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An *in vitro* adherence assay reveals that *Helicobacter pylori* exhibits cell lineage-specific tropism in the human gastric epithelium

(host–microbial interactions/adhesins/glycoproteins/gastric epithelial cell biology)

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ABSTRACT *Helicobacter pylori* is a microaerophilic bacterium found in the stomach of asymptomatic humans as well as patients with acid peptic disease and gastric adenocarcinoma. We have developed an *in situ* adherence assay to examine the cell lineage-specific nature of binding of this organism and to characterize the nature of cell surface receptors that recognize its adhesin. Fluorescein isothiocyanate-labeled *H. pylori* strains were bound to surface mucous cells present in the pit region of human and rat gastric units but not to mucous neck, parietal, or chief cell lineages present in the glandular domains of these units. Binding was abolished by proteinase K treatment of tissue sections and by pretreatment of the bacteria with bovine submaxillary gland mucin, a rich source of fucosylated and sialylated carbohydrates. Several lines of evidence suggest that binding to surface mucous cells is not dependent upon terminal nonsubstituted α2,3- and α2,6-linked sialic acids in the adhesin receptor: (*i*) binding was not inhibited by incubating *H. pylori* strains with sialylated glycoconjugates such as fetuin and free sialyllactose; (*ii*) immunohistochemical stainings using the sialic acid-specific *Sambucus nigra* and *Maackia amurensis* lectins and the cholera toxin B subunit did not detect any sialylated glycoconjugates in these epithelial cells; and (*iii*) binding was not sensitive to metaperiodate under conditions that selectively cleaved carbons 8 and 9 of terminal nonmodified sialic acids. A role for fucosylated epitopes in the glycoprotein(s) that mediate binding of *H. pylori* to surface mucous cells was suggested by the facts that this lineage coexpresses the adhesin receptor and major fucosylated histo-blood group antigens, that monoclonal antibodies specific for histo-blood group antigens H, B, and Le^b block binding, and that the lectin *Ulex europaeus* type 1 agglutinin, which is specific for α-L-fucose, also bound to the same cells that bound the bacteria. Furthermore, human colostrum secretory IgA inhibited adhesion in a metaperiodate- and α-L-fucosidase-sensitive but neuraminidase-independent fashion. The *in situ* adherence assay should be useful in further characterizing the *H. pylori* adhesin and its receptor and for identifying therapeutically useful compounds that inhibit strain-specific and cell lineage-specific binding of this human pathogen.

Helicobacter pylori is a spiral-shaped organism originally assigned to the genus *Campylobacter* (1). This genetically diverse species (2) has been estimated to infect the gastric mucosa of >60% of adult humans by the time they enter their seventh decade (3). Moreover, *H. pylori* has been implicated as a causative agent in chronic active (type B) gastritis (4), gastric and duodenal ulcers (5), and gastric adenocarcinoma (6). A large number of questions remain unanswered about how this organism contributes to these pathogenic states: assessments of cell lineage-specific patterns of binding in the stomach have not been reported, nor is the molecular basis of *H. pylori*'s tropism for the gut understood.

Electron microscopic analyses have shown that the bacteria can adhere to apical membranes of epithelial cells via small cellular projections (adherence pedestals; ref. 7). Studies in model systems such as mouse adrenal Y-1 cells (8) have suggested that surface-associated flexible fibrillar structures that surround this organism function as adhesins or colonization factor antigens to mediate *H. pylori* binding to cellular sialic acid-containing glycoproteins. Binding is inhibited by neuraminidase and fetuin. The organism also produces sialic acid-specific hemagglutinins (9). Moreover, *H. pylori* are able to bind *in vitro* to certain of the acid glycosphingolipids extracted from human stomach such as the ganglioside GM3 (NeuAcα2-3Galβ1-4Glcβ1-1Cer) and sulfatide (SO_3-Galβ1-1Cer) (10). In contrast, *H. pylori* adhesion to HeLa cells appears to be independent of sialyllactose (11).

