Structural modifications of *Helicobacter pylori* lipopolysaccharide: An idea for how to live in peace

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Abstract

In this review, we discuss the findings and concepts underlying the "persistence mechanisms" of *Helicobacter pylori* (*H. pylori*), a spiral-shaped, Gram-negative rod bacterium that was discovered as a gastric pathogen by Marshall and Warren in 1984. *H. pylori* colonizes the gastric mucosa of nearly half of the human population. Infections appear in early childhood and, if not treated, persist for life. The presence or absence of symptoms and their severity depend on multiple bacterial components, host susceptibility and environmental factors, which allow *H. pylori* to switch between pathogenicity and commensalism. Many studies have shown that *H. pylori* components may facilitate the colonization process and the immune response of the host during the course of *H. pylori* infection. These *H. pylori*-driven interactions might result from positive or negative modulation. Among the negative immunomodulators, a prominent position is occupied by a vacuolating toxin A (VacA) and cytotoxin-associated gene A (CagA) protein. However, in light of the recent studies that are presented in this review, it is necessary to enrich this panel with *H. pylori* lipopolysaccharide (LPS). Together with CagA and VacA, LPS suppresses the elimination of *H. pylori* bacteria from the gastric mucosa by interfering with the activity of innate and adaptive immune cells, diminishing the inflammatory response, and affecting the adaptive T lymphocyte response, thus facilitating the development of chronic infections. The complex strategy of *H. pylori* bacteria for survival in the gastric mucosa of the host involves both structural modifications of LPS lipid A to diminish its endotoxic properties and the expression and variation of Lewis determinants, arranged in O-specific chains of *H. pylori* LPS. By mimicking host components, this phenomenon leaves these bacteria "invisible" to immune cells. Together, these mechanisms allow *H. pylori* to survive and live for many years within their hosts.

Key words: *Helicobacter pylori*; Lipopolysaccharide; Immune response; Adaptation; Inflammation

Core tip: *Helicobacter pylori* (*H. pylori*), a Gram-negative bacterium, colonizes the gastric mucosa of at least half of the human population and possesses a unique lipopolysaccharide (LPS) structure. According to recent studies, this structure contributes to the immunomodulatory properties of LPS. The structural rearrangements of *H. pylori* LPS, especially in relation to the lipid A and O side chains, result in a unique pattern of interactions between the bacterium and the host. In this review, we report and discuss the actual findings underlying the LPS-driven "persistence mechanisms" of *H. pylori*, explaining how structural modifications may allow these bacteria to "live in peace" within a human host.
INTRODUCTION

In this review, we discuss the findings and concepts underlying the “persistence mechanisms” of Helicobacter pylori (H. pylori), a human pathogen colonizing the gastric mucosa. We focus on aspects concerning the structure, bioactivity, and immunomodulatory properties of H. pylori lipopolysaccharide (LPS) and its contribution to the development of chronic gastritis. Today, we know that LPS is the main outer membrane component of Gram-negative bacteria and that LPS has strong immunostimulatory and inflammatory capacities. Recent years have provided new insights into the H. pylori LPS structure and the receptors of the innate immune system that are involved in its recognition and signal transduction pathways. These new data allow a more precise definition of the harmful and beneficial effects of H. pylori LPS. There is a growing number of theories suggesting that H. pylori LPS has evolved during the co-existence of these bacteria with human hosts since the migrations out of Africa 60000 years ago, enabling these bacteria “to live in peace”[9].

HELICOBACTER PYLORI: BETWEEN PATHOGENICITY AND COMMENSALISM

H. pylori is a spiral-shaped, Gram-negative rod bacteria that was discovered as a gastric pathogen by Marshall et al[1]. H. pylori colonizes the gastric mucosa of nearly half of the human population. Infections appear in early childhood and, if not treated, persist for life[3]. Disease development depends on multiple bacterial virulence components, host susceptibility, and environmental factors[4]. A characteristic symptom of H. pylori infection is an excessive inflammatory response, which results in the development of pathological processes in the gastric epithelium, such as erosions, ulcers, changes in cell phenotype, excessive cell proliferation, and the secretion of proinflammatory cytokines[5-7]. The balance of bacterial factors and the host inflammatory response to H. pylori infection determines the outcome of the disease[8]. Most H. pylori-infected individuals (80% to 90%) remain clinically asymptomatic throughout their lifespan. In most cases, H. pylori colonizes the stomach for many decades before symptoms appear, which distinguishes this bacteria from bacterial pathogens that cause acute infections. However, even in asymptomatic subjects, H. pylori induces histological gastritis, due to the infiltration of the gastric mucosa by immune cells[8]. Approximately 10% of infected subjects develop symptomatic gastritis, peptic ulcer, or even gastric cancer. Despite the high prevalence of H. pylori infection in Africa and South Asia, the incidence of gastric cancer in those areas is much lower than in other regions. Such geographic differences in pathology can be explained in part by the presence of different types of H. pylori virulence factors[10-13]. Many studies have been performed to define what makes H. pylori a pathogen and, at the same time, why so many people do not suffer due to infection. Generally, penetration of H. pylori through the gastric mucosa results in the recruitment of host immune defenses to the site of infection and the development of acute inflammation. If the course of events is favorable to the host, the bacteria are eliminated, and healing begins. However, when acute inflammation fails to fight the infection, a chronic inflammatory response occurs. Among several mediators engaged in propagating gastric inflammation after H. pylori infection, cyclooxygenase-2[20], together with reactive oxygen species (ROS) and reactive nitrogen species (RNS)[15,16], may be the principal ones. Excessive ROS/RNS production correlates well with histopathological mucosal damage and with bacterial load[14,15]. The permanent recruitment of inflammatory cells to infectious foci results in progressive tissue damage. Asymptomatic H. pylori-infected individuals likely develop very weak gastric inflammation in response to H. pylori. In such conditions, the bacteria are not eliminated. A type of balance is created between the microbes and the host, which limits the recruitment of immune cells and prevents the development of gastric pathologies.

