Immune Response of Fish to Viral Infection

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ABSTRACT: This study reports on the immune responses of fish to viral infections. In this review, the innate and acquired host immune responses of fish to viral infections will be considered. These would enable a more comprehensive understanding of fish immunity to viruses. The control of these viral infections are challenging as there are only a few treatments that are effective. Furthermore, the development of vaccines for the control of viruses remains elusive. Viruses are important pathogens affecting both wild and cultured fish. Teleost fish possess an array of defence mechanisms to prevent successful infections of these pathogens which abound in the surrounding aquatic environment. These mechanisms can be grouped as innate (non-specific) and adaptive (specific) immunity. Here the general protective immune mechanisms of fish to viral infections are reviewed. The fundamental components of innate and adaptive immunity present in finfish are similar to their mammalian counterparts in many ways. Once the physical barriers are overcome by the invading pathogen, chemical and genetic barriers kick in. Complement and interferon are part of such chemical barriers. Natural Killer cells also contribute to non-specific immunity through cell-mediated cytotoxicity (CMC). B-lymphocytes and T-lymphocytes are the major players in specific immune response and are the final defence against the pathogens. Developments in genomics have caused remarkable breakthroughs in insight into immune responses in finfish. However, quite a lot of ground remains to be covered. The zebra fish is currently being studied as a model to bridge the gaps in knowledge.
[Adedeji BO, Onianwa O, Okerentugba PO, Okonko IO. **Immune Response of Fish to Viral Infection.** *Nat Sci* 2012;10(8):70-76]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 12

Keywords: Viral infections, Defence mechanisms, Complement, Interferon, Cell-mediated cytotoxicity, Innate (non-specific), Adaptive (specific) immunity

1. INTRODUCTION

The importance of fisheries to humans cannot be overemphasized as they are a good source of protein in the diet. They also contribute to a healthy ecological balance of the greater marine environment (Skouras *et al.*, 2003). The global availability of fish and fish products depends on the natural population of macroalgae, crustaceans, shellfish and finfish of fresh water and marine sources. Aquaculture provides the solution to the high demand of fish products. One of the challenges faced by aquaculture however is the effective detection and prevention of disease outbreak in fishes. These diseases have resulted in significant economic losses in the marine aquaculture industry (Ming-Wei *et al.*, 2008) as well as caused disease outbreaks in humans and other mammals. Fish contaminated with toxins are hazardous when sold as food (Talaro, 2005).

In addition to improving the yield of fish for human consumption, teleost fish have the basic components of innate and adaptive immunity found in other vertebrates. This has led to their use as models to study general host immune response to infections in vertebrates (Purcell *et al.*, 2012). Organisms that cause diseases in fish include a large number of bacteria, fungi, metazoans, protozoans and viruses. These are present in the aquatic environment and have intimate contact with the fishes. To cause an infection the pathogen would have to invade the host cells through the process of attachment and penetration. This would be followed by the replication of the pathogen in the cell through the interference of the cell's processes for production of nucleic acid and protein (Ellis, 2001). The invasion of these pathogens into the body of the fish is inhibited by physical barriers and the replication of the pathogen within the host is inhibited by natural and acquired immune mechanisms. These constitute the total immune system of the fish. Attempts to characterize the fish immune mechanisms, has met some progress. Notwithstanding, there is still a lot of uncovered ground in the knowledge of numerous immune mechanisms. The immune response of a host to a pathogen is divided into two distinct components which are the nonspecific (Innate) immune response and the specific (adapted/acquired) immune response (Wiley *et al.*, 2011).

Innate immunity is also referred to as natural immunity and is the first line of defence of the host to any foreign agent (Alvarez-Pellitero, 2008). It is of immense importance due to several reasons. The first is that it is non-specific and as acts independently of

the distinct molecular structure of the invading pathogen. Secondly, the response of these defences to invading pathogens is relatively quick. Inflammation for example occurs within 2 days of infection. Finally such responses are relatively temperature independent (Ellis, 2001). Acquired immunity usually develops after exposure to the infectious agent. It is equally important as it is specific for the particular antigen that elicited the response. Though it takes a longer time to be fully developed after the first exposure to the pathogen, subsequent exposures are usually faster and of higher magnitude than the initial response. Another disadvantage of acquired immunity is that it is temperature dependent (Ellis, 2001; Wiley *et al.*, 2011).

In this review, the innate and acquired host immune responses of fish to viral infections will also be considered. These would enable a more comprehensive understanding of fish immunity to viruses.

