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Review

Vacuolating cytotoxin A (VacA) – A multi-talented pore-forming toxin from *Helicobacter pylori*

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ABSTRACT

Helicobacter pylori is associated with severe and chronic diseases of the stomach and duodenum such as peptic ulcer, non-cardial adenocarcinoma and gastric lymphoma, making *Helicobacter pylori* the only bacterial pathogen which is known to cause cancer. The worldwide rate of incidence for these diseases is extremely high and it is estimated that about half of the world's population is infected with *H. pylori*. Among the bacterial virulence factors is the vacuolating cytotoxin A (VacA), which represents an important determinant of pathogenicity. Intensive characterization of VacA over the past years has provided insight into an ample variety of mechanisms contributing to host-pathogen interactions. The toxin is considered as an important target for ongoing research for several reasons: i) VacA displays unique features and structural properties and its mechanism of action is unrelated to any other known bacterial toxin; ii) the toxin is involved in disease progress and colonization by *H. pylori* of the stomach; iii) VacA is a potential and promising candidate for the inclusion as antigen in a vaccine directed against *H. pylori* and iv) the *vacA* gene is characterized by a high allelic diversity, and allelic variants contribute differently to the pathogenicity of *H. pylori*. Despite the accumulation of substantial data related to VacA over the past years, several aspects of VacA-related activity have been characterized only to a limited extent. The biologically most significant effect of VacA activity on host cells is the formation of membrane pores and the induction of vacuole formation.

This review discusses recent findings and advances on structure-function relations of the *H. pylori* VacA toxin, in particular with a view to membrane channel formation, oligomerization, receptor binding and apoptosis.

Contents

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1. Introduction

Given the enormous impact on human health, *Helicobacter pylori* is now among the best characterized bacterial human pathogens and intensive research efforts are devoted to the analysis of the molecular pathobiology of this organism. *Helicobacter pylori*, a Gram-negative, spiral-shaped, microaerophilic organism is able to persistently colonize the human stomach for decades and causes serious chronic diseases such as dyspepsia, gastric atrophy, peptic ulcer disease, gastric adenocarcinoma and gastric cell lymphoma (Cover and Blaser, 2009; Montecucco and Rappuoli, 2001; Suerbaum and Michetti, 2002). Striking differences are associated with gastric cancer prevalence in different geographical locations. Gastric cancer is the third leading type of cancer worldwide and it is the fifth most common cancer in Europe (Ferlay et al., 2010). The risk of developing gastric cancer is strongly correlated to the prevalence of *H. pylori*–associated atrophic gastritis (Wen and Moss, 2009). *H. pylori* is now considered as the most common bacterial infectious agent of relevance to human health (Atherton, 2006; Ferlay et al., 2010) and it is obvious that a pathogen with this impact on human health became a priority target for the extensive analysis of its molecular physiology. The clinical presentation of infections with *H. pylori* is determined by a complex interaction of multiple factors such as strain diversity, host genetic predisposition, environmental factors and nutrition (Wen and Moss, 2009). *H. pylori* is contagious and most likely transmitted *via* oral-oral, fecal-oral, and gastro-oral (mediated by a reflux of gastric juice) routes (Boehnke et al., 2015; Brown, 2000; El-Sharouny et al., 2015; Khalifa et al., 2010; Krueger et al., 2015; Rakhmanin and German, 2014; Santiago et al., 2015; Tirodimos et al., 2014; Yokota et al., 2015). Epidemiological data related to disease prevalence have to be interpreted with caution as a large proportion of infected individuals (approx. 80%) can remain symptomless over long periods of time. Moreover, diagnostic procedures appropriate for *H. pylori* infections may be limited or not available at all in some developing countries (Akguc et al., 2014; Batts et al., 2013; Shrestha et al., 2014; Taylor and Blaser, 1991; Vilaichone et al., 2014). Nevertheless, current information on the incidence of *H. pylori*-infections suggests a high burden to public health care, especially in developing countries where acquisition of the disease appears to occur at higher rates than in developed countries (Aziz et al., 2015; Weaver, 1995; Archampong et al., 2015). In addition, infections during the childhood seem to be more frequent in developing countries where up to 50% of children (5 years of age) and 90% of adults are infected (Khalifa et al., 2010). Marked geographic differences in *H. pylori* prevalence were previously attributed to differential acquisition rates during early childhood and developing countries display higher incidences of *H. pylori* infections during childhood (<10 years of age) than developed countries (Pounder and Ng, 1995).