It is known that H. pylori is a genetically diverse species[15,23]. H. pylori strains isolated from different hosts possess various virulence capacities, and their genetic diversity may also appear within the gastric niche of a single human host. Most H. pylori-related diseases depend on the expression of specific protein virulence factors, such as cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA)[7]. A recent study on H. pylori-host protein interactions confirmed that the type IV secretion system (TIVSS) can translocate CagA to gastric epithelial cells using human β1 integrin as a receptor[17]. H. pylori strains that are positive for the Cag TIVSS more successfully cause chronic infections in humans. In contrast, it has been observed that the activity of the TIVSS system may limit H. pylori colonization. The study in a mouse model has shown that an exchange of DNA between genetically heterogeneous H. pylori strains may support chronic colonization[18,19]. Other well-characterized H. pylori virulence factors include urease[20,21] and several types of outer membrane proteins, such as Hop proteins (Hsp70/Hsp90-organizing Hsp)[22] and blood antigen-binding adhesins[23]. However, the panel of H. pylori proteins is very complex and, as shown in a proteomics study, includes over 1200 compounds[24,25]. The role of the majority of these proteins in the course of H. pylori-related diseases needs to be clarified. It is interesting that different H. pylori proteins may interact with various host molecules and be involved in numerous pathogenic signaling pathways.

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Key findings

H. pylori demonstrates high affinity to the gastric mucosa and is well adapted to live in the acidic environment of the stomach. In general, the interactions of H. pylori with host cells resemble a lifelong homeostasis, but in certain patients, these microbes cause pathological changes, such as ulcers or gastric cancers. It is not clear why certain patients remain asymptomatic, whereas others develop illness. Recently, both bacterial virulence factors and host susceptibility have been recognized as contributors to H. pylori pathogenicity.

UNIQUE STRUCTURAL AND BIOLOGICAL FEATURES OF H. PYLORI LPS - A KEY PLAYER IN THE PERSISTENCE STRATEGY

Although H. pylori, as a gastric pathogen, expresses several unquestionable virulence factors that are related to the cytotoxicity of this bacterium, its most potent feature is the ability to persist for years within the gastric epithelium of the host. This unique, adaptive property occurs due to a complex mechanism of H. pylori colonization and persistence, which is mediated by proteins, glycoconjugates, and lipids exposed on the surface of this bacterium. Well-characterized adhesins in H. pylori include blood antigen-binding adhesins (BabA), which interacts with the host’s Lewis’ (Le’), and sialic acid-binding adhesin, which binds sialylated Lewis’ (Le’). There are many other H. pylori adhesion molecules, such as hemagglutinin, which interacts with sialic acid exposed on human tissues, and less-characterized adhesion molecules that interact with host extracellular matrix (ECM) molecules.[12,23,26,27] Among H. pylori virulence factors, LPS plays a special role due to its unique structural and biological properties, which differ from those of the LPSs typical of other Gram-negative bacteria. In general, these unique features concern modifications of lipid A, resulting in a reduction in the endotoxic and proinflammatory properties of H. pylori LPS[31] and the expression of O chains. These chains are structurally related to Lewis blood-group antigens, which are also expressed by human cells. This phenomenon, called molecular mimicry, most likely makes these pathogens less sensitive to recognition by the immune cells of the host.[14] The direct cytotoxic effect of H. pylori LPS on immune cells and epithelial as well as endothelial barriers and its immunomodulatory activity, mediated through different cytokines, are being investigated.

Lipid A of H. pylori LPS: A weak bioactive center

Similarly to other Gram-negative bacteria, H. pylori contains a cell envelope composed of poly- or oligosaccharides that are covalently linked to lipid A of LPS. Fresh clinical isolates produce a high-molecular-weight, smooth LPS with an O-antigen, which, in an in vitro culture, may convert into a rough form without the O side chain.[15,16] Lipid A of H. pylori LPS has a unique structure and lower biological activity compared with lipid A from other bacteria.[17-20] Probably it evolved due to adaptation to long-term infections in the gastric milieu. Structural analysis of H. pylori lipid A of the rough and smooth types has indicated that these lipids contain fatty acids that are longer than those present in lipid A of enterobacterial species. The predominant form is tetraacyl lipid A, with long acyl chains of 16 to 18 carbons and underphosphorylation, which are unusual properties for H. pylori lipid A. Similarly, studies on reference lipid A from Salmonella minnesota and E. coli have shown that specific phosphorylation patterns and fatty acid compositions and a particular degree of ac-
ylation are necessary for LPS to express its full biological activity. The underphosphorylation and underacylation of H. pylori lipid A moieties promote the lipid's low endotoxicity and biological activity. H. pylori LPSs, interpreted in the context of their lipid A, possess a significantly lower ability to induce the production of cytokines, nitric oxide, and prostaglandin E2, show diminished potential to activate natural killer (NK) cells; barely induce the expression of E-selectin; and are unable to impair the activity of suppressor T lymphocytes. These suggest that this lipid should be treated not as a typical endotoxin, but rather as a type of matrix that enables H. pylori to interact with and modulate the host environment.

