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Helicobacter pylori-Associated Gastritis in Mice is Host and Strain Specific

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The *vacA* and *cagA* geno- and phenotypes of two mouse-adapted strains of *Helicobacter pylori*, SS1 and SPM326, were determined. The SS1 strain, which had the *cagA*$^+$ and *vacA* s2-m2 genotype, induced neither vacuole formation in HeLa cells nor interleukin-8 (IL-8) production in KATO III cells. In contrast, *H. pylori* SPM326, with the *cagA*$^+$ and *vacA* s1b-m1 genotype, induced vacuoles as well as IL-8 production in vitro. Furthermore, a spontaneous mutant of SPM326, which produced a vacuolating cytotoxin but was not able to induce IL-8 production (SPM326/IL-8$^-$), was detected. C57Bl/6 and BALB/c mice were infected with these three strains to investigate the colonization pattern and the effect on the immune response in vivo. The SS1 strain colonized the stomachs of all mice in large numbers which remained constant over time. Colonization with the SPM326/IL-8$^+$ and SPM326/IL-8$^-$ strains was lesser, or even absent, and decreased over time. At 5 weeks postinoculation all three *H. pylori* strains induced a mild increase of neutrophil count in the gastric corpus of C57Bl/6 mice, which disappeared by 12 weeks. At both 5 and 12 weeks postinoculation C57Bl/6 mice colonized with SPM326/IL-8$^+$ showed an increased expression of major histocompatibility complex (MHC) class II antigen in the cardia which was accompanied by an increased number of T cells. C57Bl/6 mice that were infected with SS1 and SPM326/IL-8$^-$ did not show chronic inflammation. BALB/c mice colonized with SS1 and SPM326/IL-8$^-$ also showed an increase in neutrophil count at 5 weeks, which normalized again by 12 weeks postinoculation. At this time point SS1-infected mice showed inflammation in the corpus and antrum. At these sites an increased expression of MHC class II antigens and an increased number of T cells were observed. Although small lymphoid follicles were already observed 5 weeks after inoculation with SS1, their incidence as well as their number was increased at 12 weeks. These results show that inflammation induced by *H. pylori* depends both on the bacterial strain and the host.

Helicobacter pylori infection nearly always leads to gastritis in humans. The infection is not cleared by the gastric immune response, and after a prolonged period of time more-severe clinical symptoms, such as peptic ulcer disease, carcinoma, and lymphoma, may develop (4). Several genes of *H. pylori*, such as *vacA* and *cagA*, are associated with this development (5). *vacA*, which encodes a cytotoxin, shows mosaicism in the signal (s) sequence and the middle (m) region of the gene. In addition to the previously described s1a, s1b, and s2 alleles, recently a fourth allele, s1c, was described. The m region is now expanded to three alleles: m1, m2a, and m2b (2, 30). Several combinations of these s and m alleles are present, but s2-m1 is not. The s1-m1 and s1-m2 genotypes are associated with production of a vacuolating cytotoxin, whereas the s2-m2 genotype is considered to be noncytotoxic (2, 20, 23).

The CagA protein is about 128 kDa in size (9), and although the function of CagA is unknown, its association with peptic ulcer disease and carcinoma makes it a useful marker for virulent strains (17, 32). *cagA* is part of the pathogenicity island of *H. pylori* (1, 8). Some of the genes in this island play a role in the induction of interleukin-8 (IL-8) production by epithelial cells (1, 8, 28). IL-8 is a potent chemoattractant and activator of neutrophils and T cells and is considered to be an important contributor to inflammation (22).

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Several *H. pylori* strains are now used in animal models, such as the mouse. However, detailed information on geno- and/or phenotype is often not available for these strains. In this study we determined the *vacA* s and m genotypes of and the expression of an active cytotoxin by two mouse-adapted *H. pylori* strains. Furthermore, the presence of *cagA* was determined, as well as the ability of these *H. pylori* strains to induce IL-8 production in a gastric cell line. C57Bl/6 mice and BALB/c mice have previously been shown to have different inflammatory responses after infection with *Helicobacter felis* (21, 25). Using these two strains of mice we investigated the distribution of the *H. pylori* strains in the stomach and the effects of their colonization on the gastric immune response.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The *H. pylori* strains used in this study were SS1 (18), SPM326 (19), ATCC 43504, and 93-1184 (isolated from a patient with gastritis at the University Hospital of the Vrije Universiteit, Amsterdam, The Netherlands). Bacteria used for cytotoxin and IL-8 assays were grown in brucella broth supplemented with 5% (vol/vol) newborn calf serum (NCS) (Gibco BRL, Paisley, Scotland) at 37°C for 24 h in a microaerobic atmosphere with shaking. The bacteria that were used in animal experiments were cultured on horse blood agar plates with Dent supplement (Oxoid, Basingstoke, United Kingdom), as described (31).

Genotyping by LiPA and AFLP. DNA of *H. pylori* was extracted by the CTAB method (3). *vacA* and *cagA* genotypes of these strains were determined by PCR and a single-step reverse hybridization line probe assay (LiPA), as described previously (29, 30). In addition, SS1, SPM326, and a spontaneous mutant of the latter strain lacking IL-8 induction capacity (SPM326/IL-8$^-$) were analyzed by amplified fragment length polymorphism (AFLP). The AFLP was performed as described (16) with only minor modifications in the sequences of the fluores-

TABLE 1. Genotypes and phenotypes of different *H. pylori* strains

Strain	Genotype		Phenotype
	vacA	*cagA*	Vacuolation
ATCC 43504	ND	+[b]	+
93-1184	s2-m2	−	−
SS1	s2-m2	+	−
SPM326/IL-8$^+$	s1b-m1	+	+
SPM326/IL-8$^-$	s1b-m1	+	+

[a] ND, not determined.
[b] *cagA* PCR was performed as described by Tummuru et al. (28).

cence-labeled Eco-O primer (5′-GACTGCGTACCAATTC-3′) and the unlabeled Mse-O primer (5′-ACGATGAGTCCTGAGTA-3′).

