LIPOPOLYSACCHARIDE A VIRULENCE FACTOR OF *HELICOBACTER PYLORI*: EFFECT OF ANTIULCER AGENTS

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*Helicobacter pylori* plays a major role in the pathogenesis of gastric disease. The gastric epithelial integrity is compromised by the *H. pylori* cell wall lipopolysaccharide untoward effect on the gastric epithelial cell receptors interaction with proteins of extracellular matrix, glycoproteins of mucus coat, and bioactive peptides. These interactions cause the weakening of the mucus coat rendering the underlying epithelium vulnerable to noxious luminal contents and disrupting the regulatory feedback of somatostatin and gastrin. Moreover, *H. pylori* lipopolysaccharide induces histologic lesions typical of acute gastritis and these changes are reflected in the increased epithelial cell apoptosis. These findings thus identify cell wall lipopolysaccharide as a virulent factor responsible for the *H. pylori* effect on gastric epithelium. The effect of antiulcer agents on the interference of lipopolysaccharide with the laminin receptor was found to be most efficiently countered by ebrotidine, sulglycotide and sucralfate, whereas sulglycotide is the most potent in the reversal of the inhibitory effect of the lipoplysaccharide on mucin receptor binding. In the case of somatostatin-receptor binding, sulglycotide followed by sucralfate and ebrotidine showed the most potency in of reversing the effect of *H. pylori* lipopolysaccharide. Thus these antiulcer agents have a great promise in the treatment gastric diseases associated with *H. pylori* infection.

**Key words:** *H. pylori*, lipopolysaccharide, epithelial surface receptors, antiulcer agents, apoptosis

**INTRODUCTION**

A decade after its discovery (1, 2), the role of *Helicobacter pylori* in the development of gastroduodenal disease has been finally recognized (3, 4). *Helicobacter pylori* is a microaerophilic Gram-negative, motile sinuous or S-shaped gently spiral bacteria, which has been shown to be the causative agent of active chronic gastritis in humans, and a major causal factor in the
development of peptic ulcer disease (5, 9). The presence of bacteria in stomach has been also strongly associated with gastric cancer (10, 11).

The precise mechanism of mucosal injury and ulceration is unclear. Infection of *H. pylori* markedly alters and damages the mucosa thus affecting mucus, epithelial cells, bicarbonate secretion, gastric blood flow, and gastric and somatostatin cell function (4, 5, 12—18).

A number of disease-inducing factors have been identified in *H. pylori*, including:

- Enzymes (urease, catalase, lipase, phospholipase, protease),
- Cytotoxin,
- Cag A protein,
- Chemotactic factor,
- Heat shock protein,
- Lipopolysaccharide (LPS).

Lipopolysaccharides are characteristic cell wall constituents of Gram negative bacteria which are vital for the structure and function of the outer membrane. LPS also provides the basis for serological classification, serves as a taxonomic marker, and is a potent toxin of the bacterium. Chemically, LPS of *H. pylori* is composed of a poly — or oligosaccharide and a lipid component, called lipid A. LPS exists in two forms, high-molecular-weight smooth, S-form and low-molecular-weight rough, R-form. The high molecular-weight form possesses O side chain, and is a polymer of repeating oligosaccharide units, a core oligosaccharide and lipid A. The low molecular weight form of the LPS differs from S-form by the lack of O side chains (19—25) (Fig. 1).

Moran *et al* (4, 19, 20, 25) in a series of studies characterized the structure of R-LPS in *H. pylori*. The structure of core R-LPS of *H. pylori* contains fucose, D-mannose, D-glucose, D-galactose, two heptoses and 3-deoxy-D-manno-2-octulosonic acid. However, although the composition of the core is conserved in different strains, the molar ratios of the hexoses differ between different strains of *H. pylori*, thereby reflecting structural differences (4, 6, 25). The lipid A in LPS of *H. pylori* contains D-glucosamine with a phosphate group at carbon-1 and fatty acids as well as ethanolamine. The major fatty acids in lipid A of *H. pylori* LPS are β-hydroxypalmitic acid (3-OH-16: O), β-hydroxystearic acid (3-OH-18: O) and stearic acid (18: O) with minor amounts of myristic acid (14: O) and palmitic acid (16: O) (19, 20, 26). The presence of relatively longer β-hydroxy fatty acids in lipid A is an unusual feature which distinguishes the LPS of *H. pylori* from that of other bacterial species (eg. *E. coli* and *Brucella spp.*) (6, 24, 26).