O side chains of H. pylori LPS: The idea of molecular mimicry

Considering the role of H. pylori LPS in the course of gastroduodenal infections, several studies have analyzed the relevance of O side-chain repeating units. In contrast to other pathogens, H. pylori LPS O antigen is strongly conserved, which suggests that its role in the pathogenesis of H. pylori is not related to weak endotoxic activity. The O side chain of H. pylori can be fucosylated and thus mimics human Lewis molecules and other related blood-group antigens: LeX, LeY, and, in certain strains, Le+, LeX, LeY, sialyl-LeX, and H-1 antigens. The side chain can also mimic the antigens of blood groups A and B. Inactivation of the H. pylori LeX and LeY determinants results in the unsuccessful colonization of mice in vivo. The biosynthesis of H. pylori LPS follows a pathway that is different from all established synthesis cascades. The translocation of the O chain is accomplished by Wzk translocase, which is essential for the cell surface expression of Le antigens. During persistent infection, the diversity of H. pylori LPS tends to increase, especially in relation to poly (C) tracts in the gene encoding α-1,3-fucosyltransferases as well as a poly (C) tract and poly (TAA) repeats in the gene encoding α-1,2-fucosyltransferase. The phenomenon of LPS phase variation results in increased heterogeneity of the bacterial population which enables H. pylori to more precisely adapt to the changing environment of the gastric mucosa. Considering the similarities between LeX and LeY and LeX and LeY determinants, it has been suggested that the host Le phenotype may preferentially select for the expression of Le determinants by H. pylori. Thus, the host milieu promotes a selection of bacterial strains with particular characteristics that facilitate adaptation and survival in the gastric mucosa of the individual host and shape the bacterial community structure. An investigation of the genetic basis of H. pylori Le biosynthesis showed that despite functional similarity, low sequence homology occurs between bacterial and mammalian α-1,3/4- and α-1,2-fucosyltransferases. Thus, the expression of Le antigens in H. pylori and these antigens’ role in the mimicry phenomenon could be influenced by several factors: the regulation of fucosyltransferase genes, the activity and expression levels of functional enzymes, the preferences of the expressed enzyme for distinctive acceptor molecules, and the availability of activated sugar intermediates.

The poorly endotoxic properties of H. pylori LPS and the expression of LeX or LeY determinants in O-specific chains suggest that antigenic mimicry induced by H. pylori LPS may allow the bacterium to avoid the host immune response via the induction of potentially auto-reactive anti-LeX or anti-LeY antibodies, as well as by strengthening the adhesion process. The functional role of H. pylori Lewis antigens in the adhesion of bacteria to the human gastric mucosa has been reevaluated by studying the in
in situ adhesion of the strains with or without the expression of BabA in patients with various gastric pathologies. It was concluded that BabA is a major H. pylori adhesin that mediates H. pylori binding to gastric epithelial cells via a mucosal Le concentrated on the expression and role of H. pylori LPS in the vascular endothelium and that related strains are not able to colonize C3H/HeJ mice. However, more recent studies showed that the polymeric Le structure in the O antigen of H. pylori LPS is specifically recognized by β-galactoside-binding lectin (galectin-3), which has been identified as a gastric receptor. Previously, it was shown that the core region of LPS may bind laminin (a molecule of the host ECM) and thus contribute to the colonization process.

**Key findings**

Among H. pylori virulence factors, a special role is played by LPS. This molecule’s unique structure and low biological activity promote the survival of the bacterium in the gastric mucosa, despite the existence of various immune barriers. Underphosphorylation and underacylation of lipid A result in the low endotoxicity and biological activity of H. pylori LPS. Fucosylation of the H. pylori O antigen results in the phenomenon of antigenic mimicry, in which bacterial fucose residues imitate human Le blood-group antigens. This imitation allows the bacterium to avoid host immune responses and contributes to prolonged infection.

**HOST CELLULAR IMMUNE BARRIERS VS H. PYLORI LPS**

Pathogens may modulate host immune responses by interfering with host recognition and signal transduction mechanisms. During the co-evolution of H. pylori and its host, the bacterium evolved complex strategies to impair immune defenses while maintaining a limited, balanced inflammatory response. Several H. pylori compounds reduce the inflammatory response and the recognition of bacterial antigens by the host immune system (Figure 2). The immunomodulatory activities of VacA, CagA, γ-glutamyl transpeptidase, arginase, and several adhesins have been shown. The release of H. pylori LPS into an infectious niche may play an important role in the induction of both local and systemic inflammatory responses. This induction may be a consequence of direct LPS-cell interactions or cell-to-cell cytokine-mediated crossstalk. Changes occurring in the gastric epithelium colonized by H. pylori lead to the penetration of bacterial antigens through the basal membrane, where they bind to the ECM and, via pathogen recognition receptors (PRRs), interact with immune cells infiltrating the lamina propria and induce the production of cytokines. Moreover, the soluble antigens of H. pylori may enter the circulation and induce systemic effects.

