INFECTION AND IMMUNITY, Dec. 1993, p. 5008–5012
0019-9567/93/125008-05$02.00/0

Vol. 61, No. 12

Correlation between Vacuolating Cytotoxin Production by *Helicobacter pylori* Isolates In Vitro and In Vivo

TIMOTHY L. COVER,[1,2]* PING CAO,[1] CHRISTOPHER D. LIND,[1] KYI T. THAM,[2,3] AND MARTIN J. BLASER[1,2,4]

Departments of Medicine,[1] Microbiology and Immunology,[4] and Pathology,[3] Vanderbilt University School of Medicine, Nashville, Tennessee 37232-2605, and Department of Veterans Affairs Medical Center,[2] Nashville, Tennessee 37232-2637

Received 6 July 1993/Returned for modification 18 August 1993/Accepted 16 September 1993

Approximately 50 to 60% of *Helicobacter pylori* isolates produce a vacuolating cytotoxin in vitro. To assess cytotoxin production in vivo, we sought to determine whether infection with a Tox$^+$ *H. pylori* strain is associated with the presence of serum antitoxin antibodies. *H. pylori* isolates and serum samples were obtained from 30 patients, and serum samples were obtained from 20 uninfected patients as controls. Sera were tested by enzyme-linked immunosorbent assay for reactivity with the purified 87-kDa vacuolating cytotoxin, and the 30 *H. pylori* isolates were tested for vacuolating cytotoxin production. Supernatants from 14 (47%) of the 30 *H. pylori* isolates induced vacuolation of HeLa cells. Sera from the 30 *H. pylori*-infected patients reacted with the purified 87-kDa cytotoxin to a greater extent than sera from the uninfected controls for both immunoglobulin G (IgG) and IgA classes ($P = 0.0004$ and $P < 0.0001$, respectively). Serum IgG and IgA responses to the purified 87-kDa cytotoxin were higher among the 14 patients infected with Tox$^+$ strains than among the 16 patients infected with Tox$^-$ strains (mean optical densities ± standard errors of the means of 0.603 ± 0.11 versus 0.234 ± 0.07 [$P = 0.005$] and 0.644 ± 0.12 versus 0.341 ± 0.08 [$P = 0.04$] for IgG and IgA, respectively). Infection with a Tox$^+$ strain compared with a Tox$^-$ strain was associated with increased antral polymorphonuclear leukocyte inflammation scores ($P = 0.04$). These data indicate that cytotoxin production by *H. pylori* isolates in vitro correlates with cytotoxin production in vivo and that infection with Tox$^+$ *H. pylori* isolates may be associated with increased antral mucosal polymorphonuclear leukocyte infiltration.

Helicobacter pylori is a gram-negative bacterium that lives within the microenvironment of the mucus layer of the human gastroduodenal mucosa (12). In this niche, the organism elicits an inflammatory response in the mucosa that is manifested histologically as diffuse superficial gastritis (13). The majority of persons infected with *H. pylori* remain asymptomatic (14). However, a small percentage of *H. pylori*-infected persons develop serious complications, including peptic ulcer disease and possibly gastric carcinoma (2, 17).

There are several potential virulence factors that may contribute to the mucosal inflammation and damage associated with *H. pylori* infection (4, 12). One such agent is a cytotoxin that induces vacuolation of eukaryotic cells (7, 18). Production of the vacuolating cytotoxin by *H. pylori* isolates is associated with production of a 120- to 128-kDa protein (3, 7, 10, 23); however, the active cytotoxin is a protein that migrates at 87 kDa under denaturing conditions (5). With cell culture assays, vacuolating cytotoxin production in vitro has been detected in culture supernatants from 50 to 60% of *H. pylori* isolates but not in supernatants from the remainder of isolates (7, 15, 18). This may represent a lack of in vitro expression of cytotoxin by some *H. pylori* strains that express cytotoxin in vivo or may reflect a complete absence of functionally active cytotoxin production by some strains. To distinguish between these two possibilities, the presence of antibody responses to the cytotoxin can be used as a marker for in vivo cytotoxin expression. We recently reported that serum immunoglobulin G (IgG) from some *H. pylori*-infected persons specifically recognizes or neutralizes cytotoxin activity (6). We now have collected both *H. pylori* strains and serum specimens from 30 patients, in order to examine whether there is a correlation between serum antibody responses to the cytotoxin and expression of cytotoxin activity in vitro.

