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The Immune Battle Against *Helicobacter pylori* Infection: NO Offense

Alain P. Gobert[1,4] and **Keith T. Wilson**[1,2,3,4,5,*]

[1]Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232, USA

[2]Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

[3]Department of Cancer Biology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

[4]Center for Mucosal Inflammation and Cancer, Vanderbilt University Medical Center, Nashville, TN 37232, USA

[5]Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN 37212, USA

Abstract

Helicobacter pylori is a successful pathogen of the human stomach. Despite a vigorous immune response by the gastric mucosa, the bacterium survives in its ecological niche, thus favoring diseases ranging from chronic gastritis to adenocarcinoma. The current literature demonstrates that high-output of nitric oxide (NO) production by the inducible enzyme NO synthase-2 (NOS2) plays major functions in host defense against bacterial infections. However, pathogens have elaborated several strategies to counteract the deleterious effects of NO; this includes inhibition of host NO synthesis and transcriptional regulation in response to reactive nitrogen species allowing the bacteria to face the nitrosative stress. Moreover, NO is also a critical mediator of inflammation and carcinogenesis. In this context, we review the recent findings on the expression of NOS2 in *H. pylori*-infected gastric tissues and epithelial cells, the role of NO in *H. pylori*-related diseases and *H. pylori* gene expression, and the mechanisms whereby *H. pylori* regulates NO synthesis by host cells.

Keywords

Nitric oxide; *Helicobacter pylori*; Polyamines; Gastric cancer

*Correspondence: keith.wilson@vanderbilt.edu (K.T. Wilson)..

Infection with *Helicobacter pylori*

More than half of the world's human population carries *Helicobacter pylori*, a Gram-negative microaerophilic bacterium that specifically colonizes the stomach. *H. pylori* typically coexists with its host, who may even benefit from this colonization notably in childhood [1]. However, long-term infection may cause diseases including chronic gastritis, peptic ulcers, and gastric cancer. The persistence of the bacterium within the hostile, acidic ecologic niche of the stomach is principally due to the activity of the *H. pylori* urease that neutralizes gastric acidity by generating ammonium from urea. Moreover, the common trait of *H. pylori* strains that have increased risk of inducing gastric adenocarcinoma is the expression of specific virulence genes including the protein cytotoxin-associated gene A (CagA) [2] and the vacuolating cytotoxin A (VacA) [3] (Box1). Environmental components [4] and host factors [5] have been also shown to be involved in the outcome of *H. pylori* infection. But, whether host and pathogen genomic variations in a spatio-temporal axis are associated with the development of gastric diseases, overall the clinical outcome of *H. pylori* infection-induced gastric carcinogenesis is determined by the progression along the histologic cascade from non-atrophic gastritis to adenocarcinoma [6].

The pathogenesis of *H. pylori*-induced diseases is mediated by the infiltration, activation, and persistence of cells of the innate and chronic immune response. Furthermore, the non-specific defense program of gastric epithelial cells and macrophages against *H. pylori* leads to the production of nitric oxide (NO), a ubiquitous free radical synthesized by the enzyme NO synthase (NOS) through the oxidation of L-arginine. The activity of the inducible NOS (NOS2) enzyme is independent of Ca^{2+} and produces a high-output of NO for a long period of time; this enzyme is regulated at the transcriptional level in numerous cells types after activation by cytokines or pathogen-associated molecular patterns. The present review synthesizes knowledge on the interactions that occur between *H. pylori* and NO, with respect to expression of NOS2 in infected tissues and cells, biological significance of NO synthesis for the host and the bacterium, and occurrence of NO synthesis regulation.

Infected Gastric Tissues: Where Is NOS2?

Numerous investigations have evidenced an increase of *Nos2* mRNA expression in the gastric tissues of *H. pylori*-infected patients [7-9], independently of the *cagA* status of the bacteria [10]. The *NOS2* gene is more highly expressed in the antrum part of the stomach, where *H. pylori* colonization has been shown to be greater, than in the body [7, 11], suggesting that *NOS2* expression is directly related to the presence of the bacteria. In support of this contention, by immunohistochemistry, NOS2 is less expressed in *H. pylori*-negative gastritis than in infected patients [12], and in the gastric mucosa after the eradication of *H. pylori* by antibiotic-based triple therapy when compared to before the treatment [11, 13-15].