**H. pylori LPS: An atypical PAMP**

H. pylori, similarly to many intestinal microorganisms, has developed mechanisms to vary its LPS chemistry to subvert recognition by innate immune receptors. Bacterial LPSs are pathogen-associated molecular patterns (PAMPs), or so-called “alarmins”, which allow the recognition of bacterial invasion and trigger the innate immune response of the host. LPSs and other PAMPs, including lipopolysaccharides, peptidoglycan, flagellin, double-stranded RNA, CpG-DNA, and zymosan, are recognized by PRRs on the cells of the first line of immune defense: macrophages and dendritic cells (DCs). Toll-like receptors (TLRs) and nucleotide-binding domain, leucine-rich repeat-containing receptors (NLRs) are well-characterized PRRs that display a high degree of PAMP specificity. In contrast to the adaptive response, innate immunity is not specific. Each type of PRR can differentiate microbial components from similar structures, i.e., patterns or molecular signals, that are unique to host cells, allowing distinction between self and non-self.

The recognition of PAMPs induces the production of proinflammatory cytokines and the maturation of DCs, which in turn result in the activation of a specific immune response.

In the context of infections caused by Gram-negative bacteria, LPS constitutes the most dominant and suitable PAMP. The structure of lipid A, the expression of Le antigens, and the fucosylation patterns in H. pylori LPS may have multiple effects on immune responses. The interactions of lipid A with host cells are mediated by cellular and soluble receptors: (1) serum LPS-binding protein (LBP); (2) the membrane CD14 receptor; (3) sCD14 protein, a soluble form of the CD14 receptor; and (4) TLR4 protein, which recognize bacterial LPS via the TIR domain containing MyD88 and TIRAP adapter proteins or, alternatively, of the TRAM and TRIF proteins, leading to the activation of nuclear factor κB or IRF3/7 transcription factors. This events result in the production of proinflammatory cytokines and type I interferons (IFNs).

Studies conducted on two types of cellular systems, 70Z/3 cells expressing CD14 and an epithelial cell lineage with CD14-dependent activation, confirmed that H. pylori LPS, although much less active than E. coli LPS, stimulates cells to secrete interleukin (IL)-8 via CD14. Lower efficiency in LBP binding may be one of the explanations for the low biological activity of H. pylori LPS, which is especially observed in the activation of human monocytes. Although H. pylori LPS binds LBP less efficiently than does E. coli LPS, the level of circulating LBP significantly rises in H. pylori-infected patients with gastritis and is even more elevated in H. pylori-infected patients with coronary heart disease (CHD), most likely as a consequence of the chronic inflammatory response and endothelial dysfunction. Through LBP, LPS is detoxified in the circulation, mainly by its incorporation into low-density lipoproteins (LDLs). However, considering LPS/LBP/LDL interactions in CHD patients may serve as a mechanism that enables the deposition of H. pylori LPS in the vascular endothelium, thus enhancing the TLR4-dependent inflammatory response.
The variation in bacterial lipid A structures might be a successful strategy for escaping recognition by the innate immune system and blocking TLR4 signaling. In vitro studies showed that moieties of lipid A from H. pylori LPS are poorly recognized by human TLR4 [79]. This phenomenon is due to the presence of fewer but longer (16- to 18-carbon) acyl chains. The potential of these pathogens to cause severe chronic disease in humans is attributed, at least in part, to their relatively low ability to activate TLR4 signaling [67]. However, the advantage of this mechanism for the persistence of H. pylori is limited in the gastric milieu because human gastric epithelial cells do not express functional TLR4, which is present on the surface of monocytes and macrophages [80]. H. pylori lipid A, which is unable to trigger a signal through TLR4, can act through TLR2, expressed on both epithelial cells and macrophages, which may compensate for a lack of sufficient TLR4 signaling [81]. It has been shown that LPSs from certain H. pylori strains can antagonize TLR4. The antagonistic activity of H. pylori LPS and its binding to TLR2 might give the bacterium an advantage over the host, and this phenomenon might be associated with the clinical outcome of H. pylori infection [79]. It was shown that the presence of cag pathogenicity island (PAI) genes may be crucial for the synthesis of bioactive lipid A molecules that stimulate TLR4-mediated expression of mitogen oxidase 1 (Mox1) in gastric pit cells, which could be involved in the initiation of mucosal cell inflammatory and immune responses against H. pylori infection [82,83]. However, the genomic and molecular bases for the link-
age between α1,2-fucosyltransferase A and a weakly antigenic epitope (WA-LPS), which is expressed after the removal of a β-N-acetyl-D-glucosamine residue, a highly antigenic determinant. This antigenic conversion, resulting with the loss or lack of only one β-linked D-GlcNAc residue, may serve as an escape mechanism for *H. pylori*, enabling the bacterium to evade the host antibody-mediated immune response. In the presence of SP-D, WA-LPS possesses a stronger ability to upregulate TLR4 expression, proliferation of epithelial cells, inflammation and tumorigenesis. Whether the Leα and Leβ determinants are involved in the pathways of cytokine signaling is unclear. It has been shown that *H. pylori* LPSs, with or without LeXV determinants, differ in their ability to induce the production of inflammatory cytokines by peripheral blood mononuclear leukocytes (PBMLs). The lack of Lewis determinants in *H. pylori* LPSs results in a significant reduction in its ability to induce TNF-α secretion compared with *H. pylori* LPS containing LeXV antigenic moieties or standard *E. coli* LPS. Little is known about the recognition of bacterial Le determinants by the immune cells of the host. Successful pathogens may manipulate signaling through bacterial structures that modify TLR signaling. For instance, *Mycobacterium tuberculosis*, due to the interaction of mannose-capped lipoarabinomannan (ManLAM) with the mannose receptor, inhibits LPS-induced IL-12 production by macrophages. Additional receptors such as DC-specific intercellular adhesion molecule (ICAM)-3-grabbing non-integrin (DC-SIGN) on DCs, work together with the mannose receptor to inhibit antimicrobial and anti-inflammatory responses by modifying the effects of subsequent TLR activation.

