Mucolytic Effects of *Helicobacter pylori*

J. SAROSIEK, D. A. PEURA, R. L. GUERRANT, B. J. MARSHALL,
W. LASZEWICZ, A. GABRYELEWICZ & R. W. McCALLUM
University of Virginia Health Sciences Center, Charlottesville, Virginia, USA, and
Medical School, Bialystok, Poland

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The mechanisms associated with colonization of human gastroduodenal mucosa by *Helicobacter pylori* remain unclear. To colonize gastric-type epithelium *H. pylori* must enter the gastric lumen, resist damage by all bactericidal factors operating within the acidic gastric milieu, penetrate the mucus gel despite highly viscous and hydrophobic properties of the mucus layer, and, finally, secure optimal conditions for its further multiplication. Since the *H. pylori* microorganism has been seen freely spread throughout the entire mucus layer thickness as well as in intimate contact with surface epithelium, the interrelationship between this spiral microorganism and the mucus seems to be of paramount importance. *H. pylori* has been shown to affect adversely the chemical and physical properties of the mucus layer. Therefore, the mucus layer compromised by the presence of this microorganism may become an easy target for acid and peptic damage, which ultimately leads to mucosal pathology, inflammation and/or peptic ulcer disease.

Key words: Helicobacter pylori; hydrophobicity; lipase; mucin; mucus; phospholipase; protease; viscosity

Jerzy Sarosiek, M.D., Ph.D., Health Sciences Center, Dept. of Medicine, Division of Gastroenterology, P.O. Box 145, Charlottesville, VA 22908, USA

Mucus, a viscoelastic, highly cohesive and adhesive gel covering the surface of the epithelium within the alimentary, respiratory, and genitourinary tracts, is considered to be the vanguard of protection and to function as a link between the outer or luminal milieu and inner or cellular compartment. Mucus also serves as a barrier against invasion by various pathogenic microorganisms, while providing a suitable microenvironment for the growth of indigenous nonpathogenic microbial flora. *Helicobacter pylori* seems to contradict this paradigm. *H. pylori*, although considered a human pathogen, has found a way not only to counteract successfully physicochemical forces of the mucus gel but also to inoculate the mucus gel layer and perhaps also to utilize its physicochemical qualities to further its own pathogenetic goals. It seems to masquerade within the mucus layer as indigenous flora while inducing pathogenetic changes within the colonized mucosa.

THE ROLE OF MUCUS CONSTITUENTS IN MUCOSAL PROTECTION

One unique feature of gastric secretion is its high content of physiologic components that are potentially destructive to the stomach's own protective structures and defensive mechanisms. This makes the study of the gastroduodenal mucosal barrier fascinating and intellectually challenging.

The gastroduodenal mucosa of most humans is able to withstand a gradient of over 1,000,000 hydrogen ions combined with the strong proteolytic activity of pepsin. This implies phylogenetically and ontogenetically well developed and well balanced protective mechanisms. Within the gastroduodenal mucosa one of the best defined

and explored protective mechanisms is the ability of the epithelial cells to generate an extracellular layer of mucus. Mucus gel firmly adhering to surface epithelial structures is considered to be a primary target for all the damaging factors applied to the luminal surface, including acid and pepsin. The discovery of *H. pylori* within this mucus layer raises interesting questions concerning the effect of this microorganism on the chemical and physical properties of mucus and, ultimately, on the mucosal protective barrier.

Composition of mucus

Mucus gel can be separated into organic and inorganic components. Several endogenous organic components seem to play an important role in determining a functional equilibrium or disequilibrium between aggressive and protective factors. Although there are also numerous cations and anions present within the mucus layer, the dominant inorganic components, as hallmarks of mucosal damage and protection, are hydrogen and bicarbonate ions, respectively.

Since the mucus gel covers the surface of the epithelium, its composition is affected by both luminal and mucosal factors. Among luminal factors that may influence its composition are components of salivary secretion, food ingredients, and solubilized mucus components adsorbed secondarily to the surface of the mucus gel.

