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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-$\beta$, transforming growth factor $\beta$; 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-$\alpha$ 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-$\kappa$B, 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]

$\gamma$-glutamyl transferase is a virulence factor that contributes to production of IL8 and activation of nuclear factor-$\kappa$B 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 $\gamma$-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

more frequently with gastric cancer.[41] In vitro studies also have shown an increase in oxidative damage and apoptosis.[42,43] However, studies have shown some cells with DNA damage are less likely to undergo apoptosis, thus increasing the potential for cancer to arise from these cells.[43]

The DNA damage from the *H pylori* infection can result from oxidative stress. In vitro studies have shown cells with deficient DNA repair mechanisms that are infected with *H pylori* result in more oxidative stress and DNA damage.[44,45] In vivo work with mice deficient in part of the base excision repair mechanism also showed severe gastric lesions after *H pylori* infection.[46] The ability of *H pylori* to induce DNA strand breaks likely contributes to genomic instability and may facilitate the carcinogenesis.[47] NO can prevent the removal of DNA mutations by 8-oxoguanine glycosylase.[44] Studies have shown an increase of phosphohistone H2AX, a marker of repair for double-strand DNA breaks, after *H pylori* infection.[44] We propose (Figure 1) that ROS causes DNA damage subsequent to 8-hydroxy-2′deoxyguanosine accumulation. The loss of a base after damage would result in an abasic site that could lead to a single-strand break in the DNA. The lack of repair or continued damage may induce double-strand breaks in the DNA, although DNA strands can be induced by other means. If a cell fails to repair too many breaks, it may result in a neoplastic precursor.

Inhibition of the base excision repair and mismatch repair systems during infection allows for cellular transformation to occur. AGS cells express decreased messenger RNA expression of apurinic/apyrimidinic endonuclease 1 (APE1), which makes the cells less able to repair DNA mutations and may result in increased genomic instability.[48] APE1 is a multifunctional molecule that repairs damage DNA via its carboxy-terminus while its amino-terminus regulates transcription.[49] Initial cloning experiments discovered APE1 as the mammalian ortholog of *Escherichia coli* Xth and a DNA repair enzyme.[50,51] These early studies identified APE1 as a molecule to evaluate genomic instability. Shortly after identification as a DNA repair enzyme, APE1 also was determined to be a redox protein.[52] A recent study showed the ability of APE1 to regulate epithelial ROS via Rac1 and Nox1 after *H pylori* infection.[23] APE1 was shown to decrease the expression of Nox1 and interact with

