Immune Response Of Fish To Bacterial Infections

Adedeji OB[1], Okerentugba PO[2], Onianwa O[2], Okonko IO[2]

[1]Department of Veterinary Public Health & Preventive Medicine, University of Ibadan, Ibadan, Nigeria
[2]Department of Microbiology, University of Port Harcourt, East-West Road, P.M.B. 5323, Choba, Port Harcourt, Rivers State, Nigeria;
mac2finney@yahoo.com; iheanyi.okonko@uniport.edu.ng; Tel: +234-80-3538-0891

ABSTRACT: Teleosts possess various mechanisms which protect them from infection caused by bacterial pathogens. These mechanisms share differences and similarities to those found in other higher vertebrates. The innate and acquired immune responses are not mutually exclusive as they work together to protect the host. Components of innate immunity include physical, cellular and humoral barriers such as integumental defences, inflammation and complement. These are very effective against both Gram-positive and Gram-negative infectious bacteria. Unless physically damaged, the integument is very effective in preventing pathogens present in the aquatic environment from penetrating host tissue. B cells and T cells on the other hand make up the acquired immune response and develop only after exposure to a specific antigen. IgD, IgM and IgT are antibodies produced in telosts. Research into antibody production has huge potential for the development of novel vaccines and therapeutics. T cells in teleosts are subdivided into T-Helper cells, cytotoxic T cells and regulatory T cells also having various functions similar to those present in mammals. The aim of this review is to list current developments in research into teleost immunity, outlining the various immune mechanisms while comparing them with those present in their mammalian counterparts.

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1. BACKGROUND

An infectious disease is a change in the normal health status of a host whereby the latter is no longer capable of carrying out normal functions because of the presence of pathogenic agents (Wiley *et al.*, 2011). This definition is suitable for all manner of hosts, whether vertebrate or otherwise. Conceptually, infections occur as a result of interactions between host and pathogen and the development of such diseases are a consequence of the entry, invasion and establishment of pathogens in host tissues. A strong host immune system reduces the chances of infection and ultimately disease manifestation. The host immune system however is under strict control and mon itoring in order to avoid unwarranted damage as a consequence of the destruction of pathogens (Renshaw and Trede, 2012).

Researchers over the years have studied the human host immune system in order to gain insights into its control and function for the purpose of the development of novel therapies and vaccines to control and prevent diseases such as cancer, asthma and tuberculosis in the world today. This research has mainly been carried out using vertebrate models (mainly mice) and other *invitro* systems. Apart from challenges such as the high labour requirement and larger research space demands, such approaches have been relatively successful. A very important limitation however is the inability to carry out *invivo* imaging in these vertebrate models (Trede *et al*, 2004; Renshaw and Trede, 2012). The realization of this major limitation has resulted in increased research into other vertebrate models such as flies, worms and teleost fish.

Research into the fish immune system as a basis for gaining greater insight into the human immune system is motivated by the similarities shared between both groups of organisms. Apart from the fact that teleost fish have the same basic cellular and humoral immune responses and central organs present in other vertebrates, most of the secondary lymphoid organs (excluding the bone marrow and lymphatic nodules) possessed by these fishes are also present in mammals (Press and Evensen, 1999). Some of the lymphoid organs present in teleost fish are shown in figure 1. Differences between both groups include humoral diversities such as the presence of several C3 isoforms in fishes which are absent in man. The antibody response in fish is also relatively slower than that in humans (Tort *et al.*, 2003). Despite these differences, research into the defence mechanisms in teleost fish has caused huge breakthrough in the overall understanding of the immune system in vertebrates (Renshaw and Trede, 2012).

Insight into the mechanisms involved in fish immunity is therefore essential to fully comprehend the immune system of their higher counterparts.

Information obtained would further prove useful in the overall control of diseases affecting fish thus significantly reducing the high economic losses in the aquaculture industry (Ming-Wei *et al.*, 2008).