Cell lineage relationships in the gastric epithelium are not well defined. The best understood system is in the adult mouse where complex regional differences in cell types occur along the cephalocaudal axis of the stomach (12–15). The proximal third (forestomach) is lined with a keratinized stratified epithelium, whereas the distal two-thirds contains a glandular epithelium composed of gastric units. All gastric units contain an upper pit region lined with mucus-producing surface epithelial cells. The midportion of the gastric unit (its isthmus) is composed of proliferating and nonproliferating immature cells (13). The lower portion (gland) of these units may contain intrinsic factor- and pepsinogen-producing chief cells, acid-producing parietal cells, mucous neck cells, and various enteroendocrine cell types (zymogenic glands). They may lack chief and parietal cells and be composed primarily of mucous cells that are distinct from surface mucous cells and several enteroendocrine cell subpopulations (pure mucous glands). The cellular composition of gastric units varies as a function of their location along the cephalocaudal axis (12): zymogenic glands are located in the midportion of the stomach, whereas pure mucous glands are situated in the gastric antrum. The results of [^{3}H]thymidine labeling and electron microscopic studies of mouse gastric units have led Lee and LeBlond (13) to propose that the isthmus region contains stem cells that give rise to daughters, which undergo a bipolar, migration-dependent differentiation. Surface mucous cells migrate up the pit to the luminal surface and have a life-span of ≈3 days (14). The other cell types are thought to arise during their descent to the glands, where the differ-

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Abbreviations: FITC, fluorescein isothiocyanate; DIG, digoxigenin; UEA1, *Ulex europaeus* type 1 agglutinin; SNA, *Sambucus nigra* agglutinin; MAA, *Maackia amurensis* agglutinin.

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entiated members of some lineages have a life-span of >60 days (15).

We have developed an *in situ* assay for *H. pylori* adherence that has allowed us to define host species-specific, gastric epithelial cell lineage-specific, and bacterial strain-specific differences in attachment. In addition, a series of biochemical studies suggest that *H. pylori* adhesins interact with glycoprotein receptor(s) present in the gut epithelium and that this interaction does not require sialylated carbohydrate epitopes.

MATERIALS AND METHODS

***In Situ* Adherence Assay for *H. pylori*.** Multiple samples of adult human esophagus, stomach, duodenum, colon, kidney, cervix, endometrium, and midbrain were obtained from the surgical pathology and autopsy files of the Department of Pathology at Washington University. Only samples of nondiseased tissues were used in these experiments. The gastrointestinal tracts of 250-g Sprague–Dawley rats and 6- to 12-week-old FVB/N mice (16) were removed after sacrifice by cervical dislocation and regionally dissected as described (17). All tissues were fixed in 10% formalin or in a solution of picric acid/formaldehyde/glacial acetic acid (15:5:1; Bouin's fluid) and subsequently embedded in paraffin (17). Five-micron sections were prepared and used for hematoxylin/eosin staining (to identify the cell types present in gastric units and to verify that the tissue samples had no pathologic changes) and/or for subsequent adherence, histochemical, and/or immunocytochemical assays.

Five previously characterized clinical isolates of *H. pylori* were used. NCTC 11637 and 11638 were isolated in 1982 from patients with active chronic gastritis (18). Strain WV229 was from a patient with gastric ulcer, whereas P466 (kindly provided by R. Gilman, The Johns Hopkins University School of Medicine, Baltimore) was obtained from a patient with acute gastritis. Strain MO19 was from an asymptomatic carrier. Strains were grown at 37°C on *Brucella* agar supplemented with 10% bovine blood and 1% IsoVitalex (Becton Dickinson) under microaerophilic conditions (5% O_2/10% CO_2/85% N_2) and 98% humidity. Five days after inoculation, 1 μl of bacteria removed with a sterile loop from a plate was resuspended in 1 ml of 0.15 M NaCl/0.1 M sodium carbonate, pH 9.0 (19), by gentle pipetting. Ten microliters of a 10 mg/ml solution of fluorescein isothiocyanate (FITC, Sigma), freshly prepared in dimethyl sulfoxide, was added to the suspension, which was then incubated for 1 hr at room temperature in the dark. The bacteria were recovered by centrifugation at 3000 × *g* for 5 min, resuspended by gentle pipetting in 1 ml of phosphate-buffered saline (PBS)/0.05% Tween 20, and pelleted by centrifugation as above. The wash cycle was repeated three times. The intensity of FITC labeling of all bacterial strains was similar as judged by inspection of comparable numbers of organisms by fluorescence microscopy. Aliquots (100 μl) were taken from the final suspensions and utilized immediately or stored at −20°C until further use. No differences in attachment patterns were observed between strains labeled and used fresh and strains that were frozen and thawed once before use.

