Inhibition of Binding of *Helicobacter pylori* to Tissue Culture Cells by Anti-Lipopolysaccharides Monoclonal Antibodies

MaanAbul-Milh* , Ihsan Abdullah Kumail and Fadil Abbas Hamad,
College of Dentistry, Al-Muthanna University, Al-Muthanna, Iraq
***Corresponding author:MaanAbul-Milh, Ph.D..address: College of Dentisrty,**
Al-Muthanna University, Al-Muthanna, Iraq, e-mail address:
mamilh@hotmail.com,

Abstract

Binding of ^{35}[S]- methionine-labeled *Helicobacter pylori* to tissue culture was carried out using human colon carcinoma epithelial cells (Caco-2), and human adenocarcinoma tissue culture cells (AGS). In this research, clinical isolates (smooth) as well as type culture collection (rough) strains were used in binding and inhibition of the binding study of *H. pylori* to tissue cultures using monoclonal antibodies (MAbs) raised against bacterial lipopolysaccharides (LPS). The task of the work was to discriminate and evaluate the role of LPS as bacterial adhesin in the binding as well as the specificity of the binding of various *H. pylori* strains to cell lines. Binding of *H. pylori*, clinical isolate Hel 305(smooth strain),to Caco-2 cells was strongly inhibitedby species-specific MAbs raised against Hel 305-LPS antigen. It was also recognized that inhibition of binding of Hel 305 strain to Caco-2 cells was high effective when using species-specific MAbs in comparison with MAbs raised against LPS from type culture collection CCUG 17874 (rough strain). In the same manner, binding of rough CCUG 17874 strain to Caco-2 cell line was inhibited by species-specific LPS MAbs stronger than with MAbs raised against Hel 305 LPS. In both overlay cases, no significant inhibition with other LPS MAbs of different origins was detected, after bacterial incubation with MAbs raised against LPSs from other tested *H. pylori* strains.

Inhibition of binding of *H. pylori* Hel 305 bacteria to the AGS cell line, using own- LPS MAbs revealed lower level activity on bacterial binding to the cell line than inhibition of bacterial binding to Caco-2 cell line. In the same aspect, MAbs raised against LPS of *H. pylori* CCUG 17874 rough strain demonstrated weak inhibitory effect on the binding of Hel 305 strain to AGS cell line. Binding of CCUG 17874 *H. pylori* strain to AGS was inhibited equally well using MAbs raised against LPS from both rough and smooth strains. No inhibitory effect was detected with other species of LPS MAbs. Using anti-Lewis MAbs like anti-Lewis X and anti-Lewis Y, in the binding of *H. pylori* clinical isolate Hel 305 to Caco-2 cell line was more effective when using anti-Lewis X in comparison with anti-Lewis Y. Both anti-Lewis X and anti-Lewis Y MAbs were characterized as weak MAbs to inhibit binding of *H. ylori* Hel 305 strain to Caco-2 than MAbs raisedaginstown-LPS.

Introduction

Helicobacter pylori is recognized as the most common bacterial pathogen associated with chronic gastritis and peptic ulcers in humans with increased risk of gastric

adenocarcinoma and mucosa-associated lymphoid tissue lymphoma (7). *Helicobacter pylori* is associated with the gastric mucosa of more than half of the world's gastric and duodenal ulcers, and also gastric malignances (11, 33).

Colonization of the gastric mucosa by adhering to the mucous epithelial cells and the mucous layer lining the epithelium has been reported (16, 21). To adhere to the target tissue, the bacterium uses adhesins responsible for recognizing of the specific carbohydrate structures expressed by that tissue (13).*Helicobacter pylori* LPS exhibits extremely low endotoxic activities, including lethality, pyrogenic activity, mitogenic activity, and

induction of proinflammatory cytokine production, therefore, most infected individuals do not reveal any clinical symptoms (16, 25, 27, 28). *H. pylori* produces several putative colonization factors, including urease, adhesin, flagella, LPS and cytotoxin. Bacterial virulent factors and host susceptibility features play a role in the development of infection (22).

The best defined adhesins are the blood group-binding adhesin (BabA) with affinity to Lewis b and H type 1 antigens as well as sialic acid-binding adhesin (SabA) that binds sialyl Lewis X structure (14, 17). Lewis antigens in human represent terminal modifications on mucins, where the mucins are the crucial components of mucus, which may facilitate the binding of *H. pylori* to the gastric mucosa.

Increased of sialyl Lewis X in gastric mucosa was clearly detected in the cases of inflammation (16, 21, 29). The Lewis blood group antigens are also expressed on the O-specific chain of the lipopolysaccharide (LPS) of *H. pylori* smooth strains.

The overall architecture of *H. pylori* LPS is similar to that of LPS of other gram-negative pathogens. The low level of endotoxic activity is due to the variability in the chemical structures such as the molecular species as well as the number of fatty acid residuesof the lipid A region (26). These lipid A modifications minimize endotoxic and inflammatory properties of *H. pylori* LPS. Generally, lipid A moiety of LPS is connected to the core region (oligosaccharide), which in turn is connected to the O-antigen (O-side chain or Lewis antigen).

On the other hand, gastric epithelial cells also express Lewis antigens (23, 32), suggesting that the expression of Lewis antigens on the bacterial surface may serve as a mimicry strategy. Studies on clinical isolates (32, 34) and experimental infections in animals (4, 30, 35) support this role for bacterial Lewis antigens in immune evasion. In human infection, *H. pylori*

Lewis antigens have been linked to the severity of peptic ulcer and duodenitis (31).

Another important feature of *H. pylori* LPS is its modified lipid A structure, with reduced acylation and fewer charged groups than is typical of enterobacteria (24).

The aim of the present study is to focus on the role of lipopolysaccharides in the colonization and infection of stomach tissues with the possibility of using this finding to design treatment of gastritis, peptic and duodenal ulcers diseases.

Materials and Methods

Bacterial Cultivation and Labeling.

