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Role of the Host in Pathogenesis of *Helicobacter*-Associated Gastritis: *H. felis* Infection of Inbred and Congenic Mouse Strains

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In humans, *Helicobacter pylori* establishes a chronic infection which can result in various degrees of gastric inflammation, peptic ulcer disease, and a predisposition to gastric cancer. It has been suggested that bacterial virulence factors such as the vacuolating toxin (VacA) and the cytotoxin-associated gene product (CagA) may play a major role in determining the clinical outcome of *Helicobacter* infections. The role of host responses in these varied outcomes has received little attention. *Helicobacter felis*, which does not express CagA or VacA, causes chronic infection and inflammation in a well-characterized mouse model. We have used this model to evaluate the role of host responses in *Helicobacter* infections. BALB/c, C3H, and C57BL/6 mice were orally infected with a single strain of *H. felis*, and 2 and 11 weeks after infection, the mice were sacrificed and evaluated histologically for magnitude of *H. felis* infection, intensity and extent of inflammation, and cellular composition of the inflammatory infiltrate. All three strains of mice demonstrated comparable levels of infection at 11 weeks, but the pattern and intensity of inflammation varied from minimal in BALB/c mice to severe in C57BL/6 mice. Gastric epithelial erosions were noted in C3H mice, and mucous cell hyperplasia was observed in C3H and C57BL/6 mice. Abundant mucosal mast cells were observed in the gastric tissues of all three mouse strains. Studies using major histocompatibility complex (MHC)-congenic mice revealed probable contributions by both MHC and non-MHC genes to *Helicobacter*-induced inflammation. Thus, large variations in the severity of disease were observed after infection of different inbred strains and congenic mice with a single isolate of *H. felis*. These results demonstrate the importance of the host response in disease outcome following gastric *Helicobacter* infection.

The pathogenesis of *Helicobacter pylori*-associated gastroduodenal disease is poorly understood. *Helicobacter* infection can result in histologic gastritis with or without overt symptoms, peptic ulcer disease, and/or predisposition to gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma (16, 17, 26, 27, 29, 31, 36). It has been suggested that this diversity of outcomes in *H. pylori* infection may be due to the characteristics of the strain of *H. pylori* infecting the host. In particular, the production of the 94-kDa VacA cytotoxin and the 128- to 140-kDa CagA gene product (35) found in approximately 50% of *H. pylori* strains recovered from patients has been demonstrated to be associated with duodenal ulcers (5, 6). However, more recent results suggest that the relationship between disease and bacterial phenotype may be more complex (38). Although these and perhaps other bacterial factors may be important in the diseases associated with *Helicobacter* infections, genetic differences in the host response to *Helicobacter* infection have received less attention. One recent twin study suggests that the genetic component of susceptibility may be greater than previously believed (23). Animal models for *Helicobacter* infection include nonhuman primates, pigs, ferrets, cats, dogs, and rodents. Although there have been reports that fresh isolates of *H. pylori* can infect immunodeficient and immunocompetent mice (18, 19, 25), the most extensively characterized rodent model for gastric *Helicobacter* infection has employed *Helicobacter felis* infection in outbred Swiss and inbred BALB/c mice (2–4, 7, 9, 12, 21, 22, 28, 32). The large number of well-characterized inbred mouse strains makes this *H. felis* mouse model an attractive system in which to further explore the role of the host in *Helicobacter* infection. In this study, we compared the magnitude of bacterial colonization and the pattern and severity of inflammation in three different inbred mouse strains and in major histocompatibility complex (MHC)-congenic mice.

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MATERIALS AND METHODS

Animals and bacteria. Specific-pathogen-free female mice (7 to 8 weeks old) from the following strains were purchased from Taconic Laboratories (Germantown, N.Y.): BALB/c, C3H/HeN, and C57BL/6. MHC-congenic mice of the following strains were obtained from Jackson Laboratories (Bar Harbor, Maine): C57BL/6By (*H-2*^{b}), B6.C-H2^d/aBy, C-B10-H2^b/LiMcdJ, and BALB/cBy (*H-2*^{d}). The mice were housed in microisolators and allowed free access to autoclaved chow and water. The animals were cared for and all procedures were performed according to protocols approved by the Case Western Reserve University Institutional Use and Care of Animals Committee. In addition, the animal facility at Case Western Reserve University has been fully accredited by the American Association for Accreditation of Laboratory Animal Care. The *Helicobacter felis* strain used in this study was originally isolated from the stomach of a cat and identified on the basis of morphology, Gram stain, and urease, catalase, and oxidase production (7). Bacteria were grown on Columbia agar containing 7% horse blood and incubated microaerobically at 37°C for 5 to 7 days.