Tissue sections were deparaffinized in xylene and isopropanol, rinsed in water followed by PBS, and then incubated for 15–30 min in blocking buffer (0.2% bovine serum albumin/0.05% Tween 20, prepared in PBS). The FITC-labeled bacterial suspension was diluted 20-fold in blocking buffer and 200 μl was placed on the slide, which was then incubated for 1 hr at room temperature in a humidified chamber. Slides were subsequently washed four to six times with PBS prior to inspection.

To analyze the ability of glycoproteins or free oligosaccharides to block binding, 200-μl suspensions of FITC-labeled bacteria were preincubated for 2 hr at room temperature with the following compounds: bovine submaxillary mucin (Sigma, 500 μg/ml), fetuin (Sigma, 100 μg/ml), asialofetuin (Sigma, 100 μg/ml), human sialyllactose (Sigma, 5 μg/ml), bovine sialyllactose (Sigma, 5 μg/ml), human serum IgA (Cappel Laboratories; 500 μg/ml), and human colostrum secretory IgA (Cappel Laboratories, 15 μg/ml). Bacteria were washed once in blocking buffer before the mixture was added to tissue sections. To further characterize the receptor active domain of human colostrum secretory IgA, periodate oxidation was performed according to a protocol provided in the Glycan detection kit (Boehringer Mannheim). The IgA fraction was then washed with PBS using M_r 10,000 cutoff Centricon filters (Amicon). Human colostrum IgA was also incubated for 2 hr at 37°C with 100 milliunits of bovine kidney α-L-fucosidase or *Vibrio cholerae* neuraminidase (Boehringer Mannheim) prior to incubation with bacteria.

Two other experiments were conducted to ascertain the nature of the bacterial receptor in tissue sections. (*i*) Deparaffinized sections were treated for 2 hr at 37°C with 200 milliunits of proteinase K from *Trichiratum albus* (Boehringer Mannheim). They were subsequently washed three times in PBS, treated with blocking buffer, and overlaid with a suspension of FITC-labeled *H. pylori* strain P466 or WV229. (*ii*) Deparaffinized sections were treated with 10 mM sodium metaperiodate/50 mM sodium acetate, pH 5.5, for 10 min at 0°C to selectively cleave carbons 8 and 9 of the unsubstituted side chain of terminal sialic acids (20) or with 10 mM sodium metaperiodate/50 mM sodium acetate, pH 4.5, for 1 hr at room temperature to cleave carbon–carbon bonds between vicinal hydroxyl groups in (most) carbohydrates with a free carbon in the 3 position (21). Control sections were incubated with 50 mM sodium acetate buffer alone. After two PBS washes, the sections were reduced by adding 50 mM sodium borohydride prepared in PBS (pH 7.6). Following three washes with PBS, suspensions of FITC-labeled strain WV229, P466, or MO19 were applied and the slides were processed as described above. Control experiments were used to establish that under the "harsher" oxidation conditions, the antigenicity of proteins present in gastric epithelial cell lineages was preserved: periodate-treated sections of rat stomach were incubated with a rabbit polyclonal antiserum raised against intrinsic factor and the antigen–antibody complexes were subsequently detected by Texas red-conjugated donkey anti-rabbit IgG (22, 23). The intensity of staining of chief cells was retained in periodate-treated sections, whereas the binding of the α-L-fucose-specific *Ulex europaeus* type 1 lectin (UEA1) to surface mucous cells was completely abolished under the same conditions.

