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Differences in Surface-Exposed Antigen Expression between *Helicobacter pylori* Strains Isolated from Duodenal Ulcer Patients and from Asymptomatic Subjects

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We have analyzed possible qualitative and quantitative differences in antigen expression between *Helicobacter pylori* strains isolated from the antrum and different locations in the duodenum of 21 duodenal ulcer (DU) patients and 20 asymptomatic subjects (AS) by enzyme-linked immunosorbent assay (ELISA) and inhibition ELISA. Almost all antral and duodenal strains grown in vitro expressed the *N*-acetyl-neuroaminyllactose-binding hemagglutinin, flagellins (subunits FlaA and FlaB), urease, a 26-kDa protein, and a neutrophil-activating protein. In 75% of both the DU patients and the AS, antral *H. pylori* strains expressed either the blood group antigen Lewis y (Le^y) alone or together with the Le^x antigen. However, duodenal *H. pylori* strains of DU patients expressed Le^y antigen more frequently than corresponding strains of AS ($P < 0.05$). Presence of Le^y on *H. pylori* was related to the degree of active duodenitis ($P < 0.05$). Duodenal *H. pylori* strains isolated from AS were significantly more often Lewis nontypeable than duodenal strains of DU patients ($P < 0.01$). Presence of *H. pylori* blood group antigen-binding adhesin (BabA) was significantly higher on both antral and duodenal strains isolated from DU patients than on corresponding strains isolated from AS ($P < 0.05$). BabA-positive duodenal *H. pylori* strains isolated from DU patients were associated with active duodenitis more frequently than corresponding strains isolated from AS ($P < 0.01$). Infection with *H. pylori* strains positive for Le^y and BabA in the duodenum is associated with development of duodenal ulcer formation.

Helicobacter pylori colonizes gastric-type epithelium in the stomach and areas of gastric metaplasia in the duodenum. Infection with *H. pylori* is strongly associated with the development of acute and chronic gastritis, peptic ulcers, and gastric cancer (8, 25, 30). Although most infected individuals remain asymptomatic, about 10 to 15% develop duodenal ulceration (36). Few studies have investigated if it is *H. pylori* infection in the duodenum itself that is associated with the development of duodenal ulcer (DU). An important question is whether it is possible to differentiate between any specific properties exposed on those *H. pylori* strains colonizing the duodenum and those colonizing the antrum.

We have previously reported that the presence of cytotoxin-associated gene A (*cagA*) is markedly increased in duodenal *H. pylori* strains in DU patients compared to in asymptomatic subjects (AS) (20). We also showed that the distribution of inflammatory cells in the duodenal bulb was related to the duodenal gastric metaplasia and found a positive correlation between the extent of duodenal gastric metaplasia and duodenal *H. pylori* density (20).

Other antigens in *H. pylori* that could be associated with increased virulence or induction of symptoms are urease and neutrophil-activating protein (NAP). Urease causes ammonium formation which protects the bacteria from lethal gastric acidity but which may also have a toxic effect on human epithelial cells (22). NAP induces the production of interleukin-8, thus mediating greater infiltration of neutrophils at the site of infection (17). Additional antigens proposed to be associated with virulence of *H. pylori* infection are the HpaA protein, which has been suggested to be a colonization factor promoting adherence of the bacteria to receptors on erythrocytes and gastric epithelium containing sialic acid (14), and the *H. pylori* blood group antigen-binding adhesin (BabA), which has been suggested to mediate adhesion to Lewis b (Le^b) receptors on gastric epithelium (7, 21). There is also the flagellin of the flagellae providing motility-promoting colonization (23), as well as a 26-kDa protein that is a major protein of the bacteria (28). The lipopolysaccharide (LPS) O chains of *H. pylori* were recently shown to contain fucosylated *N*-acetyllactoseaminoglycan determinants, for example, the Le^x antigen, the Le^y antigen, or both, which are also present in human mucosa and are known as blood group antigens (34).

In this study, we sought to determine if any of a number of antigens may be associated with virulence and if these antigens are expressed in higher frequencies by *H. pylori* isolated from patients with symptoms, i.e., DU patients, than by strains from AS. Furthermore, it was investigated whether *H. pylori* strains isolated from the duodenum express different antigens than corresponding *H. pylori* strains in the antrum. Possible quantitative differences between various antigens of *H. pylori* isolated from DU patients and AS were also studied.

