Veterinary Microbiology 176 (2015) 19–31

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Differential effects of alloherpesvirus CyHV-3 and rhabdovirus SVCV on apoptosis in fish cells

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ARTICLE INFO

Article history:
Received 4 March 2014
Received in revised form 28 November 2014
Accepted 1 December 2014

Keywords:
CyHV-3
KHV
SVCV
CCB
Apoptosis
Alloherpesviridae

ABSTRACT

Whilst *Herpesviridae*, which infect higher vertebrates, actively influence host immune responses to ensure viral replication, it is mostly unknown if *Alloherpesviridae*, which infect lower vertebrates, possess similar abilities. An important antiviral response is clearance of infected cells *via* apoptosis, which in mammals influences the outcome of infection. Here, we utilise common carp infected with CyHV-3 to determine the effect on the expression of genes encoding apoptosis-related proteins (p53, Caspase 9, Apaf-1, IAP, iNOS) in the pronephros, spleen and gills. The influence of CyHV-3 on CCB cells was also studied and compared to SVCV (a rhabdovirus) which induces apoptosis in carp cell lines. Although CyHV-3 induced iNOS expression *in vivo*, significant induction of the genetic apoptosis pathway was only seen in the pronephros. *In vitro* CyHV-3 did not induce apoptosis or apoptosis-related expression whilst SVCV did stimulate apoptosis. This suggests that CyHV-3 possesses mechanisms similar to herpesviruses of higher vertebrates to inhibit the antiviral apoptotic process.

Abbreviations:
CyHV-3, Cyprinid herpesvirus 3
KHV, Koi herpesvirus
SVCV, Spring viremia of carp virus
MCP, major capsid protein
GP, glycoprotein
CCB, common carp brain cell line

1. Introduction

Throughout evolution viruses have developed various strategies to evade the immune system of the host and thus ensure their replication. One of these strategies

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http://dx.doi.org/10.1016/j.vetmic.2014.12.012
0378-1135/

targets the induction of apoptosis in infected cells which, when it occurs early in the infection, prevents viral replication and distribution (Hay and Kannourakis, 2002). The viral strategy can, for example, result in the inhibition of cellular apoptosis to ensure viral replication in the host cells, or the active induction of apoptosis in order to impair the immune response or to release progeny at the later stage of viral replication (Best and Bloom, 2004; Leu et al., 2013; Tschopp et al., 1998). The association between apoptosis and viral infection is therefore complex, either increasing or reducing host cell death. Which of these outcomes predominates during an infection seems to, at least partially, depend on the virus type, *i.e.* viruses with large genomes appear to have a higher capacity to actively influence the apoptotic process compared with viruses with small genomes (*e.g.* Roulston et al., 1999). In general RNA viruses, such as the rhabdovirus *Spring viremia of carp virus* (SVCV), have a small genome which does not appear to encode genes with the ability to influence the apoptotic process of the host. In contrast, DNA viruses, such as the herpesviruses, have large genomes, and are known to interfere with the host's immune response and apoptosis pathway by expressing homologue genes to their host (Ahne et al., 2002; van Beurden et al., 2011). This phenomenon has been intensively studied in mammalian herpesviruses (*i.e. Herpesviridae*). As reviewed by Lagunoff and Carroll (2003) it has been shown that many sequenced *γ-Herpesviridae*, such as *Epstein-Barr virus* (EBV) and *Human herpesvirus 8* (HHV-8) express a homologue of the anti-apoptotic protein Bcl-2. In addition, HHV-8 has been shown to express proteins such as LANA and vIL6 that prevent p53 and IFN-α induced apoptosis. In contrast, Herpes simplex viruses, which belong to the *α-Herpesviridae*, trigger apoptosis earlier in the infection but inhibit this process later in the infection process by expressing a variety of anti-apoptotic genes. This ability to actively influence host apoptosis has been suggested by Leu et al. (2013) to be directly correlated to the virulence of the virus since it facilitates viral replication and virus survival.