Although procedures to isolate gastric mucus are always confounded by the possible contamination with constituents of luminal origin, most mucus gel components originate within the glandular mucosa. Components originating within the gastric mucosa can be divided into the three broad categories (1–3):

1. *Secretory components*: mucus glycoprotein (mucin), secretory IgA, IgM, vitamin B_{12}-binding proteins, pepsinogens, and pepsins.

2. *Transudatory components:* serum albumin, serum glycoproteins, lipoproteins, serum IgG, IgM, and IgA.

3. *Exfoliatory components:* plasma membrane glycoproteins, phospholipids, glycosphingolipids, nucleic acids, integrins, and ligands for integrins.

Mucus gel adhering to the plasma membranes of the surface epithelium consists of approximately 70% proteins, 14% sugars, and 16% lipids (4). Mucus glycoprotein, so-called mucin, is a major constituent and a leading determinant of chemical composition and the physical properties of mucus. This glycoprotein is composed of 60–80% carbohydrates, 20–40% protein, and 0.3–0.4% covalently bound fatty acids (1, 2). Mucin exists as a polymer, with an approximate molecular weight 2×10^6, which is formed of four subunits covalently bound to a linking protein. To its protein core, rich in threonine, serine, glycine, and proline, are linked carbohydrate chains composed of *N*-acetylglucosamine, *N*-acetylgalactosamine, galactose, fucose, and sialic (*N*-acetylneuraminic) acid (2, 3). There is controversy with regard to the spacial organization of mucus molecules, and the 'coiled thread' model, 'windmill' organization with 'bottle brush' shape of subunits, and subunits rotated 120° along the linking protein are the most widely accepted three-dimensional configurations (1–3, 5). Only the last conformation, however, considers the modulating role of lipids (1).

Function of mucus

The polymeric structure of mucin and its highly hydrophilic and expanded molecular configuration enables it to form a gel. This gel provides a viscoelastic, spinnable, and permselective mucus layer, crucial for protection of the surface epithelium against exogenous or endogenously elaborated damaging luminal factors. In addition, mucus is the most physiologic lubricant. It also agglutinates and aggregates microorganisms, binds bacterial toxins, and modifies the activity of pepsin (1, 3, 16–19). Bicarbonate secreted by the glandular mucosa is trapped into the mucus gel architectural network and permits the mucus layer to maintain the pH gradient between the acidic gastric luminal milieu and the neutral epithelial cell surface (20).

Various factors, such as lipids, especially phospholipids, albumin, IgA, and prostaglandins, further enhance the protective physical properties (viscosity, retardation of hydrogen ion diffusion) of mucus (6–8). Also various ulcer-healing drugs have been shown to improve the physicochemical

properties of gastric mucus, thereby implementing more favorable conditions for restoration 'ad integrum' of the surface epithelium, damaged during ulcerogenesis (12–15). However, both viscosity and permselectivity of gastric mucus and mucin can be impaired through interaction with damaging compounds such as acetylsalicylic acid, lysophosphatidylcholine, or pepsin (9–11).

The luminal surface of the mucus gel is subject to a continuous erosive activity by various agents and factors within the gastric luminal milieu. Pepsin, especially at a low pH, remains the leading mucus-degrading factor. Since mucous cells can vigorously secrete newly synthesized mucin and restore their mucin stores, an equilibrium is maintained between degradation and the restoration of a mucus gel layer during conditions considered physiologic. This balance, however, dynamically changing with the pace of continuously modifying stimuli and challengers, may undergo disequilibrium if aggressive forces overcome protective factors. *H. pylori* may lead to such an imbalance.

HELICOBACTER PYLORI-RELATED FACTORS INFLUENCING THE MUCUS LAYER

Mucus, covering the surface epithelium of the gastroduodenal mucosa, may because of its multiple components and structural diversity serve both to repell and to attract various surface-exposed structures of the *H. pylori* microorganism. Since exfoliated epithelial cells with specific *H. pylori* adhesion receptors are continuously shed into the mucus layer, one may expect that some receptor molecules would be exposed on the surface of a mucus gel. Therefore, initial docking of *H. pylori* on the surface of the mucus gel could potentially be mediated by gel-embedded membrane fragments with intact receptor molecule. This initial stage may enable *H. pylori* to contact and to anchor within the mucus gel.

