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Infectious salmon anemia virus is a powerful inducer of key genes of the type I interferon system of Atlantic salmon, but is not inhibited by interferon

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Abstract

Infectious salmon anemia virus (ISAV) is an aquatic orthomyxovirus causing disease and high mortality in farmed Atlantic salmon (*Salmo salar*). The virus is thus apparently able to initiate replication without being hampered by the host's immune system. In this work we have studied the role of the type I interferon (IFN) system of Atlantic salmon in protection against ISAV. Real-time RT–PCR was used to study the expression of type I IFN and the IFN stimulated genes Mx and ISG15 in TO cells and live fish in response to infection with ISAV. The *in vitro* studies showed that ISAV was a powerful inducer of Mx and ISG15 genes in TO cells and that induction started relatively early during infection. In contrast, IFN transcripts were induced later than both Mx and ISG15 transcripts in the ISAV infected cells indicating that Mx and ISG15 are induced through IFN-independent pathways in the early stages of ISAV infection. A cohabitee infection trial with ISAV in Atlantic salmon resulted in high mortality, even though elevated levels of IFN, Mx and ISG15 transcripts in the head kidney and liver were observed. Immunoblotting confirmed the presence of Mx and ISG15 proteins in the liver of infected salmon. In order to evaluate whether the type I IFN system is able to inhibit replication of ISAV, TO cells were stimulated with recombinant salmon IFN-α1 (rSasaIFN-α1) and subsequently infected with virus. The rSasaIFN-α1 showed no protection of TO cells against ISAV, but full protection against IPNV. These data demonstrate that key proteins of the type I IFN system are induced during an ISAV infection, but that they are unable to inhibit the replication of ISAV *in vitro* and *in vivo*. ISAV must thus encode genes that enable the virus to counteract IFN induced antiviral proteins of the host.

Keywords: Atlantic salmon; Infectious salmon anemia virus; Interferon-α/β; Mx; ISG15; Real-time RT–PCR; Recombinant salmon IFN-α1

1. Introduction

Infectious salmon anemia virus (ISAV) represents the first orthomyxovirus characterized in teleosts and has been designated to the new virus genus *Isavirus* [1–3]. Being a major causative agent for mortal disease in farmed Atlantic salmon, the virus must have developed mechanisms to overcome the innate antiviral immune mechanisms of its host. The type I interferon (IFN) system plays a major role in the innate antiviral immune system of vertebrates. In most

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mammalian cells the IFN system is activated by recognition of viral dsRNA through intracellular receptors, which include the two RNA helicases RIG-I and MDA5 and the endosomal located Toll-like receptor 3 (TLR3) [4–6]. DsRNA is produced as an intermediate during viral replication [7]. Binding of dsRNA to RIG-I, MDA5 or TLR3 leads to activation of the transcription factors NF-κB and IRF-3 through the kinases TBK-1 and IKKε, resulting in transcription of the IFN-β gene [8–10]. The IFN superproducing cells plasmacytoid dendritic cells (pDCs) that are present in the blood, recognize viral nucleic acids through TLR7, TLR8 and TLR9. This results in induction of IFN-α/β transcription by activation of IRF-7 through the adaptor protein Myd88 [11].

The secreted type I IFNs act like alarm proteins, which bind to specific cell surface receptors associated with the Jak/STAT signaling pathway. Activation of this pathway results in induction of expression of interferon stimulated genes (ISGs), some of which encode antiviral proteins including Mx, double-stranded RNA activated protein kinase (PKR) and 2′,5′-oligoadenylate synthetase (OAS) [12].

The cloning of IFN and several typical ISGs from Atlantic salmon and other fish species has established that teleost fish possess a type I IFN system similar to mammals [13]. Type I IFN from Atlantic salmon was cloned and expressed as a recombinant protein (rSasaIFN-α1) in human cells and was shown to induce protection of salmon cells against infection by the aquatic birnavirus infectious pancreatic necrosis virus (IPNV) [14]. Moreover, both Mx and the IFN-induced gene ISG15 have been cloned from Atlantic salmon [15,16]. Antiviral activity of Mx protein is well documented in mammals [17–19], and has recently also been demonstrated against IPNV in Atlantic salmon [20]. Human and mouse Mx confer resistance against the orthomyxoviruses influenza A and B [17] and human Mx in addition possesses antiviral activity against a wide variety of other viruses [21]. The antiviral mechanism of Mx protein is still uncertain, but it has been hypothesized to interfere with viral replication by preventing intracellular trafficking of viral nucleocapsids [22,23]. ISG15 is one of the earliest and most predominant proteins to be induced in mammals following IFN-α/β stimulation, which suggests that it has an important function in the IFN system [24]. The function of ISG15 is not yet understood, but it has been shown that similar to ubiquitin, ISG15 conjugates to cellular proteins in both human [24], goldfish [25] and Atlantic salmon [16]. Some evidence for antiviral activity of ISG15 has also been presented [26–28]. Moreover, influenza B NS1 protein has been shown to bind specifically to human ISG15 and block the conjugation to cellular proteins, a phenomenon that supports an antiviral role of ISG15 [29]. Binding of Atlantic salmon ISG15 to an ISAV protein was reported recently, but the functional relevance of this phenomenon is yet unknown [16].

As a countermeasure to the IFN system, both influenza A and B viruses are reported to produce a non-structural IFN antagonist protein during replication [30–34]. An IFN antagonist is also reported in ISAV where the authors hypothesized that the function of this protein is to block transcription of ISGs, most notably Mx [34].

In this work we describe expression patterns of IFN, Mx and ISG15 genes in Atlantic salmon cells and live fish during ISAV infection. The kinetics of transcript production was monitored by real-time RT–PCR and expression of Mx and ISG15 proteins in liver was measured using immunoblotting. The present studies suggest that ISAV is a powerful inducer of these ISGs. On the other hand we demonstrate for the first time that recombinant Atlantic salmon IFN-α1 does not protect salmon cells against ISAV infection. Together these data suggest that ISGs including Mx and ISG15 have no antiviral effect against ISAV in Atlantic salmon and that ISAV must have developed mechanisms to counteract the activity of IFN-induced antiviral proteins.

2. Materials and methods

2.1. Fish

Atlantic salmon presmolts were obtained from the Aquaculture Research Station (Tromsø, Norway). The Atlantic salmon weighing approximately 50 g were kept in 300-L tanks containing fresh water at 8–9 °C. Ten days prior to injection the fish were transferred to a new tank supplied with water containing 10‰ salt.

Prior to treatment, the fish were anaesthetized with 0.04 g/L benzocaine (Sigma, St. Louis, USA). The fish were over-anaesthetized using 0.08 g/L benzocaine prior to harvest of organs.

2.2. Cell lines and virus

TO cells originating from Atlantic salmon head kidney were obtained from Dr. Heidrun Wergeland (University of Bergen, Norway). TO cells were cultivated in Eagles minimum essential medium (MEM) supplemented with