The robust immune system of teleost fish in response to viral infections has been discussed previously (Adedeji *et al.*, 2012). In this paper, we go further to list and evaluate the innate and acquired immune mechanisms used by teleost fish in response to infections caused by bacterial pathogens, most of which are a part of the normal microflora surrounding aquatic environment.

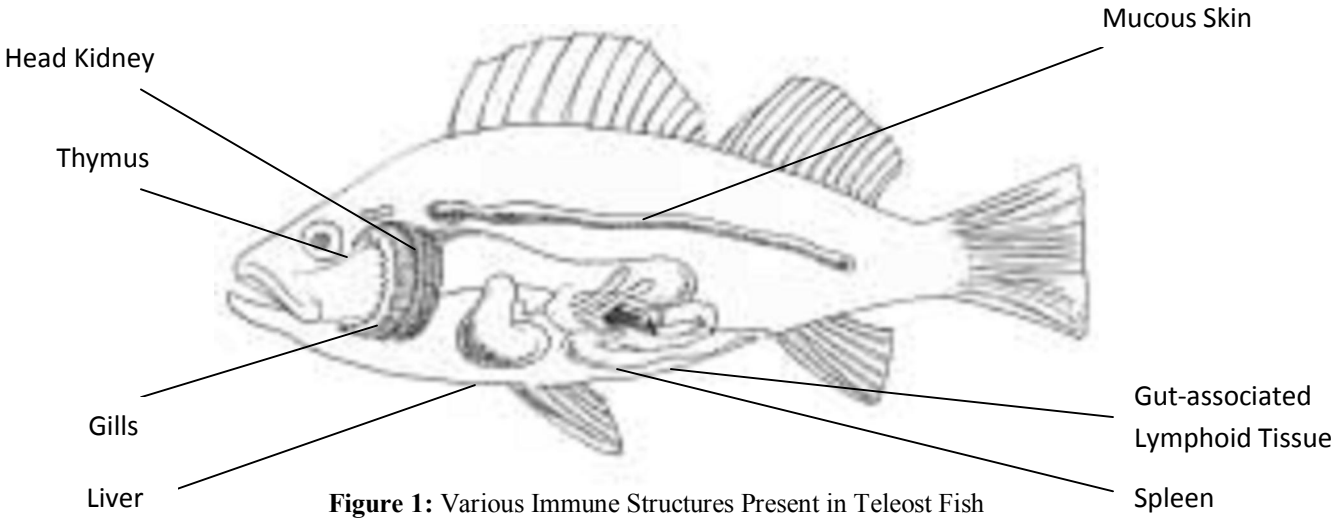

Figure 1: Various Immune Structures Present in Teleost Fish (Source: Tort *et al.*, 2003)

2. INNATE AND ACQUIRED IMMUNE SYSTEMS IN FISH

The innate immune system precedes the acquired immune system, activating and determining response nature of the latter. This natural response has advantages such as being non-specific and relatively quicker in action than the acquired response (Ellis, 2001; Tort *et al.*, 2003). Parts of the innate immune system include physical barriers as well as cellular and humoral components. The acquired (adaptive) immune response of a host on the other hand, develops after its exposure to the infectious agent. It has the advantage of being antigen-specific. The development of this system takes a longer time after the first exposure to the antigen however subsequent exposures are faster and are of higher magnitude than the initial response (Wiley *et al.*, 2011; Adedeji *et al.*, 2012). Though it is generally accepted that the immune system consists of both innate (non-specific) and acquired (specific) mechanisms, these however are not mutually exclusive of each other as they work in combination with each other to protect the host (Magnadottir, 2006). The various components of the innate and adaptive immune system and their functions in fish immunity include the following;

2.1. Integumental and Other Associated Defences

Pathogens that cause disease in fishes are present in the aquatic environment. These make contact with the epithelial surfaces of the fish in water. The epithelial surfaces of fish include the skin and gills (Narvaez *et al.*, 2010). Also antibacterial substances secreted in fish mucosal membranes include lectins, lysozyme, pentraxins and trypsin-like proteases. These as well as other epidermal secretions offer protection to the fish.