MATERIALS AND METHODS

Source of *H. pylori* isolates and clinical specimens. *H. pylori* isolates, antral biopsies, and sera were obtained from 30 different patients who underwent gastroduodenoscopy and antral biopsy. Fifteen patients were from the Department of Veterans Affairs Medical Center, Denver, Colo.; 12 were from Vanderbilt University Medical Center, Nashville, Tenn.; 2 were from the Department of Veterans Affairs Medical Center, Syracuse, N.Y.; and 1 was from the Mayo Clinic, Rochester, Minn. (Table 1). The endoscopic diagnoses were peptic ulcer disease ($n = 4$), nonulcer dyspepsia ($n = 21$), esophagitis ($n = 1$), and asymptomatic ($n = 1$); for three patients endoscopic diagnostic information was not available. Gastric specimens were cultured for *H. pylori* onum selective medium (Campy pylori agar; Remel, Lenexa, Kans.) or 5% sheep blood agar plates and incubated under microaerobic conditions, and a single isolate was picked from each plate. Isolates were identified as *H. pylori* by Gram stain morphology and by urease, oxidase, and catalase positivity. Sera also were obtained from 20 uninfected dyspeptic patients as controls; these patients were classified as uninfected on the basis of negative gastric cultures and the absence of *H. pylori*-specific serum IgG antibodies in a standardized enzyme-linked immunosorbent assay (ELISA) (19). Permission for this study was obtained from the

* Corresponding author.

TABLE 1. *Helicobacter* strains used in this study

Isolate	Location	Endoscopic diagnosis[a]	Expression of vacuolating cytotoxin activity	Reciprocal cytotoxin titer	OD[b]	Presence of *cagA* gene
87-33	Colorado	NUD, Billroth I	+	80	0.217	+
87-81	Colorado	NUD	+	≥160	0.368	+
87-91	Colorado	NUD	+	10	0.062	+
87-29	Colorado	Duodenal ulcer	+	≥160	0.782	+
87-199	Colorado	NUD, Billroth I	+	≥160	0.454	+
86-86	New York	NA	+	40	0.083	+
86-332	Minnesota	NA	+	10	0.014	+
92-18	Tennessee	NUD	+	5	0.071	+
92-19	Tennessee	NUD	+	≥160	0.229	+
92-21	Tennessee	Duodenal ulcer	+	5	0.056	+
92-29	Tennessee	NUD	+	80	0.654	+
92-25	Tennessee	Duodenal ulcer	+	≥160	0.899	+
92-26	Tennessee	NUD	+	≥160	0.641	+
92-27	Tennessee	Asymptomatic	+	≥160	0.163	+
87-90	Colorado	NUD	−	<5	0.006	−
87-226	Colorado	NUD	−	<5	0.054	+
87-225	Colorado	Esophagitis	−	<5	0.020	−
87-230	Colorado	NUD	−	<5	0.018	−
87-75	Colorado	NUD	−	<5	0.035	−
87-203	Colorado	NUD	−	<5	0.015	−
86-313	Colorado	NUD	−	<5	0.047	−
87-6	Colorado	Gastric ulcer	−	<5	0.003	+
86-385	Colorado	NUD	−	<5	0.117	−
86-338	Colorado	NUD	−	<5	0.088	−
86-63	New York	NA	−	<5	0.007	+
92-20	Tennessee	NUD	−	<5	0.046	−
92-28	Tennessee	NUD	−	<5	0.021	−
92-22	Tennessee	NUD	−	<5	0.018	+
92-23	Tennessee	NUD	−	<5	0.040	−
92-24	Tennessee	NUD	−	<5	0.010	−

[a] NUD, nonulcer dyspepsia; Billroth I, a partial gastrectomy performed for refractory peptic ulcer disease; NA, not available.
[b] OD in an ELISA for the detection of an 87-kDa vacuolating cytotoxin in concentrated culture supernatant.

Vanderbilt and Nashville Department of Veterans Affairs Medical Center Institutional Review Boards.

