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Role of the *Helicobacter pylori* Virulence Factors Vacuolating Cytotoxin, CagA, and Urease in a Mouse Model of Disease

PAOLO GHIARA,[1]* MARTA MARCHETTI,[1] MARTIN J. BLASER,[2] MURALI K. R. TUMMURU,[2] TIMOTHY L. COVER,[2] ELLYN D. SEGAL,[3] LUCY S. TOMPKINS,[3] AND RINO RAPPUOLI[1]

Immunobiological Research Institute Siena, Chiron Biocine SpA, Siena, Italy[1]; Division of Infectious Diseases, Vanderbilt University, Nashville, Tennessee 37232-2605[2]; and Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, California 94305-5092[3]

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The pathogenic role of *Helicobacter pylori* virulence factors has been studied with a mouse model of gastric disease. BALB/c mice were treated orally with different amounts of sonic extracts of cytotoxic *H. pylori* strains (NCTC 11637, 60190, 84-183, and 87A300 [CagA$^+$/Tox$^+$]). The pathological effects on histological sections of gastric mucosae were assessed and were compared with the effects of treatments with extracts from noncytotoxic strains (G21 and G50 [CagA$^-$/Tox$^-$]) and from strains that express either CagA alone (D931 [CagA$^+$/Tox$^-$]) or the cytotoxin alone (G104 [CagA$^-$/Tox$^+$]). The treatment with extracts from cytotoxic strains induced various epithelial lesions (vacuolation, erosions, and ulcerations), recruitment of inflammatory cells in the lamina propria, and a marked reduction of the mucin layer. Extracts of noncytotoxic strains induced mucin depletion but no other significant pathology. Crude extracts of strain D931, expressing CagA alone, caused only mild infiltration of inflammatory cells, whereas extracts of strain G104, expressing cytotoxin alone, induced extensive epithelial damage but little inflammatory reaction. Loss of the mucin layer was not associated with a cytotoxic phenotype, since this loss was observed in mice treated with crude extracts of all strains. The pathogenic roles of CagA, cytotoxin, and urease were further assessed by using extracts of mutant strains of *H. pylori* defective in the expression of each of these virulence factors. The results obtained suggest that (i) urease activity does not play a significant role in inducing the observed gastric damage, (ii) cytotoxin has an important role in the induction of gastric epithelial cell lesions but not in eliciting inflammation, and (iii) other components present in strains which carry the *cagA* gene, but distinct from CagA itself, are involved in eliciting the inflammatory response.

Infection of the human gastric mucosa by the gram-negative spiral bacterium *Helicobacter pylori* is associated with gastritis, peptic ulcer disease, and gastric cancer (2, 3). Although it is expensive, successful eradication of the infection obtained by treatment with antimicrobial agents, markedly alters the natural history of peptic ulceration. However, the occurrence of resistant bacterial strains may limit the effectiveness of this therapeutic approach. Because of its lower cost and historic success in the fight against infectious diseases, vaccination is a most promising strategy to combat *H. pylori* infection.

The best candidates for such a vaccine might include relevant virulence factors. *H. pylori* produces several factors that are related to the establishment and maintenance of infection. All *H. pylori* strains express a potent urease, flagella, and various adhesins, which are thought to be important for survival in the gastric environment and for tissue-specific colonization (15, 30). A subset of strains also possesses a surface-exposed immunodominant antigen (CagA), which is encoded by *cagA* (4, 34), and expresses a cytotoxin, which is encoded by *vacA* (5, 22, 25, 32), that induces cellular vacuolation in a number of different epithelial cell lines in vitro. Thus, *H. pylori* strains can be subdivided into at least two groups which either do (type I) or do not (type II) express CagA and the cytotoxin; in addition, some type I strains possess *cagA* but lack cytotoxin activity, and others lack *cagA* but express cytotoxin activity (37).

Of key importance in the development of a vaccine against *H. pylori* is the availability of suitable animal models to test relevant virulence factors for the assessment of their role in the pathogenesis and eventually to evaluate their ability to induce protective immunity for their consideration as candidate vaccine components. Presently available animal models consist of either infection models or disease models. Experimental infection by *H. pylori* of animals such as nude mice (14), primates (9), gnotobiotic pigs (10), and dogs (23) requires special conditions that may not be easily available for all laboratories. Infection of smaller hosts with other *Helicobacter* species, such as colonization of mice with *Helicobacter felis* (16) or of ferrets with *Helicobacter mustelae* (12), has allowed initial understanding of pathogenesis but does not provide information on the role of the pathogenic determinants that are specific for *H. pylori* (30).