Figure 3  Local and systemic consequences of *Helicobacter pylori*-driven immunomodulation. *Helicobacter pylori* (*H. pylori*) lipopolysaccharide (LPS) may facilitate colonization of the gastric mucosa through extracellular matrix proteins and induction of local inflammation. Increased permeability of the gastric epithelium due to the activity of *H. pylori* proteins such as urease and vacuolating cytotoxin, which contribute to the degradation of intercellular tight junctions, may result in broad interactions of soluble *H. pylori* LPS with host cells. This LPS can bind to various immune cells infiltrating the mucosal basal lamina or even enter the circulation, where the LPS may contribute to the inflammatory response via a direct influence on immune cells or through the formation of complexes with low density lipoprotein (LDLs), and possibly oxidized LDL. Released *H. pylori* LPS can also move along the gastrointestinal tract and, together with the intestinal microbiota, may induce complex immunomodulatory mechanisms. These interactions can result in an increased expression of adhesins, pathogen recognition receptors (PRRs), and proinflammatory cytokines. Different PRRs can mediate the interactions of *H. pylori* LPSs with the immune cells, including Toll-like receptor 4 or 2, dendritic cell-specific intracellular adhesion molecule-3-grabbing non-integrin, and triggering receptor expressed on myeloid cells-like receptors. The signaling pathways triggered by these receptors may cause cytokine secretion. NKT: Natural killer T lymphocytes; PMNs: Polymorphonuclear cells.
It has been shown that whole H. pylori bacteria and their LPSs bind to DC-SIGN not only via Le carbohydrates but also through galactose moieties. It was suggested that differences in H. pylori binding affinity for DC-SIGN are determined by the phase variation. The ability of H. pylori to bind DC-SIGN might allow the bacterium to modulate the T helper cell 1 (Th1)/T helper cell 2 (Th2) balance and could be crucial for the control of H. pylori infections. Activated lymphocytes more effectively control bacterial growth and might diminish inflammation in the gastric mucosa by releasing cytokines, most likely of the Th2 type. Beyond TLRs and DC-SIGN receptors, other molecules, such as triggering receptor expressed on myeloid cells (TREM)-like and nucleotide-binding oligomerization domain 1 (NOD1) receptors, are also engaged in H. pylori-driven immune responses. As shown in recent studies, the stimulation of gastric epithelial cells by H. pylori-derived NOD1 ligands leads to a production of host defense factors, such as IFN-β, IFN-γ, and IFN-γ-inducing protein 10 (IP-10), all of which regulate bacterial growth. The molecular mechanisms of NOD1-mediated mucosal host defense against H. pylori are not completely understood but are at least partially dependent on the presence of a functional TIVSS. Interestingly, TLR-dependent proinflammatory cytokine responses are remarkably enhanced in the presence of NOD1 ligands. Thus, synergetic TLR-NOD1 activation in the gastric epithelium should be considered.

**ESCAPING PHAGOCYTOSIS**

Host recognition of microbial invasion induce the recruitment of neutrophils and mononuclear cells into the lamina propria of the gastric mucosa which depends on the density and avidity of adhesion-mediated molecules expressed on leukocytes and endothelial cells. The majority of adhesins, such as ICAM-1 and vascular adhesion molecule-1, are upregulated in the antral mucosa in patients with H. pylori-related gastritis. A higher level of E-selectin was shown in the gastric mucosa of individuals infected with H. pylori strains positive for the cagPAL. These findings indicate that E-selectin and its ligands preferentially mediate the extravasation of mononuclear cells and their migration into inflamed tissues. In this context, a question arises: why are the phagocytes, not able to effectively destroy and eliminate H. pylori? It has been shown that several mechanisms allow H. pylori to alter phagocyte functions, especially by evading opsonization, actively retarding phagocytosis, affecting membrane trafficking, and inhibiting phagosome maturation. The anti-phagocytic properties of H. pylori depend on the presence of the cagPAI, sialic acid-specific and heparan sulfate-binding proteins, and on the expression of various ECM proteins. H. pylori LPS may also have various effects on phagocytes. In an in vitro study, H. pylori LPS diminished the ingestion of H. pylori by human peripheral blood polymorphonuclear leukocytes (PMNs). This anti-phagocytic effect of H. pylori LPS was dose dependent and neutralized by recombinant LPS-binding protein (rLBP). LBP is thought to primarily function in facilitating LPS-stimulated cytokine synthesis by monocytes and macrophages. However, LBP also enhances the later stages, affecting bactericidal/permeability-increasing protein (BPI) activity and opsonizing Gram-negative bacteria and thus playing a more direct role in host defense. It is possible that LBP reduces the concentration of BPI required to kill bacteria. The LPS-mediated attachment of H. pylori to laminin may affect the effectiveness of the engulfment process. This mechanism may limit the availability of bacteria to phagocytes. Another anti-phagocytic mechanism of H. pylori LPS could be associated with LPS-driven apoptotic changes in phagocytes. Previous findings implicated caspase-3 involvement in gastric mucosal inflammatory responses to H. pylori LPS and suggested the participation of nitric oxide synthase 2 in the amplification of the cell death signaling cascade. In vivo, H. pylori LPS may diminish bacteria elimination and, despite abundant granulocyte infiltration in the gastric mucosa, promote persistence of the infection.