Assessment of gastric histology. Antral biopsy specimens were available from 22 of the 30 *H. pylori*-infected patients in this study, and multiple biopsy specimens were available from 11 of these patients. Biopsy specimens were examined blindly by a pathologist and scored according to severity of acute and chronic mucosal inflammation, glandular atrophy, intestinal metaplasia, mucin depletion, epithelial degeneration, epithelial vacuolation, erosion, and foveolar hyperplasia, according to modifications of the Sydney criteria (21). Acute inflammation was scored as (i) grade 0, polymorphonuclear leukocytes being absent or rare; (ii) grade 1, focal mild polymorphonuclear infiltration (<10 per high-power field); (iii) grade 2, focal dense polymorphonuclear leukocyte infiltration (≥10 per high-power field); or (iv) grade 3, diffuse and dense polymorphonuclear leukocyte infiltration in >50% of the mucosa. Chronic inflammation was scored as (i) grade 0, few scattered mononuclear cells; (ii) grade 1, slight increase in mononuclear cell infiltration; (iii) grade 2, dense but focal mononuclear infiltration; or (iv) grade 3, dense and diffuse mononuclear infiltration in >50% of the mucosa. For 11 patients from whom multiple biopsy specimens were available, the scores from individual biopsies were combined to generate a mean score for each parameter.

Cell culture assay for vacuolating cytotoxin activity. *H. pylori* isolates were cultured for 3 days at 37°C in 50-ml volumes of brucella broth containing 5% fetal bovine serum in a microaerobic atmosphere generated by CampyPak Plus (BBL, Cockeysville, Md.). After centrifugation of the cultures, 30-ml aliquots of supernatants were concentrated 30-fold by using Centriprep-30 ultrafiltration units (Amicon, Beverly, Mass.) and passaged through 0.2-μm-pore-size cellulose acetate filters (Life Science Products, Denver, Colo.). These concentrated culture supernatants were serially diluted 1:5 to 1:160 and incubated for 24 h with HeLa cells in 96-well plates (7). Tissue culture medium was supplemented with 10 mM ammonium chloride to potentiate cytotoxin activity, as described previously (9). Cell vacuolation was quantitated spectrophotometrically by a neutral red uptake assay (8). The maximum dilution of supernatant that produced vacuolation was defined as that which induced an optical density (OD) with >2 intervals of standard deviation above that produced by medium alone. The results were verified by visual inspection of cells by light microscopy (7).

Antigen-detection ELISA for quantitation of vacuolating cytotoxin production. To detect the presence of vacuolating cytotoxin by ELISA, concentrated *H. pylori* supernatants were diluted 1:50 in 50 mM sodium carbonate buffer (pH 9.6), placed in the wells of 96-well microtiter plates, washed, and reacted with a 1:10,000 dilution of rabbit antiserum to the purified denatured 87-kDa protein (5). Antigen-antibody complexes were detected by reaction with peroxidase-conjugated anti-rabbit IgG, as described previously (5).

Antibody-detection ELISA. To detect serum antibodies to the vacuolating cytotoxin, an ELISA was performed with purified cytotoxin (15 ng per well) as the antigen (5, 6). The

cytotoxin used in these assays was purified from *H. pylori* 60190 as described previously (5), except that gel filtration chromatography was performed with a Superose 6 10/50 column (Pharmacia). Serum samples from patients were diluted 1:100, and the second antibody was peroxidase-conjugated anti-human IgG or IgA (5).

Detection of cytotoxin-associated gene (*cagA*). To determine whether *H. pylori* isolates contained the *cagA* gene, which encodes a 128-kDa protein associated with vacuolating cytotoxin production, colonies of the 30 *H. pylori* isolates were hybridized with a *cagA* probe (pMC3), as described previously (23).

Statistical methods. Results are expressed as the mean ± standard error of the mean. The distributions of ODs and histologic scores were each compared by the using Student's *t* test for independent variables. Proportions were compared by using Fisher's exact test.

