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Review article

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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 underying 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 myrystic 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

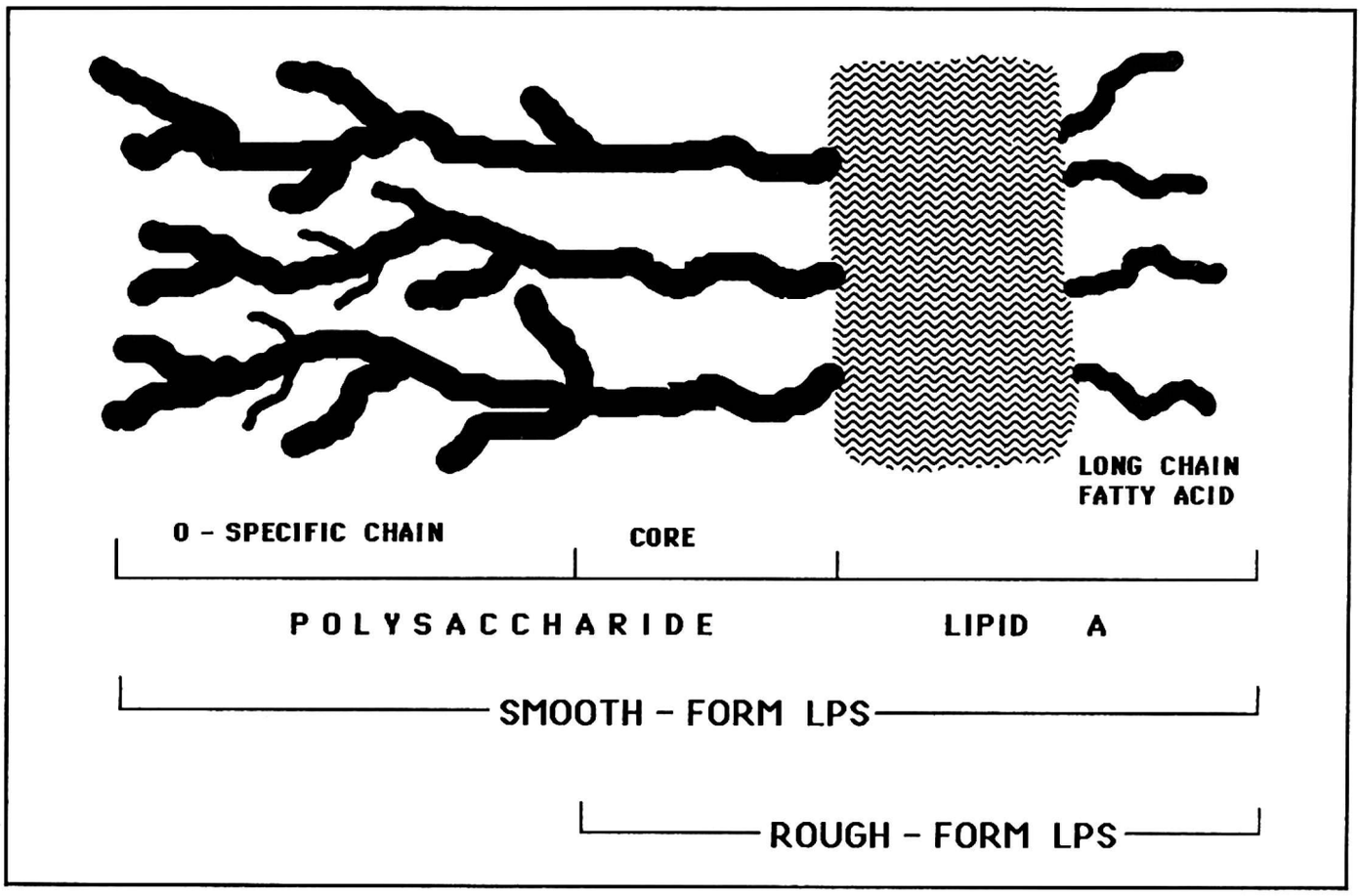


Fig. 1. Structural features of *Helicobacter pylori* lipopolysaccharide (LPS)

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^x-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.

LIPOPOLYSACCHARIDE AND LAMININ

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_2 (63—66). Enzymes and cytotoxins produces by *H. pylori* 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 *H. pylori* (62).

Ours early studies on *H. pylori* 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 *H. pylori.* 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 *H. pylori,* using gastric mucosal laminin receptor binding to laminin-coated surface, showed that a significant decrease in receptor binding occured in the presence of *H. pylori* 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).

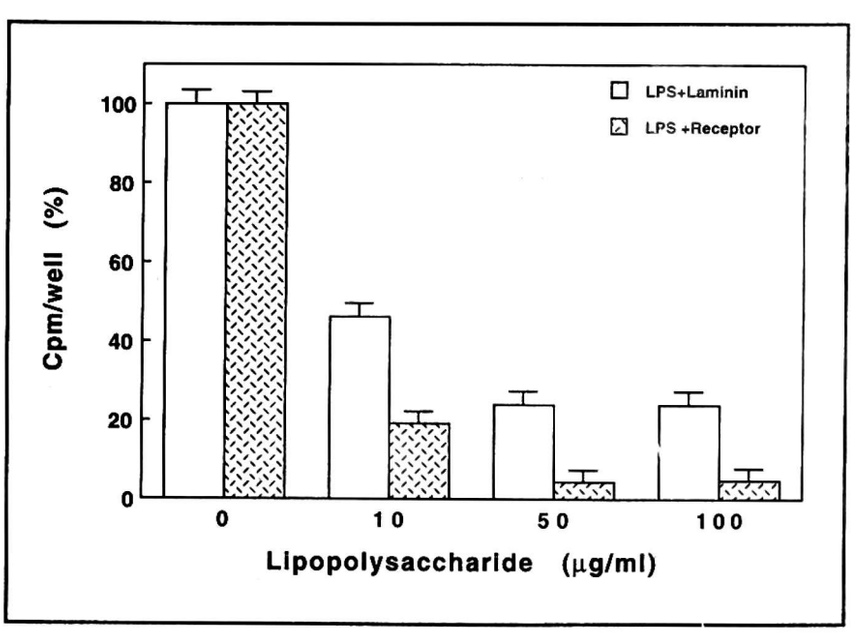


Fig. 2. Effect of *H. pylori* lipopolysaccharide on the binding of laminin receptor-containing liposomes to laminin coated surface. The liposomes containing [^{125}I] labeled receptor protein or the laminin-coated wells were preincubated for 30 min at room temperature with different concentrations of lipopolysaccharide (0—150 μg/ml) and then assayed for liposomes binding (67).