MATERIALS AND METHODS

Patients and AS. Twenty-one patients suffering from active or inactive DU disease (15 males; mean age 54 years, range 26 to 84 years) and 20 AS (14 men; mean age 50 years, range 29 to 60 years) participated in this study. The DU patients were recruited from patients seeking care for gastroduodenal disease at

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TABLE 1. Characteristics of MAbs used to determine antigens on *H. pylori*[a]

Specificity	Clone	Isotype	Ig concn (μg/ml)	Dilutions used in ELISA and inhibition ELISA	Reference
HpaA	30:1:1:6	IgG$_1$	65	1/100	5
Flagellins (FlaA+FlaB)	48:13	IgG$_1$	40	1/100	24
Urease 30-kDa subunit	8:1	IgG$_1$	42	1/100	I. Bölin and A.-M. Svennerholm, unpublished data
26-kDa protein	18:1	IgG$_1$	34	1/100	24
NAP	6:8	IgG$_1$	31	1/100	D. G. Evans and A.-M. Svennerholm, unpublished data
Le^a (type 1 chain)	T174	IgG$_1$	175	1/2,000	29, 35
Le^b (type 1 chain)	T218	IgM	810	1/2,000	29, 35
Le^x (type 2 chain)	P12	IgM	175	1/2,000	29, 35
Sialyl-Le^x	CSLEX1	IgM	50	1/2,000	29, 35
Le^y (type 2 chain)	F3	IgM	125	1/2,000	29, 35

[a] Anti-Lewis MAbs were purchased from Signet Laboratories, Inc.

the Sahlgrenska University Hospital in Göteborg, Sweden, and the AS were recruited from healthy, age-matched blood donors with no symptoms of dyspepsia at the blood bank of the Sahlgrenska University Hospital as previously described (20). Informed consent was obtained from all patients and AS who participated in the study, and the protocol was approved by the Ethical Committee of the Medical Faculty, Göteborg University, Sweden. All subjects were screened for the presence of *H. pylori*-specific antibodies in serum as described (19), and AS were recruited based on positive *H. pylori* serology. Infection was confirmed by culturing of biopsy specimens provided by gastroscopy (20).

The ABO blood group status of each subject was analyzed by a serological test in which equal volumes of serum and erythrocytes of known ABO blood groups were mixed for 10 min. Serum antibodies agglutinating specific blood groups were recorded, and the relationship between the ABO blood group status and the expression of BabA on the best-growing *H. pylori* strain in the antrum and the duodenum was analyzed for each subject.

Design of study. Initial screening for the presence of specific *H. pylori* antigens was determined by enzyme-linked immunosorbent assay (ELISA) on *H. pylori* isolated from one of the two antral biopsy specimens (except for the BabA, which was analyzed on *H. pylori* isolated from each of the two antral biopsy specimens) and on all *H. pylori* isolates that were recovered from four to five different duodenal biopsy specimens collected from each subject. Some of the antigens positive in the qualitative analyses were also tested for quantitative analyses, i.e., the concentrations of the HpaA, flagellins, urease, and the 26-kDa protein were determined on *H. pylori* strains isolated from one of the two antral strains isolated from each subject by using inhibition ELISA. Possible relationships between the presence of specific antigens on *H. pylori* and active or chronic inflammatory score in the duodenum were based on analyses of the best-growing strain from either type of the four to five duodenal biopsy specimens from each subject.

Culture conditions for *H. pylori* and reference strains. Each biopsy specimen was stored in 1 ml of saline for approximately 1 h after gastroscopy and was thereafter analyzed for the presence of *H. pylori* by disintegrating the tissue with a homogenizer (Ultra-Turrax T25; IKA-Laboratechnik) at a rate of 8,000 to 9,500 rpm for 20 to 40 s. *H. pylori* strains were cultured on 8.5% horse blood Columbia agar plates and selective agar plates supplemented with 3 μg of vancomycin per ml, 5 μg of trimethoprim per ml, 10 μg of nalidixic acid per ml, and 10 μg of nystatin per ml and were incubated under microaerobic conditions at 37°C for 3 to 6 days and stored in a specific freeze-drying medium at −80°C.

The following reference strains positive and, in some instances, negative for the different antigens were included in the study: *H. pylori* CCUG (Culture Collection, Göteborg University) 17874, a spontaneous *H. pylori* urease-negative mutant (provided by G. I. Perez-Perez, Nashville, Tenn.), *H. pylori* strains G21 and G39 (provided by N. Figura, Siena, Italy), *H. pylori* strains P466 and MO19 (provided by J. Çelik, Karolinska Institute, Stockholm, Sweden), *H. pylori* E32, *Helicobacter felis* CCUG 28540, and *Helicobacter nemestrinae* CCUG 32350. Two *Escherichia coli* reference strains, E1392 and WM73494, were cultured on 5% horse blood agar plates and incubated at 37°C for 24 h under aerobic conditions.