While there is a consensus about *NOS2* expression in gastric biopsies during *H. pylori* infection, the cellular distribution of the protein has been questioned by different studies (Figure 1, Key Figure). Fu *et al.* immunolocalized NOS2 protein in epithelium, endothelium, and lamina propria inflammatory cells of the stomach of patients in the U.S. showing *H.*

pylori gastritis [7]. Similarly, one third of Japanese patients with *H. pylori*-positive gastric ulcer exhibited NOS2 staining in both epithelium and infiltrating inflammatory cells at the margin of active gastric ulcers [16]. But in the same study, the authors showed NOS2 reactivity mainly in the lamina propria of 46% of the infected patients [16]. Similarly, other investigations have reported NOS2 only in inflammatory cells of the mucosa of *H. pylori*-infected patients, including polymorphonuclear leukocytes and mononuclear cells [12, 13, 15]. The localization of the gastric biopsies or the state of the disease may explain these differences, but also the varying antibodies used for immunohistochemistry could also be an important consideration. Interestingly, nitrotyrosine, which indicates the nitration of tyrosine by peroxynitrite ($ONOO^-$) generated from NO and O_2^-, is immunodetected in epithelial cells and macrophages, even in studies in which NOS2 was found only in inflammatory cells [12, 13, 16]. This suggests that NO is effectively synthesized from NOS2 and that reactive nitrogen intermediates (RNI) target not only the NO-producing cells, but also the cells surrounding NOS2-expressing macrophages, thus providing a rationale for a potential biological effect in the infected tissues.

Animal models have provided a strong tool to study *H. pylori*-associated inflammation and carcinogenesis. Thus, increased *Nos2* mRNA has been observed in isolated gastric macrophages of *H. pylori* SS1-infected C57BL/6 mice after 4 months [17, 18] and in macrophages and monocytes of C57BL/6 male Big Blue transgenic mice infected for 6 months with the same strain [19]. However, in the latter study the authors show that *Nos2* mRNA is not detected in mice infected for 12 months and propose that a regulation of *H. pylori* factors involved in *Nos2* induction may occur in a long-term infection [19]. The expression of *Nos2* mRNA is also induced in Mongolian gerbils infected with *H. pylori* for 2 weeks [20] or 3 months [21]. Finally, like in humans, a reduction of *Nos2* mRNA is observed with the eradication of *H. pylori* in infected INS-GAS mice [22], which are transgenic animals overexpressing gastrin that develop accelerated gastric cancer after *Helicobacter* infection. Furthermore, NOS2 colocalizes with $F4/80^+$ macrophages, but not with $CD11c^+$ dendritic cells, in *H. pylori*-infected mice [18, 23].

The Mechanism of NOS2 Induction in Host Cells by *H. pylori*

Although live *H. pylori* or its lipopolysaccharide (LPS) fails to induce NO production by human macrophages [24, 25], several groups have demonstrated *NOS2* mRNA induction in human gastric epithelial cell lines after *H. pylori* infection [26-28]. However, in several of these studies, either: (i) the level of *NOS2* induction is very low [26, 29]; (ii) the production of RNI does not reflect NOS2-dependent high-output NO generation – e.g., ~ 6 μM NO_2^- produced by AGS cells infected for 24 h with *H. pylori* [27]; or (iii) NO production is not shown in these papers [26, 28]. These investigations could also have increased our knowledge about the molecular mechanism by which *H. pylori* might induce NOS2 in epithelial cells, but there is a strong discrepancy observed between the reports. While authors show that p38 and ERK1/2 [26] and the transcription factor NF-κB [30] are involved in *H. pylori*-induced-*NOS2* in Hs746T and MKN45 cells, Cho *et al.* have demonstrated that *H. pylori* strain HP99 induces *NOS2* mRNA and protein expression in AGS cells through a mechanism implicating a Ras-AP-1 signaling pathway [27]. Interestingly, epigenetic modifications, including histone demethylation and acetylation and release of methyl-CpG-

binding protein 2 at the promoter region favors *NOS2* transcription in MKN28 cells infected with wild-type (WT) *H. pylori* or a Cag pathogenicity island^{-}/VacA^{-} mutant strain [28]. To our knowledge, only one report has shown that a combination of *H. pylori* LPS and interferon-γ (IFN-γ) stimulates *Nos2* expression at the transcriptional and protein level in the immortalized murine gastric epithelial cell line GSM06 [31].

