Surface Proteins from *Helicobacter pylori* Exhibit Chemotactic Activity for Human Leukocytes and Are Present in Gastric Mucosa

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Summary

The mechanism by which *Helicobacter pylori*, a noninvasive bacterium, initiates chronic antral gastritis in humans is unknown. We now show that *H. pylori* releases products with chemotactic activity for monocytes and neutrophils. This chemotactic activity was inhibited by antisera to either *H. pylori* whole bacteria or *H. pylori*–derived urease. Moreover, surface proteins extracted from *H. pylori* and purified *H. pylori* urease (a major component of the surface proteins) exhibited dose-dependent, antibody-inhibitable chemotactic activity. In addition, a synthetic 20–amino acid peptide from the NH$_2$-terminal portion of the 61-kD subunit, but not the 30-kD subunit, of urease exhibited chemotactic activity for monocytes and neutrophils, localizing the chemotactic activity, at least in part, to the NH$_2$ terminus of the 61-kD subunit of urease. The ability of leukocytes to chemotax to *H. pylori* surface proteins despite formyl-methionyl-leucyl-phenylalanine (FMLP) receptor saturation, selective inhibition of FMLP-mediated chemotaxis, or preincubation of the surface proteins with antiserum to FMLP indicated that the chemotaxis was not FMLP mediated. Finally, we identified *H. pylori* surface proteins and urease in the lamina propria of gastric antra from patients with *H. pylori*–associated gastritis but not from uninfected subjects. These findings suggest that *H. pylori* gastritis is initiated by mucosal absorption of urease, which expresses chemotactic activity for leukocytes by a mechanism not involving N-formylated oligopeptides.

The presence of *Helicobacter pylori* in the gastric antrum of humans is associated with chronic antral (type B) gastritis, duodenitis, and duodenal ulcer (1–6). Epidemiologic, clinical, and pathological evidence implicate *H. pylori* in the etiology of these inflammatory lesions (7–10). However, the pathophysiology of *H. pylori*–induced inflammation is poorly understood. In particular, the mechanism by which *H. pylori* recruits mononuclear and polymorphonuclear leukocytes to the inflammatory lesion is unclear since the bacteria appear not to be invasive (11). Although curved bacteria have been observed on occasion within the phagocytic vacuoles of neutrophils between gastric epithelial cells (12), invading *H. pylori* have not been identified below the basal membrane of the epithelium.

These observations suggest that *H. pylori* recruits inflammatory cells by actively releasing or passively shedding cellular components that, after absorption into the mucosa, serve as chemoattractants for mononuclear phagocytes and PMN. To investigate this hypothesis, we assessed the ability of material released by *H. pylori*, as well as extracted *H. pylori* surface proteins and *H. pylori* urease, to induce chemotaxis by human leukocytes in vitro, and then evaluated gastric biopsy specimens from patients with *H. pylori* infection for the presence of *H. pylori* chemotactic products within the antral mucosa.

Materials and Methods

Isolation of Cells. Healthy adult donors were subjected to leukapheresis and their mononuclear leukocytes separated into highly purified populations of monocytes and lymphocytes by countercurrent centrifugal elutriation (13, 14). Polymorphonuclear neutrophils were separated from the red cell pellets by brief exposure to NH$_4$Cl (ACK lysing buffer; NIH Media Unit). The purity of the monocytes was determined by morphology (96% monocytes), phenotypic analysis (>96% Leu-M3 positive, <2% Leu-1 positive), and esterase staining (>95% positive) (13). Purity of the neutrophils was confirmed by morphology (98% neutrophils). Cells were enumerated by a cell counter (Coulter Electronics, Inc., Hialeah,

FL) and suspended at a concentration of 10^6 cells/ml in Gey's balanced salt solution (Gey's BSS;[1] NIH Media Unit).

H. pylori Strains. Two strains of *H. pylori* (84-180, isolated from a patient with antral gastritis; and 84-183, isolated from a patient with antral gastritis and duodenal ulcer) from the culture collection of the Denver Veterans Administration Medical Center Campylobacter Laboratory (15) were grown in liquid media containing brain heart infusion broth (Difco Laboratories, Detroit, MI) supplemented with 0.25% yeast extract (Difco Laboratories) and 10% heat-inactivated FCS (Whittaker Bioproducts, Walkersville, MD) (16). For experiments testing whether *H. pylori* released a chemotactic stimulus, supernatant from cultures of *H. pylori* was obtained during log phase growth.