Microbiological examination of *H. pylori* strains. The typical rod-shaped morphology of *H. pylori* strains was identified by phase-contrast microscopy. The activities of urease (10), catalase (Merck, Darmstadt, Germany), and oxidase (Sigma-Aldrich Co., Tyresö, Sweden) were measured on all *H. pylori* strains. Growth of *H. pylori* was confirmed by using a specific monoclonal antibody (MAb) against HpaA in a dot blot method, as previously described (5).

***H. pylori* antigen preparations.** Whole membrane proteins were prepared from selected *H. pylori* strains, CCUG 17874 and Hel 305, by sonication followed by differential centrifugation as described previously (1). These strains were selected since they had previously been shown to be positive for most of the antigens analyzed by ELISA and in other immunological analyses. NAP (15, 17),

HpaA (5, 14), and the 26-kDa proteins (28) were recombinantly produced in *E. coli*. *E. coli* cells were transformed with an expression vector containing the *H. pylori napA* gene under the control of the T7 promoter. Cells were disrupted, and the soluble proteins were applied to a Q-Sepharose column (Pharmacia BioTech, Uppsala, Sweden) at pH 8.0 in a buffer containing 25 mM Tris-HCl and 50 mM NaCl. The recombinant NAP (rNAP) was found in the nonbinding fraction. The fractions containing the rNAP were concentrated and purified by gel filtration on a Superdex 200 column (Pharmacia). The rNAP eluted as a single peak. The recombinant HpaA (rHpaA) protein was purified from *E. coli* cells transformed with an expression vector containing the *H. pylori hpaA* gene under the control of the T7 promoter. Cells were dissolved with 1.5% Triton X-114 (TX-114; Sigma) and were subjected to two-phase partitioning (6). The detergent phase was diluted to approximately 1% TX-114 and was applied to a Q-Sepharose column (Pharmacia) at pH 8.0 in a buffer containing 50 mM Tris, 2 mM EDTA, 50 mM NaCl, and 0.1% Triton X-100. The rHpaA was found in the nonbinding fraction. The fractions containing the rHpaA were pooled and applied to a new Q-Sepharose column (Pharmacia) at pH 8.6, and the rHpaA was collected by isocratic elution with a buffer containing 10 mM Tris, 2 mM EDTA, 1 M NaCl, and 0.1% Triton X-100, pH 8.6.

Flagellins (subunits FlaA and FlaB), kindly provided by I. Bölin, were purified from strain E32 as previously described (23). The flagellin fraction was further purified by flow pressure low chromatography fractionation on a Resource Q column (Pharmacia). Urease was purified from strain E32 by using a modified version of the method described by Dunn et al. (13) and Evans et al. (16). The positive fractions were pooled, dialyzed against phosphate-buffered saline (PBS), filtered through a 0.45-μm-pore-size membrane, and purified by flow pressure low chromatography.

The purity of the proteins was confirmed by Coomassie staining of material that had undergone sodium dodecyl sulfate-polyacrylamide gel electrophoresis and by immunoblotting, using rabbit polyclonal antisera against whole *H. pylori* bacteria as well as MAbs reacting specifically with the respective protein (24).

Production of MAbs. The properties of the manufactured and in-house MAbs used in the study are shown in Table 1. MAbs against Lewis antigens were purchased (Signet Laboratories, Inc., Amsterdam, The Netherlands) and also provided as a gift from B. Appelmelk, Amsterdam, The Netherlands. The MAbs specific to the 26-kDa protein, the HpaA, and the flagellin FlaA and FlaB subunits of *H. pylori* have previously been described (5, 24).