RESULTS

Vacuolation of HeLa cells induced by *H. pylori* culture supernatants. To assess vacuolating cytotoxin production by *H. pylori* strains, concentrated culture supernatants from 30 isolates were incubated with HeLa cells for 24 h, and vacuolation then was quantitated by using the neutral red uptake assay (8). Supernatants from 14 (47%) of the isolates produced vacuolation of cells, whereas the remaining 16 (53%) did not (Table 1). The maximum dilutions of supernatants that produced vacuolation ranged from 1:5 to ≥1:160, an indication that there was considerable variability in the quantity or activity of cytotoxin produced or secreted in vitro by Tox$^+$ *H. pylori* isolates. As noted previously (23), there was an association between the presence of the *cagA* gene and expression of vacuolating cytotoxin activity ($P \leq 0.001$) (Table 1); 26 (87%) of 30 isolates were concordant for the presence of *cagA* and expression of cytotoxin activity.

ELISA for quantitation of vacuolating cytotoxin production by *H. pylori* isolates. To quantitate vacuolating cytotoxin production by an alternate method, concentrated *H. pylori* culture supernatants were diluted 1:50 and reacted in an ELISA with polyclonal antiserum to the purified 87-kDa protein from Tox$^+$ *H. pylori* 60190 (5). The 14 supernatants that induced vacuolation of HeLa cells reacted to a significantly greater extent than did the 16 supernatants that did not induce vacuolation (OD [mean ± standard error of the mean] = 0.335 ± 0.08 versus 0.034 ± 0.01, $P = 0.0004$) (Table 1), a confirmation of previous results (5). The cytotoxin activities of supernatants as quantitated in the cell culture assay and the ELISA ODs were significantly correlated ($r = 0.813$ and $P < 0.001$ by linear regression analysis).

Serologic responses to the vacuolating cytotoxin. To assess cytotoxin production by *H. pylori* isolates in vivo, we examined serum antibody responses to the purified cytotoxin in 30 *H. pylori*-infected patients and 20 uninfected controls by ELISA (5, 6). As expected, sera from the infected persons reacted with the purified cytotoxin to a significantly greater extent than sera from uninfected persons, for both the IgG (OD = 0.406 ± 0.07 versus 0.107 ± 0.01, $P = 0.0004$) and IgA (OD = 0.483 ± 0.07 versus 0.085 ± 0.01, $P < 0.0001$) classes. Among the *H. pylori*-infected persons, there was a significant association between the IgA and IgG responses to the cytotoxin ($r = 0.689$ and $P < 0.001$ by linear regression analysis) (Fig. 1). A cutoff for seropositivity in the ELISA was defined on the basis of the mean level of reactivity of uninfected control sera with the purified cytotoxin; an OD of ≥3 standard deviations above this value

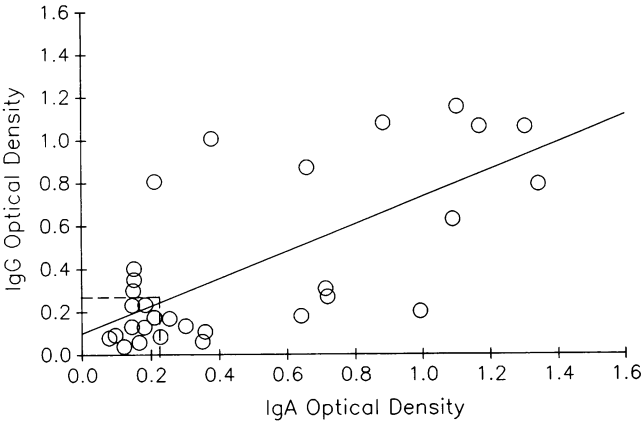

FIG. 1. Relationship between IgG and IgA serum antibody responses to the *H. pylori* vacuolating cytotoxin among *H. pylori*-infected persons. Sera from 30 *H. pylori*-infected patients were diluted 1:100 and tested for IgG and IgA reactivity with the purified 87-kDa vacuolating toxin in an ELISA. The results represent the means of at least three determinations. Cutoffs for seropositivity, based upon the testing of 20 serum samples from uninfected controls, are indicated by dashed lines. There was a significant relationship between IgG and IgA serologic responses to the cytotoxin ($r = 0.689$ and $P < 0.001$ by linear regression analysis).

was considered positive. On the basis of this cutoff, 14 (47%) of the 30 serum samples from *H. pylori*-infected persons contained IgG antitoxin antibodies, and 17 (57%) of the 30 contained IgA antibodies to the toxin.

