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Influences of immunostimulants on phagocytes in cultured fish: a mini review

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Abstract

Lower vertebrates like fish mostly depend on innate immunity as the first line of defence against invading pathogens. One of the defence mechanisms in innate immunity for fish is the use of phagocytic cells such as monocytes, macrophages, neutrophils, mast cells and dendritic cells in fighting pathogens. These cellular cells employ a variety of mechanisms in protecting fish including but not limited to; the use of pattern recognition receptors and antigen presenting cells to detect the presence of pathogens in host, neutralising them using measures including; inflammation, production of lysosomal enzymes released into the phagosome to neutralising pathogens and prevention of systemic autoimmunity by apoptosis. Some phagocytic cells play the extra role of serving as a bridge between innate and adaptive immunity in fish. Recent accumulating data showed that phagocyte cell activity in fish could be enhanced by a natural or chemical substance (immunostimulants) that stimulates the immune system in fish principally meant to enhance the immune response mechanisms. In this review, the main focus is on elucidating the distinct phagocytic mechanisms played by monocytes, macrophages, neutrophils, mast cells and dendritic cells as principal phagocytes in immune defence and how their activities are influenced by immunostimulants in cultured fish.

Key words: dendrictic cells, immunostimulants, macrophages, mast cells, monocytes, neutrophils.

Introduction

Massive development in aquaculture in recent decades has increased the interest in studies of the fish immune system and defence against diseases (Whyte 2007; Abarike *et al.* 2018). The immune system is an important physiological mechanism that protects the organism against invading pathogens. The immune system is broadly categorised as either innate or adaptive (Whang *et al.* 2011). Lower vertebrates like fish mostly depend on innate immunity as the first line of defence against invading pathogens (Ye *et al.* 2010) due to the constraints placed on the adaptive immunity (Whyte 2007). One typical innate immune defence mechanism is phagocytosis (Dussauze *et al.* 2015) by phagocyte cells which are principally dedicated to the recognition and elimination of invading organisms and damaged tissue (Whang *et al.* 2011). Phagocyte cells include monocytes, macrophages, neutrophils, mast cells (MCs), dendritic cells (DCs) and non-specific cytotoxic cells (Aoki *et al.* 2008; Uribe *et al.* 2011; Whang *et al.* 2011). Phagocyte cells protect host by ingesting (phagocytosis) harmful foreign particles as well as dead or dying cells (Uribe *et al.* 2011; Grayfer *et al.* 2014; Sirimanapong *et al.* 2014). Recent accumulating data show that phagocyte cell activity in fish could be induced by immunostimulants, a natural or chemical substance that stimulates the immune system (Vallejos-Vidal *et al.* 2016) in fish principally meant to enhance the innate immune response mechanisms (Sakai 1999) as well as inducing pronounced physiological effects on growth. Immunostimulant use has accelerated in fish culture as a result of resistant bacterial strains becoming more prevalent and difficult to treat (Bedasso 2017).

A growing number of studies have dealt explicitly with immunostimulants use in fish (Iwashita *et al.* 2015). Evidence of the beneficial use of immunostimulants in aquaculture have showed they can enhance phagocytosis by activating phagocyte cells for example, neutrophils and lymphocytes, ignite the expression of immune-related genes for instance cytokines in phagocytes, coordinate humoral immunity as well evoke antibody and complement responses in fish (Wang *et al.* 2001, 2017). However, there are no summarised reports about immunostimulant modulation of phagocyte cells in cultured fish. In addition, many aspects of immunostimulant modulation of phagocytes immune mechanisms which confer protection against many pathogens in fish are still unknown. In the present review, facts about phagocytic cells including monocytes, macrophages, neutrophils, mast cells and dendritic cells as phagocyte immune cells in fish and how their activities are modulated in cultured fish are presented. Also, some research gaps that need further addressing are pointed out.