Qualitative determination of *H. pylori* antigens. The presence of NAP, a 26-kDa protein, HpaA, urease, flagellins, and Lewis blood group antigens (Le^x, Le^y, Le^a, Le^b, and sialyl-Le^x) on the different *H. pylori* isolates was determined by optimized whole-cell ELISAs as previously described (29). Briefly, *H. pylori* isolates were grown on 8.5% horse blood Columbia agar plates, resuspended in PBS at a final concentration of ~2 × 10^9 bacteria/ml (the lower limit for detection of the antigens adjusted according to standard curves for the individual antigen). Thereafter, 100 μl of the bacterial suspensions was applied to each well of a 96-well polystyrene plate as a solid-phase antigen and was incubated at 4°C overnight. After blocking with 1% bovine serum albumin (BSA) in PBS at 37°C for 30 min and washing two times with PBS, the different MAbs described in Table 1 were diluted in 1% BSA–PBS–0.05% Tween to the lowest concentration giving clear cutoff absorbance values for positive and negative antigen expression, were added to the wells, and were incubated at 37°C for 90 min. After washing, the plates were developed with horseradish peroxidase-conjugated anti-mouse immunoglobulin M (IgM) and IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.), followed by addition of H$_2$O$_2$ and *o*-phenylene-diamine in sodium citrate buffer (pH 4.5), and were read at 450 nm. All *H. pylori* samples were run in duplicate on two different occasions. The same number of bacteria and concentration of MAb were used when testing samples and the negative and positive controls. Titers were determined as the reciprocal dilution

giving an absorbance of 0.5 above background (i.e., slightly above the mean absorbance value plus three times the standard deviations of negative control samples).

Analyses of *H. pylori* BabA using Le^b oligosaccharide probe. Agar-grown *H. pylori* strains from the antrum and duodenum (*H. pylori* isolated from two antral biopsy specimens and one to three duodenal biopsy specimens) were directly transferred to nitrocellulose filters probed with biotinylated Le^b oligosaccharides and incubated with streptavidin-alkaline phosphatases as previously described (9). The filters were developed by using the 5-bromo-4-chlor-3-indolylphosphate substrate and the nitroblue tetrazolium color reagent (BCIP/NBT tablets; Sigma). The intensity of the color reaction was observed by eye, and results were compared with those positive and negative *H. pylori* control strains, as well as *E. coli* controls.

Quantitative determination of *H. pylori* antigens by inhibition ELISA. The concentrations of HpaA, flagellins, urease, and the 26-kDa protein in different *H. pylori* isolates were measured by an optimized inhibition ELISA (24). The lower limit for detection of *H. pylori* antigens studied varied between 2 and 25 μg for individual antigens. Plates were coated with one of the following antigen preparations: whole membrane proteins of CCUG strain 17874 (25 μg/ml), 26-kDa protein (10 μg/ml), urease (2 μg/ml), or a crude flagellin preparation (2 μg/ml) and incubated at room temperature (RT) overnight. Suspensions of frozen *H. pylori* bacteria ($\sim 2 \times 10^{10}$ bacteria/ml) were serially diluted threefold in 0.1% BSA-PBS in noncoated plates. Thereafter, an equal volume of each of the specific MAbs, diluted in BSA-PBS to a concentration corresponding to 10 to 20 times the ELISA titer against the respective antigen, was added to each of the bacterial dilutions and incubated at RT for 1 h with moderate shaking. The mixtures were then transferred to the antigen-coated plates and were incubated at RT for 90 min. Plates were washed and developed as described for ELISA. The bacterial concentrations causing 50% inhibition of the binding of the respective MAb to the solid-phase antigen were determined (24). All samples and reference strains were tested in duplicate on two different occasions.

Histological examination of duodenal biopsy specimens. In each biopsy specimen, the severity and activity of chronic inflammation (infiltration of lymphocytes and plasma cells) and active inflammation (infiltration of neutrophils) were graded on a scale of 0 to 3, which correspond to no inflammation, 0; mild inflammation, 1; moderate inflammation, 2; and severe mucosal inflammation (duodenitis), 3. The severity of active inflammation was expressed as the mean score of the total number of biopsies, whereas the activity of active inflammation was expressed as the highest score observed among the different duodenal biopsies from each subject.

RESULTS

Isolation of *H. pylori* from DU patients and AS. Biopsy specimens were collected from the antrum and different locations in the duodenum from each of 21 DU patients and 20 AS. *H. pylori* was recovered from all antral biopsy specimens in each subject. *H. pylori* was positive by culture in one or more of four to five duodenal biopsy specimens collected from 20 out of 21 DU patients, that is in as many as 67 out of 94 (71%) duodenal biopsy specimens. *H. pylori* was cultured from one or more of four duodenal biopsy specimens collected from 16 AS, that is only in 41 out of 81 (51%) duodenal biopsy samples. *H. pylori* could not be cultured from any of the duodenal biopsy specimens from four AS. In some cases, duodenal strains could not be recultured after storage at −80°C.