The high metabolic activity of the microorganism may also help to restore its new adhesion molecules within the cell membrane and begin a new search for the receptor on the plasma

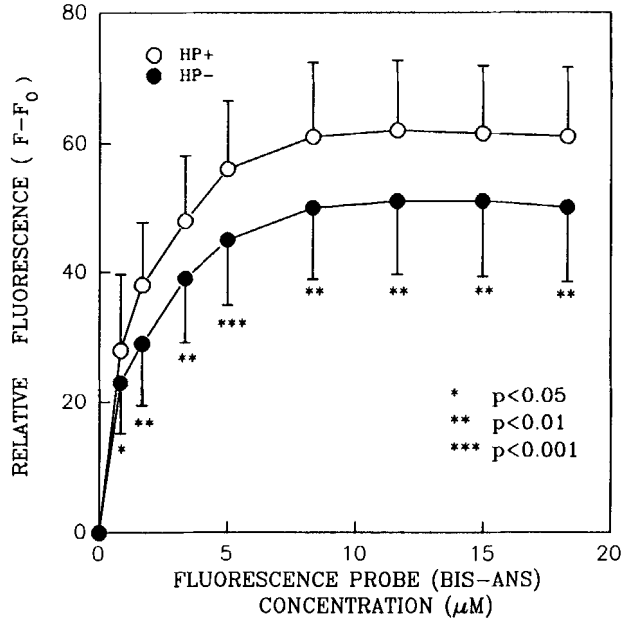

Fig. 1. Hydrophobicity of human gastric juice from patients with or without concomitant *Helicobacter pylori* colonization (mean ±SD).

membrane of an intact cell within the mucosa. Short-range forces such as hydrogen, ionic, and hydrophobic binding may also permit the microorganism to anchor within the mucus layer if the cell plasma membrane receptor density and affinity do not favor direct *H. pylori* adhesion. Hydrophobic regions of *H. pylori* have recently been described by two independent groups using hydrophobic interaction chromatography (21, 22). Moreover, strongly hydrophilic *H. pylori* surface structures have been described by salt aggregation testing, contact angle determination, and adherence to sulfonated polystyrene (21). Therefore, both hydrophilic and hydrophobic *H. pylori* membrane structures may play some role in adhesion of the microorganism to mucous cell plasma membranes and in colonization of the mucus gel.

The potential clinical role, in *H. pylori* colonization, of hydrophobic or lipophilic domains has also recently been underscored by Spychal et al. (23), who described a decrease in hydrophobicity of the surface of the mucus layer in patients with *H. pylori* infection. Furthermore, we have recently found that the gastric juice of patients with non-ulcer dyspepsia (NUD) colonized with *H. pylori* has significantly enhanced ability to bind a hydrophobic probe, BIS-ANS (1-anilinonaphthalene-8-sulfonic acid), than the gastric juice of NUD patients without *H. pylori* colonization (Fig. 1). Although our method of measuring hydrophobicity is based on recording relative fluorescence generated by BIS-ANS bound to hydrophobic binding sites and is different from the method utilized by Spychal, both suggest excessive loss of hydrophobicity from the mucus layer into the gastric lumen. This may be due to enzymes such as protease and phospholipase described in some strains of *H. pylori* (24, 25). How this relates to gastritis and peptic ulcer still remains to be determined.