The structure of lipid A, in particular the phosphorylation pattern has been shown to be responsible for the low biological and immunological activities. Despite this property, LPS of *H. pylori* still has the ability to induce several
cytokines (TNFα, IL-1β, IL-8) which play important role in acute and chronic inflammation. The lower biological and immunological activity of the LPS may allow *H. pylori* for longer survival in the gastric mucosa and, hence may aid the establishment of chronic inflammation (4, 21). LPS of *H. pylori* possesses the ability to stimulated pepsinogen secretion (27), although not all the strains induced stimulations (28). Furthermore, all fresh clinical isolates of *H. pylori* produce S-form of LPS, whereas strains which have been grown in vitro produce R-form LPS and, it was shown that in vitro culture on solid media can result in the loss of the O-specific chain and produce R-form of LPS. This loss can be prevented when strains are grown in liquid media (19, 25).

Polysaccharide O-chain of *H. pylori* LPS was extensively studied by Aspinall *et al* (29, 30) who showed that O-side chain of S-form LPS is composed mainly of fucosylated and nonfucosylated N-acetyllactosamine units, and that LPS bears some structural similarity with Lewis*-like epitope of human cell surface glycoconjugates. LPS can also bind to various extracellular matrix proteins which are component of the basement membrane (3).

This review discusses the role of *H. pylori* cell wall lipopolysaccharide in the pathogenesis of mucosal injury and ulceration, and the effect of antiulcer agents on this process.
Adherence of microorganism to host tissue is a prerequisite for colonization and development of infections and is dependent on the expression and availability of complementary molecules on both the bacterial and host cell surfaces. The integrity of gastric epithelium is maintained mainly through basement membranes. That are thin sheetlike structures that form a highly specialized part of the extracellular matrix located in the immediate proximity of the surface organ cells such as epithelial, endothelial and mesenchymal cells where they meet with the underlying interstitial connective tissue (31,32).

The basement membrane is composed of several distinct proteins, some of which are specific for these structure, such as type IV collagen, laminin, heparan sulfate proteoglycan, fibronectin and enactin. These glycoproteins and proteoglycans are known to mediate cell attachment via specific cell-surface receptors termed integrins (33, 34). These receptors in general are composed of a β-subunit that is noncovalently linked or attached to a distinct α-subunit. Both α and β-subunits are type I transmembrane glycoproteins with large amino-terminal extracellular extension, a short hydrophobic transmembrane sequence, and a short carboxy-terminal cytoplasmic domain (35).

The most abundant component of specialized extracellular matrices basement membranes is laminin. Laminin, a high-molecular weight glycoprotein (about 890—1,000 kDa) is synthesized by a variety of cell types which include endothelial, epithelial, muscle and is involved in numerous biological functions (36, 37). By interacting with the components of basement membranes such as collagen type IV (38—40), and heparan sulfate proteoglycans (41) as well as with itself (42—44), laminin plays a crucial role in determining the structure of basement membrane. Laminin also has the ability to interact with a variety of cell types through cell surface constituents, such as the 67-kDa laminin receptor (45—47), the integrin proteins, and sulfated glycolipids (48—50). Through these interactions, laminin promotes cell adhesion, growth, morphology, migration, agglutination, metastasis, and neurite outgrowth. Several surface exposed proteins of bacteria that bind laminin have been shown to exist on S. pyogenes, E. coli and S. aureus (51—54).