Experimental protocol. Confluent plates of *H. felis* were harvested, and a single dose of 5×10^7 CFU was intragastrically administered to each mouse through polyethylene tubing. Two and eleven weeks after infection, the mice were sacrificed by carbon dioxide asphyxiation. Multiple gastric biopsies from infected and control stomachs were tested for the presence of bacteria with the rapid urease test. For histologic evaluation, a longitudinal strip of tissue along the greater curvature of the stomach from the esophagus to the duodenum was fixed in buffered 10% formalin, processed, and embedded in paraffin, and 5-μm sections were cut. Sampling techniques were standardized such that each section contained 0.3 to 0.4 cm of squamous, epithelial-lined cardia, 0.9 to 1.1 cm of oxyntic epithelial-lined fundic mucosa, 0.4 to 0.6 cm of antrum, and 0.4 to 0.5 cm of duodenum. This allowed us to score a large uniform area of stomach from each case. Slides were stained with hematoxylin and eosin and Giemsa stains.

Mice were considered infected by either a positive urease test or the direct appearance of bacteria in histologic sections.

Quantitation of *H. felis*. Quantitative culturing of *H. felis* has been shown by our group and others to be unreliable, consistently underestimating the actual number of infected animals (3, 12). Therefore, the dose of *H. felis* used to infect the mice was estimated from the optical density of a suspension of organisms scraped from a confluent plate as described previously (3). Since quantitative culturing was not possible and urease testing is qualitative and lacks sensitivity, we devised a histologic scoring system using Giemsa-stained tissue sections to estimate the extent and magnitude of *H. felis* infection of mice. Extent of infection was estimated by the total number of glands containing one or more stainable *H. felis* organisms per linear centimeter of mucosa. Magnitude of infection was estimated by the maximum number of stainable *H. felis* organisms in the most densely colonized crypt.

Inflammation score. Intensity of gastric inflammation was graded on a 0 to 3 scale, using the most severely affected ×20 microscopic field and taking into account only the relative density of inflammatory cells. Longitudinal extent of inflammation was scored from 0 to 3 as follows: 1, focal (1 to 2 small foci only); 2, patchy (greater than focal but <50% of total length); 3, diffuse (>50% of total length). Vertical extent of inflammation was scored in a similar fashion: 1, basal (inflammation not extending to the surface of mucosa); 2, transmural (full-thickness involvement of mucosa); 3, deep (involvement of both mucosa and submucosa). The numerical scores for intensity, longitudinal extent, and vertical extent were added together to derive a single score which we call the inflammation score. Vacuolation of fundic epithelial cells was a prominent feature in some animals. The nature of the intracytoplasmic vacuolation was assessed in serial sections by using periodic acid-Schiff stain with and without Diastase digestion, Mucin carmine stain, and Alcian Blue stain at pH 1.0 and 2.5. Sections of colonic mucosa were used as positive controls for these stains. This characteristic was graded from 0 to 3 on the basis of its extent in the most severely affected ×20 microscopic field.

Cellular composition of the inflammatory infiltrate. Mast cells were identified on the basis of their large Giemsa-positive cytoplasmic granules and quantitated as the total number of cells per linear centimeter of mucosa. All other cellular characteristics were graded from 0 to 3 on the basis of frequency in the section as a whole. These included mononuclear cells, polymorphonuclear leukocytes, activated mononuclear cells (defined by nuclear enlargement and presence of eosinophilic nucleoli), plasma cells, crypt abscesses (polymorphonuclear leukocytes within gland lumens), and lymphoid aggregates (collections of small lymphocytes with a central core of larger mononuclear cells).

PCR amplification of CagA and VacA genes. Amplification was performed with *Taq* DNA polymerase (GIBCO BRL, Gaithersburg, Md.) according to the manufacturer's instructions. Total genomic DNA from a $CagA^+$ $VacA^+$ strain of *H. pylori* was used as the positive control, and sonicated *H. felis* was used as the test template. Reaction tubes were incubated in a thermocycler (model TC-1; Perkin-Elmer) at 94°C for 5 min to denature the template. The following amplification sequence was applied for 35 cycles; denaturing at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, 30 s. Following completion of the 35 cycles, the tubes were incubated at 72°C for an additional 10 min. The amplification products were analyzed by agarose gel electrophoresis with a 100-bp DNA ladder (GIBCO BRL) for standard molecular weights. The *H. pylori* DNA template and the following primers were a kind gift from Oravax Inc. (Cambridge, Mass.): VacA primers, *vac*A 1F (20-mer) (5′ ATggAAATA CAACAAACACA 3′) and *vac*A 1R (19-mer) (5′ CTCCAgAACCCACACgATT 3′); CagA primers, *cag*A F (22-mer) (5′ gATAACgTCgCTTCATACg 3′) and *cag*A R (22-mer) (5′ gATgATTCCTgATTgT 3′); UreB primers, HfU50 (5′ ggTCCCACTACCggggAT 3′) and HfU25 (5′ gCCTACgCgTCCCATAgCCT 3′).

