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Review

Immunity to betanodavirus infections of marine fish

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ABSTRACT

Betanodaviruses cause viral nervous necrosis in numerous fish species, but some species are resistant to infection by these viruses. It is essential to fully characterize the immune responses that underlie this protective response. Complete characterization of the immune responses against nodaviruses may allow the development of methods that stimulate fish immunity and of an effective betanodavirus vaccine. Such strategies could include stimulation of specific immune system responses or blockage of factors that decrease the immune response. The innate immune system clearly provides a front-line defense, and this includes the production of interferons and other cytokines. Interferons that are released inside infected cells and that suppress viral replication may be the most ancient form of innate immunity. This review focuses on the immune responses of fish to betanodavirus infection.

Contents

1. Introduction of betanodavirus infection

The betanodavirus, also referred to as the nervous necrosis virus (NNV), was first identified on an aquacultural farm of Japanese parrotfish (*Calotomus japonicus*) in Nagasaki, Japan during 1986. In this incident, many fish fry of 6–25 mm length died when the water temperature rose during the summer (June–July). Affected fish fry lost their balance and developed necrosis in the brain and retina. Viral particles were identified as the cause, leading to the name of nervous necrosis virus (NNV) (Yoshikoshi and Inoue, 1990). NNV is a betanodavirus of the family Nodaviridae, and has been documented as the cause of mass mortality of numerous larval-stage marine fish species (Mori et al., 1992; Nishizawa et al., 1997; Kuo et al., 2011).

An infected fish has a spiral swimming pattern, dashes underwater and floats back to the surface, darkened body color, poor appetite, and clusters near the side of a pool. Scoliosis may also occur. NNV infection is an acute infectious disease and death may occur within one week of symptom onset. Nodavirus infections are common, and NNV infections, also referred to as viral encephalopathy or retinopathy (VER), are responsible for significant fish mortalities (Munday et al., 2002).

Betanodavirus is non-enveloped and icosahedral with a diameter of 20–30 nm, and two positive-sense RNA strands known as

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RNA1 and RNA2. RNA1 encodes RNA-dependent RNA polymerase (RdRp), a mitochondrial enzyme, and is responsible for viral replication. RNA2 encodes a coat protein, which assembles to form viral particles. Mature viral particles are released extracellularly. Recent research indicated that the N-terminal region of RNA1 transcribes a subgenomic RNA3, which may encode the B protein, which facilitates intracellular viral RNA accumulation and is inhibited by host RNAi activity (Fenner et al., 2006a,b; Ou et al., 2007). Four NNV genotype strains (SJNNV, TPNNV, GNNV, and BFNNV) have been purified for the preparation of rabbit poly-antiserum and the relationship of virus strains was assessed by cross-neutralization reactions, which led to the identification of three serotypes (Mori et al., 2003). In particular, BFNNV and GNNV are serologically similar, although BFNNV occurs mainly in cold water species and GNNV occurs mainly in warm water species. This research led to the establishment of the betanodavirus genus (piscine nodavirus), a new virus genus that is specific for marine fish and is related to the alphanodavirus, an insect nodavirus (Nishizawa et al., 1995).

The red-spotted grouper nervous necrosis virus (RGNNV) has a wide host range, and infects fish in 5 orders and 12 families of fresh and salt water fish, all of which inhabit warm water. There are about 8 fish species in Taiwan that are infected by NNV, including the yellow-wax pompano (*Trachinotus falcatus*), cobia (*Rachycentron canadum*), European eel (*Anguilla anguilla*), barramundi (*Lates calcarifer*), hump-back grouper (*Cromileptes altivelis*) and Chinese catfish (*Silurus asotus*) (Chi et al., 2003). Analysis of the viral nucleic acids of RGNNV isolates in Taiwan indicated a 97% similarity to Japanese RGNNV isolates. Although NNV is best known for infecting saltwater fish, an infection of fresh water fish was reported in eel and catfish aquaculture systems in Taiwan, and the catfish mortality was 100% (Chi et al., 2003). The cause of nodavirus dispersal from saltwater to fresh water is still unknown. Barramundi (*L. calcarifer*) fry breed in saltwater in Taiwan, and are moved to fresh water at the juvenile stage, so betanodavirus-infected barramundi may be responsible, but further research is required.