The property of gastric mucosa and its ability to withstand insults by luminal contents is multicomponential in nature, and includes mucus-bicarbonate zone, cell membranes of gastric epithelium, and mucosal blood flow (55, 56). The integrity of this defenses zone is controlled by the factors affecting the synthesis, secretion and breakdown of its constituents (57—60). While this equilibrium is maintained successfully under normal physiological conditions, injury to mucosa results when aggressive forces overcome the factors that control mucosal defences. The natural niche of H. pylori is the human stomach, where it colonizes the mucus and adheres to gastric mucosal cells, especially at the intracellular junctions (61, 62). The ability of H. pylori to penetrate the mucus results from the
flagella activity and the microbial activity through elaboration of several enzymes to facilitate its movement through the mucus. Among these enzymes are protease, lipase and phospholipase A<sub>2</sub> (63—66). Enzymes and cytotoxins produced by <i>H. pylori</i> are capable of weakening the interaction between the mucosal cells and the proteins of the intracellular matrix as well as the mucus coat, causing cellular destruction and exposure of the underlying basement membrane for colonization by <i>H. pylori</i> (62).

Ours early studies on <i>H. pylori</i> in vitro showed that LPS is the main component responsible for weakening of the tissue cell integrity (67). The studies were conducted with LPS isolated from <i>H. pylori</i>. Laminin receptor was isolated from gastric epithelial cell membrane solubilized with octylglucoside followed by affinity chromatography on laminin-coupled Sepharose. The receptor protein eluted from the matrix with cation-free EDTA buffer, yielded on SDS-PAGE a single 67 kDa band. Following radioiodination, the protein was incorporated into liposomes which displayed specific affinity toward the laminin coated surface.

The finding that the isolated protein is a laminin receptor was supported by the fact that the protein bound to laminin-affinity column and was displaced by EDTA following elution of fibronectin with GRGDSP peptide (68). Furthermore, the protein exhibited properties of a membrane embedded receptor since it was easily susceptible to detergent solubilization and the incorporation into the lipid vesicles (69,70). The affinity of 67 kDa receptor protein for lipid bilayers, its cell surface localization, and the capacity to interact specifically with laminin indicated that the bound protein is indeed a gastric mucosal laminin receptor possessing features similar to laminin receptors of other tissues which bind laminin through Y1GSR peptide (71, 72). Assays of LPS from <i>H. pylori</i>, using gastric mucosal laminin receptor binding to laminin-coated surface, showed that a significant decrease in receptor binding occured in the presence of <i>H. pylori</i> LPS, a 81% inhibition at 10 µg/ml in the laminin-receptor binding, with maximum value of 96% at 50 µg/ml (Fig. 2) and (Fig. 3).

![Fig. 2. Effect of <i>H. pylori</i> lipopolysaccharide on the binding of laminin receptor-containing liposomes to laminin coated surface. The liposomes containing [125I] labeled receptor protein or the laminin-coated wells were preincubated for 30 min at room temperature with different concentrations of lipopolysaccharide (0—150 µg/ml) and then assayed for liposomes binding (67).](image-url)
Effect of antiulcer agents on the interaction between mucosa laminin receptor and *H. pylori* lipopolysaccharide

Since *H. pylori* remains in intimate contact with mucous cell surface (73—76), it is most likely that bacteria through its lipopolysaccharide disrupts the epithelial cell laminin receptor interaction with extracellular matrix laminin in the stomach. As a consequence, this leads to the loss of cellular integrity and weakening of gastric mucosal defence. Therefore, agents capable of interfering with untoward effects of *H. pylori* may be of value in the treatment of gastric disease associated with *H. pylori* infection. Among the antiulcer agents exhibiting these properties are new drugs which not only strengthen the mucosal integrity (77—80) but also display anti-*H. pylori* action (81—86). Most potent among these appears to be ebrotidine (87), which evoked essentially
complete restoration in binding of laminin to its mucosal receptor (97% at 8 µg/ml dose) followed by sulglycotide (88), the inhibitory effect of which reached the value of 92% at 40 µg/ml, and sucralfate where a 94.1% restoration in laminin binding occurred when sucralfate at 45 µl/ml was introduced to the assay system (69), (Tab. 1).