Qualitative and quantitative analyses *H. pylori* antigens. The presence of HpaA, urease, flagellins, NAP, and a 26-kDa protein was determined by different ELISAs. Almost all DU patients and AS had antral as well as at least one of the four to five duodenal strains, which expressed the different antigens studied (Table 2).

Antral *H. pylori* strains isolated from most of the DU patients and AS were tested for concentrations of HpaA, flagellins, urease, and the 26-kDa protein in inhibition ELISA (Fig. 1). The mean concentrations of HpaA, flagellins, urease, and the 26-kDa protein did not differ significantly between strains isolated from the DU and AS groups; however, the mean concentration of flagellins was somewhat higher on the *H. pylori* strains isolated from the AS ($P = 0.053$).

H. pylori isolates were analyzed for the expression of the different Lewis blood group antigens, that is, Le^x, sialyl-Le^x, Le^y, Le^a, and Le^b, by ELISAs (Table 3). On the antral isolates from DU patients as well as AS, Le^y alone or together with Le^x

TABLE 2. Number (%) of DU patients and AS with antral and duodenal *H. pylori* strains expressing indicated antigens

Antigen	Antrum		Duodenum	
	DU ($n = 21$)	AS ($n = 20$)	DU ($n = 20$)	AS ($n = 16$)
HpaA	21 (100)	20 (100)	20 (100)[a]	14 (88)[b]
Flagellins	21 (100)	20 (100)	20 (100)	15 (94)
Urease	20 (95)	19 (95)	20 (100)	14 (88)
26-kDa protein	20 (95)	20 (100)	20 (100)	14 (88)
NAP	18 (86)	18 (90)	20 (100)	16 (100)
BabA	13 (67)[c]	5 (25)[c]	13 (65)[c]	5 (31)[c,d]

[a] At least one of the duodenal isolates from each subject was positive for the respective antigen. In most cases, all individual duodenal isolates from each subject gave similar results.

[b] Two AS carriers had HpaA-negative duodenal strains determined by ELISA. However, these strains were positive for HpaA protein by a dot blot assay using a specific MAb (5).

[c] In those subjects that were negative for BabA, all individual isolates from the same subject were negative.

[d] $P < 0.05$ when comparing prevalence of BabA-positive strains in the antrum and the duodenum of DU patients as compared to in AS (Fisher's exact test).

was detected in 75% of the cases. When analyzing duodenal isolates, on the other hand, as many as 18 of 20 (90%) of the DU patients but only 7 of 16 (42%) of the AS had *H. pylori* strains which expressed any Lewis antigen ($P < 0.01$). None of the strains isolated from the antrum or the duodenum were sialyl-Le^x positive in either group. Duodenal *H. pylori* strains that only expressed the Le^y antigen were found in six (30%) of the DU patients but in none of the AS. In 64% of the DU patients and AS, the antral isolates differed from the corresponding duodenal isolates with regard to Lewis antigen profile. In some cases, 3 of 20 (15%) of the DU patients and 1 of 16 (6%) of the AS, two different Lewis phenotypes were detected on the different duodenal strains from the same subject. The results, using commercially available MAbs (Signet Laboratories), were confirmed by using MAbs provided by B. J. Appelmelk.

No major changes in the Lewis antigens of the different *H. pylori* strains were seen even after eight subcultures, although a few, 5 of 108 (5%) of the *H. pylori* strains tested for Lewis antigens, converted from being Lewis antigen negative to Lewis antigen (mostly Le^x) positive after several subcultures.

The presence of BabA was assessed on *H. pylori* isolated from both antral biopsy specimens and from one to three of the duodenal biopsy specimens of each subject. The results were almost consistent in all subjects, that is, the two antral and all the different duodenal isolates were either BabA positive or negative. In the DU patients, 13 of 21 (67%) had BabA-positive *H. pylori* strains in the antrum, and 13 of 20 (65%) had BabA-positive *H. pylori* strains in the duodenum (Table 2). Both antral and duodenal strains isolated from AS were significantly less often positive for BabA ($P < 0.05$). Thus, only 5 of 20 (25%) of the AS had BabA-positive strains in the antrum, and 5 out of 16 (31%) had BabA-positive strains in the duodenum.

Correlations of *H. pylori* BabA and Lewis antigens with human ABO blood group status and inflammation in the duodenum. BabA expression in strains isolated from either antrum or duodenum did not correlate with the blood group of the host. However, AS with blood group O were more often colonized by duodenal *H. pylori* strains that lacked BabA than by BabA-positive strains.