2.2. Gills

The gills are part of the mucosa-associated lymphoid tissue (MALT) in teleost fish. They contain populations of leucocytes and plasma cells (Press and Evensen, 1999). The gill lamellae also possess pillar cells which form part of the reticuloendothelial system (Dalmo *et al.*, 1997; Tort et al., 2003). Koppang *et al.*, (1998) also reported a high expression of Major Histocompatibility Complex class II (Class II MHC) β chain mRNA in the gills of Atlantic salmon. These all provide evidence of the role of gills in host defence.

2.3. Integument

The skin is the integument of vertebrates. It is a tough protective layer which develops as the organism grows. In teleost fish, the integument is covered with scales while in mammals it is covered with hair. The skin constitutes one of the major physical barriers in both mammals and fishes as it prevents the entry of pathogens into the host. Pathogens that successfully adhere to the cells of the

outer skin in humans are removed through the continuous shedding of the cells (Wiley *et al.*, 2011). The successful resistance of teleost fish to infection due to the presence of integumental defences have been reported. The rainbow trout was not susceptible to infection by *Aeromonas salmonicida* because of its integumental defences. The Atlantic salmon however was susceptible to infection after experiencing the same immersion challenge. Little difference in disease manifestation in both fishes was observed however when they were injected with the same lethal dose of bacteria (Secombes & Olivier, 1997, Ellis, 2001). The absence of integumental defences also played a key role in the infection of adult zebra fish with *Edwardsiella tarda* as the fish were not susceptible to infection unless they were injured before the immersion challenge (Pressley *et al.*, 2005).

2.4. Mucus

Mucosal membranes in man trap invading bacteria preventing them from attaching to epithelial surfaces to invade host tissues (Talaro, 2005). These two mechanisms are also present in fish. Increased mortality in Turbot and Ayu (*Plecoglossus altivelis*) occurred as a result of the experimental removal of the mucous membrane (Kanno *et al.*, 1989; Fouz *et al.*, 1990; Ellis, 2001). Also the crude extract of mucus from Eel fish inhibited the growth of *Salmonella paratyphi* and other human pathogens *in vitro* (Bragadeeswaran and Thangaraj, 2011).

2.5. Lysozyme

In mammals, many antimicrobial substances toxic to various bacteria surround the mucosal surfaces. These substances include lysozyme and lactoperoxidase. Lysozyme (also called *N*-acetylmuramide glycanohydrolase or muramidase) destroys bacteria through the hydrolyses of β(1 - 4) bonds connecting the *N*-acetylmuramic and *N*-acetylglucosamine biopolymers that make up the bacterial cell wall peptidoglycan while lactoperoxidase catalyses the production of superoxide radicals which are toxic to many microorganisms (Wiley *et al.*, 2011). Lysozyme has been extensively studied in fish being identified in fish mucus, ova, and serum (Ellis, 1999) and is present in all fish species studied. The c-type lysozyme is the most abundant of six types of lysozyme and has been isolated from various fishes including the Japanese flounder (*Paralichthys olivaceus*) and Rainbow trout (*Oncorhynchus mykiss*) (Dautigny *et al.*, 1991; Hikima *et al.*, 2000). Recently, three c-type lysozymes (C1, C2 and C3) were identified and expressed in Tilapia (*Oreochromis aureus*) (Gao *et al.*, 2012). These expressions were mainly in the muscle and liver. Up-regulation for C1-type mRNA lysozyme was observed in kidney and spleen, while C2- and C3-type lysozyme increases were observed in the stomach and liver thus suggesting the roles of C1-type lysozyme and C2- and C3-type lysozyme in defense and digestive functions against bacterial infection respectively.

