] Grayfer L, Belosevic M. Identification and molecular characterization of the interleukin-10 receptor 1 of the zebrafish (*Danio rerio*) and the goldfish (*Carassius auratus* L.). Developmental and Comparative Immunology. 2012;**36**:408-417. DOI: 10.1016/j.dci.2011.08.006

[126] Wei H, Wang S, Qin L, Wang X, Zhou H. Molecular characterization, 3D modeling of grass carp interleukin-10 receptor 1 (IL10R1). Engineering. 2013;**5**:214-219. DOI: 10.4236/eng.2013.510B045

[127] Monte MM, Wang T, Collet B, Zou J, Secombes CJ. Molecular characterisation of fourclass 2 cytokine receptor family members in rainbow trout, *Oncorhynchus mykiss*. Developmental and Comparative Immunology. 2015;**48**:43-54

[128] Esteban MÁ, Cuesta A, Chaves-Pozo E, Meseguer J. Phagocytosis in Teleosts. Implications of the new cells involved. Biology (Basel). 2015;**4**(4):907-922

[129] Wu L, Qin Z, Liu H, Lin L, Ye J, Li J. Recent advances on phagocytic B cells in Teleost fish. Frontiers in Immunology. 2020;**11**:824. DOI: 10.3389/fimmu.2020.00824

[130] Stuart LM, Ezekowitz RA. Phagocytosis: Elegant complexity. Immunity. 2005;**22**:539-550. DOI: 10.1016/j.immuni.2005.05.002

[131] Tauber AI. Metchnikoff and the phagocytosis theory. Nature Reviews Molecular Cell Biology. 2003;**4**:897-901. DOI: 10.1038/nrm1244

[132] Watts C, Amigorena S. Phagocytosis and antigen presentation. Seminars in

Immunology. 2001;**13**:373-379. DOI: 10.1006/smim.2001.0334

[133] Allen PG, Dawidowicz EA. Phagocytosis in Acanthamoeba: I. A mannose receptor is responsible for the binding and phagocytosis of yeast. The Journal of Cellular Physiology. 1990;**145**:508-513. DOI: 10.1002/jcp.1041450317

[134] Davies B, Chattings LS, Edwards SW. Superoxide generation during phagocytosis by *Acanthamoeba castellanii*: Similarities to the respiratory burst of immune phagocytes. The Journal of General Microbiology. 1991;**137**:705-710. DOI: 10.1099/00221287-137-3-705

[135] Moretti J, Blander JM. Insights into phagocytosis-coupled activation of pattern recognition receptors and inflammasomes. Current Opinion in Immunology. 2014;**26**:100-110. DOI: 10.1016/j.coi.2013.11.003

[136] Alberdi F Jr, Alderton MR, Coloe PJ, Smith SC. Characterization of immu-norelated peptides to porcidin P1. Immunology and Cell Biology. 1995;**73**:505-510. DOI: 10.1038/icb.1995.80

[137] Alberdi F Jr, Alderton MR, Korolik V, Coloe PJ, Smith SC. Antibacterial proteins from porcine polymorphonuclear neutrophils. Immunology and Cell Biology. 1995;**73**:38-43. DOI: 10.1038/icb.1995.6

[138] Kaplan DH, Greenlund AC, Tanner JW, Shaw AS, Schreiber RD. Identification of an interferon-gamma receptor alpha chain sequence required for JAK-1 binding. The Journal of Biological Chemistry. 1996;**271**:9-12. DOI: 10.1074/jbc.271.1.9

[139] Ganz T, Lehrer RI. Antimicrobial peptides of leukocytes. Current Opinion in Hematology. 1997;**4**:53-58. DOI: 10.1097/00062752-199704010-00009

[140] Borelli V, Banfi E, Perrotta MG, Zabucchi G. Myeloperoxidase exerts micro-bicidal activity against Mycobacterium tuberculosis. Infection and Immunity. 1999;**67**:4149-4152

[141] Sorensen OE, Follin P, Johnsen AH, Calafat J, Tjabringa GS, Hiemstra PS, et al. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. Blood. 2001;**97**:3951-3959. DOI: 10.1182/blood.V97.12.3951

