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Mechanisms of Resistance among Salmon to the Parasitic Copepod *Lepeophtheirus salmonis*

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Abstract

The purpose of this paper is to review the current knowledge of salmonid defence responses to *Lepeophtheirus salmonis*. The salmon louse *L. salmonis* is an important pest of economically valuable salmonids in seawater throughout the northern hemisphere. Treatment of salmon lice on cultured salmon often fails in regions where the parasite has developed resistance to commonly used therapeutants. The development of efficacious vaccines is hampered by limited knowledge of parasite antigens that elicit protective immunity and a poor understanding of defence responses mounted by the salmonid host. Infection kinetics indicate a wide range of susceptibilities to *L. salmonis* among salmon species: juvenile coho and pink salmon are relatively resistant whereas Atlantic and chum salmon are susceptible. Innate resistance is linked to the speed and intensity of local inflammatory reactions at the site of infection. Conversely, susceptibility is related to an absence of these reactions and in Atlantic salmon is mediated in part by hypersecretion by the parasite of prostaglandin E_2 and other compounds. Transcriptomic analysis shows that the susceptible salmonid response is characterised by cell stress, tissue remodelling and diminished immunological responsiveness during infection. In contrast, there is evidence of cell motility, somatic growth and immuncompetence among resistant salmon following infection. Future research should apply a combination of genomic, proteomic and immunological studies to better understand defence mechanisms among susceptible and resistant salmonids.

Keywords: *Lepeophtheirus salmonis*; Salmon; Secretory-excretory products; Innate immunity; Susceptibility; Immunomodulation

Introduction

Copepods belonging to the family Caligidae, referred to as sea lice, are ectoparasites of marine fishes. All sea lice share similar life cycles including nauplius stages and an infective copepodid stage that disperse in the plankton while subsisting on endogenous lipids. Once settled onto a suitable host, the copepodid moults through four chalimus stages that are tethered to the host with a frontal filament. Depending on species, the parasite further develops through one or two preadult stages to the adult stage which are untethered and mobile on the host. Much of the damage caused by parasitic copepods is related to attachment to the host (Figure 1) and feeding behaviour: the parasites graze on host tissues that range from mucus, cells of the epidermis, dermis or subcutaneous tissues. The more invasive feeding behaviours are associated with the larger developmental stages.

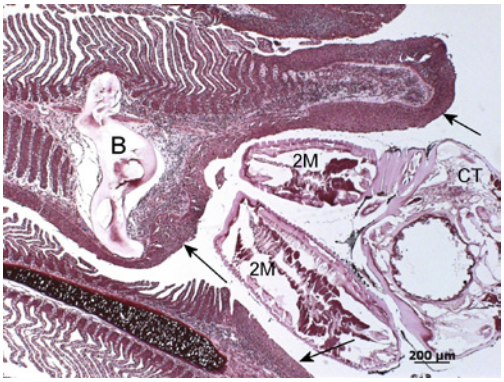

Figure 1: Light micrograph of the attachment of the lernaeopodid copepod *Salmincola californiensis* to a gill lamella of a spawning pink salmon (*Oncorhynchus gorbuscha*). The lamella is distended by the anchoring bulla (B) and epithelial hyperplasia (arrow) is evident adjacent to the site of attachment. Parasite structures also visible are the second maxillae (2M) and cephalothorax (CT). Gram stain.

Species of sea lice parasitic on salmonid fishes are well described pests in marine aquaculture and in the northern hemisphere include *Lepeophtheirus salmonis* (the salmon louse) and several species of *Caligus* [1-4]. Infections with *L. salmonis* incur annual costs to marine open netpen aquaculture in excess of 700M Euros in Norway, Scotland, Ireland and Canada [5]. In addition, infections with *Caligus rogercresseyii* are a significant economic burden to salmon aquaculture in Chile [6]. Management of sea lice infections in aquaculture depends on integrated husbandry schemes including chemotherapeutant intervention [7]. However, recent trends indicate a declining efficacy for many compounds used for treatment, suggesting that in several jurisdictions *L. salmonis* or *C. rogercresseyii* have developed tolerance or resistance to these compounds. The absence of commercially efficacious vaccines against sea lice reflects a lack of detailed knowledge of immunogenic parasite antigens that elicit protective immunity in salmonids. In addition, defence responses against parasitic copepods in fishes are poorly documented. A more thorough understanding of teleost defense mechanisms against sea lice will provide a rational basis for the development of novel management strategies. The purpose of this paper is to review the current understanding of salmonid defence responses to *L. salmonis*.