Animal models of *H. pylori*-induced acute disease have also been reported. A rat model of chemically induced gastric ulcerogenesis has been described elsewhere (24), in which ulcer healing was affected by the presence in the stomach of either intact *H. pylori* cells or bacterial filtrates. Karita et al. (14) reported that oral administration of an *H. pylori* bacterial filtrate, containing 2% fetal calf serum, induced gastric and duodenal injury in mice. We recently reported that oral treatments of BALB/c mice with either crude extracts of *H. pylori* or the purified cytotoxin induce gastric injury that resembles the pathology observed in humans (32).

In the present study, we further investigated the direct effect on murine gastroduodenal mucosae of oral treatments with crude sonic extracts obtained from different phenotypic variant strains of *H. pylori*. We have used this model to explore the pathogenic role of the major virulence factors of *H. pylori.*

* Corresponding author. Mailing address: Department of Immunology, Immunobiological Research Institute Siena, Chiron Biocine SpA, Via Fiorentina 1, 53100 Siena, Italy. Phone: 39-577-243316. Fax: 39-577-243564. Electronic mail address: GHIARA@IRIS02.Biocine.It.

TABLE 1. Genotypic and phenotypic properties of *H. pylori* strains used in this study[a]

Strain	*cagA*		*vacA* gene	Cytotoxin expression	Urease expression
	Gene	Protein			
NCTC 11637	+	+	+	+	+
60190	+	+	+	+	+
60190:v1	+	+	+[b]	−	+
60190:M22	+[b]	−	+	+	+
84-183	+	+	+	+	+
84-183:v1	+	+	+[b]	−	+
84-183:M22	+[b]	−	+	+	+
94-46	+[b]	−	+[c]	−	+
87A300	+	+	+	+	+
Ure1	+	+	+	+	+[d]
G21	−	−	+	−	+
G50	−	−	+	−	+
D931	+	+	+	−	+
G104	−	−	+	+	+

[a] The presence of genes was assessed by Southern blots; protein expression was determined by Western blots. The expression of vacuolating cytotoxin was also assessed by cell culture assays (37).
[b] Disrupted by insertion of a kanamycin resistance gene (5, 34).
[c] Disrupted by insertion of a chloramphenicol resistance gene.
[d] Mutated strain that expresses an inactive urease (26).

MATERIALS AND METHODS

Mice. Male 4- to 6-week-old BALB/c mice were purchased from Charles River (Calco, Italy) and were housed in our animal facilities in air-conditioned rooms on a 12-h light-dark schedule (illumination from 7:00 a.m. to 7:00 p.m.); standard food and water were available ad libitum.

***H. pylori* strains.** *H. pylori* type strain NCTC 11637 ($CagA^+/Tox^+$) was obtained from the University of Goteborg. Strains 60190 and 84-183 ($CagA^+/Tox^+$) and isogenic mutants derived by insertional disruptions of either the *vacA* or *cagA* gene have been previously described (5, 35). Strain 94-46 carrying insertional mutations of both *vacA* and *cagA* was obtained by transforming pCTB8:*cm* (a disrupted *vacA* gene generated by insertion of a *Campylobacter coli* chloramphenicol resistance gene [36] in the unique *Eco*RI site of plasmid pCTB8) into the isogenic *cagA* strain 84-183:M22. Strain 87A300 and its urease-negative mutant strain Ure1, which were obtained by electron microscopy-induced mutagenesis, have been previously described (26). Strains D931 ($CagA^+/Tox^-$), G104 ($CagA^-/Tox^+$), and G21 and G50 ($CagA^-/Tox^-$) are wild-type, laboratory-passaged clinical isolates and have been described previously (37). Phenotype and genotype characterizations of the variant wild-type strains of *H. pylori* have been described by Z. Xiang and A. Covacci (37). *Escherichia coli* TG1 is an $EcoK^-$ derivative of strain JM101. Table 1 summarizes the genotypes and phenotypes of the *H. pylori* strains used in this study. All strains were grown on blood agar plates at 37°C in a 5% CO_2 humidified atmosphere, as previously described (37). Bacteria were harvested from the plates by using a sterile cotton swab and were suspended in 5 ml of sterile saline at about 10^9 bacteria per ml. The suspension then was pulse-sonicated three times for 30 s with a Branson Sonifier 450 (Branson Ultrasonics, Danbury, Conn.) set at 50% capacity which was immersed in a melting-ice bath with pauses of 15 s between each pulse-sonication cycle. This bacterial lysate then was centrifuged at 20,000 × *g* for 30 min at 4°C, and the supernatant was frozen in liquid nitrogen and maintained at −80°C until use. Protein concentrations of the crude extracts were determined with the Bradford reagent (Bio-Rad) by using bovine gamma globulin as the standard. Usually, 1 mg of protein in the sonic extracts corresponded to about 10^9 bacteria. In each case, the presence of CagA, the vacuolating cytotoxin, and urease in the sonic extracts was confirmed by Western blot (immunoblot) analysis with specific rabbit antisera. The bioactivity of cytotoxin in the extracts was detected in vitro by the capacity to induce vacuolation of HeLa cells (21).

