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Review

Regulation and functions of inflammasome-mediated cytokines in *Helicobacter pylori* infection

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Abstract

Persistent stomach infection with ***Helicobacter pylori*** causes chronic mucosal inflammation (gastritis), which is widely recognized as an essential precursor to gastric cancer. The **IL-1 interleukin family cytokines IL-1β and IL-18** have emerged as central mediators of mucosal inflammation. Here, we review the regulation and functions of these cytokines in *H. pylori*-induced inflammation and carcinogenesis.

Keywords: *Helicobacter pylori*; Inflammasome; IL-18; IL-1β; NOD-like receptors; Gastric cancer

1. Introduction

Gastric cancer is the fifth most common malignancy and the third leading cause of cancer-related mortality worldwide [1]. Persistent infection of the stomach with the Gram-negative bacterium *Helicobacter pylori* causes chronic gastritis, an essential precursor to carcinogenesis. For this reason, *H. pylori* infection was announced as a class I carcinogen by the World Health Organization in 1994 [2].

Upon infection, *H. pylori* interacts with host cells within the gastric mucosa, resulting in activation of multiple innate immune signaling pathways, leading to pro-inflammatory cytokine production and immune cell recruitment [3]. The Interleukin-1 (IL-1) family comprises 11 cytokines that act as important mediators of host mucosal immune responses against microbial pathogens [4]. Two of these members, IL-1β and IL-18, have been shown to recruit innate immune cells, direct adaptive immune responses and modulate tissue homeostasis, thereby determining the outcome of *H. pylori* infection [5–7]. Moreover, genetic polymorphisms in the *IL1B* and *IL18* genes strongly correlate with an increased risk of *H. pylori*-associated diseases [8–10]. El-Omar et al. [8] showed that polymorphisms in the promoter region of the *IL1B* gene are associated with higher secretion levels of IL-1β and gastric atrophy, leading to carcinogenesis. In addition, certain *IL18* genetic variants were reported to determine the susceptibility to *H. pylori* infection in the Korean population [10]. Despite the important roles of IL-1β and IL-18 in *H. pylori*-related disease, our understanding of the cellular and molecular processes involved are still poor. This review will discuss recent findings on the regulation and physiological functions of these cytokines in *H. pylori*-induced inflammation and gastric cancer.

2. *H. pylori*-induced gastric diseases

Although *H. pylori* colonize over 50% of the human population, the majority of infections remain asymptomatic [3]. Approximately 10% of *H. pylori* infections are associated with

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peptic ulcer disease, whilst 1–3% of those infected will progress to gastric cancer, with a survival rate of under 5 years [3]. A further 0.1% of those infected will develop mucosa-associated lymphoid tissue (MALT) lymphoma, a type of cancer that affects mucosal lymphoid tissues [3]. The susceptibility and severity of disease are determined by a complex interplay between bacterial, genetic and environmental factors [3].

According to the Correa model [11], gastric adenocarcinoma arises from a spectrum of pathological events, starting from chronic active gastritis, leading to atrophic gastritis (involving the loss of specialized cell types, such as parietal cells and chief cells), intestinal metaplasia, dysplasia and ultimately, resulting in carcinoma. *H. pylori*-induced gastritis, which is the first detectable change in this stepwise process of gastric carcinogenesis, is manifested by an increased infiltration of immune cells (*i. e.* neutrophils macrophages, lymphocytes and plasma cells) to the site of infection [11]. These cellular responses are accompanied by the production of pro-inflammatory cytokines, as well as of reactive oxygen and nitrogen species: ROS and RNS, respectively [11].

It is generally thought that gastric inflammatory responses are in large part mediated by *H. pylori* delivery of virulence factors into host cells via a type IV secretion system (T4SS) [12,13]. The *H. pylori* T4SS is a pilus structure encoded by the *cag* pathogenicity island (CagPAI) that consists of approximately 30 genes [12]. *In vivo* studies in the Mongolian gerbil model showed that infection with *H. pylori* strains possessing an intact T4SS led to more severe gastric immunopathology and development of precancerous lesions than that caused by T4SS-defective strains [14]. The *H. pylori* T4SS delivers bacterial effector molecules into host cells, specifically the Cytotoxicity-associated gene A (CagA) protein [15] and cell wall peptidoglycan (PGN) fragments, known as muropeptides [16].