We have previously reported that DU patients had significantly higher ($P < 0.001$) infiltration of neutrophils, lymphocytes, and plasma cells in the duodenum than AS, as shown by

HpaA

p=0.787

Flagellins
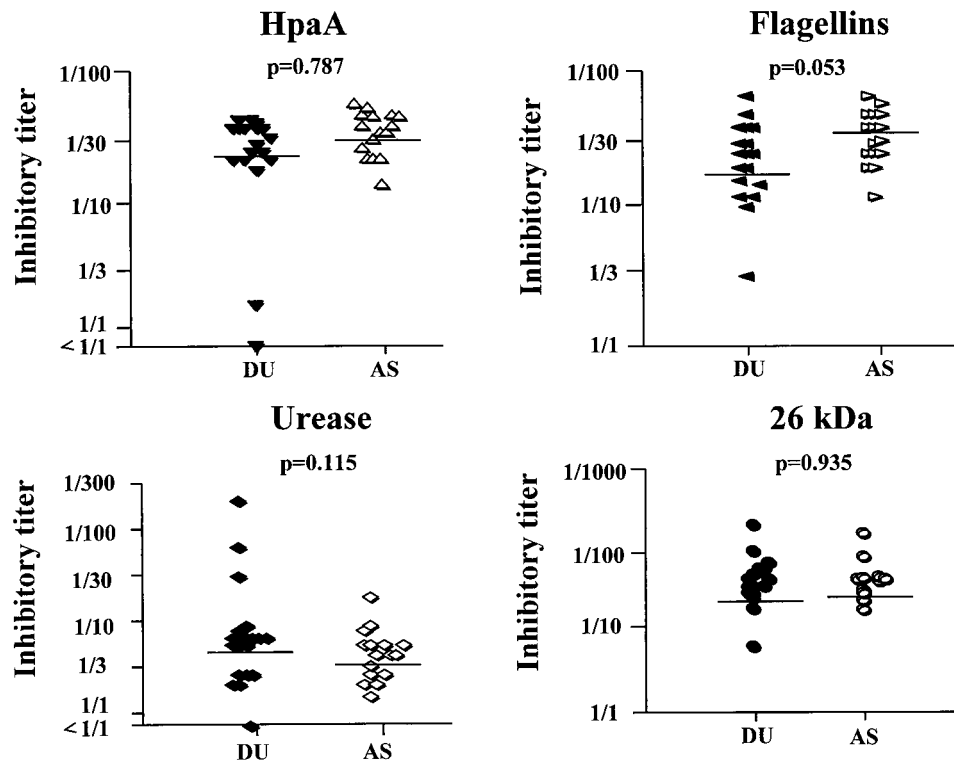
p=0.053

Urease

p=0.115

26 kDa

p=0.935

FIG. 1. Quantitative expression of different *H. pylori* antigens as determined by inhibition ELISA. Strains (one per subject) isolated from antral biopsy specimens from 16 to 18 of the DU patients (filled symbols) and 11 to 16 of the AS (open symbols) were, after adjustment to similar bacterial concentration ($\sim 2 \times 10^{10}$ bacteria/ml), analyzed for 50% inhibitory titers in inhibition ELISA by using specific MAbs. Horizontal lines indicate geometric mean titer for each group, and *P* values refer to comparisons of geometric mean values for DU patients and AS (Student's *t* test).

histopathological analysis (20). When comparing the Lewis antigen type of the *H. pylori* strain in the duodenum with the extent of active inflammation, we found that Le^y positivity was related to increased numbers of neutrophils in the duodenum ($P < 0.05$) (Fig. 2). However, there was no correlation between the expression of Le^y positivity on the duodenal *H. pylori* strains and the number of chronic inflammatory cells in the duodenum.

There was also a significant correlation between active inflammation in the duodenum and presence of strains possessing BabA in DU patients as compared to AS ($P < 0.01$),

whereas chronic inflammation in the duodenum was not related to infection with strains expressing BabA.