Assay for cytotoxin production by *H. pylori* strains. Cytotoxin production was determined as described (12). Bacterial culture supernatants were filter sterilized and stored at −20°C until use. HeLa cells (kindly provided by B. Kremer, Academic Center for Dentistry, Amsterdam, The Netherlands) were cultured in RPMI 1640 medium supplemented with 5% (vol/vol) NCS, 10 U of penicillin per ml, and 10 μg of streptomycin (Gibco BRL) per ml. Cells were harvested by trypsinization and seeded at a concentration of 3×10^2 cells/100 μl of tissue culture medium in a microtiter plate. After 16 h of culture at 37°C in 95% air and 5% CO_2 in a humidified incubator, twofold dilutions of bacterial culture supernatants in RPMI 1640 were added. After overnight incubation, the cells were washed with RPMI 1640 and a solution of saline with 0.05% neutral red was added. After 5 min the cells were washed with cold saline containing 0.2% NCS. A total of 100 cells were inspected, and if more than 50 cells showed vacuoles the *H. pylori* strain was designated as cytotoxin positive (12).

Assay for IL-8 induction in KATO III cells. KATO III cells (human gastric carcinoma cell line; kindly provided by A. van der Ende, Academic Medical Center, Amsterdam, The Netherlands) were grown in RPMI 1640 supplemented with 10% (vol/vol) NCS, 10 U of penicillin per ml, and 10 μg of streptomycin per ml. The IL-8 assay was performed as described by Sharma et al. (26), with a few modifications. Cells were seeded in 24-well tissue culture plates at 2×10^5 cells per well in a volume of 1 ml of tissue culture medium and were cultured for 2 days. Twelve hours before stimulation, the cells were washed with phosphate-buffered saline (PBS), and RPMI 1640 without NCS or antibiotics was added to the wells, to minimize the IL-8 production induced by NCS. Six to eight single colonies of each strain were expanded, and bacteria were harvested by centrifugation, washed with PBS, and resuspended at a concentration of 2×10^8 cells/ml. A bacterium:cell ratio of 100:1 was used, and supernatant (50 μl) was collected after 2 and 4 h, centrifuged at 15,000 × *g*, and stored at −20°C until further analysis of IL-8 protein by enzyme-linked immunosorbent assay (ELISA). The type strain of *H. pylori*, ATCC 43504, that was previously reported to induce IL-8 production in KATO III cells was used as a positive control (10). Strain 93-1184, which is *cagA* negative, and serum-free tissue culture medium were used as negative controls.

IL-8 ELISA. IL-8 protein concentrations were determined with an IL-8 sandwich ELISA kit (CLB, Amsterdam, The Netherlands) and expressed as picograms per milliliter. The detection limit for IL-8 was 1 pg/ml. Concentrations of IL-8 were determined from a standard curve (range, 1 to 240 pg/ml).

Inoculation of mice with *H. pylori*. Female 6-week-old specific-pathogen-free C57Bl/6 and BALB/c mice (Harlan CPB, Zeist, The Netherlands) were housed under conventional conditions in our animal facilities. The mice had free access to food and water. The Animal Care Committee of the Vrije Universiteit of Amsterdam approved all animal experimentation described. The inoculation with bacteria was done as described previously (18, 19). The mice received bacteria three times in a 5-day period. A volume of 0.15 ml of bacterial suspension containing approximately 10^9 CFU/ml in PBS was given orally with a feeding needle. Control mice received sterile PBS. Animals were killed at various times postinoculation by cervical dislocation, and their stomachs were removed.

In the first experiment a total of 48 C57Bl/6 and 48 BALB/c mice were inoculated with the *H. pylori* strains SS1, SPM326/IL-8$^+$, and SPM326/IL-8$^-$ (16 mice of each strain for each *H. pylori* strain; see Table 2). The control group consisted of eight C57Bl/6 mice and eight BALB/c mice. Gastric colonization with *H. pylori* was assessed after 5 and 12 weeks. The stomachs of eight mice from each group inoculated with an *H. pylori* strain and four control mice were resected at each time point and divided longitudinally into two halves. One half of the stomach was frozen immediately and was used for immunohistochemistry, whereas the other half was used to assess colonization in a semiquantitative way. The stomach tissue was gently rubbed over the surface of freshly prepared blood agar plates (31) containing Dent supplement (Oxoid) and 75 mg of bacitracin per liter. After 3- to 5-day incubation at 37°C in a microaerobic atmosphere, the numbers of *H. pylori* colonies present on the plates were counted. *H. pylori* was identified by Gram's stain and detection of positive reactions in urease, oxidase, and catalase tests. The presence of *H. pylori* in Giemsa-stained sections was also determined microscopically.

The second experiment was conducted to determine the colonization patterns of the different *H. pylori* strains in the stomach. At 4 weeks postinoculation the stomachs of 12 C57Bl/6 mice (4 mice per *H. pylori* strain) were divided horizontally into three parts: the small zone between the nonglandular and glandular parts of the stomach, the corpus, and the antrum. Culture of bacteria and counting of colonies were performed as described above.

Immunohistochemistry. Longitudinal sections (thickness, 8 μm) of the stomach that contained mucosal tissue from the nonglandular part of the stomach, cardia, corpus, and antrum were picked up on gelatin-coated slides, air dried, and fixed in pure acetone for 10 min. A two-step immunoperoxidase method was used as described previously (31). Consecutive sections were stained with monoclonal antibodies RA3-6B2 (which recognizes B220 on B cells), 59-AD-22 (which recognizes Thy-1 on T cells), and M5.114 (which recognizes major histocompatibility complex [MHC] class II antigens) (31). These monoclonal antibodies were raised in our laboratory from hybridoma cell lines. At least three sections per mouse were investigated for the presence of inflammatory cells. Sections of the spleen were included on every slide as a positive control. Neutrophils were recognized by staining of endogenous peroxidase.

