

# Innate and adaptive immunity in teleost fish: a review

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**ABSTRACT:** The immune system of fish is very similar to vertebrates, although there are some important differences. Fish are free-living organisms from the embryonic stage of life in their aquatic environment. They have mechanisms to protect themselves from a wide variety of microorganisms. Consequently, fish rely on their innate immune system for an extended period of time, beginning at the early stages of embryogenesis. The components of the innate immune response are divided into physical, cellular and humoral factors and include humoral and cellular receptor molecules that are soluble in plasma and other body fluids. The lymphoid organs found in fish include the thymus, spleen and kidney. Immunoglobulins are the principal components of the immune response against pathogenic organisms. Immunomodulatory products, including nucleotides, glucans and probiotics, are increasingly used in aquaculture production. The use of these products reduces the need for therapeutic treatments, enhances the effects of vaccines and, in turn, improves the indicators of production. The aim of this review is to provide a review of the immune system in fish, including the ontogeny, mechanisms of unspecific and acquired immunity and the action of some immunomodulators.

**Keywords:** fish; immune response; immunomodulators

**CRP** = C-reactive protein; **ICM** = intermediate cell mass; **IHNV** = infectious hematopoietic necrosis virus; **IL** = interleukin; **INF** = interferon; **IPNV** = infectious pancreatic necrosis virus; **ISAV** = infectious salmon anaemia virus; **LPS** = lipopolysaccharide; **MHC** = Major histocompatibility complex; **MMCs** = melanomacrophage centers; **SAA** = serum amyloid protein; **TCR** = T cell receptor; **TLR** = toll-like receptor; **TNF** = tumour necrosis factor; **VHSV** = viral haemorrhage septicaemia virus

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## 1. Introduction

The immune system of fish is physiologically similar to that of higher vertebrates, despite certain differences. In contrast to higher vertebrates, fish are free-living organisms from early embryonic stages of life and depend on their innate immune system for survival (Rombout et al., 2005). Nonspecific immunity is a fundamental defence mechanism in fish. In addition, it plays a key role in the acquired immune response and homeostasis through a system of receptor proteins. These receptor proteins identify molecular patterns that are typical of pathogenic microorganisms, including polysaccharides, lipopolysaccharide (LPS), peptidoglycan bacterial DNA, viral RNA and other molecules that are not normally on the surface of multicellular organisms. This response is divided into physical barriers and cellular and humoral immune response. These immunological parameters include growth inhibitors, lytic enzymes, the classic complement pathways, the alternative and lectin pathway, agglutinins and precipitins (opsonins and primary lectins), antibodies, cytokines, chemokines and antibacterial peptides. Various internal and external factors can influence innate immune response parameters. Temperature changes, stress management and density may have suppressive effects on this type of response, while several food additives and immunostimulants can enhance their efficiency (Magnadottir, 2006, 2010).

The aim of this review is to provide some background on the immune system of fish, describing the ontogeny and core components, the nonspecific and adaptive immune mechanisms and elements that promote their actions as immunomodulators.

## 2. Ontogeny of the immune system

The ontogeny of the immune system of teleost fish has been studied in various species, including rainbow trout (*Oncorhynchus mykiss*), catfish (*Ictalurus punctatus*), zebrafish (*Danio rerio*) and grouper (*Acanthistius brasilianus*). Teleosts have been described as primitive models of hematopoiesis. The first hematopoietic organ is called the intermediate cell mass (ICM). In kili (*Pseudepiplatys annulatus*) and rainbow trout, hematopoiesis exists for a short period in the yolk sac and then shifts to the ICM (Zapata et al., 2006). With respect to the development of the organs involved in the immune

response, the anterior kidney and thymus are considered to be complete before hatching occurs in rainbow trout and Atlantic salmon (*Salmo salar*) (Razquin et al., 1990); this has not been clarified at the end of incubation in many marine species, including bream (*Sparus aurata*), grouper and turbot (*Scophthalmus maximus*). Despite these differences, the sequence of lymphomyeloid organ development in marine teleosts is as follows: kidney, spleen and thymus (Mulero et al., 2007), but larval spleen has a greater erythropoietic function than lymphopoietic function (Nakanishi, 1986; Schroder et al., 1998). Cells of myeloid lineage can be found around the yolk sac from 24 hours post fertilization (hpf) but are not seen in the ICM. Myeloblasts and neutrophilic myelocytes are observed between 34 and 48 hpf. On the other hand, numerous erythrocytes circulate in the blood vessels, and mature granulocytes are found in the blood system in the connective tissue that surrounds the yolk sac at 34 hpf and adjacent to the first renal tubules at 48 hpf. In addition, young myelocytes and neutrophils migrate through blood vessel walls during the same period. Mature neutrophils are described in various tissues at 72 hpf (Willett et al., 1999).