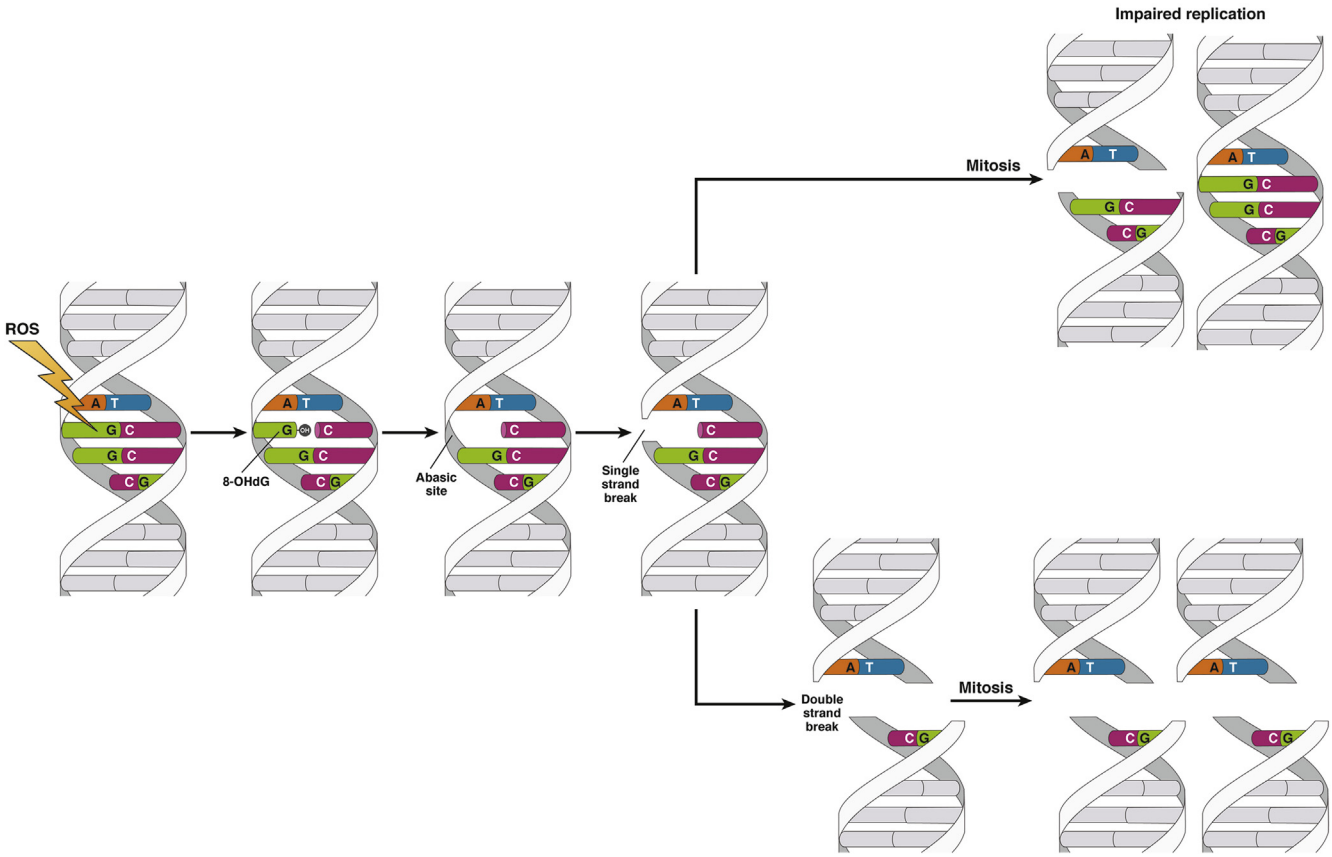


**Figure 1. Oxidative stress may result in DNA damage.** ROS produced during *H pylori* infection can cause DNA damage. The oxidation of the DNA often occurs on deoxyguanosine, resulting in 8-hydroxy-2′deoxyguanosine (8-OHdG). This base then can be lost as a result of the damage, causing an abasic site. This abasic site produces a single-strand break in the DNA if not repaired. Double-strand breaks can develop from single-strand breaks or from other sources such as chemicals or collapsed replication forks. Both single- and double-strand breaks can increase chromosomal instability and lead to errors during replication that may increase the chances of a tumor formation.

