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Cytotoxin Production by *Campylobacter pylori* Strains Isolated from Patients with Peptic Ulcers and from Patients with Chronic Gastritis Only

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A total of 66.6% of *Campylobacter pylori* strains isolated from patients with peptic ulcers produced a cytotoxin active against mammalian cells in vitro, versus 30.1% of strains isolated from patients with chronic gastritis of various degrees of severity only. This difference was statistically significant and suggests that the toxic substance could be involved in the development of peptic ulcers.

Campylobacter pylori is associated with chronic gastritis and peptic ulcers (1). The pathogenicity characteristics of the organism are not well known. *C. pylori* strains can degrade gastric mucin (3) and produce a cytotoxin whose detrimental effect on the mucosa is still to be proved (2).

To see whether the toxic activity of *C. pylori* could be associated with ulcerative lesions of gastroduodenal mucosa, we compared the frequencies of cytotoxin production by campylobacter strains isolated from patients with peptic ulcers or chronic gastritis only.

We examined 180 patients who underwent diagnostic gastroduodenal endoscopy and who were not receiving treatment with drugs potentially active against *C. pylori*. We obtained from each patient five biopsies from the gastric antrum and the edges of ulcers (when present). One biopsy sample was examined histologically with hematoxylin-eosin stain. The severity of gastritis was assessed by the degree of round and polymorphonuclear cell infiltrates and the presence of erosions. The other biopsy samples were cultured on Columbia agar with 7% horse blood and the Skirrow mixture of antibiotics, smeared, and tested for rapid urease activity. Smears were stained with Gram stain and acridine orange. Suspected colonies on plates were identified as *C. pylori* if organisms were slender or spiral gram-negative rods; oxidase, catalase, urease, and DNase positive; hippurate negative; susceptible to cephalothin; and resistant to nalidixic acid (30-μg disks). Strains were stored at −70°C in Wilkins-Chalgren broth with 20% glycerol. For the cytotoxin production test, strains were thawed and streaked onto Columbia blood agar. Brucella broth with 10% fetal calf serum was inoculated and incubated at 37°C in a microaerophilic atmosphere at 150 oscillations per min for 48 h. Broth culture filtrates (0.22-μm-pore filters) of strains were added to CHO, Vero and, in 61 cases, HeLa cells in vitro at dilutions of 1:2, 1:4, 1:10, and 1:20. Uninoculated broth served as a negative control, and a culture filtrate of *C. pylori* CCUG 17874 served as a positive control. Cells were examined after 24 and 48 h of incubation for the presence of the typical toxic effect (intracytoplasmic vacuolization) at one or more dilutions.