Table 1. Effect of gastroprotective agents on the inhibition of gastric mucosal laminin receptor by H. pylori lipopolysaccharide.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Optimal concentration (µg/ml)</th>
<th>Laminin-receptor binding restoration (%)</th>
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<tbody>
<tr>
<td>Ebrotidine</td>
<td>8.0</td>
<td>97.0</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>45.0</td>
<td>94.1</td>
</tr>
<tr>
<td>Sulglycotide</td>
<td>40.0</td>
<td>92.0</td>
</tr>
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</table>

Ref. 69, 87, 88

With all agents, preincubation the lipopolysaccharide prior to laminin-receptor binding assay led to nearly complete restoration in the binding of laminin receptor. Thus, these agents clearly demonstrate the ability to render the lipopolysaccharide factor of H. pylori virulent action ineffective.

However, the mechanism involved in the interaction between H. pylori LPS and laminin appears to be complex. Laminin receptor, possesses two binding sites, one for protein and another for carbohydrate that interacts with galactoside sugars. Laminin binds with high affinity to the receptor at the protein binding site. Binding of carbohydrate at the sugar binding site, however lowers the affinity at the protein binding site resulting in release of bound protein (47). Thus the structure of lipopolysaccharide containing carbohydrate moiety potentially interacts with the carbohydrate binding site of the laminin receptor and blocking binding of laminin.

**LIPOPOLYSACCHARIDE AND MUCIN**

Gastric mucins are recognized as an essential component of mucosal defence against a variety of luminal insults. Affinity of mucins toward gastric epithelium has been recognized for long as a prerequisite for mucus coat formation. The maintenance of the integrity of this systems depends upon the tenacity of interaction between the epithelial cell surface and the mucin components of the mucus layer. This interaction has been thought to occur through a variety of hydrophobic, hydrogen and ionic bonds involving both glycosylated and nonglycosylated regions of mucus glycoprotein molecule and the surface of epithelium (89—91).
Our studies, provided evidence that the interaction of mucin with gastric epithelium involves a specific cell surface receptor. The receptor protein from the gastric mucosal cell membrane displays an apparent molecular weight of 97 kDa and shows specific affinity towards surface coated with gastric mucin. The interaction between mucin and the receptor has been shown to require the presence of carbohydrate chains in mucin, as deglycosylation of the mucin caused a drastic reduction of 87% in binding (92). Since *H. pylori*, through its cell-wall lipopolysaccharide has been shown to interfere with gastric mucin synthesis and the interaction between the mucosal cells and the proteins of extracellular matrix, (93, 105) the effect of this bacterial LPS on the interaction of mucin with its mucosal receptor was assessed. The data revealed that the binding of mucin to the receptor was, indeed susceptible to the interference by *H. pylori* LPS (94). This inhibitory effect was dose dependent and attained a maximum 91% at 30μg/ml of LPS from *H. pylori* (Fig. 4).

![Fig. 4. Effect of *H. pylori* lipopolysaccharide on the binding of mucin to its gastric epithelial cell membrane receptor. The [125I] labeled receptor protein (3μg) was preincubated for 1h at room temperature with different concentrations of lipopolysaccharide (0—80μg/ml) and then assayed for mucin binding. The effect of lipopolysaccharide at concentration of 10μg/ml and above was significant at p>0.05 (94).](image)

The results from this study provide strong evidence that the interference by *H.pylori* LPS with receptor-binding site for mucin could account for the alterations in mucus coat with *H. pylori* infection, such as loss of mucus gel continuity, its patchy appearance and reduction in thickness (95—97).