Statistical analysis. Differences between IL-8 concentrations and colonization levels were determined by unpaired *t* test and were considered significant when the *P* value was <0.05.

RESULTS

Genotypes and phenotypes of the *H. pylori* strains. The genotypes of the strains used in this study were determined by LiPA and are summarized in Table 1. The strains 93-1184 and SS1, which showed the s2-m2 *vacA* genotype, did not induce vacuolation in HeLa cells. The other strains induced vacuolation in approximately 50% of the cells. The SS1 strain was the only *cagA*-positive *H. pylori* strain that did not produce a vacuolating cytotoxin.

The ability of these strains to induce IL-8 production was tested in KATO III cells. The results of this test are presented in Fig. 1. ATCC 43504 and SPM326 were the only strains that induced significantly elevated levels of IL-8 in KATO IIII cells after 4 h of incubation. Surprisingly, the *cagA*-positive strain SS1 was not able to induce IL-8 production in KATO III cells. One of eight single colonies of strain SPM326 was not able to induce IL-8. This strain was named SPM326/IL-8$^-$. The *vacA* genotype and phenotype of this strain were tested and were shown to be identical to those of the wild-type strain, SPM326/IL-8$^+$ (Table 1). To further ensure that strain SPM326/IL-8$^-$ originated from the wild-type strain, both strains were analyzed by AFLP. Identical patterns were obtained for the two SPM326 strains, whereas the SS1 strain showed a different pattern (results not shown).

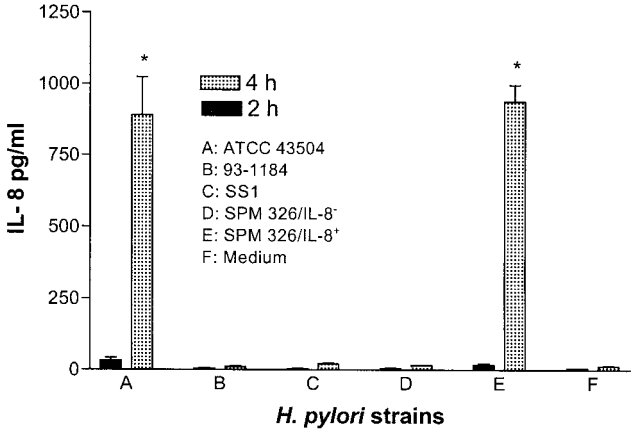


FIG. 1. *H. pylori*-induced IL-8 production by KATO III cells after 2 and 4 h. Each value is the mean ± standard deviation of four determinations obtained in two independent experiments. *, $P < 0.009$, compared with the medium control or strain 93-1184.

TABLE 2. Colonization of C57Bl/6 and BALB/c mice by three *H. pylori* strains

Mouse strain	*H. pylori* strain (no. of inoculated mice)	Wks postinoculation	No. of mice positive for *H. pylori*/total no. of mice	Log CFU/stomach[b]
C57Bl/6	SS1 (16)	5	8/8	4.17 ± 0.12
		12	8/8	NA[a]
	SPM326/IL-8$^+$ (16)	5	7/8	2.64 ± 0.58†
		12	2/8	1.32 ± 0.76*
	SPM326/IL-8$^-$ (16)	5	6/8	3.75 ± 0.18†‡
		12	2/8	3.71 ± 0.18‡
BALB/c	SS1 (16)	5	8/8	4.21 ± 0.17
		12	8/8	3.98 ± 0.41
	SPM326/IL-8$^+$ (16)	5	0/8	0
		12	0/8	0
	SPM326/IL-8$^-$ (16)	5	3/8	3.87 ± 0.03†
		12	5/8	2.81 ± 0.14*†

[a] NA, not available. *H. pylori* was detected for eight of eight mice in Giemsa-stained sections.

[b] †, $P < 0.009$, SS1 infection compared with SPM326/IL-8$^+$ and SPM326/IL-8$^-$ at the same time point in the same mouse strain. *, $P < 0.03$, infection at 5 weeks compared with at 12 weeks; ‡, $P \leq 0.001$, infection with SPM326/IL-8$^+$ compared with SPM326/IL-8$^-$.

Colonization of C57Bl/6 and BALB/c mice with three *H. pylori* strains. The total numbers of bacteria present in the stomachs of C57Bl/6 and BALB/c mice were determined as part of a study in which the immune response was investigated. The greatest number of colonies was observed in mice inoculated with the SS1 strain, which colonized all C57Bl/6 and BALB/c mice (Table 2). No difference in bacterial density was observed between these two mouse strains during the 12-week period of the experiment. The SPM326/IL-8$^+$ strain colonized seven of eight C57Bl/6 mice at 5 weeks, and this number decreased to two of eight mice at 12 weeks, accompanied by a severely decreased number of bacteria. Surprisingly, SPM326/IL-8$^+$ was not able to colonize BALB/c mice. Even after repeated in vivo passages in C57Bl/6 mice SPM326/IL-8$^+$ did not colonize BALB/c mice. The number of C57Bl/6 mice colonized with SPM326/IL-8$^-$ decreased in a way similar to the decrease observed for the parent strain, but the bacterial density was significantly higher. In contrast with the parent strain, SPM326/IL-8$^-$ was able to infect BALB/c mice. The number of mice which were colonized with this strain was greater at 12 weeks than at 5 weeks, but the bacterial density decreased over time (Table 2).