H. pylori Constituents. To test whether *H. pylori* constituents have chemotactic activity and are present within the antral mucosa of infected patients, *H. pylori* surface proteins and *H. pylori* urease were prepared as previously described (17–19). Briefly, *H. pylori* cells were harvested in 0.15 M NaCl and centrifuged at 3,000 g for 25 min at 25°C. Bacterial pellets were resuspended in an equal volume of sterile distilled water, vortex mixed for 45 s, and again centrifuged at 3,000 g. The supernatant, which contained the water-extracted surface proteins, was stored at −20°C. Urease was isolated by water extraction and then purified by size exclusion chromatography and anion exchange chromatography (17). The protein contents of the water-extracted surface proteins and the purified urease were determined as previously described (17). In addition, 20 residue peptides based on the known sequence of the NH_2-terminal portion of the 61- and 30-kD subunits of *H. pylori* urease (17, 20) were synthesized on an automated synthesizer (430; Applied Biosystems, Inc., Foster City, CA) using t-Boo chemistry as developed by Merrifield (21). Amino acid composition of the synthesized peptides was assessed using an amino acid analyzer (Beckman Instruments, Inc., Palo Alto, CA) and by analytic HPLC.

Chemotaxis Assay. Monocytes and neutrophils were assayed for chemotactic activity in microchamber plates (Neuroprobe, Rockville, MD) as previously described (22). The chemotactic stimuli were suspended in Gey's BSS and placed in the bottom wells of the microchamber plates, which were separated from the upper wells by a polycarbonate filter (Neuroprobe) with 5.0-μm pores for monocytes and 3.0-μm pores for neutrophils. Each assay contained test stimulus, Gey's BSS alone for a negative control, and FMLP (Peninsula Laboratories, Belmont, CA), and C5a desArg (derived from endotoxin-activated human serum) (23) for positive controls. After a 90-min incubation (monocytes) or a 60-min incubation (neutrophils) at 37°C in a humidified atmosphere with 5% CO_2, the polycarbonate filters were removed, fixed, and stained with Diff-Quik (American Scientific Products, Columbia, MD). Cells that had migrated through the filters from the top chambers were counted with an image analyzer (Optomax Inc., Hollis, NH). Chemotactic activity is defined as the mean (± SD) number of cells that migrated through the filter pores in three standard fields for each of triplicate filters.

To determine whether *H. pylori* components induced gradient-directed (chemotactic) vs. random (chemokinetic) locomotion, migratory activity of monocytes and neutrophils to *H. pylori* surface proteins was evaluated by checkerboard analysis as previously described (24). In these experiments, increasing concentrations of surface proteins were placed in wells above the filter (same wells as the cells) and in wells below the filter in a checkerboard pattern

in order to discriminate between cell movement to a chemoattractant and stimulus-induced random motion.

Inhibition Studies. The chemotactic activity of the *H. pylori* culture supernatant, extracted surface proteins, and purified urease was assayed before and after incubation for 30 min with rabbit antisera to either *H. pylori* (strain 84-183) whole bacteria (15), urease cut from a nondenaturing gel (18), or the 61-kD urease subunit cut from a denaturing two-dimensional gel (18). To determine whether the chemotactic activity of the *H. pylori* surface components was due to the presence of N-formylated oligopeptides, the ability of leukocytes to migrate in response to *H. pylori* surface proteins was tested before and after the cells had been incubated for 30 min with either FMLP (10^{-6} M) to saturate available FMLP receptors or sulfoxone (100 μg/ml) to selectively inhibit FMLP-directed chemotaxis (25). In addition, the surface proteins were tested for chemotactic activity before and after a 30-min incubation with antisera to FMLP (kindly donated by Dr. W. A. Marasco, Dana Farber Cancer Institute, Boston, MA) (26).

Gastric Tissue Specimens. Specimens of gastric antral tissue were obtained during endoscopy from two patients with duodenal ulcerations. The antral biopsies, which were from within 2 cm of the pylorus, were positive for urease activity by CLO test (Tri-Med Specialties Inc., Overland Park, KS), showed Gram-negative curved rods overlaying the epithelium, and grew microorganisms on chocolate agar under microaerobic conditions that were identified by standard morphological features and biochemical activities (positive urease, catalase, oxidase) to be *H. pylori*. Antral biopsy specimens that did not contain urease activity or curved organisms, and that did not yield *H. pylori* on culture, were obtained from three subjects with esophageal but not gastric disease to serve as control tissue. All patients gave informed written consent for the clinically indicated endoscopic procedure and for obtaining the biopsies. Biopsy specimens were snap frozen in embedding medium (O. C. T. compound; Miles Laboratories, Elkhart, IN) and sectioned (8 μm) for immunochemical staining. Parallel specimens were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin or carbol fuchsin to evaluate for the presence of gastritis and *H. pylori*.

Immunocytochemical Staining of Gastric Tissue. To determine whether *H. pylori* surface proteins were absorbed into the gastric mucosa, snap-frozen sections of the gastric biopsy specimens from *H. pylori*–positive and –negative subjects were stained with appropriate antibodies using the horseradish peroxidase–labeled avidin-biotin procedure (ABC Vecta Stain Kit; Vector Laboratories Inc., Burlingame, CA) (27). Immunocytochemical staining for *H. pylori* surface proteins and urease was performed using polyclonal rabbit antibodies at concentrations of 1:100 to 1:500 to *H. pylori* whole bacteria (strain 84-183) and the 61-kD subunit of *H. pylori* urease after blockade of endogenous peroxidase in the tissue by H_2O_2. Each experiment contained sections that were incubated with an irrelevant antibody (anti–astrocyte glial fibrillary acidic protein [anti-GFAP]), normal rabbit serum, and sections incubated with either antibody alone or peroxidase alone. After immunochemical staining, the sections were counterstained with methyl green.