*Effect of antiulcer agents on the interaction between mucin mucosal receptor and *H. pylori* lipopolysaccharide*

Since the disruption of the interaction between mucin and its epithelial cell surface receptor by *H. pylori* leads to the loss of the pre-epithelial element of mucosal defence, agents capable of counteracting this disruptive effect of *H. pylori* LPS may be of value in the treatment of gastric disease associated with this bacterial infection. Studies into the effect of antiulcer agents on the
inhibition of mucin-mucosal receptor interaction by *H. pylori* LPS provide strong evidence that sulglycotide, meets this criterion as it possesses the ability to render the *H. pylori* LPS ineffective. The results indicate that sulglycotide when preincubated with LPS caused reversal of the inhibitory effect on mucin-receptor binding. This effect was dose dependent and nearly complete restoration 94% in mucin-receptor binding occured at 50 μg/ml sulglycotide (Fig. 5, 6). These findings demonstrated the effectiveness of sulglycotide in preventing the loss of mucus coat continuity disrupted by *H. pylori* and a great promise in the treatment disease associated with *H. pylori* infection (98).

Fig. 5. Effect of sulglycotide on binding of mucin to its gastric epithelial cell membrane receptor in the presence and absence of *H. pylori* lipopolysaccharide. Sulglycotide (0—70 μg/ml) was first incubated for 1 h at room temperature with lipopolysaccharide (30 μg) followed by 1 h incubation with mucin receptor and then assayed for mucin binding using mucin-coated (5 μg/50 μl) nitrocellulose discs. Following washing, the bound [125I]-labeled receptor was quantitated in a gamma counter (98).

We also conducted studies on the effect of this drug on the interaction of *H. pylori* LPS with mucin and its mucosal receptor. The results obtained from those experiments showed that sucralfate is capable of counteracting the disruptive effect of LPS on epithelial cell membrane mucin receptor. The restoration effect of sucralfate on mucin-receptor binding was found to be dose-dependent and reached a maximum 75% at 60 μg/ml dose of the drug (99) (Fig. 7). Ebrotidine, a new H₂-receptor antagonist with many features similar to ranitidine and cimetidine (100), yet in contrast to ranitidine and cimetidine, displays gastroprotective effects through stimulation of mucosal blood flow, and enhancement of physicochemical properties of gastric mucus glycoprotein (79, 101—104). The results from ours study showed that the effect of ebrotidine was dose dependent and at its optimum concentration of 60 μg/ml, a 51% restoration in mucin-receptor binding was achieved (105), (Fig. 8). Thus sulglycotide, sucralfate and ebrotidine *in vivo* may be able to prevent the loss of mucus coat occurring in gastric disease associated with *H. pylori* infection.
**Fig. 6.** Mechanism of the reversal of interference by *Helicobacter pylori* with mucin-gastric mucosal receptor interaction by sulglycotide.

**Fig. 7.** Effect of sucralfate on the binding of mucin to its gastric epithelial cell membrane receptor in the presence of *H. pylori* lipopolysaccharide. Sucralfate (0—100 µg/ml) was first incubated for 1 h at room temperature with lipopolysaccharide (30 µg/ml) followed by 1 h incubation with mucin receptor and then assayed for mucin binding using mucin-coated (5 µg/50 µl) nitrocellulose discs (99).
Fig. 8. Effect of ebrotidine on the binding of mucin to its gastric epithelial cell membrane receptor in the presence of *H. pylori* lipopolysaccharide. Ebrotidine (8—80 μg/ml) was first incubated for 1 h at room temperature with lipopolysaccharide (30 μg/ml) followed by 1 h incubation with mucin receptor and then assayed for mucin binding. The effect of ebrotidine at concentration of 60 μg/ml and above was significant at \( p > 0.05 \) (105).

**LIPOPOLYSACCHARIDE AND SOMATOSTATIN RECEPTOR**

Somatostatin is a biologically active peptide secreted by antral mucosal C-cells and exerts its effect on gastrin release by the G-cells (106—108). The clinical data indicate that the infection by *H. pylori* is accompanied by enhanced gastrin release and hypergastrinemia due to the impairment in feedback inhibition by somatostatin (109, 110).