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Host susceptibility

Appropriate defence responses mounted by the host determine copepod survival and therefore the severity of the infection. Thus, infection dynamics compared among groups of salmon in controlled environments may be useful for inferring differences in the ability to mount a defensive response, and these differences may form the basis of selective breeding programmes [8]. Differential susceptibility to *L. salmonis* occurs among salmon species. On salmon farms in Ireland, rainbow trout (*Oncorhynchus mykiss*) carried fewer *L. salmonis* than did Atlantic salmon [9] and in Japan, fewer *L. salmonis* occurred on coho salmon (*Oncorhynchus kisutch*) compared with rainbow trout, despite concurrent exposures to the parasite from wild chum salmon (*Oncorhynchus keta*) [10]. While the mean abundance of lice declined both on sea trout (*Salmo trutta*) and Atlantic salmon, a higher mean abundance was maintained on the sea trout eight weeks following a laboratory exposure, suggesting greater susceptibility [11]. Similarly, parasites were lost more rapidly from coho salmon compared with Atlantic salmon or rainbow trout [12,13] and matured more slowly on coho salmon than on Atlantic salmon or rainbow trout [13]. More recently, juvenile chum salmon were shown to retain a higher intensity of infection with *L. salmonis* compared with pink salmon (*Oncorhynchus gorbuscha*), from which the parasite was rapidly rejected following laboratory exposures [14,15]. Juvenile pink salmon acquire and retain a natural resistance to *L. salmonis* before they reach a mean weight of 1g, despite inadequate nutrition [16,17]. Taken together, these observations indicate susceptibility to *L. salmonis* varies among species of anadromous salmonids and the rapid rejection of parasites indicates a relatively robust innate resistance in coho and pink salmon. Using these criteria, rainbow trout and Chinook salmon have intermediate resistance whereas the natural resistance of Atlantic and chum salmon and sea trout is limited.

Although Atlantic salmon are highly susceptible to infection with *L. salmonis* [12,13], intraspecific heterogeneity in susceptibility occurs among distinct spawning stocks [18] and among full-sib families [19-21]. The heritability of lice counts in the latter studies ranged from 0.07 to 0.33, indicating a genetic basis for the differences observed among families. Similarly, a heritability of 0.22 was calculated for counts of the related copepod *Caligus elongatus* among full-sib Atlantic salmon families [22]. Susceptibility to *L. salmonis* in Atlantic salmon has been linked to a major histocompatibility (MH) class II genotype (Sasa-DAA-3UTR) [23]. However, a subsequent QTL analysis provided only weak support for this relationship [24] and suggested a better understanding of innate mechanisms of resistance to *L. salmonis* is necessary to explain differential susceptibility. Previous exposure to *L. salmonis*, severity of the exposure and co-infection with *C. elongatus* can influence susceptibility to *L. salmonis*. Thus, the importance of reproducible controlled challenges for assessing the genetic basis for susceptibility to *L. salmonis* in Atlantic salmon has been emphasised [20,25].

Defence mechanisms against salmon lice

Innate histological responses: The skin and its mucous secretions form a natural barrier and constitute the first lines of defence against salmon lice [26]. The capacity of the integument to cope with damage associated with copepod feeding [27] and to respond in a way that limits the infection is an important determinant of host resistance. Natural infections on Atlantic salmon are associated with feeding damage at the site of attachment that ranges from hyperplasia, sloughing of cells, oedema and inflammation to scale loss and haemorrhage [28]. These lesions are not unlike those caused by *Lepeophtheirus pectoralis* in the skin of flounder (*Platichthys flesus*) [29].

