Adherent properties of *Helicobacter pylori* to human epithelial cells

Zheng-Xiang Wang, Hou-Feng Shen, Hong-Ju Chen

Zheng-Xiang Wang, Hou-Feng Shen, Hong-Ju Chen, Department of Microbiology and Immunology, Yangzhou University Medical College, Yangzhou 225001, Jiangsu Province, China

**Author contributions:** All authors contributed equally to the work.

**Supported by** Jiangsu Natural Science Foundation (No. BK93155315).

**Original title:** China National Journal of New Gastroenterology (1995-1997) renamed World Journal of Gastroenterology (1998-)

**Correspondence to:** Dr. Zheng-Xiang Wang, Yangzhou University Medical College, Yangzhou 225001, Jiangsu Province, China

**Telephone:** +86-514-7312921

**Received:** July 26, 1996
**Revised:** October 1, 1996
**Accepted:** January 1, 1997
**Published online:** March 15, 1997

**Abstract**

**AIM:** To study the properties and factors of *Helicobacter pylori* (*H. pylori*) adherence to human epithelial cells.

**METHODS:** The adherent properties of human epithelial cells were studied using a group of isolated *H. pylori* strains, anti-*H. pylori* monoclonal antibodies and varied pH environment in *in vitro* adherence model with HEp2 cells.

**RESULTS:** *H. pylori* YC 11A was able to adhere to HEp2 cells specifically and its adherence efficiency reached the highest (81%) within 3 h after incubation with HEp2 cells. There was no significant difference between adherence in air and in 5% carbon dioxide. The monoclonal antibodies specific to *H. pylori* predominant antigens did not inhibit activities on adherence of *H. pylori* to HEp2 cells. The pH value significantly affected the adherence process and the optimal pH was 3.0-4.6.

**CONCLUSION:** *H. pylori* specifically adheres to HEp2 cells, and pH value significantly affects this process. A high level of anti-*H. pylori* predominant antibodies in serum may have no protective activities against *H. pylori* infection.

**Key words:** *Helicobacter pylori*; Epithelial cells; Antibodies; Monoclonal antibodies; Hydrogen ion concentration

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wang ZX, Shen HF, Chen HJ. Adherent properties of *Helicobacter pylori* to human epithelial cells. *World J Gastroenterol* 1997; 3(1): 35-37. Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i1/35.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i1.35

**INTRODUCTION**

*Helicobacter pylori* (*H. pylori*) is a pathogen of nearly all duodenal ulcers and most gastric ulcers and is associated with an increased risk of gastric adenocarcinoma[1-2]. *H. pylori* has been found to intercellular junctions as well as on the surface of natural cells in *vivo*, but never inside the cells for its poor invasive properties, yet its adherent properties are rarely identical and could generate the characteristic histopathological lesions. This study aims to develop an *in vitro* model of adherence of *H. pylori* and analyze the properties and the factors of adherence of *H. pylori* to human epithelial cells.

**MATERIALS AND METHODS**

**Strains and cells**

The *H. pylori* strains used were isolated initially from patients with chronic active gastritis or digestive ulcers and stored at 70 °C[3-5]. HEp2, an epithelial cell line, was obtained from the Chinese Academy of Preventive Medicine and has passed 23 generations in culture.

**Adherence tests**

HEp2 cells were grown in 24 well microplates (Nunc, Roskilde, Denmark) with cover slips in 1.5 mL of Delbacco’s modified Eagle’s medium with 10% fetal calf serum without antibiotics to obtain a subconfluent monolayer. The bacteria were cultured for 48-72 h on Skirrow’s blood medium at 35 °C under 5% O2, 10% CO2 and 85% N2 and were gently harvested in brucella broth to give a cell density of 10.7/mL. The HEp2 cell slips were washed three times with Hank’s solution, one time with 0.2 mol/L (pH3.6) citrate buffer, followed by addition of 0.9 mL of 0.2 mol/L (pH3.6) citrate buffer and 0.1 mL of the bacteria suspension. The microplates were then reincubated within microaerobic condition for 4 h and subsequently washed 5 times with strong agitation with 0.9% saline solution to remove nonadherent bacteria and fixed with 2.5% glutaraldehyde solution for 15 min at room temperature. The slides were stained and examined under light microscope.

