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Vibrio cholerae modulates the immune defense of human gut mucosa

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av medicine doktorsexamen framläggs till offentligt försvar i Astrid Fagraeus hörsal A103, byggnad 6E, Umeå Universitet, tisdagen 5 juni, kl 13.00.
Avhandlingen kommer att försvaras på engelska.

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Abstract

The key function of innate immunity is to sense danger signals and initiate effective responses as a defense mechanism against pathogens. Simultaneously, effector responses must be regulated to avoid excessive inflammation with resulting tissue damage. microRNAs (miRNAs), are small endogenous molecules, that has recently gained attention as important regulatory elements in the human inflammation cascade. The control over host miRNA expression may represent a previously uncharacterized molecular strategy exploited by pathogens to mitigate innate host cell responses.

Vibrio cholerae is a Gram-negative bacterium that colonizes the human small intestine and causes life-threatening secretory diarrhea, essentially mediated by cholera toxin (CT). It is considered a non-invasive pathogen and does not cause clinical inflammation. Still, cholera is associated with inflammatory changes of the small intestine. Furthermore, CT-negative strains cause gastroenteritis and are associated with extra-intestinal manifestations, suggesting that other virulence factors than CT are involved in the pathogenesis.

The innate immune response to *V. cholerae* is poorly investigated and the potential role of microRNA in cholera had not been studied before. Therefore, this thesis explores the role of intestinal epithelial cells in response to *V. cholerae* infection with a focus on regulatory miRNA as a potential contributor to the pathogenesis. The *in vivo* material was small intestinal biopsies from patients suffering from *V. choleare* infection. As an *in vitro* model for *V. cholerae* attack on intestinal epithelium, we used tight monolayers of T84 cells infected with the bacterium and its released factors. We analyzed changes in levels of cytokines, immunomodulatory miRNA and their target genes.

We showed that miRNA-146a and miRNA-155 reached significantly elevated levels in the intestinal mucosa at acute stages of disease in *V. cholerae* infected patients and declined to normal levels at the convalescent stage. Low-grade inflammation was identified at the acute stage of *V. cholerae* infection, which correlated with elevated levels of regulatory microRNA. Furthermore, outer membrane vesicles (OMVs) released by the bacteria were shown to induce miR-146a and live bacteria induced miR-155 in intestinal epithelial cells. In addition, OMVs decreased epithelial permeability and caused mRNA suppression of pro-inflammatory cytokines, including immune cell attractant IL-8 and CLL20, and the inflammasome markers IL-1β and IL-18. These results propose that *V. cholerae* regulates the host expression of miRNA during infection and may set the threshold for activation of the intestinal epithelium.

Moreover, we showed that *V. cholerae* also harbors inflammatory-inducing capabilities, by secreting a pore-forming toxin, *Vibrio cholerae cytolysin* (VCC). By using genetically modified strains as well as soluble protein in challenge experiments, VCC was found to be solely responsible for the increased epithelial permeability and induction of several pro-inflammatory cytokines in intestinal epithelial cells. In contrast to OMVs, VCC displayed strong upregulation of the pro-inflammatory cytokines IL-8, TNF-α, CCL20, IL-1β and IRAK2, a key signaling molecule in the IL-1 inflammasome pathway. This suggest that VCC is an important virulence factor in the *V. cholerae* pathogenesis, particularly in CT-negative strains. Furthermore, we showed that the bacterium could control the inflammatory actions of VCC by secreting the PrtV protease, which degraded VCC and consequently abolished inflammation.

Keywords
Vibrio cholerae, intestinal epithelial cell, duodenum biopsy, acute cholera, immunomodulation, microRNA, OMV, cytokine, T84 monolayer, miR-146, miR-155, VCC, PrtV, hemolysis, IL-8, TNF-α, IL-1β, IL-18, CCL20, IRAK2, inflammation

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