Comparative controlled laboratory studies among salmon species provide valuable insights into the occurrence and mechanisms of host resistance. For example, histological changes in the epithelia of gill and fin differed among Atlantic, Chinook and coho salmon and evolved over time following a laboratory exposure to *L. salmonis* copepodids [12]. Coho salmon gill showed acute inflammation at the site of parasite attachment with a cellular infiltrate that consisted predominantly of neutrophils with some lymphocytes. In contrast, inflammation in Chinook and Atlantic salmon gill was mild although the cellular infiltrate also included neutrophils and lymphocytes. At louse attachment or feeding sites on fins of coho salmon, mild inflammation of the dermis with an infiltrate consisting predominantly of neutrophils was observed between one and five days, whereas similar lesions were not observed in fins of Atlantic or Chinook salmon. From 10 to 20 days after exposure, a more chronic inflammation was observed in coho salmon consisting of extensive epithelial hyperplasia with a mixed inflammatory infiltrate of neutrophils, macrophages and some lymphocytes. Some copepods were entirely or partially encapsulated within the hyperplastic lesion. In contrast, little or no histopathological changes were evident in the fins of Chinook or Atlantic salmon at this time. Implantation of cortisol into coho salmon reduced the severity of the inflammatory response and delayed the rejection of *L. salmonis* [12]. Conversely, dietary immunostimulants (e.g. CpG oligodeoxynucleotide [ODN]) caused greater than 40% reduction in parasite intensity in Atlantic salmon seven to 10 days after exposure to *L. salmonis* (M. Fast, personal communication). A mild to moderate inflammatory infiltrate and epithelial cell hyperplasia was also observed at the site of parasite attachment in treated fish.

Together, these data support a hypothesis that resistance to *L. salmonis* is mediated in part by an aggressive and localised inflammatory reaction in response to the attached copepod. Furthermore, the more rapid rate of rejection of parasites from the coho salmon and immunostimulant-treated Atlantic salmon appears to be directly related to the severity of this reaction. In contrast, the relative susceptibility of Atlantic and Chinook salmon appears to be related to the limited abilities of these fish to develop similarly aggressive histological responses. Epidermal thickness and number of mucus cells in the epidermis was not affected by infection with *L. salmonis* in coho salmon, Atlantic salmon or rainbow trout [13], indicating that the inflammatory lesions described above are confined to the site of sea lice attachment and feeding. In related work, inflammatory lesions, including ulcerative necrosis, haemorrhage, fibrin deposition and mixed leucocyte infiltration, were similar at *L. salmonis* attachment sites in gill and fin integument of juvenile pink and chum salmon [15]. The more rapid rejection of the parasites from pink salmon indicates that further research is needed to better describe differences in the histological responses of juvenile pink and chum following exposure to *L. salmonis*.

Expression of immune-related genes may provide insight into the occurrence and timing of inflammatory processes occurring at the site of infection or systemically with respect to host defence. The expression of cyclo-oxygenase-2 (COX-2), interleukin-1β (IL-1β), tumour necrosis factor alpha (TNFα) and major histocompatibility class I and II (MH-I, MH-II) was measured in intact head kidney or in cultured adherent head kidney leucocytes of Atlantic salmon following infection with relatively few (8 to 11 per fish) *L. salmonis* [30]. Although perturbation in the expression of several genes was observed and

indicated the onset of systemic inflammation, conflicting observations from the intact kidney and cultured cells in addition to the absence of expression data from the skin failed to suggest possible response mechanisms. In a subsequent trial, the expression of IL-1β, TNFα, COX-2, transforming growth factor β (TGFβ), MH-I and MH-II was increased in head kidney of Atlantic salmon following two exposures to *L. salmonis*, 14 days apart [31]. In a comparative study, a more rapid and elevated expression of IL-8 was observed in skin and head kidney of pink but not chum salmon seven days after exposure to *L. salmonis* copepodids [15]. Similarly, expression of TNFα was elevated in the head kidney of pink but not chum salmon suggesting a relatively rapid inflammatory response is preferentially elicited in pink salmon and may play a role in the early rejection of the parasite from juvenile pink salmon. In contrast, expression of IL-1β was only elicited in the skin of chum salmon and only 28 days after exposure, possibly reflecting the later persistence of the parasite on the chum salmon.

Innate humoral responses: The occurrence of defence-related compounds in the epidermal mucus of teleosts has been known for over 40 years [32] and more recent advances have been reviewed [33]. Although soluble compounds associated with the mucus of various marine fish species differ in their ability to trigger host-seeking behaviour or enzyme secretion from *L. salmonis* [34-36], very little is known about the inhibitory properties of inducible or constitutive soluble mucus components with respect to copepod survival on salmon. Fast et al. [13] concluded that effectors of host resistance to *L. salmonis* occurred in mucus because of the absence of significant differences in physiological parameters in the plasma of susceptible and resistant salmonids following infection with *L. salmonis*. However, concentrations of alkaline phosphatase (AP), lysozyme and protease in the mucus were not correlated with the rapid decline in copepod intensity observed on coho salmon [13], indicating a more detailed assessment of changes in mucus biochemistry following exposure to parasitic copepods is required.