**MODULATION OF NK CELL ACTIVITY**

There is a growing set of data concerning the interplay of H. pylori antigens with NK cells and its consequences in the course of infection. The main function of NK cells, which are large granular lymphocytes representing the innate immune system, is to promote major histocompatibility complex (MHC)-unrestricted killing of both target cells infected by viruses or bacteria and tumor cells. NK cell-dependent responses are especially important in defense against pathogens that induce extensive cell damage, apoptosis, or cancerogenic changes. H. pylori bacteria fulfill all of these criteria. The predominance, heterogeneity, and distribution of NK cells at different sites within the gastric mucosa reflect a potential functional role for these cells during H. pylori infections. CD8 CD16 CD56bright NK cells in the gastric mucosa of H. pylori-infected subjects have likely adapted to respond to such bacterial infections. NK cells may respond by robust IFN-γ secretion for both direct H. pylori stimulation and indirect antigen presenting cell (APC)-dependent cytotoxic activation. Regarding the anti-phagocytic properties of H. pylori LPS, it is unclear how this LPS influences NK cell activity compared with the effects of other surface H. pylori antigens and standard E. coli LPS. It was found that H. pylori LPS, but not E. coli LPS or H. pylori glycine-acid extract (GE) of surface antigens, downregulated the natural cytotoxic activity of peripheral blood lymphocytes, which also correlated with a diminished ability of PBMLs to produce IFN-γ and IL-2 and a retained but very low secretion of IL-10. H. pylori LPS-driven downregulation was more intense in H. pylori-infected individuals. The activity of H. pylori LPS, but not GE or E. coli LPS, was related to a decrease in the cytotoxic properties and propagation of NK cells (CD3 CD56+). The H. pylori LPS-driven downregulation
of lymphocyte cytotoxicity was also accompanied by the expansion of natural killer T (NKT) (CD3+CD56+CD16+) cells exclusively in H. pylori-infected donors. This might be explained by the previous in vivo stimulation of immune cells with H. pylori antigens, including LPS, and the adaptive-like properties of NKT cells. The abundant population of NK cells in H. pylori-infected individuals expresses the CD56+CD16+ phenotype, exhibiting regulatory rather than cytotoxic activity. Lindgren et al. suggested that CD56+CD16+ NK cells present in the gastric mucosa respond to bacterial infections by cytokine production, which influences the course of innate defense. Thus, the response of NK cells to H. pylori most likely depends on the type of antigenic challenge. H. pylori antigens may differ in their ability to induce a different cytokine network, which in turn may positively (by GE antigens) or negatively (by LPS) modulate NK cell cytotoxic and secretory activities. The H. pylori LPS-mediated decrease in lymphocyte cytotoxicity is accompanied by a lack of CD3+CD56+CD16+ NK cell proliferation, inhibition of proinflammatory cytokine expression, and intense suppression of IL-10-producing NK cells. These results are particularly interesting in the context of the results obtained for E. coli LPS, which was found to stimulate the propagation of human CD56+CD3+ NK cells, followed by a substantial increase in cytolytic activity and IFN-γ production. This expansion might be driven by monocytic-derived DCs. Using a guinea pig model, it was confirmed that H. pylori GE antigens stimulated the cytotoxic activity of lymph node lymphocytes in vitro, whereas H. pylori LPS did not possess such potential and thus might have promoted the development of chronic infection.

T LYMPHOCYTES AND ADAPTIVE IMMUNE RESPONSES

LPSs have various effects, mainly on myeloid cell lineages, including induction of acute phase responses, leukocytosis, moderate fever, infiltration of defense cells into the site of infection, and activation of antimicrobial mechanisms. If not excessive, this response is crucial for the elimination of pathogens. The role of LPS in the development of an antimicrobial humoral response was evaluated in a mouse model where LPS induces the polyclonal activation of B lymphocytes, and secretion of antibodies with different specificities was observed. The mechanisms of LPS involvement in adaptive cellular antimicrobial immunity are not yet known. In several studies, LPS has been shown to stimulate human T lymphocytes to proliferate and secrete the Th1-type cytokines IFN-γ, IL-12, and TNF-α. However, T cell activation depends on accessory myeloid cells that provide costimulatory signals. Although all of these findings support the hypothesis that LPS could be involved in cellular adaptive antimicrobial immunity, it has been shown that humans may be divided into LPS responders and non-responders. The type of LPS-driven cell response may have implications for the outcomes of infections with Gram-negative bacteria, including H. pylori. It has been shown that H. pylori LPS itself possesses a very weak, if any, capacity to stimulate the proliferation of PBMLs from H. pylori-infected dyspeptic patients or the lymphocytes of guinea pigs with induced H. pylori infection. However, in the presence of IL-2, a lymphocyte growth factor, H. pylori lysates or LPS can effectively stimulate human lymphocytes. The role of the interaction between IL-2 and the macrophage CD14 surface antigen, serving as the LPS-binding site, in the activation of human monocytes was reported by Bosco et al.