We have also observed differences in the physical property of gastric mucus in patients with NUD with or without *H. pylori* colonization. Both groups of selected patients showed the same proteolytic profile of gastric juice. As is shown in Fig. 2, patients whose gastric mucosa is colonized by *H. pylori* have viscosity of mucus in the gastric juice significantly lower than that of *H. pylori*-negative patients. It seems, therefore, that the

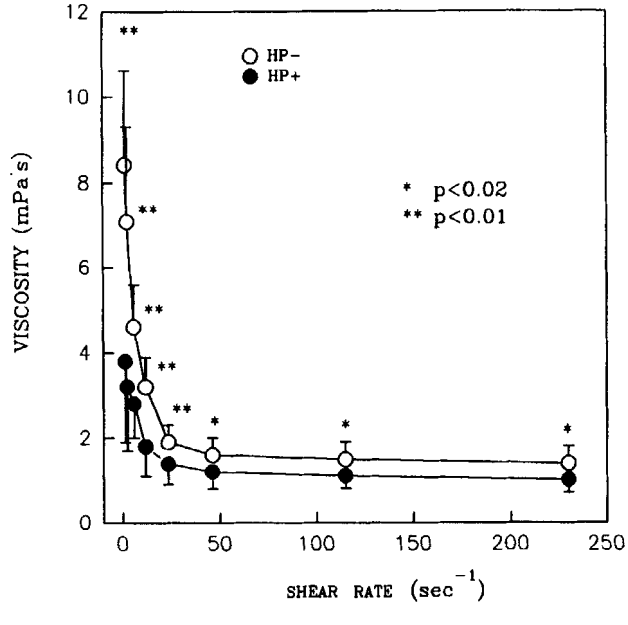

Fig. 2. The viscosity of the gastric mucus from patients with (HP+) and without (HP−) *Helicobacter pylori* colonization within the mucosal compartment (mean±SD).

gastric mucosal barrier in patients with *H. pylori* is physically compromised by *H. pylori*-related pathogenetic mechanisms. The decrease in the viscosity of gastric mucus may at least partly explain why the gastroduodenal mucus gel thickness in *H. pylori*-positive patients with NUD was significantly impaired as compared with *H. pylori*-negative NUD patients. In those with confirmed *H. pylori* infection the thickness of the mucus layer (mean ± SD) was 0.093 ± 0.033 mm in duodenal, 0.085 ± 0.027 mm in antral, and 0.105 ± 0.033 mm in corporal mucosa. In those without concomitant *H. pylori* colonization the thickness of the mucus gel was 0.162 ± 0.045 mm, 0.175 ± 0.067 mm, and 0.161 ± 0.064 mm in duodenum, antrum, and corpus, respectively. These differences were statistically significant (26).

As we have recently shown, *H. pylori* exhibits either predominantly diffuse or predominantly focal adherence to the surface of human gastric epithelium isolated from patients with NUD and maintained in culture for 3–14 days (27). We evaluated our samples in an inverted microscope equipped with Hoffmann modulation contrast and differential interference contrast (Axiomat-Zeiss). We could clearly see that during physical contact between *H. pylori* and the surface of mucous cells, mucin granules were released, acting as a major repelling force on the surface of epithelium. Final adhesion of *H. pylori*, mediated by an intimate contact of *H. pylori* and the cell membrane or through flagella (SEM magnification, × 7,500–15,000) (Figs. 3 and 4), occurred only when cells were depleted of their mucin stores and reduced in size by approximately 40–50%. Some *H. pylori* became entrapped by mucin granules (Figs. 3 and 5). Coaggregation, in which many *H. pylori* microorganisms bind to those already attached to the epithelial cell surface in a focal pattern of adhesion, can also be seen (Fig. 5).

Considering all available data, we should like to present a scheme by which the *H. pylori* microorganisms may mediate potential damage to the gastric mucosal barrier, especially to a mucus layer (Fig. 6). There are two types of *H. pylori*-related epithelial damage: direct and indirect.

