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Characterization and evaluation of sex-specific expression of suppressors of cytokine signaling (SOCS)-1 and -3 in juvenile yellow perch (*Perca flavescens*) treated with lipopolysaccharide

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ABSTRACT

The suppressor of cytokine signaling (SOCS) proteins are a family of intracellular proteins that are centrally involved with vertebrate growth, development and immunity via their effects as negative feed-back regulators of cytokine (and hormone) signaling. The genes for SOCS-1 & -3 were cloned, sequences analyzed and expression patterns examined in the commercially-important teleost, yellow perch (*Perca flavescens*). The deduced (mature) proteins for yellow perch (yp)SOCS-1 and (yp)SOCS-3 consist of 211 and 205 amino acids, respectively. Functional domains such as the Src homology-2 (SH2) and SOCS-box were present in ypSOCS-1 and ypSOCS-3 and these domains were well conserved between teleost species. Sequence analysis showed that ypSOCS-1 & -3 share highest homology (among similar teleost sequences), to the stickleback (*Gasterosteus aculatus*) SOCS-1 & -3 protein homologs. To investigate sex-specific expression of the ypSOCS-1 and ypSOCS-3 mRNAs, juvenile male and female yellow perch were immunologically challenged with a single injection (10 μg/g bw) of lipopolysaccharide (LPS) and tissues (gill, head kidney, kidney, liver and spleen) were sampled over a 48-h time-course. Quantitative real-time PCR analysis showed that ypSOCS-1 & -3 were expressed in all tissues examined and at all sampling time-points. LPS injection significantly induced ypSOCS-1 & -3 mRNA levels in gill, head kidney, liver, kidney and spleen, with maximal induction occurring at 6 h post-injection in each tissue. By 48-h post-injection, expression levels for ypSOCS-1 & -3 mRNAs approached, or reached, control levels in all tissues examined. While there were statistical interactions among variables (treatment, time and sex) for ypSOCS-1, we only found a main effect of sex on SOCS-3 mRNA expression in head kidney with higher copy numbers occurring in males than in females treated with LPS. Sexually-dimorphic expression of SOCS-1 or -3 mRNA has not been examined, or described, in a teleost. Our findings suggest the involvement of the SOCS genes in the yellow perch immune response and that differences among the sexes are evident and should be explored further.

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1. Introduction

Cytokines are secreted proteins that have indispensable roles in mediating the immune and inflammatory responses in complex organisms [1]. Following the binding of a cytokine (many of the type I and II members) to its receptor, a signal transduction cascade is initiated that involves cytoplasmic tyrosine kinases called janus kinases (JAK) and transcription factors from the family of proteins called signal transducers and activators of transcription (STATs). The JAK–STAT cytokine signaling cascade elicits biological responses that ultimately enable the tissues and cells of an organism to adjust appropriately to particular external and internal cues [2–5]. While different cytokines (and their receptors) utilize various combinations of the JAK and STAT family members for signaling specificity [for review see Refs. [4,6–8]], there is potential for overlapping actions and excessive cytokine signaling, which can lead to chronic inflammation and disease [5,9,10]. Consequently, each level of the JAK-STAT pathway represents a point of feed-back control whereby the duration, magnitude and sensitivity to cytokine signaling can be regulated within a cell. Another group of intracellular proteins, called suppressors of cytokine signaling (SOCS), inhibit this cytokine receptor signal transduction pathway by serving as negative feed-back regulators. The SOCS proteins share a number of common structural regions including the

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N-terminal Src homology 2 (SH2) and kinase inhibitory region (KIR) domains and the C-terminal SOCS-box, which enable these proteins to negatively regulate the JAK–STAT signaling pathway in various ways (e.g., inhibiting JAK docking, STAT transphosphorylation and increased signaling protein degradation) [10–15].

The SOCS family of proteins/genes was initially described in mammals and consists of SOCS-1 through SOCS-7 and a cytokine-inducible SH2-containing protein (CISH) [16,17]. Much is known regarding the function of SOCS-1 and SOCS-3 in mammals and their involvement in controlling the signaling of cytokines and hormones that affect immunity, disease, growth, development and reproduction [10,11,13–15,18–20]. The involvement of SOCS proteins in these important biological processes has been most vividly demonstrated using loss-of-function (non targeted knock-outs) animal models. SOCS-1 deficient mice were reported to have displayed growth retardation, multi-organ inflammation and degradation, heightened sensitivity to sub-lethal doses of lipopolysaccharide (LPS) and early mortality [21–25]. SOCS-3-deficient mice display poor embryonic growth and embryonic lethality that was attributed to excessive erythropoiesis, poor development of embryonic vessels and placental trophoblasts [25,26]. While other SOCS knock-out models exist, perhaps the most dramatic of the SOCS deletion models is that of SOCS-2 null mice. Deletion of the SOCS-2 gene in mice results in an over-growth (gigantism) phenotype that is characterized by increased body weight and length, increased organ size and increased sensitivity to growth hormone administration [19,27]. Conversely, SOCS-2 knockout mice have been shown to exhibit adverse phenotypes involving a range of neural abnormalities as well as reduced life-span [20,28–30].