However, the effect of *H. pylori* on murine macrophages has been extensively studied. The novelty of the *H. pylori*–innate immune system response crosstalk resides mainly in the fact that *H. pylori* LPS is not effective in inducing macrophage NO generation [25, 32, 33], in contrast to the endotoxin of other Gram-negative bacteria [32, 34]. When live *H. pylori* is in direct contact with murine macrophages, a strong NOS2 induction and a high level of NO production are observed in the cells [32, 35]. In this context, different mutant strains of *H. pylori* lacking CagA, the type IV secretion system, VacA, catalase, the outer membrane proteins AlpAB, or urease induce the same level of NO production than the parental strain [25, 36, 37], suggesting that more than one bacterial product can stimulate NOS2 expression after phagocytosis; however, when *H. pylori* and macrophages are physically separated by a filter, a urease mutant fails to activate NOS2 [36], demonstrating that urease released by *H. pylori* is a potent inducer of NO production (Figure 1).

Role of High-output NO Production on *H. pylori*-related Pathological Processes

Its ability to freely diffuse across the producing cells and its strong reactivity as a radical molecule confers to NO a critical role in homeostasis and pathophysiological processes. Although it has been reported that the level of gastritis and the Th1 and innate responses are similar in WT and *Nos2*-deficient mice during *H. pylori* infection [38, 39], several lines of evidence point toward an effect of NO on *H. pylori*-mediated carcinogenesis (Figure 1). First, histological analyses have revealed increased NOS2 protein level, nitrotyrosine immunostaining, and oxidative DNA damage in patients with gastric cancer compared to individuals with *H. pylori* gastritis [12, 40]; moreover, cancer cells express more *NOS2* than noncancerous foveolar epithelial cells or mucosal neck cells, mainly in older patients [40]. Similarly, it has been observed that *Nos2* expression in macrophages parallels the mutation frequency in gastric epithelial cells in Big Blue transgenic mice [19]. Second, in WT mice treated with the carcinogen N-methyl-N-nitrosourea and then infected with *H. pylori* for 50 weeks, NOS2 is expressed in adenocarcinoma and inflammatory cells [41]; moreover gastric adenocarcinoma incidence is significantly reduced by more than 50% in *Nos2*$^{-/-}$ mice compared to WT animals [38, 41]. Accordingly, DNA fragmentation is observed in WT mice, but not in *Nos2*$^{-/-}$ mice infected with *H. pylori* SS1, despite the same level of acute inflammation [38]. Third, a long (CCTTT) repeat (> 13) in the 5′ promoter region of the NOS2 gene, which increased mRNA expression, has been associated with an increased risk of gastric cancer in *H. pylori*-infected Japanese patients [42, 43], providing a rationale for the involvement of NO in *H. pylori*-associated carcinogenesis.

NO and certain RNI are considered to be potent mutagens. When levels of RNI surpass the antioxidant capacity, they can cause nitrosative and nitrative damage to nucleic acids,

proteins, and lipids. Hence, *H. pylori* gastritis is associated with enhancement of gastric gland epithelial cell content of 8-nitroguanine [44, 45], one of the major products formed by the reaction of guanine with $ONOO^-$ [46], which facilitates G:C→A:T transversions in DNA and is therefore potentially mutagenic (Figure 1). Interestingly, this effect on DNA results in one of the most common mutations in the p53 tumor suppressor gene in early phases of human gastric carcinogenesis [47]. However, further studies are required to determine the causal relationship between NO-dependent DNA damage and gastric cancer during *H. pylori* infection.

Apoptosis is increased in human gastric epithelial MKN45 [48] and AGS [29] cells pretreated with IFN-γ and then infected with *H. pylori*; a NOS inhibitor blocks apoptosis, which led to the conclusion that *H. pylori*-induced apoptosis is NO-dependent [29, 48]. However, the level of *NOS2* mRNA is not convincing and NO generation is not shown in these papers. Nonetheless, *in vivo* experiments showing that infected *Nos2*$^{-/-}$ mice have less gastric mucosal apoptosis than WT [38] demonstrate that NO could play a role in *H. pylori*-induced cell death in mice. On the contrary, it has been shown that an NO donor, S-nitroso-N-acetyl-D,L-penicillamine, inhibits *H. pylori*-induced caspase-3 activity and apoptosis in MKN-45 epithelial cells [30], demonstrating that exogenous NO decreases apoptosis. Macrophage apoptosis in response to *H. pylori* is mediated by the generation of hydrogen peroxide through the arginase-2 (ARG2)-ornithine decarboxylase (ODC)-spermine oxidase metabolic pathway [49, 50] and is NO-independent [49]. Thus, the role of NO in *H. pylori*-induced apoptosis appears to cell and model specific.

