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Prostaglandin E_2 modulation of gene expression in an Atlantic salmon (*Salmo salar*) macrophage-like cell line (SHK-1)

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Abstract

Following lipopolysaccharide (LPS)-stimulation of Atlantic salmon (*Salmo salar*) macrophage-like SHK-1 cells, prostaglandin E_2 (PGE_2) exhibited dose-dependent inhibition of the antigen presenting molecules major histocompatability class I and II and the pro-inflammatory cytokine interleukin-1β gene expression. Prostaglandin E_2 was found to be stimulatory towards cyclooxygenase-2 (COX-2) expression at higher concentrations (1×10^{-6} and 1×10^{-8} M) and inhibitory at lower concentrations (1×10^{-10} and 1×10^{-12} M) after 4 h exposure. After 24 h exposure, however, LPS-induced COX-2 expression decreased and was completely inhibited by all PGE_2 concentrations (1×10^{-6}–1×10^{-10} M). Incubation of SHK-1 cells with LPS alone had no effect on tumour necrosis factor α (TNFα)-like gene or transforming growth factor β-like gene expression after 4 h, however, LPS and PGE_2 showed a synergistic effect on TNFα-like gene expression after 24 h. This study provides evidence for the existence of a PGE_2-mediated negative feedback mechanism in the control of PGs through down-regulation of COX-2, as well as for inflammatory responses by the down-regulation of both COX-2 and IL-1β. The differential regulation of immune-related genes under these conditions further demonstrates the usefulness of the SHK-1 cell line for studying aspects of salmonid immunology.
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1. Introduction

The development of continuous cell lines derived from teleost leucocytes has provided fish immunologists with the ability to study different aspects of cellular immunity and infection conveniently in vitro. The SHK-1 cell line is a continuous cell line derived from Atlantic salmon (*Salmo salar*) head kidney leucocytes and characterized as having some of the same properties as the macrophage [1]. As these cells are able to phagocytose fish pathogens but do not exhibit bactericidal activity or a macrophage appearance, they have been likened to a precursor of

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a melanomacrophage or dendritic leucocyte. The melanomacrophage is a macrophage that contains melanin but retains the ability to phagocytose, even at low temperatures [1]. In order to better characterize this cell line we must obtain a comprehensive understanding of how different physiological conditions regulate SHK-1 cell gene expression. Previous work has shown that these cells express both major histocompatibility class I (MH class I) and II (MH class II) genes constitutively [2]. Antigen presenting cells (APCs) and T-cell interactions are also regulated by the release of cytokines. Cytokines such as interleukin-1β (IL-1β) and tumour necrosis factor α (TNFα), for instance, recruit inflammatory leucocytes to sites of injury [3,4] and promote phagocytosis of foreign particles, which are then presented to T-cells in conjunction with MH molecules. In contrast, cytokines such as transforming growth factor β (TGFβ) are anti-inflammatory and can inhibit APC phagocytosis [5,6].

The role of eicosanoids (oxygenated derivatives of polyunsaturated fatty acids) in immune modulation has been studied in several fish species [7]. However, despite the work done on leucocyte proliferative responses [8] and generation of plaque forming cells [9] in rainbow trout (*Oncorhynchus mykiss*), little work has been applied to other species and nothing has been pursued with respect to effects on gene expression.

Prostaglandin E_2 is a common eicosanoid produced by monocytes and thrombocytes from arachidonic acid (AA) and it is involved in numerous biological processes such as vasodilation, cellular proliferation, leucocyte activation and neutrophil chemotaxis and accumulation in inflammatory sites. The production of PGE_2 during inflammatory events is a result of AA metabolism by the inducible form of cyclooxygenase (COX-2). Over the past decade, the immunomodulatory effects of PGE_2 have been investigated in numerous systems including: rat models of allergic asthma [10], mast cell cytokine synthesis [11,12], and host–parasite interactions [13,14]. In mammals, PGE_2 is known to down-regulate the pro-inflammatory cytokines tumour necrosis factor α (TNFα), interleukin-12 (IL-12), IL-2, and interferon γ (IFN-γ) [15–18], and have no effect on others such as IL-4 and IL-5 [15]. The role of prostaglandins in fish gene regulation, however, is as yet unstudied.

We investigated the effects of PGE_2 administration on the expression of TNFα and IL-1β in LPS-stimulated and non-stimulated Atlantic salmon head kidney derived cell line cells (SHK-1) using quantitative real-time PCR. To provide further evidence of the applicability of these cells to the study of the salmonid immune system, expression of the other immunologically important genes MH class I, MH class II and COX-2 were also studied. These data build upon our knowledge of the characteristics of the SHK-1 cell line, as well as the regulatory role of PGE_2 in immune responses of fish.

2. Materials and methods

2.1. Cell culture

SHK-1 cells were cultured at 18 °C in 75 cm^2 tissue-culture-treated flasks (Costar), in L-15 medium (500 ml with 300 mg/l L-glutamine) supplemented with 500 μl gentamicin sulphate (50 mg/ml in distilled water), 365 μl 2-mercaptoethanol (55 mM in Dulbeco's phosphate buffered saline) and 5% fetal bovine serum (FBS). All media components were purchased from Gibco. Confluent flasks were passaged weekly by dividing cells and medium evenly between two flasks and adding an equal volume of new media to each flask. Cells used in this study were between passages 58 and 60.

2.2. Cell stimulation

SHK-1 cells were seeded at approximately 4.0×10^6 cells/flask in L-15 medium supplemented as described above. Cell stimulation followed the same procedure as Zou et al. [19] and Brubacher et al. [20]. Briefly, following a 48-h period after passaging, to minimize any manipulation-induced gene expression, 10 ml of fresh media without lipopolysaccharide (LPS) or prostaglandin E_2 (PGE_2), or with LPS and without PGE_2, or without LPS and with PGE_2 was added to each flask. Lipopolysaccharide was added to obtain a final concentration of 1.7 μg/ml and PGE_2 was added to give final concentrations of 3.3×10^{-6}, 3.3×10^{-8}, 3.3×10^{-10}, and 3.3×10^{-12} M. Treatments were carried out in triplicate and cells were induced under these conditions for 4 h prior to RNA

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