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Adhesion of *Helicobacter pylori* to Polarized T_{84} Human Intestinal Cell Monolayers Is pH Dependent

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Epithelial cells, which form tight polarized monolayers on porous substrates, constitute ideal model systems to study bacterial adhesion and invasion. The binding of *Helicobacter pylori* to the apical membrane of T_{84} cells, an epithelial cell line derived from a human colon carcinoma, was assessed biochemically and morphologically. Attachment was rapid, and binding remained constant over time, with a significant ($P < 0.01$, Mann-Whitney U test) ca. fourfold increase at pH 5.4 (76% ± 22%) compared with pH 7.4 (18% ± 7%). In contrast, adhesion of enteropathogenic *Escherichia coli* was not enhanced at pH 5.4. The transepithelial electrical resistance of the T_{84} cell monolayers was not affected by pH or by *H. pylori*. Following binding, *H. pylori* induced a reorganization of the brush border as reflected by actin condensation, facilitating the intimate association of the bacteria with the apical plasma membrane. *H. pylori* was not internalized, as shown by confocal microscopy. Some bacteria, found in deep invaginations of the apical membrane, were probably inaccessible to gentamicin, thus accounting for the observed tolerance to the antibiotic. These data provide the first evidence that an acidic environment favors *Helicobacter* adhesion and that binding is followed by survival of the bacteria in pockets of the apical membrane.

Helicobacter pylori has developed efficient strategies to bind to cell surfaces and survive in the harsh acidic environment of the stomach. Following binding, the brush border architecture is disrupted, with an intimate contact between the microorganism and the plasma membrane (8, 18).

The mechanisms of binding of *H. pylori* have been analyzed in vitro with cell lines including HEp-2, HEL, CHO, HeLa, mouse adrenal Y-1, human buccal, and human intestinal undifferentiated 407 cells (8, 9, 12, 14, 29), as well as KATO-III cells, a cell line derived from a gastric carcinoma (6–8, 17, 31, 35). All these cell lines, however, lack a brush border at their apical membrane, in contrast to gastric epithelial cells.

Whether intact viable *H. pylori* cells are internalized by gastric epithelial cells remains controversial. Some authors have described microorganisms in vivo in the gastric lamina propria (2, 38) and present as antigens in the endocytic compartment of gastric epithelial cells (25), but most histopathological studies of biopsy specimens obtained from *H. pylori*-infected humans have not provided evidence for bacterial invasion. Internalization has been described in vitro (10, 26), but the cultured eukaryotic cells used in these experiments were not polarized, and therefore these experiments are difficult to interpret. In addition, the use of nonpolarized cells did not allow any tests of whether *H. pylori* was able to disrupt intercellular contacts between adjacent epithelial cells.

The aims of this study were to examine the binding of *H. pylori* in vitro to the luminal surface of polarized epithelia exhibiting a brush border and to elucidate whether an acidic pH affects binding or facilitates bacterial internalization and translocation, or both. We selected for our binding studies a highly differentiated and polarized human cell line, morphologically resembling human gastric surface cells and able to assemble a brush border when cultured on permeable filters and the Madin-Darby canine kidney (MDCK) epithelial cells.

In this paper, we demonstrate that the binding of *H. pylori* to the apical cell surface of T_{84} cells is morphologically similar, if not identical, to the binding observed in human biopsy specimens. Interestingly, the binding on polarized epithelial is pH dependent and induces a local disorganization of the brush border. We also show that tolerance of *H. pylori* to gentamicin, once the bacteria are bound to the cell surface, does not necessarily require internalization of the bacteria.

MATERIALS AND METHODS

Assay to quantitate adherent and intracellular bacteria. The following bacterial strains were used in this study: *H. pylori* 1101 (Institute of Microbiology, Lausanne University, Lausanne, Switzerland), isolated from a patient suffering from functional dyspepsia and shown to have erosive gastritis upon endoscopy; strain 69A, isolated from a patient with nonulcer dyspepsia (Department of Medical Microbiology, Amsterdam University, Amsterdam, The Netherlands); strain 888-0, isolated from a patient with a duodenal ulcer (Department of Medical Microbiology and Immunology, Hamburg University, Hamburg, Germany); and *Escherichia coli* HB101 and the enteropathogenic *E. coli* strains E2348/69 (20), EAF^+ eae^+ (JPN15.pMAR7) (19), and EAF^- eae^- (JPN15.96) (21).