When the four viral strains were isolated, RGNNV was found in warm water fish, Tiger puffer nervous necrosis virus (TPNNV) and Barfin flounder nervous necrosis virus (BFNNV) were found in cold water fish, and Striped Jack nervous necrosis virus (SJNNV) were found in warm and cold water fish. Iwamoto et al. (2000) identified the optimal temperatures for viral replication in the different strains (RGNNV: 25–30 °C, TPNNV: 20 °C, BFNNV: 15–20 °C, and SJNNV: 20–25 °C). Upon nodavirus challenge, red-spotted grouper was more easily infected at a high water temperature than low water temperature (Tanaka et al., 1998). Temperature also affects the infection rate of seven-band grouper (Fukuda et al., 1996). Chi and colleagues reported that a grouper nodavirus isolate infected fish cells more rapidly at 20–24 °C than at 28–30 °C, as determined by the rate of viral nucleic acid and protein replication and virus count (Chi et al., 1999). Nodavirus infects warm water fish in Southeast Asia, but outbreaks have also been reported in cold water aquaculture systems in Japan and northern Europe (Nishizawa et al., 1995; Starkey et al., 2001; Johnson et al., 2002). Nodaviruses isolated from cold water species did not affect warm water species, and nodaviruses isolated from warm water species did not affect cold water species (Totland et al., 1999). These results may be explained by host specificity or temperature specificity of the virus. Marine fish immunity to betanodaviruses is not fully understood, and further research is needed. During the process of betanodavirus infection, the virus and host appear to have complex interactions. Some studies showed that many betanodavirus strains have broad host ranges, and are particularly common in groupers and sea bass, although there were significant effects of host and temperature (Chi et al., 1999; Iwamoto et al., 2004; Bandín and Dopazo, 2011). Despite their common features, the different genotypes of betanodaviruses apparently evolved to infect a wide variety of hosts (results summarized in Table 1).

2. Immunology of betanodavirus infection

Fish, like humans, have immune systems consisting of specialized immune cells, receptors, and chemical messengers. The functional components of the fish immune system have the potential to form a highly organized defense against nodavirus infection. In general, the immune system is very complex and is integrated with built-in control and failsafe mechanisms so that some components can provide backup if other components fail. Viral infection of a fish leads to changes in the expression of immune system genes, similar what occurs in humans. Fish immunologists have focused on the anti-viral activities of interferons during the past 6 years. As with humans, the two types of immune responses in fish can be classified as humoral immunity and cellular immunity. Humoral immunity is characterized by the production of antibodies (specific immunoglobulins) that are secreted by B cells following antigen-mediated B cell activation. Cellular immunity is mediated by activated T cells, which secrete cytokines and directly kill pathogens.

In acute nodavirus infections, which usually become persistent in infected grouper, there is a balance of induction and inhibition of immune responses. Generally speaking, this is regarded as immune evasion. Nodaviruses seek to evade the host's protective systems so that they can replicate and transmit progeny to other cells or hide from surveillance by the immune system in a latent condition (Vossen et al., 2002). Immune evasion can be achieved by interruption of various host responses and inhibition of innate responses of the infected fish, such as production of the interferon-induced antiviral protein Mx (Wu and Chi, 2006). Another clear example is porcine reproductive and respiratory syndrome virus (PRRSV). This virus evades humoral immunity, by undergoing antigenic variation or encoding proteins that induce polyclonal activation of immune cells; this gives rise to hyperproduction of immunoglobulins that are not necessarily protective against the virus (Butler et al., 2008). The hepatitis C virus (HCV) has a somewhat novel mechanism of immune evasion. The virus encodes highly glycosylated envelope proteins, and this glycosylation can mask the neutralizing epitopes, thus rendering the antibody response ineffective (Helle et al., 2007). In some infections, viral proteins can target effector molecules by encoding homologous cytokines or cytokine receptors, having acquired such genes through modification or capture of host cellular genes, or inhibit the complement-mediated killing of virus-infected cells. A better understanding of how this virus subverts the immune response will provide valuable information that may help the development of rapidly acting biotherapeutics to use in response to an outbreak of betanodavirus.

Host antibody production after grouper infection by betanodavirus is an important response. Antibodies can directly neutralize viruses and prevent damage, or can target infected cells and initiate cell-mediated cytotoxicity (CMC). Several reports demonstrated that nodavirus infection increases the number of T cells in fish and also increases the expression of several cytotoxic-T lymphocyte (CTL)-related genes (Chang et al., 2011a; Scapigliati et al., 2010; Øvergård et al., 2012; Chaves-pozo et al., 2012). Recently, Chaves-Pozo and colleagues reported that nodavirus infection induces CMC activity in resistant gilthead seabream and in susceptible European sea bass (Chaves-Pozo et al., 2012). These studies indicated that nodavirus infection up-regulated NCCRP-1, a non-specific cytotoxic cell receptor that has an important function in target cell recognition and activation of cytotoxicity (Jaso-Friedmann et al., 2001).