In another study, it was shown that downregulation of H. pylori LPS-driven PBML proliferation was determined by the susceptibility of host cells to bacterial LPS and could have been a result of the direct influence of H. pylori LPS on non-adherent lymphocytes or a consequence of an indirect effect of the LPS preparations on monocyte-derived macrophages, known to be the main targets of bacterial LPS. These results revealed that in the milieu of H. pylori LPS, but not E. coli LPS, only mature macrophages from LPS responders expressed inhibitory activity toward lymphocytes which could result from changes in cytokine production; the expression of surface costimulatory molecules; the production of non-protein mediators, such as oxygen free radicals; and changes in TLR4 expression on macrophages. H. pylori LPS not only activates phagocytes to release oxygen free radicals but also increased nicotinamide adenine dinucleotide phosphate oxidase and TLR4 expression on gastric epithelial cells leading to elevation of oxidative stress. It has been shown that elimination of regulatory lymphocytes from the population of H. pylori-specific memory T cells increased their proliferation in response to H. pylori antigens and abolished the effect of anergy. It could be speculated that H. pylori LPS affects the macrophage-lymphocyte interactions contributing to prolonged H. pylori infection. Immunosuppression induced by H. pylori protein components, such as VacA and CagA antigen, has also been suggested to be involved in the chronic nature of H. pylori infections. These bacteria may alter the Th1/Th2 balance to evade the immune response of the host. Th1 polarization of the gastric immune response may be induced by different H. pylori components, including LPSs, enhancing IFN-γ and IL-12 secretion and inhibiting IL-2 production and cell proliferation, which may be necessary for Th2 responses. It has also been shown that H. pylori modulates the Th1/Th2 cell balance through a phase-variable interaction between LPS and DC-SIGN. Lewis-positive H. pylori variants were shown to bind DC-SIGN C-type lectin present on gastric DCs, and this interaction blocked Th1 cell development, whereas Lewis-negative H. pylori variants that were not involved in binding to DC-SIGN induced a strong Th1 response.

A growing number of reports suggest that the Th17 cells identified by their production of IL-17 are involved in H. pylori induced inflammation. H. pylori colonization and H. pylori-induced inflammation were more...
intense in wild-type mice than in IL-17-deficient mice, indicating that IL-17 may not contribute to protection, instead playing a role in the development of inflammation. In another study, anti-IL-17 treatment resulted in a reduced H. pylori burden and lower inflammation in the stomach, suggesting that IL-17 neutralization contributes to bacterial clearance and prevents inflammatory processes. It has been also shown that H. pylori specific tumor infiltrating lymphocytes (TILs) of gastric mucosa produce IL-17. The results obtained in animal models are in line with findings based on real-time PCR and Western blotting analyses of human gastric biopsies, in which the upregulation of IL-17 occurred at both the RNA and the protein levels in H. pylori-infected biopsies compared with uninfected biopsies. The direct implication of LPS or any other H. pylori antigen in IL-17 induction has not been clarified yet. However, regardless of the presence or absence of Th1 or Th17 cells, the results described here support the suggestion that gastritis due to H. pylori is the result of complex interactions between at least several different T cell subsets.

Key findings
What distinguishes H. pylori from other gastric pathogens is its ability to avoid and modulate host immune mechanisms to maintain balanced inflammation in the mucus layer of the stomach. These mechanisms include the manipulation or downregulation of TLR signaling pathways, SP-D binding, and modulation of the network of cytokines secreted by immune cells. All of these mechanisms are determined by the unique structure of H. pylori LPS, and mainly the low acylation of lipid A and phase variation in relation to the O antigen. The diminished elimination of H. pylori due to the interference of its LPS in the process of phagocytosis and its negative modulation of NK cell cytotoxic and cytokine activities are also being considered. Although the involvement of H. pylori LPS in the mechanisms of adaptive immune responses is not yet known, the H. pylori LPS-driven alterations in lymphocyte proliferation and macrophage-lymphocyte interactions may contribute to the lifelong persistence of H. pylori infection.