H. pylori was grown on agar plates made of a brain heart infusion (BioMérieux, Paris, France) containing 0.25% yeast extract (Difco Laboratories, Detroit, Mich.) and 10% sheep blood and supplemented with 0.4% *Campylobacter* selective complement (Skirrow supplement, SR 69; Oxoid Ltd., Basingstoke, United Kingdom), maintained in a microaerophilic atmosphere (85% N_2, 10% CO_2, 5% O_2) for 2 days, harvested under sterile conditions into brain heart infusion, and finally diluted in Dulbecco's modified Eagle's medium plus Ham's F12 (DMEM-Ham's 1:1) (pH 5.4 or 7.4) without antibiotics at 10^8 bacteria ml^{-1}. *E. coli* was grown on standard Luria-Bertani agar plates.

T_{84} cells were grown to confluency on Transwell polycarbonate membrane filters (diameter, 12 mm; pore size, 0.4 μm, [Costar, Cambridge, Mass.]) in DMEM-Ham's 1:1 without antibiotics; MDCK cells were grown in DMEM. In about 10 days, the transepithelial electrical resistance monitored with Milicell-ERS (Millipore Corp., Bedford, Mass.) reached ~1,500 Ωx cm^2.

Bacteria were suspended at ~2×10^7 ml^{-1}, and 0.5 ml was added to each

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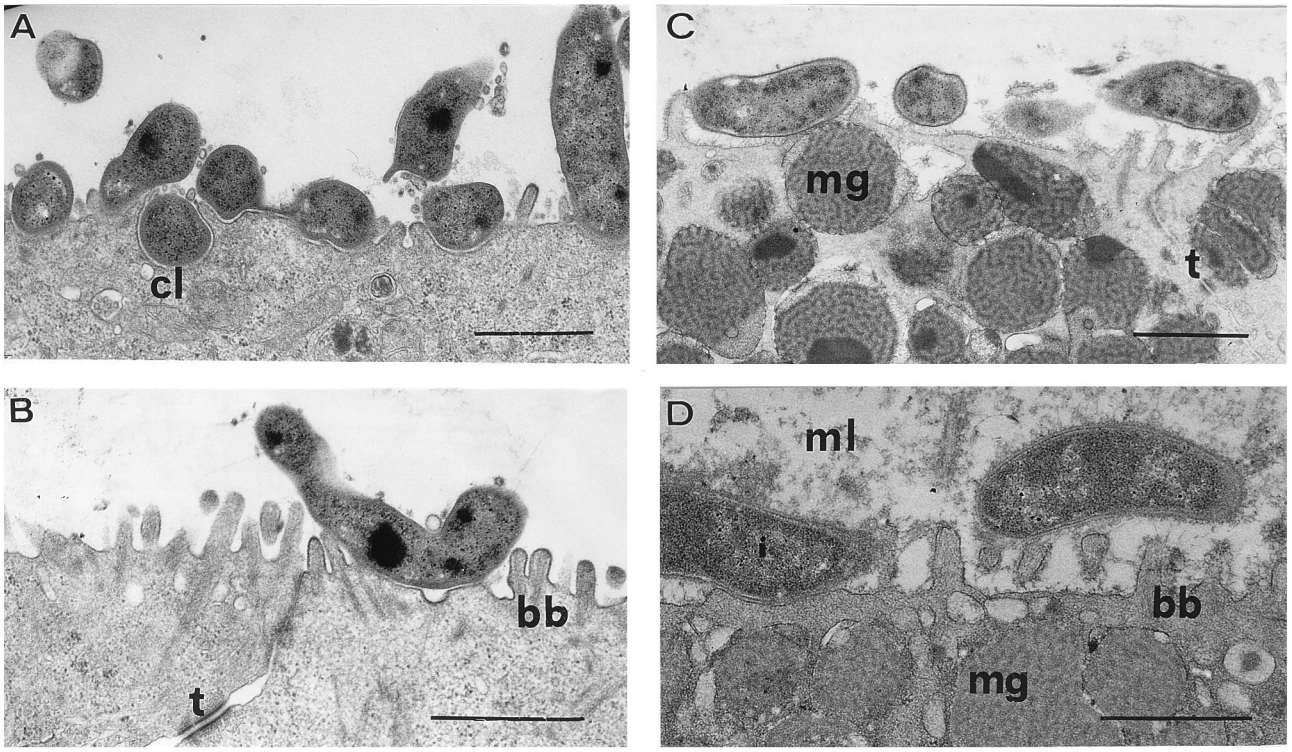