To estimate the factors affecting the adherence, the adherence tests were carried out in air, in varied pH or in the system containing 0.1 mL of 1:10 monoclonal antibodies specific to *H. pylori* predominant antigens[5].

**RESULTS**

The results obtained for *H. pylori* YC 11A adherence to HEp2 are shown in Table 1. The adherence of *H. pylori* to HEp2 began 5 min after incubation and peaked at the 3rd hour. There was no significant difference between adherence in air or in microaerobic atmosphere (*P* > 0.01).

*H. pylori* YC-11A started to adhere to HEp2 with its terminal portion, and after a long time of incubation, it could adhere to every part of the surface of HEp2, yet adherence to apicals of HEp2 cells was more frequent (Figure 1).
The adherence efficiency obtained with 11 strains of *H. pylori* isolates is listed in Table 2. The pH of adherence environment remarkably affected the adherence of *H. pylori* YC-11A to HEp2 cell (Figure 2). The optimal adherent pH was 2.6-4.6 and the maximum adherence efficiency was obtained with pH at 3.0. The results of inhibition of monoclonal antibodies specific to *H. pylori* on adherence are listed in Table 3 and there was no inhibited activity at pH3.6 in microaerobic atmosphere.

**DISCUSSION**

To colonize luminal mucus, *H. pylori* adheres to the apical plasma membrane of the epithelial cell surface in the antrum *in vivo* by the specific compounds on its surface. These specific structures include flagella and adhesins. All the eleven strains of *H. pylori* isolates showed different adherent efficiency, indicating that the expression level of adhesin and mobility by various isolates differed.

Current evidence suggested that there are a number of adhesins on the surface of *H. pylori*. These include fibrillar hemagglutinins and M (microbial) selectins. Fibrillar hemagglutinin specifically binds sialylactose in structure, and immunogenity and immunogenicity and expression of a gene encoding an adhesin subunit protein of *Helicobacter pylori*.

Evans BG, Karjalainen TK, Evans DJ, Graham IV, Lee CH. Cloning, nucleotide sequence, and expression of a gene encoding an adhesin subunit protein of *Helicobacter pylori*. *J Bacteriol* 1991; 18: 118-119

Wang ZX, Shen HF, Chen HJ and Tong K. Establishment and preliminary characterization of the hybridoma cell lines secreting anti-*Helicobacter pylori* monoclonal antibodies. *J Infect Dis* 1995; 172: 99-104

The monoclonal antibodies used were a cluster of antibodies specific to the predominant antigens of *H. pylori*, but they all had no inhibitory actions on adherence of *H. pylori* and even promoted adherence of *H. pylori*. These results further indicated that a high level of antibodies in human serum against *H. pylori* predominant antigens might not benefit the clearance of *H. pylori* infection in gastric mucus and may be a factor for persistence of *H. pylori* infection.

**REFERENCES**

1. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; 1: 1311-1315 [PMID: 6145023 DOI: 10.1016/S0140-6736(84)91816-6]
2. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. *Helicobacter pylori* infection and the risk of gastric carcinoma. *N Engl J Med* 1991; 325: 1127-1131 [PMID: 1890020 DOI: 10.1056/NEJM199110173251603]
3. Wang ZX, Wang XL and Wu Y. A singular procedure for culture isolation of *Helicobacter pylori*. *J Med Microbiol* 1993; 41: 674-683 [PMID: 7678592]
4. Evans BG, Karjalainen TK, Evans DJ, Graham IV, Lee CH. Cloning, nucleotide sequence, and expression of a gene encoding an adhesin subunit protein of *Helicobacter pylori*. *J Bacteriol* 1991; 175: 674-678 [PMID: 5768592]
5. Lingwood CA, Wasfy G, Han H, Huesca M. Receptor affinity purification of a lipid-binding adhesin from *Helicobacter pylori*. *Infect Immun* 1991; 61: 2474-2478 [PMID: 8508882]
Helicobacter pylori bind to common lipid receptors in vitro. *Infect Immun* 1993; 61: 2632-2638 [PMID: 8500901]

Tacket CO, Losonsky G, Link H, Hoang Y, Guesry P, Hilpert H, Levine MM.

Protection by milk immunoglobulin concentrate against oral challenge with enterotoxigenic Escherichia coli. *N Engl J Med* 1988; 318: 1240-1243 [PMID: 3283555 DOI: 10.1056/NEJM198805123181904]