Mouse inoculations. Mice (three to six per group) were fasted overnight before each oral treatment and before sacrifice but could have free access to water. Treatments were always performed at the same time, usually between 9:00 and 11:00 a.m. Every 48 h, through a sterile flexible gastric gavage, the mice received an oral inoculum of 0.25 ml of saline alone or containing different amounts of crude extracts from *H. pylori* strains. The mice were allowed to have free access to food and water 2 h after the treatment. The mice were sacrificed 48 h after each oral administration, and their stomachs were removed. The forestomach, containing nonsecreting epithelium, was eliminated and the remaining organ containing oxyntic, antral, and duodenal mucosae was opened with scissors along the lesser curvature, fixed overnight with 4% buffered formalin, dehydrated, and embedded in paraffin. Serial sections (5 to 7 μm) were cut such that an average tissue thickness of at least 500 μm could be examined. Tissues were oriented to examine oxyntic, antral, and duodenal mucosae in the same section.

Tissue staining. After rehydration, the sections were stained with hematoxylin and eosin or with periodic acid-Schiff's (PAS) reagent and were counterstained with hematoxylin. After dehydration and mounting, the sections were observed by an individual blinded to the treatments, and 10 random 200× fields per section were scored on the basis of the extent of the histopathological lesions observed. Both inflammatory reactions and epithelial lesions were separately assessed by a modification of previously published methods (11, 13). Epithelial damage scoring (EDS) was performed according to the following scale: 1 = no lesion; 2 = disarray of columnar cells and vacuolation; 3 = diffuse microerosions and epithelium disaggregation; 4 = erosive lesion, denudation of basal membrane, and ulceration. With the inflammation score (IS), the amounts of inflammatory cells were graded as follows: 1 = none visible; 2 = scattered mononuclear and polymorphonuclear cells in the lamina propria and submucosa; 3 = definite increase in subepithelial areas of lamina propria; 4 = marked infiltration of lamina propria and focal abscesses. The mean EDS and IS of triplicate sections per mouse then were calculated, and results are expressed as means ± standard errors of the means (SEM) for three to six mice per group.

Quantitation of mucin layer. To assess the thickness of the mucin layer overlying the gastric epithelium, triplicate sections per mouse including oxyntic, antral, and duodenal mucosae were stained with PAS reagent and counterstained with hematoxylin. Quantitation of the mucin layer present was then accomplished by using an image analysis software program (VIDAS; Kontron, Munich, Germany) that allowed the calculation of areas (in square micrometers) of PAS positivity of 200× fields observed through a ZVS-47E CCD video camera (Optronics Engineering, Goleta, Calif.) connected to the microscope (Axiophot; C. Zeiss). Total PAS-positive areas on triplicate sections per mouse were then determined, and the results are expressed as means ± SEM (in square micrometers) for three mice per group.

RESULTS

Induction of gastric damage by sonic extracts of cytotoxic strains of *H. pylori*. Mice were treated orally with 0.25 ml of saline alone or saline containing 100 μg of protein of the sonic extract from the cytotoxic NCTC 11637 strain, and the treatment was repeated every 48 h for as many as eight times. At time zero and 48 h after each treatment, a group of three to six mice was killed, and each stomach was processed for histology as described and the examined sections were scored for the extent of gastric damage induced by these treatments. A small induction of gastric damage was already apparent after one treatment; gastric damage reached its highest level after two treatments and did not significantly change with subsequent treatments (not shown). Mice treated with saline alone did not show any significant pathology (not shown). For all of the subsequent experiments, the treatment thus consisted of only two oral administrations of sonic extracts, 48 h apart, and histopathological changes were determined 48 h after the last treatment.

Then mice were treated twice orally with saline alone or with saline containing from 10 to 5,000 μg of crude extract from strain NCTC 11637. A dose of 10 μg of the extract did not induce any significant change, but a dose of 50 μg per mouse was able to induce a significant increase in EDS only. The maximal amount of gastric damage was obtained with doses of 500 μg per mouse (not shown).

Figure 1 illustrates gastric mucosae from a control mouse treated with saline alone and from a mouse treated with 500 μg of sonic extract from strain NCTC 11637. For the mouse treated with sonic extract, infiltration of inflammatory cells at the base of the gastric glands and epithelial erosions are evident. Infiltrated cells were mainly of the mononuclear type, although some polymorphonuclear leukocytes were also found. In some sections, it also was possible to observe epithelial ulcerations and marked infiltration of inflammatory cells (Fig. 1). Similar results were obtained when the same amounts of sonic extracts obtained from other cytotoxic strains (87A300, 60190, and 84-183) were administered to mice (data not shown).