FIG. 1. Adherence of *H. pylori* to human gastric mucosa and to T_{84} cells. Electron micrographs of T_{84} cells exposed to *H. pylori* for 2 h (A and B) and of a gastric mucosal biopsy specimen obtained from a *H. pylori*-positive patient (C and D). Dense concentrations of microfilaments can be observed below the epithelial cell cytoplasmic membrane beneath adherent bacteria. Abbreviations: bb, brush border; t, tight junctions; mg, mucus granule; ml, mucus layer; cl, cup-like structures. Bar, 1 μm.

T_{84}-seeded well (surface area, 1.13 cm^2). The monolayers were then incubated at 37°C under 5% CO_2 for 1, 2, 8, or 24 h. The bacterial count in the inoculum was estimated by plating dilutions of *H. pylori* (10^{-3}, 10^{-5}, and 10^{-7}) on serum plates (GC agar; Gibco BRL, Paisley, Scotland) supplemented with 10% horse serum, Inotec, and 1% IsoVitaleX (Baltimore Biological Laboratories, Baltimore, Md.).

To analyze the ability of glycoproteins to block binding, bacteria were added to the T_{84} cells with or without a 30-min preincubation in the presence of calf serum fetuin or asialofetuin at 100 μg ml^{-1} and bovine submaxillary gland mucin, or human colostrum secretory immunoglobulin A (IgA), or human serum IgA at 500 μg ml^{-1}.

(i) Adherent bacteria. At the end of the incubation period, the monolayers were washed six times with 0.9% NaCl and three times with phosphate-buffered saline (PBS) (no Ca^{2+} or Mg^{2+}). No viable bacteria were recovered in the wash fluid after seven washes. The cells were then lysed by a 10- to 15-min incubation at 37°C in the presence of 0.5 ml of trypsin-EDTA added to the apical and the basolateral sides. The bacteria were dispersed by vigorous pipetting and serial dilutions (10^{-3} and 10^{-5}) in 0.9% NaCl and were plated on serum plates.

(ii) Gentamicin-tolerant bacteria. The cells and bacteria were washed nine times with PBS in the presence of 100 μg of gentamicine per ml. They were then incubated for 4 h in culture medium in the presence of 100 μg of gentamicin per ml. The gentamicin solution was washed off, and the cells were lysed and processed as described above.

To analyze the effect of antibiotics on *H. pylori* when bound to epithelial cells, ampicillin, tetracycline, erythromycin, clarythromycin, streptomycin, and kanamycin were added during incubation at concentrations of 20 μg ml^{-1} (40 to 400 × MIC).

Preparation of bacteria and monolayers for microscopy. (i) Human biopsy specimens. Human antral biopsy specimens were taken by esogastroduodenoscopy from *Helicobacter*-positive subjects without gastroduodenal ulcers.

(ii) Electron microscopy. T_{84} monolayers and human biopsy specimens were washed four times with 0.9% NaCl and five times with PBS (no Ca^{2+} or Mg^{2+}), fixed for 2 h with 2.5% glutaraldehyde in 0.1 M sodium cacodylate, postfixed with 2% osmium tetroxide in 0.1 M cacodylate, dehydrated, and embedded in Epon 812 (Polysciences, Inc., Washington, Pa.). Thin sections were stained with uranyl acetate and lead citrate and examined on a Philips CM10 transmission electron microscope.