2.6. Proteases

Proteases play a very important role in various organisms. Their function in fish immunity includes the cleavage of bacterial proteins as well as the activation of various immunological components such as complements, antimicrobial peptides and immunoglobulins (Morrissey, 1998; Yoshikawa *et al.*, 2001; Cho *et al.*, 2002a,b). They are generally classified based on their chemical nature into four distinct groups which are the cysteine, serine, aspartic and metalloproteases. All of these groups have been identified in fish such as the rainbow trout (*Oncorhynchus mykiss*), European eel (*Anguilla anguilla*) and Japanese eel (*Anguilla japonica*) and have been demonstrated to be effective in the control of various fish pathogens including *Listonella anguillarum* (Hjelmeland *et al.*, 1983; Aranishi and Nakane, 1997a,b; Cho *et al.*, 2002a,b; Fast *et al.*, 2002). Subramanian *et al.*, (2007) investigated protease activity in other fish including haddock (*Melanogrammus aeglefinus*), brook trout (*Salvelinus fontinalis*) and koi carp (*Cyprinus carpio* sub sp. koi) and also compared enzyme levels in the various fishes. Of all fish studied, Koi carp had the highest level of Cathepsin B (a cysteine protease).

2.7. Lectins

Lectins are proteins (other than enzymes and antibodies) with high specificity for sugar molecules. Though their presence in fish has always been known, it is only recently their contribution to host immunity is being discovered. In addition to various synthetic and regulatory functions, they also participate in important roles in innate immunity such as inflammation, opsonisation and complement activation (Arason, 1996; Ellis, 2001). Defence lectins can bind to surfaces of various infectious agents. They have been detected in ova, larva, egg and mucus and Lectins are classified based on their carbohydrate specificity which is facilitated by the Carbohydrate Recognition Domain (CRD). Ewart *et al.* (1999) isolated a soluble mannose binding lectin from the serum of the Atlantic salmon and demonstrated its binding to *Vibrio anguillarum* and *Aeromonas salmonicida* thus hinting at a possible function in pathogen recognition. The lectin also increased phagocytosis and bactericidal activity of the

host against *A. salmonicida* (Ottinger *et al.,* 1999). Research into lectin activity in the serum of blue gourami, *Trichogaster trichopterus* (Pallus), revealed that increasing concentrations of *N*-Acetyl galactosamine binding-lectin (BGL) enhanced the phagocytic activity of macrophages against *Aeromonas hydrophila* (Fock *et al.*, 2001) Though a great number of other fish defence lectins have been isolated and identified but their specific immunological activity has not been determined (Russell and Lumsden, 2005).

2.8. Antibacterial Peptides

These are low molecular weight (<10kDa), broad spectrum cationic, antibiotic proteins (Boman, 1995). They play a very important role in host immunity to bacterial infection. Though their existence in vertebrates and invertebrates has been known for about 30 years, studies into their specific immunological activity against fish pathogens have been conducted only within the last decade. One of the earliest reports was made by Smith *et al.* (2000) where the antibacterial activity of a heat-stable, hydrophobic, cationic peptide (<3kDa) was demonstrated against Gram-positive bacteria, *Planococcus citreus* (believed to be pathogenic for fresh water fish) and *Micrococcus luteus*. The protein however showed no muramidase activity. The gene encoding pleurocidin, an antibacterial peptide produced in the skin of the winter flounder, is encoded by a gene that is expressed as early as 13 days post-hatch thus hinting at the possibility of a role in the protection of fry (Douglas *et al.*, 2001). Defensins are a unique group of cationic peptides of molecular weight 3-6kDa. They have good activity against bacteria, fungi and viruses (Bals *et al.*, 1998; Weinberg *et al.*, 2006; Krishnakumari *et al.*, 2009). They are classified as α-, β- and θ- depending on their cysteine disulphide bonding. β-defensins have been isolated from a variety of teleost fish including the rainbow trout, olive fish, *Paralichthys olivaceus* and zebra fish, *Danio rerio* (Zou *et al.*, 2007; Falco *et al.*, 2008; Nam *et al.*, 2010). Apart from their traditional antimicrobial roles, they also act as lymphocytes, monocytes and dendritic cells. Jin *et al.*, (2010) isolated and characterised a β-defensin produced in the pituitary and testis of the orange-spotted grouper, *Epinephelus coioides* therefore suggesting a role of the protein in endocrine regulation.

