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ORIGINAL RESEARCH

Adherent properties of *Helicobacter pylori* to human epithelial cells

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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% oxygen. 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

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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 in 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 ℃[3,4]. 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 ℃ under 5% O_2, 10% CO_2 and 85% N_2 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 under microaerobic condition for 8 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 coincubation 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).

Table 1 Levels of sIL-2R

Time	Adherence efficiency (%)	
	In microaerobic conditions	In air
5 min	3 ± 2	4 ± 2
40 min	16.5 ± 4.0	14.0 ± 3.5
1.5 h	38 ± 5	34 ± 6
3 h	81 ± 3	78 ± 4
4 h	84 ± 5	76 ± 3
5 h	82 ± 4	81 ± 4

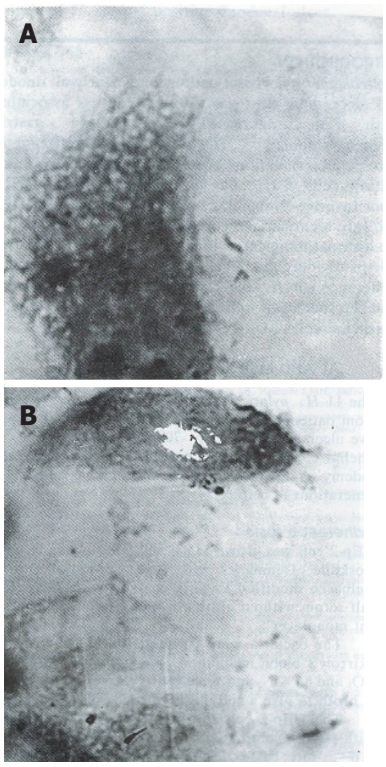

Figure 1 Adherence of *Helicobacter pylori* YC 11A to HEp2 cells (1000 ×). A: Incubation for 40 min; B: Incubation for 5 h.

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 hemagglutinin[6] and M (microbial) selectins[7]. Fibrillar hemagglutinin specifically binds sialyllactose[6]. M selectin is similar to exoenzyme S from *Pseudomonas aeruginosa* in structure, and immunogenity and monoclonal antibodies against this adhesin prevent the attachment of *H. pylori in vitro* to its lipid receptors—gangliotetraosyceramide, gangliotriaosylceramide and phosphatidylethanolamine[8]. Yet, the gastric acidic environment has not been considered. Adherence of *H. pylori* to HEp2 cell was pH restricted and the low pH benefited the adherence, suggesting that the binding properties of adhesins of *H. pylori* to its receptor and the natural properties of the adhesins and their receptors possibly possess specificities, which differ greatly from those of other enteropathogens, such as enterotoxingenic *Escherichia coli*, whose virulence can be easily neutralized by

Table 2 Adherent efficiency of different *Helicobacter Pylori* strains to HEp2 cells

Strains	Adherence efficiency (%)
H. pylori YC-1	74
H. pylori YC-2	76
H. pylori YC-3	52
H. pylori YC-4	64
H. pylori YC-5	61
H. pylori YC-6	58
H. pylori YC-7	71
H. pylori YC-8	85
H. pylori YC-9	80
H. pylori YC-11A	81
H. pylori YC-11B	79

H. pylori: Helicobacter pylori

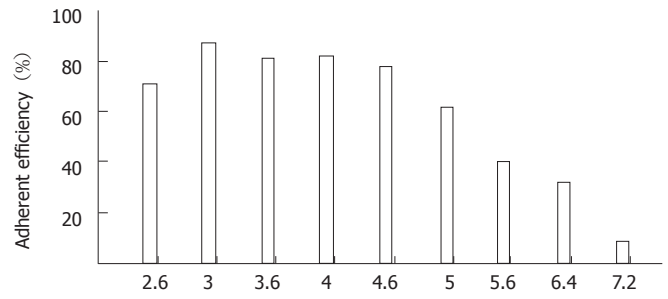

Figure 2 Effect of pH on adherence of *Helicobacter pylori* to HEp2 cells.

Table 3 Monoclonal antibodies inhibited adherence of *Helicobacter pylori* YC-11A to HEp2 cells

Monoclonal antibody	Adherence efficiency (%)
21A5-3	81
22C6-3	84
23C2-2	78
31A10-1	83
31A11-3	74
31B1-1	76
31B1-2	80
31D12-2	78
Control	81

antibodies specific to its adhesin[9]. *H. pylori* infection stimulates immune response, leading to a much higher level of antibodies in sera[4]. The monoclonal antibodies used were a cluster of antibodies specific to the predominant antigens of *H. pylori*[5], but they all had no inhibitory actions on adherence of *H. pylori* and even more 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* in gastric mucus and may be a factor for persistence of *H. pylori* infection.

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