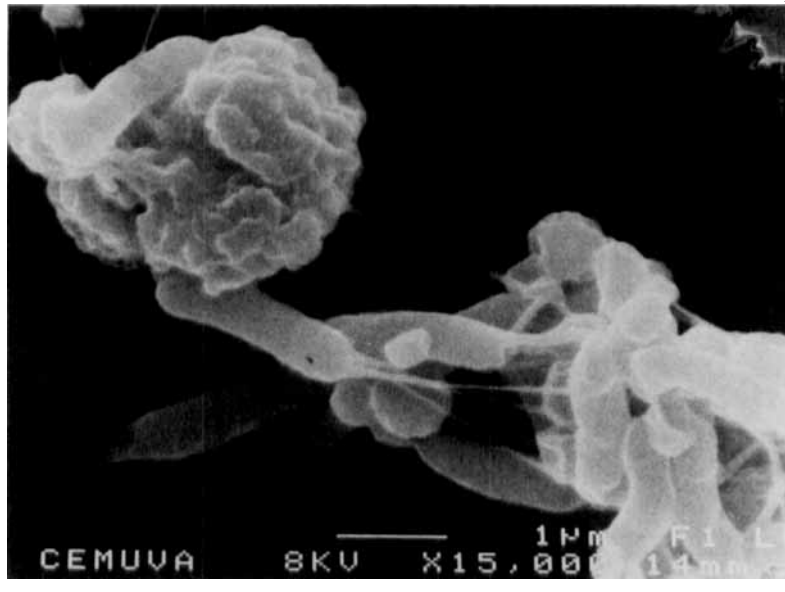

Fig. 3. Scanning electron microscopy (× 15,000) of the human gastric epithelial cell with depletion of its mucin stores and diminished size. There are two *Helicobacter pylori* adhering to the cell through cell membrane structures and one *H. pylori* adhering to the cell through its flagella. In the vicinity of the cell some of *H. pylori* microorganisms seem to be entrapped by mucin granules released by the cell.

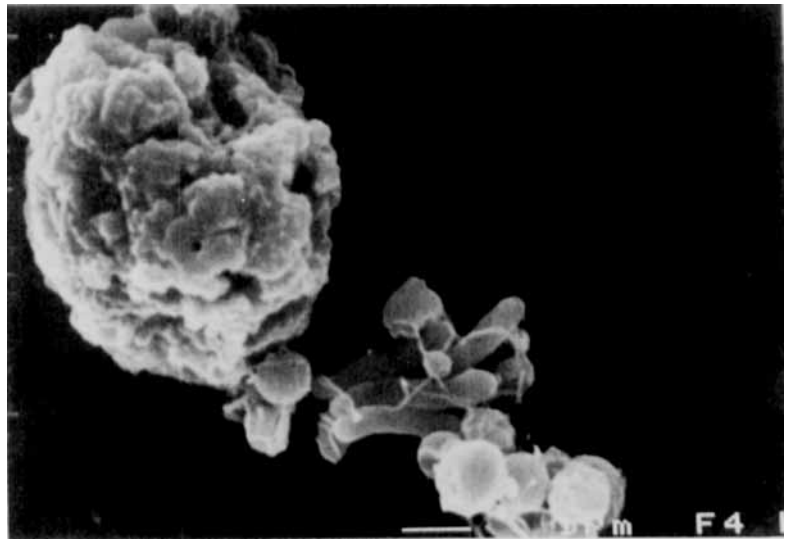

Fig. 4. Scanning electron microscopy (× 9000) of the human gastric epithelial cell releasing its mucin granules on the physical contact between *Helicobacter pylori* and the cell membrane. There are two types of adhesion of *H. pylori* to the cell, one mediated by cell membranes and the other by flagella. Coaggregation of *H. pylori* in the proximity of the cell may be partially mediated by mucin granules entrapping microorganisms by thin threads of viscoelastic mucus due to spinnability.