2.9. Inflammatory Responses

Inflammation is a very important non-specific reaction of the host immune system of vertebrates. Generally, it is an internal response to any traumatic occurrence in the host tissues. This event, also triggered by the entry of a pathogen, is so powerful it gives the host good opportunity to maintain stability and recover from injury but could be detrimental to host tissue if chronic (Talaro, 2005). Inflammation is mediated by a variety of molecules including cytokines such as interleukins and the Tumour Necrosis Factor (TNF). In addition to the initiation of the pro-inflammatory cytokine cascade, these cytokines also recruit macrophages and stimulate adaptive immune response. Interleukin-1β (IL-1β) has been isolated from a considerable number of teleost fish including rainbow trout, carp and zebrafish (Accession # AY340959) (Zou *et al.*, 1999; Fujiki *et al.*, 2000; Pressley *et al.*, 2005). The Tumor Necrosis Factor-α (TNF-α) has been identified in all taxonomic groups of teleost fish such as rainbow trout, brook trout, carp, Japanese flounder and zebrafish (Accession # AY427649) (Bobe and Goetz, 2001; Hirono *et al.*, 2000; Laing *et al.*, 2001; Saeij *et al.*, 2003; Pressley *et al.*, 2005). Recently the inflammatory response of zebra fish to infection by the intracellular bacterial pathogen, *E. tarda* was demonstrated (Pressley *et al.*, 2005). This organism causes edwardsiellosis (a generalized septicaemia often associated with poor water quality and stress) in many fish species and also infects a variety of higher organisms including amphibians and reptiles (Plumb *et al.*, 1999). Expression levels of IL-1β and TNF-α were assessed by real-time PCR and found to be significantly upregulated in the infected zebra fish and embryos.

2.10. Complement

The complement (also referred to as the C factor) is a complex and multiple-duty system. It is part of innate immunity and derives its name from its function which is basically to complement immune reactions. Complement functions include the facilitation of phagocytosis, bridging of innate and adaptive immunity and the disposal of wastes including dead host cells (Wiley *et al.*, 2011). The complement system which consists of more than 35 soluble plasma proteins is activated through three pathways which are the classical, alternative and lectin pathways (Uribe *et al.*, 2011). The complement system has been shown to be present in vertebrates including teleost fish such as common carp and catfish (Matsuyama *et al.*, 1992; Jenkins and Ourth, 1993; Endo *et al.*, 1998; Nonaka and Smith, 2000; Zardakis *et al.*, 2001). Lipopolysaccharide, a major constituent of the cell wall of Gram-negative bacteria can activate the complement system via the alternative pathway which eventually leads to the lysis of the bacteria (Ellis, 2001; Wiley *et al.*, 2011). In comparison with the classical pathway however, the alternative pathway is not as effective against many bacteria pathogens in fish (Boesen *et al.*,

1999ab; Ellis, 1999). The C3 protein molecule in teleost fish is similar to that found in a number of other vertebrate species being composed of a disulphide-linked two chain glycoprotein having a thioester bond (Magnadottir *et al.*, 2005).

2.11. Phagocytes

Phagocytic cells (monocytes, neutrophils, macrophages and dendritic cells) play very important roles in the defence of the host during the early period of infection. Phagocytes survey the host for antigens, destroying them through a process known as phagocytosis. This process can be executed through two mechanisms namely the opsonin – dependent and opsonin – independent recognition (Talaro, 2005; Wiley *et al.*, 2011). Phagocytosis is very important in teleost fish as the latter are poikilothermic. Changes in temperature however do not significantly affect the activities of the phagocytes (Blazer, 1991; Lange and Magnadottir, 2003; Magnadottir *et al.*, 2005). Neutrophils and macrophages are the most important phagocytes in teleosts (Secombes and Fletcher, 1992). These cells possess lysozyme and a number of other lytic enzymes in their lysosomes (Fischer *et al.*, 2006; Uribe *et al.*, 2011).