H. pylori Response to NO Exposure

Pathogenic bacteria have elaborated strategies to resist the direct effect of oxidative and nitrosative stress. Intestinal pathogenic *Escherichia coli* or *Salmonella enterica* express NO sensors that directly regulate the transcription of genes implicated in virulence [51, 52] and resistance to large amount of RNI [53, 54]. Therefore, intra- or extra-cellular pathogenic bacteria exhibit a strong resistance to NO challenge [51, 55]. However, *H. pylori* is more sensitive to NO and RNI, including $ONOO^-$, than other enteric pathogens [56, 57]; moreover, it has been shown *in vitro* that activated macrophages inhibit the growth of *H. pylori* through an NO-dependent pathway [35, 58, 59]. This anti-proliferative effect may be mediated by the irreversible inhibition of *H. pylori* respiration by NO and $ONOO^-$ [60]. Of note, the *H. pylori* urease subunit, UreA, can be S-nitrosylated by RNI, leading to the inhibition of enzymatic activity [61]. Interestingly, Kuwara *et al.* have reported that CO_2 formed by urease activity reacts with ONOO– to form nitrosoperoxycarbonate ($ONOOCO_2^-$), which is converted to the non-toxic metabolite, NO_3^- (Figure 2), thus suppressing the deleterious effect of RNI on *H. pylori* survival [57].

The susceptibility of *H. pylori* to NO is sustained by the lack of the major NO sensors, e.g. NsrR or NorR, harbored by other pathogenic bacteria [53, 62] and lack of detoxification proteins such as Hmp [63]. However, sublethal doses of RNI may impact the transcriptome of *H. pylori*, in ways that may allow *H. pylori* to escape some of the effects of RNI. The two-component system CrdS–CrdR is activated when *H. pylori* is exposed to NO donors and the Δ*crdS/R* strains are more susceptible to NO than the WT [56], suggesting that CrdS is a

sensor for nitrosative stress and that CrdR responds to this challenge. The NO-induced CrdSR-dependent genes include, notably, those encoding proteins involved in iron transport [56], but no genes specifically involved in direct NO detoxification; thus maintaining iron homeostasis may be a way for *H. pylori* to defend against nitrosative stress (Figure 2). However, the mechanism by which CrdS responds to NO remains unknown.

Justino *et al.* have identified two *H. pylori* proteins that are potent reducer of NO: First, the protein Hp0013, renamed NADPH-dependent NO reductase of *H. pylori* (NorH), metabolizes NO through an NADPH-dependent enzymatic activity and allows the resistance of *H. pylori* to *S*-nitrosoglutathione (GSNO) and the NO donor dipropylenetriamine-NONOate [64]. Second, the gene *frxA* (HP0642) encodes a GSNO reductase that is transcriptionally induced by various NO donors, but reduces only GSNO into glutathione (GSH), supporting the concept that this enzyme plays a major function in the survival of phagocytized *H. pylori* [65]. Consequently, NorH and FrxA attenuate the NO-dependent *H. pylori* killing by macrophages *ex vivo* and also favor the survival of *H. pylori in vivo* [64; 65] (Figure 2), further indicating that NO effectively attenuates *H. pylori* growth in the stomach.

Two thioredoxins (Trx) exist in *H. pylori*: TrxA (or Trx1) and TrxC (also called Trx2). While TrxC is more involved in preventing oxygen-dependent damage [66], TrxA dampens the nitrosative attack on *H. pylori* [67, 68]. The deletion of *trxA* yields a diminution of stomach colonization by *H. pylori* [66]. Interestingly, a proteomic analysis revealed that 38 proteins are regulated in *H. pylori* exposed to the NO donor sodium nitroprusside [69]. Among those induced by NO, more than half correspond to antioxidant and stress proteins [69], as expected. Notably, the Trx reductase TrxR, also identified as TrxB, is upregulated by NO and that the deletion of the gene encoding this protein enhances the susceptibility of *H. pylori* to RNI [69]. Moreover, TrxA is also a reductant for the *H. pylori* peroxiredoxin AhpC [67], which converts ONOO– into NO_2^- [70], further demonstrating the essential role of the TrxA/TrxR pathway in RNI resistance (Figure 2). This is reinforced by the fact that *H. pylori* lacks the glutathione system, which is essential for cellular thiol:disulfide balance and survival under oxidative stress in many Gram-negative bacteria [71].