By means of PAS staining, we also observed a marked loss of

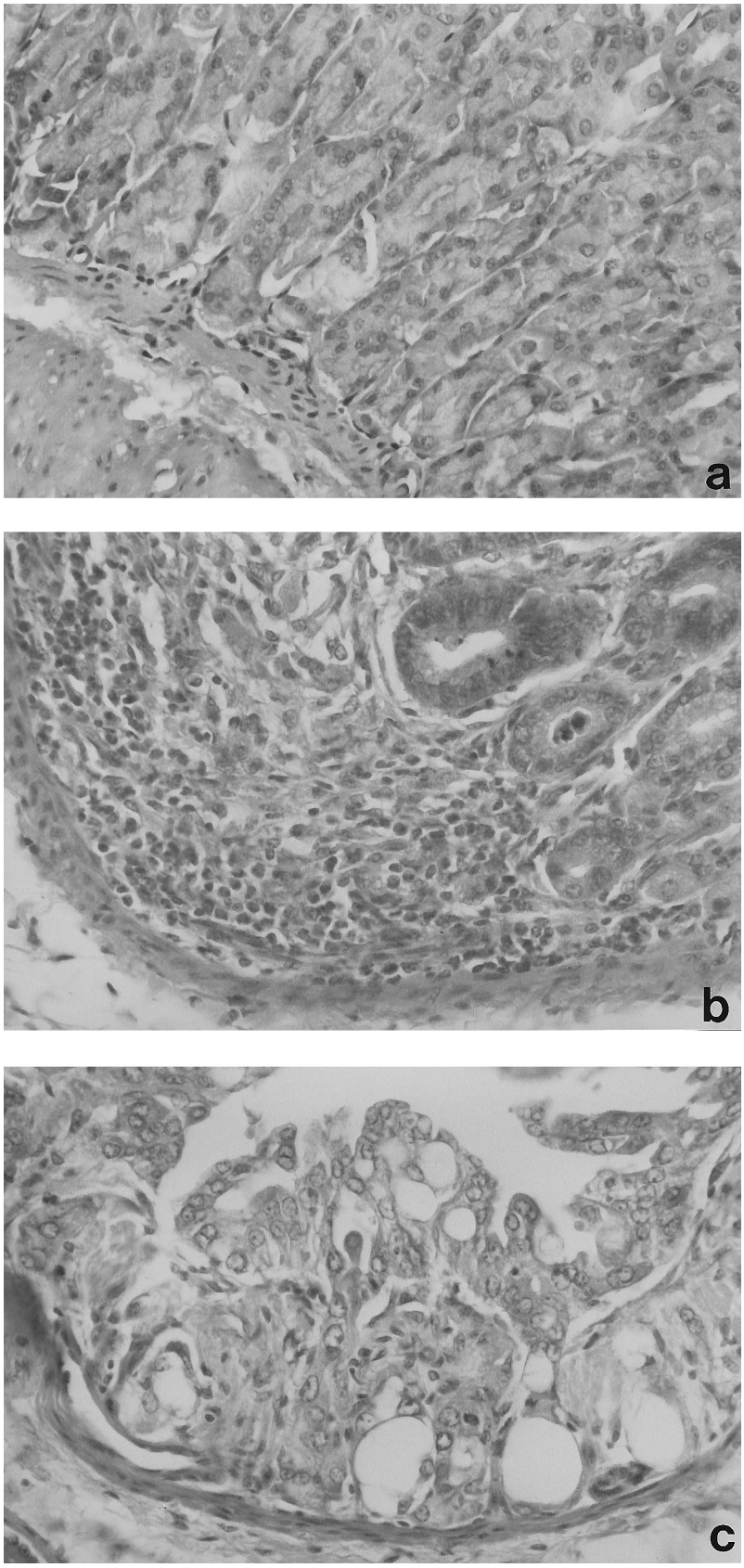

FIG. 1. Hematoxylin and eosin staining of mouse stomach tissues. (a) Gastric oxyntic mucosa of a control saline-treated mouse; (b and c) gastric mucosa of a mouse treated with 500 μg of a sonic extract of strain NCTC 11637 (original magnification, ×400).

FIG. 2. PAS staining of mouse stomach tissues. (a) Gastric oxyntic mucosa of a saline-treated mouse; (b) gastric mucosa of a mouse treated with 500 μg of a sonic extract from *H. pylori* NCTC 11637 (original magnification, ×400).

mucin after the same treatment. Figure 2a shows the PAS staining of oxyntic mucosa from a saline-treated mouse, and Fig. 2b shows the same mucosal region from a mouse treated with 500 μg of NCTC 11637 sonicate. To further characterize the effect on mucin depletion, the amounts of PAS staining were determined quantitatively for sections obtained from mice treated with saline alone or with saline containing different amounts of crude extract from strain NCTC 11637. The minimal effective dose producing a significant ($P < 0.05$) effect was 100 μg per mouse, and the effect was maximal at 1,000 μg per mouse (Fig. 3).

