EFFECTS OF THE SECRETORY/EXCRETORY PRODUCTS OF *LEPEOPHTHEIRUS SALMONIS* ON ATLANTIC SALMON LEUKOCYTE MIGRATION

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ABSTRACT

Lepeophtheirus salmonis evades its host, Atlantic salmon, by inhibiting inflammatory responses; however, the mechanisms of this activity are not well characterized. Studies from other arthropod ectoparasites have identified chemokine-binding proteins in the saliva and salivary gland extracts that inhibit neutrophil recruitment and activation *in vitro* and *in vivo* by binding to chemokines such as leukotriene B4 (LTB4). To determine if *L. salmonis* is using a similar mechanism to evade the host's immune response the effects of *L. salmonis* secretory/excretory products (SEPs) on LTB4 stimulated leukocytes *in vitro* were examined. Results showed an increase in leukocyte recruitment in response to LTB4 immune stimulation; yet, pre-incubation of LTB4 with SEPs for 1 hour significantly reduced cell migration compared to LTB4 stimulation alone. This data suggests that *L. salmonis* inhibits inflammatory responses in Atlantic salmon by secreting proteins with chemokine inhibiting activity. Furthermore, we measured the effects of SEPs on the host's response to chitin, a component of the exoskeleton of arthropods known to induce inflammation and IL-8 production, *in vivo*. Histological analysis of epithelial tissues collected 6 hours post chitin exposure showed a significant increase in leukocyte recruitment to chitin + dopamine seawater (CDSW) treatment compared to the no injection control. SEPs + chitin and SEPs only treatments were similar, with fewer leukocytes present at injections sites. These treatments were not significantly different from positive controls. To gain a better understanding of the chemotactic pathways disrupted by SEPs, the expression of genes involved in neutrophil activation and cellular chemotaxis in epithelial tissues collected from the *in vivo* chitin challenge was assessed. Data showed CDSW significantly upregulated IL-8 gene expression relative to the vehicle control at 24h post challenge. When SEPs were administered in combination with chitin, IL-8 expression was not significantly different from the CDSW and chitin only treatments. Results suggest SEPs are not inhibiting IL-8 transcription in response to chitin. While histologically fewer leukocytes are migrating in the chitin + SEP and SEP only treatments, transcriptionally it was shown there is no change in IL-8 expression between chitin and chitin + SEP treatments. The *in vivo* data suggests SEPs are directly interacting with leukocytes inhibiting the release of IL-8, or are directly interacting with IL-8 inhibiting its ability to interact with immune cells and stimulate an inflammatory response in Atlantic salmon.

KEYWORDS

Parasite, leukocyte, SEPs, cytokine, immune evasion

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