Adaptive immune responses: Salmonids appear to mount a weak adaptive immunological response to infection with caligid copepods. No *L. salmonis*-specific antibodies were detected in sera obtained from pen-reared rainbow trout following an eight week natural infection with approximately 10 *L. salmonis* per fish. In contrast, sera from pen-reared Atlantic salmon, exposed to as many as 200 *L. salmonis* per fish for two years, recognised approximately five antigens in an *L. salmonis* homogenate compared with at least 38 parasite antigens recognised by immunised rabbits or fish [37,38]. The relatively poor response elicited during infection is a likely result of limited exposure of the salmon immune system to copepod antigens, some of which are associated with gut epithelium [37,39], during attachment and feeding on the skin. The available evidence does not indicate that salmon develop protective immunity as a result of a previous infection with *L. salmonis* [40] and it is not known whether the response elicited by injection with louse homogenates is protective.

Immunomodulation

Following the onset of Atlantic salmon aquaculture, anecdotal evidence indicated that salmon infested with *L. salmonis* are at greater risk of infections with other disease-causing agents and that the risk may be related to increased opportunities for secondary colonisation by pathogens via louse-induced skin lesions, immunomodulation caused by stress or other physiological changes in the host or from pathogens transmitted by sea lice [1]. Although there are limited data demonstrating increased susceptibility of salmonids to secondary infection as a result of a primary salmon louse infection, the intensity of infection with *Loma salmonae*, a microsporidian parasite of the gills, was elevated in rainbow trout following a previous exposure to *L. salmonis*, compared to non-lice-infected controls [41]. The physiological consequences of salmon louse infection are better documented and include a generalised stress response characterised by an increased plasma cortisol titre [15,31,42-47]. In fish, plasma cortisol titres vary inversely with disease resistance by suppressing B lymphocyte function [48]. Infection with *L. salmonis* increases the susceptibility of rainbow trout to additional stressors [49]. Exposure to *L. salmonis* was associated with depressed respiratory burst and phagocyte activities in cultured adherent head kidney leucocytes coincident with elevated plasma cortisol in rainbow trout or in the absence of a cortisol response in Atlantic salmon [13,42,46]. This suggests that impairment of cell mediated immunity is also a consequence *L. salmonis* infection, and may be mediated by cortisol.

The possibility that *L. salmonis* modulates the host immune response by secreting prostaglandin E_2 (PGE_2) was indicated by the observation of elevated serum PGE_2 in louse infested salmon [1]. Subsequent work confirmed the presence of PGE_2 in dopamine-elicited *L. salmonis* secretory/excretory products (daSEP) [50] however the increase in plasma of infected salmon was not statistically significant [31]. Unfractionated daSEP was shown to up-regulate major histocompatibility (MH) class I gene expression in lipopolysaccharide (LPS)-stimulated Atlantic salmon head kidney leucocytes, but had no effect on the expression of IL-1β or COX-2 gene expression in these cells [51]. In contrast, several HPLC-derived fractions of the daSEP, as well as the unfractionated daSEP, down-regulated expression of IL-1β and COX-2 in LPS-stimulated salmon SHK-1 cells, an immortalised cell line derived from Atlantic salmon head kidney [51,52]. Proteolytic activity in the daSEP was observed in a higher proportion of lice following incubation with mucus from the susceptible Atlantic salmon compared with those incubated in the mucus of the less susceptible coho salmon or winter flounder (*Pseudopleuronectes americanus*) [36], suggesting that *L. salmonis* SEP may play a role in the increased susceptibility of Atlantic salmon to this parasite. However, more research is needed to explore the roles and consequences of the *L. salmonis* excretory/secretory products among salmon belonging to a broader range of susceptible and resistant species.