Comparison of gastric lesions induced by phenotype variant strains of *H. pylori*. To relate the expression of cytotoxic activity and the induction of gastric pathology, mice were treated with 500 μg of sonic extracts of wild-type phenotypic variant strains with different patterns of expression of CagA and/or the cytotoxin. The gastric damage scores obtained are shown in Fig. 4. To rule out possible nonspecific effects of oral treatments with crude gram-negative bacterial extracts, a group of mice was also treated with the same amount of sonic extract of *E. coli* TG1. No significant gastric damage was induced by this crude extract, and the histological sections of the gastric mucosae obtained from these mice were indistinguishable from those obtained from saline-treated mice. Type I strains NCTC 11637, 60190, and 84-183 are reported as positive control strains that induced a high level of gastric damage.

To determine whether the *H. pylori* components that caused gastric damage and inflammation were heat labile, a group of mice was treated with the same amounts of a sonic extract from NCTC 11637 strain that was boiled for 15 min before oral administration. No gastric damage was produced by the heat-treated sonicates, which indicates that heat-stable components such as lipopolysaccharide are not responsible for causing gastric pathology (not shown).

Extracts from the two noncytotoxic type II strains, G21 and G50, induced increases in the EDS and IS values that were not significantly different from those induced by treatment with saline alone.

Treatment with an extract of strain D931, expressing only the CagA protein but not the cytotoxin, induced less gastric injury than did sonic extracts of the cytotoxic strains NCTC 11637, 60190, and 84-183. The damage consisted mainly of induction of inflammatory foci in the lamina propria, with few signs of epithelial erosion. In contrast, sonic extract from strain G104, expressing the cytotoxin protein but not CagA, caused gastric damage mainly consisting of epithelial erosions and ulcerations with limited inflammatory reaction.

The effect of 500 μg of sonic extract from noncytotoxic strain G21 per mouse on the mucin layer was then quantitatively determined and compared with that induced by the NCTC 11637 extract. Sonic extracts from both strains reduced the thickness of mucin to similar extents (not shown). Similar effects on mucin were observed after treatments with all of the other phenotype variant strains studied (not shown). Thus, the effect on the mucin layer induced by the extracts was independent of their effect on EDS and IS values. Treatment with a sonic extract of *E. coli* TG1 or with boiled sonic extract from strain NCTC 11637 did not induce any significant change in the thickness of the mucin layer (not shown).

Pathogenic role of CagA, cytotoxin, and urease. To define the role played by particular virulence factors of *H. pylori* in the induction of the observed pathology, mice were treated with

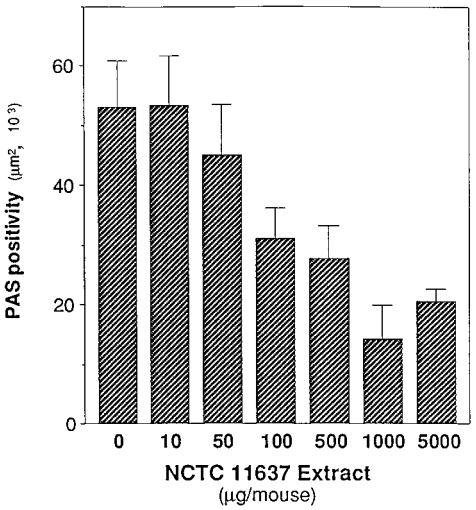

FIG. 3. Quantitation of the dose-dependent effect on PAS positivity. The amounts of PAS positivity in the sections were determined with the aid of an image analysis software package as described in Materials and Methods. Results are expressed as means ± SEM (in square micrometers) of PAS-positive areas obtained from histological sections of three mice per groups as described in Materials and Methods. The results shown are representative of three different experiments with similar results.

sonic extracts obtained from various mutant strains, each lacking the expression of specific virulence factors (Fig. 5). We used saline-treated mice as negative controls and mice treated with sonic extracts of NCTC 11637 as positive controls. Disruption of *vacA* in strain 84-183 (84-183:v1) by insertion of a kanamycin cassette (5) caused a marked decrease in the capability of the strain to induce epithelial erosions and ulcerations; however, gastric inflammatory infiltration was still observed (Fig. 5). In contrast, disruption of *cagA* (84-183:M22) by a similar insertion (35) did not cause any significant change in the capability of the strain to induce gastric damage or inflammation. Parallel results were obtained with strain 60190 and its derivative isogenic mutants lacking CagA or cytotoxin expression (not shown).