Statistical analysis. Comparisons of bacterial colonization or inflammation scores among experimental groups were done by analysis of variance with Fisher's protected *t* test. The nonparametric Mann-Whitney test was also done and resulted in the same conclusions (not shown).

RESULTS

Groups of 14 to 16 inbred mice were infected with a single dose of *H. felis* as described above. At 2 weeks and again at 11 weeks postinfection, approximately half of the animals in each group were sacrificed and evaluated for infection. Similar numbers of unmanipulated control animals were sacrificed and evaluated at the same time points. As discussed in Materials and Methods, quantitative culture of *H. felis* has been unreliable and is further complicated by the patchy nature of the infection. Urease testing provides a qualitative result but in our hands is less sensitive than microscopy. Both of these methods also utilize small biopsy specimens and are subject to sampling errors. Therefore, standardized longitudinal histologic sections of the entire greater curvature of the stomach were Giemsa stained and used to score the extent and magnitude of infection (see Materials and Methods). In this analysis, both the number of glands per centimeter containing one or more spiral organisms and the maximum number of organisms in the most densely colonized gland were determined for both the fundus and the antrum of each animal. All three inbred mouse strains and the congenic mice were infected after a single dose of *H. felis*, and organisms were observed in both the antrum and fundus.

TABLE 1. *H. felis* infection and inflammation in inbred mice[a]

Mouse strain	Time p.i. (wks)[b]	*H. felis*-positive glands/cm[c]	*H. felis* max. organisms/gland[d]	Inflammation score[e]
BALB/c	2	4.0 ± 3.7 ‡	2.6 ± 3.5 ‡	1.1 ± 0.5 †‡
C3H	2	4.4 ± 4.3 ‡	4.6 ± 4.2 ‡	4.5 ± 2.7 *
C57BL/6	2	35.0 ± 27.7 *†	22.7 ± 17.4 *†	4.3 ± 2.2 *
BALB/c	11	10.2 ± 6.8	8.7 ± 6.6	0.9 ± 0.7 †‡
C3H	11	6.0 ± 5.3	8.1 ± 6.0	2.8 ± 2.3 *
C57BL/6	11	16.0 ± 19.6	12.4 ± 10.3	6.2 ± 0.9 *

[a] *, $P < 0.01$ versus BALB/c; †, $P < 0.01$ versus C3H; ‡, $P < 0.01$ versus C57BL/6. Data are means ± standard deviations.

[b] p.i., postinfection.

[c] *H. felis*-positive glands/cm, the number of glands per centimeter of fundic mucosa which contain spiral-shaped bacteria by Giemsa stain consistent with appearance of *H. felis.*

[d] *H. felis* max. (maximum) organisms/gland, the number of spiral-shaped bacteria in the most densely populated fundic gland.

[e] Inflammation score (maximum = 9) equals the sum of the grade of intensity of inflammation in the most inflamed area (0 to 3) and the longitudinal extent (0 to 3) and depth (0 to 3) of inflammation. Longitudinal extent and depth of inflammation were scored for the entire section.

Level of infection. At 2 weeks postinfection, seven of seven BALB/c mice, six of seven C3H mice, and seven of seven C57BL/6 mice were positive for spiral bacteria (a representative section is shown in Fig. 1a). None of the control, uninfected mice were *H. felis* positive by either urease or histology. The level of antrum colonization tended to be higher and more variable than fundic colonization in all three strains of mice (results not shown). Therefore, we also evaluated *H. felis* colonization in the fundus. C57BL/6 mice consistently had 5- to 10-fold-higher colonization in the fundus than did the other two strains (Table 1). This increase applied to both the number of glands infected ($P < 0.01$) and the maximum number of organisms per gland ($P < 0.01$).

At 11 weeks postinfection, although the overall prevalence of infection remained high (nine of nine BALB/c, six of seven C3H, and eight of eight C57BL/6 mice), colonization levels had shifted somewhat. Compared with the 2-week groups, the extent of fundic colonization remained stable in C3H mice, increased slightly in BALB/c mice, and decreased in C57BL/6 mice. Despite the relative decrease in fundic colonization for C57BL/6 mice, this strain continued to have the highest overall levels of colonization of the three strains, both in terms of *H. felis*-positive glands per centimeter and maximum number of organisms per gland.