On the other hand, the first appearance of IgM in lymphocytes varies considerably among fish species (Magnadottir et al., 2005). However, the first appearance of B-lymphocytes and immunoglobulins is late in marine species compared to fresh water species, and larvae have reached about 20–30 mm in length when IgM is first expressed (Chantanachookhin et al., 1991; Magnadottir et al., 2005). In addition, Dalmo (2005) mentioned that the transfer of maternal antibody to eggs and embryos has been demonstrated in several species. Further, Magnadottir et al. (2005) suggest that the primary role of maternal antibodies is to protect the eggs against vertical transfer of certain pathogens or that maternal IgM may aid phagocytosis or the activation of complement pathways in early developmental stages; IgM may even function simply as a nutritional yolk protein.

Finally, complement component C3 has been found in unfertilised eggs in the spotted wolfish (*Anarhichas minor* Olafsen) indicating maternal transfer (Ellingsen et al., 2005). Using immunohistochemistry techniques, C3 has been found in several different organs and tissues of developing cod and halibut (Lange et al., 2004a,b). These studies suggest that complement may play a role in the generation of different organs and not only in the defence against invading pathogens (Dalmo, 2005).

### 3. Lymphoid organs

The thymus, kidney (anterior and middle) and spleen are the largest lymphoid organs in teleosts (Zapata et al., 2006). In freshwater teleosts, the thymus is the first organ to become lymphoid, but before this event, the anterior kidney may contain hematopoietic progenitors, but not lymphocytes (Lam et al., 2004).

#### 3.1. Thymus

This organ has two lobes, is homogeneous, and is represented by a thin sheet of oval lymphoid tissue that is arranged subcutaneously in the dorsal commissure of the operculum and is lined by mucous tissue of the pharyngeal epithelium (Ellis, 2001). The structure that characterizes the thymus of fish is a capsule that surrounds the lymphoid bark tissue. Basically, the thymus can be considered as an aggregation of macrophages that promote the encapsulated proliferation of T cells (Davis et al., 2002). The differentiation of the thymic structure is highly variable in teleosts, and in many species, it is not possible to observe a clear differentiation between the cortex and medulla that is found in higher vertebrates (Bowden et al., 2005). Furthermore, myeloid cells and eosinophilic granular cells can be found in this organ. Other studies described the appearance of focal epithelial nests, known as Hassal's corpuscles (Zapata et al., 2006). The thymus is responsible for the production of T cells. In zebrafish, thymus development involves neural crest cells derived from the neuroectoderm, which migrate to the third and fourth pharyngeal pouches and interact with the endoderm (Trede et al., 2001). The early development of the thymus has been studied in several teleost fish species, and development time differs between species according to the effects of temperature on growth. The relationship between growth and development is dynamic, with physiological age expressed as degree days, and is not a factor for differences in historical temperature (Bowden et al., 2005).

#### 3.2. Kidney

The kidney in teleost fish is the equivalent of the bone marrow in vertebrates and is the largest site of haematopoiesis until adulthood (Zapata et al.,

2006). In rainbow trout, the kidney is well-developed after hatching, when it mainly produces red blood cells and granulocytes. Studies suggest the presence of lymphoid cells that release IgM between 12 and 14 days post fertilization (dpf) (Castillo et al., 1993) and have also shown two variants of IgM by ELISA in embryos eight days before the end of the incubation period (Sanchez et al., 1995). This finding suggests that a source of B cells exists before the end of the incubation period in the kidney or in other hematopoietic sites. Structurally, the anterior kidney is composed of a network of reticular fibres that provide support for lymph tissue and are found scattered among hematopoietic system cells that line the sinusoid reticuloepithelium (Press et al., 1994). The main cells found in the anterior kidney are macrophages, which aggregate into structures called melanomacrophage centers (MMCs), and lymphoid cells, which are found at all developmental stages and exist mostly as Ig<sup>+</sup> cells (B cells) (Press et al., 1994). Reticular cells play an important role in supplying the interactions necessary for the function of lymphoid cells (Press et al., 1994) and endothelial cells of sinusoids. The latter system is the main component for blood filtering due to its ability to perform endocytosis (Danneving et al., 1994).

#### 3.3. Spleen

The spleen is composed of a system of splenic ellipsoids, MMCs and lymphoid tissue. In most species, ellipsoids are clustered together and are organized around the other two components (Ferguson, 1989). The ellipsoids are thick-walled capillaries that open in the pulp and result from the division of the splenic arterioles. The cells along the walls are actively involved in the macrophage phagocytosis of antigens. Usually in the form of antibodies or metabolic products, antigens may be detained for long periods of time, which has an important role in immunological memory. The spleen in zebrafish remains a small organ that contains large amounts of eritroblast at 30 days post fertilization. At three months, when lymphoblasts are evident in the spleen, emerging ellipsoids are involved in the capture of antigen. A similar developmental pattern has been described for other teleost spleen, such as that of the Atlantic salmon, grouper and catfish (Dos Santos et al., 2000; Petrie-Hanson and Ainsworth, 2000).