(iii) Immunofluorescence/confocal microscopy. *H. pylori* cells were washed in PBS and labeled with pKH26-GL (Sigma Chemical Co, St Louis, Mo.) as specified by the supplier. Bacteria (5×10^5) washed in DMEM-Ham's supplemented with 10% fetal calf serum were added to each T_{84} cell-containing filter. After incubation for 1 to 2 h, the bacteria were directly counted under the microscope or fixed with 3% formaldehyde in PBS for confocal microscopy analysis. Epithelial cells were permeabilized with 2% Triton X-100 for 4 min and processed for immunofluorescence. Villin was detected with a polyclonal antibody (30) diluted 1:250 and fluorescein-conjugated anti-IgG antibody (Amersham International, Amersham, United Kingdom). Preparations were examined under a Polyvar photomicroscope and by confocal laser scanning microscopy (CLSM 410; Zeiss).

Statistical methods. Experiments were run in triplicate, and the results were expressed as means and standard deviations. Significance was estimated with a Mann-Whitney U test when applicable.

RESULTS

***H. pylori* attaches to T_{84} cells and induces effacement of the brush border.** *H. pylori*, added to the apical side of confluent T_{84} monolayers, became intimately associated with the brush border as seen in gastric mucosa biopsy specimens from infected patients (compare Fig. 1A and B, and compare Fig. 1C and D). Cup-like invaginations and occasional adherence pedestals were observed (34). The bacterial membrane was closely apposed to the epithelial cell membrane, and the brush border architecture was altered at the site of contact. In both gastric and T_{84} cells, some bacteria were seen in deep invaginations, but engulfment usually did not result in formation of intracellular vacuoles (Fig. 1).

To quantitate adhesion, about 10^6 T_{84} cells were plated per filter, and at confluency the monolayers were exposed to 1×10^5, 1×10^6, or 2×10^7 bacteria at neutral pH. After 1, 2, or 4 h of incubation at 37°C, adherence was measured by counting viable bacteria. At neutral pH, adhesion, proportional to the number of bacteria plated on the monolayers, was rapid (Table

TABLE 1. Effect of inoculum size on adherence to polarized T_{84} cells

Incubation period (h)	% adherent to monolayer after inoculation (CFU) of[a]:		
	1×10^5	1×10^6	2×10^7
1	2 ± 0.3	1.4 ± 0.1	5.0 ± 1.6
2	2.3 ± 1.9	1.0 ± 0.3	9.0 ± 3
4	9.0 ± 2.6	2.0 ± 0.1	15.0 ± 5.4

[a] The results are expressed as the percentage of the inoculum that adhered to the monolayer at the end of the incubation period ($n = 3$ for each time point).(Results are means ± standard deviations.)

1). In all subsequent experiments, we used 10^7 to 10^8 bacteria per well.

***H. pylori* adhesion is pH dependent.** The binding of live *H. pylori* labeled with the fluorescent marker pKH26 to T_{84} cell monolayers was 4 times higher at pH 5.4 than pH 7.4, irrespective of the *H. pylori* strain tested (Fig. 2A). In addition, the pH did not affect the baseline transepithelial electric resistance, which remained above 1000 Ωx cm^2 at both pH values (Fig. 2B). The adhesion process was rapid, with many bacteria already attached after 5 to 10 min (data not shown), and