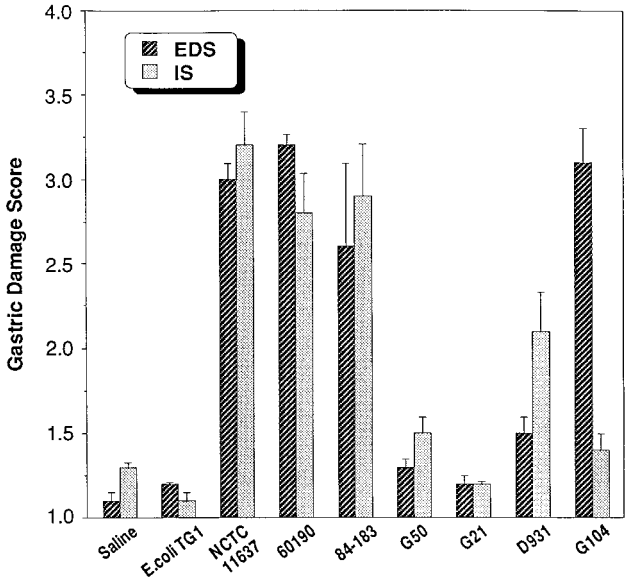

FIG. 4. Gastric damage induced by different phenotype variant strains of *H. pylori*. Mice received two oral treatments of saline alone or of saline containing 500 μg of sonic bacterial extracts. Mice were sacrificed 48 h after the last treatment, and histopathological scoring of the gastric damage was performed. Data are expressed as means ± SEM for six mice per group. The results shown are representative of two different experiments with similar results.

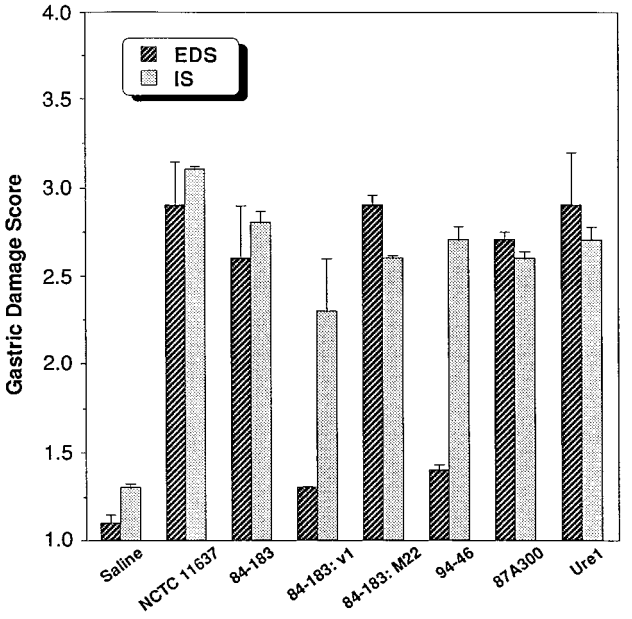

FIG. 5. Gastric damage induced by sonic extracts of mutant strains of *H. pylori*. Mice were treated twice orally with saline alone or saline containing 500 μg of sonic extracts of the specified bacterial strains. Data are expressed as means ± SEM of data obtained from three mice per group. The results shown are representative of three different experiments with similar results.

The gastric damage induced by sonic extracts of strain 94-46, bearing disruptions of both *vacA* and *cagA*, consisted of marked inflammation without epithelial erosions, which is similar to what was observed with the isogenic *vacA* mutant strain lacking only cytotoxin expression.

Finally, mice treated with sonic extracts of strain Ure1, which lacks an active urease but which has normal expression of both CagA and cytotoxin, had gastric damage scores that were essentially identical to those obtained with the parent strain, 87A300.

All of the mutant strains induced mucin depletion that was indistinguishable from that induced by the wild-type parent strains (not shown).

DISCUSSION

H. pylori strains that can be isolated from human gastroduodenal biopsies can be classified in at least two groups, termed type I and type II, on the basis of genotypic and phenotypic differences (31, 37). Type II *H. pylori* strains produce urease, flagella, a number of adhesins, and heat shock proteins, among other constituents. Type I bacteria, in addition to the above properties, bear a highly immunogenic protein called CagA and also express the cytotoxin (encoded by *vacA*) that is involved in vacuole formation in epithelial cells. Type II bacteria also possess *vacA* but fail to express cytotoxin activity in vitro.

Infection of human gastroduodenal mucosae by *H. pylori* results in infiltration of the lamina propria by mononuclear and polymorphonuclear leukocytes. Other common histopathological findings are marked reduction or loss of the mucin layer and epithelial erosive lesions that range from the focal loss of

integrity of a few cells to extensive desquamation of the epithelial cell layer and exposure of the basal membrane to the gastric environment (11, 29, 33).