Immunohistochemical Studies. The cellular distribution of sialylated oligosaccharides was examined in human stomach by incubating sections with the following lectins: (*i*) fluorescein-, rhodamine- (List Biological Laboratories, Campbell, CA), or peroxidase- (Sigma) conjugated cholera toxin B subunit (5 μg/ml) (24); (*ii*) digoxigenin (DIG)-conjugated *Sambucus nigra* lectin (SNA, 10 μg/ml, Boehringer Mannheim) (25); and (*iii*) DIG-conjugated *Maackia amurensis* lectin (MAA) (10 μg/ml, Boehringer Mannheim) (26). DIG-conjugated lectins were detected with peroxidase-conjugated monoclonal mouse anti-DIG antibody (500 milliunits of peroxidase per ml, Boehringer Mannheim).

Fucosylated blood group antigens were detected with mouse monoclonal antibodies directed against the histo-blood group antigens A, B, and H (final concentrations = 1 μg of protein per ml; Dakopatts, Glostrup, Denmark) or Le^a and Le^b (10 μg/ml; Immucor, Norcross, GA). Antigen–antibody complexes were visualized with fluorescein- or rhodamine-conjugated rabbit anti-mouse immunoglobulins (30 μg/ml; Dakopatts). The distribution of fucosylated gly-

coconjugates was also assessed by FITC-conjugated UEA1 (5 μg/ml; Sigma).

RESULTS AND DISCUSSION

Analysis of Cell Lineage-Specific Binding of *H. pylori* Using an *in Situ* Adherence Assay. Five clinical isolates of *H. pylori* were labeled with FITC following 5 days of growth on rich media under microaerophilic conditions and then overlaid on sections of formalin-fixed human stomach. Strains NCTC 11637, NCTC 11638, WV229, and P466, all recovered from patients with dyspeptic syndrome, bound to surface mucous cells situated in the upper pit and luminal surface (Fig. 1 *A* and *B*). Mucous neck cells located in the upper portions of the glandular segment of gastric units were negative, indicating that these bacteria were able to distinguish between two differentiated mucus-producing cell lineages present in the stomach (27). No binding to parietal or chief cells was noted in zymogenic glands. Strain MO19, the only isolate tested that was recovered from an asymptomatic "healthy" carrier, did not bind at detectable levels to any human gastric epithelial cell lineage (Fig. 1*C*).

Strains WV229 and P466 did not adhere to the squamous epithelium of human esophagus but did adhere under the reaction conditions employed to esophageal submucosal glands and their ducts and to duodenal villus-associated enterocytes (Fig. 1*D*). We also observed weak binding to enterocytes situated in the colonic homolog of small intestinal villi—the surface epithelial cell cuff that surrounds each crypt orifice (Fig. 1*E*). The intensity of staining (i.e., density of adherent organisms) was considerably less throughout the cephalocaudal axis of the intestine than it was in the stomach epithelium.

