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Induction of interferon system genes in Atlantic salmon by the imidazoquinoline S-27609, a ligand for Toll-like receptor 7

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Abstract Imidazoquinolines represented by imiquimod and its derivative S-27609, have previously been shown to have potent antiviral and antitumor activity in several mammalian models. Much of the antiviral properties of imidazoquinolines have been ascribed to induction of IFN-α in peripheral blood mononuclear cells most notably plasmacytoid dendritic cells. Toll-like receptor (TLR) 7 has been identified as the receptor for imiquimod and S-27609 in mammals.

In this work we show that S-27609 induces expression of IFN-α1/α2, Mx, ISG15 and IFN-γ in organs of Atlantic salmon, which suggests that salmon responds to S-27609 through a TLR7-like receptor. The kinetics of gene expression in liver and head kidney induced by S-27609 was compared with that induced by the double-stranded RNA poly I:C, which is a ligand for TLR3 and the RNA helicase MDA5. In liver, S-27609 and poly I:C both induced transcripts for IFN-α1/α2, Mx and ISG15 with a peak at about 24 h. In head kidney, poly I:C induced IFN-α1/α2 and Mx with one peak at about 24 h. In contrast, S-27609 induced a biphasic increase of IFN-α1/α2 and Mx transcripts in head kidney with a minor peak at 14–24 h and another increase starting at 72 h. The other major difference in gene induction by the two stimulants was that S-27609 induced much lower levels of IFN-α1/α2 than poly I:C during the early time stages (14–48 h) both in liver and head kidney. The difference in induction of IFN-α1/α2 by S-27609 and poly I:C may be a consequence of different cell targets for the two stimulants where S-27609 primarily induces IFNs through immune cells whereas poly I:C induces IFNs in most nucleated cells.
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Introduction

IFN-α/β play a critical role in the innate immune defence against viruses in vertebrates. Mammalian host cells produce IFN-α/β upon recognition of viral nucleic acid by various intracellular receptors whereupon secreted IFN-α/β protect other cells from viral infection [1]. Protection is achieved by binding of IFN-α/β to its receptor, which is present on most nucleated cells and this results in induction of several hundred interferon stimulated genes (ISGs) some of which encode antiviral proteins [2].

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The main viral sensors in most mammalian nucleated cells are the RNA helicases RIG-I and MDA5, which recognize viral single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA) [1,3—7]. Many cells also recognize viral dsRNA through Toll-like receptor 3 (TLR3), which is located in endosomal membranes [1,8]. Binding of virus-derived nucleic acids to RIG-I, MDA5 or TLR3 results in a coordinated activation of the transcription factors NF-κB and IRF-3 subsequently leading to transcription of the IFN-β gene. The synthetic dsRNA poly I:C, which is often used to imitate a viral infection, triggers IFN-β production in mammals through MDA5 and TLR3, but not through RIG-I [3,5,8].

Plasmacytoid dendritic cells (pDCs) have been shown to produce particularly high levels of IFN-α and recognize viruses through mechanisms that are different from most other body cells [1,9]. The viral sensors in pDCs are TLR7, which recognize viral single-stranded RNA and TLR9, which recognize unmethylated CpG-rich DNA that is present in both viral and bacterial genomes [3]. Binding of ligands to TLR7 and TLR9 recruit the adaptor protein MyD88, which results in activation of IRF-7 and transcription of multiple IFN-α genes.

Interestingly, the first ligands discovered for TLR7 were not viral ssRNA, but synthetic low molecular compounds called imidazoquinolines represented by imiquimod, S-27609 and R-848 [10,11]. Later R-848 was also shown to be a ligand for TLR8 [12]. Imidazoquinolines were first shown to have potent antiviral and antitumor activity in several animal models and imiquimod is now an approved treatment for external genital warts caused by human papilloma virus infection [13,14]. Much of the antiviral properties of imidazoquinolines have been ascribed to induction of IFN-α in peripheral blood mononuclear cells (PBMC), and recently, pDCs were confirmed to be the predominant cells in the blood that produces IFN-α in response to imidazoquinolines [15].