Correlation between vacuolating cytotoxin production in vitro and serologic responses to the toxin. We next sought to determine whether infection with a Tox$^+$ *H. pylori* strain was associated with the presence of serum IgG and IgA responses to the cytotoxin. *H. pylori* isolates were classified as Tox$^+$ ($n = 14$) or Tox$^-$ ($n = 16$) on the basis of the activity of the culture supernatants in the HeLa cell assay. Sera from patients infected with Tox$^+$ strains reacted with the purified toxin in the IgG ELISA to a significantly greater extent than sera from patients infected with Tox$^-$ strains (OD = 0.603 ± 0.11 versus 0.234 ± 0.07, $P = 0.005$) (Fig. 2A). In an alternate analysis, there was also a significant correlation between cytotoxin production in vitro, as quantitated in the antigen detection ELISA, and IgG serum ELISA ODs ($P = 0.01$, $r = 0.47$). However, when the Tox$^-$ strains were excluded from the analysis, there was no significant relationship between the magnitude of cytotoxin production by the 14 Tox$^+$ strains in vitro and the intensity of the serum IgG antibody responses ($P = 0.27$, $r = 0.32$). On the basis of the cutoff defined with sera from uninfected persons, 11 (79%) of the 14 serum samples from patients infected with Tox$^+$ strains were positive for antitoxin IgG versus 3 (19%) of 16 serum samples from patients infected with Tox$^-$ strains ($P = 0.003$, Fisher's exact test). In two of the three cases in which antitoxin IgG accompanied infection with an apparently Tox$^-$ strain, the isolates were *cagA*$^+$. In the IgA ELISA, sera from patients infected with Tox$^+$ strains also reacted with the purified cytotoxin to a greater extent than did sera from patients infected with Tox$^-$ strains (0.644 ± 0.12 versus 0.341 ± 0.08, $P = 0.04$) (Fig. 2B). There was a trend toward higher rates of antitoxin IgA seropositivity among patients infected with Tox$^+$ strains than among patients infected with Tox$^-$ strains (10 [71%] of 14 versus 7 [44%] of 16, $P = 0.12$). Overall, serum IgG antitoxin antibodies