2. VIRAL DISEASES IN FISHES

The presence of viruses in fish pathology has been known since the pre-1950s (Wolf, 1988) though the actual causative agents of the diseases was not proven until the technique involving the isolation of piscine viruses in fish cell lines was established (Wolf, 1962; Gravell and Malsberger, 1965). This technique of isolation of fish viruses *in vitro* became the yardstick for the detection of viruses in various species of fish such as the Atlantic salmon and rainbow trout. Some of the significant pathogens that affect finfish include *Aquabirnavirus* (the etiological agent of infectious pancreatic necrosis (IPN) in farmed trout), *Betanodavirus* (the etiological agent of viral nervous necrosis (VNN)), Infectious salmon anemia virus (ISAV) (the etiological agent of infectious salmon anemia (ISA) in the Atlantic salmon (*Salmo salar*)), Salmon alphavirus (SAV) (the etiological agent of Pancreas Disease (PD)), Infectious hematopoietic necrosis virus (IHNV) (the etiologic agent of infectious hematopoietic necrosis (IHN) in most salmonid fish species), Epizootic hematopoietic necrosis virus (EHNV) (the etiologic agent of The disease associated with EHNV is termed epizootic hematopoietic necrosis (EHN)), and Viral hemorrhagic septicemia virus (VHSV) (the etiologic agent of viral hemorrhagic septicaemia in farmed rainbow trout) (Crane and Hyatt, 2011). The IHNV is one of three rhabdoviruses reportable to The World Organization for Animal Health (OIE) (The other two being the spring viremia of carp virus (SVCV) and viral hemorrhagic septicemia virus (VHSV)) (Purcell *et al.*, 2012). Viral diseases have caused huge losses in aquaculture. Some viruses affect mainly young fish while others could cause disease at the fishes at every stage of development. Some fish could also be persistent carriers of these viruses and shed them continuously into the aquatic environment, thus serving as a continual source of infection to other fishes (Ellis, 2001). In natural conditions, the diseases caused by some pathogens are mild as observed in the case of herpes virus infections (Hanson *et al.*, 2011). The nature of such diseases however, is worse in immune-compromised hosts. The control of viral infections is challenging as there are only a few treatments that are effective. Furthermore, the development of vaccines for the control of viruses remains elusive (Crane and Hyatt, 2011).

3. ZEBRA FISH IMMUNOLOGICAL MODEL

History has witnessed the use of vertebrate models and *in vitro* systems as essential tools to gain insight into the molecular dynamics of host immune response to pathogen invasion. Such models have been predominantly mouse models and immune cell culture. The limitations encountered with these mammalian models have led to the development of non-mammalian models as tools to further understand immune responses to infection (Renshaw and Trede, 2012). The use of the zebrafish (*Danio rerio)* model as a tool for biological study has expanded beyond the borders of genetic dissection of early vertebrate development to that of organogenesis and disease processes (including cancers) since its first splash 30 years ago. The immune system of the Zebra fish has received considerable attention in recent years due to the relative ease to image cell movements and organogenesis in the entire vertebrate organism. These advantages, coupled with the increasing recognition that teleosts have an immune system similar to that of mammals as it includes both innate and adaptive immunity, makes them good models for immunity studies (Yoder *et al.*, 2002; Trede *et al.*, 2004). There is still a lot of ground to cover in the analysis of the immunity in the Zebra fish. Areas such as the complete characterisation of differences and similarities between zebrafish and mammalian immune systems as well as the development of well-designed screening methods to identify potent compounds for the therapeutic manipulation of immunity are yet to be covered. Other finfish in which studies of immune response has been carried out include the Atlantic Salmon (*Salmo salsa*), rainbow trout and channel catfish (*Ictalurus punctatus*) (Robertsen *et al.*, 2003; Long *et al.*, 2004).

4. INNATE RESPONSE

4.1. Inteferon

Interferons (IFNs) are low molecular weight, pH-resistant cytokines produced in response to viral infections (Wiley *et al.*, 2011). The quick induction of

IFN is an essential ingredient of any innate immune response to viral infection as it determines the viral pathogenesis and disease development (Deonarain *et al.*, 2004). Interferon production is usually induced by the presence of double-stranded RNA (dsRNA) and is produced rapidly after infection causing a large percentage of scientists to believe that it provides a degree of infection till the specific immune system is activated. Double stranded RNA (dsRNA) is produced by most viruses during replication. These molecules can be detected by hosts and this leads to the consequent production of the cytokine (Jacobs and Langland, 1996). After the transcription of the interferon gene, interferon is produced. The cytokine then diffuses out of the cell and makes it way to uninfected cells where it induces the production of proteins such as 2′,5′-oligoadenylate synthetase, protein kinase P1 and Mx proteins that block viral replication and degrade viral RNA (Stark *et al.*, 1998; Talaro, 2005). Polyinosinic:polycytidylic acid (Poly I:C), a synthetic analog of viral dsRNA, has been reported to produce interferon-like activity and induce Mx gene expression *in vivo* and *in vitro* in fish (Ellis, 2001; Saint-Jean and Pérez-Prieto, 2007). The exposure of fish and fish cell lines to IFN-containing supernatants has resulted in protection against several viruses (Rogel-Gaillard *et al.*, 1993). Analytical methods used in the detection and monitoring of interferon responses in fish involve the detection of Mx gene mRNA or Mx proteins through RT-PCR and labelled antibodies respectively (Robertsen *et al.*, 1997; Trobridge *et al.*, 1997; Trobridge *et al.*, 1997). Nygaard *et al.*, (2000) suggested the use of Mx gene and Mx RNA as molecular markers for the production of type 1 interferon after demonstrating the induction of Mx protein by dsRNA in fish occurs through interferon. Within the past decade, Type 1 interferon genes have been cloned and sequenced in Atlantic salmon, channel catfish and zebra fish (Altman *et al.*, 2003; Robertsen *et al.*, 2003; Long *et al.*, 2004).