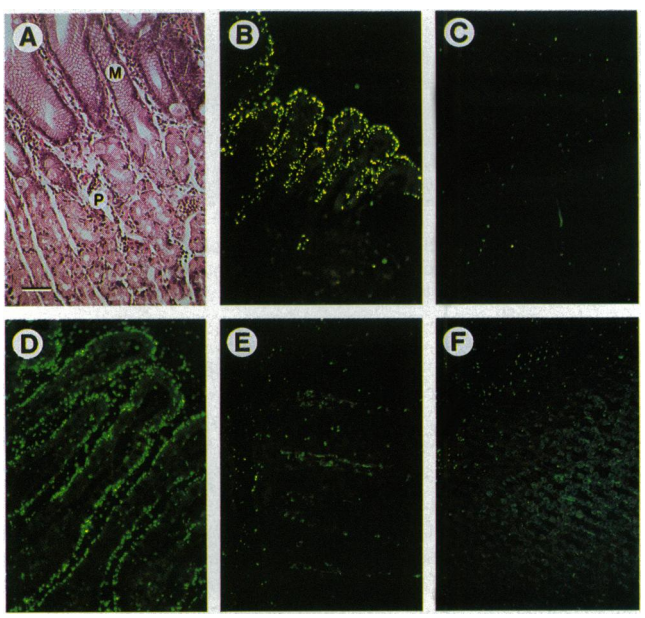

FIG. 1. *In situ* assay for binding of *H. pylori* to gut epithelial cell lineages. (*A*) Section of human stomach stained with hematoxylin/eosin showing the uppermost portion of gastric units in the zymogenic zone. Surface mucous (M) and parietal (P) cells are indicated. (*B* and *C*) Sections of human adult stomach incubated with FITC-labeled *H. pylori* strains WV229 (*B*) and MO19 (*C*). Strain WV229 is associated with surface mucous cells located in the upper pit of gastric units and their associated luminal surfaces. Strain MO19 is not bound to any cell lineage. (*D* and *E*) Incubation of strain WV229 with sections of adult human duodenum (*D*) and colon (*E*). FITC-labeled bacteria stain villus-associated epithelial cells (including enterocytes). Colonocytes located in the upper portion of colonic crypts and their surface epithelial cuffs are very weakly stained. Incubation of sections of human stomach, intestine, and colon with strains NCTC 11637, NCTC 11638, and P466 produced results comparable to those shown in *B*, *D*, and *E* (data not shown). (*F*) Section from the zymogenic zone of an adult Sprague–Dawley rat stomach incubated with strain WV229. Note that although the staining is much weaker than that in the human stomach, this strain of *H. pylori* is associated with surface mucous cells but not with cell lineages associated with the isthmus or glandular domains of rat gastric units. (Bar = 50 μm.)

Control experiments indicated that *H. pylori* P466 and WV229 did not bind to any epithelial cell populations represented in kidney, cervix, or endometrium. Surveys of the central nervous system including the midbrain also failed to produce a signal above background (data not shown).

The *in situ* adherence assay was used next to ascertain whether the cell lineage-specific and *H. pylori* strain-specific patterns of binding occurred in other mammalian species. Strains WV229, P466, and MO19 were incubated with sections of stomach, small intestine, and colon from adult Sprague–Dawley rats and FVB/N mice and stomach from dogs. Strain MO19 was not bound in detectable amounts to tissue sections prepared from any of these three species (data not shown). As in the human stomach, strains WV229 and P466 bound to surface mucous cells in the rat but not to any other differentiated epithelial cell population located in the zymogenic or pure mucous zones (Fig. 1*F*). The intensity of binding was significantly weaker than that observed with human gastric surface mucous cells (compare Fig. 1 *B* and *F*). Unlike in human sections, bacteria adhered to the stratified squamous epithelium of the rat esophagus and forestomach, whereas the small intestinal epithelium was negative with the exception of Brunner's glands (data not shown). The forestomach of the mouse was decorated with adherent bacteria, whereas the remainder of the stomach (i.e., its zymogenic, mucoparietal, and pure mucous zones; ref. 12) and the epithelial and mesenchymal components of the intestine did not yield a signal with FITC-labeled strains WV229 and P466. Finally, no binding of either of the two strains was observed in dog stomach (data not shown).

Biochemical Studies of the Interaction Between *H. pylori* and Gastric Epithelial Cell Lineages. To characterize the nature of the interactions between *H. pylori* and the surface mucous cells, we initially tested the ability of a series of compounds to inhibit binding of FITC-labeled strains P466 and WV229 to sections of human stomach (and proximal small intestine). When calf serum fetuin (a glycoprotein rich in sialylated oligosaccharides including sialyllactose, ref. 28), asialofetuin, soluble sialyllactose prepared from bovine milk (85% NeuAcα2,3Galβ1,4Glc and 15% NeuAcα2,6Galβ1,4Glc), and human milk sialyllactose (15% NeuAcα2,3 and 85% NeuAcα2,6) were preincubated with these two strains, no subsequent reduction in adherence was noted (data not shown). These findings are in agreement with the work of Fauchère and Blaser (11), who observed sialyllactose-independent attachment of *H. pylori* to HeLa cells. However, preincubation of the bacteria with 0.5% bovine submaxillary gland mucin, a rich source of fucosylated and sialylated carbohydrates (29), completely inhibited binding of bacteria to surface mucous cells (Fig. 2 *A* and *B*) and to duodenal, villus-associated, epithelial cells. Human colostrum secretory IgA was also a potent inhibitor: 15 μg/ml (≈40 nM) fully blocked adherence. This glycoprotein carries a highly varied set of N- and O-linked oligosaccharides (30) and has been reported to "nonspecifically" protect gut mucosa against environmental antigens (31). Several observations suggested that this inhibition of *H. pylori* binding by human colostrum secretory IgA was not due to a protein A–Fc receptor-like interaction (32): (*i*) inhibition was abolished by pretreating the secretory IgA with metaperiodate, (*ii*) preincubation at 85°C for 30 min did not affect its inhibitory activity, and (*iii*) α-L-fucosidase treatment of the IgA fraction markedly reduced blocking activity, whereas neuraminidase treatment