The development of good animal models is essential for understanding the pathogenesis of *H. pylori* infection and for the development of vaccines.

We have previously reported that oral administration to mice of a sonic extract of the cytotoxic type I strain NCTC 11637 was able to induce acute lesions in the gastric mucosa (32). We have used the same animal model here to further assess the pathogenic role of some of the major virulence factors of *H. pylori*. It has to be pointed out that in this mouse model of acute mucosal injury, the delivery of virulence factors to the gastric mucosa, consisting in pulse-administration of bacterial extracts, is likely much different from the targeted slow release occurring during an infection. However, we could observe a gastric injury that shares some similarity with that observed in patients that are chronically infected by *H. pylori* (11, 29, 33). The histopathological damage induced by this treatment consists of epithelial erosion and ulceration, infiltration of the lamina propria by inflammatory cells, and marked reduction of the PAS-stainable mucin layer (Fig. 1 and 2).

The extent of the observed effects was dependent on the number of treatments; a single dose was able to induce an increase in gastric damage score, but the full response could be achieved with a second treatment repeated after 48 h (data not shown). The finding that repeated administrations did not worsen the degree of gastric damage suggests that the gastric mucosa can adapt to the particular injurious stimuli. The extent of gastric injury was dependent on the dose administered and was maximal with 500 μg of sonicate.

It is calculated that a chronically infected human stomach can carry 10^7 to 10^{10} *H. pylori* cells; in acute infections, the load is probably even higher. In a recently developed mouse model of *H. pylori* infection and disease (19), the range of bacteria recovered from the stomach was 5×10^3 to 2×10^4/100 mg of gastric mucus. In the present study, the minimal active amount of sonicate given orally to mice corresponded to about 5×10^7 bacteria. Although this may seem a high dose for the mouse, it has to be considered that the delivery of virulence factors may be targeted much more efficiently by live bacteria during infection than by the administration of a sonicate.

To assess the role of virulence factors in gastroduodenal damage, we used phenotypic variant strains which either express or do not express CagA and/or vacuolating cytotoxin (37) (Fig. 4). Only sonic extracts of type I bacteria induced severe gastric damage. These results confirm and extend our previous observations (32) and are in agreement with the clinical finding that patients with the most severe gastroduodenal pathologies are infected with type I bacteria (4).

Interestingly, by using the phenotypic variant strains that express either CagA or cytotoxin, we could dissociate the induction of inflammatory infiltration from the induction of epithelial cell derangements. In fact, moderate inflammation with only mild signs of epithelial cell injury was induced by treatment with strain D931, which expresses only CagA and not cytotoxin. On the other hand, sonic extract of strain G104, expressing the cytotoxin but not CagA, could induce marked epithelial injury with minimal inflammatory cell infiltration. It is noteworthy that in a previous study, which employed the same two strains, D931 and G104, the $CagA^+$ phenotype has been associated with in vitro induction on human epithelial cell lines of the chemotactic cytokine interleukin 8 (IL-8) (6). Thus, although the IL-8 gene is not present in mice (1), factors expressed by strains carrying the *cagA* gene may likely be responsible for induction of phagocyte chemotaxis.

All strains were capable of inducing depletion of the mucin layer, indicating that neither the cytotoxic nor the $CagA^+$ phenotype is associated with this phenomenon.

To better characterize the role played by the different virulence factors in the observed gastric pathology, we then used genetically manipulated *H. pylori* strains in which the genes encoding CagA, cytotoxin, or urease have been disrupted or mutated. The histopathological data show that the cytotoxin is directly responsible for most of the gastric epithelial injury induced by the sonic extracts. This result confirms the pivotal role of cytotoxin in the induction of gastric pathology and further extends our previous observation that the oral treatment of mice with purified cytotoxin induces marked gastric epithelial derangement without evident signs of inflammation (32).

The result obtained with phenotypic variant strain D931, expressing only CagA and not cytotoxin, suggested that the induction of inflammation could be associated with the expression of CagA (Fig. 4). However, disruption of the *cagA* gene did not affect the capability of the extracts to cause either inflammation or epithelial damage (Fig. 5). Moreover, both the $CagA^+/Tox^+$ wild-type strains and the $CagA^-/VacA^-$ isogenic mutant strain 94-46 induced greater inflammation than the $CagA^-/Tox^-$ wild-type strains G50 and G21. These results suggest that other factors, distinct from the CagA molecule, that are present in strains carrying the *cagA* gene are responsible for eliciting inflammation in the gastric mucosa. These factors are presumably still expressed even when the *cagA* gene is disrupted. This observation is in agreement with recent data (7, 27) showing similar results regarding the in vitro induction of synthesis of the neutrophil chemotactic cytokine IL-8 (1).