Severity of inflammation. Previous reports of *H. felis*-associated gastritis have noted a relatively mild form of chronic active inflammation dominated by lymphocytes (12, 13, 22, 28). These studies were done with BALB/c mice and outbred mice and rats. In the current experiments, we observed a much broader spectrum of inflammatory response which varied with the strain of the mouse (Fig. 1b to 1d). Although both the antra and the fundi of mice of each mouse strain showed inflammatory changes compared with those of the uninfected controls, the degree of inflammation was almost always more severe in

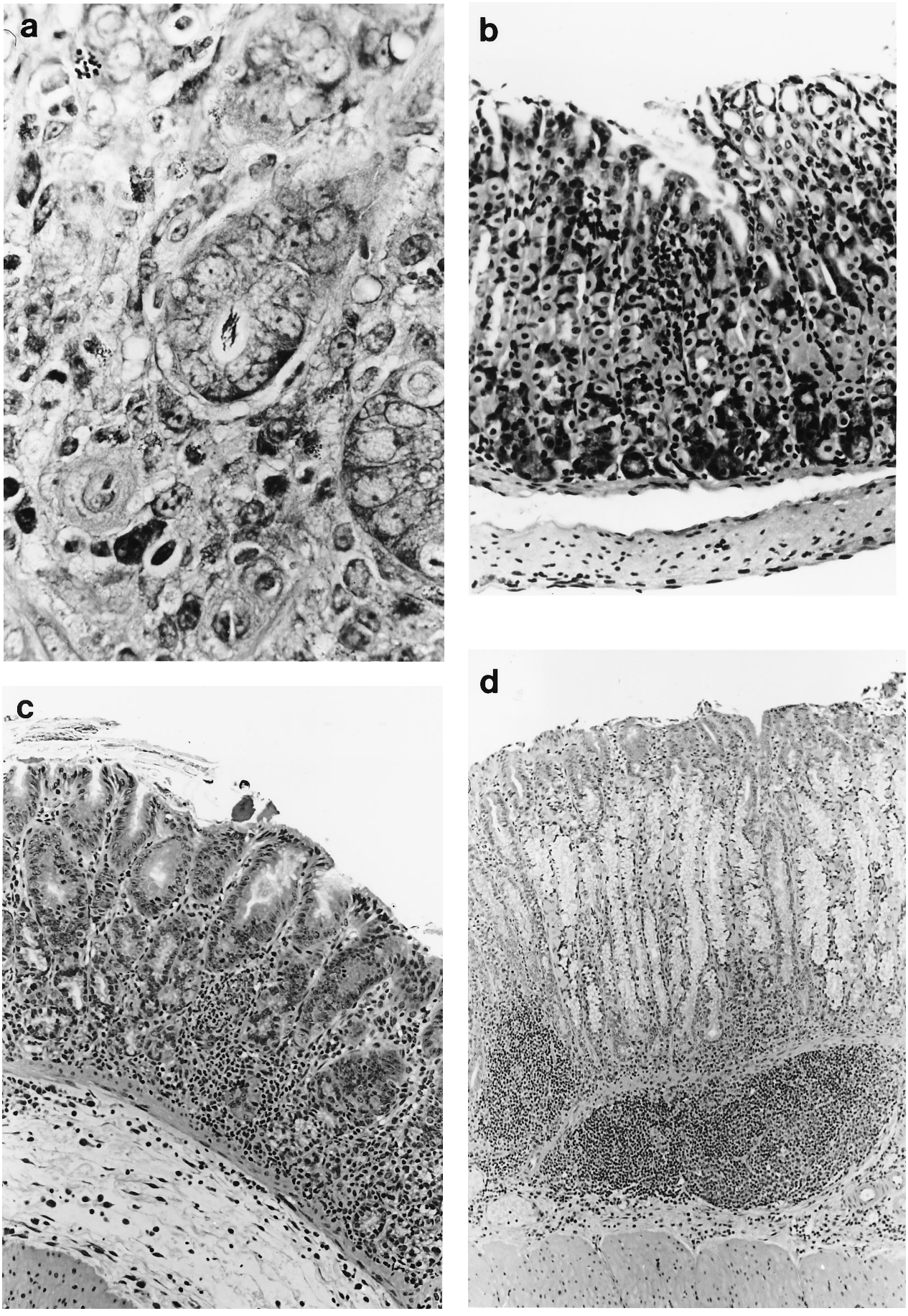
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TABLE 2. Cellular composition of inflammatory infiltrate in inbred strains of mice infected with *H. felis*[a]