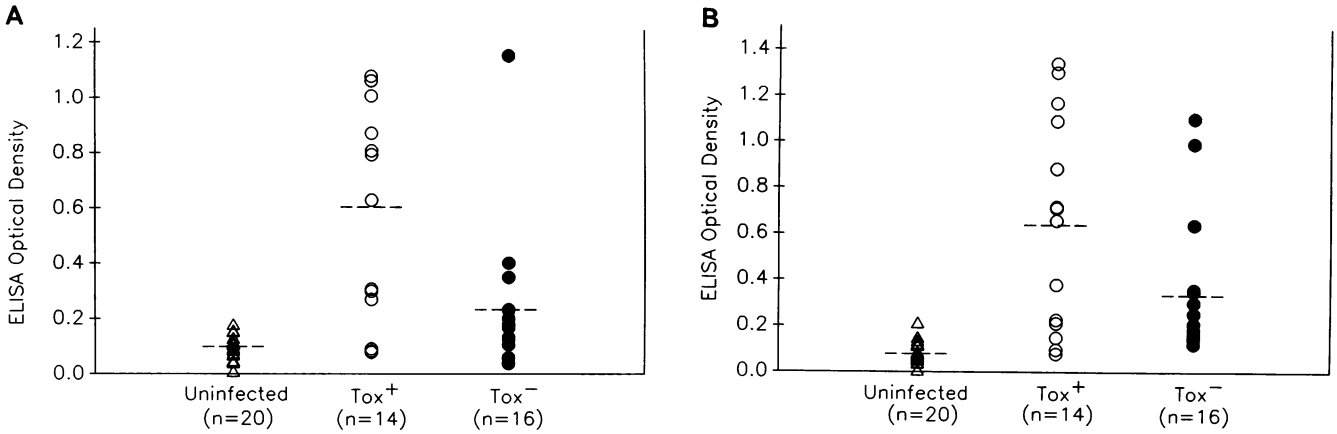

FIG. 2. Correlation between vacuolating cytotoxin production by *H. pylori* isolates in vitro and serologic responses to the cytotoxin. Sera from 30 *H. pylori*-infected patients and 20 uninfected controls were diluted 1:100 and tested for reactivity with the purified 87-kDa vacuolating cytotoxin in an ELISA. The results represent the means of at least three determinations. Mean ODs for each group of sera, are indicated by horizontal dashed lines. IgG (A) and IgA (B) responses to the cytotoxin were greater among patients infected with Tox^+ strains than among patients infected with Tox^- strains (ODs of 0.603 ± 0.11 versus 0.234 ± 0.07 [$P = 0.005$] and 0.644 ± 0.12 versus 0.341 ± 0.08 [$P = 0.04$], respectively).

correlated better than IgA with the cytotoxin phenotype of the infecting strain.

As a control, the 30 serum samples from *H. pylori*-infected patients were also tested for reactivity with pooled sonicated *H. pylori* antigens, as described previously (19). Sera from patients infected with Tox^+ strains and patients infected with Tox^- strains did not differ significantly in reactivity with these antigens (OD = 0.784 ± 0.09 versus 0.896 ± 0.06, $P = 0.31$). Thus, the difference in antibody responses between these groups of sera was specific for *H. pylori* cytotoxin. In addition, this experiment demonstrated that one of the three patients who were infected with a Tox^+ strain but who lacked IgG antibodies to the cytotoxin also lacked IgG antibodies to the pool of sonicated *H. pylori* antigens.

Histologic correlates of vacuolating cytotoxin production. Crabtree et al. have reported that local antibody responses to the cytotoxin-associated 128-kDa protein are associated with increased antral polymorphonuclear leukocyte inflammation scores (11). Therefore, we sought to test the hypothesis that infection with a Tox^+ *H. pylori* strain is associated with antral polymorphonuclear inflammation. We compared the histologies of antral biopsy specimens from 9 patients infected with Tox^+ strains and specimens from 13 patients infected with Tox^- strains. There was a trend toward higher acute (polymorphonuclear) inflammation scores among patients infected with a Tox^+ strain than among those infected with a Tox^- strain (1.83 ± 0.31 versus 1.15 ± 0.21, $P = 0.04$, one-tailed independent t test). There were no differences between the two groups in chronic mucosal inflammation, glandular atrophy, intestinal metaplasia, mucin depletion, epithelial degeneration, epithelial vacuolation, erosion, or foveolar hyperplasia scores. As reported in previous studies (15, 16, 22), patients in this study with duodenal ulcer disease or a history of duodenal ulcer disease (on the basis of the presence of a Billroth I anastomosis) were infected with cytotoxin-producing strains more commonly than patients with nonulcer dyspepsia (5 [100%] of 5 versus 6 [32%] of 19, $P = 0.02$).

DISCUSSION

Using cell culture and antigen detection methodologies, several studies have indicated that there is considerable strain-to-strain variation in the quantity or activity of vacuolating cytotoxin produced or secreted by *H. pylori* isolates (7, 15, 18). In the present study, cytotoxin activity was undetectable in the tissue culture assay for 53% of *H. pylori* isolates, and there were ≥32-fold differences in the titers of cytotoxin activity detectable in supernatants from Tox^+ strains. To determine whether the heterogeneity in cytotoxin production by *H. pylori* isolates in vitro is relevant in infected persons, we used serologic responses to the cytotoxin as an indicator of cytotoxin production in vivo. Thus, we tested the hypothesis that infection with Tox^+ *H. pylori* strains is associated with the presence of serum antitoxin antibodies. The results of this study indicate that IgG antitoxin antibodies are present in sera from patients infected with Tox^+ *H. pylori* strains significantly more frequently than in sera from patients infected with Tox^- strains. These data indicate that there is heterogeneity among *H. pylori* isolates with respect to cytotoxin production in vivo as well as in vitro. It will be useful in future studies to extend these observations by immunohistochemical staining of gastric biopsy specimens.