These findings underline that the resistance of *H. pylori* to RNI is mediated by unique mechanisms, including in comparison with other pathogenic bacteria of the gastrointestinal tract.

Regulation of NO Production by *H. pylori*

Years of co-evolution between *H. pylori* and the host has forced the bacterium to adapt to the immune response, and more particularly to limit macrophage NO production by mechanisms involving the regulation of NOS2 gene expression and L-arginine-dependent pathway (Figure 3).

The enzyme heme oxygenase-1 (HO-1) is induced in *H. pylori*-infected macrophages through a CagA-dependent process involving signal transduction through p38-NRF-2, and in gastric myeloid cells of patients with *H. pylori* gastritis or of mice experimentally infected

[72]. HO-1 expression results in an attenuation of *NOS2* mRNA expression [72]. Consequently, macrophages from HO-1 null mice produce more NO and animals are less colonized by *H. pylori* [72].

H. pylori also induces ARG2, but not arginase-1, through an NF-κB-dependent pathway [73]. Arginases are enzymes that catabolize L-arginine into urea and L-ornithine and are in competition with NOS2 for substrate availability. In *H. pylori*-infected macrophages, blocking ARG2 activity with a specific inhibitor results in increased NO production [58], highlighting that the induction of the arginase metabolic pathway by the bacterium is a first way of controlling NO production. Moreover, *H. pylori* stimulates the synthesis of asymmetric dimethylarginine (ADMA), an endogenous NOS2 inhibitor [74]; *H. pylori*-infected patients have increased ADMA in the gastric tissue and serum, and this level is reduced with *H. pylori* eradication [75, 76]. Thus, treatment of rat duodenum with an *H. pylori* water extract leads to ADMA synthesis and inhibits NO-mediated alkaline secretory response to luminal acid [74], thus potentially increasing gastric mucosal injury.

Importantly, the arginase product L-ornithine is converted by ODC into the first polyamine putrescine, which is then catabolized to spermidine and spermine. Although ODC is mainly regulated at the post-transcriptional level in many cell types, *Odc* mRNA expression is increased in murine macrophages infected *in vitro* with *H. pylori* [73] and in gastric macrophages of infected mice and humans [17], and this favors the synthesis of the three polyamines [17, 73]. Spermine blocks NOS2 translation in murine macrophages, without regulating the expression of the gene [77]. The pharmacological inhibition of ODC by difluoromethylornithine (DFMO) or *Odc* gene silencing results in increased NOS2 protein expression and NO generation, and consequently *H. pylori* killing [77]. Of note, not only ODC is an enzyme that controls NO production; macrophages from *Arg2*-deficient mice express more NOS2 protein, produce more NO, and are more effective in killing *H. pylori* than WT macrophages [18].

The intracellular bioavailability of L-arginine depends on the expression of the L-arginine transporter solute carrier family 7 (cationic amino acid transporter, y+ system), member 2 (SLC7A2), also called cationic amino acid transporter 2. The expression of this transporter is induced in *H. pylori*-infected macrophages *in vitro* and gastric macrophages of mice and humans with *H. pylori* gastritis, thus increasing L-arginine uptake [17]. Moreover, increased intracellular L-arginine concentration favors NOS2 translation upon *H. pylori* infection, independent of phosphorylation/dephosphorylation of the eukaryotic translation initiation factor alpha [78]. All together, these data indicate that *H. pylori* should activate NO production by macrophages.

However, spermine synthesized by the ARG2/ODC metabolic pathway, or added exogenously, inhibits L-arginine uptake, and consequently NOS2 translation [17]. Blocking *Odc* mRNA expression or ODC activity favors L-arginine entry in macrophages, but does not regulates *Slc7a2* mRNA expression or SLC7A2 protein level [17], providing a rationale for the assumption that spermine is an inhibitor of SLC7A2 activity. From this, we speculate that the induction of SLC7A2 is an innate response of macrophages to favor NO production in order to eliminate pathogens, whereas the production of polyamines is an undesirable

effect stimulated by *H. pylori* to counteract NOS2 translation. In this context, the identification of the bacterial and cellular signals leading to macrophage ODC induction by *H. pylori* would be helpful to develop strategies aimed at restoring NO-dependent macrophage function (see Outstanding Questions).