Mouse strain	Time p.i. (wks)[b]	Mononuclear cells	Activated mononuclear cells	Polymorphonuclear leukocytes	Crypt abscesses	Plasma cells	Lymphoid aggregates
BALB/c	2	1.0 ± 0.0	0.0 ± 0.0 †	0.0 ± 0.0 ∫	0.0 ± 0.0 †	0.0 ± 0.0	0.0 ± 0.0
C3H	2	1.6 ± 0.6	1.0 ± 0.6 ‡*	0.6 ± 1.0	1.1 ± 0.7 *‡	0.0 ± 0.0	0.0 ± 0.0
C57BL/6	2	0.9 ± 0.7	0.0 ± 0.0 †	1.4 ± 1.4 ∞	0.1 ± 0.4 †	0.1 ± 0.4	0.3 ± 0.8
BALB/c	11	1.0 ± 0.0	0.0 ± 0.0 †	0.1 ± 0.3 ‡	0.0 ± 0.0 †	0.4 ± 0.5 ‡	0.6 ± 0.5 ‡
C3H	11	1.4 ± 0.8	0.3 ± 0.5 ‡*	0.4 ± 0.5	0.4 ± 0.8 *‡	0.4 ± 0.5 ‡	1.0 ± 0.8 ‡
C57BL/6	11	1.4 ± 0.9	0.0 ± 0.0 †	2.0 ± 0.8 *	0.2 ± 0.5 †	1.4 ± 0.7 *†	1.4 ± 0.9 *†

[a] ∞, $P < 0.05$ versus BALB/c; ∫, $P < 0.05$ versus C57BL/6; *, $P < 0.01$ versus BALB/c; †, $P < 0.01$ versus C3H; ‡, $P < 0.01$ versus C57BL/6. Relative frequency for each cellular characteristic was semiquantitatively graded from 0 to 3. Data are means ± standard deviations.

[b] p.i., postinfection.

the fundus, in agreement with previous studies with outbred mice (12).

The intensity of inflammation was graded on a semiquantitative scale of 0 to 3 by examination of the most severely affected ×20 microscopic field for each section (Table 1). Longitudinal and vertical extents of inflammation in the entire section were also scored from 0 to 3, and the three scores were added together to give an overall inflammation score reflecting severity. At 2 and 11 weeks postinfection, all three strains had evidence of gastric inflammation the magnitude of which did not, however, show an obvious correlation with bacterial load. Both C3H and C57BL/6 mice had significantly more severe inflammation than did BALB/c mice ($P < 0.01$). Only the C57BL/6 mice developed progressive chronic inflammation at 11 weeks postinfection; BALB/c mice continued to show only a minimal inflammatory response, and the C3H mice showed a reduced level of inflammation at 11 weeks postinfection.

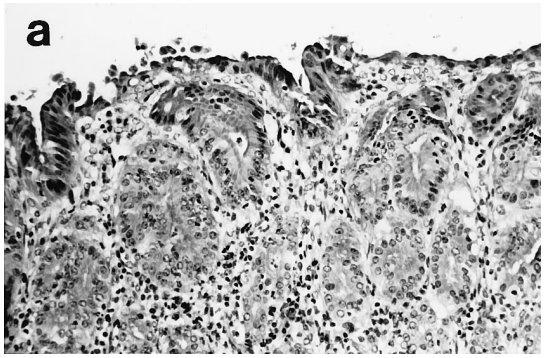
The inflammatory response in C3H mice at 2 weeks and in C57BL/6 mice at both 2 and 11 weeks had significant effects on mucosal architecture. One dramatic change was a variation in mucosal thickness due to mucous cell hyperplasia (see below) and cellular infiltration. Mucosal thickness in the three strains was equivalent in uninfected mice (not shown) but increased dramatically with inflammation (Fig. 1b to 1d). Other signs of mucosal disruption included glandular distortion and necrosis, fibrosis of the lamina propria, and erosions of the surface epithelium (Fig. 2a). These erosions were seen only in C3H mice. The presence of epithelial erosions prompted us to test our *H. felis* strain for the presence of the CagA and VacA genes. PCR amplification of *H. felis* DNA demonstrated a lack of these two genes (data not shown), whereas PCR amplification of the urease gene gave a fragment of the expected size. As an additional control, a cytotoxin-positive *H. pylori* strain was positive for CagA and VacA genes.