The strains used in this work were stored at - 80 °C in tryptic soy broth containing 15% glycerol (by volume). The bacteria were initially cultured on gonococcal agar base-Campylobacter medium agar under humid (98%) microaerophilic conditions (5–7 % O2, 8–10% CO2, and 83–87% N2) for 48–72 h at 37 °C. The bacterial motility and purity were checked by phase-contrast microscopy. For cultivation of *H. pylori*

on agar medium in order to be labeled with radioactive ^{35}[S]-methionine, a semi-solid Brucella agar (Difco) supplemented with 10% heat-inactivated fetal calf serum and enriched with 0.5% IsoVitaleX was used. The agar medium was inoculated by streaking with bacterial material from the agar plate, and the new plate was

sprinkled with ^{35}[S]-methionine (100 µCi/plate). The incubation conditions and preparation of bacterial cells were done as described above.

Most of the experiments of the present work were performed on *H. pylori* cells grown in agar cultures.

Caco-2 tissue culture cells.

Human adenocarcinoma tissue culture cells (Caco-2), obtained from the American Type Culture Collection strains. Cells were cultured in polystyrene tissue-culture flasks in an atmosphere of $C0_2$ 5% in air and 98% humidity at 37°C. The cell medium contained minimum essential medium (MEM, Invitrogen Life Technologies), 10% fetal bovine serum, MEM-Nonessential Amino Acid, and antibiotic-antimycotic drugs. Confluent growth was obtained when flasks were incubated for 8-10 days. The culture medium was changed every other day. Upon reaching confluency, cells were then trypsinized and seeded onto 24-well plates at 1×10^5 cells/well then incubated under identical conditions for 72 hours. Cells, thereafter were washed twice with phosphate-buffered saline, PBS (pH 7.4), overlaid with ^{35}[S]-methionine-labeled *H. pylori* bacteria. Plates were then incubated under above conditions for 2 hours, washed again with PBS 3 times to remove non-adherent bacteria, thereafter treated with trypsin, the bacteria were then collected in groups (in triplicates) in order to measure cpm values in scintillation counter.

AGS tissue culture cells.

Human adenocarcinoma tissue culture cells (AGS), were grown to a confluence monolayer in tissue culture flasks. AGS cell line was cultured in RPMI 1640 medium (Invitrogen, Grand Island, NY) containing 10% fetal bovine serum at 37°C in a humidified environment and 5% CO_2, as described elsewhere. Each AGS monolayer was washed twice with phosphate-buffered saline, PBS (pH 7.4), treated with trypsin, inoculate 24-well plates and incubated till confluency under above conditions, then be overlaid with ^{35}[S]-methionine-labeled bacteria in suspension in phosphate-buffered saline, PBS (pH 7.4). Incubation, treatment with

trypsin, measurements of cpm in scintillation counter were carried out as described previously.

Extraction and isolation of LPS.

Lipopolysaccharides (LPS) from *H. pylori* strains was extracted and isolated by the method of Darveau and Hancock (9), with some modifications. Briefly, disrupted cells were treated with DNase, RNase, pronase, and sodium dodecyl sulfate (SDS) were subjected to $MgCl_2$ precipitation and high-speed centrifugation. These LPS preparations contained less than 1% protein as determined by a dye-binding assay (Bio-Rad Laborato- ries, Richmond, Calif.), and no bands were detected after silver staining of sodium dodecyl sulfate-polyacrylamide gels. LPS hydrolysis; ten milligrams (dry weight) of LPS was hydrolyzed at 100°C for 2 h in 1ml of 1% (vol/vol) acetic acid previously saturated with nitrogen. Lipid A (insoluble) and polysaccharides (soluble) were separated by centrifugation at 12,000 x g for 10 min after neutralization with 5 N NaOH. The polysaccharidic fraction (also referred to as detoxified LPS) was used .

Antigen preparations.

Total membranes and Triton X-100 insoluble outer membranes were produced as described previously (1). Briefly, bacteria were scraped from plates of confluently growing *H. pylori*, sonicated, and subjected to low-speed centrifugation to remove intact bacteria. Total membranes were pelleted by centrifugation for 30 min at 10,000 x g. Outer membranes were prepared by suspending the total membranes in Triton X-100, followed by ultracentrifugation to pellet the insoluble outer membranes. Lipopolysaccharide (LPS) was prepared by the hot phenol-water

extraction method according to the procedure of Westphal and Jann (38), followed by repeated ultracentrifugation.

Production of LPS MAbs.

Monoclonal antibodies (MAbs) against *H. pylori* E50 were produced as described previously(9a) by immunizing BALB/c mice with LPS. In the initial screening, the 23 hybrids reacting in the highest titers with the *H. pylori* membranes were selected. Of these, nine stable antibody-producing hybridomas were cloned and expanded by cultivation in100 ml of Iscove's medium-10% fetal calf serum in tissue culture bottles (Nunc). Culture fluids from established, antibody-secreting hybridomas were harvested and frozen in aliquots at -30°C until further tested, and the specific *H. pylori*-antibody-producing cells were frozen in liquid nitrogen for long-term storage.

The isotypes of the different MAbs were determined by means of single radialimmunodiffusion with mouse immunoglobulin (Ig) isotype-specific antisera(IgM, IgA, IgG1, IgG2a, IgG2b, and IgG3) by the method of Mancini et.al.(18). The Ig concentration was determined by the same method by assaying an appropriate dilution of the MAb in isotype-specific anti-mouse Ig; preparationswith known contents of the respective mouse Igisotype were used as standards.

ELISA.

The enzyme-linked immunosorbent assay (ELISA) used for screening of the MAbs was performed as described previously (34), with 25mg of total membrane preparations, 10 mg of LPS, and 5mg of flagellin protein per ml for coating.

Results

Comparison of H. pylori binding and inhibition of binding data between culture collection strain and two clinically isolated strains to Caco-2 cells demonstrating differences when cross applications in species-specific manner.

In Table 1, the presented data shows two clinical *H. pylori* strains Hel 305 and E50 were compared with type culture collection strains CCUG 17874 on the basis of MAbs specificities. Culture collection strain as well as other two clinical isolates were incubated with all three MAbs separately. The inhibition results demonstrated in Table 1 clearly indicated that the species-specific MAbs revealed high effectivity with related strain than LPS-MAbs from other unrelated *H. pylori* strains. The inhibition of binding of clinical Hel 305 to Caco-2 was strongest 86% when using species-specific MAbs, weak and very weak when using LPS- MAbs from E50 and CCUG 17874, repectively applied in the assay.