DISCUSSION

Few studies have demonstrated a correlation between specific antigens on *H. pylori* and the development of DU in humans. However, in animal models, specific surface-exposed and/or other antigens on *H. pylori* strains isolated from the antrum (12), e.g., urease, *cagA*, and the vacuolating cytotoxin, have been suggested to be associated with the

TABLE 3. Number (%) of DU patients and AS with antral and duodenal *H. pylori* strains expressing different Lewis antigens

Lewis antigen	Antrum			Duodenum		
	DU ($n = 21$)	AS ($n = 20$)	*P* values	DU ($n = 20$)	AS ($n = 16$)	*P* values
Le^x	0 (0)	0 (0)	NS^f	1 (5)^a	1 (6)	NS
Le^y	4 (19)^b	7 (35)	NS	6 (30)	0 (0)	<0.05^d
Le^{xy}	12 (57)^c	8 (40)	NS	7 (35)	3 (19)	NS
Le^a	0 (0)	0 (0)	NS	2 (10)	0 (0)	NS
Le^b	1 (5)	2 (10)	NS	1 (5)	3 (19)	NS
Le^{ab}	1 (5)	0 (0)	NS	1 (5)	0 (0)	NS
Le negative (nontypeable)	3 (14)	3 (15)	NS	2 (10)	9 (58)	<0.01^e

^{a} At least one of the duodenal isolates from each subject was positive for the respective antigen. In most cases, all individuals duodenal isolates from each subject gave similar results.
^{b} Two of four patients had antral *H. pylori* strains coexpressing the Le^{ab} antigens.
^{c} Two of 12 patients had antral *H. pylori* strains coexpressing the Le^a or the Le^b antigens.
^{d} $P < 0.05$ when comparing the higher presence of Le^y-positive strains in the duodenum of DU patients to that in AS (Fisher's exact test).
^{e} $P < 0.01$ when comparing the higher frequency of Le-negative (nontypeable) strains in the duodenum of AS to that in DU patients (Fisher's exact test).
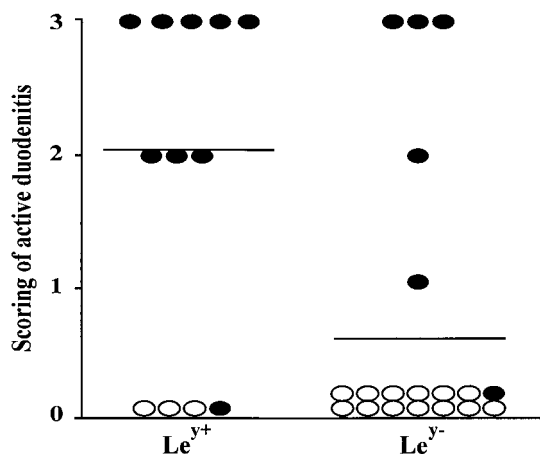^{f} NS, not significant.

FIG. 2. The relationship between Le^y expression on *H. pylori* strains and inflammation in the duodenum. *H. pylori* strains (the best growing strain from each DU patient) isolated from the duodenum of DU patients (filled symbols) and AS (open symbols). Five patients with Le^y-positive *H. pylori* strains who had been treated with antiulcer medication (from 1 week to 2 months prior to gastroscopy) were excluded from the analysis, since such treatments have been shown to lower the activity of chronic gastritis as well as to significantly decrease the accumulation of inflammatory infiltrates and release of toxic products in the antrum of DU patients and AS (31). The highest score of duodenitis of several duodenal biopsy specimens collected from the duodenum in the same subject is shown on the vertical axis. Lines indicate average values for each group. Le^y type of the *H. pylori* strain in the duodenum was related to increased numbers of neutrophils in the duodenum ($P < 0.05$, Fisher's exact test).

development of ulcer (18). We have been focused on studying if there are any antigens exposed on *H. pylori* isolated from both the antrum and the duodenum of patients with DU that differ from those exposed on strains isolated from AS.

Even if the genome of *H. pylori* seems to be rather heterogeneous (33), we found that most of the antigens studied, i.e., HpaA, urease, flagellins (subunit FlaA and FlaB), NAP, and a 26-kDa protein, remained constant to a high degree in almost all *H. pylori* strains, irrespective of whether they had been isolated from the antrum or the duodenum from DU patients or AS. Even though these antigens do not seem to be linked to the severity of inflammation, they are important for survival and colonization of *H. pylori* in the host (12, 14, 16, 17, 23, 28).