Results

H. pylori Release Products That Recruit Human Leukocytes. Since whole bacteria themselves are not chemotactic and since infection with *H. pylori* in the gastric antrum is associated with cellular infiltration, we first investigated whether the mechanism of this infiltration could involve the release of prod-

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[1] *Abbreviations used in this paper:* anti-GFAP, anti–astrocyte glial fibrillary acidic protein; BSS, balanced salt solution.

ucts from *H. pylori* capable of stimulating leukocyte recruitment. Supernatants from cultures of *H. pylori* in log phase were evaluated for the ability to recruit human monocytes and neutrophils. As shown in Fig. 1, the supernatant from cultures of *H. pylori* strain 84-183 elicited a vigorous migratory response by both monocytes (*A*) and neutrophils (*B*), whereas control media at a dilution of 1:2 had no such activity. Cell migration was detected at a dilution of 1:128 and increased in a dose-dependent manner to >200 cells/field at a dilution of 1:2. Similar results were obtained with the culture supernatant from *H. pylori* strain 84-180 (data not shown). The magnitude of these responses was similar to or exceeded that induced by FMLP (10^{-8} M) (monocytes, 85 ± 18 cells/field; and neutrophils, 204 ± 36 cells/field; for three experiments). These findings suggested that live *H. pylori* release material(s) with chemotactic properties for human leukocytes.

Recruitment Activity of Products Released by H. pylori Is Inhibited by Antisera to H. pylori Whole Bacteria and Urease. Cell wall turnover is a characteristic of many Gram-negative microorganisms (28). Therefore, we next investigated whether the chemotactic material released by *H. pylori* was, at least in part, surface proteins by determining whether the chemotactic activity could be inhibited with antibodies to whole *H. pylori* bacteria or *H. pylori* urease. Preincubation of supernatant from cultures of *H. pylori* (84-183) in log phase with 20% rabbit antisera to either *H. pylori* whole bacteria (anti-WB) or *H. pylori* urease (anti-U) caused a significant reduction in the chemotactic activity of the supernatant for both monocytes (Fig. 1 *A*, *inset*) and neutrophils (Fig. 1 *B*, *inset*). In contrast, preincubation of culture supernatant with anti-GFAP or preimmune rabbit serum (see legend to Fig. 1) did not affect the chemotactic activity of the supernatant.

These observations suggested that *H. pylori* release cellular components, in particular urease, that are capable of recruiting monocytes and neutrophils.
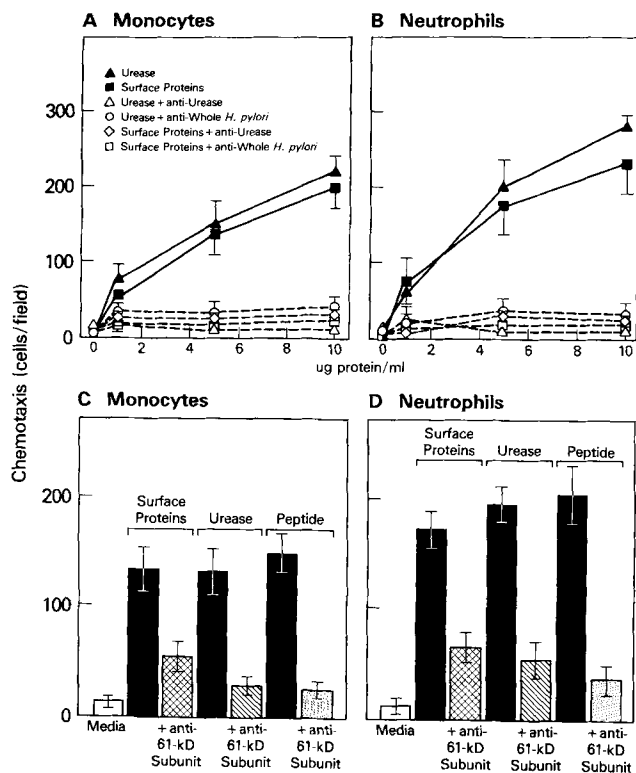
Confirmation That H. pylori Surface Proteins and Urease Express Recruitment Activity for Human Leukocytes. To confirm that *H. pylori* surface proteins and urease are capable of recruiting leukocytes, we next tested the ability of water-extracted surface proteins and highly purified urease to serve as recruitment factors. As shown in Fig. 2, *H. pylori* surface proteins and urease elicited dose-dependent migratory responses by both monocytes (*A*) and neutrophils (*B*). Specificity of these components for leukocyte recruitment was confirmed by the ability of 20% antisera to either *H. pylori* whole bacteria or purified urease to completely inhibit their recruitment activity (Fig. 2, *A* and *B*). Moreover, antiserum to whole bacteria inhibited the chemotactic activity of urease, and antiserum to urease inhibited the chemotactic activity of the surface proteins (Fig. 2, *A* and *B*), suggesting that urease was the chemotactic factor in *H. pylori* surface proteins. Preincubation of the surface proteins and urease with anti-GFAP or preimmune rabbit serum did not reduce leukocyte recruitment (see legend to Fig. 2). These findings confirmed that *H. pylori* surface proteins were capable of stimulating migration by monocytes and neutrophils, and implicated urease as the chemotactic factor within the surface proteins.