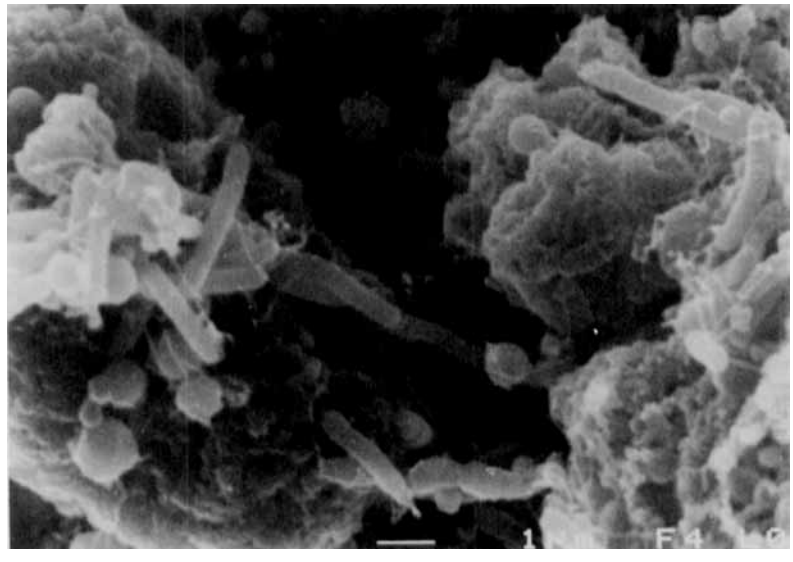

Fig. 5. Scanning electron microscopy (× 7500) of the human gastric epithelial cells with focal adhesion of *Helicobacter pylori* to the surface of epithelium. *H. pylori* microorganisms adhering to the cell surface tend to coaggregate within mucin granules released by the cell.

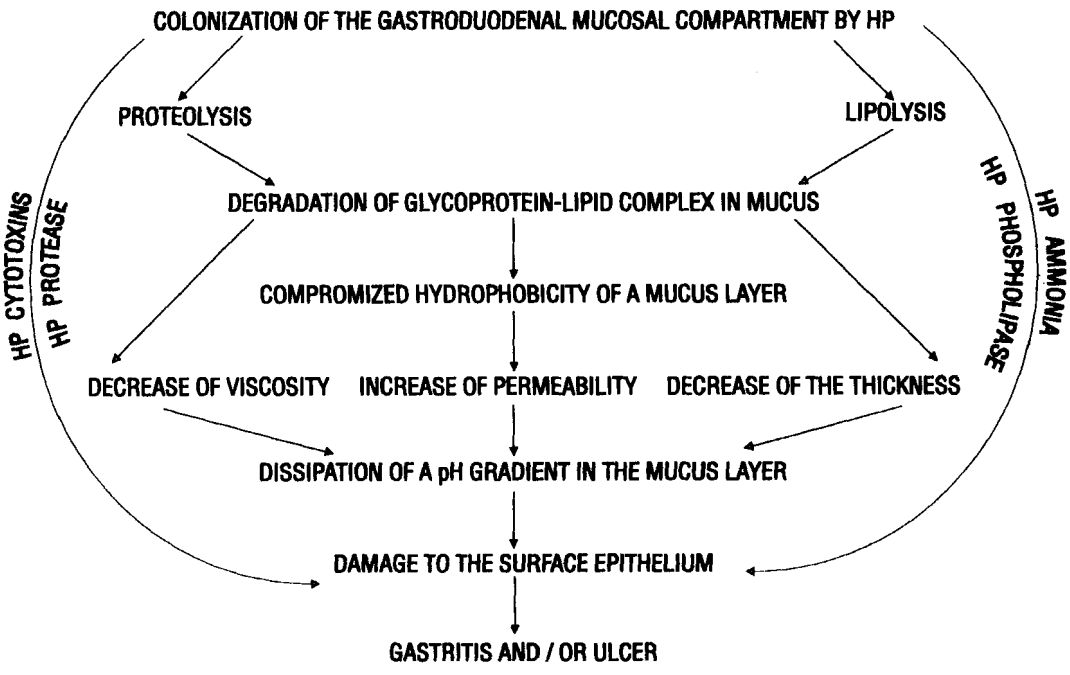

Fig. 6. The direct and indirect effects of *Helicobacter pylori* on the gastric mucosal barrier.

