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Sexual maturation and administration of 17β-estradiol and testosterone induce complex gene expression changes in skin and increase resistance of Atlantic salmon to ectoparasite salmon louse

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ABSTRACT

The crustacean ectoparasitic salmon louse (*Lepeophtheirus salmonis*) is a major problem of Atlantic salmon aquaculture in the Northern hemisphere. Host–pathogen interactions in this system are highly complex. Resistance to the parasite involves variations in genetic background, nutrition, properties of skin, and status of the endocrine and immune systems. This study addressed the relationship between sex hormones and lice infection. Field observation revealed a sharp reduction of lice prevalence during sexual maturation with no difference between male and female fish. To determine if higher resistance against lice was related to sex hormones, post-smolt salmon were administered control feed and feeds containing 17β-estradiol (20 mg/kg) and testosterone (25 mg/kg) during a 3-week pre-challenge period. After challenge with lice, counts were reduced 2-fold and 1.5-fold in fish that received 17β-estradiol and testosterone, respectively. Gene expression analyses were performed from skin of salmon collected in the field trial and from the controlled lab experiment at three time points (end of feeding-before challenge, 3 days post challenge (dpc) and 16 dpc) using oligonucleotide microarray and qPCR. Differential expression was observed in genes associated with diverse biological processes. Both studies revealed similar changes of several antibacterial acute phase proteins; of note was induction of *cathelicidin* and down-regulation of a *defensin* gene. Treatment with hormones revealed their ability to modulate T helper cell (Th)-mediated immunity in skin. Enhanced protection achieved by 17β-estradiol administration might in part be due to the skewing of Th responses away from the prototypic anti-parasitic Th2 immunity and towards the more effective Th1 responses. Multiple genes involved in wound healing, differentiation and remodelling of skin tissue were stimulated during maturation but suppressed with sex hormones. Such opposite regulation suggested that these processes were not associated with resistance to the parasite under the studied conditions. Both studies revealed regulation of a suite of genes encoding putative large mucosal proteins found exclusively in fish. Marked decrease of erythrocyte markers indicated reduced circulation while down-regulation of multiple zymogen granule membrane proteins and transporters of cholesterol and other compounds suggested limited availability of nutrients for the parasites.

1. Introduction

A crustacean ectoparasitic copepod salmon louse (*Lepeophtheirus salmonis*) is one of the major problems of commercial Atlantic salmon aquaculture, causing direct economic losses, reduction of fish welfare and fitness, and imposing negative pressures on wild stocks (Torrissen et al., 2013; Jackson et al., 2013). Although the salmon louse is ubiquitous in nature, heavy infestations commonly establish in fish farms with high stock densities (Costello, 2006). Parasiticide treatments remain the main measure of lice control; however, their efficacy has been rapidly reducing over the last years (Jones et al., 2013). Much effort is currently being invested in alternative approaches including selective breeding and developing functional feeds with immune stimulants, repellents and other additives. Despite progress, these measures are still unable to substitute pharmaceuticals.

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Development of biological methods for prevention and mitigation needs better understanding of host pathogen interactions and mechanisms of salmon resistance to the parasite, which may depend on multiple factors including the genetic background, nutrition, properties of skin, ability to heal wounds and the immune system responses (Wagner et al., 2008; Gharbi et al., 2009; Gjerde et al., 2011; Igboeli et al., 2013; Purcell et al., 2013). Teleost skin is a dynamic organ expressing great variability in physical properties, cell types and hormonal systems involved (reviewed by Rakers et al., 2010; Esteban, 2012). A wide range of susceptibilities to *L. salmonis* exists among salmonids; coho and pink respond with aggressive inflammation and subsequent rejection of the parasite (Johnson and Albright, 1992; Braden et al., 2012, 2015; Sutherland et al., 2011) while in Atlantic, sockeye and chum salmon, there is a delayed or weakened inflammatory response to infection (Skugor et al. 2008; Tadiso et al., 2011; Krasnov et al. 2012; Braden et al., 2012, 2015; Sutherland et al., 2011, 2014), which permits heavier parasitic abundance. The study of Holm et al. (2014) suggested that a thicker epidermis and larger mucus cell size were not associated with resistance in Atlantic salmon. In contrast, the development of structural skin complexity, including the formation of scales may at least partly explain resistance to salmon louse in post-emergent pink salmon (Jones et al., 2008). With respect to molecular pathways involved in response to skin damage in fish, a microarray study of Vieira et al. (2011) in sea bream is of note. Processes implicated in skin and scale regeneration are also modulated in skin of lice-infected salmonids (cell cycle, proliferation, adhesion, immune response and antioxidant activities) (Skugor et al., 2008; Tadiso et al., 2011; Krasnov et al., 2012; Braden et al., 2012; Sutherland et al., 2014). Suppressed wound healing appears to be a feature of Atlantic, chum and sockey salmon in response to lice (Skugor et al., 2008; Braden et al., 2012, 2015; Krasnov et al., 2012). Knowledge accumulated so far suggests that mounting of an appropriate immune response (reviewed in Fast, 2014), together with appropriate regulation of tissue remodelling and wound repair, as well as iron sequestration (Sutherland et al., 2014) play important contributing roles in the mechanisms for resistance against *L. salmonis*.