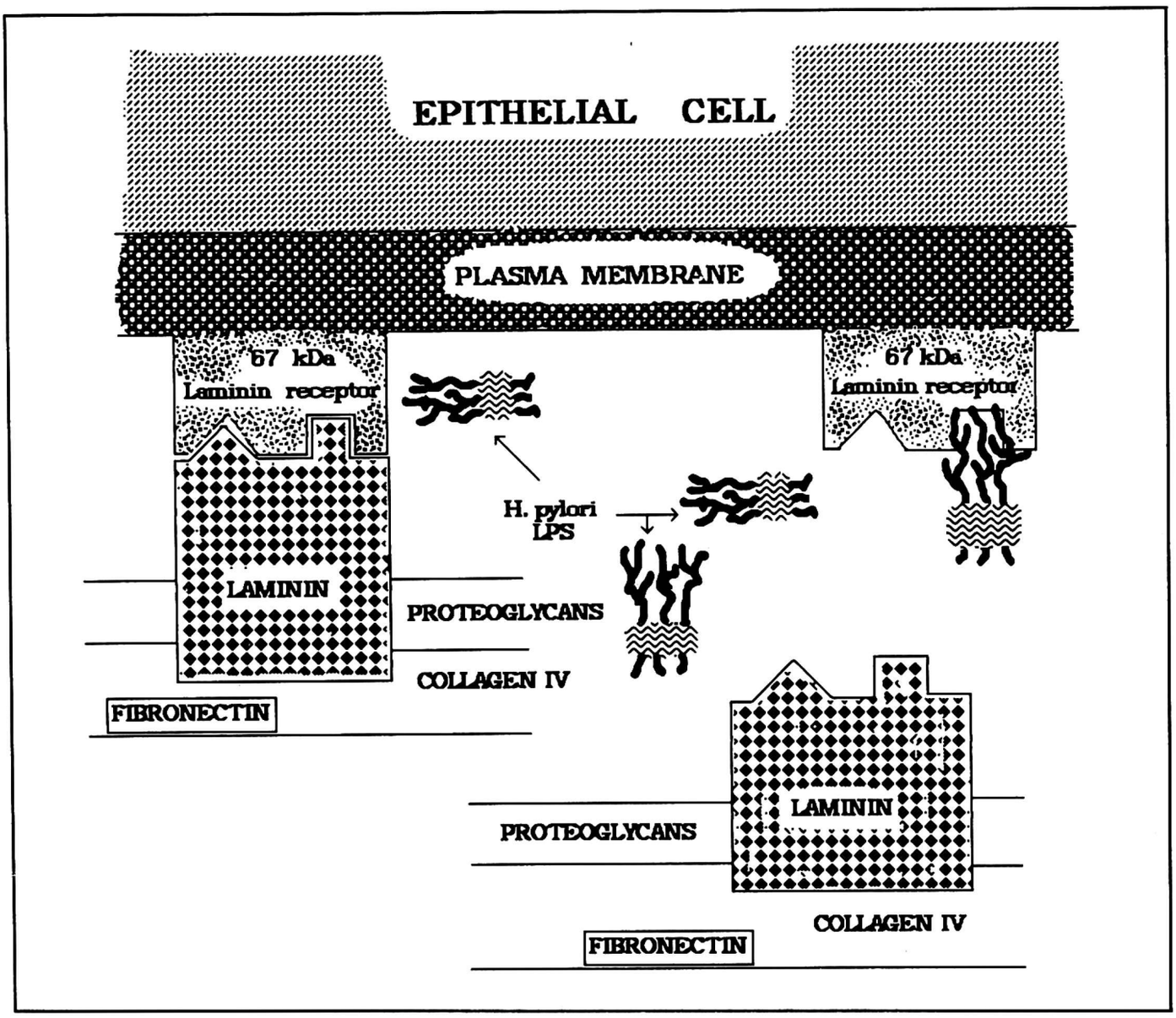


Fig. 3. Model of hypothetical interaction of *H. pylori* lipopolysaccharide with surface receptor proteins. Lipopolysaccharide interacts with laminin receptors and blocking binding of laminin leading to disruptive basement membrane.

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.

Agent	Optimal concentration (μg/ml)	Laminin-receptor binding restoration (%)
Ebrotidine	8.0	97.0
Sucralfate	45.0	94.1
Sulglycotide	40.0	92.0

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 show 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 30 μg/ml of LPS from *H. pylori* (*Fig. 4*).

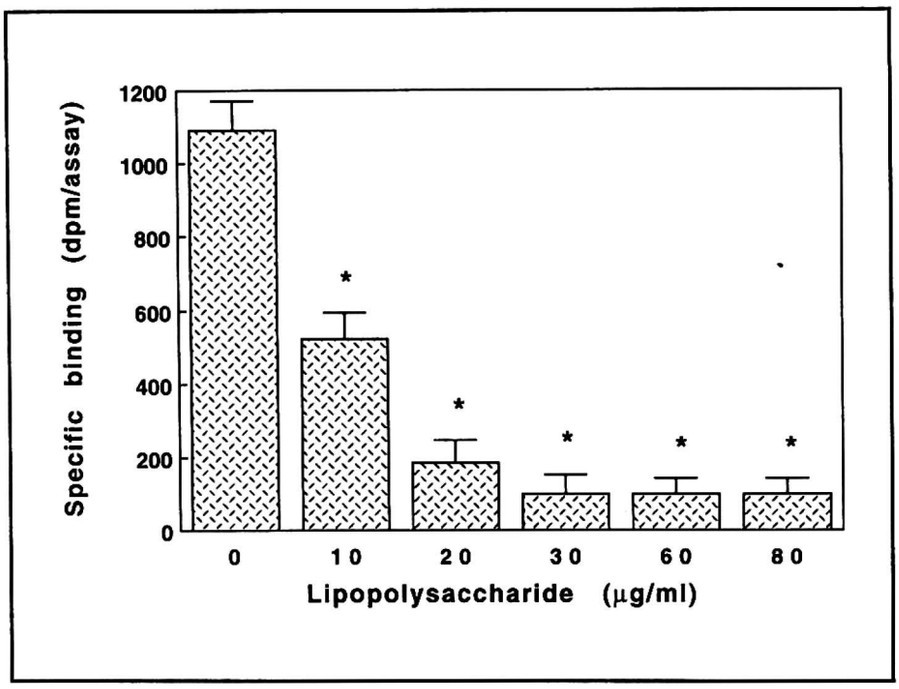


Fig. 4. Effect of *H. pylori* lipopolysaccharide on the binding of mucin to its gastric epithelial cell membrane receptor. The [^{125}I] labeled receptor protein (3 μg) was preincubated for 1 h at room temperature with different concentrations of lipopolysaccharide (0—80 μg/ml) and then assayed for mucin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

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).

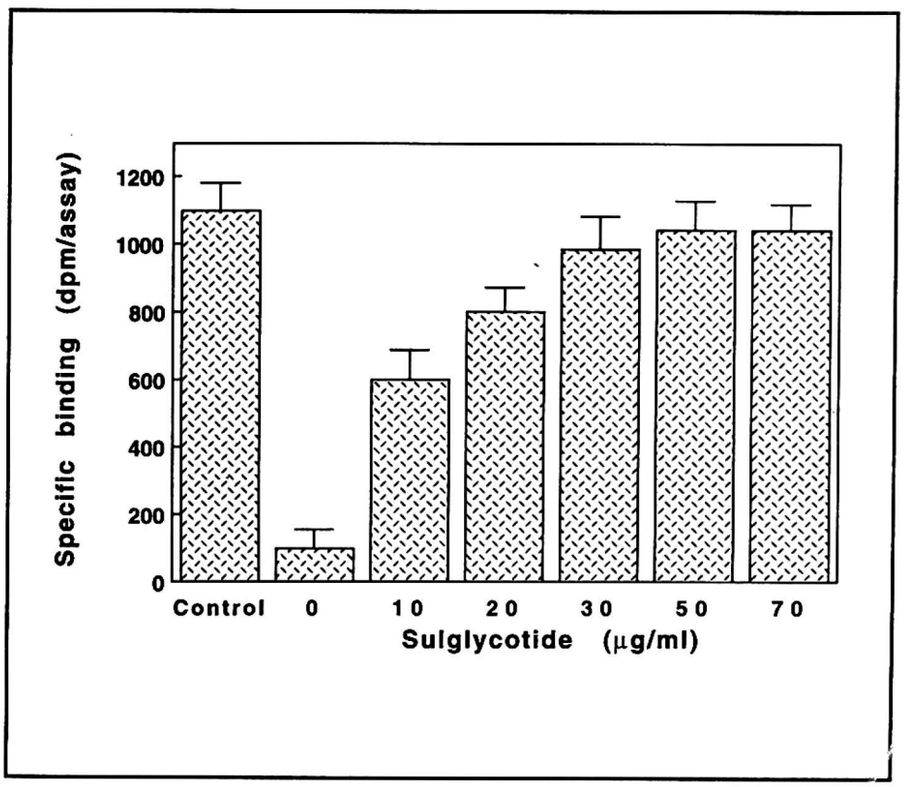


Fig. 5. Effect of sulglycotide on binding of mucin to its gastric epithelial cell membrane receptor in the presence and absence of *H. pylori* lipopolysaccharide. Sulglycotide (0—70 μg/ml) was first incubated for 1 h at room temperature with lipopolysaccharide (30 μg) followed by 1 h incubation with mucin receptor and then assayed for mucin binding using mucin-coated (5 μg/50 μl) nitrocellulose discs. Following washing, the bound [^{125}I]-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 H_2-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.