#### 4. Cells involved in immune response

Fish possess lymphocyte populations that are analogous to T cells, B cells, cytotoxic cells (similar to natural killer cells), macrophages and polymorphonuclear leukocytes. The immune system of teleosts has sub-populations of T lymphocytes that exhibit differential responses to mitogens, B cell acute allograft reactions, mixed leukocyte reactions and cooperative interactions between T cells, B cells and macrophages that are essential for the production of antibodies. Moreover, elasmobranch and teleost fish are the most primitive groups that possess the Major Histocompatibility Complex (MHC) and T cell receptor (TCR) (Manning and Nakanishi, 1996).

#### 5. Nonspecific immunity

In fish, the innate response has been considered an essential component in combating pathogens due to limitations of the adaptive immune system, their poikilothermic nature, their limited repertoire of antibodies and the slow proliferation, maturation and memory of their lymphocytes (Whyte, 2007). It is commonly divided into three compartments: the epithelial/mucosal barrier, the humoral parameters and the cellular components. The epithelial and mucosal barrier of the skin, gills and alimentary tract is an extremely important disease barrier in fish, being constantly immersed in media containing potentially harmful agents (Magnadottir, 2010). This type of response requires a series of mechanisms that involve humoral factors, cell and tissue, antimicrobial peptides and complement factors. Humoral factors may be cellular receptors or molecules that are soluble in plasma and other body fluids (Magnadottir, 2006; Subramanian et al., 2007; Subramanian et al., 2008)

##### 5.1. Physical barriers

Flakes, skin mucus and gills act as the first barrier to infection (Ingram, 1980; Shepard, 1994; Ellis, 2001). The mucus of fish contains lectins, pentraxins, lysozymes, complement proteins, antibacterial peptides and immunoglobulin M (IgM), which have an important role in inhibiting the entry of pathogens (Alexander and Ingram, 1992; Rombout et al., 1993; Aranishi and Nakane, 1997; Boshra et al., 2006; Saurabh and Sahoo, 2008). In addition, the epidermis

is able to react to different attacks (thickening and cellular hyperplasia), and its integrity is essential for osmotic balance and to prevent the entry of foreign agents (Hibiya, 1994). On the other hand, defending cells are present, such as lymphocytes, macrophages and eosinophilic granular cells (Sveinbjornsson et al., 1996; Ellis, 2001; Fischer et al., 2006).

##### 5.2. Nonspecific cellular cytotoxicity

In mammals, nonspecific response situations are mainly executed by cytotoxic cells, known as natural killer cells. Although the nonspecific cytotoxic cells of catfish are morphologically distinct from the large granular lymphocytes of mammals, they are suggested to be functionally similar (Evans and Jaso-Friedmann, 1992). These cells are able to eliminate a range of spontaneously xenogeneic targets, including parasites in fish and traditional targets of natural killer cells in mammals (Hasegawa et al., 1998). Unlike the natural killer cells of mammals, the nonspecific cytotoxic cells of catfish are agranular, small lymphocytes that are commonly found in lymphoid tissues, such as the anterior kidney and spleen, but are rarely found in the blood (Shen et al., 2002). In addition to the case in catfish, nonspecific cytotoxic cells have shown activity in other fish species, including rainbow trout (Greenle et al., 1991), common carp (*Cyprinus carpio*) (Hinuma et al., 1980; Suzumura et al., 1994), damselfish (*Dascyllus albisella*) (McKinney and Achmale, 1994) and tilapia (*Oreochromis* spp.) (Faisal et al., 1989).

##### 5.3. Antimicrobial peptides

Studies of the integument and integument secretions of fish (Hellio et al., 2002) have demonstrated an important role for this system in host defence against viruses and bacteria (Ellis, 2001; Chinchar et al., 2004; Maier et al., 2008). These peptides have been found in the mucus, liver and gill tissue of teleost fish (Birkemo et al., 2003). These low molecular weight polypeptides have the ability to break down bacterial walls (Ellis, 2001).

##### 5.4. Phagocytosis

Phagocytosis is one of the most important processes in poikilothermic animals because it is

the process that is least influenced by temperature (Blazer, 1991; Lange and Magnadottir, 2003; Magnadottir et al., 2005). The main cells involved in phagocytosis in fish are neutrophils and macrophages (Secombes and Fletcher, 1992). These cells remove bacteria mainly by the production of reactive oxygen species during a respiratory burst. In addition, neutrophils possess myeloperoxidase in their cytoplasmic granules, which in the presence of halide and hydrogen peroxide kills bacteria by halogenation of the bacterial cell wall. Moreover, these cells have lysozymes and other hydrolytic enzymes in their lysosomes (Fischer et al., 2006). Similarly, macrophages can produce nitric oxide in mammals and can be as potent as antibacterial agents, peroxy nitrites and hydroxyl groups (Secombes and Fletcher, 1992).

