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REVIEW

Oxidative Stress Resulting From *Helicobacter pylori* Infection Contributes to Gastric Carcinogenesis

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SUMMARY

Helicobacter pylori is known to induce a chronic immune response including persistent oxidative stress in the stomach. This response results in DNA damage that eventually can lead to gastric cancer.

Helicobacter pylori* is a gram-negative, microaerophilic bacterium that infects the stomach and can lead to, among other disorders, the development of gastric cancer. The inability of the host to clear the infection results in a chronic inflammatory state with continued oxidative stress within the tissue. Reactive oxygen species and reactive nitrogen species produced by the immune and epithelial cells damage the host cells and can result in DNA damage. *H pylori* has evolved to evoke this damaging response while blunting the host's efforts to kill the bacteria. This long-lasting state with inflammation and oxidative stress can result in gastric carcinogenesis. Continued efforts to better understand the bacterium and the host response will serve to prevent or provide improved early diagnosis and treatment of gastric cancer. *(Cell Mol Gastroenterol Hepatol 2017;3:316–322; http://dx.doi.org/10.1016/j.jcmgh.2017.02.002)

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Gastric cancer, which is the third leading cause of cancer deaths worldwide,[1] largely is caused by *Helicobacter pylori*, a gram-negative, microaerophilic bacterium that infects half of the world's population. In addition to gastric carcinogenesis, *H pylori* also contributes to the development of peptic ulcers, chronic gastritis, and mucosa-associated lymphoid tissue lymphoma.[2] Although the human immune system is capable of creating a robust innate and adaptive immune response to the infection, it usually fails to clear *H pylori* completely, thereby resulting in a persistent infection. This prolonged infection results in chronic inflammation, oxidative stress, and DNA damage.[3–5]

There are several *H pylori* virulence factors that contribute to its ability to evade the immune system and disrupt the host's cells. One of the most studied factors is cytotoxin-associated gene A (CagA), which is injected into the host cell where it can affect the cell's shape, motility, and proliferation.[6–10] Vacuolating cytotoxin A (VacA) is another well-studied virulence factor that is a toxin secreted by *H pylori* and able to induce inflammatory cytokines after entering the host cell.[11] In addition, VacA has several mechanisms to help the bacteria evade immune response such as the disruption of phagosome maturation and the creation of fused phagosomes called *megasomes*, which prevent the destruction of the bacteria contained within.[12,13] Although not as well understood, blood group antigen binding adhesion (BabA) is another virulence factor that is known to induce inflammatory gene transcription and skew the immune response from T helper 2 to T helper 1 with a weakened interleukin (IL)33 response. These are a few of the virulence factors that *H pylori* uses to maintain a prolonged proinflammatory response while evading self-destruction.

The Correa et al[14] model hypothesizes that normal gastric mucosa can develop gastritis, which progresses to dysplasia, and, finally, the development of cancer. There are many factors that contribute to the initiation of gastritis and the progression to cancer such as host gene polymorphisms, dietary factors, and *H pylori* strain infection among others. This review summarizes the host's response to generate oxidative stress after *H pylori* infection and the resulting DNA damage that may contribute to the development of gastric cancer.

Oxidative Stress Generation

Host Response

The presence of *H pylori* results in reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by the host in the gastric mucosa. Although there are many cell types that can contribute to the production of ROS/RNS, including the epithelial cells, it is primarily the neutrophils that contribute the greatest amount.[15] Nicotinamide adenine dinucleotide phosphate (NADPH oxidase [Nox]) on

Abbreviations used in this paper: **APE1, apurinic/apyrimidinic endonuclease 1; BabA, blood group antigen binding adhesion; CagA, cytotoxin-associated gene A; iNOS, inducible nitric oxide synthase; IL, interleukin; NADPH, nicotinamide adenine dinucleotide phosphate; NapA, neutrophil activating factor A; Nox, nicotinamide adenine dinucleotide phosphate oxidase; OH, hydroxyl radical; O_2^-, superoxide; RNS, reactive nitrogen species; ROS, reactive oxygen species; TGF-β, transforming growth factor β; VacA, vacuolating cytotoxin A.**

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the cell membrane catalyzes the ROS production to kill bacteria.[12,16] During this process, Nox is activated to receive an electron from NADPH, which is donated to oxygen to create superoxide (O_2^-). Then O_2^- is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase catalysis. H_2O_2 then can be converted to the more toxic hypochlorous acid. In addition, H_2O_2 reacts with O_2^- to form hydroxyl radicals (OH). Combined, these ROS usually kill any bacteria within the neutrophil. However, the separation between neutrophils in the tissue and bacteria in the lumen make it difficult to kill all of the *H pylori* present. Consequently, the ongoing attempt to do so is thought to result in the chronic–active inflammation and damage to the gastric mucosa during the course of the prolonged infection.