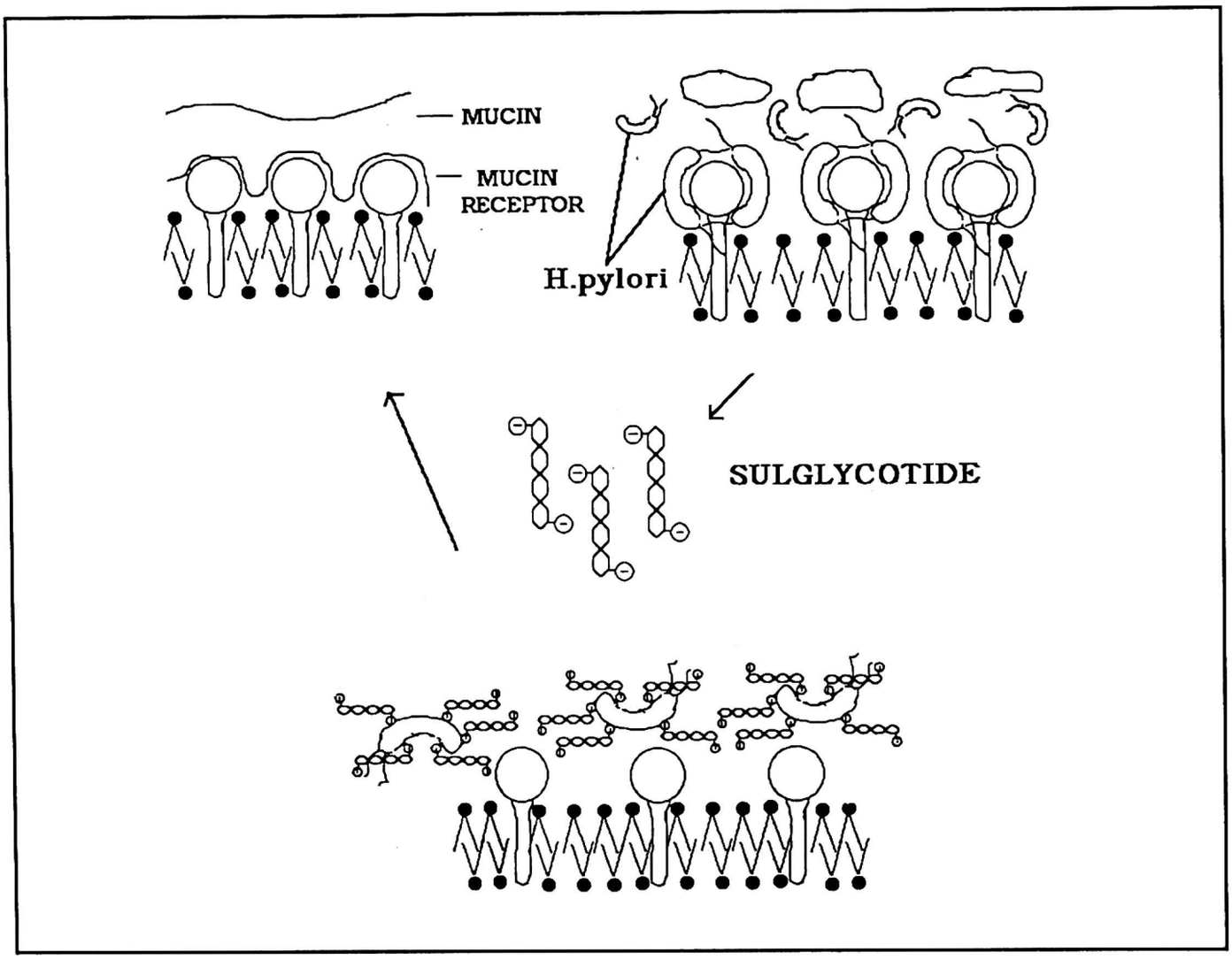


Fig. 6. Mechanism of the reversal of interference by *Helicobacter pylori* with mucin-gastric mucosal receptor interaction by sulglycotide.

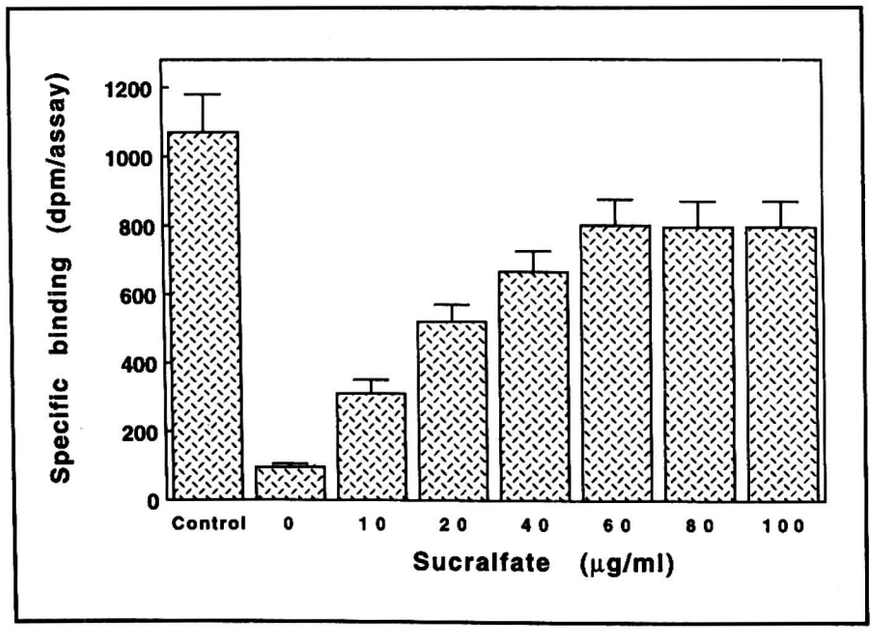


Fig. 7. Effect of sucralfate on the binding of mucin to its gastric epithelial cell membrane receptor in the presence of *H. pylori* lipopolysaccharide. Sucralfate (0—100 μg/ml) was first incubated for 1 h at room temperature with lipopolysaccharide (30 μg/ml) followed by 1 h incubation with mucin receptor and then assayed for mucin binding using mucin-coated (5 μg/50 μl) nitrocellulose discs (99).

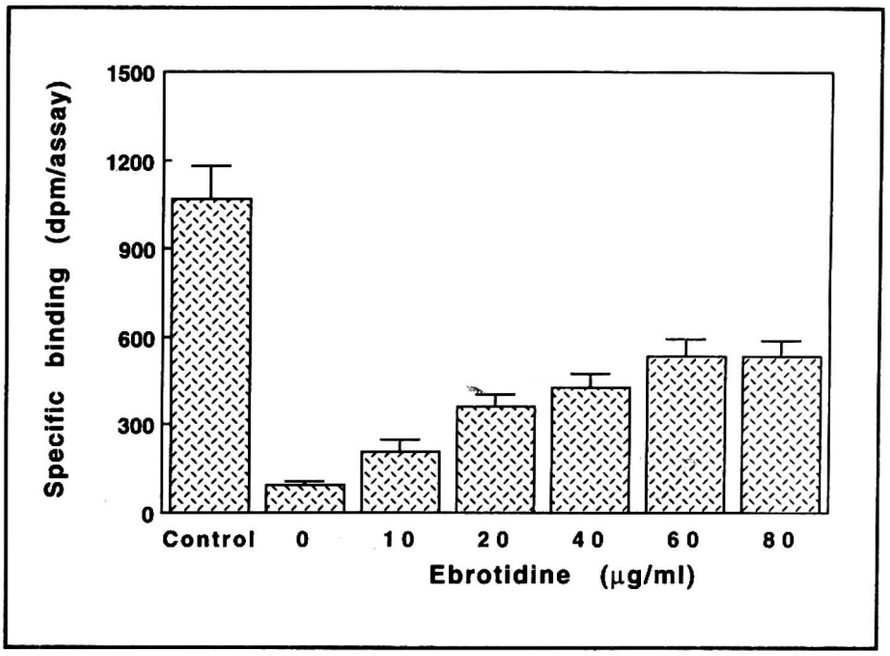


Fig. 8. Effect of ebrotidine on the binding of mucin to its gastric epithelial cell membrane receptor in the presence of *H. pylori* lipopolysaccharide. Ebrotidine (8—80 μg/ml) was first incubated for 1 h at room temperature with lipopolysaccharide (30 μg/ml) followed by 1 h incubation with mucin receptor 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 *at 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 *at 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 *at 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-Tryp 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).

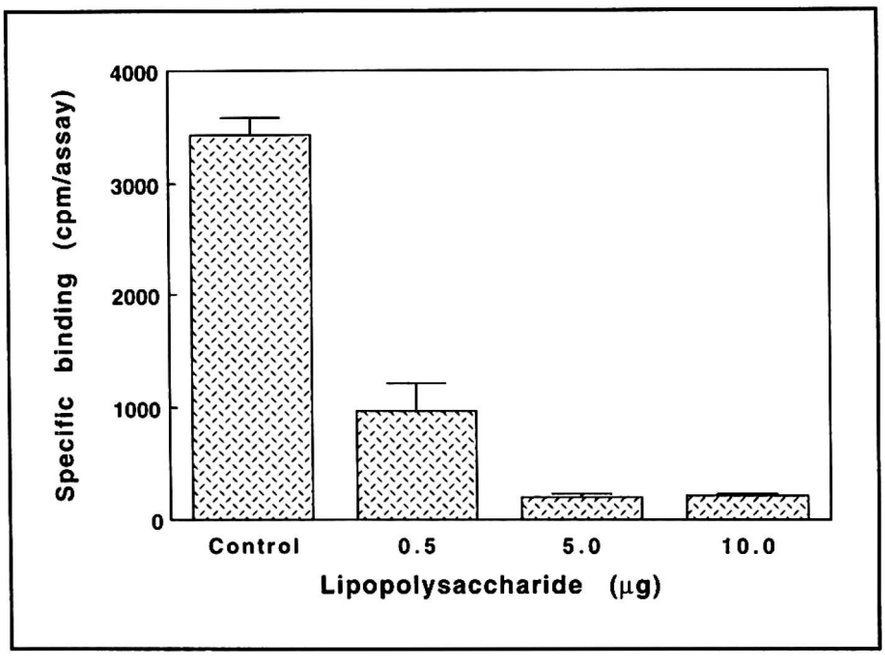


Fig. 9. Effect of *H. pylori* lipopolysaccharide on the binding of [^{125}I-Tyr11] SRIF to gastric mucosal somatostatin receptor. The receptor protein (8 μg) samples were preincubated for 30 min at 37°C with different concentrations of lipopolysaccharide (0—100 μg/ml) and then assayed for SRIF binding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

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 H_2-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.