### 5.5. Complement

The complement system in teleosts, as well as that in higher vertebrates, can be activated in three ways: the classical pathway, which is triggered by antibody binding to the cell surface (Holland and Lambris, 2002), the alternative pathway, which is independent of antibodies and is activated directly by foreign microorganisms, and the lectin pathway, which is activated by the binding of a protein complex consisting of mannose/mannan-binding lectin in bacterial cells (Sakai, 1992). However, the mechanisms and molecules involved in this system in teleosts are not well understood, with the exception of the genetic sequence of the mannose-binding lectin protease that is associated with serum (Matsushita et al., 1998; Nikoskelainen et al., 2002). Studies suggest that the alternative complement pathway is of great importance in the innate immune response in teleost fish (Yano, 1996). The activity of complement-opsonized teleosts has been documented in a variety of species, such as common carp (Matsuyama et al., 1992), catfish (Jenkins and Ourth, 1993) and salmonids (Lammens et al., 2000). Salmonid antibodies, in the presence of complement proteins, are able to neutralize enveloped viruses, including the infectious hematopoietic necrosis virus (IHNV) and the viral haemorrhagic septicaemia virus (VHSV) (Lorenzen and La Patra, 1999). In bacterial infections, complement activation by lipopolysaccharide found in the cell wall of gram-negative pathogenic bacteria stimulates C5a factor production, a pow-

erful chemokine to macrophages and neutrophils that have receptors for C3, the central complement molecule which is a part of all three pathways, and which remains attached to the bacteria, favouring phagocytosis (Jenkins and Ourth, 1993). Teleost C3 is composed of a disulfide-linked two-chain (a and b) glycoprotein containing a thioester bond comparable to C3 from several vertebrate species (Magdanottir et al., 2005).

### 5.6. Tumor necrosis factor (TNF)

Several studies in fish have provided direct evidence suggesting that TNF- $\alpha$  and - $\beta$  are important activators of macrophages. Studies in rainbow trout, turbot, sea bream (*Sparus aurata*), goldfish (*Carassius auratus*) and catfish have shown that TNF causes the activation of macrophages, leading to increased respiratory activity, phagocytosis and nitric oxide production (Yin et al., 1997; Mulero and Meseguer, 1998; Tafalla et al., 2001).

### 5.7. Interferon (INF)

INF $\alpha$  and  $\beta$  are cytokines with a nonspecific antiviral function that is based on the inhibition of nucleic acid replication within infected cells. INF plays an important role in the defence against viral infection in vertebrate host cells, which secrete INF $\alpha$ / $\beta$  upon recognition of viral nucleic acid (Robertsen, 2006). These INFs protect other cells from viral infection by binding to different receptors, which results in the induction of several hundred genes that are stimulated by INF (ISGs). Some of these genes encode antiviral proteins, such as protein MX (MX), protein kinase dsRNA activated (PKR) and 2,5-oligoadenylate synthetase (OAS) (De Veer et al., 2001; Samuel, 2001). Two interferons (INF $\alpha$ -1 and INF $\alpha$ -2) have been cloned from the Atlantic salmon and were characterized with respect to their sequence, gene structure, promoters and induction of antiviral activity of interferon-stimulated genes (ISG) (Killeng et al., 2007; Rokenes et al., 2007). The INF $\alpha$ -1 Atlantic salmon induces the expression of MX and ISGs, and both have similar properties to INF $\alpha$ / $\beta$  and INF $\gamma$  in mammals (Rokenes et al., 2007). Furthermore, INF $\alpha$ -1 in Atlantic salmon induces a potent antiviral activity against infectious pancreatic necrosis virus (IPNV) in cells. However, this cytokine does not protect Atlantic salmon cells

against infectious salmon anaemia virus (ISAV), suggesting that this virus must have developed mechanisms to counteract the antiviral activity of INF (Killeng et al., 2007). Moreover, INF $\alpha$ -2 expressed recombinantly in Atlantic salmon was also confirmed to confer antiviral activity against IPNV (Ooi et al., 2008). Some authors suggest that rainbow trout have at least three INFs (Zou et al., 2007). Rainbow trout (INF1 (rtl INF1) and INF2 (rtl INF2) show sequences similar to INF $\alpha$ -1 and INF-2 in Atlantic salmon. Rainbow trout INF 3 (rtl INF 3) is related to INF $\alpha$  in mammals and fish, as shown by the conservation of four cysteine residues. The rtl INF1 and 2 proteins have been shown to regulate the production of MX, inhibiting the proliferation of VHSV in cell lines from the gonad of rainbow trout (GTR 2). Moreover, rtl INF 3 has been proven to be a poor inducer of antiviral activity. It is worth noting that these three INFs in rainbow trout show differential expression across cells and tissues, suggesting that they have different functions in the fish immune system (Zou et al., 2007).

### 5.8. Interleukins (IL)

IL-1 in mammals is comprised of 10 ligands and 10 protein receptor molecules and plays an important role in inflammation and host defence (Dinarello, 1997). IL-1 $\beta$  has been detected in 13 teleost fish species and is involved in the regulation of immunity through the stimulation of T cells. The function of IL-1 $\beta$  in these fish species is analogous to mammalian IL-1 $\beta$  (Mathew et al., 2002; Magnadottir, 2010). In teleost fish, IL-1 receptors have been cloned and sequenced from the rainbow trout and Atlantic salmon. The expression of the IL-1 receptor in salmon appears to be constitutive in all tissues tested and is regulated in the anterior kidney, spleen, liver and gills after stimulation with LPS and TNF- $\alpha$ , suggesting a role for the IL-1 receptor in regulating IL-1 $\beta$  during the inflammatory response (Sangrador-Vegas et al., 2000; Subramaniam et al., 2002).