The rationale of immunostimulants use in fish aquaculture

The major setback to the development of aquaculture is the emergence of various diseases (Abarike *et al.* 2018) in the culture systems. Various efforts have been made to outwit fish diseases including the use of antibiotics, development of vaccines and the use of other chemotherapeutics (Sakai 1999). Although vaccines to a great extent have contributed to curbing disease in fish culture, there are many challenges concerning their use for instance, vaccines are often too expensive (Harikrishnan *et al.* 2011), unpractical for wide spread use in fish farms, and also a single vaccine has a specific effect against only one type of pathogen (Plant & LaPatra 2011) but limits the effectiveness for wide range of pathogens due to the complex antigenic structure (Ardó *et al.* 2008). On the other hand, the use of antibiotics in aquaculture poses the threat of antibiotic resistant bacteria, the accumulation of antibiotic residue from lower food chain animals to higher food chain animals and negative effects on the indigenous microflora of juveniles or adult fish (Misra *et al.* 2006). These have resulted in the ban on the use of antibiotics (Patterson & Burkholder 2003).

As have been indicated in previous studies, there is still the need to prevent and control a broad spectrum of fish diseases arising in aquaculture systems (Defoirdt *et al.* 2011; Mehana *et al.* 2015). Recently, the fore of research is the use of immunostimulants, as suitable alternative to strengthen fish immune systems in aquaculture (Abarike *et al.* 2018). An immunostimulant is a natural or chemical substance that stimulates the immune system by specific (vaccines or antigens) or non-specific (irrespective of antigenic specificity) routes (Vallejos-Vidal *et al.* 2016). Immunostimulants can be divided into several groups depending on their sources: bacterial, algae-derived, animal-derived, nutritional factors as immunostimulants, hormones and recombinant cytokines (Shahbazi & Bolhassani 2017). The above-mentioned immunostimulants can be derived from natural sources or synthetically made with different chemicals to have different modes and mechanism of action (Vasudeva Rao *et al.* 2006; Petrunov *et al.* 2007). Administration of immunostimulants alone or in combination with vaccination has emerged as a promising approach to prevent or control fish diseases (Sahoo 2007). Immunostimulants can be applied in vaccine formulations to improve good antibody responses without adverse side effects (Wang *et al.* 2017). The use of immunostimulant, as dietary supplements can improve the innate immune system of fish providing increased resistance to pathogens during stressful conditions such as changes in water temperature, grading and transfer amongst others (Bricknell & Dalmo 2005). In additions, the use of immunostimulants are greatly encouraged because they can be obtained from natural sources in large amounts and from cheap sources making them the most cost effective dietary supplement for promotion of growth, enhancement of immune response and resistance to diseases and above all without any environmental and hazardous problems (Jian & Wu 2004; Bricknell & Dalmo 2005; Citarasu 2010).

Immunostimulant effect on phagocyte cells

Morphologically different types of cells have been described as being phagocytic in cultured fish (Vallejos-Vidal *et al.* 2016). Immunostimulants enhancement of phagocyte cell activity in fish activates an innate immune response, for instance, the inflammatory response before adaptive response such as antibody production (Nayak 2010). Phagocytosis represents the first cellular line of defence preserved in vertebrates and invertebrates (Bachère, 1995; Roth & Kurtz 2009; Uribe *et al.* 2011; Chi *et al.* 2014). It also represents the process by which cells engulf recognised particles (i.e. bacteria, other microorganisms, aged red blood cells and foreign matter), through binding to their surface and internalising them into phagosomes, formed around the engulfed materials (Carbone & Faggio 2016). Fishes treated with immunostimulants usually show enhanced phagocytic cell activities (Sakai 1999). Immunostimulants enhance the immune system and the activities of phagocytic cells of fish through several means: enhancement of mitogenic activities (Hardie *et al.* 1991; Siwicki *et al.* 1996), activation of complement activities (Li & Lovell 1985; Engstad *et al.* 1992), and enhancement of antibody production (Thompson *et al.* 1993), increased number of phagocytes (Sakai 1999) and superoxide anion production (Watanuki *et al.* 2006). A typical example is the significant increase in blood neutrophil activity and promotion of phagocytosis by the neutrophils in bovine at the correct concentration using *Lonicera japonica* as immunostimulant (Hu *et al.* 1992). Several studies have reported that oral administration of chitin (Sakai *et al.* 1992) and yeast products (MacroGard, Vitastim and *Saccharomyces cerevisiae*) (Jeney *et al.* 1997) increased the phagocytic capability of the cells in rainbow trout. It has been further reported that the extracellular activity was very high in fish fed with dietary glucan (Jeney *et al.* 1997). Dietary *Spirulina platensis* have been reported to enhanced the phagocytic activity and superoxide anion production in kidney phagocytic cells in carp, *Cyprinus carpio* (Watanuki *et al.* 2006).