The role of urease in induction of gastric damage has also been assessed. Urease seems to be a good candidate since (i) it is produced in abundance and is released by the bacterium; (ii) in vitro proinflammatory activities have been previously reported (17, 18); (iii) during infection it is actually present in deep tissues (lamina propria), whereas *H. pylori* cells are absent (17); (iv) it has been suggested to play a role in the induction of gastric damage (20, 28); and (v) its synthesis appears to be nutritionally regulated (8).

In the mouse model used here, urease activity is not clearly associated with the induction of either inflammation or epithelial gastric damage. In fact, sonic extract from mutant Ure1 is able to induce gastric damage that is indistinguishable from that obtained with the parent strain 87A300. The mutant Ure1 expresses the same amounts as the wild-type strain of the two urease subunits A and B, but they cannot assemble in an active A_6B_6 configuration (26). Although we cannot rule out the possibility that the unassembled subunits may still cause some effect, this possibility is also very unlikely considering our previous observation that purified active urease, given orally to mice, did not induce any significant histopathological change (32). However, it must be considered that targeted delivery of urease to deep tissues occurs in natural infections with live organisms (17) and likely does not occur with sonicates.

Taken together, the results reported in the present paper have shown that the virulence factors expressed by type I strains are able to cause severe gastric pathology in a small animal model and are consistent with the observation that this subset of *H. pylori* strains is associated with more severe pathology in humans (4, 31). The animal model of *H. pylori*-induced disease described here will be useful to further dissect the pathogenesis of infection by this important human pathogen.

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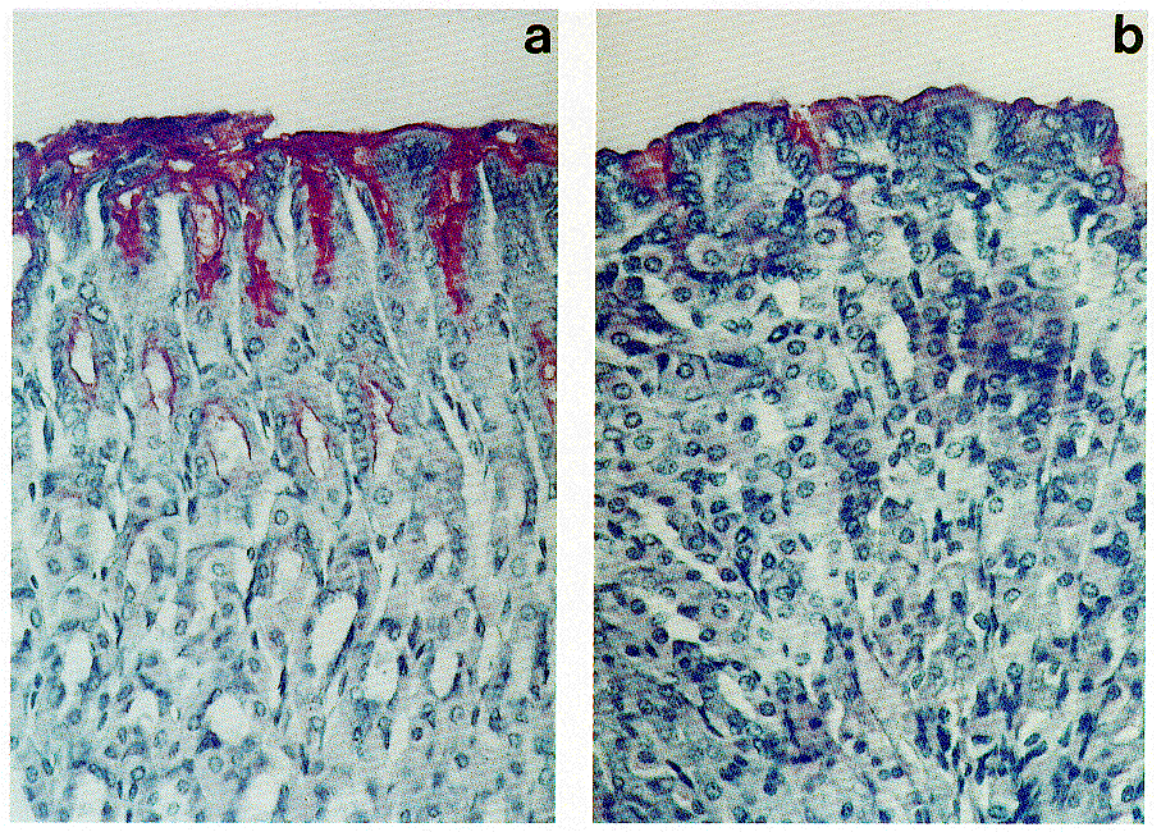
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