### 5.9. Others cytokines and chemokines

As described above, TNF- $\alpha$  and IL-1 $\beta$  are cytokines involved in the induction of inflammatory responses to Gram negative bacteria in fish. In addition, it has been demonstrated that IL-6 is

also involved in the cascade leading to an inflammatory response for this type of bacteria (Savan and Sakai, 2006). Further, cytokines involved in leukocyte differentiation, including granulocyte colony stimulating factor (CSF) (Arma et al., 2004), macrophage-CSF (Hanington et al., 2007) and IL-7 (Kono et al., 2008), are all identified in fish. On the other hand, chemokines are also present and in some fish species considerable numbers of genes have now been identified (DeVries et al., 2006); however, many have no clear homologues in other vertebrate groups and their function remains to be determined (Secombes, 2008).

### 5.10. Protease inhibitors

Several protease inhibitors are present in the serum and other body fluids of fish (Bowden et al. 1997). The main function of protease inhibitors is to maintain body fluid homeostasis. These molecules are involved in acute phase reactions and defence against pathogens that secrete proteolytic enzymes (Magdanottir, 2010). The most widely studied of the protease inhibitors is the  $\alpha$ -2 macroglobulin, which has a high specificity for inhibiting the physical encapsulation of protease (Armstrong and Quigley, 1999).

### 5.11. Lysozyme

Lysozyme is a bacteriolytic enzyme that is widely distributed throughout the body and is part of the nonspecific defence mechanisms in most animals. In salmonids, lysozyme has been detected in serum, secretions, mucous membranes and tissues rich in leucocytes, mainly the kidney and intestine (Grinde et al., 1988; Lie et al., 1989). Apparently, the main sources of lysozyme are monocytes/macrophages and neutrophils. However, recent studies have detected this enzyme in the granules of the eosinophilic granular cells of the intestine (Sveinbjornsson et al., 1996). The bactericidal action of this enzyme involves the hydrolyzation of the peptidoglycan of bacterial cell walls resulting in cell lysis. Lysozyme was initially associated with the defence against Gram-positive bacteria, but has been found to lyse Gram-negative bacteria as well. Furthermore, this enzyme is known to trigger an opsonin of the complement system and phagocytic cells (Magnadottir, 2006).

### 5.12. Natural antibodies

Natural antibodies are produced in fish at a level that is regulated in the absence of antigenic stimulation of cells that are equivalent to B1 cells (Boes, 2000). These natural antibodies are found in high levels in the serum of fish, where they provide immediate and broad protection against bacterial and viral pathogens, making these factors key components of nonspecific immunity. Natural antibodies are also linked to adaptive immunity. Teleost fish are capable of generating specific IgM-type natural antibodies against various antigens. The intensity of this response, however, has been shown to vary between different species and environmental conditions (Whyte, 2007).

### 5.13. Pentraxins

C-reactive protein (CRP) and serum amyloid protein (SAA) are present in the body fluids of vertebrates and invertebrates and are commonly associated with the acute phase response of inflammation (Bayne and Gerwick, 2001; Wu et al., 2003). The expression of CRP has been reported in several teleost fish species, including rainbow trout (Hoover et al., 1998), catfish (Szalai et al., 1992), Atlantic salmon, common cod (*Gadus morhua*), halibut (*Hippoglossus hippoglossus*) and dog fish (*Hoplias malabaricus*) (Lund and Olafsen, 1998). The levels of these proteins are increased upon tissue injury, trauma or infection. These proteins play an active role in the immune system (Cook et al., 2003), activation of the classical complement pathway (De Haas et al., 2000) and the removal of apoptotic cells (Nauta et al., 2003). Different stimuli, such as tissue damage, trauma or infection, have been shown to generate various patterns of CRP production in teleost fish, in which either the level of CRP is decreased in serum (negative acute phase protein) (Szalai et al., 1994; Liu et al., 2004) or the level of CRP is increased in serum (positive acute phase protein) (Kodama et al., 2004). Although the pentraxins of teleosts have a recognized role in defence mechanisms, studies performed by Cook et al. (2003, 2005) have demonstrated their role in immune function through the activation of complement-mediated lysis in sheep erythrocytes. These authors note that the phagocytosis of beads soaked with pentraxins possibly occurs through the action of a surface receptor expressed in phagocytes.