Negative inhibitory effect on the binding of *H. pylori* E50 was shown when using unspecific LPS-MAbs from rough and smooth strains.

Binding of ^{35}[S]- labeled H. pylori 305 clinical isolate to Caco-2 epithelial cells monolayer after bacterial treatment with various monoclonal antibodies.

Binding results presented in Fig.1 and Table 1 demonstrate binding of *H. pylori* Hel 305 to Caco-2 epithelial cells after been treated with various monoclonal antibodies (MAbs). Tissue cultures wells were overlaid with ^{35}[S]- labeled bacteria revealed no reduction in the binding activity to Caco-2 cells, when incubated with species unspecific MAbs raised against LPS of a number of *H. pylori* strains. The negative inhibitory effect with unspecific MAbs was shown in column 2 using MAbs raised against LPS from *E. coli*. Binding of ^{35}[S]- labeled Hel 305 clinical isolate untreated with MAbs bacteria as in column 1, treated with anti-LPS from *E. coli* used as negative control as in column 2, while treated with species

unspecific MAbs was shown in all columns except 6 and 7 columns (from left). Binding results demonstrated in columns 6 and 7,were carried out after incubation with MAbs HP17-3:4 and Hel73-9:10 raised against LPS from culture collection strain CCUG 17874 and clinical isolate Hel305, respectively. Weak inhibitory effect of MAbs on the binding of clinical isolate Hel 305 Caco-2 cells was shown in column 6 after treatment with MAbs raised against LPS from CCUG 17874 and strong inhibitory effect was detected with species-specific MAbs as in column 7.

Binding of ^{35}[S]- labeled H. pylori 305 clinical isolate to human adenocarcinoma AGS cells monolayer after bacterial treatment with various monoclonal antibodies.

Results presented in Fig.2 demonstrate binding and inhibition of binding to AGS tissue culture cell line reveal different and weaker binding-monster in comparison with Caco-2 cells. Identical batch of MAbs was used in order to discriminate inhibitory effects between bindings do different cell lines. Still inhibitory of binding of clinical isolate Hel 305 to AGS cells shows the best among performed overlay assays with species-specific MAbs application. Very weak inhibitory effect was observed when MAbs raised against LPS from *H. pylori* CCUG 17874 and *H. pylori* E50 were used to inhibit binding of Hel305 clinical isolate to AGS cells. Inhibitory effect of E50 LPS-MAbs on binding of Hel 305 was also variable as demonstrated in standard deviation of column 5.

Binding of ^{35}[S]- labeled H. pylori culture collection strain to human Caco-2 epithelial cells cell line after bacterial incubation with various monoclonal antibodies.

Binding of *H. pylori* type culture collection CCUG 17874 to Caco-2 cells demonstrated different binding panel than what was demonstrated with the clinical isolate Hel 305 to the same cells. In Fig.3 and Table 1, binding

data shows generally weak inhibition of the binding as presented in the columns 4, 6, 7 and 8 with little stronger inhibition effect was detected with the species-specific MAbs HP17-3:4. MAbs raised against LPS from Hel 305 demonstrated less affectivity of inhibition than type culture collection CCUG 17874 species-specific monoclonal antibodies.

Binding of ^{35}[S]- labeled H. pylori culture collection strain to human adenocarcinoma AGS cell line after bacterial treatment with various monoclonal antibodies.

In Fig4, the presented binding data was also different with respect to the *H. pylori* binding strain and tissue culture cells. Binding activity of type collection strain CCUG 17874 to AGS cells was almost identical, as in colums 6 and 7, after incubation with own-specific MAbs and Hel 305-LPS MAbs before overlay tissue culture cell-monolayers.

Binding of ^{35}[S]- labeled H. pylori Hel 305 clinical isolate to human Caco-2 epithelial cell line after bacterial incubation with anti-Lewis and anti-LPS monoclonal antibodies.

H. pylori Hel 305 and Caco-2 cells were used as a good model in the evaluation of inhibition activity of anti-Lewis X and anti-Lewis Y on the binding of bacteria. Specific-MAbs raised against whole LPS showed strongest inhibitory effect, while anti-Lewis X MAbs were also effective to reduce binding of bacteria to tissue culture, but less effective than LPS specific MAbs. Weak reduction in binding of Hel305 to Caco-2 was detected when bacteria previously incubated with anti-Lewis Y monoclonal antibodies.

Discussion

Helicobacter pylori is a gram-negative and microaerophilic bacterium that is recognized as a major cause of chronic gastritis, peptic ulcer, and gastric

cancer (8, 19). This work describes the structure, attributes and properties of *Helicobacter pylori* lipopolysaccharides (LPS), and their potential role in pathogenesis.

Inhibition of binding of *H. pylori* to tissue culture Caco-2 and AGS cell receptors indicates clearly to the role determined by LPS, O-side chain and less with the core region of LPS. This conclusion is based on our results of inhibition assay of *H. pylori* binding to tissue cultures Caco-2 and AGS cells using LPS MAbs. Smooth strain with complete O-side chain structure of LPS, presented always by wild type bacteria, was involved mainly in the binding to Caco-2 and AGS cells. Due to the inhibition study analysis, species-specific MAbs raised against LPS reduced binding of bacteria to less than 20% . Weak inhibitory effect was detected with other LPS MAbs prepared from different origins. This phenomenon may be correlated to the difference in the LPS polysaccharide sequence or might also indicate to the involvement of core region of LPS. Rough strains like type culture collection were weakly inhibited by anti-LPS MAbs, which support further the important role of O-side chain in the binding as well as inhibition of that binding with even species-specific MAbs.

Interestingly, we observed the results of anti-Lewis X and anti-Lewis Y MAbs inhibitory effect of the bacterial binding to tissue culture cells, which demonstrate similar phenomenon. Strong inhibitory effect was detected with anti-Lewis X MAbs on binding of the smooth strain to Caco-2 tissue culture cells, but weaker effect than recognized by application of anti-whole LPS specific MAbs. Much weaker inhibition of smooth strain using anti-Lewis Y MAbs in the binding to Caco-2 tissue culture cells, which may represent structurally different binding site adhesin than Lewis X adhesin.