However, imidazoquinolines also induce other cytokines in mammals such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-1β and IL-6 in PBMC [16] and TNF-α, IL-6 and IL-12 in macrophages [10]. Imiquimod and R-848 were also found to stimulate T cells to produce IFN-γ in human PBMC and mouse spleen cell cultures, but this was determined to be an indirect effect mediated by induction of IL-12 and IFN-α in monocytes/macrophages [17]. The cytokine response to imidazoquinolines is likely to be initiated by cells that express TLR7 and/or TLR8 and these receptors are expressed exclusively by cells of the immune system. TLR7 is expressed by B cells, macrophages and neutrophils in addition to pDCs [10,18—20]. TLR8 is expressed by monocytes, myeloid dendritic cells and neutrophils [18,20].

The studies above suggest that poly I:C stimulates most nucleated cells whereas imidazoquinolines only stimulate immune cells expressing TLR7 or TLR8, in particular pDCs. Accordingly, imidazoquinolines and poly I:C can be used to stimulate different parts of the innate antiviral immune system and this should be reflected in the cytokine response they induce.

The cloning of IFNs and several typical ISGs from Atlantic salmon and various other fish species have established that teleost fish possess an innate IFN system similar to mammals [21]. Two innate IFNs were identified in salmon and were named SasaIFN-α1 and SasaIFN-α2 because their sequences were most similar to mammalian IFN-α. Recombinant salmon IFN-α1 was shown to induce both Mx and ISG15, two of the most prominent antiviral ISGs, in Atlantic salmon [22,23]. Moreover, salmon IFN-α1 induced protection against infectious pancreatic necrosis virus in salmon cells [22]. Salmon also possesses IFN-γ, which in mammals have a major role in adaptive cellular immune responses [21]. Information about receptors for viral nucleic acids in fish is also emerging. Genetic evidence suggests that fish possess homologs of most if not all TLRs found in mammals. In zebrafish, 19 putative TLR variants have been identified including TLRs with sequence similarity to TLR3, TLR7, TLR8 and TLR9 [24,25]. Moreover, TLR3 has been cloned from rainbow trout [25], and TLR8 and TLR9 has been cloned from Atlantic salmon [26]. Induction of IFN, Mx and ISG15 by ligands for TLR3 (poly I:C) and TLR9 (CpG oligonucleotides) in salmonid cells or live fish have also been documented in several reports [22,27—29]. On the other hand, very little is known about the immunomodulatory effects of ligands for TLR7 and TLR8 in fish, although R-848 was recently shown to upregulate expression of IFN-α1 in head kidney of rainbow trout [30].

In this work we demonstrate that the imidazoquinoline S-27609 induces SasaIFN-α1/α2, Mx, ISG15, and IFN-γ in Atlantic salmon and compare the expression of these genes after stimulation with S-27609 and poly I:C. The results support the presence of a TLR7-like receptor in teleost fish.

Materials and methods

Fish

Atlantic salmon presmolts were kept at the Aquaculture Research Station (Tromsø, Norway) in 300 L tanks supplied with running fresh water at 10 °C. Fish weighing 40—55 g were used for detecting Mx expression by Northern blotting, immunoblotting and immunocytochemical staining. Fish weighing 75—80 g were used for measuring gene expression by Real Time RT-PCR. Prior to treatments fish were anaesthetised in 0.005% benzocaine (Sigma, St Louis, MO, USA). Fish were sacrificed using 0.01% benzocaine prior to harvest of organs. Procedures involving fish and their care were conducted in strict accordance with established guidelines in agreement with national and international conventions and regulations.

Stimulation of Atlantic salmon with S-27609 and poly I:C

Polyinosinic polycytidylic acid (poly I:C) was obtained from Amersham Pharmacia Biotec, Uppsala, Sweden. S-27609 (4-amino-α, α, 2-trimethyl-1H-imidazo[4, 5-c]quinoline-1-ethanol) was a gift from Dr. Richard L. Miller, 3 M Pharmaceuticals, St. Paul, MN, USA. Atlantic salmon presmolts were injected intraperitoneally (i.p) with 10 mg kg^{-1} of either poly I:C or S-27609, both dissolved in phosphate buffered saline (PBS). Control fish were injected 10 ml kg^{-1} of PBS. For each group, head kidney, liver, heart, spleen and gills were harvested from five fish at 24, 48, 72 and 96 h post injection (p.i.). Harvested organs were immediately frozen in liquid nitrogen and later stored at −80 °C until use.

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