### 5.14. Transferrin

Iron is an essential element in the establishment of infection by many pathogens, but the availability of iron in the tissue fluids of vertebrates is extremely low due to its high affinity for the blood protein transferrin. Only bacteria with high affinity systems for iron absorption are able to maintain sufficient iron levels to grow *in vivo* (Ellis, 2001; Stafford and Belosevic, 2003). Transferrin is a globular glycoprotein with a high iron chelator activity. This protein is the major iron ion transport protein in animals and plants. Transferrin has a high degree of genetic polymorphism in all species and is found in the serum and secretions of all vertebrates. Pathogenic bacteria have developed a set of mechanisms to obtain iron from the host, even from transferrin. However, because of the high degree of genetic polymorphism and the ability of certain bacterial pathogens to obtain iron from transferrin, this protein may be restricted to certain genotypes. In coho salmon (*Oncorhynchus kisutch*), three genotypes have been identified with respect to transferrin: AA, AC and CC. In some strains of coho salmon that originate in Oregon, increasing resistance against bacterial kidney disease has been associated with the C allele of transferrin (Suzumoto et al., 1997). However, the latter has not been confirmed for two strains of coho salmon that are native to British Columbia (Withler and Evelyn, 1990).

## 6. Specific immunity

The specific immune response occurs through mechanisms that involve a complex network of specialized cells, proteins, genes and biochemical messages that provide the means necessary for the body to respond specifically to antigens, antibodies and effector cells with high specificity and affinity.

### 6.1. Antibodies

The predominant immunoglobulin in teleosts is a tetramer of the IgM class and contains eight antigenic combining sites (Acton et al., 1971). Some teleosts have a monomer of IgM in their serum, although the factors leading to its expression are still unknown (Wilson and Warr, 1992). The binding affinities of monomeric and tetrameric IgM in rainbow trout are similar, but tetrameric IgM activates

complement more effectively than the monomeric form due to a structural difference in the Fc portion of the molecule (Elcombe et al., 1985). IgD was the second immunoglobulin isotype identified in fish, specifically catfish, due to sequence similarity with IgD in mammals, its location immediately under the IgM gene and its expression in B cells (Wilson et al., 1997). Moreover, the concentration of IgM in the serum of salmonids is extremely low compared to that of other teleosts such as Japanese eel (*Anguilla japonica*) (Uchida et al., 2000), cyprinids (Vilain et al., 1984) and some Perciformes (Scapigliati et al., 1997). However, the amounts of IgM in the serum of brown trout (*Salmo trutta*) and rainbow trout that are infected or acclimated to high temperature (19 °C) reach values similar to those of the common cod and haddock (*Melanogrammus aeglefinus*). In addition, IgM levels in salmon and atlantic cod (*Gadus morhua*) vary with size (Sanchez et al., 1993; Magnadottir et al., 1999), temperature (Sanchez et al., 1993) and water quality season (Olesen and Jorgensen, 1986; Magnadottir et al., 2001). Teleost antibodies are found in the skin (Hatten et al., 2001), intestine (Rombout et al., 1986), gill mucus (Lumsden et al., 1993), bile (Jenkins et al., 1994) and systemically in the plasma. The immune response of the skin and gills is important because these organs are in direct contact with the environment. Specific antibodies can be generated in the skin (Cain et al., 2000), intestine, (Jones et al., 1999) and gills (Lumsden et al., 1993) without necessarily generating a systemic response.

## 6.2. Immunological memory

Fish develop a memory response before a second exposure to an antigen (Arkoosh and Kaattari, 1991; Whittington et al., 1994). Rainbow trout respond to suboptimal doses of both T lymphocytes in an antigen-dependent and independent manner after an initial exposure to the same antigen (Arkoosh and Kaattari, 1991). It is remarkable that it takes two exposures before the fish responds to the second administration of T-dependent antigens, whereas T-independent antigen requires only one exposure. Additionally, while response is faster and of larger magnitude than the primary response, the number of antigen-specific B cells in the spleen is directly proportional to the frequency of B-cell specific antigen precursors. This finding suggests that the secondary response is caused by the expansion of the pool of memory B cells (Kattari, 1992) and not a specific

difference in the antibodies. However, the response to suboptimal levels of antigen suggests that perhaps B cells with high affinity receptors were selected as memory B cells (Morrison and Nowak, 2002).

## 6.3. Cellular Cytotoxicity

The leukocytes of fish are capable of generating cellular cytotoxicity reactions, which has been demonstrated in several fish species. However, the cells that are responsible for cell-mediated cytotoxicity in fish are difficult to characterize due to the lack of appropriate tools for cellular and molecular recognition (Fisher et al., 2006). In mammals, the adaptive immune response implemented by CD8+ cytotoxic T lymphocytes has been shown to be critical in combating various viral infections. These lymphocytes recognize and kill cells with virus-derived peptides using class I MHC molecules. Several studies indicate that this mechanism of cell death also exists in fish. The sequencing of fish homologues of MHC class I and CD3+ T cells suggests that CD8+ MHC class I presentation is similar to that which occurs in higher vertebrates (Fisher et al., 2006). Furthermore, we found a direct monoclonal antibody against the recombinant protein Onmy-UBA\*501, the MHC class I molecule of rainbow trout, which are expressed in the same cell types as classical mammalian MHC mammalian class I molecules (Dijkstra et al., 2003). Like other vertebrates, fish acquire immunocompetency during ontogeny. In sea bass (*Dicentrarchus labrax* L.), T cells appear early in larval development, at least five days after the start of incubation (Dos Santos et al., 2000). Fisher et al. (2006) have demonstrated in rainbow trout that transcripts of CD8+ T cells and MHC class I are detectable one week after insemination, while TCR mRNA in T cells appears one week after insemination. MHC class I molecules and CD8 are expressed from the larval stage, suggesting that very young fish can be vaccinated to develop cellular immunity. In fact, up to 14 days after the end of incubation young trout can destroy skin allografts, a process accompanied by infiltration of cytotoxic T lymphocytes (Tatner and Manning, 1983).