It has been reported that LPS antigens due to their position are necessary for the establishment of infection, because mutant strains defective in LPS O-antigen synthesis or for Lewis X/Y expression fail to colonize mice stomach (2, 15).

Interesting is the fact that the long structure of LPS with polysaccharide chain makes easy for bacteria to reach the target surface, before any other adhesin on the bacterial cell, in order to initiate the first step in the colonization process, thereafter other adhesins may take over to make the interaction more solid and persistent.

The chemistry and biology of *H. pylori* lipopolysaccharides (LPS) have been

extensively studied. Aspinall et al. (6) and Monteiro et al. (20) determined the structures of the O-polysaccharides of *H. pylori* LPS and found in them to be the same as the Lewis X (Lex) and Lewis Y (Ley) determinants of human cell surface.

H. pylori LPS possess unique biological properties. *H. pylori* LPS has, in general, low immunological activity and this property may aid the persistence of infection.

It has been reported that the structures of LPS isolated from a variety of *H. pylori* strains have been determined chemically, therefore it is very important to mention here, that there is an evidence regarding expression of Lewis antigens on the bacterial surface, that may contribute to adherence of *H. pylori* to gastric epithelial cells (5, 10), and play a role in tissue tropism (12, 13).

Our work support the finding regarding the important role of LPS in the binding and colonization of human gastric tissue by *H. pylori* using LPS MAbs as well as anti-Lewis X and anti-Lewis Y, which will be a helpful in the designing of medicine strategy in order to prevent infection.

Our study on binding and inhibition of binding clearly demonstrates , that Caco-2 tissue culture cells expresses receptors quantitatively and qualitatively on behalf of recognition by *H. pylori* LPS these receptors. In order to study the receptors expressed by the tissue cultures, one may suggest to prepare glycolipids, phospholipids, proteins and glycoproteins from tissue culture cells.

These products whether they are proteins or glycoproteins, can be tested by overlay assay with labeled-bacteria on Western-Blot or glycolipids and phospholipids separated on thin-layer chromatograms, respectively.

H. pylori LPS, in part, mediates the binding of the bacterium to laminin, and interferes with gastric cell receptor-laminin, thereby potentially contributing to the loss of mucosal integrity (36).

Appelmelk et al. (3) suggested that the mimicry of Lewis antigens by this bacterium raised titers of autoantibodies to Lewis antigens in infected individuals. As these antigens are present in the gastric mucosa, the expression of Lewis antigens on the bacterial surface may camouflage the bacterium and aid survival of *H. pylori.*

*H. pylori*LPS might induce anti-$Le^{x/y}$antibodies that bind to the bacteria but also to the gastric epithelial cells; when followed by complement fixation which may lead to tissue injury.

Conclusions

1. Inhibition of binding of *H. pylori* strains to tissue culture cells is effective in the species-specific manner.

2. Caco-2 tissue culture cells are better target than AGS cells for binding of *H. pylori* clinical isolates as well as type culture collection, which may be correlated to a less and/or different receptor active components expressed by the latter.

3. Inhibitory effect of MAbs raised against LPS from own rough (type culture collection) and smooth (Hel 305) strains on the binding of *H. pylori* rough strain to AGS cell line was almost identical.

4. Lewis X antigen is part of the LPS, which is involved in the binding of *H. pylori* to Caco-2 cells.

1 2 3 4 5 6 7 8 9

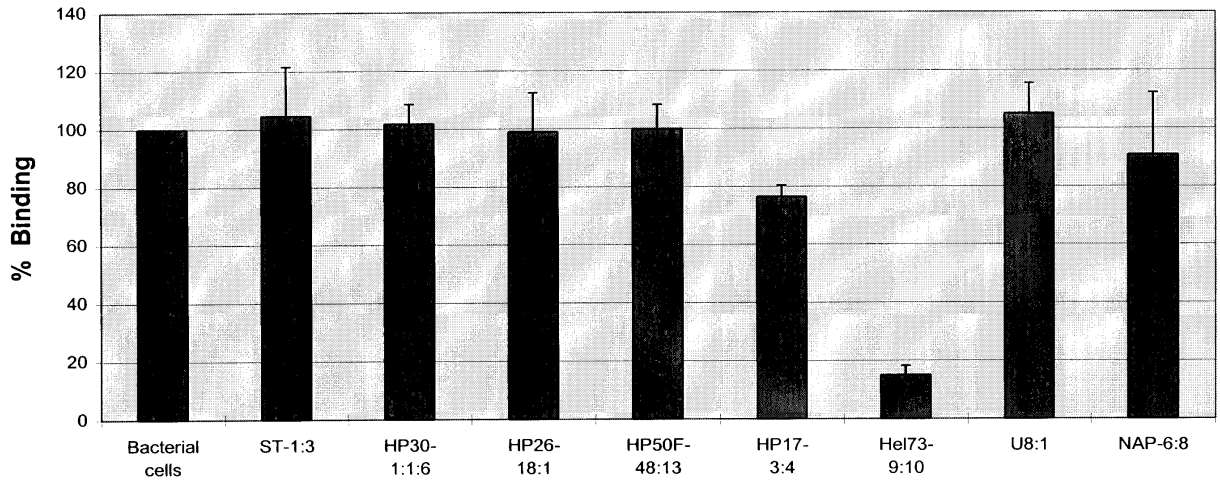

Figure 1

Inhibitory effect of various MAbs on the binding percentage of *Helicobacter pylori* clinical isolate Hel 305 to Caco-2 epithelial cells.