4.2 Complement

Research into the activation of complement in fish as a response to IHNV and VSHV viral infections has been reported (Lorenzen *et al.*, 1999). Though the results show that the classical pathway is involved in the *in vitro* neutralization of the rhabdoviruses, the exact mechanism by which complement aids viral neutralization remains unclear till this present day (Lorenzen and LaPatra, 1999; Purcell *et al.*, 2012). The possibility of a requirement of enveloped viruses in order to activate complement components has been hinted at as antibodies do not require complement to neutralize non-enveloped viruses (Ellis, 2001).

4.3 Natural Killer (NK) Cells and other non-specific cytotoxic cells (NCC)

Natural killer cells as a group of cytotoxic cells contribute immensely to innate immunity in higher vertebrates. Though NK cells develop from the same lymphoid progenitor and as such share similar expression of some surface receptors such as CD8 found in T cells, their action is nonspecific. They protect the host through the lysing of infected cells and tumour cells (Renshaw and Trede, 2012). They are part of host defence in the early period of infection with viruses and bacteria protecting the host pending the complete development of Cytotoxic T-Cells (Tc) cells to Cytotoxic T-Lymphocytes (CTL) (Wiley *et al.*, 2011). The action of non-specific Cell-Mediated cytotoxic (CMC) activity in various genera of fish has been clearly demonstrated. The infection of AS and RTG-2 cells (cell lines derived from tissue culture of the respective Atlantic salmon and rainbow trout) with Infectious Pancreatic Necrosis Virus (IPNV) resulted in an increase in the cytotoxic effect of kidney leukocytes for the respective cell lines (Moody *et al.*, 1985; Yoshinaga *et al.*, 1994). Evidence of CMC activity in channel catfish (*Ictalurus punctatus*), common carp (*Cyprinus carpio*) and crucian carp (*Carassius carassius*) has also been reviewed (Fischer *et al.*, 2006; Nakanashi *et al.*, 2011). Both nonspecific cytotoxic cells (NCC) and NK-like cells are responsible for non-specific CMC in channel catfish (Evans and Jaso-Friedman, 1994; Shen *et al.*, 2003; Shen et al., 2004).

4.4 Glucan-Induced Immunity

β-glucans are polycassharides of glucose linked by glycosidic bonds. They are found in the cell walls of bacteria, plants and fungi (Brown and Gordon, 2003). The immunostimulatory activities of β-glucans fish and other animals have been reported (Ai *et al.*, 2007; Masuda *et al.*, 2009). Glucan-injected rainbow trout was more resistant to immersion challenge with IHNV than controls injected with saline; though neutralizing antibody production was significantly lower (LaPatra *et al.*, 1998). Kim *et al.*, (2009) reported the detection of Mx gene expression in grass carp infected with grass carp hemorrhage virus (GCHV) within 12 hours of post-infection. Mx gene expression remained at high levels on the 10th day. Persistence of the Mx gene expression may be as a result of a combined effect of the virus infection and the presence of β-glucan. The exact mechanism through which β-glucan mediates anti-viral activity in fish is still unknown.

4.5 Genetic Resistance

Genetic resistance is an important factor in host defence against diseases as clearly shown in the

susceptibility of some organisms when compared to others within the same population (Talaro, 2005; Wiley *et al.*, 2011). Past research has demonstrated resistance to viral infections as a result of mutations in genetic sequences. Brown *et al.*, (1994) demonstrated how a mutation in the P antigen (cellular receptor) could lead to resistance against the parvovirus B19. Also mutations in chemokine receptor 5 (CCR5) has been shown to be the basis for resistance to HIV-1 (Dean *et al.*, 1996; Liu *et al.*, 1996; Samson *et al.*, 1996). This clinical variability within populations is the basis for current research into genetic factors responsible for defence against infections (Alcais *et al.*, 2009). Dorson *et al.*, (1994) challenged hybrids of brook trout and rainbow trout with VSHV and reported reduced mortality and viraemia with the hybrids when compared to the rainbow trout controls. Interferon production levels were also low, thus suggesting the possibility that the resistance had nothing to do with the activity of interferon. A collection of double haploid fish clones and isogenic derived fibroblast-like cell lines from rainbow trout also displayed a wide range of susceptibility to VHSV infection (Verrier *et al.*, 2012).