## 6.4. Cytokines involved in adaptive immunity

Cytokines have been described in adaptive immunity in fish and with the recent discovery of CD4



in teleosts in it seems likely these cytokines will drive the activation and differentiation of T helper cell subsets to release different cytokine repertoires (Secombes, 2008). As stated above, type I and type II IFN are present that could potentially drive Th1 cell differentiation, with IFN- $\gamma$  a potential effector of Th1 responses altogether with IL-12 (Yoshiura et al., 2003; Nascimento et al., 2007), IL-15 (Wang et al., 2007) and IL-18 (Huising et al., 2004; Zou et al., 2004). In relation to Th2-type cytokines, a molecule which is claimed to be a homologue of IL-4 has been found recently; however, it has no obvious similarity to known vertebrate IL-4 genes (Li et al., 2007). However, the presence of the transcription factor that drives Th2 cell differentiation, GATA3, suggests that Th2 responses may exist in fish, although this remains to be proven (Secombes, 2008). Finally, both IL-10 (Zou et al., 2003; Pinto et al., 2006) and TGF- $\beta$  (Hardie et al., 1998) are present in fish, and thus may form the effectors of potential T regulatory cells.

## 7. Environmental effect on immune response

The immune system and response of fish can be greatly influenced by various external factors like temperature, light, water quality, salinity and different stress inducers (Magnadottir, 2010).

Decreases in temperature, which are important due to the poikilothermic nature of fish, affect the rate of their physiological functions (Hayward et al., 2009; e.g., enzymatic activity). In the rainbow trout, photoperiod has also been shown to impact the immune response through a reduction in the number of circulating leukocytes that is caused by increasing daylight hours; in turn, leukocytes are increased to reduce the hours of exposure to this factor. An increase in the photoperiod can also generate increases in the activity of lysozyme and the circulating levels of IgM. Furthermore, oxygen levels in the environment may modulate the immune response; hypoxia depresses the respiratory burst activity of macrophages and lowers the levels of circulating antibodies, which in turn, are elevated by hyperoxia (Watts et al., 2001; Bowden, 2008). On the other hand, increased levels of suspended solids in the fish environment help to raise hematocrit levels to compensate for the diminished ability of the gills to capture oxygen. This factor also increases the activity of lysozyme and the levels

of circulating IgM as a response to elevated levels of pathogens that are expected in an environment rich in particles. Increased salinity also affects immune parameters by increasing the activity of lytic enzymes, the respiratory burst of macrophages and the circulating levels of IgM. However, the reason why these changes are associated with salinity is currently unknown, but some authors suggest that increases in salinity levels cause an increased burden of pathogens from the environment to generate a more favourable osmotic environment (Bowden, 2008). Changes in environmental pH levels show conflicting results for immune system parameters, such as levels of lysozyme and IgM in the circulation. Finally, stress in fish as a result of population density associated with cultivation and production management can increase circulating cortisol levels, generating a decrease in specific and non-specific immunity and, therefore, making the fish prone to opportunistic pathogens (Brydges et al., 2009; Ramsay et al., 2009).

## 8. Compounds that modulate the immune system in fish

Another approach to immunoprophylactic control is the use of probiotics and immunostimulants including specific dietary manipulation (Magnottir, 2010). These compounds are defined as chemicals, drugs, stress generators or other actions that raise nonspecific defence mechanisms or the specific immune response (Anderson, 1992) and include nucleotides, glucans and probiotics. These treatments are primarily aimed at enhancing the innate system and therefore are of value as general preventive measures in aquaculture (Magnadottir, 2010).

### 8.1. Nucleotides

Nucleotides are composed of a purine or pyrimidine base, a ribose or deoxyribose sugar and one or more phosphate groups. The term nucleotide in this context refers not only to a specific form of the compound, but also to all forms that contain purine or pyrimidine bases (Rudolph, 1994). These compounds are essential in many physiological and biochemical functions, among which are included the encoding and decoding of genetic information, the mediating of energy metabolism and cell signals, and also forming components of coenzymes, al-