Distribution of *H. pylori* in the stomachs of C57Bl/6 mice. The distribution of *H. pylori* strains SS1, SPM326/IL-8$^+$ and SPM326/IL-8$^-$ in the stomachs of C57Bl/6 mice was studied at 4 weeks postinoculation. The numbers of colonies cultured from the cardia, corpus, and antrum are shown in Table 3. The SS1 strain showed an equal distribution of bacteria over the three parts of the stomach. SPM326/IL-8$^+$ and SPM326/IL-8$^-$ colonized the stomach in fewer numbers than the SS1 strain. Surprisingly, both SPM326 strains showed the highest density of bacteria in the cardia, with numbers decreasing from the cardia to the antrum (Table 3).

Immunohistological analysis of C57Bl/6 and BALB/c mice infected with *H. pylori*. Serial stomach sections of control and infected mice were investigated for the presence of neutrophils and B and T cells and for expression of MHC class II antigens. Mast cells were detected in Giemsa-stained sections by the presence of large Giemsa-stain-positive granules in the cytoplasm. At 5 weeks postinoculation SS1-, SPM326/IL-8$^+$-, and SPM326/IL-8$^-$-colonized C57Bl/6 mice showed mild increases of neutrophil counts in the submucosa and deep mucosa of the corpus. A small lymphoid follicle, primarily consisting of B cells, was observed in the cardia of one of eight SS1-infected mice. Besides increased numbers of neutrophils in the corpus, in three of seven SPM326/IL-8$^+$-colonized C57Bl/6 mice an increase in the number of T cells was also present in the mucosa of the cardia. In the same area MHC class II antigen expression was increased. In control mice MHC class II antigens were expressed only by small cells, which were located between the gastric glands, whereas in SPM326/IL-8$^+$-infected mice MHC class II antigen expression was extended to the cells of the glands. B cells or lymphoid follicles were absent. After 12 weeks the number of neutrophils had normalized in all infected C57Bl/6 mice. In C57Bl/6 mice infected with SPM326/IL-8$^+$ an increase of MHC class II antigen expression and T-cell number similar to that observed at 5 weeks was observed. No inflammation was observed in SS1- or SPM326/IL-8$^-$-infected C57Bl/6 mice.

All SS1- and SPM326-IL-8$^-$-infected BALB/c mice showed an increase in neutrophil count (Fig. 2) similar to that observed in C57Bl/6 mice at 5 weeks postinoculation. However, in some SS1-infected mice the neutrophils not only were present in the submucosa but also extended to the upper part of the corpus mucosa. In SPM326/IL-8$^-$-infected BALB/c mice the neutrophils present in increased numbers were located in the antrum. The neutrophil response decreased over time in a way similar to the decrease observed in C57Bl/6 mice. One of eight SS1-infected BALB/c mice showed a follicle in the cardia at 5 weeks. At 12 weeks postinoculation, areas with increased MHC class II antigen expression were present in both the corpus and the antrum of SS1-colonized BALB/c mice (Fig. 3A). At these locations increased numbers of T cells were present (Fig. 3B). The number of SS1-infected mice with lymphoid follicles increased from one mouse (of eight mice) at 5 weeks to five mice (of 8 mice) at 12 weeks postinoculation. These small lymphoid follicles were observed in the deep mucosa of the cardia and occasionally also in the corpus (Fig. 3C), whereas these structures were absent in control mice. Most follicles consisted primarily of B cells. Chronic inflammation was not observed in SPM326/IL-8$^-$-infected BALB/c mice.

During the 12-week period of infection mast cell numbers were comparable in infected and control mice (results not shown). No vacuolation of epithelial cells or ulceration was observed in the mice. Edema was occasionally observed in infected mice of both strains (Fig. 3C).

TABLE 3. Distribution of *H. pylori* in the cardia, corpus, and antrum of C57Bl/6 mice after 4 weeks of infection

No. of colonies	Mice infected with *H. pylori* strain[b]: SS1 ($n = 4$)			SPM326/IL-8$^+$ ($n = 4$)			SPM326/IL-8$^-$[a] ($n = 4$)		
	Ca	C	A	Ca	C	A	Ca	C	A
$1–10^2$					2	3			2
$10^2–10^3$				4	2		3	3	1
$10^3–10^5$	4	4	4						

[a] Three of 4 mice were colonized.

[b] Ca, cardia equivalent (every part of the stomach was cultured separately); C, corpus; and A, antrum.