Agent	Optimal concentration (μg/ml)	Somatostatin--receptor binding restoration (%)
Sucralfate	70.0	92.5
Sulglycotide	50.0	94.9
Ebrotidine	20.0	84.0

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 cytokins (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 *at al.* (119) showed that the increased cell proliferation in *H. pylori*-associated gastritis is accompanied by an increased epithelial apoptosis. Jones *at 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

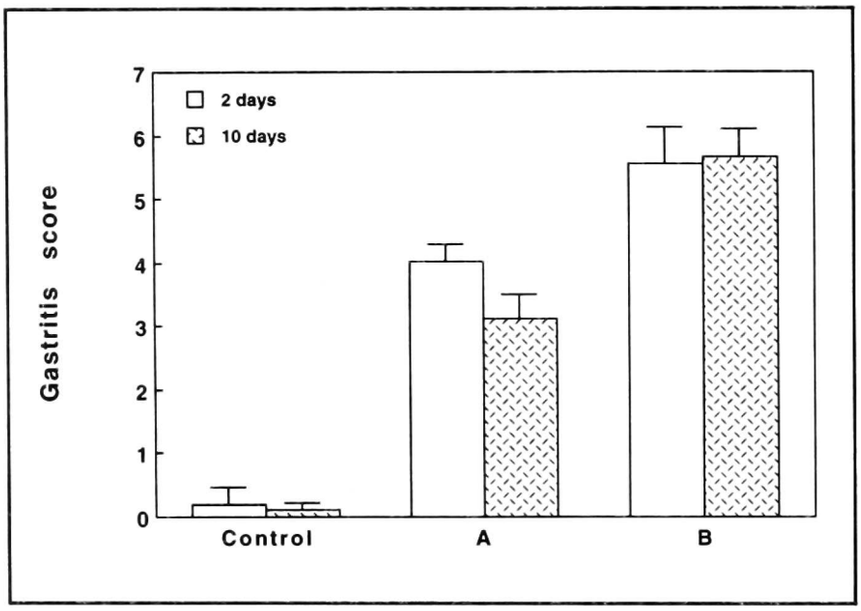
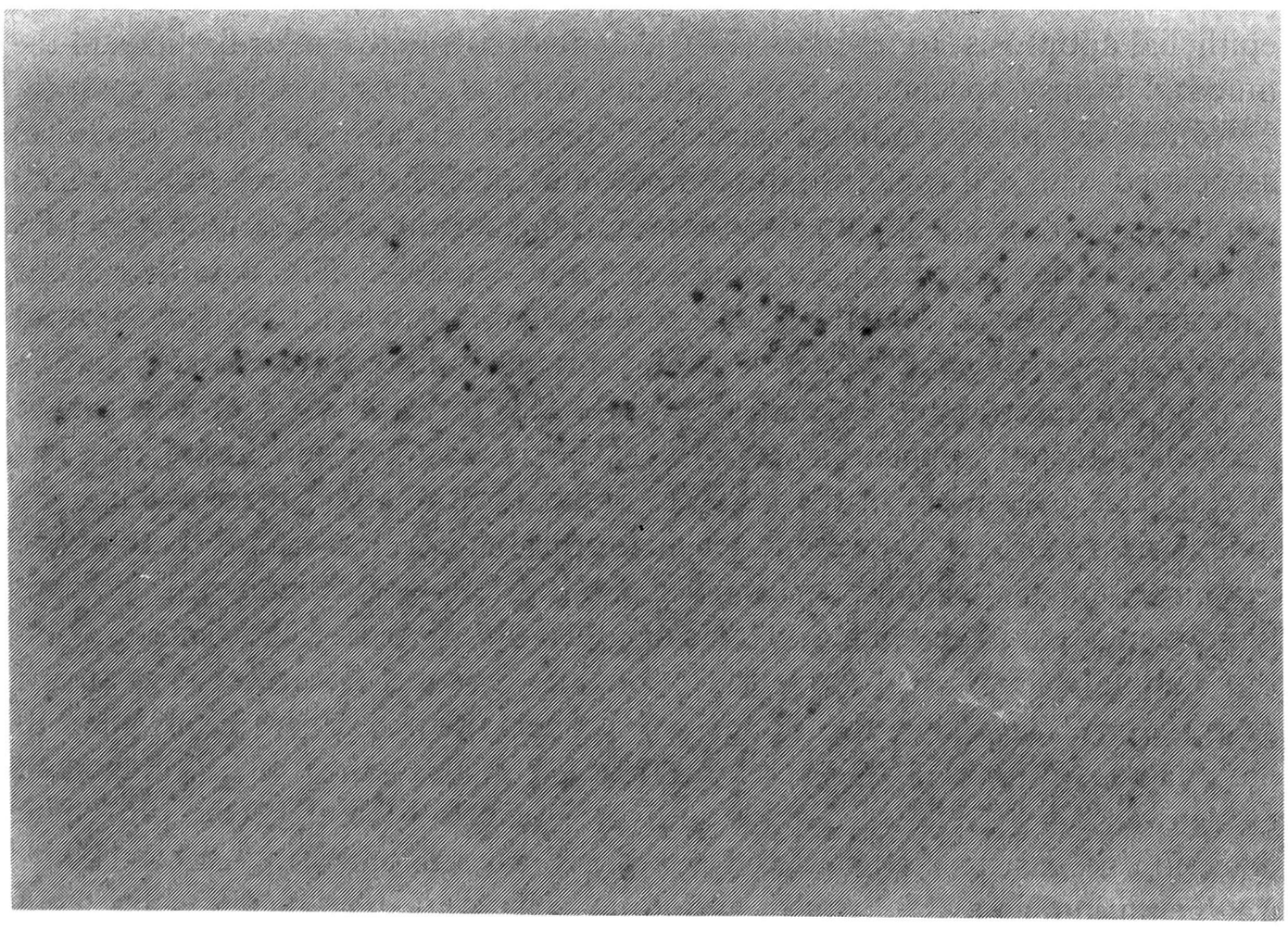


Fig. 10. Effect of *H. pylori* lipopolysaccharide on the acute gastritis scores in rats subjected to intragastric surface epithelial application of the lipopolysaccharide at 50 μg (A) and 200 μg (B) dose and examined 2 and 10 days after the treatment (123).

A



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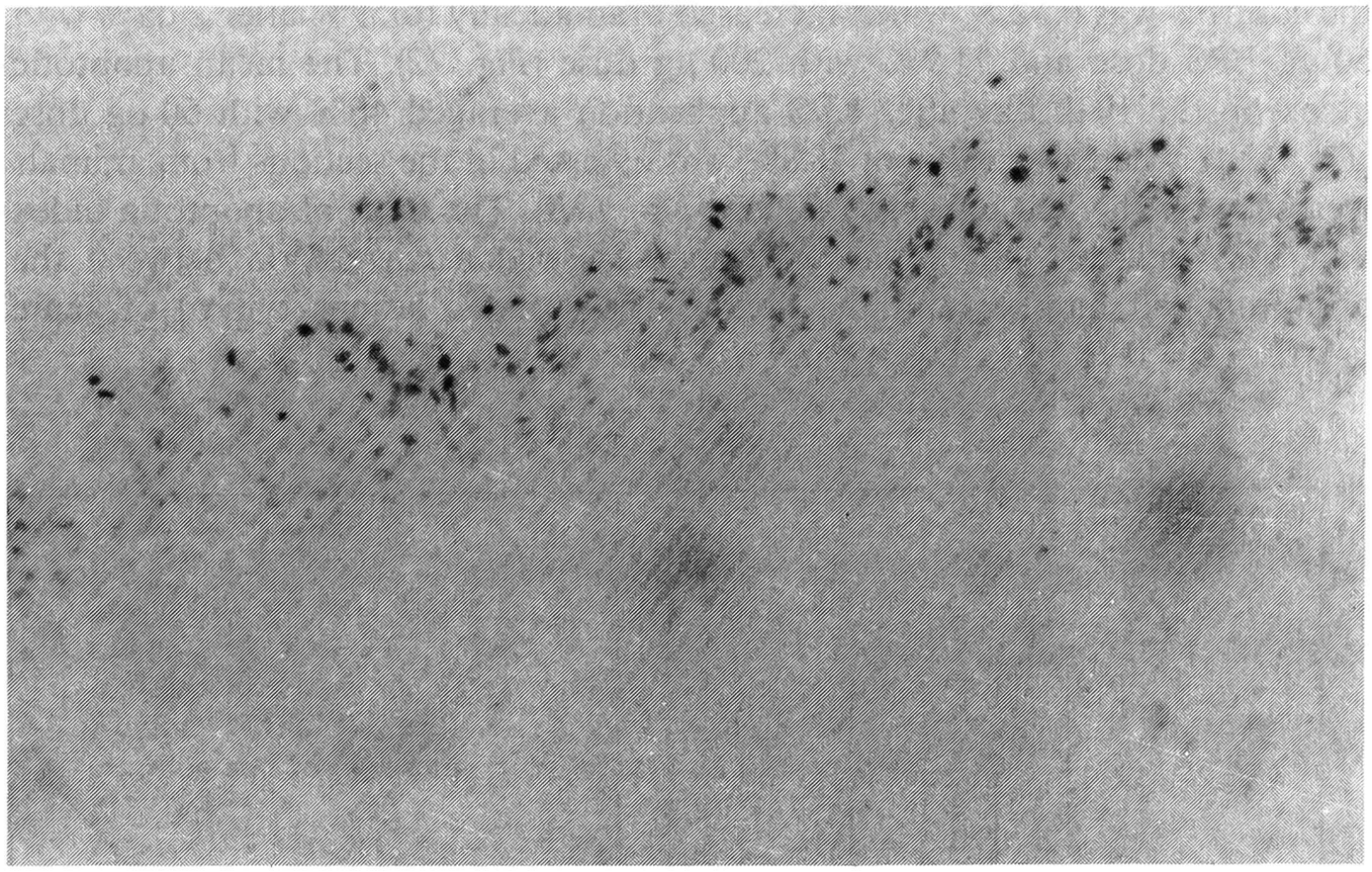