Character of inflammation. Finally, the cellular composition of the inflammatory cell response was determined in the three inbred strains of *H. felis*-infected mice. These results are presented in Table 2. Consistent with published reports, mononuclear lymphocytes were observed in the gastric inflammatory cell infiltrate of BALB/c mice at both 2 and 11 weeks. Similar numbers of mononuclear cells were observed in C3H and C57BL/6 mice. Only in C3H mice and primarily at 2 weeks postinfection were a significant ($P < 0.01$) number of activated mononuclear cells observed (Fig. 2b). This is the same mouse strain and time point for which erosions and crypt abscesses were observed (Fig. 2a and Table 2). In addition to the mononuclear cells, small numbers of neutrophils were observed in C3H mice at both time points, and moderate (2 weeks) to large (11 weeks) numbers of neutrophils were noted in C57BL/6 mice. Neutrophils in C3H mice were primarily associated with crypt abscesses, while those in C57BL/6 mice tended to localize in the lamina propria (Fig. 2b and d). Neither plasma cells nor lymphoid aggregates were observed to an appreciable degree in any mice at 2 weeks postinfection. In contrast, at 11 weeks small (BALB/c and C3H) to moderate (C57BL/6) ($P < 0.01$ versus the other two strains) numbers of plasma cells and small (BALB/c) to moderate (C3H) and high (C57BL/6) ($P < 0.01$ versus the other two strains) numbers of lymphoid aggregates were observed in the gastric mucosae of infected mice. The appearance of plasma cells in the gastric mucosa at 11 weeks coincided with a 10-fold or greater rise in *H. felis*-specific serum antibody titers in all strains of mice (results not shown), although no effort was made to determine the specificity of the plasma cells located in the mucosa.

Inflammation was associated with vacuolation of fundic glandular cells in C3H and C57BL/6 (but not BALB/c) mice (Fig. 2e). Mucin carmine, Alcian Blue (pH 1.0 and 2.5), and periodic acid-Schiff stains (Fig. 2e, inset) revealed that this vacuolation represented mucous cell hyperplasia, a lesion also known as pyloric metaplasia in humans (11). Mucous cell hyperplasia was most severe in C57BL/6 mice at 11 weeks (Table 3), paralleling their overall higher levels of colonization and inflammation (Table 1).

Another prominent feature of the inflammatory response to *H. felis* infection was the large number of mast cells observed in the gastric mucosae of infected mice from all three strains (for an example, see Fig. 2c). We took advantage of our Giemsa-stained whole-stomach sections to quantitate these mast cells (Table 3). The number of mast cells, as measured by the average number of mast cells per linear centimeter, was small in C3H mice, intermediate in BALB/c mice, and significantly larger in C57BL/6 mice ($P < 0.05$) at 11 weeks postinfection. The paucity of mast cells was most striking in C3H animals at 11 weeks. No consistent relationship between the number of