In accordance with these *in vitro* and *ex vivo* experiments there is (i) increased NOS2 protein by flow cytometry, (ii) enhanced NO production, shown by measurement of NO release and by *in situ* nitrotyrosine staining, and (iii) decreased *H. pylori* colonization in *Arg2* knockout mice or in animals treated with DFMO [17, 18]. However, the genetic ablation of *Arg2* results in increased gastritis [18], whereas a reduction of gastric inflammation and histological damage is observed with DFMO treatment [17]. However, this could be due to a collateral effect of DFMO, which reduces *H. pylori* growth [79]. This issue should be resolved by the analysis of *H. pylori* infection in myeloid-specific *Odc* knockout mice, which is ongoing in our laboratory.

The last stratum of regulation of host NO production by *H. pylori* corresponds to the direct effect of the bacterial arginase, RocF, initially described to be involved in protection of the bacteria against acidic conditions [80]. A WT strain of *H. pylori*, but not the arginase mutant *rocF*$^{-}$, depletes L-arginine from the extracellular milieu in conditions that mimics the L-arginine concentration *in vivo*, e.g. 0.1-0.2 mM. Thus, the production of NO is enhanced when macrophages are co-cultured with a *rocF* mutant strain of *H. pylori* compared to the parental WT strain, without affecting *Nos2* mRNA expression [35]. Consequently, the arginase-deficient *H. pylori* is more effectively killed by an NO-dependent pathway than the WT strain [35], emphasizing the critical role of arginase in *H. pylori* persistence in the gastric tissues. Not only does the bacterial arginase compete with the host NOS2 for L-arginine availability, but the depletion of L-arginine also results in reduced NOS2 translation since this amino acid is essential for the expression of the NOS2 protein [78]. As a striking example of the evolutionary arms race, it has been demonstrated that NO inhibits the activity of RocF [68], which should favor NOS2 translation and NO production (Figure 3); but *H. pylori* limits this effect of NO through the protein TrxA that acts as an anti-nitrosative chaperone for RocF [68] (Figure 2).

Hence, *H. pylori* has elaborated strategies to dampen NO production by macrophages, principally by decreasing L-arginine bioavailabilty for host cells. It will be of interest to now determine whether the same regulatory mechanisms occur in gastric epithelial cells.

Concluding Remarks

A particular crosstalk, which reflects thousands of years of coevolution [81], exists between *H. pylori* and host-derived NO. On one hand, the expression of NOS2 in myeloid cells through a pathway activated by the *H. pylori* urease, a unique feature in the bacterial kingdom, could be considered as a mechanism developed by the host to respond to a highly abundant protein of a bacterium exhibiting a low endotoxic activity [82]. On the other hand, *H. pylori*, which possesses a limited arsenal to fight NO challenge, has elaborated strategies to block NOS2 expression and NO production by the host in order to enhance its own survival. Furthermore, because evidence suggests that NO is involved in gastric cancer

development, the limitation of NO production by *H. pylori* could even be envisioned as a collateral evolutionary process to reduce carcinogenesis and increase life expectancy of the infected host.

Box 1

H. pylori CagA and VacA

CagA is a bacterial factor that is part of the *cag* pathogenicity island and is injected into human epithelial cells through a type IV secretion system. CagA is then sequentially phosphorylated by the host c-Src and Abl kinases [83] and dysregulates the homeostatic signal transduction of gastric epithelial cells [84]. This results in persistent inflammation and malignancy by loss of cell polarity, modulation of apoptosis, and chromosomal instability [2].

VacA contributes to *H. pylori* pathogenesis by regulating inflammatory process [85] and by damping autophagic cell death, thus favoring gastric colonization and oxidative damage [3]. Although the contribution of VacA to gastric dysplasia has not been directly demonstrated using animal models, epidemiological studies have emphasized a correlation between the *vacA* gene structure and severity of *H. pylori*-related diseases. More precisely, the signal region s1 and the middle regions m1 of the *vacA* gene are present in strains associated with increased risk for developing peptic ulcers and/or gastric cancer, compared to s2 or m2 strains.

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Outstanding Questions

What are the *H. pylori* factors involved in the expression of ARG2 and ODC, which counter the induction of NOS2?

What is the evolutionary benefit for *H. pylori* of limiting NO production rather than resisting RNI?

By which molecular mechanisms do RNI contribute to carcinogenesis during *H. pylori* infection? Can further evidence for the role of NOS2-derived NO in humans be determined by investigation of populations at high-risk versus low-risk for gastric cancer?

Trends Box

Macrophages are an important source of NOS2-dependent NO production in gastric tissues of *H. pylori*-infected humans and in experimental animal models.