C

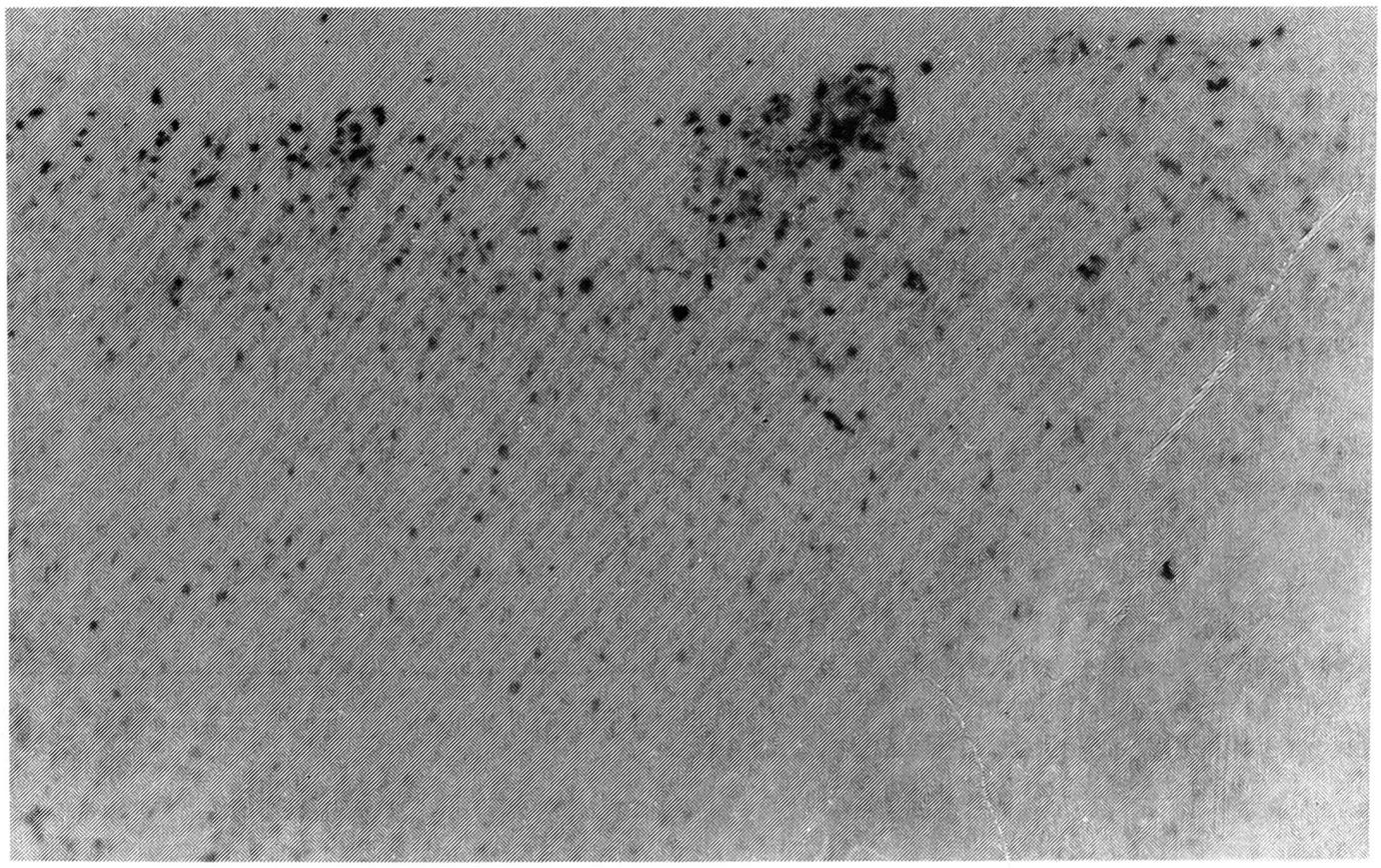

Fig. 11. Gastric mucosa epithelial cell apoptosis shown with the aid of direct immunoperoxidase detection of digoxigenin-labeled genomic DNA. Gastric mucosa at 2(B) and 10(C) days after the intragastric surface epithelial application of *H. pylori* lipopolysaccharide at a 50 μg(B) and a 200 μg(C) dose. (A)-normal gastric mucosa (control). (Magnification, ×200) (123).

(*Fig. 11 B, C*). 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*).

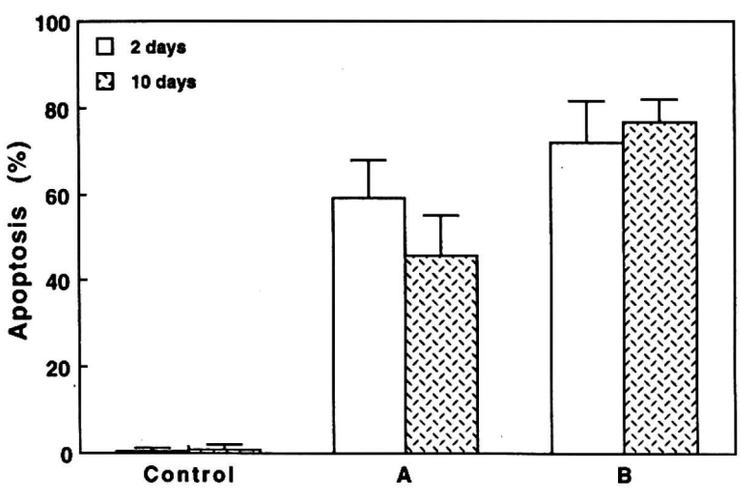

Fig. 12. Effect of *H. pylori* lipopolysaccharide on gastric epithelial cell apoptosis. The animals were subjected to intragastric surface epithelial application of lipopolysaccharide at 50 μg (A) and 200 μg (B), and examined 2 and 10 days after the treatment. The mucosal sections were counted, and the number of positive cells was expressed as the apoptotic index (%) (123).

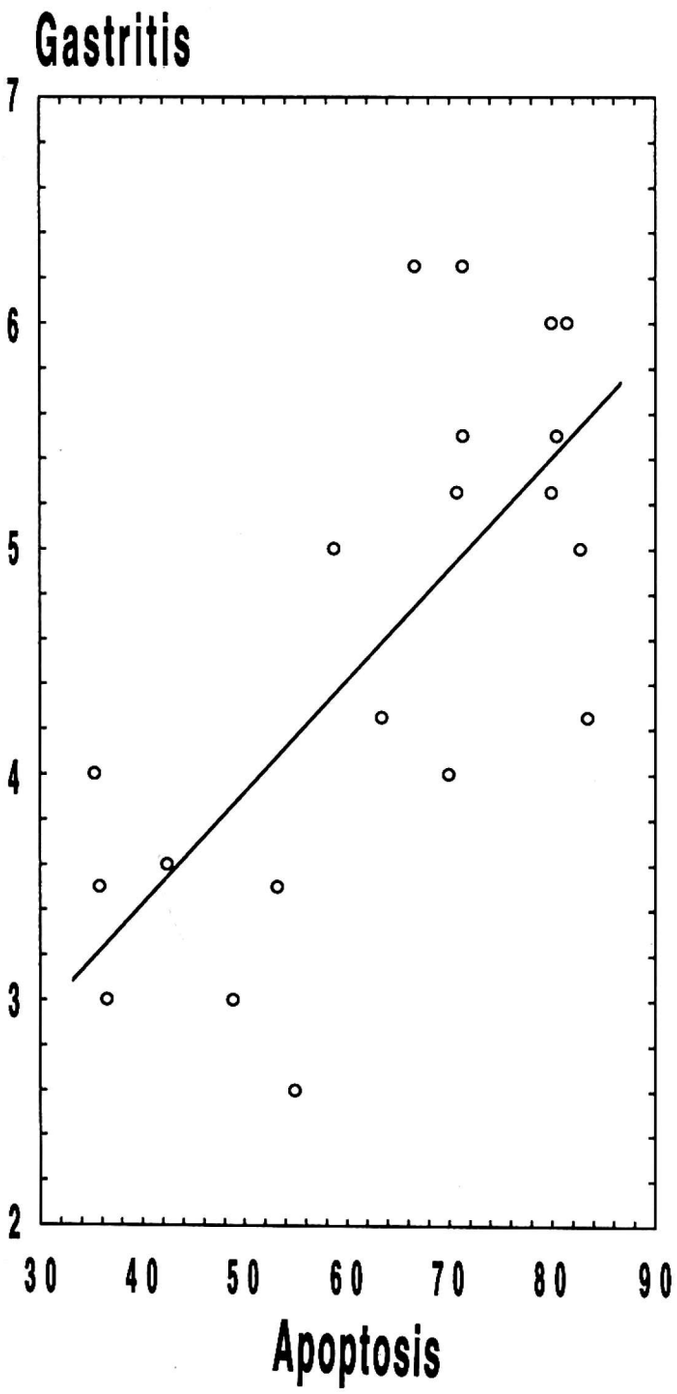

Fig. 13. Correlation between the *Helicobacter pylori* lipopolysaccharide-induced epithelial cell apoptosis and the degree of histologic lesions typical of acute gastritis (123).