[142] Canton J, Khezri R, Glogauer M, Grinstein S. Contrasting phagosome pH regulation and maturation in human M1 and M2 macrophages. Molecular Biology of the Cell. 2014;**25**:3330-3341. DOI: 10.1091/mbc.E14-05-0967

[143] Rabinovitch M. Professional and non-professional phagocytes: An introduction. Trends in Cell Biology. 1995;**5**:85-87

[144] Briggs RT, Drath DB, Karnovsky ML, Karnovsky MJ. Localization of NADH oxidase on the surface of human polymorphonuclear leukocytes by a new cytochemical method. Journal of Cell Biology. 1975;**67**:566-586. DOI: 10.1083/jcb.67.3.566

[145] Jorgensen JB, Robertsen B. Yeast beta-glucan stimulates respiratory burst activity of Atlantic salmon (*Salmo salar* L.) macrophages. Developmental and Comparative Immunology. 1995;**19**:43-57. DOI: 10.1016/0145-305X(94)00045-H

[146] Stafford JL, Galvez F, Goss GG, Belosevic M. Induction of nitric oxide and respiratory burst response in activated goldfish macrophages

requires potassium channel activity. Developmental and Comparative Immunology. 2002;**26**:445-459. DOI: 10.1016/S0145-305X(01)00087-8

[147] Sepulcre MP, Lopez-Castejon G, Meseguer J, Mulero V. The activation of gilthead seabream professional phagocytes by different PAMPs underlines the behavioural diversity of the main innate immune cells of bony fish. Molecular Immunology. 2007;**44**:2009-2016. DOI: 10.1016/j.molimm.2006.09.022

[148] Boltana S, Donate C, Goetz FW, Mackenzie S, Balasch JC. Characterization and expression of NADPH oxidase in LPS-, poly(I:C)- and zymosan-stimu-lated trout (*Oncorhynchus mykiss* W.) macrophages. Fish and Shellfish Immunology. 2009;**26**:651-661. DOI: 10.1016/j.fsi.2008.11.011

[149] Sharp GJE, Secombes CJ. The role of reactive oxygen species in the killing of the bacterial fish pathogen Aeromonas salmonicida by rainbow trout macrophages. Fish and Shellfish Immunology. 1993;**3**:119-129. DOI: 10.1006/fsim.1993.1013

[150] Ardó L, Jeney Z, Adams A, Jeney G. Immune responses of resistant and sensitive common carp families following experimental challenge with Aeromonas hydrophila. Fish and Shellfish Immunology. 2010;**29**:111-116. DOI: 10.1016/j.fsi.2010.02.029

[151] John M, Qiao-Wen X, Nathan C. Nitric oxide and macrophage function. The Annual Review of Immunology. 1997;**15**:323-350. DOI: 10.1146/annurev.immunol.15.1.323

[152] Stafford JL, Neumann NF, Belosevic M. Products of proteolytic cleavage of transferrin induce nitric oxide response of goldfish macrophages. Developmental and Comparative Immunology. 2001;**25**:101-115. DOI: 10.1016/S0145-305X(00)00048-3

[153] Stafford JL, Wilson EC, Belosevic M. Recombinant transferrin induces nitric oxide response in goldfish and murine macrophages. Fish and Shellfish Immunology. 2004;**17**:171-185. DOI: 10.1016/j.fsi.2004.03.001

[154] Taylor MW, Feng GS. Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. The FASEB Journal. 1991;**5**:2516-2522. DOI: 10.1096/fasebj.5.11.1907934

[155] Yuasa HJ, Takubo M, Takahashi A, Hasegawa T, Noma H, Suzuki T. Evolution of vertebrate indoleamine 2,3-dioxygenases. The Journal of Molecular Evolution. 2007;**65**:705-714. DOI: 10.1007/s00239-007-9049-1

[156] MacKintosh C, Ferrier DEK. Recent advances in understanding the roles of whole genome duplications in evolution. F1000 Research. 2017;**6**:1623