Since *H. pylori* urease is composed of two polypeptides migrating at 61 and 30 kD (17), we next sought to localize the chemotactic activity within the urease molecule. Antiserum to the gel-purified 61-kD subunit significantly inhibited the chemotactic activity of the surface proteins and purified urease for both monocytes (Fig. 2 *C*) and neutrophils (Fig. 2 *D*). In addition, a synthetic 20–amino acid peptide based on the

A Monocytes **B Neutrophils**

H. pylori **Culture Supernatant (CS) (dilution)**
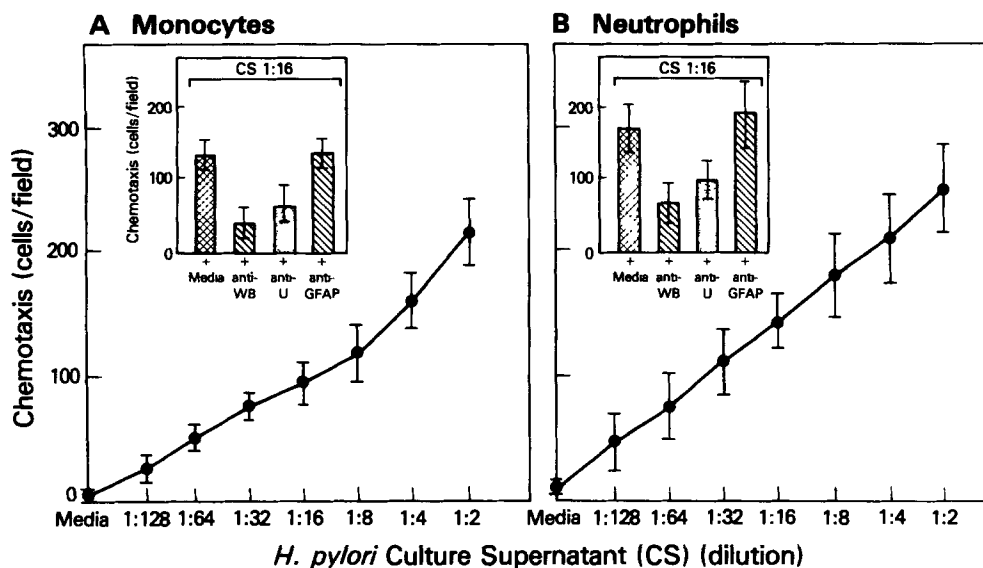
Figure 1. Leukocyte recruitment activity of *H. pylori* culture supernatant. Dilutions of supernatant harvested from cultures of *H. pylori* strain 84-183 during log phase growth were assayed for chemotactic activity for (*A*) monocytes and (*B*) neutrophils. (*Insets*) Inhibition of recruitment activity of *H. pylori* culture supernatant by antisera to whole bacteria or. urease or antibodies to GFAP. *H. pylori* strain 84-183 culture supernatant (*CS*) at a concentration of 1:16 was incubated with 20% concentrations of media, antiserum to *H. pylori* whole bacteria (*anti-WB*), antiserum to *H. pylori* urease (*anti-U*), or irrelevant antibodies to astrocyte glial fibrillary acidic protein (*anti-GFAP*, 1:25 dilution), and then assayed for migration activity by monocytes (*A*, *inset*) and neutrophils (*B*, *inset*). Preincubation of culture supernatant with 20% preimmune rabbit serum also did not inhibit chemotactic activity (monocytes, 168 ± 21 cells/field; and neutrophils, 159 ± 30 cells/field). Values are the mean of three separate experiments (except for the experiment in which culture supernatant was preincubated with anti-U, for which *n* = 1 due to limited availability of the antiserum).

519   Mai et al.

A Monocytes

▲ Urease
■ Surface Proteins
△ Urease + anti-Urease
○ Urease + anti-Whole H. pylori
◇ Surface Proteins + anti-Urease
□ Surface Proteins + anti-Whole H. pylori