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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REFERENCES

1. Warren JR, Marshall BJ. Unidentified curved bacillus on gastric epithelium in active chronic gastritis. *Lancet* 1983; 1: 1273—1275.
2. Marshall BJ, Warren JR. Unidentified curved bacilli in stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; 1: 1311—1315.
3. Valkonen KH, Wadstrom T, Moran A. P. Interaction of lipopolysaccharides of *Helicobacter pylori* with basement membrane protein laminin. *Infect Immun* 1994; 62: 3640—3648.
4. Moran AP. Pathogenic properties of *Helicobacter pylori. Scand J Gastroenterol* 1996; 31: Suppl. 215: 22—31.
5. Lin S-K, Lambert JR. *Helicobacter pylori* in ulcerogenesis. *Scand J Gastroenterol* 1995; 30; Suppl. 210: 64—69.
6. Conrad RS, Kist M, Schlecht S, Galanos C. Extraction and biochemical analysis of *Helicobacter pylori* lipopolysaccharides. *Curr. Microbiol.* 1992; 24: 165—169.
7. Konturek PCh, Konturek SJ. Role of *Helicobacter pylori* infection in gastro-duodenal secretion and in pathogenesis of peptic ulcer and gastritis. *J Physiol Pharmacol* 1994; 45: 333—350.
8. Tytgat GNJ, Noach L. Rauws AJ. *Helicobacter pylori. Scand J Gastroenterol 1991;* 26: Suppl. 187: 1—8.
9. Goodwin CS, Armstrong JA, Chilveres T. *et al.* Transfer of *Campylobacter pylori* and *Campylobacter mustelae* to *Helicobacter* gen. nov. as Helicobacter pylori comb. nov. and *Helicobacter mustelae* comb. nov., respectively. *Int J Syst Bacteriol* 1989; 39: 397—405.
10. The EUROGAST Study Group. An International association between *Helicobacter pylori* infection and gastric cancer. *Lancet* 1993; 341: 1359—1362.
11. Forman D. *Helicobacter pylori* and gastric cancer. *Scand J Gastroenterol* 1996; 31: Suppl. 214: 31—33.
12. Graham DY, Opekun A, Lew GM, Evans DJ. Jr, Klein PD, Evans DG. Ablation of exaggerated meal-stimulated gastrin release in duodenal ulcer patients after clearance of *Helicobacter* (*Campylobacter*) *pylori* infection. *Am J Gastroenterol* 1990; 95: 394—398.
13. Queiroz DM, Mendes EN, Rocha GA. Effect of *Helicobacter pylori* eradication on antral gastrin and somatostatin immunoreactive cell density and gastrin and somatostatin concentrations. *Scand J Gastroenterol* 1993; 28: 858—64.
14. Lee A, Finaly M. Lambert JR, Saviono R, Soveny C., Midolo P. Antral gastrin and somatostatin cells in *Helicobacter pylori* subjects: restitution of D cell number with eradication. *Gastroenterology* 1993; 104: A1046.
15. McColl KE, El-Omar E. Effect of *H. pylori* infection on gastrin and gastric acid secretion. In: *Helicobacter pylori*: basic mechanism to clinical cure. Hunt RH, Tytgat G.H. (ed.) 1994; pp 245—56.
16. Cover TL, Dooley CP, Blaser MJ. Characterization of and human serologic response to proteins in *Helicobacter pylori* broth culture supernatants with vacuolizing cytotoxin activity. *Infect Immun* 1990; 58: 603—610.
17. Murakami A T, Yanai H. Effect of *Helicobacter pylori* on gastric mucosal blood flow. In: Proceedings of World Congress of Gastroenterology, Sydney, 1990.
18. Lambert JR, Lin SK, Aranda-Michel J. *Helicobacter pylori. Scand J Gastroenterol* 1995; 30: Suppl. 208: 33—46.

19. Moran AP, Helander IM, Kosunen IU. Compositional analysis of *Helicobacter pylori* rough-form lipopolysaccharides. *J. Bacteriol* 1992; 174: 1370—1377.
20. Moran AP. Cell surface characteristics of *Helicobacter pylori. FEMS Immun Med Microbiol* 1995; 10: 271—280.
21 Muotiala A, Helander IM, Pyhala L, Kosunen TU, Moran AP. Low biological activity of *Helicobacter pylori* lipopolysaccharide. *Infect Immun* 1992; 60: 1714—1716.
22. Brade H, Brade L, Rietschel ET. Structure-activity relationships of bacterial lipopolysaccharide (endotoxins). Current and future aspects. *Zentralbl Bakteriol Mikrobiol Hyg. A* 1988; 268: 151—179.
23. Weckesser J, Mayer H. Different lipid A types in lipopolysaccharides of phototrophic and related non-phototrophic bacteria. *FEMS Microbiol Rev* 1988; 54: 143—154.
24. Rietschel ET, Brade L, Holst O *et al.* Molecular structure of bacterial endotoxin in relation to bioactivity. In: Endotoxin research series Vol. 1. Cellular and molecular aspects of endotoxin reactions. Nowotny A, Spitzer JJ, Ziegler EJ (ed.) 1990; pp. 15—32.
25. Moran AP, Walsh EJ. Expression and electrophoretic characterization of smooth-form lipopolysaccharide of *Helicobacter pylori. Acta Gastroenterol Belg* 1993; 56 (S): 97.
26. Mattsby-Baltzer I, Mielniczuk Z, Larsson L, Lindgren K. Goodwin S. Lipid A in *Helicobacter pylori. Infect Immun* 1992; 60: 4383—4387.
27. Cave TR, Cave DR. *Helicobacter pylori* stimulates pepsin secretion from isolated rabbit gastric glands. *Scand J Gastroenterol* 1991; 26: Suppl. 181: 9—14.
28. Young GO, Stemmet N, Lastovica A *et al. Helicobacter pylori* lipopolysaccharide stimulates gastric mucosal pepsinogen secretion. *Aliment Pharmacol Ther* 1992; 6: 169—177.
29. Aspinall GO, Monteiro MA, Pang H, Walsh EJ, Moran AP. Lipopolysaccharide of the *Helicobacter pylori* type strain NCTC 11637 (ATCC 43504): structure of the O antigen chain and core oligosaccharide regions. *Biochemistry* 1996; 35: 2489—2497.
30. Aspinall GO, Monterio MA. Lipopolysaccharides of *Helicobacter pylori* strains P466 and MO19: structures of the antigen and core oligosaccharide regions. *Biochemistry* 1996; 35: 2498—2504.
31. Yurchenco PD, Schittny JC. Molecular architecture of basement membranes. *FASEB J* 1990; 4: 1577—1590.
32. Milan SB, Haskin Ch., Zardeneta G., Chen D., Magnuson V. L., and Klebe R. J. Cell adhesion protein in oral biology. *Oral Biol Med* 1991; 2(4): 451—491.
33. Timpl R. Structure and biological activity of basement membrane proteins. *Eur J Biochem* 1989; 180: 487—502.
34. Ruoslahti E, Hayman EG, Pierschbacher MD. Extracellular matrices and cell adhesion. *Arteriosclerosis* 1985; 5: 581—594.
35. Akiyama SK, Nagata K, Yamada KM. Cell surface receptors fo extracellular matrix components. *Biochim Biophys Acta* 1990; 1031: 91—110.
36. Chung AE, Jaffe R, Freemann IL, Vergnes JP, Braginski JE, Carlin B. Properties of basement membrane-related glycoprotein synthesized in culture by a mouse embryonal carcinoma-derived cell line. *Cell* 1979; 16: 277—281.
37. Kleinman HK, Cannon FB, Laurie GW *et al.* Biological activities of laminin. *J Cell Biochem* 1985; 27: 317—325.
38. Liotta LA, Rao CN, Wewer UM. Biochemical interactions of tumor cells with the basement membrane. *Annu Rev Biochem* 1986; 55: 1037—1057.
39. Woodley DT, Rao CN, Hassell JR, Liotta LA, Martin GR, Kleinman HK. Interactions of basement membrane components. *Biochem Biophys Acta* 1983; 761: 278—283.
40. Rao CN, Marghulies IM, Liotta LA. Binding domain for laminin on type IV collagen. *Biochem Biophys Res Commun* 1985; 128: 45—52.