FIG. 1. Strain-specific differences in the inflammatory response to *H. felis*. (a) Cluster of six Giemsa-positive spiral bacteria consistent with *H. felis* within a gland from a BALB/c mouse with minimal inflammation. Magnification, ×200. (b) Infected BALB/c mouse at 2 weeks postinoculation with minimal focal inflammation just below the surface epithelium (hematoxylin and eosin stain [H&E]; magnification, ×100). (c) Infected C3H/HeN mouse at 2 weeks postinoculation with severe active gastritis (H&E; magnification, ×45). (d) Infected C57BL/6 mouse at 2 weeks postinoculation with severe acute and chronic inflammation, marked mucus cell hyperplasia, and numerous lymphoid follicles in the mucosa and submucosa (H&E; magnification, ×25).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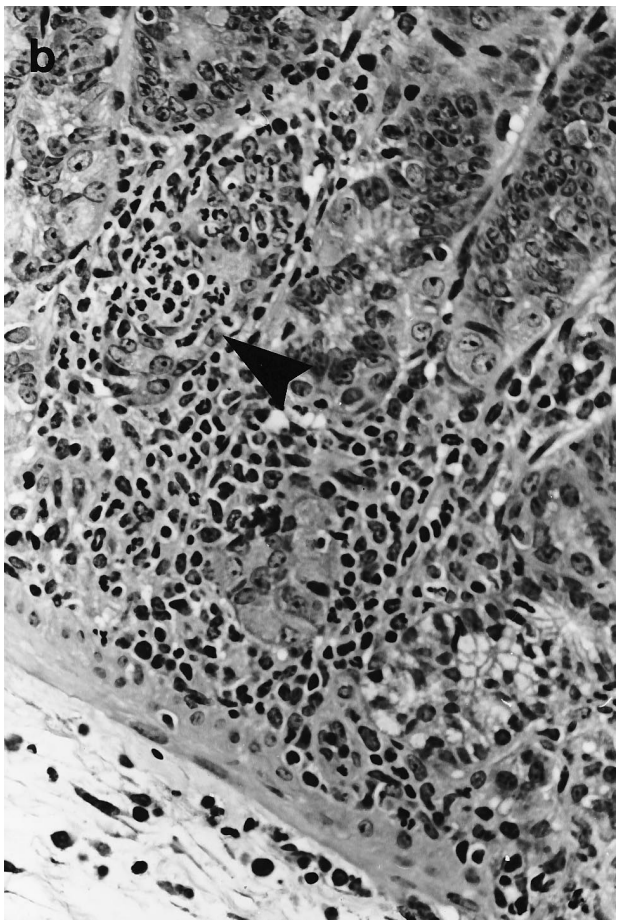
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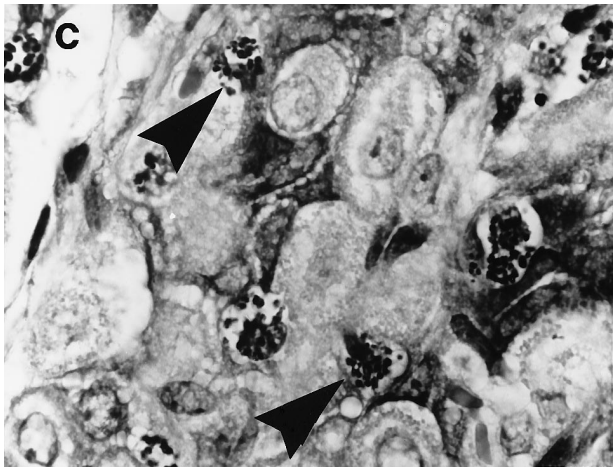
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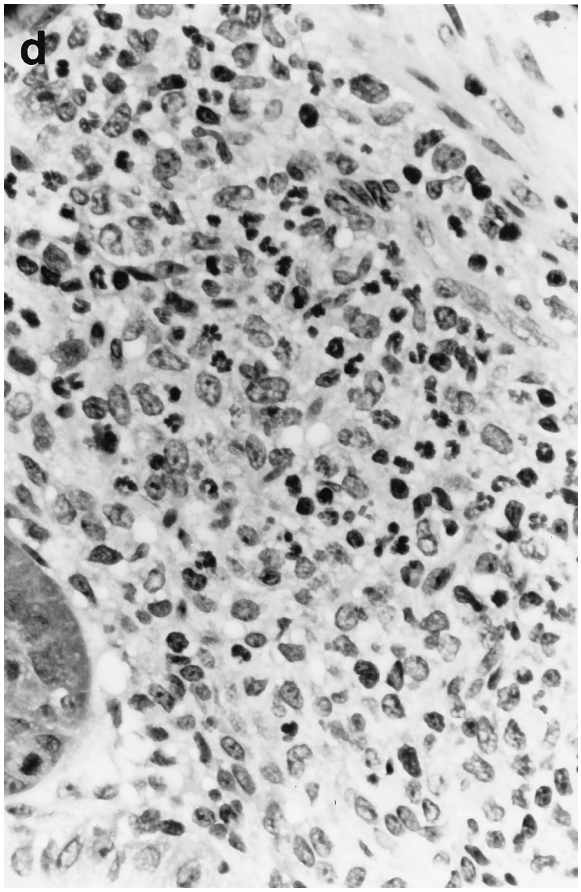
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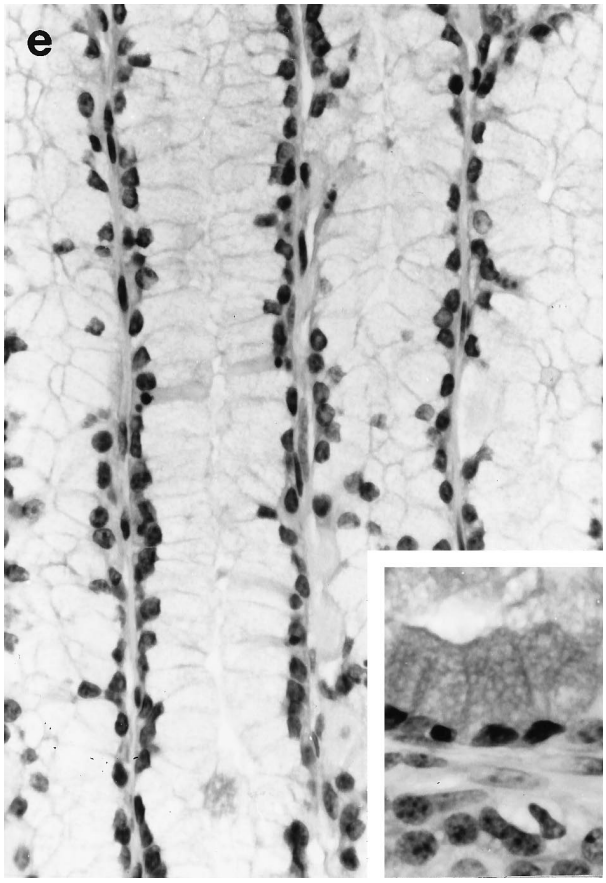
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TABLE 3. Gastric mast cells and mucous cell hyperplasia in *H. felis* infection of inbred mice[a]

