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Induction of rainbow trout MH class I and accessory proteins by viral haemorrhagic septicaemia virus

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ABSTRACT

Major histocompatibility (MH) class I receptors are glycoproteins which play a critical role during responses to intracellular pathogens by presenting endogenous peptides to cytotoxic T cell lymphocytes (CD8+). To date, little is known about MH class I regulation at the protein level during viral infections in fish. In this study, we characterised the MH class I pathway response to polyinosinic–polycytidylic acid (poly I:C) and upon infection with viral haemorrhagic septicemia virus (VHSV) genotype IVa using the rainbow trout monocyte/macrophage cell line RTS11. A 14-day challenge with VHSV IVa at 14 °C demonstrated enhanced expression of the class I heavy chain, β2 microglobulin (β2M) and tapasin, while the expression of other accessory molecules ERp57 and calreticulin remained unchanged. However, when infection occurred at 2 °C no change in expression levels of any of these molecules was observed. β2M accumulated in the media of RTS11 over time, however the β2M concentrations were 2 fold higher in cultures infected with VHSV 14 days post infection. Strikingly, when cells were maintained at 2 °C the secretion of β2M was significantly reduced in both infected and non-infected cultures. These results indicate that VHSV infection alters the kinetics of β2M release as well as the expression of MH class I and suggests that cellular immunity against VHSV can be compromised at low temperatures which may increase host susceptibility to this virus during the winter.

Introduction

The major histocompatibility class I receptor is expressed on the cell surface of all nucleated cells in vertebrates and is responsible for the display of foreign peptide fragments derived from intracellular pathogens. In order to assure the stability of the MH class I receptor during export and on the cell surface, a stringent and specialized I pathway in the endoplasmic reticulum (ER) has evolved to facilitate the optimal folding of MH class I with a diverse pool of peptides. This process is facilitated in mammals by the assembly of the peptide loading complex which involves multiple protein interactions including the heterodimer MH class I-β2m as well as ERp57, tapasin, calreticulin and an endogenous peptide (Wearsch and Cresswell, 2008; Rizvi and Raghavan, 2010; Chapman and Williams, 2010.

The first teleost major histocompatibility (MH) gene fragments were isolated from carp (Hashimoto et al., 1990) and later genomic linkage studies mapped a unique architecture in which the major histocompatibility class I and class II loci do not reside on the same chromosome as found in mammals (Stet et al., 2003; Phillips et al., 2003). However, the genes for the proteasomal subunits: low molecular mass polypeptide, low molecular mass polypeptide (LMP2, LMP2/δ), TAP heterodimer and tapasin (TAPBP.A) are linked to the teleost class Ia locus (Phillips et al., 2003; Landis et al., 2006), which highlights their role as a functional co-operative unit in antigen presentation. In mammals, genes encoding the class Ia pathway are regulated by the action of the interferons (IFN), which are rapidly induced during viral infections. Receptor binding of IFN on the cell surface of both immune and non-immune cells activates a JAK-STAT pathway which subsequently turns on the expression of many IFN stimulated genes including the MH class I pathway genes (Goodbourn et al., 2000; Robertsen, 2006). Studies in salmonid fish have demonstrated a similar IFN mediated response which resulted in the transcriptional induction of the MH class I pathway during in vivo infections with infectious hematopoietic virus and infectious salmon anaemia virus. In addition, promoter analysis of these genes in trout revealed putative regulatory factor and IFNγ binding site supporting their activation by IFN (Hansen and La Patra, 2002; Jørgensen et al., 2006).

Viral haemorrhagic septicaemia virus is the cause of viral disease that affects a large number of freshwater and marine species in the northern hemisphere, causing major economic impact in

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cultured fish such as rainbow trout (Skall et al., 2005). It is classified in the Novirhabdovirus genus within the Rhabdoviridae family. VHSV contains a non-segmented, single-stranded RNA genome which encodes five basic structural proteins comprising nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), RNA polymerase (L), and a single non-structural protein, called non-virion (NV) protein (Purcell et al., 2012). Four major genotypes of VHSV have been identified based on phylogenetic tree analyses of the N and G sequences: I, II, III and IV (Purcell et al., 2012). Genotype IV is further subdivided into VHSV IVa and VHSV IVb. Genotype IVa is endemic to a wide range of Pacific fish species and causes a low level of mortality in rainbow trout (Kahns et al., 2012). VHSV replicates optimally at 15 °C (Winton et al., 2007) but has been recently shown to be capable of replicating at 4 °C (Vo et al., 2013).

Although, much progress has been made in the recent years in understanding the anti-viral mechanisms against this virus both in vitro and in vivo, little is known about the regulation of the MH class I antigen presentation machinery at the protein level during the course of rhabdoviral infection in fish or possible temperature effects on that process which contribute to viral pathogenicity. Interestingly, a study conducted with the murine lymphoma RMA-S cell line demonstrated that at a low physiological temperature for murine cells, 26 °C, MH class I molecules can dimerize with β2m and exit the Golgi to the plasma membrane, but do not contain a bound peptide and therefore fail to present antigen to cytotoxic T cells (Ljunggren et al., 1990). This finding raises an interesting question about how antigen presentation is maintained during viral infection in poikilothermic animals, such as bony fish. It has been previously demonstrated in carp that expression of the class I heavy chain-β2m heterodimer is abolished at 6 °C in peripheral blood leucocytes (PBL) (Rodrigues et al., 1998). This study showed that surface expression of the MH class I molecule can be restored after 6 days when the fish are returned to 12 °C and that the lack of expression at 6 °C was mediated by down regulation of β2m transcription (Rodrigues et al., 1998). However, a study with rainbow trout and Atlantic salmon showed that the cellular levels of β2m can be maintained at 2 °C in both species and demonstrated persistent surface expression of β2m in PBL at this temperature (Kales et al., 2006).