B Neutrophils

C Monocytes

D Neutrophils

Figure 2. (*A* and *B*) Leukocyte recruitment activity of *H. pylori* surface proteins and urease. Surface proteins and urease from *H. pylori* strain 84-183 were assayed before and after incubation with 20% antisera to *H. pylori* whole bacteria or urease for the ability to recruit monocytes (*A*) or neutrophils (*B*). Values are the mean of three separate experiments. (Controls for *A*) Urease (5 μg/ml) incubated with anti-GFAP (1:25 dilution) or 20% preimmune adult serum did not inhibit chemotaxis (116 ± 18 and 117 ± 9 cells/field); surface proteins (5 μg/ml) incubated with anti-GFAP or preimmune rabbit serum also did not inhibit chemotaxis (97 ± 12 and 95 ± 30 cells/field). (Controls for *B*) Urease (5 μg/ml) incubated with anti-GFAP (1:25 dilution) or 20% preimmune rabbit serum did not inhibit chemotaxis (201 ± 25 and 234 ± 27 cells/field); surface proteins (5 μg/ml) incubated with anti-GFAP or preimmune rabbit serum also did not inhibit chemotaxis (180 ± 42 and 177 ± 27 cells/field). (*C* and *D*) Localization of *H. pylori* recruitment activity using *H. pylori* surface proteins, urease, and a 20-residue peptide synthesized from the NH₂ terminus of the 61-kD subunit of urease as chemoattractant. Chemotaxis for each constituent (5 μg/ml) was assayed before and after incubation with 20% antiserum to the 61-kD subunit of the urease molecule. Values are the mean of two experiments. (Controls for *C*) Surface proteins, urease, and peptide incubated with anti-GFAP (1:25 dilution) did not inhibit chemotaxis (98 ± 24, 132 ± 24, and 150 ± 36 cells/field); surface proteins, urease, and peptide incubated with 20% preimmune rabbit antiserum also did not inhibit chemotaxis (138 ± 19, 129 ± 27, and 158 ± 12 cells/field). (Controls for *D*) Surface proteins, urease, and peptide incubated with anti-GFAP (1:25 dilution) did not inhibit chemotaxis (170 ± 57, 196 ± 63, and 194 ± 59 cells/field); Surface proteins, urease, and peptide incubated with 20% preimmune rabbit serum also did not inhibit chemotaxis (204 ± 19, 215 ± 13, and 209 ± 30 cells/field).

known sequence of the NH₂-terminal portion of the 61-kD polypeptide subunit (17, 20) exhibited chemotactic activity that also could be inhibited by antiserum to the 61-kD subunit (Fig. 2, *C* and *D*). Incubation of surface proteins, urease, and peptide (1–10 μg/ml) with either anti-GFAP or preimmune rabbit serum had no inhibitory effect on the chemotactic ac-

tivity of these *H. pylori* components (see legend to Fig. 2). In contrast, the same concentrations of a synthetic 20–amino acid peptide of the NH₂-terminal portion of the 30-kD subunit exhibited no chemotactic activity for monocytes or neutrophils (data not shown). Antiserum to the 30-kD subunit is not yet available for inhibition studies. These observations localized the migratory activity of *H. pylori* surface proteins to, at least in part, the NH₂ terminus of the 61-kD subunit of the urease molecule.

Recruitment Activity of H. pylori Components Is Due to Chemotactic, Not Chemokinetic, Activity. To determine whether the migratory activity of monocytes and neutrophils in response to *H. pylori* components was due to gradient-directed migration (chemotaxis) and not stimulated random locomotion (chemokinesis), a checkerboard analysis of monocyte and neutrophil migration to surface proteins was performed. As shown in Table 1, the migration responses of monocytes to a gradient of higher concentrations of *H. pylori* surface proteins in the opposite (lower) wells were in nearly all cases greater than the responses of monocytes to higher concentrations of surface proteins in the same (upper) wells. Similarly, the migration of neutrophils was greater in the presence of a concentration gradient established by larger amounts of surface proteins in the lower wells than in the upper wells. These results indicate that the migration of monocytes and neutrophils to *H. pylori* surface proteins was the result of chemotactic and not chemokinetic activity.

Absence of N-formylated Oligopeptides in the Chemotactic Products Released by H. pylori. Since the cell walls of many Gram-negative bacteria contain N-formylated oligopeptides, structural components with chemotactic activity (26), we investigated whether the presence of these peptides contributed to the chemotactic activity of the *H. pylori* constituents. After incubation of neutrophils with optimal doses of either FMLP to saturate FMLP receptors or sulfoxone to selectively inhibit FMLP-directed chemotaxis (25), migratory response to further stimulation by FMLP was almost completely inhibited; however, chemotaxis to the surface proteins remained intact (Fig. 3). In addition, preincubation of the surface proteins with antiserum to FMLP did not reduce the ability of the surface proteins to recruit monocytes or neutrophils, whereas incubation of FMLP with the antiserum completely inhibited FMLP-mediated chemotaxis (Table 2). Moreover, comparison of the amino acid sequence of urease (20) with that of FMLP showed no sequence homology. Thus, the chemotactic activity of *H. pylori* cellular components and urease was not due to the presence of FMLP.