41. Laurie GW, Bing JT, Kleinman HK *et al.* Localization of binding sites for laminin, heparan sulfate proteoglycan and fibronectin on basement membrane (type IV collagen. *J Mol Biol* 1986; 205—216.
42. Del Rosso M, Cappelleti R, Viti M, Vannucchi S, Chiarugi V. Binding of the basement-membrane glycoprotein laminin to glycosaminoglycans. An affinity-chromatography study. *Biochem J* 1981; 199: 699—704.
43. Yurchenco PD, Tsilibary EC, Charonis AS, Furthmayr H. Laminin polymerization *in vitro*. Evidence for a two-step assembly with domain specificity. *J. Biol Chem* 1985; 260: 7636—7644.
44. Timpl R, Rohde H, Robey PG, Rennard SI, Foidart JM, Martin GR. Laminin- a glycoprotein from basement membranes. *J Biol Chem* 1979; 254: 9933—9937.
45. Rao CN, Goldstein IJ, Liotta LA. Lectin-binding domains on laminin. *Arch Biochem Biophys* 1983; 227: 118—124.
46. Rao CN, Castronova V, Schmitt C, Wewer UM, Claysmith AP, Liotta LA, Sobel M. Evidence for a precursor of the high-affinity metastasis-associated murine laminin receptor. *Biochemistry* 1989; 287: 7476—7486.
47. Mecham RP. Receptors for laminin on mammalian cells. *FASEB J* 1991; 5: 2538—2546.
48. Gehlsen KR, Dillner L, Engvall E, Ruoslahti E. The human laminin receptor is a member of the integrin family of cell adhesion receptors. *Science* 1988; 241: 1228—1229.
49. Tomaselli KJ, Damsky CH, Reichardt LF. Purification and characterization of mammalian integrins expressed by rat neuronal cell line (PC12): evidence that they function as alpha/beta heterodimeric receptors for laminin and type IV collagen. *J Cell Biol* 1988; 107: 1241—1252.
50. Roberts DD, Rao CN, Magnoni JL, Spitalnik SL, Liotta LA, Ginsburg V. Laminin binds specifically to sulfated glycolipids. *Proc Natl Acad Sci U.S.A.* 1985; 82: 1306—1310.
51. Switalski LM, Murchison R, Timpl RC, Hook M. Binding of laminin to oral and endocarditis strains of *viridans streptococci. J Bacteriol* 1987; 169: 1095—1101.
52. Switalski LM, Speziale P, Hook M, Wadstrom T, Timpl R. Binding of *Streptococcus pyogenes* to laminin. *J Biol Chem* 1984; 259: 3734—3738.
53. Lopes JD, Reis M, Brentani RR. Presence of laminin receptors in *Staphylococcus aureus. Science* 1985; 229: 275—277.
54. Westerlund B, Merenmies J, Rauvala H *et al.* The O75 adhesin of uropathogenic *Escherichia coli. Microbiol Pathol* 1987; 3: 117—128.
55. Szabo S, Pihan G, Triers S. 1986 Alteration in blood vessels during gastric injury and protection. *Scand J Gastroenterol* 1986; 21: Suppl. 125: 92—96.
56. Flemstrom G. Gastric and duodenal mucosal bicarbonate secretion; in: Physiology of the Gastrointestinal Tract, L. R. Johnson (*ed.*) 1987; pp 1011.
57. Silen W. Gastric mucosal defense and repair; In: Physiology of the Gastrointestinal Tract, L. R. Johnson (*ed.*) 1987; pp 1055.
58. Slomiany A, Yano S, Slomiany BL, Glass GB. Lipid composition of the gastric mucous barrier in the rat. *J Biol Chem* 1978; 253: 3785—3791
59. Slomiany A, Takagi A, Slomiany BL. The synthesis and secretion of gastric mucus, glycoprotein by mucosal cells cultured in the presence of ethanol. *Arch Biochem Biophys* 1985 236: 654—661.
60. Slomiany BL, Piasek A, Sarosiek J, Slomiany A. The role of surface and reformed intracellular mucus in gastric mucosal protection against hydrogen ion. *Scand J Gastroenterol* 1985; 20: 1191—1196.
61. Zalesna G, Tsukada H, Okazaki K, Slomiany BL, Slomiany A. Synthesis and initial processing of gastric apomucin. *Biochem Int.* 1989; 18: 775—784.
62. Lee A, Hazell SL. *Campylobacter pylori* in health and disease: an ecological perspective. *Microb Ecol Health Dis* 1988; 1: 1—16.

63. Slomiany BL, Bilski J, Sarosiek J *et al. Campylobacter pyloridis* degrades mucin and undermines gastric mucosal integrity. *Biochem Biophys Res Commun* 1987; 144: 307—314.
64. Sarosiek J, Slomiany A, Slomiany BL. Evidence for weakenning of gastric mucus integrity by *Compylobacter pylori. Scand J Gastroenterol* 1988; 23: 585—590.
65. Slomiany BL, Nishikawa H, Piotrowski J, Okazaki K, Slomiany A. Lipolytic activity of *Campylobacter pylori*: effect of sofalcone. *Digestion* 1989; 43: 33—40.
66. Slomiany BL, Kasinathan C, Slomiany A. Lipolytic activity of *Campylobacter pylori*: effect of colloidal bismuth subcitrate (De-Nol). *Am J Gastroenterol* 1989; 84: 506—510.
67. Slomiany BL, Piotrowski J, Sengupta S, Slomiany A. Inhibition of gastric mucosal laminin receptor by *Helicobacter pylori* lipopolysaccharide. *Biochem Biophys Res Commun* 1991; 175: 963—970.
68. Pytela R, Pierchbacher D, Rouslahti E. Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor. *Cell* 1985; 40: 191—198.
69. Piotrowski J, Yamaki K, Slomiany A, Slomiany BL. Inhibition of gastric mucosal laminin receptor by *Helicobacter pylori*: effect of sucralfate. *Am J Gastroenterol* 1991; 86: 1756—1770.
70. Rao CN, Margulies I, Tralka TS at all. Isolation of a subunit of laminin an its role in molecular structure and tumor cell attachment. *J Biol Chem* 1982; 257: 9740—9744.
71. Malinoff HL, Wicha MS. Isolation of a cell suface receptor protein for laminin from murine fibrosarcoma cells. *J Cell Biol* 1983; 96: 1474—1479.
72. Graf J, Ogle RC, Robey FA *at al.* A pentapeptide from the laminin B1 chain mediates cell adhesion and binds the 67,000 laminin receptor. *Biochemistry* 1987; 26: 6896—6900.
73. Slomiany BL, Piotrowski J, Samanta A, VanHorn K, Murty VL, Slomiany A. *Compylobacter pylori* colonization factor shows specificity for lactosylceramide sulfate and GM3 ganglioside. *Biochem Int* 1989; 19: 929—936.
74. Graham DY. *Campylobacter pylori* and peptic ulcer disease. *Gastroenterology* 1989; 96: 615—625.
75. Hazell SL, Lee A, Brady L, Hennessy W. *Campylobacter pyloridis* and gastritis: association with intercellular spaces and adaptation to an environment of mucus as important factors in colonization of the gastric epithelium. *J Infect Dis* 1986; 163: 658—663.
76. Slomiany BL, Murty VLN, Piotrowski J. et al. In: *Helicobacter pylori* and gastric mucus integrity. Menge H, Gregor M, Tytgat GN *et al.* (*eds.*) 1990, pp 37—51.
77. Piotrowski J, Yamaki K, Morita M, Slomiany A, Slomiany BL. Ebrotidine a new H_2-receptor antagonist with mucosal strengtheninig activity. *Biochem Int* 1992; 26: 659—667.
78. Slomiany BL, Liu J, Keogh J. Piotrowski J, Slomiany A. Enhancement of gastric mucosal epidermal growth factor and platelet-derived growth factor receptor by sucralfate. *Gen Pharmacol* 1992; 23: 715—718.
79. Slomiany BL, Piotrowski J, Murty VL, Slomiany A. Mechanism of ebrotidine protection against gastric mucosal injury induced by ethanol. *Gen Pharmacol* 1992: 23: 719—727.
80. Piotrowski J, Czajkowski A, Yotsumoto F, Slomiany A, Slomiany BL. Effect of ebrotidine on gastric mucosal EGF and PDGF receptor expression. *Biochem Mol Biol Int* 1993; 30: 1127—1134.
81. Slomainy BL, Piotrowski J, Mojtahed H, Slomiany A. Ebrotidine effect on the proteolytic and lipolytic activities of *H. pylori. Gen Pharmacol* 1992; 23: 203—206.
82. Piotrowski J, Czajkowski A, Yotsumoto F, Slomiany A, Slomiany BL. Sulglycotide effect on the proteolytic and lipolytic activities of *H. pylori* toward gastric mucus. *Am J Gastroenterol* 1994; 89: 232—236.
83. Slomiany BL, Piotrowski J, Slomiany A. Effect of sucralfate on the degradation of human gastric mucus by *H. pylori* protease and lipases. *Am J Gastroenterol* 1992; 87(5): 595—599.