Both direct and indirect weakening of the mucosal barrier may generate the optimal conditions required for *H. pylori* colonization and replication. During direct contact between the *H. pylori* microorganism and the cell, membrane structures are exposed to extremely high concentrations of potential cytotoxins, ammonia generated by *H. pylori* urease, proteases, and phospholipases, inevitably leading to cell damage. The injury induced may sometimes far exceed expected changes, leading to a total disruption of the mucosal barrier. This exaggerated damage may lead to the elimination of the organism or force *H. pylori* to move to surrounding, less damaged areas to ensure survival.

Numerous *H. pylori* reside in the mucus gel, however, freely spread throughout the entire mucus layer. Damaging factors secreted by the microorganism into the surrounding milieu lead through lipolysis and proteolysis to degradation of the glycoprotein–lipid complex in mucus. This compromises the hydrophobicity of the mucus layer, with a subsequent decrease of viscosity, attenuation of the mucus gel thickness, and facilitation of the back-diffusion of hydrogen ion. Such changes may subsequently cause dissipation of the pH gradient in the mucus layer, exposing the surface epithelium to excessive hydrogen ions. These in turn may lead to the damaging impact of hydrogen ion and pepsin on epithelium already compromised by the direct effect of *H. pylori*, with the subsequent development of inflammation and/or ulcer.

FUTURE IMPLICATIONS

There are numerous questions that need to be answered:

1. Will the changes in hydrophobicity, viscosity, and the thickness of the mucus layer clear, diminish, or remain unchanged after eradication of *H. pylori*? If abnormalities persist, are they genetically determined, thus predisposing the mucosa to recolonization or related to environmental or iatrogenic factors?

2. Does the mucus layer prevent or facilitate colonization by *H. pylori*? What are the factors within the mucus layer facilitating or preventing

colonization of the gastroduodenal mucosa by *H. pylori*?

3. Would mucolytic drugs or the agents modifying the physicochemical properties of the gastroduodenal mucus help to eradicate *H. pylori* or promote further damage?

4. Does *H. pylori* require the mucus blanket to protect itself?

5. Does the gastroduodenal mucus layer protect the mucosa, *H. pylori*, or both?

Answers to these questions will assist us in our future design of preventive and therapeutic regimens.