Transcriptomic Responses to *L. salmonis*

The high economic value of salmonid fishes has stimulated an international effort to sequence the Atlantic salmon genome [53]. Genomics tools, including microarrays, which result from such sequencing efforts, generate large volumes of quantitative gene expression data and have provided insight into salmonid ontogeny and the responses of salmonids to environmental stimuli including infectious agents [54,55]. Microarray studies, verified by quantitative reverse transcriptase polymerase chain reactions (qRT-PCR), have begun to explore transcriptomic responses following exposure to *L. salmonis* [56,57]. In Atlantic salmon, numerous physiological pathways are dysregulated not only in skin but also in spleen and head kidney and the regulation of gene expression is influenced by the developmental stage of the parasite. Despite the early sensing of infection at three days post-exposure (upregulation of immunoglobulin-related genes in spleen and head kidney, upregulation of IL-1 receptor type 1, CD4, β-2-microglobulin, IL-12β, CD8α and arginase 1 in intact skin), the immune-related responses later declined and were replaced by those associated with immune hypo-responsiveness and cell stress [56]. The transcriptomic data suggested that in Atlantic salmon, *L. salmonis*

infection results in chronic stress, impaired wound healing and immunomodulation [56]. A subsequent paper [57] confirmed the early immune responsiveness of Atlantic salmon to *L. salmonis* and indicated that the transcriptomic response was biphasic, coinciding with the transition from the infective copepodid to the chalimus developmental stage. As in the earlier study, there was evidence of transient upregulation of early innate and adaptive immune response genes that were later replaced by transcripts indicating chronic inflammatory processes. An examination of gene expression among varying sizes of juvenile pink salmon following exposures to *L. salmonis* [58] helped further define a transcriptional basis for resistance and susceptibility to *L. salmonis*. The susceptible 0.3g pink salmon showed evidence of an inhibition of cell proliferation (somatic growth), cell stress and inflammation with tissue remodeling, whereas transcripts in the resistant 2.4g pink salmon showed evidence of somatic growth, cell motility and immune responsiveness. Microarray studies provide considerable resolution on the mechanisms by which salmon respond to *L. salmonis* and have shown that systemic and chronic inflammation combined with reduced capacity for wound healing and inappropriate partitioning of limited energy resources are hallmarks of the infection in a susceptible host. In a pilot study, the expression of 14 defence-associated transcripts was measured at the attachment sites of adult *L. salmonis* in juvenile pink, chum and Atlantic salmon [59]. Locally elevated expression of MH class II, IL-6, C-reactive protein, matrix metalloproteinase 13, IL-1β and COX-2 in skin of resistant pink salmon compared with the more susceptible chum and Atlantic salmon, suggested these molecules as candidate markers of resistance.

Future Developments

Comparative transcriptomic research is required to further elucidate markers of resistance and susceptibility to *L. salmonis* in salmon. It is evident that the susceptibility of Atlantic salmon is based partly on the modulatory activities of parasite secretory/excretory products (SEPs) and that the parasite responds to mucous fractions from the salmon by increasing the production of SEPs. The application of novel genomic tools, including sea lice microarrays [60], in combination with the capacity for host genomics already described, will permit improved understanding of host-parasite interactions. In particular, defence mechanisms associated with the range of susceptibility observed among species of Pacific salmon and among families and stocks of Atlantic salmon require elucidation. The pathological and immunological significance of genetic differences between Pacific and Atlantic Ocean types of *L. salmonis* [61] will form a novel context for much of the required research.

Some unexpected and exciting recent discoveries also require further research. Tadiso et al. [57] showed that expression of immunoglobulin T (IgT) was significant in skin of infected Atlantic salmon by 15 days post exposure to *L. salmonis*. Immunoglobulin T (tau heavy chain) is an isotype newly discovered in teleosts which functions in the mucosal immune response analogous to IgA in mammals and is secreted in salmonids in response to parasites [62]. The occurrence of elevated tau-chain transcripts suggests that an IgT response to *L. salmonis* may be a useful biomarker of immune responsiveness. Further research is required to elucidate the occurrence of an IgT during *L. salmonis* infections in Pacific salmon and to verify its role in conferring protective immunity. In addition, the unexpected observation that exposure to *L. salmonis* enhances somatic growth among resistant pink salmon [16,58] requires careful examination. In this context, it will be important to understand the bioenergetic and ecological significance of the apparent bimodal response to *L. salmonis* displayed by pink salmon: resistant juveniles actively reject the parasite whereas sub-adults and adults typically support large parasite burdens on the high seas [63,64]. Future research on salmon louse host interactions will benefit from an integration of novel genomic approaches with functional studies in proteomics, cell biology and immunology.

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