WHAT ABOUT ANTI-H. PYLORI LPS ANTIBODIES AND AUTOACTIVE RESPONSES?
It has been hypothesized that H. pylori infections induce antibodies that are potentially autoreactive. Half of H. pylori-infected individuals are positive for the production of serum autoantibodies potentially crossreacting with gastric parietal cells. The correlation between the eradication of H. pylori and a subsequent decrease in serum anti-H. pylori IgG levels, resulting in the regression of autoimmune gastritis, seems to confirm this suggestion. Candidates for the antigens responsible for the molecular mimicry that causes autoreactivity include H. pylori Hsp60, Le antigens and H^+K^+ATPase. Opinions on a possible role for antigenic mimicry in the interaction between H. pylori Hsp60 and the human gastric mucosa are varied because this antigen was not found to be involved in the autoreactive response observed in individuals infected with H. pylori. However, in the sera of H. pylori-infected patients, elevated titers of anti-H. pylori LPS antibodies have been detected. The epitope specificity of human anti-LPS antibodies has been widely discussed. Although controversial, certain data suggest that long-term H. pylori infections could induce autoreactive anti-Lewis antibodies that cross-react with the gastric mucosa, contributing to the development of gastric pathologies. However, sera from H. pylori-infected patients containing anti-canicular autoantibodies, despite intense binding to Le^+ or Le^+positive H. pylori strains, still express antigastic autoreactivity. It cannot be excluded that anti-Le^+ and anti-Le^+ antibodies occur naturally under physiological conditions because these antibodies are detected in the majority of H. pylori-uninfected individuals. However, the elevated levels of these antibodies have been detected more frequently in the sera of H. pylori-infected than uninfected individuals, which suggests that infections with these bacteria may promote specific anti-Le antibody production. Anti-Le^+ antibodies, depending on their isotype, may play a different role during H. pylori infections. The readiness of the host to respond to Le determinants using IgG, but not IgM, could be a risk factor for H. pylori-related pathologies. Anti-Le^+ IgM was found to facilitate the phagocytosis of Le^+positive, but not Le^+negative, strains, suggesting a protective role. In contrast, increased levels of anti-Le^+ and anti-Le^+ antibodies of the IgG class, either soluble or bound in immune complexes (ICs), might contribute to the pathogenesis of chronic H. pylori infections.
It is worth mentioning that ICs containing H. pylori antigens have been detected in the glomeruli of patients with membranous nephropathy and coexisting H. pylori infection. Le-anti-Le ICs are also suspected to play a role in the development or the maintenance of the inflammatory response in H. pylori-infected subjects with CHD. It has been shown on a murine model that immunization of animals with H. pylori induces anti-Le^+ antibodies that cross-react with the gastric epithelium, and particularly with the gastric H^+K^+ATPase, the proton pump localized to the parietal cell canaliculi. However, the cross-reactive antibodies are specifically directed toward peptidopeptide and not sugar, epitopes. In another study using a mouse model, it was shown that chronic H. pylori infection stimulated the production of antibodies interacting with the parietal cell canaliculi that were coupled with synthetic Le antigens. Recent studies have confirmed that anti-Le antibodies are present in most patients with H. pylori infection, including those with gastric cancer. The intensity of the anti-Le response is independent of the host Le phenotype but related to the bacterial Lewis phenotype. The role of anti-Le antibodies during H. pylori infections remains unclear. It has been suggested...
that \textit{H. pylori} anti-Le\textsuperscript{XV} antibodies are locally produced and rapidly bind to gastric mucosal epitopes. Thus, these antibodies do not appear in serum. In healthy humans, cellular barriers, innate immunity, and an efficient physiological clearance system work together to neutralize gastrointestinal LPS. For instance, nutritional lipids guarantee the maintenance of relatively low systemic levels of LPS\textsuperscript{[11]}\textsuperscript{[13]}\textsuperscript{[14]} However, evidence of increased permeability of the epithelial and endothelial barriers in the milieu of \textit{H. pylori} products, such as urease and VacA, may suggest the need for a change in the approach to \textit{H. pylori} LPS immunogenicity\textsuperscript{[15]}\textsuperscript{[16]}. In this case, the strong binding of cholesterol by \textit{H. pylori} surface components should also be taken into account\textsuperscript{[13]}\textsuperscript{[14]}.

**Key findings**

The role of autoimmune responses in the pathologies associated with \textit{H. pylori} infection must also be taken into consideration. The molecular mimicry involved in auto-reactivity might be directly induced by \textit{H. pylori} surface antigens, of which LPS is the most prominent cross-reactive candidate. Gastric pathologies can be induced by anti-Le antibodies that cross-react with Le determinants exposed on the gastric epithelium. Le-anti-Le ICs are also suspected to be associated with the maintenance of \textit{H. pylori}-related inflammatory responses.

**CONCLUSION**

The data summarized in this review support the suggestion that \textit{H. pylori} LPS evolved during the co-existence of these bacteria with their host, enabling the bacteria to “live in peace”. This phenomenon at least partially depends on the immunomodulatory properties of \textit{H. pylori} LPS and its influence on host cell barriers and the condition and responsiveness of immune cells. Many studies have shown that \textit{H. pylori} components may facilitate the colonization process, and especially the immune response of the host, during the course of \textit{H. pylori} infection. This \textit{H. pylori}-driven interaction might result from positive or negative modulation. Among the negative immunomodulators, a prominent position is occupied by a vacuolating toxin and CagA protein. However, in light of the recent studies presented in this review, it is necessary to enrich this panel with \textit{H. pylori} LPS. Together with CagA and VacA, this LPS suppresses the elimination of \textit{H. pylori} bacteria from the gastric mucosa by interfering with the activity of innate and adaptive immune cells. In particular, the LPS diminishes the inflammatory response and affects the adaptive T lymphocyte response, thus facilitating the development of chronic infections. The complex strategy of \textit{H. pylori} bacteria for surviving in the gastric mucosa of the host involves both structural modifications of LPS lipid A to diminish its endotoxic properties and the expression and variation of Lewis determinants, arranged in \textit{O}-specific chains of \textit{H. pylori} LPS. By mimicking host components, this phenomenon leaves these bacteria “invisible” to immune cells. Taken together, these mechanisms allow \textit{H. pylori} to survive and live for many years within their hosts.

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