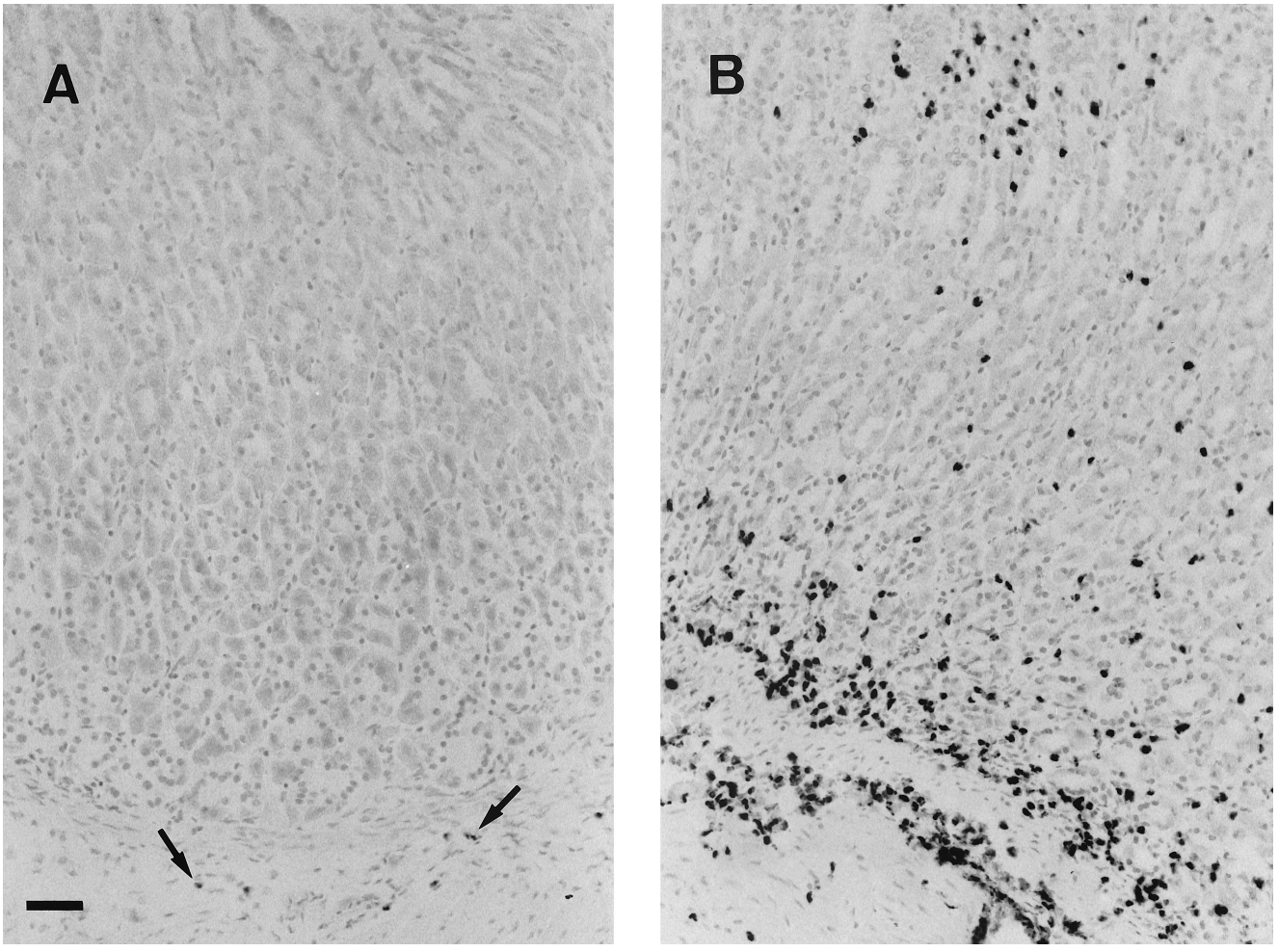


FIG. 2. Neutrophil staining in sections from BALB/c mice. (A) A few neutrophils are present in the submucosa of a control mouse (arrows). (B) A mouse colonized by the SS1 strain shows increased numbers of neutrophils in the submucosa and mucosa of the corpus at 5 weeks postinoculation. Bar = 20 μm.

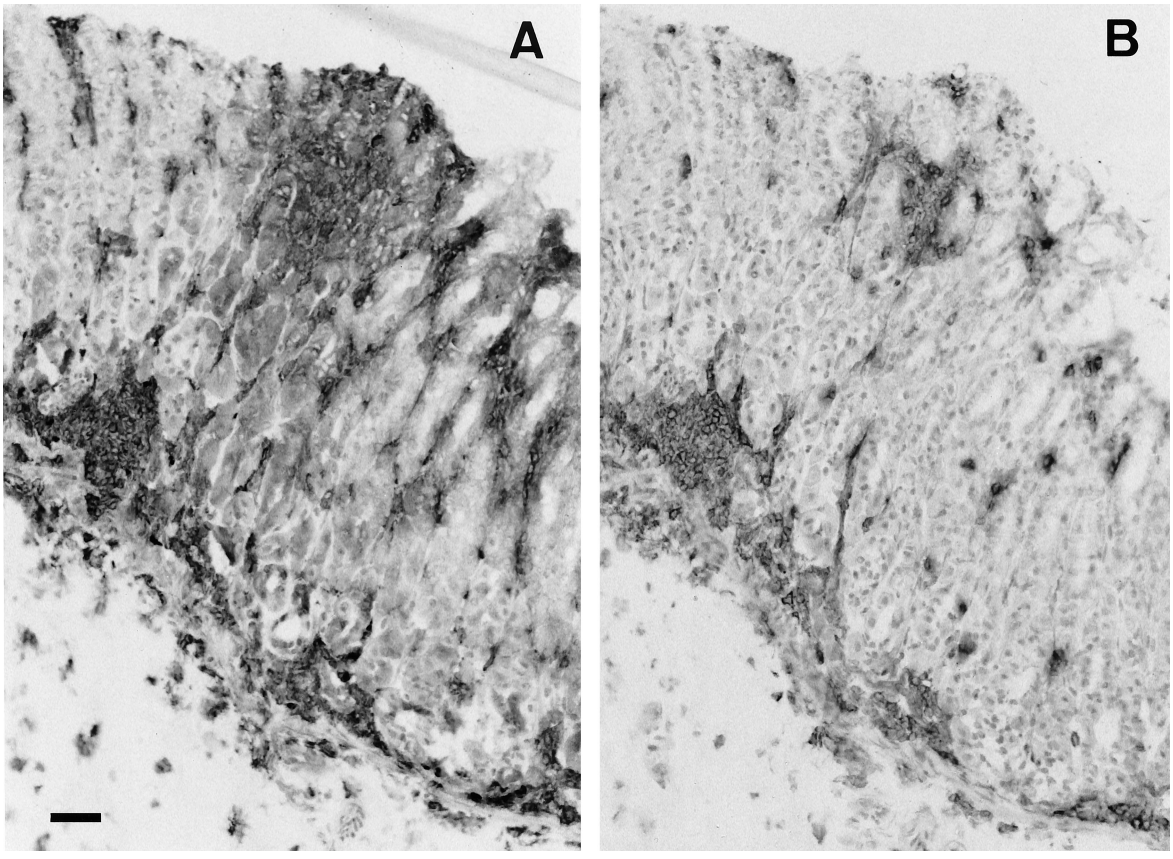
DISCUSSION

In this study the *vacA* and *cagA* genotypes of two mouse-adapted *H. pylori* strains as well as the phenotypic expression of these genes were determined. C57Bl/6 and BALB/c mice were infected with these strains, which differed in cytotoxin expression and ability to induce IL-8 production, and the patterns of colonization and gastric immune response were compared.