C. pylori was associated with peptic ulcers and with moderate to erosive gastritis (Table 1). Of the 125 *C. pylori*

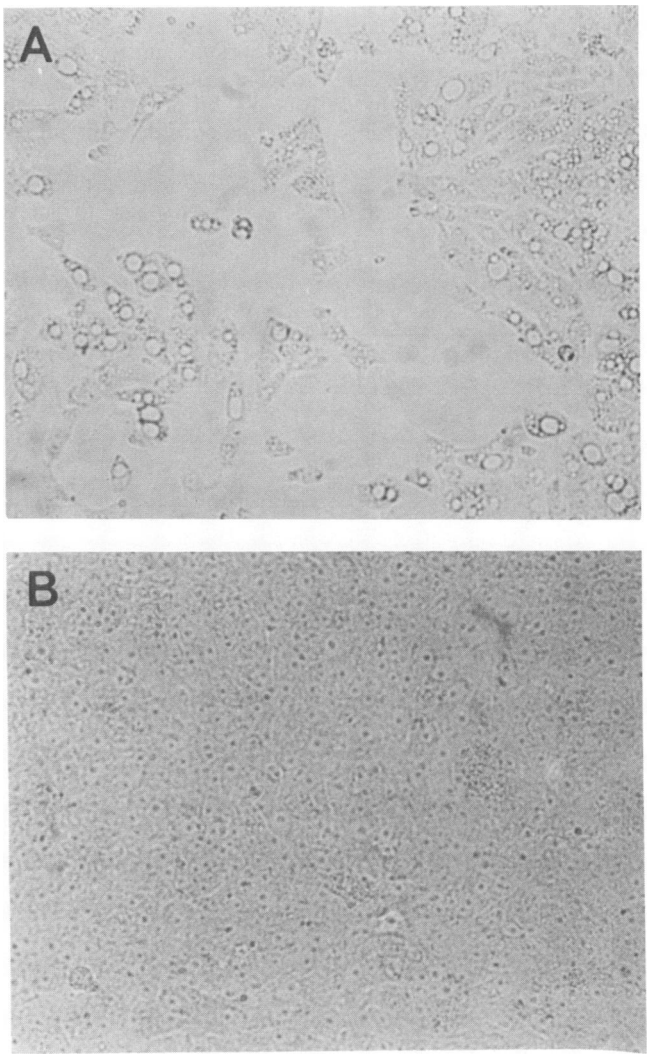

FIG. 1. Cytotoxic effect in Vero cells. (A) Vero cells exposed to cytotoxic *C. pylori* culture filtrate for 24 h. (B) Normal Vero cells.

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TABLE 1. Prevalence of *C. pylori* in 180 adult patients who underwent diagnostic gastroduodenal endoscopy

Diagnosis	No. (%) of patients who were:	
	C. pylori positive	*C. pylori* negative
Ulcer		
Duodenal	22 (100)	0
Gastric	19 (95)	1 (5)
Combined	4	0
Jejunal	0	2
Gastritis		
Mild	8 (47)	9 (53)
Moderate	19 (76)	6 (24)
Severe	34 (87)	5 (13)
Erosive	20 (87)	3 (13)
Atrophic	4 (40)	6 (60)
Adenocarcinoma	0	7
Normal mucosa	0	11
Total	130 (72.2)	50 (27.8)

strains obtained in cultures (in five cases, *C. pylori* was identified by direct microscopy but was not cultured), some strains did not survive after thawing, and others did not produce evident turbidity in brucella broth with fetal calf serum. A total of 77 strains were thus considered in all for the toxigenicity assay.

A total of 32 filtrate samples (41.5%) were cytotoxic for one or more cell lines. CHO cells proved to be the most responsive since they revealed the highest number of positive samples: 28 (36.3%) of the 77 strains tested. Vero cells showed cytopathic effects in 22.0% of the cases examined, and HeLa cells showed cytopathic effects in 21.3% of the cases examined. However, each cell line revealed two cytotoxin-producing strains which were negative on CHO cells. Cytotoxic activity was mostly detected at low filtrate dilutions ($\leq$1:4). At $\geq$1:10 dilutions, 17.8, 17.6, and 15.3% of positive samples produced cytotoxic effects on CHO, Vero, and HeLa cells, respectively. At a 1:20 dilution only one specimen caused vacuolization (on CHO cells). Vero cells were the cell line with which intracytoplasmic vacuolization was most easily visible (Fig. 1).

TABLE 2. Cytotoxigenicity of *C. pylori* strains isolated from 53 patients with chronic gastritis only

Type of gastritis	No. of strains tested	No. (%) of cytotoxic strains
Mild	4	0
Moderate	13	3 (23.0)
Severe	18	6 (33.3)
Erosive	18	7 (38.8)

Of the 77 strains tested for cytotoxin production, 24 were from patients with ulcers (and chronic gastritis) and 53 were from patients without ulcers but with chronic gastritis of various degrees of severity. A total of 16 *C. pylori* strains isolated from patients with ulcers (66.6%) and 16 strains obtained from patients with chronic gastritis only (30.1%) produced a cytotoxic substance. This difference was statistically significant at $P < 0.01$ (by the chi-square method, 1 df) and could indicate that the toxic substance plays a causative role in the development of peptic ulcers. As far as the role of the cytotoxin in the formation of gastritis is concerned, no definite results could be obtained, since the differences reported in Table 2 were not significant.

In conclusion, it is reasonable to suppose that cytotoxigenicity is involved in the development of peptic ulcerations. Studies which could reveal the in vivo production of a toxic substance(s) by *C. pylori* organisms could elucidate this matter.

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