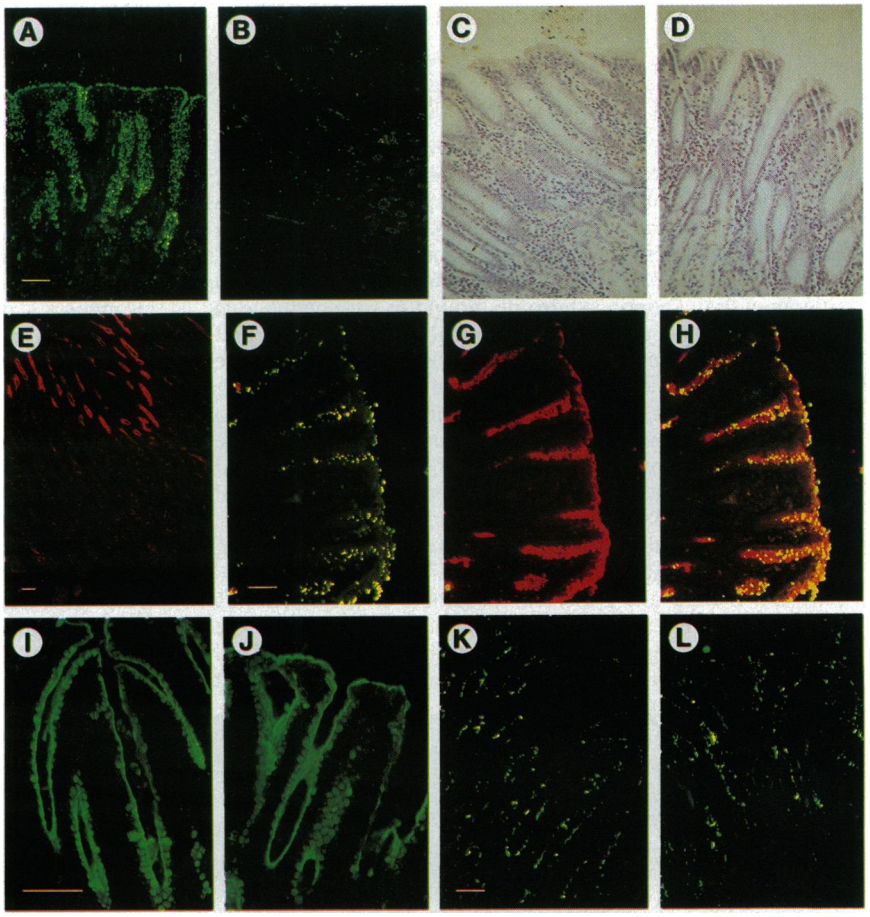

FIG. 2. Characterization of a putative *H. pylori* adhesin receptor as a glycoprotein that lacks sialyllactose. (*A* and *B*) Sections of human stomach containing gastric units with zymogenic glands were incubated with FITC-labeled *H. pylori* strain WV229 (*A*) or with bacteria that had been treated with a 0.5% solution of bovine submaxillary gland mucin (*B*). The mucin preparation produces marked reductions in binding. (*C* and *D*) Sections of human stomach were incubated with DIG-labeled MAA (*C*) or SNA (*D*). DIG-conjugated lectins were detected with a peroxidase-conjugated monoclonal mouse anti-DIG antibody. Note that none of the epithelial cell lineages produced a signal above background when incubated with these lectins that recognize sialic acid-containing carbohydrate epitopes. Control experiments employing the monoclonal antibody alone produced no staining (data not shown). (*E*) Adjacent section of human stomach incubated with rhodamine-conjugated cholera toxin B subunit. Mucus-producing cells located in the isthmus or upper portion of the glandular domain of gastric units react with this lectin. The B subunit does not bind to surface mucous cells located in the pit. (*F–H*) Sections of human stomach were incubated with FITC-labeled *H. pylori* strain P466 (*F*) and a mouse monoclonal antibody directed against the fucosylated blood group antigen H (visualized with rhodamine-conjugated rabbit anti-mouse IgG in *G*). (*H*) Double exposure showing that surface mucous cells coexpress the bacterial adhesin receptor and the blood group antigen. Note the reduction in adherence of bacteria to surface mucous cells (*F*) when compared to sections not treated with this monoclonal antibody (e.g., *A*). Similar reductions in binding were obtained using mouse monoclonal antibodies directed against fucosylated blood group antigens B and Le^b (data not shown). Nonimmune mouse IgG failed to produce this effect (data not shown). (*I*) Section of human stomach was incubated with FITC-conjugated UEA1. The lectin binds to surface mucous cells. (*J*) Pretreatment of a section of human stomach with sodium metaperiodate (pH 5.5) at 0°C for 10 min produces no appreciable reduction in binding of UEA1. (*K* and *L*) The binding of *H. pylori* to surface and pit mucous cells (*K*) was also unaffected by the sodium metaperiodate pretreatment (*L*). (Bars = 50 μm.)