However, mechanism by which *H. pylori* infection results in increased serum gastrin is not known. Several explanation to this point exist. Haruma *et al* (109) suggested, that for this event is responsible lower D-cell density or D:G cell ratio in the antral mucosa, but results reported by Graham *et al.* (107) who have shown that eradication of *H. pylori* does not change D-cell number or D:G ratio and suggested that the hypergastrinemia in *H. pylori* infection relates to the functional activity of G-cells. Queizor *et al.* (13) shown in antral biopsy specimens from patients with duodenal ulcer infected by *H. pylori*, that eradication of *H. pylori* increased significantly somatostatin concentration, however number of G-cell was unchanged, level of gastrin after eradication decreased significantly, and suggested that the function, but not the number of D-cell could be affected by *H. pylori* infection. The recent study by Konturek *et al.* (110) also shown that in patients *H. pylori*-positive basal luminal level of somatostatin was significantly lower to compared to the control group, and eradication of *H. pylori* restored the level of somatostatin.

The results from our study provide evidence that *H. pylori*, through LPS, interferes with the binding of somatostatin to its specific mucosal cell membrane receptor, and this receptor is adversely affected by *H. pylori* LPS (111).

The gastric somatostatin receptor, isolated from solubilized mucosal cell membranes by affinity chromatography on Affi-Gel-bound (D-Trypt 8)
SRIF-14, was found to display an apparent molecular weight of 61 kDa, bond specifically to SRIF-14, and showed a particular sensitivity to the interference by *H. pylori* LPS. The inhibitory effect of the LPS on somatostatin-receptor binding, as demonstrated by preincubation experiments, results from the interaction of LPS with the receptor protein rather than somatostatin, and attained maximum of 94.1% inhibition at 50 µg/ml of LPS (111) (Fig. 9). Thus, the observed interference by LPS with the receptor-binding site for somatostatin could account for the reported loss of regulatory feedback from D-cells to G-cells with *H. pylori* infection (7, 13, 112).

![Fig. 9. Effect of *H. pylori* lipopolysaccharide on the binding of [125I-Tyr11] SRIF to gastric mucosal somatostatin receptor. The receptor protein (8 pg) samples were preincubated for 30 min at 37°C with different concentrations of lipopolysaccharide (0—100 µg/ml) and then assayed for SRIF binding (111).](image)

**Effect of antiulcer agents on the interaction between mucosa somatostatin receptor and *H. pylori* lipopolysaccharide**

Drugs assessed for the interference by *H. pylori* LPS with the somatostatin binding site on its mucosal receptor include ebrotidine, a new H₂-blocker with gastroprotective and anti-*H. pylori* properties (77, 100—103), sulglycotide, a potent cytoprotective agent derived from gastric mucins by process of carbohydrate chain sulfation (124), and sucralfate, a basic aluminium salt of sucrose octasulfate, showed that these agents are capable of counteracting the disruptive effects of *H. pylori* LPS on somatostatin-receptor binding (113, 114). The results revealed that preincubation of the LPS with those agents prior to the receptor-somatostatin binding assay caused a dose-dependent reversal of the inhibitory effect of *H. pylori* LPS on the binding. The rate of restoration in receptor-somatostatin binding was proportional to drugs concentration. The reversal of the inhibitory effect of *H. pylori* LPS was attained a maximum of 84% at 20 µg/ml of ebrotidine, 92.5% at 70 µg/ml of sucralfate and 94.9% at 50 µg/ml of sulglycotide (Tab. 2.). Thus, these properties of ebrotidine, sucralfate and sulglycotide offer a great potential in the treatment of gastric disease associated with infection by *H. pylori*. 
Table 2. Effect of gastroprotective agents on the inhibition of gastric somatostatin receptor by *H. pylori* lipopolysaccharide.

<table>
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<tr>
<th>Agent</th>
<th>Optimal concentration (µg/ml)</th>
<th>Somatostatin-receptor binding restoration (%)</th>
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<tbody>
<tr>
<td>Sucralfate</td>
<td>70.0</td>
<td>92.5</td>
</tr>
<tr>
<td>Sulglycotide</td>
<td>50.0</td>
<td>94.9</td>
</tr>
<tr>
<td>Ebrotidine</td>
<td>20.0</td>
<td>84.0</td>
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Ref. 113, 114.