Detection of H. pylori Surface Proteins and Urease in Gastric Antrum from Patients Infected with H. pylori. Although *H. pylori* is noninvasive, the release of chemotactically active products during *H. pylori* infection could explain the recruitment of leukocytes to the gastric antrum if these products are absorbed into the mucosa. Therefore, we attempted to identify *H. pylori* cellular components in the antral mucosa of patients infected with *H. pylori*. Biopsy specimens of antral mucosa from two patients harboring *H. pylori* in their gastric antra were evaluated for the presence of surface proteins and urease by im-
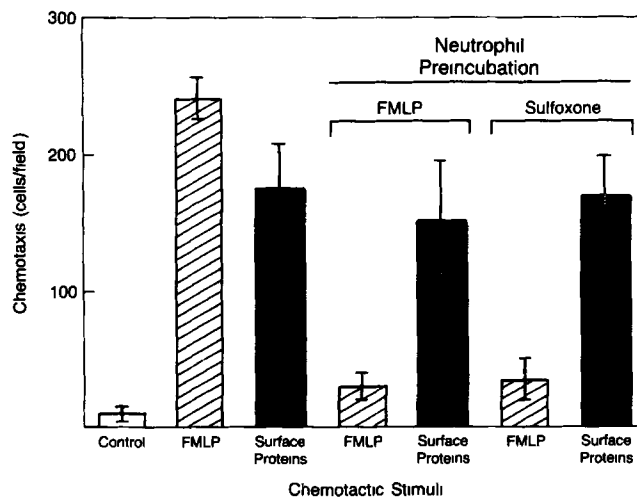
Table 1. *Checkerboard Analysis of Cell Migration to H. pylori Surface Proteins*

		H. pylori surface proteins above filter (µg/ml)					
		0	0.1	1.0	10	20	50
	µg/ml			Monocytes			
H. pylori surface	0	**47 ± 6***	11 ± 1	54 ± 10	17 ± 2	47 ± 7	39 ± 3
proteins below filter	0.1	55 ± 2	**50 ± 7**	10 ± 1	68 ± 11	26 ± 4	110 ± 7
	1.0	162 ± 22	89 ± 10	**40 ± 13**	32 ± 2	35 ± 8	28 ± 4
	10	267 ± 13	203 ± 27	170 ± 21	**47 ± 5**	85 ± 10	13 ± 3
	20	218 ± 12	250 ± 19	189 ± 24	88 ± 13	**55 ± 11**	54 ± 6
	50	174 ± 6	150 ± 24	146 ± 12	82 ± 18	62 ± 6	**84 ± 6**
				Neutrophils			
H. pylori surface	0	**33 ± 6**	54 ± 9	9 ± 1	4 ± 1	15 ± 2	36 ± 5
protein below filter	0.1	254 ± 22	**62 ± 4**	32 ± 6	11 ± 2	21 ± 3	30 ± 5
	1.0	267 ± 9	79 ± 5	**42 ± 5**	9 ± 2	8 ± 1	34 ± 7
	10	157 ± 13	143 ± 11	245 ± 34	**38 ± 4**	23 ± 4	40 ± 5
	20	339 ± 23	179 ± 36	164 ± 34	222 ± 11	**140 ± 17**	60 ± 7
	50	362 ± 11	217 ± 31	155 ± 29	120 ± 8	239 ± 24	**15 ± 2**

* Bold numbers represent number of cells that migrated when concentration of surface proteins above and below filter was equivalent.

munoperoxidase staining using the antisera to *H. pylori* whole bacteria and the 61-kD subunit of urease at a concentration of 1:200. As shown in the tissue sections in Fig. 4, deposits of peroxidase-positive material were identified throughout the lamina propria with the antiserum to whole bacteria (Fig. 4 *A*), indicating the presence of cellular components. The components appeared to be in close proximity to crypt

Figure 3. Inhibition of FMLP- but not *H. pylori*–mediated chemotaxis. Neutrophils preincubated with or without FMLP or sulfoxone as described in Materials and Methods were assayed for chemotactic activity to FMLP $(10^{-8}$ M) and *H. pylori* surface proteins (5 µg/ml). Values are from a representative experiment (n = 2).

epithelium and in some fields within cells. In parallel studies, urease also was identified in the lamina propria, close or adjacent to crypt epithelium (Fig. 4 *B*). Whole *H. pylori* bacteria, however, were not identified microscopically in the lamina propria. Antral biopsy specimens from subjects not infected with *H. pylori* showed no staining for either cellular components (Fig. 4 *C*) or the urease subunit (Fig. 4 *D*). Antral biopsy specimens from the same patients incubated with anti-GFAP or normal rabbit serum also were negative for peroxidase-staining material (data not shown). These findings indicate that cellular components, including urease, which exhibit chemotactic activity in vitro, are present within the lamina propria of antral biopsy specimens from patients with *H. pylori*-induced gastritis.