Animal models, nitrosative damage in gastric epithelial cells of *H. pylori*-infected patients, and molecular epidemiology demonstrate that NO is involved in *H. pylori*-mediated carcinogenesis. Several unique proteins, such as the NO reductase, NorH, and biochemical pathways, including metal acquisition or urease-dependent synthesis of CO_2, confer to *H. pylori* a partial resistance to reactive nitrogen intermediates (RNI).

The lack of major effectors allowing resistance to high concentrations of RNI in *H. pylori* is compensated by a strong ability to inhibit host cell NO production. *H. pylori* dampens macrophage NO synthesis by transcriptional and translational regulation of NOS2 expression and control of L-arginine substrate availability.

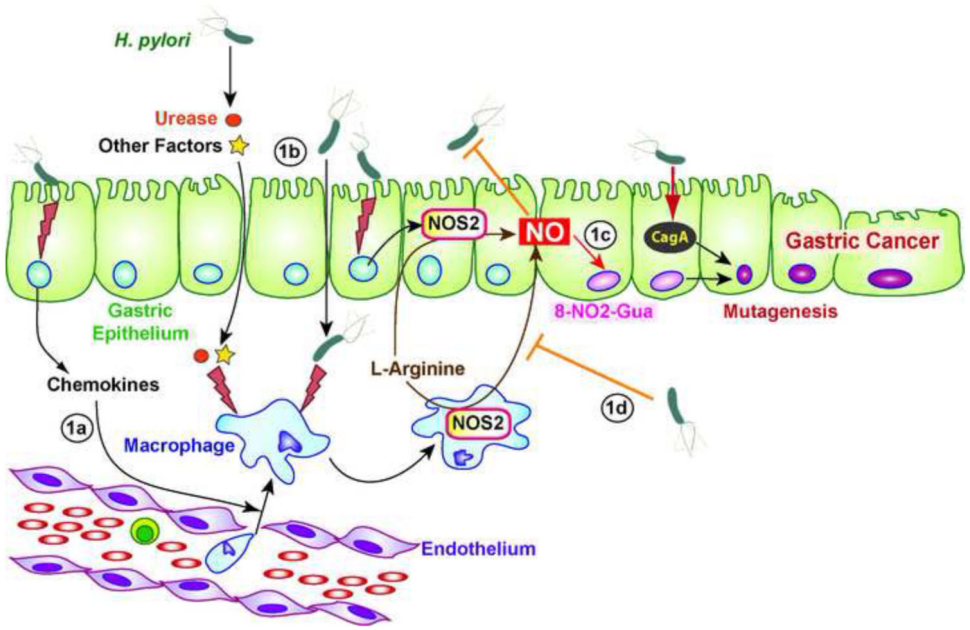

Figure 1.

Key Figure: *H. pylori* Infection in an NO World. *H. pylori* stimulates gastric epithelial cells and thus induces an innate response characterized by the production of chemokines, which leads to the recruitment of myeloid cells, including macrophages, in the gastric mucosa (1a). *H. pylori*-derived factors, such as urease, or the bacterium itself stimulate NOS2 expression in macrophages and in gastric epithelial cells (1b). NO releases by these activated cells has a cytotoxic effect on *H. pylori* and provokes the formation of 8-nitroguanine (8-NO2-Gua; 1c). The cellular changes induced by the oncoprotein CagA and the mutagenesis reinforced by the activity of 8-NO2-Gua may contribute to the development of gastric cancer. Ultimately, *H. pylori* regulates NO production by numerous strategies (1d).