84. Piotrowski J, Murty VL, Slomiany A, Slomiany BL. Susceptibility of *Helicobacter pylori* to antimicrobial agents: effect of sulglycotide. *Biochem Mol Biol Int* 1995; 35: 467—472.
85. Piotrowski J, Piotrowski E, Slomiany A, Slomiany BL. Susceptibility of *Helicobacter pylori* to antimicrobial agents: effect of ebrotidine and ranitidine. *J Physiol Pharmacol* 1996; 46: 463—469.
86. Slomiany BL, Piotrowski J, Majka J, Slomiany A. Sucralfate affects the susceptibility of *H. pylori* to antimicrobial agents. *Scand J Gastroenterol* 1995; 30: Suppl. 210: 82—84.
87. Piotrowski J, Morita M, Slomiany A, Slomiany BL. Inhibition of gastric mucosal laminin receptor by *H. pylori* lipopolysaccharide; effect of ebrotidine *Biochem Int* 1992; 27: 131—138.
88. Piotrowski J, Czajkowski A, Yotsumoto F, Slomiany A, Slomiany BL. *H. pylori* lipopolysaccharide inhibition of gastric mucosal laminin receptor: effect of sulglycotide. *Gen Pharmacol* 1993; 24: 1467—1472.
89. Slomiany BL, Slomiany A. Role of mucus in gastric mucosal protection *J Physiol Pharmacol* 1991; 42: 147—161.
90. Slomiany BL, Sarosiek J, Slomiany A. Gastric mucus and th mucosal barrier. *Dig Dis* 1987; 5: 125—145.
91. Neutra MR, Forstner JF. Gastrointestinal mucins synthesis secretion and function. In: Physiology of the Gastrointestinal Tract. Johnson L. R. (*ed.*) Vol. 2, 1987 pp. 975—1009.
92. Slomiany BL, Piotrowski J, Czajkowski A, Murty VL, Slomiany A. Characterization of gastric mucosal mucin receptor. *Biochem Mol Biol Int* 1993; 31: 745—753.
93. Liau YH, Lopez RA, Slomiany A, Slomiany BL. *Helicobacter pylori* lipopolysaccharide effect on synthesis and secretion of sulfated gastric mucin. *Biochem Biophys Res Commun* 1992; 184: 1411—1417.
94. Piotrowski J, Slomiany A, Slomiany BL. Inhibition of gastric mucosal mucin receptor by *H. pylori* lipopolysaccharide. *Biochem Mol Biol Int* 1993; 31: 1051—1059.
95. Marshall BJ. *Campylobacter pyloridis* and gastritis. *J Infect Dis* 1986; 153: 650—657.
96. Blaser NJ. Gastric *Campylobacter*--like organisms, gastritis and peptic ulcer disease. *Gastroenterology* 1987; 93: 371—383.
97. Graham DY. *Campylobacter pylori* and peptic ulcer disease *Gastroenterology* 1989; 96: 615—625.
98. Piotrowski J, Majka J, Murty LV, Czajkowski A, Slomiany A, Slomiany BL. Inhibition of gastric mucosal mucin receptor by *Helicobacter pylori* lipopolysaccharide; effect of sulglycotide. *Gen Pharmacol* 1994; 25: 969—976.
99. Slomiany A, Piotrowski J, Slomiany BL. Sucralfate counteracts the inhibition of gastric mucosal mucin receptor by *Helicobacter pylori* lipopolysaccharide. *Scand J Gastroenterol* 1995; 30: Suppl. 210: 77—81.
100. Anglada L, Marquez M, Sacristan A, Ortiz JA. Inhibitors of gastric acid secretion: N-sulphonyl formamidines ina series of new histamine H_2-receptor antagonists. *Eur J Med Chem* 1988; 23: 97—100.
101. Brzozowski T, Majka J, Konturek SJ. Gastroprotective and ulcer-healing activities of a new H_2-receptor antagonist, ebrotidine. *Digestion* 1992; 51: 27—36.
102. Konturek SJ, Brzozowski T, Drozdowicz D, Majka J. Ebrotidine, a novel H_2-receptor antagonist with local gastroprotective activity. *Eur J Gastroenterol Hepat* 1992; 3: 941—947.
103. Slomiany BL, Lopez RA, Liau YH, Slomiany A. Effect of ebrotidine on the synthesis and secretion of gastric sulfomucin. *Gen Pharmacol* 1993; 24: 611—617.
104. Gabryelewicz A, Konturek SJ, Butruk G *et al.* Comparison of the efficacy and safety of ebrotidine and ranitidine in the treatment of duodenal ulcer. *Gastroenterology* 1994; 106: A79.
105. Slomiany BL, Piotrowski J, Murty VL, Slomiany A. Inhibition of gastric mucosal mucin receptor by *H. pylori* lipopolysaccharide: effect of ebrotidine. *Gen Pharmacol* 1995; 26: 1553—1558.

106. McHenry L, Vuyyuru L, Schubert ML. *Helicobacter pylori* and duodenal ulcer disease: the somatostatin link? *Gastroenterology* 1993; 104: 1573—1575.
107. Graham DY, Lew GM, Leckago J. G-cell and D-cell numbers in *Helicobacter pylori* infection: effect of *H. pylori* eradication. *Gastroenterology* 1993; 104: 1655—1660.
108. Tarnasky PR, Kovacs TO, Sytnik B. Asymptomatic *H. pylori* infection impairs pH inhibition of gastrin and acid secretion during second hour of peptone meal stimulation. *Dig Dis Sci 1993;* 38: 1681—1687.
109. Haruma K, Sumii K, Okamoto S, Yoshihara M, Sumi M, Kajyama G, Wagner S. *Helicobacter pylori* infection is associated with low antral somatostatin content in young adults; implication for the pathogenesis of hypergastrinemia. *Scand J Gastroenterol* 1995; 30: 550—553.
110. Konturek JW, Bielanski W, Konturek SJ, Domschke W. Eradication of *Helicobacter pylori* restores the inhibitory effect of gastric paracrine somatostatin on gastric acid secretion in duodenal ulcer patients. *Gastroenterology* 1997; 112: A181.
111. Piotrowski J, Majka J, Slomiany A, Slomiany BL. *Helicobacter pylori* lipopolysaccharide inhibiton of gastric mucosal samatostatin receptor. *Biochem Mol Biol Int* 1995; 36: 491—498.
112. Moss SF, Legon S, Bishop A. Polak JM, Calam J. Effect of *Helicobacter pylori* on gastric somatostatin in duodenal ulcer disease. *Lancet* 1992; 340: 930—932.
113. Piotrowski J, Skrodzka D, Slomiany A, Slomiany BL. Reversal of gastric somatostatin receptor inhibition by *H. pylori* lipopolysaccharide with ebrotidine and sulglycotide. *Gen Pharmacol* 1997; 28: 705—708.
114. Piotrowski J, Slomiany A Slomiany BL. *Helicobacter pylori* lipopolysaccharide inhibition of gastric somatostatin receptor: effect of sucralfate. *Biochem Mol Biol Int* 1997; in press.
115. Robert ME, Weinstein WM. *Helicobacter pylori*-associated gastric pathology. *Gastroenterol Clin North Am* 1993; 22: 59—72.
116. Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death *via* specific labelling of nuclear DNA fragmentation. *J Cell Biol* 1992; 119: 493—501.
117. Hall PA, Coates PJ, Ansari A, Hopwood D. Regulation of cell number in the mammalian gastrointestinal tract: the importance of apoptosis. *J Cell Sci* 1994; 107: 3569—3577.
118. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *Science* 1995; 267: 456—1462.
119. Moss SF, Calam J, Agarawal B, Wang S, Holt PR. Induction of gastric epithelial apoptosis by *Helicobacter pylori. Gut* 1996; 38: 498—501.
120. Jones NI, Yeger H, Cutz E, Sherman PM. *Helicobacter pylori* induces apoptosis of gastric antral epithelial cells *in vivo. Gastroenterology* 1996; 110: A933.
121. Stolte M, Edit S. *Helicobacter pylori* and the evolution of gastritis. *Scand J Gastroenterol* 1996; 31: Suppl. 214: 13—16.
122. Piotrowski J, Skrodzka D, Slomiany A, Slomiany BL. *Helicobacter pylori* lipopolysaccharide induces gastric epithelial apoptosis. *Biochem Mol Biol Int* 1996; 40: 597—602.
123. Piotrowski J, Piotrowski E, Skrodzka D, Slomiany A, Slomiany BL. Induction of acute gastritis and epithelial apoptosis by *Helicobacter pylori* lipopolysaccharide. *Scand J Gastroeterol* 1997; 32: 203—211.
124. Psilogenis M, Aberico P, Bianchi G, Nazzari M, Sulglycotide. In: Drugs in Gastroenterology. Braga PD (*ed.*) 1994; pp. 232—244.

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