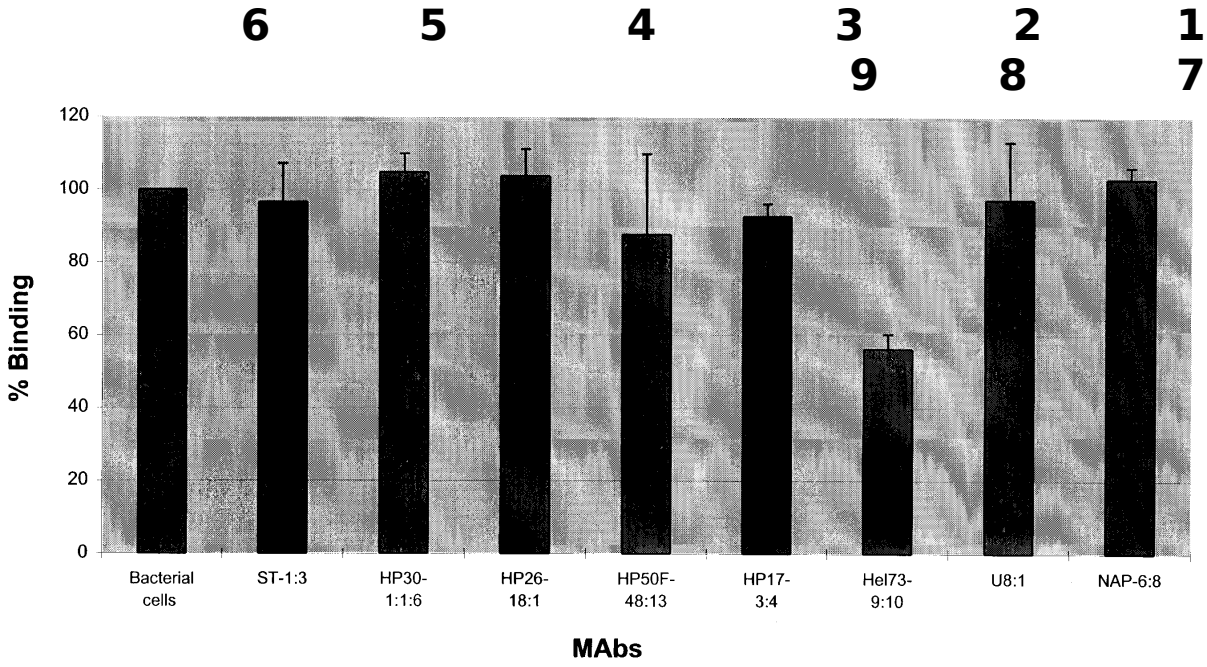

Figure 2

Inhibitory effect of various MAbs on the activity of the binding percentage of *Helicobacter pylori* clinical isolate Hel 305 to human adenocarcinoma AGS cells

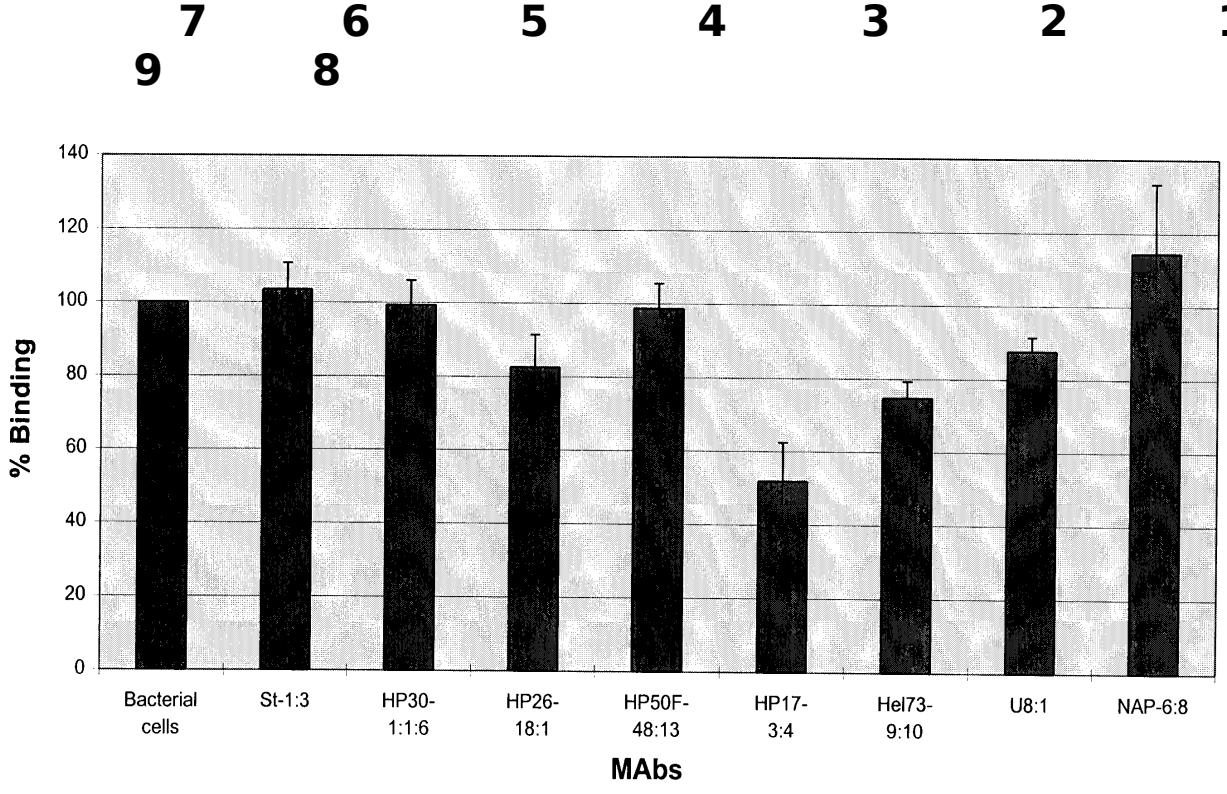

Figure 3

Inhibitory effect of various MAbs on the binding percentage of *Helicobacter pylori* culture collection CCUG 17874 to Caco-2 epithelial cells.