The presence of *H pylori* results in the influx of phagocytic cells in an effort to clear the infection. Macrophages and neutrophils phagocytize the bacteria in an attempt to kill the organism with ROS/RNS. In addition, the host neutrophils and epithelial cells also express a critical enzyme, the inducible nitric oxide synthase (iNOS), which produces NO.[17] NO reacts with metals and O_2^- to produce peroxynitrite, a strong oxidant. *H pylori* infection results in the formation of ROS and RNS by increasing the immune cell expression of Nox and iNOS.[8] Patients infected with *H pylori* have increased levels of ROS along with increased levels of NO-derived metabolites, indicating the activation of iNOS.[5,18–20] In vivo studies with iNOS-deficient mice show decreased gastric cancer incidence after infection with *H pylori* compared with wild-type mice.[21]

In addition to the phagocytic cells attempting to clear *H pylori*, there is recent evidence that gastric epithelial cells also express Nox, however, the details remain unclear.[22,23] The NADPH subunit Nox1 is expressed in gastric tissues and likely contributes to the ROS production during *H pylori* infection. ROS is produced at a much lower level in the epithelial cells compared with the phagocytic cells of the immune response and contributes to redox-sensitive signaling and may not directly kill *H pylori*.[24] In addition, dual oxidases located on the gastric epithelial cells are known to produce H_2O_2 in response to infection, also contributing to the ROS levels.[25] The combination of the phagocytic and epithelial cell ROS production creates an oxidative stress environment that contributes to the gastric carcinogenesis.

H pylori *Virulence Factors*

H pylori strains contain multiple virulence factors that may contribute to the host's production of oxidative stress. The presence of *cagA* in a strain results in an increased risk of gastric carcinogenesis compared with individuals infected with CagA-negative strains.[26] Increased hydrogen peroxide levels and oxidative DNA damage are seen with CagA-positive strains.[27,28] In addition, there is an increase in tumor necrosis factor-α and IL8, which are inflammatory and oxidative stress markers.[29] Although the precise mechanism CagA uses for carcinogenesis has not yet been defined, it is clear that these actions can contribute to the development of gastric cancer.[30]

Another virulence factor that may increase the chance for the development of gastric cancer is VacA. VacA is capable of inducing an influx of Ca^{2+} and the generation of ROS that results in the activation of nuclear factor-κB, thereby increasing proinflammatory immune response.[31]

H pylori has the ability to both recruit neutrophils and protect itself from oxidative bursts with the aid of virulence factors urease, neutrophil activating factor A (NapA), and the enzyme catalase. Urease and NapA recruit neutrophils to the site of infection and induce the oxidative burst from the neutrophils once they arrive.[32] Contributing to the survival of *H pylori* while creating a chronic inflammatory state, the neutrophils are less likely to undergo apoptosis, and *H pylori* located in the lumen is protected from the oxy-radicals released by NapA and catalase.[23]

BabA is an adhesion protein that is well characterized. BabA-positive strains induce a strong IL8 and weak IL33 cytokine response.[33,34] This immune response drives a proinflammatory response without eventually killing the bacteria. Also important is the correlation between BabA positivity and DNA damage.[35] Another adhesion is sialic acid–binding adhesion, which induces oxidative bursts in granulocytes.[36]

γ-glutamyl transferase is a virulence factor that contributes to production of IL8 and activation of nuclear factor-κB while stimulating the production of H_2O_2 from the gastric epithelium.[37] It also is known that treatment of primary gastric cells and the AGS cancer cell line with γ-glutamyl transferase results in DNA damage from oxidative stress.[37] The multiple ways of inducing the host immune response combined with the damage resulting from the oxidative stress response can initiate the steps toward carcinogenesis.

Moreover, *H pylori* also is able to protect itself from the host immune response by inducing apoptosis of macrophages. In vitro macrophages stimulated by the lipopolysaccharide of *H pylori* produce polyamine, which suppress their iNOS and induces apoptosis.[38] Within the gastric epithelial cells, the polyamine is used to create H_2O_2. *H pylori* also is thought to produce $O_2^{\cdot-}$, which is moderately cytotoxic and likely originates from the mitochondrial respiratory chain of electrons.[39] Although $O_2^{\cdot-}$ is harmful, the reaction of H_2O_2 and metals is much more potent. *H pylori* is capable of inducing a host response and then manipulating it to create a tolerant, prosurvival environment for the bacteria, which produces a chronic inflammatory environment that is harmful to the host.

Host Damage and Gastric Cancer

H pylori was the first bacterial pathogen to be recognized as a carcinogen.[40] The long lag time between the initial infection and carcinogenesis combined with the late-stage diagnosis results in a low 5-year survival rate.[1] As previously mentioned, *H pylori* is capable of inducing a prolonged inflammatory state that contributes to carcinogenesis.[3] CagA-positive strains are capable of inducing an oxidative stress response in vitro and these strains are associated