had no detectable effect. In comparison, human serum IgA did not inhibit binding at concentrations as high as 4.5 μM (data not shown). Pretreatment of sections of human stomach with proteinase K also produced marked decrease in the binding of the two *H. pylori* strains (data not shown). Together, these results raised the possibility that binding is mediated by a fucosylated rather than a sialylated glycoprotein receptor expressed on surface mucous cells.

The suggestion that binding of *H. pylori* adhesin(s) to the surface mucous cell population of human stomach does not depend on sialic acid epitopes in a cellular receptor is supported by the results of two additional experiments. First, the distribution of sialic acid-containing complex carbohydrates in the human gastric mucosa does not correlate with the bacterial patterns of adherence. MAA is specific for NeuAcα2,3Galβ1, 4GlcNAc epitopes (26), whereas *S. nigra* agglutinin recognizes NeuAcα2,6Gal/GalNAc structures (25). Binding of the MAA and SNA lectins was confined to the submucosal compartment of the human stomach: they did not react with members of any gastric epithelial cell lineage (Fig. 2 *C* and *D*). The cholera toxin B subunit recognizes sialic acid linked to internally positioned galactose—i.e., GalNAcβ1,4(NeuAcα2,3)Gal-β (24). This lectin did not bind to surface mucous cells but rather was confined to mucous neck cells located in the upper glandular domains of gastric units (Fig. 2*E*). Control experiments demonstrated (*i*) MAA binding to surface mucous cells in the dog stomach, (*ii*) binding of SNA to surface mucous and parietal cells, and (*iii*) no binding of cholera toxin B subunit to any gastric epithelial cell lineage in this species (data not shown), suggesting that these lectins can be used to define fundamental differences in the differentiation program of the surface mucous cell lineage between dog and human—differences that may account for their distinct abilities to bind *H. pylori*. Second, when tissue sections were incubated with 10 mM metaperiodate/sodium acetate, pH 5.5, at 0°C (20), selective loss of SNA binding to surface mucous cells in dog stomach was seen (data not shown). Using these periodate oxidation conditions, we found that there was no reduction in adherence of any of the binding strains to human stomach or small intestinal epithelial populations compared to control sections that had been treated with the sodium acetate buffer alone (data not shown).