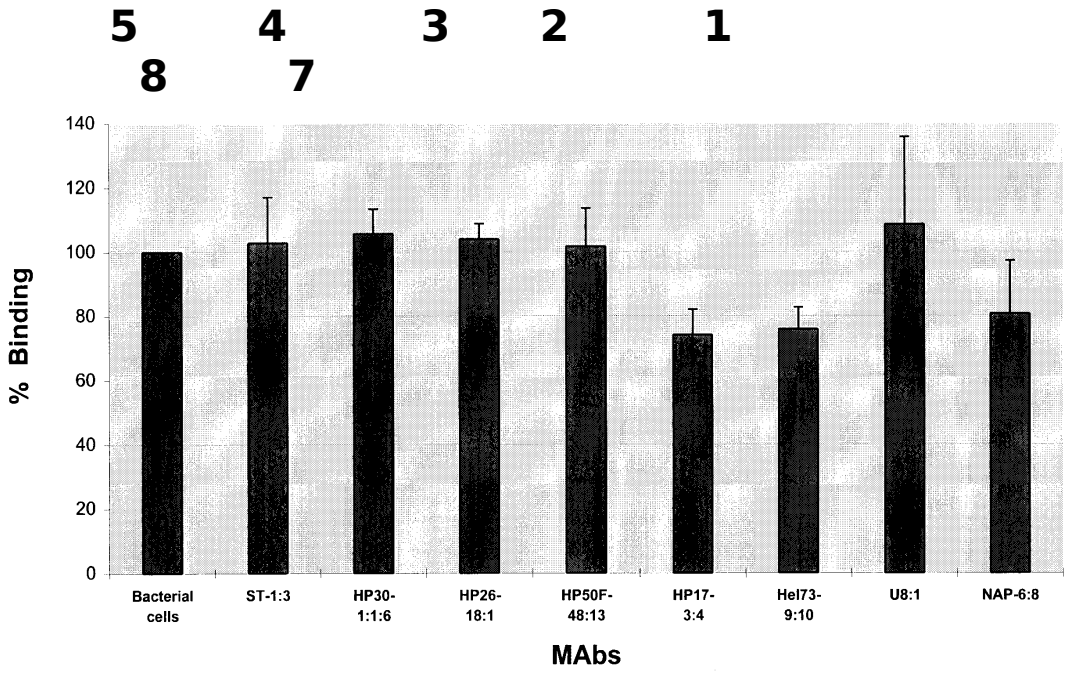

Figure 4

Inhibitory effect of various MAbs on the binding percentage of culture *collection Helicobacter pylori* CCUG 17874 to human adenocarcinoma AGS cells.

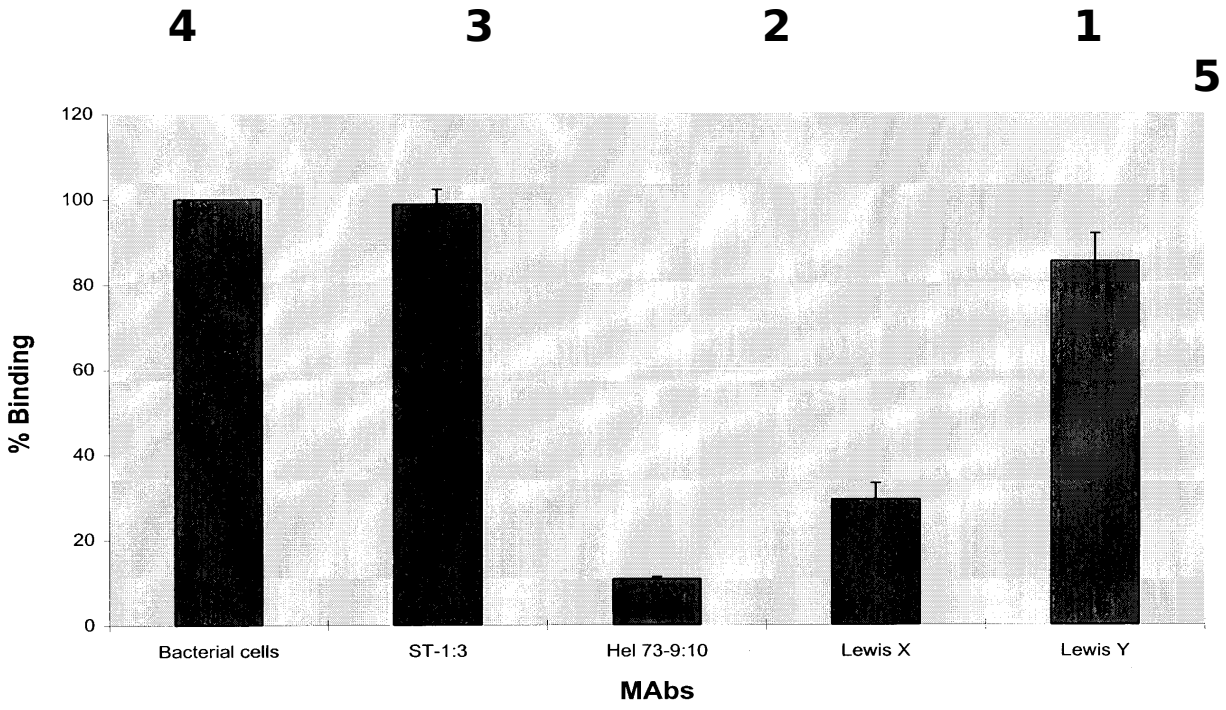

Figure 5

Inhibitory effect of various anti-LPS MAbs and anti-Lewis antigens X and Y on the binding percentage of clinical isolate *Helicobacter pylori* Hel 305 to Caco-2 epithelial cells.

Table 1

H. pylori strain	% inhibition of bacterial binding by anti-LPS MAbs[a]		
	Hel 73-9:10	HP17-3:4	HP50-3:9
CCUG 17874	25 ± 4.6	48 ± 10.47	0
E50	55 ± 14.7	ND	81 ± 4.7
Hel 305	86 ± 3.14	24 ± 3.9	0

Inhibitory effect of various monoclonal antibodies on the binding of different *H. pylori* strains to Caco-2 epithelial cells. (%): the mean value of binding results from three experiments ± *standard deviation*.

Reference

1.Achtman, M., M.Schwuchnow, R.Helmuth, S.Morelli, andP.A.Manning. 1978. Cell-cell interactions in conjugating *E. coli*: conmutants and stabilization of mating aggregates. Mol Gen Genet 164: 171–183.

2. Altman E., Chandan V., Larocque S., Aubry A., Logan S.M., Vinogradov E.,Li J. 2008: Effect of the HP0159 ORF mutation on the lipopolysaccharidestructure and colonizing ability of *Helicobacter pylori*. FEMS Immunol Med Microbiol 53:204-213.