Less is known about the relationship between host resistance to lice and the endocrine status. In view of evidence gathered from mammalian studies, the endocrine system (and in particular sex steroids) has a great potential to influence the course of parasitic infections (reviewed in Nava-Castro et al., 2012; Klein, 2004). Stress and associated immunosuppression have been discussed as factors that may affect resistance to the parasite (Johnson and Albright, 1992). The increased secretion of cortisol in fish suppresses metabolism involved in growth and immunity, similar to what happens in mammals (Barton, 2002). Elevation in cortisol level is often reported in lice trials with susceptible salmonid species. For example, Bowers et al. (2000) demonstrated correlations between the stress indicators, including cortisol and the number and life stage of copepods in Atlantic salmon and a similar result was observed in chum salmon (Jones et al., 2007; Sutherland et al., 2014). Cortisol-injection caused massive down-regulation of genes related to the wound healing cascade in skin of Atlantic salmon (Krasnov et al., 2012), in line with the well-defined role of cortisol in wound healing in mammals (Christian et al., 2006). In two older studies, cortisol administered as implants resulted in more severe *L. salmonis* infections in the resistant coho salmon (Johnson and Albright, 1992), and Atlantic salmon and Arctic charr (*Salvelinus alpinus*) (Mustafa and MacKinnon, 1999). The latter study also revealed an inverse relationship of cortisol and thyroid hormones by cortisol injections in Atlantic salmon. Moreover, it was shown that treatment with iodized feed resulted in higher plasma levels of thyroid hormones (tri- and tetra-iodothyronine, T3 and T4, respectively), higher T3/T4 ratios, lower cortisol levels, and significantly lower mean intensities of lice than controls in these two species. The thyroid system is a major mediator of basal metabolic rate in animals and in fish, it is an important regulator of early development, flatfish metamorphosis (reviewed in Power et al., 2001), and metamorphosis that occurs during smoltification in salmonids (see references in Harada et al., 2008). A broad expression pattern of the thyroid hormone receptor (TR) genes, TRα and TRβ, in brain, gill, liver, muscle, kidney, ovary, skin and eyeball of coho salmon (Harada et al., 2008), suggests complex signalling and involvement of the thyroid system in a wide range of physiological processes. A recent proteomic study by Ibarz et al. (2013) revealed that 17β-estradiol (E2) accelerated skin wound healing in gilthead sea bream, which was associated with increased production of collagen, actin and myosin components in regenerating skin. Maturational changes in skin structure associated with the reproductive cycle can be linked to the plasma levels of androgens. Intraperitoneal implants of 11-ketotestosterone increased epidermal and dermal thickness, and reduced the number of superficial goblet cells, while testosterone (T) positively affected only the epidermal thickness in brown trout (Pottinger and Pickering, 1985). Considerable thickening of the epidermis and increased goblet cell density during the spawning period were documented in Atlantic salmon (Burton et al., 1985) and winter flounder, with a larger increase observed in females (Burton and Fletcher, 1983). Seasonal and gender differences in the skin epidermal thickness and number of different secretory cells are observed in other teleosts. An increase in the number of mucus-secreting goblet cells was inversely correlated to the number of the secretory sacciform cells in sexually mature male brown trout and Arctic charr (Pickering and Fletcher, 1987) and males of two sculpin species (Halačka et al., 2012). Increased number of sacciform cells in brown trout may play a protective role in response to infestation by the ectoparasitic flagellate, *Icthyobodo* sp. (Pickering and Fletcher, 1987), while increased epidermal thickness and number of goblet cells might be inversely related to immunity. Changes in skin morphology and production of mucus likely represent adaptations related to activities that occur during the spawning period in many teleosts, e.g. fighting, nest construction, egg guarding, and aerating of eggs in salmonids. Such trade-offs between reproductive and immune functions are documented in many different contexts in vertebrates (Martin et al., 2008). Furthermore, among vertebrates, males often exhibit higher parasite susceptibility and greater infection rates than their female counterparts, particularly during the breeding season when concentrations of male sex hormones are elevated (Klein, 2004). Differential effects of male and female sex steroid hormones on non-reproductive tissues, including immune cells, could explain some of these differences. It is generally accepted that in mammals, estrogens exert mainly pro-inflammatory effects while androgens are primarily immunosuppressive (see references in Chaves-Pozo et al., 2012). Cook et al. (1994) demonstrated that E2 stimulated salmon lymphocyte proliferation in *in vitro* assays, while T, but not E2, significantly reduced the number of antibody-producing lymphocytes; the magnitude of induced immunosuppression by T was significantly greater in combination with cortisol than when either hormone was administered alone. Sex steroids also affect immune responses in birds, but the data often appear contradictory (reviewed by Koutsos and Klasing, 2008). The findings in teleosts also range from stimulatory to suppressive effects for both female and male sex steroids (reviewed by Chaves-Pozo et al., 2012). This can be partly explained by their highly context-dependent action, which is, among other factors, influenced by the presence of the pathogen and its type. In sea bream, 11-ketotestosterone increased the expression of *IL-1*β on acidophilic granulocytes (equivalents of mammalian neutrophils) only upon stimulation with the genomic DNA of *Vibrio anguilarium*, while T showed stimulatory effects