As expected, a large proportion (79%) of the patients in this study infected with Tox^+ *H. pylori* strains mounted an IgG antibody response to the vacuolating cytotoxin. In previous studies, we have demonstrated a strong correlation between the presence of IgG antitoxin serum antibodies and cytotoxin-neutralizing activity (6). The presence of serum IgG antitoxin antibodies in 3 (19%) of the 16 patients infected with apparently Tox^- *H. pylori* strains may reflect simultaneous infection with both Tox^+ and Tox^- *H. pylori* strains; however, infection with multiple strains is reported to be uncommon (20). An alternate explanation is that these three patients may have been infected with *H. pylori* strains that produced levels of cytotoxin below the threshold of detection in the in vitro cell culture assay but sufficient to elicit an antibody response in vivo. The presence of *cagA* (cytotoxin-

associated gene) in two of these three isolates supports this hypothesis. In addition, the ELISA data in this study indicate that there is overlap between the reactivity of anti-87-kDa protein serum with Tox^- and some Tox^+ *H. pylori* supernatants (Table 1); this suggests that some apparently Tox^- strains may produce small quantities of cytotoxin. Also consistent with this hypothesis is the observation that a DNA probe for the cytotoxin gene (*vacA*) hybridizes with all *H. pylori* strains studied to date, including both Tox^+ and Tox^- isolates (3a).

Although the number of gastric antral biopsy specimens examined in this study was small, the results suggest that infection with Tox^+ strains is associated with increased antral polymorphonuclear inflammation scores. In a study correlating serologic responses to the cytotoxin with gastric histology, a similar result was also observed (18a). Moreover, serologic responses to the 128-kDa CagA (cytotoxin-associated) protein have been associated with increased polymorphonuclear infiltration of the gastric mucosa (11). Taken together, these data suggest that Tox^+ (and $CagA^+$) *H. pylori* strains may synthesize products in the gastric environment that either are not produced or are produced in decreased quantities by Tox^- (and $CagA^-$) strains. We speculate that the prominent polymorphonuclear inflammation associated with Tox^+ $cagA^+$ *H. pylori* infection may be related to the increased incidence of peptic ulcer disease among patients infected with Tox^+ $cagA^+$ strains (1, 7, 11, 15, 16, 22).

ACKNOWLEDGMENTS

This work was supported in part by NIH R29 DK45293-01 and the Medical Research Service of the Department of Veterans Affairs. We thank Beverly Hosse for technical assistance.

REFERENCES

1. **Blaser, M. J.** 1992. Hypotheses on the pathogenesis and natural history of *Helicobacter pylori*-induced inflammation. Gastroenterology **102:**720–727.
2. **Correa, P.** 1992. Human gastric carcinogenesis: a multistep and multifactorial process—first American Cancer Society Award Lecture on cancer epidemiology and prevention. Cancer Res. **52:**6735–6740.
3. **Covacci, A., S. Censini, M. Bugnoli, R. Petracca, D. Burroni, G. Macchia, A. Massone, E. Papini, Z. Xiang, N. Figura, and R. Rappuoli.** 1993. Molecular characterization of the 128 kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. Proc. Natl. Acad. Sci. USA **90:**5791–5795.

3a. **Cover, T. L., M. K. R. Tummuru, and M. J. Blaser.** 1993. Conservation of genetic sequences for the vacuolating toxin among all *Helicobacter pylori* strains. Acta Gastro-Enterol. Belg. **56**(Suppl.):47.