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REFERENCES

1. Slomiany BL, Sarosiek J, Slomiany A. Gastric mucus and the mucosal barrier. Dig Dis 1987, 5, 125–145
2. Allen A. Structure and function of gastrointestinal mucus. In: Johnson LR, editor. Physiology of the gastrointestinal tract. Raven Press, New York, 1981, 617–639
3. Neutra MR, Forstner JF. Gastrointestinal mucus: Synthesis, secretion, and function. In: Johnson LR, editor. Physiology of the gastrointestinal tract. 2nd ed. Raven Press, New York, 1987, 975–1009
4. Slomiany BL, Piasek A, Sarosiek J, Slomiany A. The role of surface and intracellular mucus in gastric mucosal protection against hydrogen ion: compositional differences. Scand J Gastroenterol 1985, 20, 1191–1196
5. Carlstedt I, Sheehan JK. Macromolecular properties and polymeric structure of mucus glycoprotein. Ciba Found Symp 1984, 109, 157–166
6. Sarosiek J, Slomiany A, Takagi H, Slomiany BL. Hydrogen ion diffusion in dog gastric mucus glycoprotein: effect of associated lipids and covalently bound fatty acids. Biochem Biophys Res Commun 1984, 118, 523–531
7. Sarosiek J, Slomiany BL, Jozwiak Z, Liau YH, Slomiany A. Rheological properties of gastric mucus glycoproteins: contribution of associated lipids. Ann NY Acad Sci 1984, 435, 575–577
8. Sarosiek J, Murty VLN, Nadziejko C, Slomiany A, Slomiany BL. Prostaglandin effect on the physical properties of gastric mucin and its susceptibility to pepsin. Prostaglandins 1986, 32, 635–646
9. Sarosiek J, Mizuta K, Slomiany A, Slomiany BL. Effect of acetylsalicylic acid on gastric mucin viscosity, permeability to hydrogen ion and susceptibility to pepsin. Biochem Pharmacol 1986, 35, 4291–4295
10. Slomiany BL, Sarosiek J, Liau YH, Laszewicz W, Murty VLN, Slomiany A. Lysolecithin affects the viscosity, permeability and peptic susceptibility of gastric mucin. Scand J Gastroenterol 1986, 21, 1073–1079
11. Sarosiek J, Slomiany A, Slomiany BL. Retardation of hydrogen ion diffusion by gastric mucus constituents: effect of proteolysis. Biochem Biophys Res Commun 1983, 113, 1053–1060
12. Gabryelewicz A, Sarosiek J, Laszewicz W. The effect of ranitidine on gastric mucus and pepsin secretion in patients with peptic ulcer. Acta Med Pol 1981, 16, 226–234
13. Sarosiek J, Bilski J, Tsukada H, Slomiany A, Slomiany BL. Effect of solon on gastric mucus viscosity, permeability to hydrogen ion and susceptibility to pepsin. Digestion 1987, 37, 238–246
14. Bilski J, Sarosiek J, Murty VLN, et al. Enhancement of the lipid content and physical properties of gastric mucus by geranylgeranylacetone. Biochem Pharmacol 1987, 36, 4059–4065
15. Slomiany BL, Liau YH, Carter SR, Sarosiek J, Tsukada H, Slomiany A. Enzymatic sulfation of mucin in gastric mucosa: effect of solon, sucralfate and aspirin. Digestion 1987, 38, 178–186
16. Proust JE, Tchaliovska SD, Ter-Minassian-Saraga L. Mucin thin-film as a model of the tear film rupture. Science 1984, 98, 319–328
17. Smart JD, Kellaway IW, Worthington HEC. An in-vitro investigation of mucosa-adhesive materials for use in controlled drug delivery. J Pharm Pharmacol 1984, 36, 295–299
18. Cohen PS, Rossol A, Cabelli VJ, Yang SL, Laux DC. Relationship between the mouse colonizing ability of a human fecal EC strains and its ability to bind a specific colonic mucus gel protein. Infect Immun 1983, 40, 62–69
19. Edwards PAW. Is mucus a selective barrier to macromolecules? Br Med Bull 1978, 34, 55–56
20. Williams SE, Turnberg LA. Demonstration of a pH gradient in mucus adherent to rabbit gastric mucosa: evidence for a 'mucus-bicarbonate' barrier. Gut 1981, 22, 94–96
21. Smith JI, Drumm B, Neumann AW, Policova Z, Sherman PM. In vitro surface properties of the newly recognized gastric pathogen Helicobacter pylori. Infect Immun 1990, 58, 3056–3060
22. Pruul H, Goodwin CS, McDonald PJ, Lewis G, Pankhurst D. Hydrophobic characterization of Helicobacter (Campylobacter) pylori. J Med Microbiol 1990, 32, 93–100
23. Spychal RT, Goggin PM, Marrero JM, et al. Surface hydrophobicity of gastric mucosa in peptic ulcer disease. Relationship to gastritis and Campylobacter pylori infection. Gastroenterology 1990, 98, 1250–1254
24. Sarosiek J, Slomiany A, Slomiany BL. Evidence of

weakening of gastric mucus integrity by *Campylobacter pylori*. Scand J Gastroenterol 1988, 23, 585–590
25. Sarosiek J, Slomiany A, VnHorn K, Zalesna G, Slomiany BL. Lipolytic activity of Campylobacter pylori: effect of Sofalcone. Gastroenterology 1988, 94, A399
26. Sarosiek J, Marshall BJ, Peura DA, Hoffman S, Feng T, McCallum RW. Gastroduodenal mucus gel thickness in patients with Helicobacter pylori: a method for assessment in biopsy specimens. Am J Gastroenterol 1991, 86. In press
27. Sarosiek J, Marshall BJ, Hoffman S, et al. The attachment of Helicobacter pylori to human gastric epithelium in vitro: a model for the study of the pathomechanism of colonization. Gastroenterology 1991, 100, A155