3. Appelmelk B. J., I. Simoons-Smit, R. Negrini et al., 1996. “Potential role of molecular mimicry between *Helicobacter pylori*lipopolysaccharide and host Lewis blood group antigens in autoimmunity”. Infect. Immun. 64:2031–2040.

4. Appelmelk B.J., Monteiro M.A., Martin S.L. *et al*. 2000. Why *Helicobacter pylori* has Lewis antigens. Trends Microbiol 12:565–570.

5. Appelmelk B.J., Monteiro M.A., Martin S.L., Moran A.P., Vandenbroucke-Grauls CM. 2000. Why *Helicobacter pylori* has Lewis antigens. TrendsMicrobiol8:565-570.

6. Aspinall G. O., Monteiro M. A., Pang H., Walsh E. J., Moran A. P. 1996. Lipopolysaccharide of the *Helicobacter pylori* type strain NCTC 11637 (ATCC 43504): structure of the O antigen chain and core oligosaccharide regions. Biochem 35:2489–2497.

7. Basso D., Plebani M., Kusters J.G**.** 2010. Pathogenesis of *Helicobacter pylori*infection. Helicobacter 15:14 –20.

8. Cover T. L. and M. J. Blaser. 1996. “*Helicobacter pylori* Infection, a paradigm for chronic mucosal inflammation: pathogenesis and implications for eradication and prevention,” Advances Intern Med 41: 85–117

9.Darveau R.P., Hancock R.E. 1983. Procedure for isolation of bacterial lipopolysaccharides from both smooth and rough *Pseudomonas aeruginosa* and *Salmonella typhimurium* strains.J Bacteriol 15:831-838.

9a. De St Groth., S. F.,and D.Scheidigger.1980.Production of monoclonalantibodies. Strategy and tactics. J Immunol Methods35:1–21.

10. Edwards N.J., Monteiro M.A., Faller G., Walsh E.J., Moran A.P., Roberts I.S.,High N.J. 2000. Lewis X structures in the O antigen side-chain promote adhesion of *Helicobacter pylori* to the gastric epithelium. MolMicrobiol 35:1530-1539.

11. Eslick G.D. 2006. Helicobacter pylori infection causes gastric cancer? A review of the epidemiological, meta-analytic, and experimental evidence. World J Gastroenterol 12:2991–2999.

12. Falk P., Roth K.A., Boren T., Westblom T.U., Gordon J.I., Normark S. 1993. Anin vitro adherence assay reveals that *Helicobacter pylori* exhibits cell lineage-specific tropism in the human gastricepithelium. ProcNatlAcadSci USA, 90:2035-2039.

13. Gonzalez-Valencia G., Munoz-Perez L., Morales-Espinosa R., Camorlinga-Ponce M., Munoz O., Torres J. 2008. Lewis antigen expression by *Helicobacter pylori* strains colonizing different regions of the stomach of individual patients. J ClinMicrobiol 46:2783-2785.

14. Ilver D., Arnqvist A., Orgen J. et al. 1988. *Helicobacter pylori*adhesin binding fucosylatedhisto-blood group antigens revealed by retagging. Science 279:373–377.

15. Logan S.M., Conlan J.W., Monteiro M.A., Wakarchuk W.W., Altman E.2000.Functional genomics of *Helicobacter pylori*: identification of a beta-1,4 galactosyltransferase and generation of mutants with altered lipopolysaccharide. MolMicrobiol 35:1156-1167.

16. Magalhaes A. and Reis C.A. 2010. *Helicobacter pylori* adhesion to gastric epithelial cells is mediated by glycan receptors. Braz J Med Biol Res 43:611–618

17. Mahdavi J., Sonden B., Hurtig M. et al. 2002. *Helicobacter pylori* SabAadhesin in persistent infection and chronic inflammation. Science 297:573–578.

18. Mancini G., Carbonara A.O., Heremans J.F. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochem 2:235-254.

19. Marshall B. J. and J. R.Warren. 1984. “Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration,” The Lancet 1: 1311–1315.

20. Monteiro M. A., D. Rasko, D. E. Taylor, and M. B. Perry. 1998. “Glucosylated N-acetyllactosamine O-antigen chain in the 4 ISRN Microbiology lipopolysaccharide from *Helicobacter pylori* strain UA861”. Glycobiology 8: 107–112.

21. Moran A.P., Gupta A., Joshi L. 2011. Sweet-talk: role of host glycosylation in bacterial pathogenesis of the gastrointestinal tract. Gut 60:1412–1425.

22. Monteiro, M. A. 2001. Helicobacter pylori: a wolf in sheep’s clothing:the glycotype families of *Helicobacter pylori*lipopolysaccharides expressing histo-blood groups: structure, biosynthesis, and role in pathogenesis. AdvCarbohydChemBiochem 57, 99–158.

23. Mollicone R., Bara J., Le.Pendu J.,Oriol R. 1985.Immunohistologic pattern of type 1(Lea, Leb) and type 2 (X, Y, H) blood grouprelated antigens in the human pyloric and duodenal mucosae. Lab Invest 53:219-227.

24. Moran A. P., Lindner B., Walsh E.J. 1997. Structural characterization of the lipid A component of *Helicobacter pylori* rough- and smooth-form lipopolysaccharides. J Bacteriol 179 :6453-6463.

25. Muotiala A., Helander I.M., Pyhala L., Kosunen T.U., Moran A.P.1992.Low biological activity of *Helicobacter pylori* lipopolysaccharide. InfectImmun 60:1714 –1716.

26. Netea M.G., van Deuren M., Kullberg B.J., Cavaillon J.M., Van der MeerJ.W. 2002. Does the shape of lipid A determine the interaction of LPS withToll-like receptors? Trends Immunol 23:135–139.

27.Nielsen H., Birkholz S., Andersen L.P., Moran A.P**.** 1994. Neutrophil activation by *Helicobacter pylori* lipopolysaccharides. J Infect Dis 170:

135–139.

28. Perez-Perez G.I., Shepherd V.L., Morrow J.D., Blaser M.J. 1995. Activationof human THP-1 cells and rat bone marrow-derived macrophages by *Helicobacter pylori* lipopolysaccharide. Infect Immun 63:1183–1187.