The induction of vacuoles in HeLa cells by *H. pylori* SPM326, which has *vacA* genotype s1b-m1, and the lack of vacuolization by the *vacA* s2-m2-possessing strain SS1 are consistent with the data of other studies (2, 20). However, recently it has become clear that the ability of the m1 or m2 cytotoxin to induce vacuoles strongly depends on the cell line that is used in the assay (20, 22). Whether *H. pylori* strains with the s2-m2 genotype are able to induce vacuoles in other cell lines remains to be established.

The presence of *cagA* is associated with IL-8 production by epithelial cells (10). Although both mouse-adapted strains were *cagA* positive, only SPM326 induced a significantly higher level of production of IL-8 in KATO III cells compared with a *cagA*-negative *H. pylori* strain. In addition, a spontaneous mutant of SPM326 which induced vacuolization but did not induce IL-8 production (SPM326/IL-8$^-$) was isolated. It has been shown that mutations in several genes of the *cag* pathogenicity island affect IL-8 production by epithelial cells (1, 8, 28). Whether one of these genes is mutated in the SS1 and SPM326/IL-8$^-$ strains is currently under investigation.

Whether these three *H. pylori* strains with different cytotoxin and IL-8 induction phenotypes also showed different properties in vivo was investigated in C57Bl/6 and BALB/c mice. Culture of *H. pylori* from gastric biopsy specimens was found to be more sensitive to assess colonization than analysis of Giemsa-stained sections by microscopy; this result is in agreement with data reported by Shomer et al. (27). The SS1 strain showed the greatest number of colonies in culture (10^4 to 10^5 CFU/stomach) and was easily detected in Giemsa-stained sections, whereas both SPM326 strains showed fewer colony numbers and were hardly detectable in sections. The detection limit for *H. pylori* in sections was approximately 10^3 bacteria/mouse stomach. The SS1 strain colonized both mouse strains in large numbers, whereas SPM326/IL-8$^+$ and SPM326/IL-8$^-$ colonized in smaller numbers and SPM326/IL-8$^+$ was not able to colonize BALB/c mice at all. Although SPM326 has been reported to show a constant colonization rate in CD1 mice for up to 52 weeks (14), we observed a decrease in the numbers of SPM326/IL-8$^+$-and SPM326/IL-8$^-$-colonized C57Bl/6 mice as well as in bacterial density over time. These results suggest that



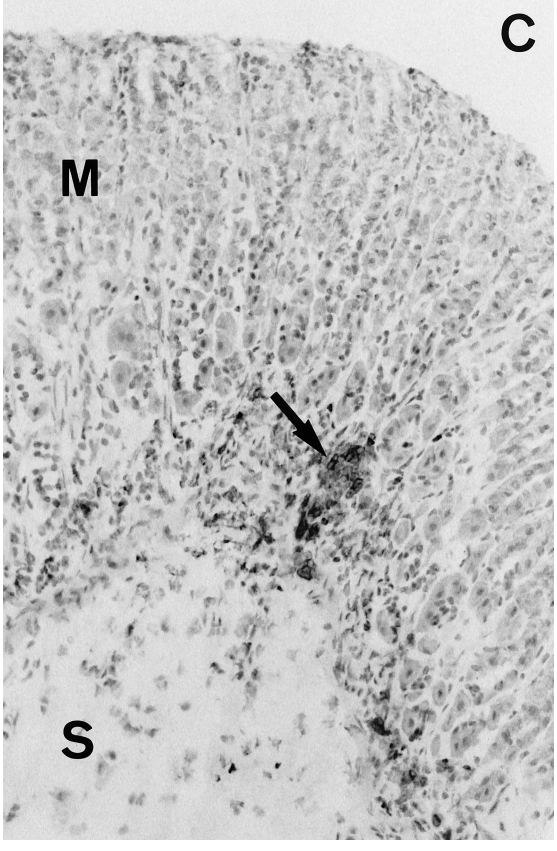


FIG. 3. SS1-infected BALB/c mouse at 12 weeks postinoculation. (A) A moderate increase in MHC class II expression is observed in the upper mucosa (dark staining). (B) At the same location an increased number of T cells is present. (C) A few B cells indicate the location of a small lymphoid follicle in the deep mucosa (arrow). Note the presence of edema in the submucosa (S). M, mucosa. Bar = 20 μm.

the SPM326 strain is more susceptible to host factors than the SS1 strain.

The distribution of *H. pylori* in the mouse stomach was assessed to investigate whether colonization correlated with the gastric immune response. In both C57Bl/6 and BALB/c mice the number of neutrophils was increased at 5 weeks but not at 12 weeks postinoculation. The presence of neutrophils in the corpus or antrum was associated neither with the highest density of bacteria nor with the IL-8 induction phenotype of the colonizing *H. pylori* strain. This may not be very surprising, because mice do not express a homologue of human IL-8 (7). However, these results show that activation of neutrophils in mice occurs via mechanisms other than those observed in humans.

In contrast with the active inflammation, the presence of chronic inflammation was correlated with the highest bacterial density. In SS1-infected BALB/c mice chronic inflammation was present in the cardia, corpus, and antrum, whereas in SPM326/IL-8^+-infected C57Bl/6 mice chronic inflammation was restricted to the cardia. Although SS1-infected BALB/c mice showed a density of bacteria similar to that for SS1-infected C57Bl/6 mice, chronic inflammation was absent in the last group. These observations are not in agreement with those reported by Lee et al. (18), who observed the opposite phenomenon: chronic inflammation was present at an earlier time

point in SS1-infected C57Bl/6 mice than in infected BALB/c mice. This discrepancy could be due to the higher level of colonization with SS1 in C57Bl/6 mice reported by Lee et al. (18).