Immunostimulatory effects on monocytes

Monocytes are a type of white blood cells produced by the bone marrow from hematopoietic stem cell precursors. They are mostly short lived as they undergo spontaneous apoptosis on a daily basis (Parihar *et al.* 2010; Saha & Geissmann 2011; Fejer *et al.* 2015). The specific and distinctive roles played by monocytes seem not to be elucidated. Very often this may be because monocytes are starting cells (i.e. cells which often metamorphosis into other cells) (Geissmann *et al.* 2010; Yona *et al.* 2012; Bain *et al.* 2013; Jones & Ricardo 2013; Fejer *et al.* 2015). The phagocytic abilities of monocytes lies in the ability of their isoform (IFNGR1 and IFNGR2) receptors to bind with highly pleiotropic pro-inflammatory and anti-viral receptors of the cytokine interferon-gamma (Gao *et al.* 2009; Grayfer & Belosevic 2009; Chen *et al.* 2015; Li *et al.* 2015). Monocyte expressions in organisms are also mediated by gene markers and some types of proteins. In fishes which lack these receptors, protein-like genes such as lipopolysaccharide-binding protein act as mediators in stimulating an immune response of monocytes (Lu *et al.* 2014). Monocyte production, development and functioning in cells of fish are governed by a variety of cell signalling genes, e.g. cytokine and chemokine genes, for example, CSF-1b and IFN-γ (Korenaga *et al.* 2013) and chemotaxin 2 (LECT2) and PaCXCL8l (Chen *et al.* 2014). Immunostimulants can trigger enhanced monocyte activity. Oral administration of Garlic to rats (Iranloye 2002), and *Echinacea* to tilapia (Aly *et al.* 2008), vitamin A in *Channa punctatus* (Syed Ali Fathima *et al.* 2012), lipopolysaccharides (LPS) in rainbow trout (Nya & Austin 2009) have been proven to modulate monocyte activity. Immunostimulant like levamisole failed to enhance the phagocytic activity of monocytes in tilapia (Bedasso 2017). That notwithstanding, extensive search of literature showed that immunostimulant influences, specifically on fish monocyte activity are scanty. It seems the properties of monocytes such as their short-lived nature or their differentiation into other cells could be the reason why they are not focused on. However, given the roles they play in innate immune response, a second look at their regulation by immunostimulants will help expand our knowledge.