Other observations support a role for fucosylated epitopes in the glycoprotein(s) that mediate binding of *H. pylori* to surface mucous epithelial cells in the human stomach. The receptor sites for FITC-labeled strains P466 and WV229 are coexpressed in members of this epithelial cell lineage together with the fucosylated histo-blood group antigens H, B, and Le^b (e.g., see Fig. 2 *F–H*). Moreover, bacterial binding was reduced in sections that had been preincubated with monoclonal antibodies specific for either of the three blood group antigens (e.g., see Fig. 2 *F–H*). UEA1, which is specific for α-L-fucose, also bound to the same cells that contained *H. pylori* receptors (Fig. 2*I*). The metaperiodate oxidation reaction conditions employed above had no effect on UEA1 binding (Fig. 2*J*) or on *H. pylori* binding (Fig. 2 *K* and *L*). Harsher cleavage conditions (i.e., reducing the pH to 4.5, increasing the incubation time to 1 hr, and raising the incubation temperature to 20°C) were required to ablate UEA1 binding and the binding of the monoclonal blood group

H antibody to human surface mucous cells. These conditions also resulted in loss of cholera toxin B subunit binding and adherence of *H. pylori* strains WV229 and P466 (data not shown). Together, these results imply that *H. pylori* binding to UEA1-positive, fucosylated blood group antigen-positive, surface mucous cells in the human stomach is not dependent upon terminal nonsubstituted sialic acid residues. [α-L-Fucose-dependent adhesion to intestinal epithelial cells by *Campylobacter jejuni* and C. *coli* has been reported previously (33).] We cannot completely exclude involvement of a sialic acid in the postulated cell-associated adhesin receptor given the modest size of our lectin panel. However, the distribution of MAA and SNA lectin and cholera toxin B subunit binding sites plus the failure of fetuin and soluble sialyllactose to inhibit binding argue strongly against involvement of the α2,3-linked sialic acid residues that had been invoked in previous studies (8–10)—studies that employed cells that are not known targets for the organisms *in vivo*.

Perspectives. The *in situ* adherence assay described in this report should prove useful because it provides (*i*) a phenotypic parameter for characterizing clinical isolates of *H. pylori* and correlating their binding capacity with acid peptic disease and/or gastric neoplasia (e.g., compare the results obtained with WV229 and MO19), (*ii*) a way of characterizing structure/activity relationships in gastric (and intestinal) epithelial cell receptors for *H. pylori* adhesin(s), and (*iii*) a functional assay for identifying therapeutically useful compounds that inhibit strain- and cell lineage-specific binding of *H. pylori*.

The adherence assay suggests that binding of *H. pylori* strains is limited to surface mucous cells in the human gastric epithelium. This raises two obvious questions. (*i*) Given the rapid, upward, pipeline-like migration and perpetual exfoliation of these cells (14), how is this organism able to establish and maintain colonization of the stomach? (*ii*) What is the functional relationship, if any, between binding of *H. pylori* to surface mucous cells and the development of acid peptic disease and gastric neoplasia? Rapid cellular translocation and exfoliation of surface mucous cells represent a potential pathway for clearance of this organism. The inhibitory effect on bacterial binding of human colostrum secretory IgA is also of interest in this context, since it might be a natural scavenging mechanism that prevents colonization by this human pathogen at early stages of life. Our observation that *H. pylori* strains adhere to esophageal submucosal glands and glandular ducts raises the possibility that these habitats represent a "silent" colonization site for this bacterium, which may then seed the stomach. It is unclear at present how much molecular cross-talk exists between the components of this system. For example, does an inflammatory response triggered by binding of the adhesin (5) affect the cellular differentiation program of surface mucous cells so as to alter either cell migration rates, the integrity of junctional complexes between cells (34), the process of exfoliation, and/or the production of receptors for bacterial lectins? Alterations in surface mucous cells have been reported to be associated with *H. pylori* colonization: mucus content is reduced and binding of the sialic acid-specific lectin *Limax flavus* agglutinin is increased (35). Though little is known about the differentiation programs of gastric epithelial cell lineages, the *H. pylori* adhesin may represent an excellent probe for defining and perturbing these programs. Such an analysis may not only provide insights about the pathogenesis of acid peptic disease and cellular transformation associated with *H. pylori* infection, but may also help describe the role of its adhesin's receptor in regulating the biology of normal gastric epithelial cell populations.

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