The chronic inflammation in the cardia of SPM326/IL-8$^+$-infected C57Bl/6 mice was characterized by increased numbers of T cells in an area in which MHC class II antigen expression was extended to epithelial gland cells. In SS1-infected BALB/c mice a similar colocalization of T cells and MHC class II antigen expression by gland cells was observed, but this was found in the corpus and antrum. In addition, lymphoid follicles were present in the deep mucosa of the cardia and corpus and their presence was not correlated with MHC class II antigen expression by gland cells. The early signs of chronic gastritis in SS1-infected BALB/c mice clearly show that differences exist between *H. felis* infection and *H. pylori* infection, as in *H. felis*-infected BALB/c mice chronic inflammation occurs only after 22 months (11). Although the chronic inflammation of SS1-infected BALB/c mice does not mimic the active and chronic inflammation observed in humans, it could be used as a model to study the interaction between T cells and the gastric epithelium.

The absence of chronic inflammation in SPM326/IL-8$^-$-infected C57Bl/6 and BALB/c mice makes this mutant an interesting strain that could be used to unravel the mechanism of induction of inflammation. This strain might be mutated in its adherence to epithelial cells. The attachment of the bacterium to epithelial cells is very important in the process of cytokine production (24). The importance of attachment of *H. pylori* cells to epithelial cells has also been studied in a mouse model, i.e., transgenic mice that express Lewis b, which has been reported to be an adhesin for *H. pylori* (6), in the gastric glands. These transgenic mice show a more severe inflammatory response than nontransgenic mice and produce autoantibodies after infection with *H. pylori* (15).

The cytotoxin of *H. pylori* is able to induce vacuolation of cells in vitro (2, 20, 23), and the oral administration of sonic extracts of cytotoxin-positive *H. pylori* strains or purified cytotoxin induces gastric lesions in mice (13). However, under these experimental conditions the concentration of cytotoxin is probably much higher than under the physiological conditions of colonization with living bacteria, as no epithelial damage or vacuolation was observed in any of the *H. pylori*-infected mice.

In conclusion, this study showed that the mouse-adapted *H. pylori* strains with the *vacA* s1 genotype induced vacuolation in HeLa cells. The IL-8-inducing ability of these strains could not be predicted from the *cagA*-positive genotype. The infection of two strains of mice with *H. pylori* strains with different *vacA* and *cagA* phenotypes showed that the inflammatory response depended on both the host and the *H. pylori* strain.