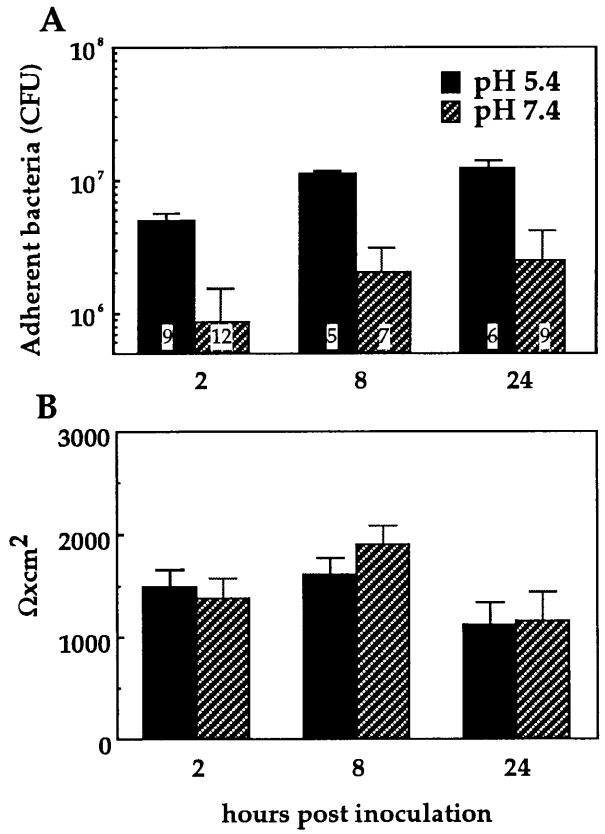

FIG. 2. Adherence of *H. pylori* to T_{84} cells is pH dependent. (A) T_{84} cells were grown in DMEM-Ham's at pH 5.4 or 7.4 on polycarbonate filters to form a polarized epithelium with high transepithelial resistance. The cells were then infected with *H. pylori*. After 2, 8 or 24 h, T_{84} cells were extensively washed (see Materials and Methods) and lysed. Diluted lysates were plated on agar, and adherent bacteria were counted as CFU after 3 days of incubation at 37°C. The number of triplicate experiments performed is indicated inside the bars. Results are expressed as the mean and standard deviation. (B) Measures of transepithelial electrical resistance expressed in ohms-square centimeters. Solid bars, pH 5.4; dashed bars, pH 7.4.

TABLE 2. Adherence of *H. pylori* to T_{84} cells is specific[a]

Strain	% of inoculum that were adherent at:	
	pH 5.4	pH 7.4
H. pylori 69A	75 ± 20	20 ± 5
E. coli HB101	0.18 ± 0.05	0.29 ± 0.13
E. coli E2348/69[b]	2.4 ± 0.85	2.75 ± 0.21
E. coli EAF^+ eae^+	1.74 ± 0.78	1.78 ± 1.05
E. coli EAF^- eae^-	0.8 ± 0.35	0.84 ± 0.44

[a] T_{84} cells were grown as described in the legend to Fig. 2 at pH 5.4 or 7.4 and infected with 2×10^7 CFU of *H. pylori* or *E. coli*. After 4 h of incubation, adherent bacteria were determined by counting the CFU. The results of two to five independent experiments run in triplicates are expressed as a percentage of the inoculum (mean ± standard deviation).
[b] Enteropathogenic *E. coli* strain.

saturation was reached within 2 h at both pH values (Fig. 2A). All binding sites were saturated with approximately 10^7 bacteria, with 50 to 70% of the inoculum bound at pH 5.4. Bacterial recovery was the same at both pH values, indicating that pH did not affect survival and/or growth (data not shown). MDCK cells were also incubated with labeled *H. pylori* at both pH values, and the number of adherent bacteria on intact monolayers after 2 h was again fourfold larger at pH 5.4 ($1.7 \times 10^5 \pm 3.6 \times 10^4$ CFU) than at pH 7.4 ($4.8 \times 10^4 \pm 3.6 \times 10^4$) (mean and standard deviation for four experiments. $P = 0.018$ by the Mann-Whitney U test). Although the overall binding of *H. pylori* to MDCK cells was lower than that to T_{84} cells, the same pH dependency was observed.

To further characterize the nature of the interaction between *H. pylori* and T_{84} cells at each pH, we used compounds that were shown to inhibit the binding of *H. pylori* to human gastric mucosa on plastic sections (5). Calf serum fetuin, a glycoprotein rich in sialylated oligosaccharides (sialylactose) and asialofetuin at a concentration of 100 μg/ml, bovine submaxillary gland mucin, rich in fucosylated and sialylated carbohydrates, and human colostrum secretory IgA and human serum IgA at concentrations of 500 μg/ml failed to block the binding of *H. pylori* to T_{84} cells. Preincubation of the bacteria for 30 min with these compounds before being applied to the T_{84} cells did not block binding either. This suggests that blood group antigens or other cell surface glycoconjugates that are accessible on plastic sections are not available for interaction at the surface of intact cell monolayers and probably in situ (data not shown).