Monocyte/macrophage cell lines have been useful tools to study many aspects of immunology including antigen processing and presentation. The monocyte/macrophage RTS11 cell line was developed from the spleen of rainbow trout and demonstrated characteristics of antigen-presenting cells (Brubacher et al., 2000; DeWitte-Orr et al., 2007; Martin et al., 2007; Kawano et al., 2010; Ganassin and Bols, 1998; Pham et al., 2013; Kales et al., 2007; Sever and Bols, 2013). Thus in this study, RTS11 cell line was used to study the effect of temperature on the regulation of MH class I antigen presentation in response to VHSV IVa both at 14 °C and 2 °C to better understand the regulation of antigen presentation mechanisms in the cold winter months in which VHS outbreaks have been reported.

Materials and methods

Fish cell lines

The rainbow trout spleen monocyte/macrophage cell line RTS11 (Ganassin and Bols, 1998) was grown in Leibovitz's L-15 medium (Hyclone, ThermoFisher) with 20% fetal bovine serum (FBS, PAA) and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, ThermoFisher) in 25 cm^2 culture flasks at 20 °C and sub-cultured every 10–14 days. The EPC cell line derived from fathead minnow (Winton et al., 2010) was maintained in L-15 medium with 10% FBS and antibiotics in 75 cm^2 culture flasks at 26 °C and sub-cultured every 7 days by trypsin (Lonza).

Propagation of VHSV

VHSV genotype IVa isolated from Pacific herring (Kocan et al., 1997) was routinely propagated on permissible EPC cultures in L-15 with 2% FBS at 14 °C. Virus-containing supernatants were collected on day 10 when severe cytopathic effects were observed. The supernatants were centrifuged at 4500 × *g* for 5 min at 4 °C, filtered through 0.2-μm membranes (Pall Corporation), aliquoted and frozen at −80 °C until used. Viral titers were determined by $TCID_{50}$/mL assays using EPC cell line for VHSV IVa, as previously described (Pham et al., 2013).

Infection of RTS11 cells with viruses

L-15 with 2% FBS was used for all infection experiments. Approximately 3×10^6 RTS11 cells/well were seeded in six-well tissue culture Falcon plates and subsequently infected with 1.5×10^7 $TCID_{50}$/mL of VHSV IVa at either 2 °C or 14 °C for 14 days. Control cultures had the same number of cells in L-15 medium with 2% FBS without virus. Cells were harvested on selected days, washed with ice-cold PBS twice and then pelleted at 500 × *g* for 4 min at 4 °C and immediately frozen at −80 °C until all samples were collected.

In vitro stimulation with poly I:C

About 2×10^6 RTS11 cells were seeded in L-15 with 10% FBS into 25 cm^2 Falcon flasks and treated up to 72 h with 50 μg/mL of poly I:C in PBS (Sigma Aldrich), for control samples the same amount of vehicle (PBS) was added.

Western blot

Cell pellets were lysed in 1% NP-40 lysis buffer containing 150 mM NaCl and 50 mM Tris [pH 8.0] supplemented with 1× of protease inhibitor cocktail (Roche). Protein concentrations were determined by the BCA protein assay (Thermo Fisher Scientific). Cell lysates were separated on a 12% acrylamide gel and transferred to nitrocellulose membranes (Bio-Rad) overnight. Membranes were blocked with 5% skim milk in TBS-T (10 mM Tris, 100 mM NaCl, and 0.1% (v/v) Tween-20) and probed with rabbit polyclonal antibodies raised against trout recombinant proteins as follows: MH class I heavy chain (1:500) (Kawano et al., 2010), β2m (1:500) (Kales et al., 2006), MH class II beta chain (1:500) (Kawano et al., 2010), ERp57 (1:1000),(Sever and Bols, 2013), calreticulin (1:500) (Kales et al., 2007) and tapasin antibody (1:200) generated against the c-terminal domain of trout Tapasin (Sever et al., 2013). Membranes were subsequently probe with 1:30,000 goat anti-rabbit alkaline phosphatase (Sigma Aldrich) and detected using NBT/BCIP (Roche) according to the manufacturer's instruction.

Deglycosylation

RTS11 cells were lysed with 1% NP-40 containing 150 mM NaCl, 50 mM Tris [pH 8.0] followed by centrifugation for 15 min at 13,000 rpm at 4 °C. Cell lysates were concentrated using vivaspin 500 with 3 kDa MWCO (GE healthcare) according to manufacturer's instruction. De-glycosylation with Endo H and *N*-glycosidase F (New England Biolabs) was performed as previously described (Kales et al., 2007). Enzyme treated and untreated protein lysates were loaded on a 15% gel SDS PAGE gel and blotted with either 1:500 of anti MH class I or calreticulin antibody.

Reverse transcription and PCR

RNA was extracted from 2×10^6 RTS11 cells using RNeasy extraction kit according to manufacturer's instructions (Qiagen)