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Frequent occurrence of apoptosis is not associated with pathogenic infectious pancreatic necrosis virus (IPNV) during persistent infection

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Abstract

Infectious pancreatic necrosis virus (IPNV), a member of the genus *Aquabirnavirus* and family Birnaviridae, is an unenveloped icosahedral virus with two segments of double-stranded RNA. IPNV causes acute infection in salmonid fry and fingerlings with high mortality. However, this mortality is low as the age increases and survivors become IPNV-carrier fish. In this study, IPNV persistent infection was established in rainbow trout with no clinical signs or mortality. TUNEL staining and immunohistochemistry showed that IPNV antigen-positive cells did not have an apoptotic nucleus in almost all tissue sections and leucocyte smears, indicating that apoptosis was not induced in IPNV antigen-positive cells. The IPNV genome detected by in situ RT-PCR was more frequent than detection of the IPNV antigen by immunohistochemistry in the kidney, spleen, and liver. This result implies that the successive replication would not occur in many IPNV-infected cells. Further, apoptotic cells were predominant in the tissue sections where the signal-positive cells were frequently detected. Therefore, the presence of apoptosis in this study might be associated with host defense mechanisms, which eliminates IPNV-infected cells by the recognition of IPNV genome at the early stage of infection.

Keywords: Rainbow trout (*Oncorhynchus mykiss*); Infectious pancreatic necrosis virus (IPNV); Apoptosis; In situ RT-PCR; TUNEL staining

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1. Introduction

Apoptosis is defined as genetically controlled cell death by cellular self-destruction in response to physiological stimuli. Cells undergoing apoptosis show characteristic morphological changes, including shrinkage, blebbing of the plasma membrane, chromatin condensation and DNA fragmentation [1].

In a virus–host cell interaction, from the pathological stimulus, the host cellular defense mechanism induces premature apoptosis to eliminate virus-infected cells before the progeny viruses spread to neighboring cells [2]. Viruses inhibit or at least delay host cell-induced premature apoptosis [3–6]. In such a strategy, host cells may simultaneously permit persistent infection [7]. However, for acute infection, many viruses also actively induce apoptosis at the late stage of infection. Thus, viruses have evolved distinct mechanisms from host cells to regulate apoptosis by two alternative infection modes, acute and persistent infection.

Infectious pancreatic necrosis virus (IPNV), a member of the genus *Aquabirnavirus* and family Birnaviridae, is an unenveloped icosahedral virus about 60 nm in diameter with two segments (segments A and B) of double-stranded RNA [8]. The typical histopathological sign of IPN is severe necrosis of pancreatic acinar tissue, and sometimes in the adipose tissue and hematopoietic tissue of the kidney, the gut and the liver [9]. IPNV causes acute infection in salmonid fry and fingerlings with high mortality, but this mortality is low as the age increases and survivors become IPNV carriers, which is very serious because of both horizontal and vertical transmissions [10].

IPNV controls apoptosis and is pathogenic for the chinook salmon embryo cell line (CHSE-214) [11–13]. IPNV-induced apoptosis precedes detectable necrotic change that is currently viewed as necrosis in vitro [14]. In contrast, the role of apoptosis is not sufficiently understood in vivo. For example, in rainbow trout showing clinical signs of IPN, apoptotic cells are in muscle lesions and the lamina propria of the intestine, but not found in the main target organ, the pancreas [15]. Therefore, apoptosis may not be the main mechanism responsible for cell death in IPN [15]. Accordingly, the ability of IPNV to control apoptosis may have an important role in persistent infection rather than in acute infection in vivo.

In this study, to examine the role of apoptosis in IPNV-carrier rainbow trout, we simultaneously detected DNA fragmentation by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining and IPNV antigens by immunohistochemistry. In situ reverse transcription polymerase chain reaction (RT-PCR) was used to examine the frequency of IPNV at the genome level compared with the expression level of IPNV antigen.

2. Materials and methods

2.1. Fish

Pathogen-free rainbow trout (*Oncorhynchus mykiss*) (4.95–12.91 g) were maintained in 600 l tanks with flow-through water at 19 ± 0.5 °C and were fed a commercial diet.

2.2. Virus and antiserum

CHSE-214 cells were cultured at 20 °C in DMEM/Ham's F-12 medium (Sigma) supplemented with 10% fetal bovine serum (FBS, Hyclone) and 100 μg ml^{-1} kanamycin. IPNV Sp strain was propagated in CHSE-214 cells for experimental infection. Rabbit serum against Y-6 strain which is the other aquabirnavirus isolated from yellowtail *Seriola quinqueradiata* with ascites in Japan [16,17] was used in this study.