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Persistence of betanodavirus in Barramundi brain (BB) cell line involves the induction of Interferon response

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Abstract

The BB cell line derived from the brain tissue of a barramundi (*Lates calcarifer*) that survived nervous necrosis virus (NNV) infection is persistently infected with NNV. To elucidate whether interferon (IFN) plays a role in the mechanism of NNV-persistent infection in BB cell line, a virus-negative control cell line was obtained by treating BB cells with NNV-specific rabbit antiserum for 5 subcultures. After the treatment, NNV titer or RNA or capsid protein was no longer detected in the cured BB (cBB) cells. Expression of Mx gene, encoding a type I IFN-inducible antiviral protein, was found in BB cells and cBB cells following NNV infection, but not in NNV-free cBB cells. Moreover, expression of Mx gene and antiviral activity against NNV were induced in cBB cells by the treatment with MAb-neutralized BB cell supernatant. Furthermore, NNV persistent infection was induced again in cBB cell culture if multiplicity of infection (MOI) was low ($\leqq 1$). These experimental results indicated that IFN-like cytokines existed in the culture supernatant of BB cells, and IFN-induced response played an important role in protecting the majority of cells from virus lytic infection and regulating NNV persistence in the BB cell line.

Keywords: Fish nodavirus; Betanodavirus; Nervous necrosis virus; Persistent infection; Interferon; BB cell line

1. Introduction

Viral nervous necrosis (VNN) disease is a worldwide disease among many economically important fish species, and causes mass mortality of fish at the larval stage [1,2]. The pathological characteristic of VNN disease is the vacuolation of the brain and retina [3]. Clinical signs of diseased fish are abnormal swimming behavior and a dark body surface. The causative agent is nervous necrosis virus (NNV), a non-enveloped icosahedral RNA virus with a diameter of 20–34 nm and two-single strands of positive-sense RNA without poly A tail [4,5]. RNA1 encodes RNA-dependent RNA polymerase, and RNA2 encodes capsid protein. It belongs to the piscine nodavirus (betanodavirus) of *Nodaviridae*.

Betanodavirus has been detected in fish surviving VNN disease without clinical and pathological syndromes [6]. To date, knowledge about NNV-persistent infection is very limited. A novel cell line BB was established from the brain

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tissue of a barramundi (*Lates calcarifer*) that survived VNN disease. The BB cell line was confirmed to have NNV-persistent infection due to the detection of infectious viral particles in its culture supernatants, and the identification of capsid protein in a few BB cells of each subculture by immunochemical staining [7].

Persistent infections are usually caused by the production of defective-interfering (DI) viral particles or the existence of temperature sensitive (Ts) mutants [8]. However, DI particles and Ts mutants do not cause NNV persistent infection in BB cell line [7]. Another type of persistent infection occurs when a small fraction of cells are infected by few progeny viruses at any given time due to the presence of IFN-like substances in the culture supernatant [8,9].

Interferons are secreted proteins (cytokines) that induce an antiviral state in cells and play a major role in the defense against virus infection in vertebrates [10,11]. The antiviral action of mammalian type I IFN (IFNα/β) is mediated by a 2-step signaling pathway. During the first step, virus-infected cells recognize viral dsRNA generated during viral replication by Toll-like receptor 3 (TLR 3) which triggers activation of the transcription factors IRF-3 and NFκB that translocate and attach to specific sites in the IFNβ promoter. The first IFNβ is then produced and secreted from the virus-infected cells. The second step involves the binding of the secreted type I IFN to the type I IFN receptors on the cell membrane of other uninfected cells. The antiviral effect is then established via the JAK-STAT signal transduction pathway resulting in expression of Mx and other antiviral proteins [10,11].

The cloned fish IFNs have the characteristic properties of type I IFNs, and antiviral activity due to type I IFN has been demonstrated in a number of fish species in vitro and in vivo [12]. Although little is yet known about the IFN-signaling system of fish, STAT1 has been cloned from zebrafish, and the zebrafish STAT1 was able to rescue type I IFN-signaling functions in a STAT1-deficient human cell line, indicating that cytokine-signaling mechanisms are likely to be conserved between fish and mammals [11]. Furthermore, Mx proteins have been used as putative markers for type I IFN production in fish [13–15], and antiviral activity of Mx protein from Atlantic salmon and Japanese flounder has been demonstrated [11].

To determine whether the persistence of NNV in BB cell line involved the induction of IFN response, the expression of Mx gene in BB cells was examined and the antiviral activity in the culture supernatant of BB cells was assayed.

2. Materials and methods

2.1. Cell lines and viruses

The GF-1 cell line [16] and the BB cell line [7] were used in this study. GF-1 cells were used for the titration and identification of NNV in the culture supernatants from BB cells. GF-1 cells were maintained in Leibovitz's L-15 medium supplemented either with 5% fetal bovine serum (FBS) for routine subcultures or 1% FBS for NNV titration, and incubated at 28 °C. BB cells were cultured using L-15 medium supplemented with 20% FBS, and also incubated at 28 °C. An NNV strain B00GD, isolated from NNV-infected Barramundi (*Lates calcarifer*) [1], was used in this study.

2.2. Curing NNV-persistent infection in BB cells by NNV-specific rabbit antiserum

One set of BB cells was continuously subcultured for 5 generations with medium containing 0.2% NNV-specific rabbit antiserum, and restored to normal culture medium without antiserum from the 6th subculture. The BB cells after antiserum curing were designated as cBB cells.

2.3. Titration of the culture supernatants from BB and cBB cells

The culture supernatants collected from 18 subcultures of BB and cBB cells were titrated in GF-1 cells. The supernatants were serially 10-fold diluted and inoculated into 96 well-tissue culture plates pre-seeded with GF-1 cells. The 50% tissue culture infectious dose ($TCID_{50}$) per ml were determined on the 6th day of infection.

2.4. Detection of NNV by RT-PCR and semi-nested PCR

BB and cBB cells were separately scraped from one 25-cm^2 tissue culture flask and re-suspended in 200 μl culture supernatants. The cell suspension was then mixed with 500 μl RNA extraction buffer (4 M guanidine thiocyanate, 25 mM sodium citrate dihydrate, 0.5% sodium lauroyl sarcosine), 70 μl 3 M sodium acetate (NaOAc) (pH 4.2),