Considerably less is known of the role of SOCS genes in teleosts, but current analysis has revealed a greater number of SOCS genes (including duplicates) that may have additional/new functions [31,32]. Recent studies have documented the effects of immune stimulation on SOCS (1–9) gene expression and their regulation by immune cytokines in teleosts [31–39]. By contrast, even less is known of the involvement of SOCS genes in growth [40,41], and nothing has been reported on sex-specific differences (e.g., growth or immunity) of SOCS expression in teleosts.

Yellow perch (*Perca flavescens*) are a commercially- and ecologically-important teleost in North America and are highly prized as a recreational and food fish. There has always been a strong demand for yellow perch in the Midwest U.S., which has traditionally been met by a commercial wild-capture fishery. Recent declines in wild stocks of the Great Lakes region of the U.S. and Canada, and restrictions on capture fisheries, has fueled the need for aquaculture production as a means to meet increasing demand for yellow perch [42,43]. However, expansion of yellow perch aquaculture has been limited by the slow growth of this species in aquaculture settings. Additionally, growth in yellow perch is sexually-dimorphic wherein females grow larger and faster than males and such size discrepancies pose further difficulties with production of this species [44–48]. Consequently, effort has been devoted to understand the basis of sex-specific differences in growth and development in yellow perch [47–53]. Given these sex-specific differences in yellow perch physiology and morphology, and our desire to better understand the biochemical pathways that potentially link growth and immune function in yellow perch, we took the initial step to characterize the expression of SOCS-1 and SOCS-3 in this species. We hypothesized that males and females would respond differently to challenge with the pathogen associated molecular pattern (PAMP) molecule, lipopolysaccharide (LPS). Accordingly, the genes for SOCS-1 & -3 were cloned, sequences analyzed, and sex-specific expression patterns examined in immune relevant tissues (gill, head kidney, kidney, liver and spleen) of juvenile male and female yellow perch challenged with LPS injection.

2. Material and methods

2.1. Animals, husbandry and injection of LPS

Yellow perch (*P. flavescens*) were obtained from broodstocks maintained at the School of Freshwater Sciences (Milwaukee, WI). The original broodstock was derived from gametes taken from wild perch from the Choptank River (Chesapeake Bay, MD) and was bred in captivity for at least two generations. Juvenile male (22.8 ± 0.14 g) and female (36.9 ± 0.33 g) perch of the same age and cohort were placed into 12 × 120 L tanks (24 animals/tank consisting of a mixed sex population) and acclimated for a period of 3 weeks. Tanks were supplied with dechlorinated municipal water at 18.5 ± 1.5 °C throughout the experiment. During this period, animals were fed a standard 1.5 mm slow sinking diet (Ziegler Finfish Starter, Slow Sinking, 1.5 mm, Gardners, PA) to satiation twice daily. During the experimental period, food was withheld for a period of 18 h prior to injection through the end of the sampling time-course. Experimental treatments were randomly allocated to each tank (see below) with each sampling time point consisting of either a separate control or LPS-treated tank of mixed-sex ($N = 24$ per tank) animals. Prior to injection, animals were anesthetized with MS-222 (100 mg/L), weighed and measured. At this time, animals received intraperitoneal injections of either sterile-filtered (0.2 μm syringe filter) phosphate-buffered saline (PBS-Control, pH 7.4 and osmolality of 290 milliOsmolals), or PBS-lipopolysaccharide (LPS, SIGMA, serotype O26:B6, 1 mg/ml in PBS) at a dose of 10 μg/g body weight. Injection volume was held constant at 10 μL/g body weight. At 3, 6, 12, 24 and 48 h after injection (PBS or LPS), animals were anesthetized with MS-222 (100 mg/L) and randomly sampled from each tank (control or LPS) until approximately 8 males and 8 females had been collected from the larger population within each tank/treatment. Additionally, we incorporated two separate time 0 control groups. The first group of time 0 control animals consisted of males and females (termed Control male and females in Figs. 6 and 7) that were immediately removed from a control-designated experimental tank at the beginning of the study, anesthetized and sampled for tissues. This was to determine basal levels of ypSOCS expression in animals with minimal human activity and handling disturbance. A second group of time 0 control animals consisted of males and females (termed LPS male and females in Figs. 6 and 7) that were immediately removed from a LPS-designated experimental tank, handled, anesthetized and sampled for tissues on a separate day. This was done to control for potential effects of cumulative human activities and handling on ypSOCS expression in animals held on the experimental system. For tissue sampling, anesthetized animals were decapitated, sex determined and tissues (gill, liver, spleen, head kidney and kidney) were harvested, flash frozen in liquid nitrogen and stored at −80 °C. Animals were maintained and euthanized in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Wisconsin-Milwaukee.

2.2. RNA isolation

Total RNA from each tissue (10–100 mg) was extracted with Tri Reagent (Molecular Research Center, Inc.) according to the manufacturer's protocol [54,55]. Prior to RNA extraction, larger tissue samples (liver, gill and head kidney) were first pulverized in liquid nitrogen. Smaller tissues (kidney and spleen) were homogenized directly using a mixer mill (Retsch, Newtown, PA). RNA stocks were stored as pellets in 100% ethanol at −80 °C. A portion of each pellet