4. **Cover, T. L., and M. J. Blaser.** 1992. *Helicobacter pylori* and gastroduodenal disease. Annu. Rev. Med. **43:**135–145.
5. **Cover, T. L., and M. J. Blaser.** 1992. Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. J. Biol. Chem. **267:**10570–10575.
6. **Cover, T. L., P. Cao, U. K. Murthy, M. S. Sipple, and M. J. Blaser.** 1992. Serum neutralizing antibody response to the vacuolating cytotoxin of *Helicobacter pylori*. J. Clin. Invest. **90:**913–918.
7. **Cover, T. L., C. P. Dooley, and M. J. Blaser.** 1990. Characterization of and human serologic response to proteins in *Helicobacter pylori* broth culture supernatants with vacuolizing cytotoxin activity. Infect. Immun. **58:**603–610.
8. **Cover, T. L., W. Puryear, G. I. Pérez-Pérez, and M. J. Blaser.** 1991. Effect of urease on HeLa cell vacuolation induced by *Helicobacter pylori* cytotoxin. Infect. Immun. **59:**1264–1270.
9. **Cover, T. L., S. G. Vaughn, P. Cao, and M. J. Blaser.** 1992. Potentiation of *Helicobacter pylori* vacuolating toxin activity by nicotine and other weak bases. J. Infect. Dis. **166:**1073–1078.
10. **Crabtree, J. E., N. Figura, J. D. Taylor, M. Bugnoli, D. Armellini, and D. S. Tompkins.** 1992. Expression of 120 kilodalton protein and cytotoxicity in *Helicobacter pylori*. J. Clin. Pathol. **45:**733–734.
11. **Crabtree, J. E., J. D. Taylor, J. I. Wyatt, R. V. Heatley, T. M. Shallcross, D. S. Tompkins, and B. J. Rathbone.** 1991. Mucosal IgA recognition of *Helicobacter pylori* 120 kDa protein, peptic ulceration, and gastric pathology. Lancet **338:**332–335.
12. **Dick, J. D.** 1990. *Helicobacter* (*Campylobacter*) *pylori*: a new twist to an old disease. Annu. Rev. Microbiol. **44:**249–269.
13. **Dixon, M. F.** 1991. *Helicobacter pylori* and peptic ulceration: histopathological aspects. J. Gastroenterol. Hepatol. **6:**125–130.
14. **Dooley, C. P., H. Cohen, P. L. Fitzgibbons, M. Bauer, M. D. Appleman, G. I. Pérez-Pérez, and M. J. Blaser.** 1989. Prevalence of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons. N. Engl. J. Med. **321:**1562–1566.
15. **Figura, N., P. Guglielmetti, A. Rossolini, A. Barberi, G. Cusi, R. A. Musmanno, M. Russi, and S. Quaranta.** 1989. Cytotoxin production by *Campylobacter pylori* strains isolated from patients with peptic ulcers and from patients with chronic gastritis only. J. Clin. Microbiol. **27:**225–226.
16. **Goosens, H., Y. Glupczynski, A. Burette, J.-P. Lambert, L. Vlaes, and J.-P. Butzler.** 1992. Role of the vacuolating toxin from *Helicobacter pylori* in the pathogenesis of duodenal and gastric ulcer. Med. Microbiol. Lett. **1:**153–159.
17. **Hentschel, E., G. Brandstatter, B. Dragosics, A. M. Hirschl, H. Nemec, K. Schutze, M. Taufer, and H. Wurzer.** 1993. Effect of ranitidine and amoxicillin plus metronidazole on the eradication of *Helicobacter pylori* and the recurrence of duodenal ulcer. N. Engl. J. Med. **328:**308–312.
18. **Leunk, R. D., P. T. Johnson, B. C. David, W. G. Kraft, and D. R. Morgan.** 1988. Cytotoxic activity in broth-culture filtrates of *Campylobacter pylori*. J. Med. Microbiol. **26:**93–99.

18a. **Pérez-Pérez, G. I., P. Correa, B. Ruiz, T. L. Cover, and M. J. Blaser.** 1993. Correlation, between tissue and humoral responses to *Helicobacter pylori* infection. Acta Gastro-Enterol. Belg. **56**(Suppl.):52.

19. **Pérez-Pérez, G. I., B. M. Dworkin, J. E. Chodos, and M. J. Blaser.** 1988. *Campylobacter pylori* antibodies in humans. Ann. Intern. Med. **109:**11–17.
20. **Prewett, E. J., J. Bickley, R. J. Owen, and R. E. Pounder.** 1992. DNA patterns of *Helicobacter pylori* isolated from gastric antrum, body, and duodenum. Gastroenterology **102:**829–833.
21. **Price, A. B.** 1991. The Sydney system: histological division. J. Gastroenterol. Hepatol. **6:**209–222.
22. **Tee, W., J. R. Lambert, M. Pegorer, and B. Dwyer.** 1993. Cytotoxin production by *Helicobacter pylori* more common in peptic ulcer disease. Gastroenterology **104:**A789.
23. **Tummuru, M. K. R., T. L. Cover, and M. J. Blaser.** 1993. Cloning and expression of a high-molecular-mass major antigen of *Helicobacter pylori*: evidence of linkage to cytotoxin production. Infect. Immun. **61:**1799–1809.