REFERENCES

1. **Akopyants, N. S., S. W. Clifton, D. Kersulyte, J. E. Crabtree, B. E. Youree, C. A. Reece, N. O. Bukanov, E. S. Drakez, B. A. Roe, and D. E. Berg.** 1998. Analyses of the cag pathogenicity island of *Helicobacter pylori*. Mol. Microbiol. **28:**37–53.
2. **Atherton, J. C., P. Cao, R. M. Peek, M. K. Tham, T. L. Cover, and M. J. Blaser.** 1995. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*: association of specific *vacA* types with cytotoxin production and peptic ulceration. J. Biol. Chem. **270:**17771–17777.
3. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. A. Smith, J. G. Seidman, and K. Struhl.** 1987. Current protocols in molecular biology. Greene Publishing Associates and Wiley-Interscience, New York, N.Y.
4. **Blaser, M. J., and J. Parsonnet.** 1994. Parasitism by the "slow" bacterium *Helicobacter pylori* leads to altered gastric homeostasis and neoplasia. J. Clin. Investig. **94:**4–8.
5. **Blaser, M. J.** 1995. Intrastrain differences in *Helicobacter pylori*: a key question in mucosal damage? Ann. Med. **27:**559–563.
6. **Boren, T., P. Falk, K. A. Roth, G. Larson, and S. Normak.** 1993. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science **262:**1892–1895.
7. **Callard, R. E., and A. J. G. Gearing.** 1994. The cytokine facts book. Academic Press, London, United Kingdom.
8. **Censini, S., C. Lange, S. Xiang, J. E. Crabtree, P. Ghiara, M. Borodovsky, R. Rappuoli, and A. Covacci.** 1996. *Cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc. Natl. Acad. Sci. USA **93:**14648–14653.
9. **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.
10. **Crabtree, J. E., S. M. Farmery, I. J. D. Lindley, F. Figura, P. Peichl, and D. S. Tompkins.** 1994. CagA/cytotoxic strains of *Helicobacter pylori* and interleukin-8 in gastric epithelial cell lines. J. Clin. Pathol. **47:**945–950.
11. **Enno, A., J. L. O'Rourke, C. R. Howlett, A. Jack, M. F. Dixon, and A. Lee.** 1995. MALToma-like lesions in the murine gastric mucosa after long-term infection with *Helicobacter felis*. A mouse model of *Helicobacter pylori*-induced gastric lymphoma. Am. J. Pathol. **147:**217–222.
12. **Figura, N.** 1996. Culture of *Helicobacter pylori* in broth, determination of vacuolising activity of bacterial broth cultures and neutralisation of the vacuolising activity, p. 224–233. *In* A. Lec and F. Mégraud (ed.), *Helicobacter pylori*: techniques for clinical diagnosis and basic research. W.B. Saunders Company Ltd., London, United Kingdom.
13. **Ghiara, P., M. Marchetti, M. J. Blaser, M. R. Tummuru, T. L. Cover, E. D. Segal, L. S. Thompkins, and R. Rappuoli.** 1995. Role of the *Helicobacter pylori* virulence factors vacuolating cytotoxin, CagA, and urease in a mouse model of disease. Infect. Immun. **63:**4154–4160.
14. **Ghiara, P., M. Rossi, M. Marchetti, A. di Tommaso, C. Vindigni, F. Ciampolini, A. Covacci, J. L. Telford, M. T. de Magistris, M. Pizza, R. Rappuoli, and G. del Giudice.** 1998. Therapeutic intragastric vaccination against *Helicobacter pylori* in mice eradicates an otherwise chronic infection and confers protection against reinfection. Infect. Immun. **65:**4996–5002.
15. **Guruge, J. L., P. G. Falk, R. G. Lorenz, M. Dans, H. P. Wirth, M. J. Blaser, D. E. Berg, and J. I. Gordon.** 1998. Epithelial attachment alters the outcome of *Helicobacter pylori* infection. Proc. Natl. Acad. Sci. USA **95:**3925–3930.
16. **Koeleman, H., J. Stoof, D. J. Biesmans, P. H. M. Savelkoul, and C. M. J. E. Vandenbroucke-Grauls.** 1998. Comparison of ARDRA, RAPD, and AFLP fingerprinting for identification of *Acinetobacter* genomic species and typing of *Acinetobacter baumannii*. J. Clin. Microbiol. **36:**2522–2529.
17. **Kuipers, E. J., G. I. Perez-Perez, S. G. M. Meuwissen, and M. J. Blaser.** 1995. *Helicobacter pylori* and atrophic gastritis: importance of the *cagA* status. J. Natl. Cancer Inst. **87:**1777–1780.
18. **Lee, A., J. O'Rourke, M. Corazon de Ungria, B. Robertson, G. Daskalopoulos, and M. F. Dixon.** 1997. A standardized mouse model of *Helicobacter pylori* infection: introducing the Sydney strain. Gastroenterology **112:**1386–1397.
19. **Marchetti, M., B. Aricò, D. Burroni, N. Figura, R. Rappuoli, and P. Ghiara.** 1995. Development of a mouse model of *Helicobacter pylori* infection that mimics human disease. Science **267:**1655–1658.
20. **Miehlke, S., A. Meining, A. Morgner, E. Bayerdörffer, N. Lehn, M. Stolte, D. Y. Graham, and M. F. Go.** 1998. Frequency of *vacA* genotypes and cytotoxin activity in *Helicobacter pylori* associated with low-grade gastric mucosa-associated lymphoid tissue lymphoma. J. Clin. Microbiol. **36:**2369–2370.
21. **Mohammadi, M., R. Redline, J. Nedrud, and S. Czinn.** 1996. Role of the host in pathogenesis of *Helicobacter*-associated gastritis: *H. felis* infection of inbred and congenic mouse strains. Infect. Immun. **64:**238–245.
22. **Oppenheim, J. J., C. O. C. Zachariae, N. Mukaida, and K. Matsushima.** 1991. Properties of the novel proinflammatory supergene intercrine cytokine family. Annu. Rev. Immunol. **9:**617–648.
23. **Pagliaccia, C., M. de Bernard, P. Lupetti, X. Ji, D. Burroni, T. L. Cover, E. Papini, R. Rappuoli, J. L. Telford, and J.-M. Reyrat.** 1998. The m2 form of the *Helicobacter pylori* cytotoxin has cell type-specific vacuolating activity. Proc. Natl. Acad. Sci. USA **95:**10212–10217.
24. **Rieder, G., R. A. Hatz, A. P. Moran, A. Walz, M. Stolte, and G. Enders.** 1997. Role of adherence in interleukin-8 induction in *Helicobacter pylori*-associated gastritis. Infect. Immun. **65:**3622–3630.
25. **Sakagami, T., M. Dixon, J. O'Rourke, R. Howlett, F. Alderuccio, J. Vella, T. Shimoyama, and A. Lee.** 1996. Atrophic gastric changes in both *Helicobacter felis* and *Helicobacter pylori* infected mice are host dependent and separate from antral gastritis. Gut **39:**639–648.
26. **Sharma, S. A., M. K. R. Tummuru, G. G. Miller, and M. J. Blaser.** 1995. Interleukin-8 response of gastric epithelial cell lines to *Helicobacter pylori* stimulation in vitro. Infect. Immun. **63:**1681–1687.
27. **Shomer, N. H., C. A. Dangler, M. T. Whary, and J. G. Fox.** 1998. Experimental *Helicobacter pylori* infection induces antral gastritis and gastric mucosa-associated lymphoid tissue in guinea pigs. Infect. Immun. **66:**2614–1618.
28. **Tummuru, M. K. R., S. A. Sharma, and M. J. Blaser.** 1995. *Helicobacter pylori picB*, a homologue of the *Bordetella pertussis* toxin secretion protein, is

required for induction of IL-8 in gastric epithelial cells. Mol. Microbiol. **18:**867–876.
29. **van Doorn, L. J., C. Figueiredo, R. Rossau, G. Jannes, M. van Asbroeck, J. C. Sousa, F. Carneiro, and W. G. V. Quint.** 1998. Typing of *Helicobacter pylori vacA* gene and detection of *cagA* gene by PCR and reverse hybridization. J. Clin. Microbiol. **36:**1271–1276.
30. **van Doorn, L.-J., C. Figueiredo, R. Sanna, S. Pena, P. Midolo, E. K. W. Ng, J. C. Atherton, M. J. Blaser, and W. G. V. Quint.** 1998. Expanding allelic diversity of *Helicobacter pylori vacA*. J. Clin. Microbiol. **36:**2597–2603.
31. **van Doorn, N. E. M., E. P. van Rees, F. Namavar, and J. de Graaff.** 1998. Local cellular immune response in the acute phase of gastritis in mice induced chemically and by *Helicobacter pylori*. J. Med. Microbiol. **47:**863–870.
32. **Weel, J. F. L., R. W. M. van der Hulst, Y. Gerrits, P. Roorda, M. Feller, J. Dankert, G. N. Tytgat, and A. van der Ende.** 1996. The interrelationship between cytotoxin-associated gene A, vacuolating cytotoxin, and *Helicobacter pylori*-related diseases. J. Infect. Dis. **173:**1171–1175.

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