3. SPECIFIC IMMUNE SYSTEM

3.1. B cells

These cells derive their name from a special gland in chickens called the *bursa of Fabricus* (the site of B cell maturation). B cells play important roles in the specific immune system (humoral immunity). Together with the T cells, they make up the third line of defence differentiating into specialized antibody-producing plasma cells and memory cells after activation (Talaro, 2005). Research into B cells development and function in zebrafish has not been as successful as that of T cells primarily because of visual limitations for the former develop in the kidney as compared to the latter which develop in the thymus (Renshaw and Trede, 2011). Li *et al.* (2006) demonstrated a phagocytic activity of B cells from teleost fish. This discovery fails to conform to the present paradigm which dictates this important activity being restricted to special phagocytes such as monocytes and macrophages and supports the idea that B cells evolved from an ancestral phagocyte. The discovery also suggests a deeper relationship between B cells and macrophages. Fletcher and Grant (1969) were the first to report the presence of antibodies in teleosts and until recently, IgM was generally believed to be the only functional antibody. The present decade however has witnessed the discovery of two additional immunoglobulins, IgD (Edholm *et al.*, 2010) and IgT/IgZ (Danilova *et al.*, 2005 and Hansen *et al.*, 2005). There is still considerable ground left uncovered in research into teleost B cells and immunoglobulins. More effort is required if novel vaccines are to be developed.

3.2. T cells

T cells are part of the specific immune system of vertebrates. Unlike B cells they have to make direct contact with antigens in order to eliminate the latter (Talaro, 2005; Wiley *et al.*, 2011). There are various subsets of T cells and their particular activity is dependent on the nature of the CD receptor they possess. Three major subsets of T cells include T-Helper cells, cytotoxic T cells and regulatory T cells. Their functions include the stimulation of antibody production by B cells, destruction of infected cells and the prevention of autoimmune responses. Genetic evidence of the presence of the three subsets listed in zebra fish is available though functional assays are lacking (Renshaw and Trede, 2012; Adedeji *et al.*, 2012). Cytotoxic T lymphocytes (CTLs) which mediate specific cell-mediated cytotoxicity have been identified in a variety of teleost fish including channel cat fish, rainbow trout and Ginbuna crucian carp (Stuge *et al.*, 2000; Fischer *et al.*, 2003; Somamoto *et al.*, 2006). Greater insight into cell-mediated cytotoxicity will be achieved with the development of appropriate tools for cellular and molecular recognition. Various studies hint at the similarities between the mechanisms of cytotoxicity in mammals and fish (Fischer *et al.*, 2006; Uribe et al., 2011).

4. CONCLUDING REMARKS

The immune system of teleosts is rich with defence mechanisms that are highly effective against pathogenic bacteria. Such mechanisms have similarities with those found in other higher vertebrates and consist of both innate and adaptive systems. This has huge potential for increased understanding of the immune system of mammals and would contribute immensely to the development of novel therapies and vaccines against bacterial infections in humans. Insights into fish immunity would also help in the prevention of fish diseases thus reducing annual losses in aquaculture. Various factors could affect the strength of these mechanisms however. Factors such as trauma and light, salinity and temperature are important to the proper functioning of the immune system. Injuries increase the chances of successful penetration of pathogens into host tissues. Environmental changes such as temperature change affect enzymatic activity but have little or no effect on phagocytic action. There are still areas of teleost immunity left unexplored. This is partly due to technologies that are still developing. With the emergence of new assays, limitations would be beaten.

Correspondence to:
Iheanyi O. Okonko
Department of Microbiology,
University of Port Harcourt,
PMB 5323 Uniport Post Office, Choba,
East-West Road, Port Harcourt, Nigeria;
E-mail:mac2finney@yahoo.com;
iheanyi.okonko@uniport.edu.ng
Tel.: +234 803 538 0891

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