The acidic pH dependency of adhesion is specific to *H. pylori*. To determine if the acidic pH dependency of bindings to intestinal cell monolayers was specific to *H. pylori*, we incubated *E. coli* at the same multiplicity of infection as *H. pylori* and monitored binding at both pH values. Binding of the laboratory strain *E. coli* HB101 and the enteropathogenic *E. coli* E2348/69, EAF^+ eae^+, and EAF^- eae^- to T_{84} cells was not enhanced at acidic pH (Table 2). The acidic pH dependency of binding to intestinal-cell monolayers thus appears to be a property specific to *H. pylori.*

***H. pylori* is not internalized by T_{84} cells despite gentamicin tolerance.** To assess internalization of *H. pylori* in T_{84} cells, we used gentamicin, an antibiotic known to kill extracellular but not intracellular microorganisms (10, 24), and scored the appearance of *H. pylori* in T_{84} cells. Within 1 h, approximately 0.03 and 0.05% of the cell surface-bound bacteria were gentamicin resistant at pH 5.4 (Fig. 3A) and pH 7.4 (Fig. 3B), respectively. These numbers remained approximately constant over the next 3 h, whereas the increase observed later, at pH 5.4 only, probably reflected bacterial multiplication (Fig. 3A).

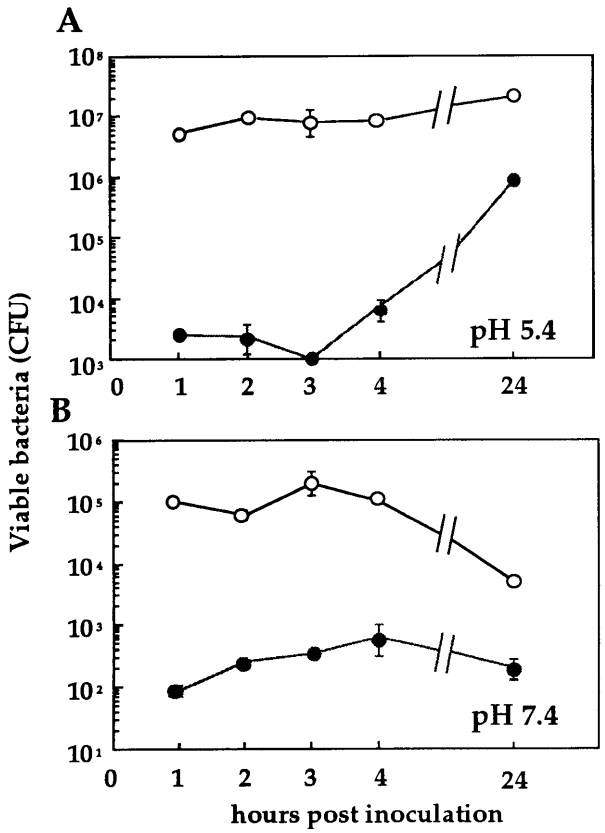

FIG. 3. Gentamicin-tolerant *H. pylori*. T_{84} cells and *H. pylori* organisms were incubated at pH 5.4 or 7.4 in the absence of gentamicin (open circles) or in the presence of 100 μg of gentamicin per ml (solid circles) as described in Materials and methods. Total viable bacteria (open circles) or gentamicin-tolerant bacteria (solid circles) are expressed as CFU after plating of the cell lysates.

No viable bacteria could be recovered following extensive washing of the monolayers, indicating that adhesion of the bacteria was tight. Following cell lysis, however, the bacteria remained susceptible to gentamicin at 10 and 100 μg/ml, indicating that they did not acquire resistance but, rather, became tolerant to the antibiotic. To facilitate access of gentamicin to putative bacteria in the intercellular space, the intercellular junctions were opened with ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) (data not shown). However, the number of gentamicin-tolerant bacteria did not change after EGTA treatment; therefore, tolerance to gentamicin could not be explained by passage of bacteria through tight junctions and sequestration in the intercellular space.

The tolerance of *H. pylori* to gentamicin treatment prompted us to test the effect of other antibiotics on epithelial cell-bound *H. pylori*. Ampicillin, tetracycline, erythromycin, clarithromycin, streptomycin, and kanamycin at 20 μg/ml had the same effect on T_{84}-bound *H. pylori* as on free bacteria (data not shown). These results exclude a general growth phase tolerance induced by binding.