Seventy percent of Western *H. pylori* strains and nearly 100% of East Asian strains harbor the *cagA* gene, encoding a protein that plays major pathogenic roles during *H. pylori* infection [12,17]. Upon entry into host cells, intracellular CagA undergoes tyrosine phosphorylation at EPIYA sites [17]. Phosphorylated CagA molecules interact with several host proteins, including: the oncogenic tyrosine phosphatase, SHP2; growth factor receptor-bound protein 2 (GRB2); and C-terminal Src kinase (CSK) [17]. Together, these protein interactions lead to activation of mitogen-activated protein kinase/extracellular-signal-regulated kinase (MEK/ERK) signaling pathways, which mediate pro-inflammatory cytokine production, mitogenic responses, as well as changes in epithelial cell junction integrity and polarity [18]. *cagA*-positive *H. pylori* strains were reported to enhance the risk of severe gastritis, atrophic gastritis, peptic ulcer disease and distal cancer [19]. Furthermore, studies using CagA transgenic mice showed that tyrosine phosphorylation of this bacterial oncoprotein were required for the development of gastric neoplasia in this model [20].

Despite the importance of CagA/CagPAI in inflammation, there is evidence that CagPAI-negative strains are also able to initiate gastric immunopathology, suggesting the involvement of T4SS-independent mechanisms [16,21]. In addition to the T4SS, Gram-negative bacteria constantly produce and shed outer membrane vesicles (OMVs) as a bacterial stress response mechanism that facilitates the delivery of bacterial factors into host cells [22,23]. *H. pylori* OMVs contain various cell wall-associated components, including lipopolysaccharide (LPS), PGN, as well as a large variety of outer membrane proteins [24,25]. These OMVs were shown to induce pro-inflammatory and autophagic responses in epithelial cells via a signaling pathway regulated by nucleotide oligomerization domain 1 (NOD1) [22], a sensor of Gram-negative type PGN [16].

H. pylori expresses outer membrane proteins, the blood group antigen-binding adhesion, BabA, and the sialic acid-binding adhesion, SabA, both of which play important roles in the pathogenesis of *H. pylori* infection by promoting adhesion of the bacteria to host epithelial cells [26]. SabA can also bind to human neutrophil receptors, thereby inducing phagocytosis activity and ROS production in these cells. Production of ROS has been reported to cause mucosal and DNA damages associated with *Helicobacter*-induced carcinogenesis [26].

Taken together, *H. pylori* interacts and delivers its virulence factors into epithelial cells in the gastric mucosa via the actions of its T4SS, OMVs or, possibly, by alternative mechanisms yet to be defined. These processes initiate inflammation, as well as modulate gastric epithelial cell survival responses, which are recognized as hallmarks of carcinogenesis. *H. pylori* also trigger the production of pro-inflammatory cytokines by host cells, such as IL-1β and IL-18. These potent cytokines have emerged as central mediators of *H. pylori*-associated gastric pathogenesis [5,27]. Nevertheless, much of the evidence in this area has arisen from gene polymorphism studies, with the roles of these inflammatory mediators in *H. pylori*-induced chronic inflammation and carcinogenesis yet to be fully defined by mechanistic studies. The following sections of this review will address two key questions: 1) how does *H. pylori* promote IL-1β and IL-18 production in host cells; and 2) to what extent do these cytokines contribute to disease pathogenesis?

3. Regulation of IL-1β and IL-18 production in response to *H. pylori* infection

3.1. Transcriptional regulation of IL1B *and* IL18 *genes and production of precursor forms*

Under normal conditions, *IL1B* gene expression is undetectable in hematopoietic cells, whereas *IL18* is constitutively expressed in cells of both hematopoietic and non-hematopoietic origins [28,29]. A clinical study by Basso et al. [6] showed that *IL1B* gene expression in the gastric mucosa of *H. pylori*-positive patients was higher than that in uninfected subjects. Two independent studies reported that *IL18* gene transcription was increased in the antral mucosa but not in the corpus of *H. pylori*-positive patients, as compared with uninfected individuals