Discussion

The studies reported here provide the first evidence that two strains of pathogenic *H. pylori* release products with chemotactic activity for monocytes and neutrophils. The ability of antisera to whole bacteria and urease to inhibit the chemotactic activity in *H. pylori* culture supernatants implicated surface proteins, specifically urease, as the chemoattractant released by the bacteria. The chemotactic activity of this bacterial product was confirmed by showing that surface proteins extracted from *H. pylori* as well as *H. pylori* urease purified from the surface proteins, were capable of inducing migratory responses by monocytes and neutrophils. Moreover, the inhibition of surface protein–mediated chemotaxis by antisera to urease implicated urease as the chemotactic factor within *H. pylori* surface proteins. That the purified urease did not

Table 2. *Leukocyte Chemotaxis to H. pylori Surface Proteins and FMLP in the Absence and Presence of Antiserum to FMLP*

Chemoattractant	Antiserum (dilution)	Chemotaxis	
		Monocytes	Neutrophils
		cells/field ± SEM	
1. Media	Media	26 ± 4	11 ± 2
2. H. pylori surface proteins*	Media	192 ± 27	253 ± 24
3. H. pylori surface proteins*	Anti-FMLP (1:100)	189 ± 12	280 ± 72
4. FMLP (10^{-8} M)	Media	132 ± 6	161 ± 25
5. FMLP (10^{-8} M)	Anti-FMLP (1:100)	15 ± 1	21 ± 4
6. Media	Anti-FMLP (1:100)	24 ± 3	15 ± 1

* *H. pylori* surface proteins (5 μg/ml).

induce a more vigorous chemotactic response than the surface proteins could be related to the linearization during purification. Furthermore, leukocyte migration to a synthetic 20–amino acid peptide from the NH_2-terminal portion of the 61-kD, but not the 30-kD, subunit of urease localized the chemotactic activity of urease, at least in part, to the NH_2 terminus of the 61-kD subunit of the urease molecule. The presence of chemotactic responses despite FMLP receptor saturation, selective inhibition of FMLP-mediated chemotaxis, and preincubation of the surface proteins with anti-FMLP indicated that chemotaxis was not due to the presence of FMLP in the *H. pylori* components. Checkerboard analysis using *H. pylori* surface proteins as stimuli confirmed that the migratory responses were indeed due to chemotaxis and not chemokinesis.

The ability of *H. pylori* urease to recruit human leukocytes does not exclude the possibility that the bacterium releases other products with both chemotactic and inflammatory properties. For example, *H. pylori* also release 82- and 128-kD proteins associated with vacuolizing cytotoxin activity into broth culture supernatants (29). These proteins have not yet been purified in order to evaluate their chemotactic activity. However, since *H. pylori* strain 84-180, which does not produce cytotoxin, was as chemotactic as strain 84-183, a cytotoxin-producing strain, it seems unlikely that the cytotoxin is a chemotactic factor.

Urease is a major component of *H. pylori* surface proteins. This multisubunit polypeptide with molecular mass >300 kD was chemotactic for both monocytes and neutrophils, indicating that urease is likely not only a potential virulence factor (30, 31) but an important chemotactic factor as well. Although previous investigators have identified chemotactically active NH_2 terminus–blocked methionyl peptides released by *Streptococcus sanguis* and *Escherichia coli* (32, 33), there is only one prior report that a bacterium (*Staphylococcus aureus*) produces a chemotactic factor other than FMLP (34). Consequently, *H. pylori* is the first Gram-negative bacterium

observed to release chemotactic factors other than N-formylated oligopeptides.

Since *H. pylori* is a noninvasive microorganism, we searched for its components in gastric antral mucosa. Within the lamina propria of antra from subjects infected with *H. pylori*, but not antra from uninfected control subjects, *H. pylori* components and specifically the 61-kD urease subunit were identified by immunocytochemical staining. These bacterial constituents were present in the proximity of phagocytic cells with morphological features of tissue macrophages. These cells play a critical role in mediating inflammatory responses through the production of inflammatory cytokines and the processing of antigenic material for presentation to T and B lymphocytes.