The vacuolating cytotoxin VacA represents an important determinant of pathogenicity with highly complex interactions between *H. pylori* and the epithelial cells of the gastric mucosa which have now become a paradigm for host–pathogen association. Virulence factors are generally defined as molecules produced by pathogens that contribute to colonization, attachment to host cells, evasion of the host immune response and consumption of nutrients. Among the major *H. pylori* virulence factors are flagellin, urease, catalase, mucinase, lipase, neutrophil activating protein (NAP), outer membrane proteins (OMP), lipopolysaccharides, cytotoxin cytotoxin-associated gene pathogenicity island (cagPAI) and the vacuolating cytotoxin VacA (Essawi et al., 2013; Rieder et al., 2005). Some actions of the VacA toxin are apparently antagonized by opposing effects mediated through CagA (Argent et al., 2008). Highly pathogenic ("*type I*") strains of *H. pylori* display a constant association of VacA and CagA and it was speculated that CagA-VacA interaction would be required to promote long-term colonization of the stomach by ameliorating the detrimental effects of the bacterial virulence factor (Oldani et al., 2009). The cagPAI covers approximately 40 kb and comprises 30 genes including a type IV bacterial secretion system utilized by the CagA protein, which contributes to a pro-inflammatory response. However, recent studies have shown that CagA plays only a minor role, if any, during the release of pro-inflammatory cytokines such as interleukin-8 (Fischer et al., 2001; Kusters et al., 2006).

Regulatory effects exerted by CagA depend on tyrosine phosphorylation by host kinases. Accumulation of unphosphorylated CagA triggers an anti-apoptotic mechanism at the mitochondria without affecting the intracellular trafficking of the toxin, whereas phosphorylated CagA apparently prevents VacA to reach its intracellular target compartments (Oldani et al., 2009). Although it seems plausible that the proinflammatory and anti-apoptotic effects of CagA are contributors to the development of peptic ulcer and gastric cancer, the progress of gastroduodenal diseases is probably attributable to a multitude of bacterial virulence factors as well as various host factors (de Bernard and Josenhans, 2014). It is interesting to note that the cagPAI pathogenicity island is not present in all strains of *H. pylori* and humans infected with cagPAI-negative strains apparently remain symptomless (Ferreira et al., 2014).

While there is a number of excellent reviews published recently on host-pathogen interactions of *H. pylori* (Backert and Tegtmeyer, 2010; Bornschein and Malfertheiner, 2014; Cid et al., 2013; Yamaoka and Graham, 2014), this review will focus mainly on structure-mechanism relations of the VacA toxin from *H. pylori*. For a comprehensive overview, the reader is directed to previous reviews summarizing the history of key findings obtained for VacA (Boquet and Ricci, 2012; Isomoto et al., 2010; Palframan et al., 2012). We apologize to authors whose work we have failed to cite owing to space constraints.

2. VacA – structure

The VacA cytotoxin represents a multifunctional protein of about 860 amino acid residues which displays structural, mechanistic and functional features unrelated to other known bacterial toxins (Cover and Blanke, 2005). The *vacA* gene is present as sole chromosomal copy in all strains investigated to date and can vary in length from 3.86 to 3.94 kbp. The gene sequences encoding VacA show considerable variations and some strains were identified which ostensibly express a functionally inactive form of the toxin (Cover et al., 1994). The toxin can interact with a variety of mammalian cells and thereby exerts pleiotropic effects which include **i**) the formation of pores in the membrane of the target cell leading to leakage of ions and small molecule nutrients (Czajkowsky et al., 2005); **ii**) the generation of large intracellular