29. Sheu B.S., Sheu S.M., Yang H.B. et al. 2003. Host gastric Lewis expression determines the bacterial density of *Helicobacter pylori* in babA2 genopositive infection. Gut 52:927–932.

30. Sheu S., Sheu B., Yang H. *et al* .2007. Anti-Lewis x antibody promotes *Helicobacter pylori* adhesion to gastric epithelial cells. Infect Immun 75:2661–2667.

31. Svennerholm A.-M. 2000. Differences in surface-exposed antigen expression between *Helicobacter pylori* strains isolated from duodenal ulcer patients and from asymptomatic subjects. J ClinMicrobiol 38:3436-3441.

32. Taylor D.E., Rasko D.A., Sherburne R., Ho C., Jewell L.D. 1998. Lack of correlation between Lewis antigen expression by *Helicobacterpylori* and gastric epithelial cells in infected patients. Gastroenterol 115:1113-1122.

33. Wang C., Yuan Y., Hunt R.H. 2007. The association between *Helicobacter pylori* and early gastric cancer: a meta-analysis. Am J Gastroenterol 102:1789–1798.

34. Wirth H.P., Yang M., Peek R.M. Jr, Hook-Nikanne J., Fried M., Blaser M.J. 1999. Phenotypic diversity in Lewis expression of *Helicobacter pylori* isolates from the same host. J Lab Clin Med133:488-500.

35. Wirth H.P., Yang M., Sanabria-Valentin E., Berg D.E., Dubois A., Blaser M.J. 2006. Host Lewis phenotype-dependent *Helicobacter pylori* Lewis antigen expression in rhesus monkeys. Faseb J 20:1534-1536.

36. Moran A. P. 1996. The role of lipopolysaccharide in helicobacter pylori pathogenesis. Aliment PharmacolTher 10: 39-50.

37. Lopez-Vidal Y., Klemm P., Svennerholm A-.M. 1988. Monoclonal antibodies against different epitopes on colonization factor antigen I of enterotoxin-producing Escherichia coli.J ClinMicrobiol 26:1967-1972.

38. Westphal, O., and K. Jann.1965. Bacterial lipopolysaccharide: extraction with phenol-water and further applications of the procedure. *In* R. Whitler (ed.), Methods in carbohydchem 5: 83–92. Academic Press, New York.

ملخص البحث

الدراسه الحاليه تقييم دورالزوائد الكاربوهيدراتيه الدهنيه المتعددة السكر(بالليبوبوليسكرايد) لبكتريا التهاب المعده والاثنى عشري (هيليكوبكتر بيلوري) في التساق هذه البكتريا بخلايا المعده مسببة المرض. لدراسة هذه الظاهره تم تسليط الضوء على قابلية الأجسام المضادة الوحيدة النسل الخاصة بهذه الزوائد (بالليبوبوليسكرايد) في منع التساق بكتريا هيليكوبكتر بيلوري بمزارع الانسجه هذه مثال خلايا القولون المبطنه السرطانيه وخلايا المعديه السرطانيه. لبحث دوراللليبوبوليسكرايد فيما اذا كانت كاملة البناء من عدمه بالالتساق بالمزارع النسيجيه استخدمت نماذج بكتبريه وحشيه كاملة بالليبوبوليسكرايد(ناعمه) ومختبريه غير كاملة بالليبوبوليسكرايد(خشنه). لقد لوحظ ان قوة منع الالتساق للنوع الناعم من هذه البكتريا بخلايا القولون السرطانيه قويه عند استعمال الأجسام المضادة الوحيدة النسل الخاصة بالنوع الناعم لمركب بالليبوبوليسكرايد. أن قوة منع التساق بكتريا التهاب المعدة بالخلايا السرطانية بواسطة الأجسام المضادة الوحيدة النسل الخاصة بالليبوبوليسكرايد الكاملة هي أكثر بكثير من قوة منع أجسام المضادة لنوع بالليبوبوليسكرايد الغير الكاملة للنوع الخشن (ألمختبري) من البكتريا ضد التساق النوع الوحشي (الكامل بالليبوبوليسكرايد) من البكتريا عند اختبارها مع النسيج الخلوي ذاته. وبنفس ألطريقه تكون قوة منع التساق النوع الخشن من البكتريا بواسطة الأجسام المضادة لنوع بالليبوبوليسكرايد الخشن هي اقوي من قوة منع الأجسام المضادة الخاصة بالنوع الناعم لليبوبوليسكرايد لمنع التساق البكتريا المختبريه (النوع الخشن) لذات الخلايا النسيجيه نفسها. اختبارات تدرس قوة منع التساق بكتريا المعده والاثني

عشري لخلايا الادينوكارسينوما المعديه السـرطانيه اجريـت لغـرض تسـليط الضـوء مـن جهة النسيج الخلوي كهـدف للالتسـاق والاصـابه. درجـة منـع التسـاق البكتريـا الوحشـيه (النوع الناعم) وكذلك النوع ألمختبري (النـوع الخشـن) بخلاياالادينوكارسـينوما المعـديه السرطانيه باستعمال أجسام المضادة المتخصصه للناعم او الخشن منالليبوبوليسكرايد كل على حده امتازت بان التساقها ومنع االتساقها اضعف من خلاياالقولون السـرطانيه. اضافة لذلك فان قوة منع الالتساق بيـن النـوعين يكـاد يكـون متسـاويا بغـض النظرعـن مصدر الأجسام المضادة بخصوص الناعم او الخشن. لم يلاحظ اي نشاط لبقية الأجسام المضادة المستعمله بهذا البحث يمنع التساق البكتريا بالمزارع الخلويه.

استعمال الأجسام المضادة الوحيدة النسل الخاصة بمؤلد المضاد (الانـتي جيـن) لـويس من الانواع أكس أو واي لمنع التساق النوع الوحشي (الناعم) من بكتريا التهـاب المعـده والاثني عشري لخلايا القولون السرطانيه وجدنا ان الجسم المضاد نوع أكس كـان أكـثر فعـاليه مـن النـوع واي ولكنهـم جميعـا اضـعف مـن أجسـام المنـاعه الخاصـة بالليبوبوليسكرايد للبكتريا نفسها.