The pathophysiology of *H. pylori*–associated inflammation, in particular, the mechanism by which this noninvasive bacterium induces mucosal inflammation, has been poorly understood. However, the findings reported here, together with our previous observations that *H. pylori* surface proteins are capable of activating human monocytes (19), offer important insights into this mechanism. These findings suggest the following sequence of events. After ingestion of the bacteria and colonization of the gastric mucosa, *H. pylori*, like many other microorganisms, shed cellular components, including surface proteins such as urease. These products become solubilized in the gastric mucus and then are absorbed across the antral epithelium into the lamina propria where the urease recruits monocytes and neutrophils. Resident and recruited mononuclear phagocytes then phagocytize the foreign material and become activated (19), releasing inflammatory cytokines such as TNF-α and IL-1, and reactive oxygen intermediates such as superoxide anion (19). TNF itself exhibits potent chemotactic and proinflammatory activities (35) that likely amplify the recruitment and activation of leukocytes. These cell products, and possibly other factors released by the newly recruited monocytes and neutrophils, may induce and perpetuate the tissue inflammatory lesion associated with *H. pylori*.
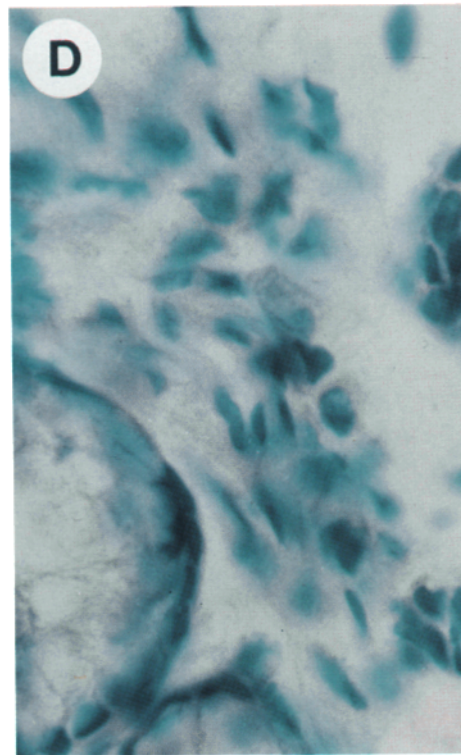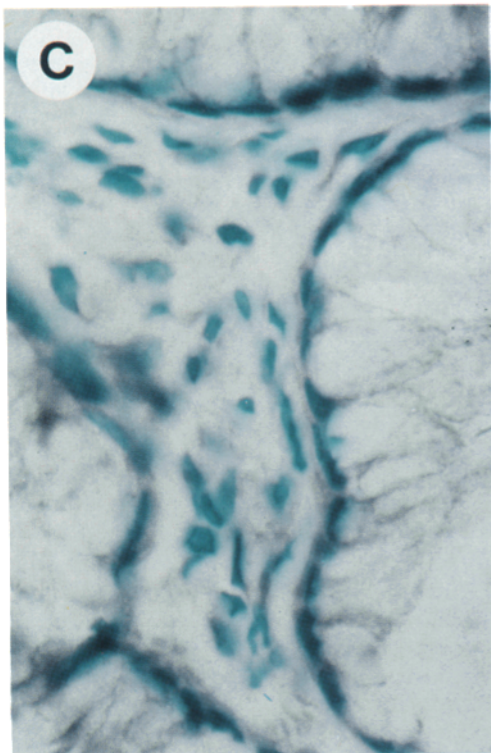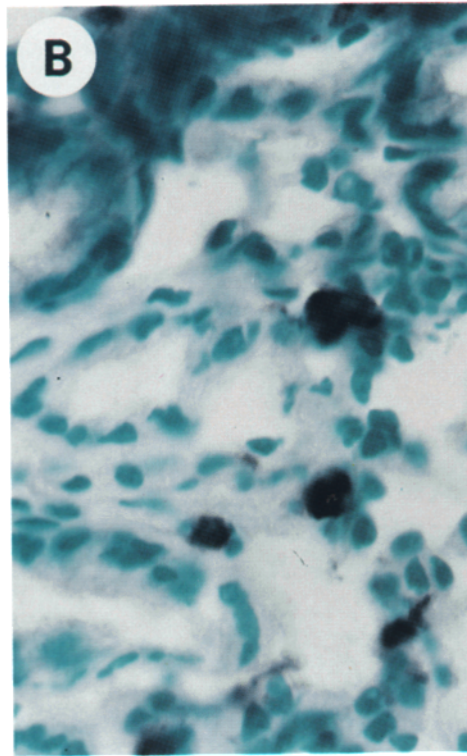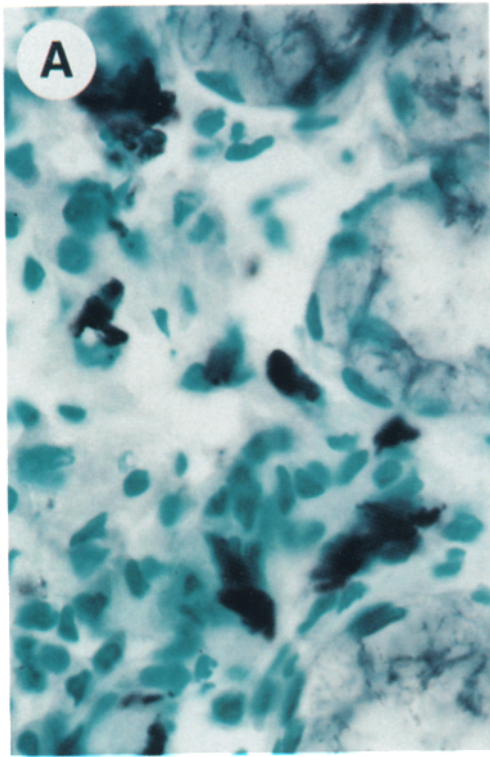
Figure 4. Immunocytochemical localization of *H. pylori* cellular components and urease in sections of antral mucosa. Representative sections of antral mucosa from a subject infected with *H. pylori* (*A* and *B*) and an uninfected control subject (*C* and *D*) were stained with antibody to *H. pylori* whole bacteria (surface components) (*A* and *C*) or to the 61-kD subunit of *H. pylori* urease (*B* and *D*). The black precipitate identifies reaction product (×100).

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