5. ACQUIRED RESPONSE

5.1. T Cells

T cells play huge roles in cell-mediated immune response. They are specific in action and contribute to the activation of B cells. Unlike B cells (which will be discussed later) which produce antibodies to control foreign antigens, the whole T cell is in direct contact with the antigen to destroy it (Talaro, 2005; Wiley *et al.*, 2011). Various subsets of T cells exist. CD8+ killer T cells control cancer cells and destroy virus-infected cells. CD4+ helper T cells assist B cells in antibody production and CD4+CD25+ regulatory T cells prevent autoimmune responses. There is genetic evidence that all three types are found in the zebra fish (Renshaw and Trede, 2012). Various studies on specific cell-mediated cytotoxicity have been carried out in species such as channel catfish, ginbuna crucian carp and rainbow trout. This has brought about increased understanding of fish T cells. Finfish possess many T cell associated genes including genes that code for T cell receptor chains as well as other T cell associated co-receptors and cytokines (Fischer *et al.*, 2006, Laing and Hanson, 2011). Various research findings also suggest similarities between CD8+ cytotoxic T lymphocytes (CTLs) in fish and those of higher vertebrates (Stuge *et al.*, 2000; Fischer *et al.*, 2003). A lack of tools such as monoclonal antibodies (mAbs) have however, slowed down research into the identification and characterization of CTLs in fish (Nakanishi *et al.*, 2011).

5.2. B Cells

The contribution of B cells to defence against infections is majorly through humoral immunity. These unique lymphocytes are produced in the *Bursa of Fabricius* in birds. After activation, B cells form plasma cells and memory cells. The plasma cells produce antibodies which contribute to the destruction of antigens. They can also act as antigen presenting cells (Talaro, 2005; Wiley *et al.*, 2011). Advances in genomics and functional studies have caused an increase in the understanding of fish B cells. The discovery of the phagocytic nature of B cells in teleost fish hints at the possibility of the lymphocytes playing important roles in the innate immune system (Li *et al.*, 2006). Antibodies induced in finfish through infection and/or vaccinations are critical to the achievement of long term adaptive immunity of fish rhabdoviruses (Lorenzen and LaPatra, 1999). The presence of immunoglobulin isotypes such as IgD, IgM and IgT (IgZ in zebrafish) have been shown in teleost fish (Hordvik *et al.*, 2002; Danilova *et al.*, 2005; Hansen *et al.*, 2005). Current research indicates that IgM-expressing B cells respond to antigenic stimulation in systemic tissues while IgT-expressing B cells are important to the mucosal immune response (Zhang *et al.*, 2010).

6. CONCLUDING REMARKS

Downstream interferon activated Mx gene has also been identified in zebrafish, grouper, salmon, trout, and halibut upon infection with aquatic viruses (Chen et al., 2006; Kibenge et al., 2005; Lin et al., 2005; McBeath et al., 2006) suggesting the importance of the interferon regulatory pathway including RNA-activated protein kinase (PKR) and the 2-5A proteins during viral infection. At present, zebrafish is rapidly becoming a valuable molecular genetics model in understanding vertebrate organogenesis and disease development (Glass and Dahm, 2004; Yee and Pack, 2005). To date, several viruses are known to infect zebrafish, such as spring viraemia of carp virus (SVCV), a member of the Rhabdoviridae, that causes significant mortality in common carp (*Cyprinus carpio*) (Sanders et al., 2003) and snakeheadrhabdovirus (SHRV) which was shown to cause mortalities exceeding 40% in zebrafish (Phelan et al., 2005).

Teleost fish possess a rich immune response to viral infections. The innate immune system is sufficient for a healthy host population with low infection pressure. This however becomes compromised due to conditions of aquaculture which could cause physical trauma leaving the fish open to attack from pathogens. The adaptive immune system is therefore a strong and effective support under such

conditions. This however takes a longer time to develop. Thus the survival of the fish to attack by viral pathogens is greatly determined by contributions from both innate and adaptive immune responses.

There is still a lot to learn about the immune system, and the present challenge of re-emerging infectious diseases in fish and mammals in both the developed and developing world drives a continuing need for increased understanding. Present work on zebrafish models is promising to revealing novel insights into infectious diseases.

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6/15/2012