Immunostimulatory effects on macrophages

Macrophages have been thought to be the first-cell type of immune defence cells in fish and other animals that protect them against the disease-causing organism. Macrophages emerge first as monocytes precursors in the bone marrows released as circulatory blood monocytes before migrating into other tissues and differentiating into macrophages to replenish local populations or in response to infection or any form of inflammation (Fejer *et al.* 2015). Macrophages facilitates phagocytosis of harmful foreign particles by engulfing and releasing lysosomal enzymes that lyse them to neutralise their effects (Henson & Hume 2006; Ovchinnikov 2008; Nayak 2010; Jones & Ricardo 2013; Gregory *et al.* 2014). Although macrophages are naturally occurring in the cells and tissues, special dietary treatments can stimulate or repressed their production and activity. Linolenic acids and probiotics dietary treatment of feed fed to fish have been reported to stimulate phagocytic activity of head kidney macrophages in juvenile Nile tilapia (Chen *et al.* 2013, 2016) in grouper, *Epinephelus coioides* (Chiu *et al.* 2010) rainbow trout (Irianto & Austin 2002), *Labeo rohita*, Ham (Kumar *et al.* 2008). For years now, it has been demonstrated that macrophages can be activated by the presence of microbial stimuli, with slight variation across different fish species. Given the influences of dietary treatments and the use of immunostimulants to activate macrophages, such treatments could enhance the activities of macrophages, probably increasing the preparedness of fish to pathogen invasion, hence, improve resistance to diseases as have been showed for example in the use of LPS (Roberts *et al.* 1997) and β-glucan (Meena *et al.* 2013). However, different immunostimulants appear to have different effects on fish species. For instance, LPS could induce macrophage activity in trout (Goetz *et al.* 2004) whiles β-glucan despite its wide application did not affect trout macrophage (Douxfils *et al.* 2017). All in all current data on the use of some common immunostimulants may require the need for further extensive research to understand their underlining mechanism, especially about specific species.

Immunostimulatory effects on neutrophils

Neutrophils represent approximately 40–75% of leucocytes and are mainly produced in response to inflammation (Vazquez Rodriguez *et al.* 2017). In a comprehensive review, (Keightley *et al.* 2014) neutrophils have been said to be key for controlling and neutralising infection at wound sites and clearing debris. Also, they likely to have other immunomodulatory functions that directly contribute to regenerative processes (Dezfuli *et al.* 2013; Biller-Takahashi & Urbinati 2014). Their chemotaxis, phagocytosis and destruction of intracellular and extracellular pathogens demonstrate their important role in innate immunity against pathogens and parasitic infection (Stakauskas *et al.* 2007; Havixbeck & Barreda 2015). Immunostimulant modulation of neutrophil phagocytosis have been reported for vitamin A in *C. punctatus* modulated significant neutrophil activity against *Aeromonas hydrophila* (Syed Ali Fathima *et al.* 2012), β-glucan in *Lutjanus guttatus* fed against dactylogyrid monogeneans (Del Rio-Zaragoza *et al.* 2011). A mixture of polysaccharides isolated from the cell walls of *S. cerevisiae* known as zymosan stimulated macrophages and induced the release of cytokines from neutrophils (Shahbazi & Bolhassani 2017). Once the infectious threat has been neutralised, neutrophils must be removed for successful inflammation resolution to occur. This happens either by apoptosis, engulfment by other phagocytes (Havixbeck & Barreda 2015) and reverse migration away from injury site (Henry *et al.* 2013). While several studies examining the modulation of neutrophils in fish such as carp, zebrafish and fathead minnows by immunostimulants indicate promising limitation to the dissemination of a broad range of pathogens (Havixbeck & Barreda 2015), excessive stimulation can lead to immunosuppression compromising the immune system in fish (Saeij *et al.* 2003).

Immunostimulatory effects on mast cells

Mast cells (MCs) are bone marrow-derived and lymphoid tissue-derived cells and require stem cell factor for their survival. They may release biologically active cytokines, chemokines, vasoactive agents, proteases, heparin (glycosaminoglycan), biogenic amines (histamine as the inflammatory messenger and a role in local immune response) and an array of neurotransmitters (mediators) as immune defence mechanisms (Baccari *et al.* 2011). MCs are important as initiators and effectors of innate immunity and regulate the adaptive immune responses. They have been described in all classes of vertebrates and seem to be morphologically and functionally similar (Mulero *et al.* 2007). Current understanding shows that the MC is a critical component of the piscine immune response and they perform additional roles in fish that are not directly linked with the immunological functions, for example in oncology and tissue repair (Sfacteria *et al.* 2015). An extensive search of the literature reveals that studies exploring the effects of MC stimulation using immunostimulants in fish are few. That notwithstanding, amongst the first application of immunostimulant in an attempt to stimulate MCs concerned the use of probiotic bacteria highlighted in a review by Prykhozhij and Berman (2014) where bacteria such as *Escherichia coli* and their products were demonstrated to be highly effective agents to activate fish MCs and induce recruitment of neutrophils to locations where MCs are present. Aside, nonfish, for example, in mouse, probiotic bacterial including *Bifidobacterium bifidum, Lactobacillus casei* and *E. coli*, have been reported to modulate MCs during an immune response (Kim *et al.* 2005). From these few studies, probiotics have been suggested for the treatment of many disease conditions due to their ability to modulate the functions of MCs. However, the molecular mechanisms behind these actions of probiotics have not been fully elucidated. Also, this kind of studies may be beneficial in immune fish studies.