The biological function of Lewis antigens expressed by *H. pylori* is still unknown. We found a correlation between Le^y-positive strains and active inflammatory score (neutrophils) in the duodenum ($P < 0.05$) (Fig. 2). It is therefore possible that strains colonizing the duodenum of ulcer patients may survive and evade inflammatory responses due to specific expression of Le^y *H. pylori* antigen mimicking the Le^y antigen expressed on the duodenal epithelium (data obtained from immunohistochemical staining of duodenal tissue sections [unpublished data]). A potential inflammatory effect of the Le^y antigen has also been suggested by Muguruma et al. (27), who showed a relationship between increased Le^y antigen expression on the epithelial cells and chronic inflammatory liver diseases, and also that the plasma levels of Le^y increased significantly with the severity of inflammation. McGowan et al. (26) also recently showed that growth of *H. pylori* in acidity may result in induction of certain LPS determinants on bacteria, since an isogenic strain lacking *wbcJ* (a gene with predicted homology to known bacterial O antigen biosynthesis involved in the conversion of GDP-mannose to GDP-fucose) failed to express either O antigen or Le^x and Le^y antigens. Thus, *H. pylori* growing in the

duodenum may survive the hostile environment by developing special features, e.g., certain Lewis antigens to withstand the increased exposure to gastric acidity, including the alteration of its LPS structures. We also show that *H. pylori* strains isolated from the duodenum of DU patients, who have reduced bicarbonate secretion and an increased gastric acid output in the duodenum compared to AS, more frequently express Lewis antigens on the LPS, that is Le^x and Le^y, than corresponding strains from AS ($P < 0.01$). Thus, duodenal strains from AS that often are nontypeable for Lewis antigens may be more sensitive to the higher acid load in the duodenum, which may explain the significantly lower numbers of *H. pylori* in AS than in corresponding regions of DU patients that we have previously reported (20).

Lewis antigen conversion in *H. pylori* strains has been reported and may be due to different levels of glycosyltransferase expression and also due to the fact that *H. pylori* strains may turn glycosyltransferase genes on or off, depending on host and/or growth factors during the infection (3), which may enable the bacteria to survive for a long time in gastric-like epithelium (4). We found that Lewis antigens on *H. pylori* were fairly stable even after several subcultures of the bacteria and repeated analyses. However, 5% of all *H. pylori* isolates tested converted from being Lewis antigen negative to Lewis antigen positive after several subcultures on agar plates. Our results from genetic analyses confirm that in these instances when *H. pylori* strains from the antrum and duodenum express different Lewis antigens, they also differ with regard to their DNA fingerprints (32). This suggests that the same patient may be colonized by a combination of *H. pylori* strains.

It has been claimed that the expression of Le^x and Le^y by *H. pylori* isolates is related to *cagA* status (35). However, we were unable to find any relationship between any of the secreted antigens or surface antigens studied, including Le^x and Le^y, with the previously demonstrated *cagA* genotype of the respective strain (20). This is puzzling, since we have previously reported that *H. pylori* density in the duodenum is associated with *cagA*$^+$ genotype as well as with active duodenitis (20). This paradox may be explained by the fact that duodenal *cagA*$^+$ strains cause active inflammation. However, colonization is not dependent on *cagA* status, since Le^y-positive strains seem to survive irrespective of whether they are *cagA*$^+$ or *cagA*.

In this study, we show that significantly higher numbers of both the antral and duodenal strains isolated from DU patients had BabA adhesin than corresponding strains from the AS, supporting the notion that colonization by BabA-positive strains is associated with ulcer formation (21). A previous study by Borén et al. (7) showed that strains with BabA preferentially bound to Le^b receptors, which are more often expressed in hosts with blood group O than in individuals with other blood groups. We did not find any such relationship between blood group and BabA-positive *H. pylori* infecting strains. Presence of BabA on the duodenal strains from the DU patients was related to both presence of *cagA* on the strain and to amount of neutrophils in the duodenal mucosa. A correlation between *cagA* on both duodenal and antral *H. pylori* strains and the bacterial binding to Le^b receptors on freshly collected gastric tissues from blood group O individuals has also been shown (21). However, the relationship between *cagA* and BabA was shown to not have any biological significance, since deletion of the entire *cag* pathogenicity island did not affect binding capacity.

In summary, our study shows that there seem to be antigens on *H. pylori* that differ between strains isolated from DU patients and AS. For example, Le^y was significantly more often detected in duodenal strains isolated from DU patients than

from AS, and a relationship between Le^y and active inflammation in the duodenum was found. We have previously demonstrated an association between presence of *cagA* and duodenitis (20). In this study, we found a correlation between presence of BabA and active duodenitis. Thus, Le^y and BabA may be relevant markers to predict DU development following *H. pylori* infection.

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