Although there have been many studies on the association between viral infections and apoptosis in mammalian systems very little is known about these mechanisms in lower vertebrates, particularly fish. Common carp (*Cyprinus carpio* L.) is a host for two highly contagious viruses: *Cyprinid herpesvirus 3* (CyHV-3) and *Spring viraemia of carp virus*. CyHV-3, commonly called Koi herpesvirus (KHV) (Hedrick et al., 2000), is a member of the *Alloherpesviridae* family of herpesviruses (Waltzek et al., 2005), it is a double stranded DNA virus, with a genome size of 295 kb, encoding 155 predicted open reading frames (Davison et al., 2013). CyHV-3 genome encodes proteins potentially involved in immune evasion mechanisms such as tumour necrosis factor receptor homologues (encoded by ORF4 and ORF12) and an interleukine-10 (IL-10) homologue (encoded by ORF134) (Aoki et al., 2007; Ouyang et al., 2013). In recent publications the effect of this virus on the innate immune response of its primary host *C. carpio* were highlighted (Adamek et al., 2012, 2013, 2014a,b; Pionnier et al., 2014; Rakus et al., 2012; Syakuri et al., 2013). As part of these studies it was shown that CyHV-3 inhibits *in vitro* up-regulation of type I interferons (Adamek et al., 2012), the cytokines, which have been closely associated with the induction of apoptosis in mammals (Tanaka et al., 1998). We therefore hypothesised that CyHV-3 could influence host apoptosis and thus facilitate its replication.

SVCV causes mortality in farmed and wild carp in Europe and North America, and also affects other cyprinids in which it tends to be less virulent (Garver et al., 2007). This virus has been identified as a member of the *Rhabdoviridae* family in the order of the *Mononegavirales* and the genus *Spirivivirus* (ICTV, 2013). SVCV, in accordance with most members of the *Rhabdoviridae* family, has a genome that is composed of one molecule of non-segmented, linear, single stranded negative-sense RNA encoding 5 genes (Ahne et al., 2002). Although two independent studies have shown that SVCV infection of the EPC cell line *in vitro* induces apoptosis at the morphological level (Björklund et al., 1997; Kazachka et al., 2007), the mechanism by which SVCV induces apoptosis at the molecular level still requires elucidation.

This manuscript aimed to study for the first time the influence CyHV-3 on the apoptotic process both *in vivo* and *in vitro*. It was shown that unlike SVCV, CyHV-3 did not induce apoptosis in CCB cells. Moreover, *in vitro* CyHV-3 infection did not induce genes encoding for classical apoptosis-related proteins (*i.e.* p53, Caspase 9, Apaf-1, IAP) as well as iNOS and type I IFN, whilst *in vivo* the genetic apoptosis pathway was only induced 14 days post infection. Possible factors influencing the differential apoptosis response during CyHV-3 and SVCV infections are discussed.

2. Material and methods

2.1. Fish

Common carp (*C. carpio* L.) of the Polish line K (Irnazarow, 1995) were reared in the facilities at the Laboratory of Fish Disease at the National Veterinary Research Institute in Pulawy, Poland. Carp were kept in two 800 l tanks at 21 ± 1 °C under a 12/12 h light/dark cycle and were allowed to acclimate for 4 weeks prior to the infection. Feeding occurred daily with commercial carp pellets (Aller Aqua, Poland) at 3% body weight/day. No mortality occurred during this acclimatisation period.

2.2. In vivo CyHV-3 challenge

CyHV-3 (local Polish isolate) was isolated at the Laboratory of Fish Disease, National Veterinary Research Institute in Pulawy, Poland from infected common carp in 2005 (passage No. 4) as described by Rakus et al. (2012). The virus was propagated in cells of the *C. carpio* brain (CCB) cell line (Neukirch et al., 1999; Neukirch and Kunz, 2001), which were cultured in minimum essential medium (MEM) (Gibco, Germany) enriched with 4.5 g/l glucose (D-glucose monohydrate), 10% fetal calf serum, penicillin (200 i.u./ml), streptomycin (0.2 mg/ml), and 1% non-essential amino acid solution (all Sigma Aldrich, Germany). Culturing was carried out at 22 °C with 5% CO_2 in a humid atmosphere (Thermo Scientific Heraeus CO_2 Incubator).