Immunostimulatory effects on dendritic cells

Dendritic cells (DCs) are the most potent professional antigen presenting cells and play a key role in innate immunity because of their distinct abilities to stimulate naive T-lymphocytes and to initiate primary immune responses (Banchereau *et al.* 2000). Previous studies have shown that activation and migration of DCs are critical for the induction of primary immune responses (Lanzavecchia & Sallusto 2000). Dendritic cells are specialised antigen presenting cells that can induce immunity and tolerance as well as bridge innate and adaptive immunity in mammals and fish (Lanzavecchia & Sallusto 2000). This link between the ancient innate immune system and the more evolutionarily recent adaptive immune system is of particular interest in cultured fish, the oldest vertebrates to have both innate and adaptive immunity. It is unknown whether DCs coevolved with the adaptive response, or if the connection between innate and adaptive immunity relied on a fundamentally different cell type early in evolution (Bassity & Clark 2012). DCs upon encountering a pathogen or its associated elements get activated to engulf and traffic foreign antigen to spleen and kidneys tissues where stimulation of antigen-specific T-lymphocytes (Lugo-Villarino *et al.* 2010) occurs. In trout and zebra fish, DCs stimulate greater proliferation than B cells or macrophages, demonstrating their specialised ability to present antigens (Bassity & Clark 2012). Kim *et al.* (2007) suggested that a maturation step is essential for DCs to initiate T cell immunity. Numerous factors induce DC maturation, for instance (i) pathogen-related molecules such as LPS (ii) pro- and anti-inflammatory signals such as tumour necrosis factor-α and prostaglandins; (iii) T cell-derived signals and other simple chemicals also induce DC maturation, especially Langerhans cells (Banchereau *et al.* 2000; Kim *et al.* 2013). In comparison with mammalian DCs, in which immature

DCs lose their phagocytic ability upon activation with toll-like receptor -ligands, in rainbow trout fish, DCs can opsonized beads in a rather limited capacity (Bassity & Clark 2012). As far as our search for literature is concerned, reports on the phagocytic abilities of DCs in cultured fish are again few and those reported are of limited phagocytic capacities. This might mean that emphasis on DCs in innate immunity should be more on their roles as specialised antigen presenting cells between innate and adaptive immunity.

Conclusion

The varied mechanism used by monocytes, macrophages, neutrophils, MCs and DCs in innate immunity shows their importance and contributions to defend and maintain the health of cultured fish. Their roles principally span from, surveying all cells of fish for pathogens, phagocytosis of pathogens and self-danger cells (autophagy), containing pathogens through inflammation, serving as activators of other immune cells in response to diseases and repair of wounds/injuries that might arise from the fight against pathogen invasion. These cellular cells have coordinated activities in maintaining the general health of fish. Immunostimulants have been shown to modulate the phagocytic capacity, ignite the expression of immune-related genes, coordinate humoral immunity as well evoke antibody and complement responses to prevent and control diseases of phagocyte cells in fish. However, there is the need for further studies to understand better immunostimulants mechanism of action and interactions with the above-mentioned phagocytic cells in cultured fish.

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Author's contribution

J. Tang and J. Cai conceived the idea, F.K.A. Kuebutornye gathered literature, E.D. Abarike, drafted the manuscript, J. Jichang and Y. Lu, proofread the manuscript.

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