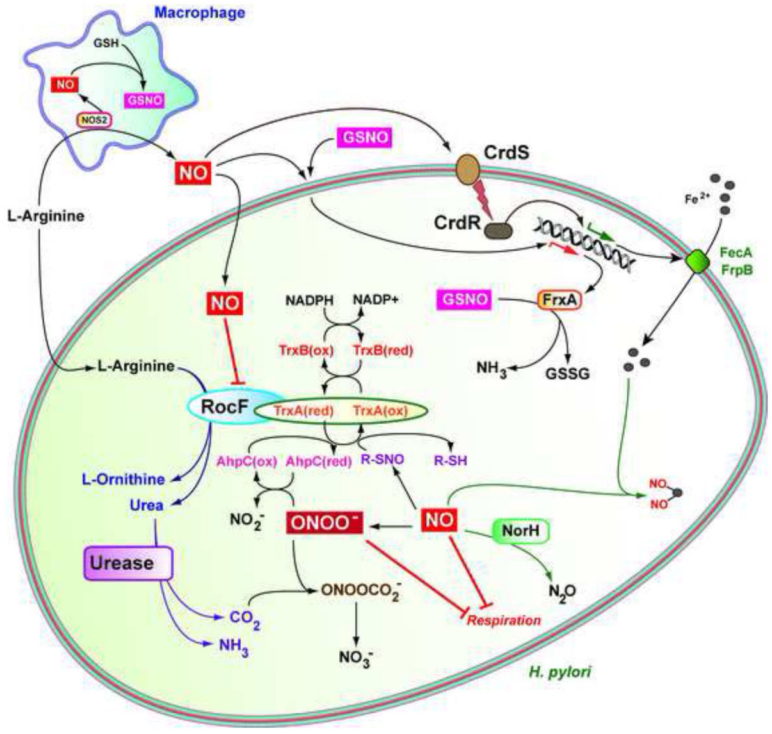

Figure 2.

RNI Detoxification in *H. pylori*. High-output NOS2-dependent NO production by macrophages is released outside the cells and may affect the bacteria at distance from producing cells or is converted in GSNO to target phagocytized *H. pylori*. RNI inhibit *H. pylori* respiration and the activity of the arginase RocF, thus decreasing the synthesis of the urease substrate, urea. *H. pylori* counteracts the deleterious effect of RNI using the NO reductase NorH, the GSNO reductase FrxA, the thioredoxin TrxA that also serves as a chaperone for RocF, and the CrdSR-dependent upregulation of FecA and FrpB that are involved in iron transport. $ONOO^-$ detoxification occurs through the urease-dependent production of CO_2 and the peroxiredoxin AhpC.

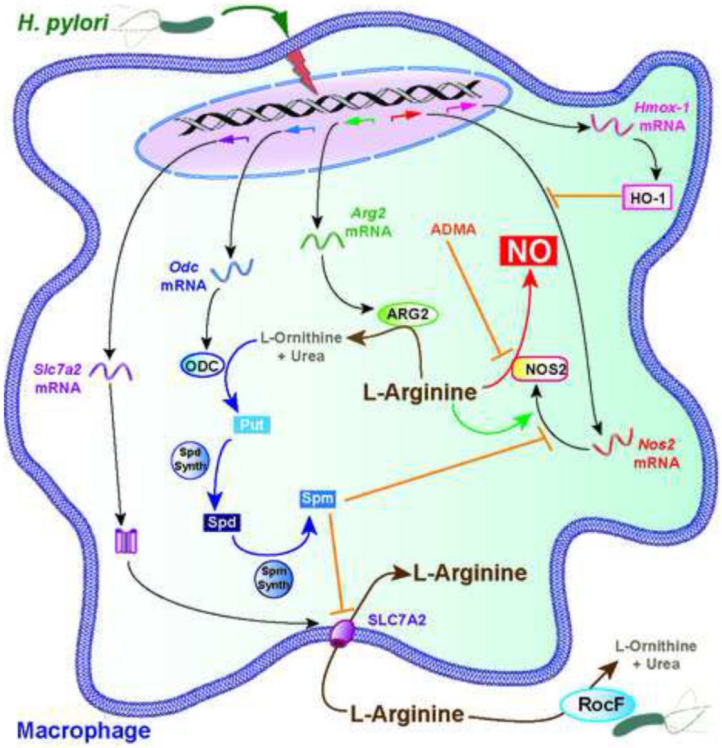

Figure 3.

Regulation of Macrophage NO Production by *H. pylori*. The infection of murine macrophages by *H. pylori* results in the expression of *Slc7a2*, *Odc*, *Arg2*, *Hmox-1* (the gene encoding HO-1), and *Nos2* mRNA. L-arginine uptake is supported by SLC7A2, promotes NOS2 translation, and is required for NO production. ARG2 and *H. pylori* arginase RocF deplete intracellular and extracellular L-arginine, respectively, thus decreasing the synthesis of NOS2 and generation of NO. Spermine (Spm), which is synthesized through the ARG2-ODC pathway, inhibits SLC7A2-dependent L-arginine uptake and thus NOS2 translation and activity. Asymmetric dimethylarginine (ADMA) is generated during *H. pylori* infection and is a natural NOS2 inhibitor. Abbreviations: Put, Putrescine; Spd, Spermidine.