losteric effectors and cellular agonists (Carver et al., 1995; Cosgrove, 1998). Nucleotide supplementation of fish was introduced by the studies of Burrells et al. (2001a,b), which indicated that dietary supplementation with nucleotides confers increased resistance on salmonids to viral, bacterial and parasitic diseases and improves the effectiveness of vaccination and the ability to osmoregulate. Further, the binding of these compounds to Toll-like receptor 9 (TLR-9) is thought to stimulate the activity of intracellular bacterial DNA and trigger innate and adaptive responses (Dalmo, 2005). With respect to the innate immune response, it is known that dietary nucleotides may influence the activity of macrophages (Gil, 2002) and natural killer cell (Carver et al., 1990). Sakai et al. (2001) demonstrated in common carp that exogenous nucleotides may influence the humoral and cellular components of the innate immune system by increasing the complement lytic activity of enzymes and the production of the superoxide anion by anterior kidney phagocytes. Li et al. (2004) reported that hybrid striped bass (*Morone saxatilis*) that were fed diets supplemented with nucleotides had a higher production of oxidative radicals from the neutrophils in their blood compared to fish that were fed a normal diet. In addition, nucleotides also influence the activity of lymphocytes and immunoglobulin production (Jyonouchi et al., 1993; Navarro et al., 1996). Ramadan et al. (1994), were the first to observe the effect of dietary supplementation with nucleotides, and described a stimulatory effect on the humoral and cellular immune response in tilapia after intramuscular injection or bath with formalin-killed *Aeromonas hydrophila*. Antibody titres and lymphocyte mitogenic responses of fish supplemented with nucleotides were significantly higher in fish that were fed the basal diet. Similar phenomena have been observed in rainbow trout (Burrells et al., 2001b; Leonardi et al., 2003), hybrid striped bass (Li et al., 2004) and Atlantic salmon (Burrells et al., 2001b). However, the mechanisms of action of these compounds are still not well understood.

## 8.2. Glucans

One of the most used substances in immunostimulation experiments in fish are various forms of  $\beta$ -glucans from different sources, normally introduced in the feed but also by intraperitoneal injection or as a vaccine adjuvant (Dalmo and Bogwald,

2008). Glucan-linked glucans  $\beta$ 1-3 and  $\beta$ 1-6 are the major components of the cell walls of yeasts and mycelial fungi. These polysaccharides are not antigenic to animals (Ballou, 1982), but have been proven to be potent activators of nonspecific mechanisms of antibacterial defences in fish (Di Luzio, 1985; Sherwood et al., 1987). The injection of glucans generates improvements in the fish innate immune response by increasing the activity of macrophages, complement activation and levels of lytic enzymes (Jeney and Anderson, 1993; Jorgensen et al., 1993; Ai et al., 2007). Furthermore, several studies have shown that injection of glucans improves antibacterial defences and efficiency of vaccination (Yano et al., 1991; Rorstad et al., 1993). Similarly, when administered orally, these compounds are capable of stimulating the immune response and disease resistance (Siwicki et al., 1994). Engstad and Robertsen (1995) inoculated Atlantic salmon with an *Aeromonas salmonicida* vaccine that contained glucans in its formulation; six weeks after inoculation, there was a greater antibody response in the vaccine-inoculated fish compared to the fish that were vaccinated with a preparation that contained only the bacteria.

## 8.3. Probiotics

Probiotics are organisms or substances that contribute to the intestinal microbial balance. Fuller (1989) defined probiotics as live microbial feed supplements which exert beneficial effects on the host animal by improving its intestinal microbial balance. Research on the use of probiotics in aquatic animals has increased the demand for sustainable aquaculture (Gatesoupe, 1999). Many of the probiotics used in aquaculture belong to the Lactobacillus, the genus *Bacillus*, photosynthetic bacteria or yeasts, although other genera or species have also been employed, including nitrifying bacteria, streptococci and Roseobacter (Wang et al., 2008). These have been tested in aquaculture with generally good results (Panigrahi et al., 2005; Aly et al., 2008; Ma et al., 2009). The benefits of supplements include improvements in feed values, contribution to enzymatic digestion, inhibition of pathogenic microorganisms, anti-mutagenic and anti-carcinogenic activity, growth-promoting factors and an augmentation of the immune response (Wang and Xu, 2006; Wang, 2007). Studies have shown that the use of *Bacillus* sp. in cropping

systems has improved the water quality, survival, growth rate and health status of juvenile giant prawn (*Penaeus monodon*) and also reduced the amount of pathogenic vibrios (Dalmine et al., 2001). Moreover, probiotics may also stimulate the innate defence through TLR, other cellular receptors or humoral factors (Magnadottir, 2010).

## 9. Concluding remarks

In the last years, great progress has been made in understanding the immune response of fish which has benefitted the growing aquaculture industry worldwide. One critical element of this area of research has been established: the immunomodulation of larval fish. The production of fish larvae is often hampered by high mortality rates and economic loss due to infectious diseases. As is well known, in fish the innate response has been considered an essential component in combating pathogens due to limitations of the adaptive immune system. Therefore, the immunomodulation of larval fish has been proposed as a potential method for improving larval survival by increasing the innate responses of developing animals until their adaptive immune response is sufficiently developed to mount an effective response to the pathogen (Dalmo, 2005). This is a crucial point in further research that is currently focused on increasing the survival of larval fish. Finally, Dalmo (2005) suggest that the development of strategies to control pathogen load and immuno-prophylactic measures must be implemented further to realise the full potential of the production of marine fish larvae and thus improve the overall production of adult fish

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