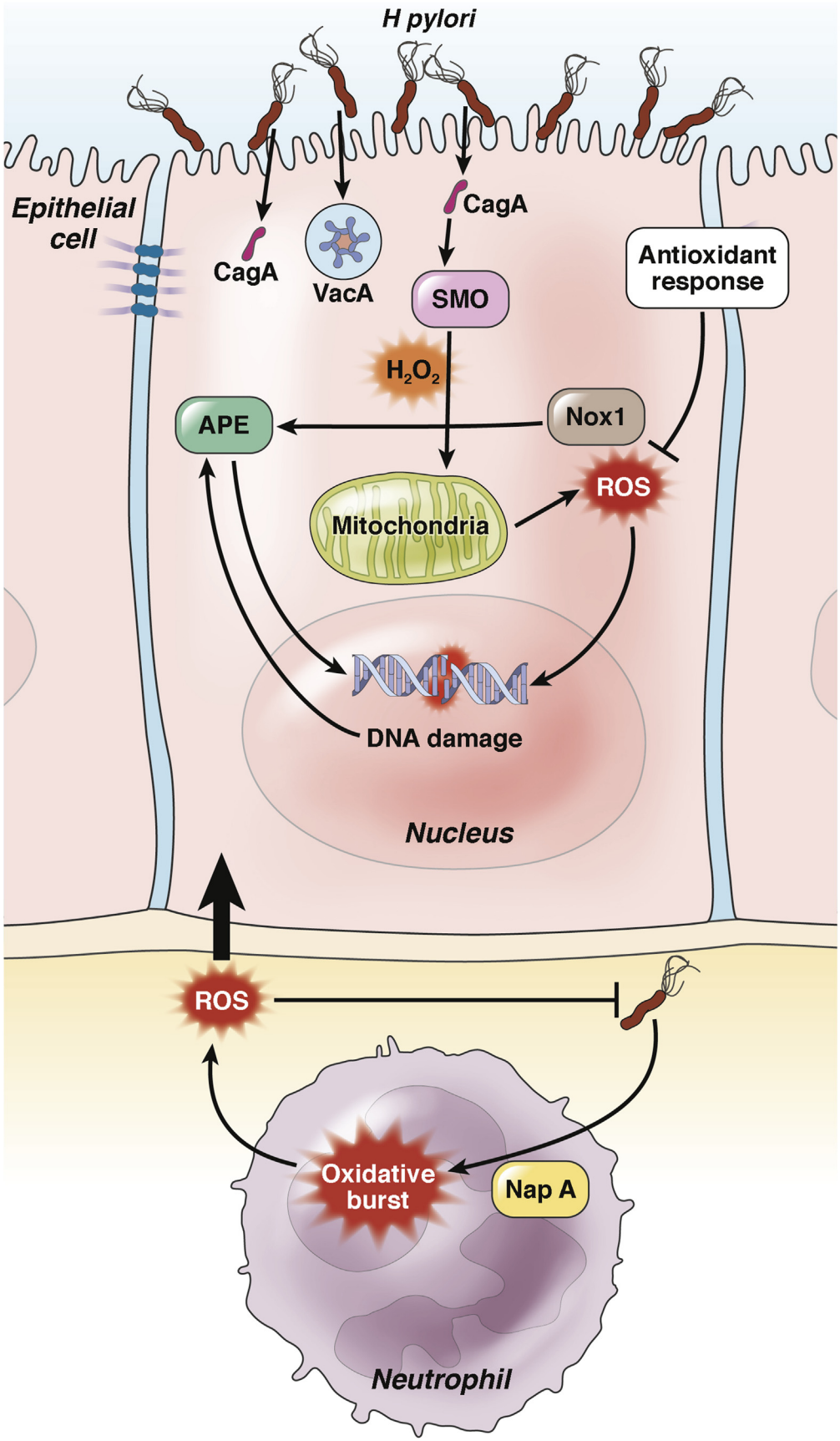


**Figure 2. *H pylori* infection leads to oxidative stress.** *H pylori* infection results in ROS production by the immune and epithelial cells in an attempt to kill the bacteria. Virulence factors from *H pylori* such as CagA are injected into the epithelial cell while VacA is secreted from the bacteria and trapped in an intracellular vesicle. The virulence factors trigger multiple cellular responses including the production of intrinsic ROS. The ROS result in DNA damage in the epithelial cells, activating APE1, which then translocates to the nucleus to regulate gene transcription and to attempt to repair the DNA. Spermine oxidase (SMO) also is activated and results in DNA damage, as well as acting on the mitochondria membrane. Immune cells recruited to the area by virulence factors including NapA release extrinsic ROS in an attempt to clear the infection, resulting in more damage to the area.

Rac1 to prevent the formation of the NADPH oxidase complex, thus limiting ROS production. If this multifunctional molecule is impaired, both the feedback loop to control ROS and DNA repair are unable to contain the negative effects of the *H pylori* infection that may contribute to gastric cancer. Further studies, especially in vivo, are needed to evaluate the protecting effects of APE1 from DNA damage because the ability of the cells to maintain genomic integrity is critical to preventing carcinogenesis.

Another source of damage within the epithelial cells during *H pylori* infection is spermine oxidase, which is an enzyme in the pathway to produce spermidine.[53] During this process, $H_2O_2$ also is produced, which results in the depolarization of the mitochondrial membrane, thereby activating caspase-mediated apoptosis.[43,54] Studies have shown an increase in spermine oxidase is correlated with increased DNA damage.[42] In addition, the increased apoptosis can result in an increase in proliferation in the localized area that also can contribute to gastric carcinogenesis. An in-depth review can be found by Chaturvedi et al.[55]

Transforming growth factor-$\beta$1 (TGF-$\beta$1) is a multifunctional cytokine that is known to regulate proliferation and cell differentiation, among other cellular processes, and is involved in the regulation of the immune response. Studies have shown that the severity of gastritis can be correlated with increased expression of TGF-$\beta$1 and that gastric mucosal biopsy specimens infected with *H pylori* have higher *TGF-$\beta$1* gene expression compared with uninfected samples.[56,57] Although overexpression of TGF-$\beta$ can be correlated with an increased immune response, underexpression also is harmful in *H pylori* infection. When TGF-$\beta$ is suppressed, it is unable to prevent the $H_2O_2$ release from macrophages, which results in an uncontrolled respiratory burst.[58] In addition, TGF-$\beta$ stimulates the induction of $Foxp3^+$ Treg cells that inhibit lymphocyte activation and favors persistent *H pylori* infection and the harmful results.[59,60] A recent study showed that TGF-$\beta$1 induced by *H pylori* infection results in activation of the epithelial–mesenchymal transition pathway and the development of gastric cancer stem cells.[61] A better understanding of the interactions between ROS and TGF-$\beta$ will help clarify its contributions to carcinogenesis.

Animal models can be useful to evaluate infection in the complexity of a living organism. Previous studies have used the Big Blue mouse model to assess the DNA damage from infection with *Helicobacter* because this models allows for the removal of a lambda vector to measure the mutations. These studies demonstrated increased genetic point mutations indicating oxidative stress occurring as early as 6 months post infection. Infection also was correlated with hyperplasia, neutrophil infiltration, and mutated p53 status.[62,63] An additional study also showed the increased point mutations from oxidative stress along with gastric lesions and a proinflammatory immune response after infection.[64] These studies suggested that long-term infection with *Helicobacter* can result in a proinflammatory immune response along with oxidative stress, which may contribute to gastric neoplasia.

## Conclusions

*H pylori* infection results in a chronic inflammatory response by the host. The chronic oxidative stress produced by cells in an attempt to eradicate the bacteria results in a harmful microenvironment for the host rather than an effective means to eliminate the pathogen. Continued host efforts to clear the bacteria merely result in an increased chance of carcinogenesis. The oxidative stress produced results not only in DNA damage, but also prevents DNA repair mechanisms from functioning properly (Figure 2). This is in addition to the increased apoptosis and subsequent cell proliferation also resulting from oxidative stress along with the development of cancer stem cells. Continued study of this process and the resulting steps to cancer are required to fully understand the mechanisms at work and, perhaps, develop an effective gastric cancer prevention or early treatment.

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The authors disclose no conflicts.

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