Confocal laser scanning and electron microscopy analysis also failed to detect intracellular and/or intercellular *H. pylori*. T_{84} monolayers incubated for 2 to 24 h with pKH26-labeled bacteria were extensively washed, fixed, and permeabilized as described in Materials and Methods. After permeabilization of the cells, the entire monolayer on the filter was incubated with an antibody directed against villin, an actin-associated protein restricted to the brush border cytoskeleton. The cellular localization of *H. pylori* and villin was then analyzed by confocal laser scanning microscopy (Fig. 4). Labeled *H. pylori* cells were found associated with the apex of the intestinal cells, in closed vicinity to villin labeling. Typically, the intensity of villin labeling was decreased at sites of *H. pylori* adhesion, suggesting a rearrangement of the F-actin network. This was confirmed by electron microscopy (Fig. 1A and B), which showed a focal loss of typical brush border microvilli around the adherent bacteria. Occasionally, a strict colocalization of *H. pylori* and villin was observed by confocal laser scanning microscopy, suggesting that some bacteria were located in deep invaginations of the apical membrane. *H. pylori*, however, was not found inside the intestinal cells. More than 12,000 intestinal cells were analyzed by observing serial horizontal confocal laser scanning microscopy optical sections from the filter to the apex of the intestinal monolayer (data not shown). The bacteria were always detected in the last two or three apical sections at or just above the level of villin labeling but not in deeper sections. Since the internalization frequency appears lower than 1/12,000, it is likely that *H. pylori* is noninvasive in T_{84} cells.

DISCUSSION

In the present study, we have analyzed the binding properties of *H. pylori* to a polarized human epithelial cell line presenting a brush border and organized tight junctions like the gastric epithelium.

Binding to human intestine cells has already been demonstrated in situ (11). When analyzing the cell lineage-specific binding of *H. pylori*, using an in situ adherence assay on slides containing human cells, Falk et al. observed binding to enterocytes situated in the colonic homolog of small intestine villi but not to kidney, cervix, or endometrium cells (11). Although the density of adherent organisms was much lower on intestinal epithelium than on the stomach epithelium, these results suggested the presence of specific receptors on intestinal cells.

In vivo, when found outside of the stomach, *H. pylori* is associated mainly with heterotopic gastric mucosa, such as gastric metaplasia in the duodenum, esophagus, and rectum. The presence of gastric-like mucosa, however, is not sufficient for *H. pylori* to bind, since *H. pylori* is rarely, if ever, present in the heterotopic gastric mucosa of Meckel's diverticula (13). Binding and colonization are thus likely to be the consequence

FIG. 4. Adhesion of *H. pylori* to T_{84} cells viewed under confocal laser scanning microscopy. A computed transverse *x-z* section of villin-labeled T_{84} cells (green) incubated with pKH26 labeled-*H. pylori* (red) is shown. Bar, 10 μm.

of a multifactorial process, dependent upon the presence of specific receptors and a local selective environment.

Polarized and differentiated epithelial cells derived from gastric antrum that form tight monolayers on permeable substrates would constitute the ideal in vitro system. Such cells, however, are yet not available, and the human intestinal T_{84} and CaCo-2- cell monolayers still represent the best systems to study bacterium-epithelial cell interactions and the factors that modulate these interactions.

The tight binding of *H. pylori* induces a local disruption of the brush border in T_{84} cells similar to that observed in gastric epithelial cells, reflected by the decrease of villin labeling, indicating a reorganization of the F-actin network and actin-associated proteins. It is known that bacterial adhesion to the epithelium of mucosal surfaces is required for the expression of the virulence program of pathogenic bacteria (3, 15). Binding promotes cross talk between the microorganism and the epithelial cells (15, 37) with the release of toxins by the microorganism (39) and the expression of adhesins (27). Recently, there has been considerable effort to characterize the pathogenic properties of the human gastric pathogen *H. pylori*, and an intimate association of this bacteria with the cell surface, reminiscent of the "attaching-and-effacing" process seen for enteropathogenic *H. pylori* (16), has been described (5, 6, 23, 25, 34).

Since the adhesion process takes place in an environment with a rapidly changing and frequently acidic pH (32), we examined the effect of pH on adhesion. On average, there was a four- to fivefold increase in the number of adherent bacteria at acidic pH compared with neutral pH. This effect was observed only with *H. pylori* but not with the control organism, *E. coli*. Several factors could theoretically be responsible for the observed pH effect. For example, low pH could affect the de novo expression of the bacterial adhesins or the host receptors. However, this is unlikely because the pH effect on binding was rapid and appeared within 10 min after the pH switch. These data are consistent with the hypothesis that direct or conformational binding is modified by pH.