LIPOPOLYSACCHARIDE AND APOPTOSIS

Apoptosis is an essential part of the cycle of cellular turnover in many tissues, including the gastrointestinal tract. In contrast to necrosis, apoptosis occurs in single cells in response to the expression of specific cellular genes (115) and cytokines (116—118). Tissue integrity is maintained when the rate of cell loss by apoptosis is balanced by the rate of new cells production by proliferation. However, change in the rate of apoptotic cell loss may contribute to disease characterized by abnormalities of tissue growth (117).

In *H. pylori* infection there is increased epithelial proliferation and little or no necrotic cells death. Moss *et al.* (119) showed that the increased cell proliferation in *H. pylori*-associated gastritis is accompanied by an increased epithelial apoptosis. Jones *et al.* (120) also demonstrated similar findings in the mucosal biopsy specimens from patients with *H. pylori*-associated gastritis.

Since *H. pylori* induced gastritis appears to abrogate the processes associated with cell cycle progression, cellular proliferation, and programmed cell death (118, 121, 122), we have explored further the effect of *H. pylori* LPS on the gastric epithelial cell apoptosis and gastritis. The data from our experiments showed that the virulence factor responsible for the induction of apoptosis by *H. pylori* is a cell wall component lipopolysaccharide (123).

The studies were conducted with groups of rats subjected to intragastric surface epithelial application of LPS at 50 µg and 200 µg/per animal. The histological assessment of the mucosal tissue and the apoptotic epithelial cell index was performed on two and ten days after the LPS treatment. Histological examination of the mucosa showed infiltration of lamina propria with lymphocytes and the edema of plasma cells with hyperemia extending from the lamina propria to the surface of the mucosa. The mean grade of gastritis on 2 days after the 50 µg LPS application was 4.0 ± 0.3 and after 10 days decreased to 3.1 ± 0.4 whereas in the case of 200 µg dose of LPS the mean grade was 5.6 ± 0.6 after 2 days and 5.7 ± 0.5 at 10th day (Fig. 10). The detection of gastric epithelial cell apoptosis, carried out using in situ DNA fragmentation assay, in which apoptotic cells were identified in the fixed
mucosal sections, was by a direct immunoperoxidase detection of digoxigenin-labeled genomic DNA. The results of microscopic assessment showed only occasionally the presence of apoptotic cells in the surface epithelium from the control animals (Fig. 11A), whereas in the *H. pylori* lipopolysaccharide-treated animals numerous apoptotic cells were identified not only in the superficial epithelium but also deeper in the glands.

Fig. 10. Effect of *H. pylori* lipopolysaccharide on the acute gastritis scores in rats subjected to intragastric surface epithelial application of the lipopolysaccharide at 50 µg (A) and 200 µg (B) dose and examined 2 and 10 days after the treatment (123).
Fig. 11. Gastric mucosa epithelial cell apoptosis shown with the aid of direct immunoperoxidase detection of digoxigenin-labeled genomic DNA. Gastric mucosa at 2 (B) and 10 (C) days after the intragastric surface epithelial application of *H. pylori* lipopolysaccharide at a 50 µg (B) and a 200 µg (C) dose. (A) - normal gastric mucosa (control). (Magnification, ×200) (123).
The mean apoptotic index in the mucosal sections obtained from the controls was 0.32%, which increased dramatically to 59% after 2 days with 50 µg LPS dose and 71.9% with 200 µg dose (Fig. 12). The mean apoptotic index on the 10th day after LPS application averaged 46% with 50 µg dose (Fig. 12A), whereas a value of 76.8% was assessed in the sections from animals treated with the 200 µg dose of LPS (Fig. 12B). The data of apoptotic index and LPS induced inflammatory changes showed a correlation between the apoptotic cell number and the degree of histologic lesions typical of acute gastritis \( r = 0.71 \) (\( p < 0.004 \)) (Fig. 13).

These findings thus identify the cells wall lipopolysaccharide, is a causative factor responsible in \( H. \) pylori induced gastric epithelial apoptosis. Thus the \( H. \) pylori lipopolysaccharide is a major virulent factor which plays a crucial role in the pathogenesis fo \( H. \) pylori-associated gastric disease.
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