The bacterial components mediating adhesion of *H. pylori* to the gastric epithelial cell surface have not been well characterized. Multiple adhesion determinants are probably involved in the binding of the organism to gastric surface receptors (36), including hemagglutinins, the 63-kDa exoenzyme H, the 31-kDa outer membrane protein, and the 20-kDa NANA-binding protein (33). Recently, it has been documented that *H. pylori* binds to lactosylceramide with a requirement for hydroxy fatty acid (22). Gastric cells, as well as most intestinal cells and probably T_{84} cells, express this glycolipid on their luminal surface (22). *H. pylori* adhesion to tissue culture cells has also been correlated to the phosphatidylethanolamine content of the cell membrane (8), and phosphatidylethanolamine in lipid extracts from T_{84} cells promoted the binding of *H. pylori* in an assay involving a thin-layer chromatography overlay binding (8). It remains to be established which of these components mediating adherence is responsible for the pH effect observed in our experiments.

It has been claimed that terminal sialic acid and L-fucose residues associated with the carbohydrate moiety of proteins and lipids are implicated in bacterial adhesion, acting as cell surface receptors for bacterial adhesins. We have therefore tested whether components rich in sialic acid and fucose, such as fetuin or mucin, were able to compete for the binding of *H. pylori* to T_{84} cells. No competition was observed, suggesting that these components are not accessible on the surface of intact cell monolayers, in contrast to what was reported for sections of plastic-embedded human gastric tissue (5).

H. pylori invasiveness has been analyzed on biopsy material obtained from *H. pylori*-infected humans. In most in vivo studies, *H. pylori* appears noninvasive, although in a few reports, the organism was found within the lamina propria of the gastric epithelium (2, 4, 38). When epithelial cell lines were used, the results were also conflicting, but part of the discrepancy may be due to differences in methodology. Invasiveness in intracellular organisms is usually assessed by the gentamicin assay, in which the number of viable intracellular bacteria is measured by protection from gentamicin, a nonpermeant antibiotic. Internalization based on gentamicin resistance was documented in HEp-2 cells (10), but viable intracellular organisms could not be recovered from the same infected cells by another group (26), despite the use of similar experimental approaches. On the basis of this assay, we have suggested that *H. pylori* is invasive and becomes internalized in MDCK or T_{84} cells (28). In this study, we have correlated gentamicin resistance with morphological detection of intracellular microorganisms. We confirmed that upon binding, some *H. pylori* become tolerant to gentamicin, but we failed to identify intracellular bacteria by confocal and electron microscopy. We also showed that some bacteria are found in deep plasma membrane invaginations, which might appear as vacuoles depending on the plane of the section. A careful analysis by colocalization of apical plasma membrane markers and *H. pylori* by using confocal microscopy and serial electron microscope sections have failed to document intracellular bacteria in T_{84} cells. If escape from gentamicin killing is not the result of bacterial internalization, we have to postulate that the observed tolerance is due to inaccessibility of the drug to the bacterial porins that allow the antibiotic to cross the bacterial membrane. We can rule out a growth phase-induced tolerance, since the T_{84}-bound *H. pylori* cells are susceptible to treatment with ampicillin, tetracycline, erythromycin, clarythromycin, streptomycin, and kanamycin. All these antibiotics can cross the T_{84} cell membrane. Tight binding and invagination of *H. pylori* might be sufficient to restrict the access of gentamicin and thus render the bacteria tolerant to the antibiotic. These invaginations have not been observed more frequently in the vicinity of the tight junctions, and the transepithelial resistance of the cell monolayers was not affected upon binding of *H. pylori*. These observations argue against the capacity of *H. pylori* to open tight junctions and to penetrate the lamina propria by an intercellular route.

In conclusion, we provide the first evidence that acidic pH favors *H. pylori* adhesion and that binding is followed by survival of the bacteria in pockets of the apical membrane. The cross talk between *H. pylori* and epithelial cells and the signal transduction pathway that triggers brush border disassembly can now be studied in vitro as for other bacterial pathogens (1). This model system should also facilitate the isolation of the genes involved in adherence.

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