Mouse strain	Time p.i. (wks)[b]	Mast cells/cm[c]	Mucous cell hyperplasia[d]
BALB/c	2	68 ± 95	0.1 ± 0.4 ∫
C3H	2	43 ± 42	1.0 ± 1.1
C57BL/6	2	96 ± 132	1.4 ± 1.1 ∞
BALB/c	11	63 ± 20 ∫	0.1 ± 0.3 ‡
C3H	11	22 ± 13 ‡	0.8 ± 0.4 ‡
C57BL/6	11	112 ± 63 ∞†	2.0 ± 0.9 *†

[a] ∞, $P < 0.05$ versus BALB/c; ∫, $P < 0.05$ versus C57BL/6; *, $P < 0.01$ versus BALB/c; †, $P < 0.01$ versus C3H; ‡, $P < 0.01$ versus C57BL/6.
[b] p.i., postinfection.
[c] The number of Giemsa-positive granuolated mucosal mast cells per centimeter of fundic mucosa was determined for each mouse, and the mean ± standard deviation is given.
[d] The grade of mucous cell hyperplasia was semiquantitatively graded on a 0 to 3 scale (mean ± standard deviation).

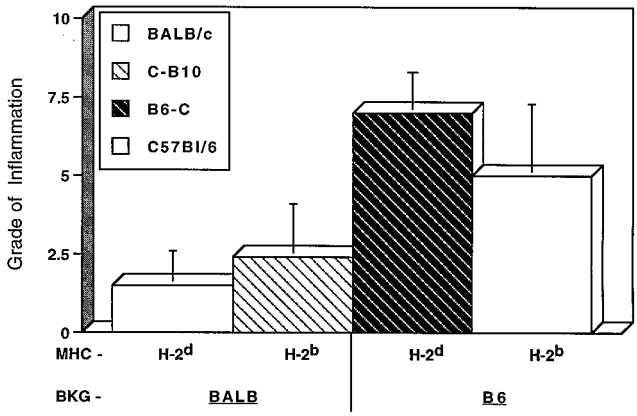

FIG. 3. Susceptibility to *H. felis*-induced gastric inflammation in MHC-congenic mice. Overall grade of inflammation was scored as described in the text. Values for mean ± standard deviation are plotted. BKG, background.

mast cells and the overall intensity of inflammation was found. However, the number of mast cells did seem to correlate with the number of bacteria present. On the basis of the striking differences observed in the gastric inflammation of different inbred strains of mice, particularly BALB/c mice versus C57BL/6 mice, we decided to determine the contribution of MHC genes to disease susceptibility. Therefore, MHC-congenic mice were obtained on BALB and B6 genetic background bearing *H-2*^{b} or *H-2*^{d} haplotypes. These mice were simultaneously infected as described before and sacrificed 2 months after infection. Gastric inflammation in these mice was given an overall grade of 0 to 10 on the basis of all of the above-mentioned characteristics. As shown in Fig. 3, once again mice bearing the B6 background (C57BL/6 and B6-C mice) developed a significantly more severe gastric inflammation than those bearing the BALB background (BALB/c and C-B10 mice) ($P < 0.01$). Therefore, this study showed that susceptibility to *H. felis*-induced gastritis is primarily dependent on the genetic background of the mouse. However, in the susceptible genetic background (B6), congenic mice bearing the *H-2*^{d} haplotype of MHC (B6-C mice) were even more inflamed than those bearing the *H-2*^{b} gene (C57BL/6 mice) ($P < 0.01$). Among mice bearing the low-inflammatory genetic background (BALB), the differences observed in gastric inflammation of *H-2*^{b} and *H-2*^{d} partners were not statistically significant ($P = 0.33$). This suggests that MHC genes may independently increase disease susceptibility as observed for the B6 background.

Collectively, mice with the B6 background, bearing *H-2*^{d} MHC genes, developed the most severe gastric pathology among the different congenic and inbred strains of mice.

DISCUSSION

The prevalence of *H. pylori* infection in adults has been estimated at between 50 and 90%. However, only a relatively small minority of these infected individuals appear to progress to peptic ulcer disease. One hypothesis to explain the discrepancy between prevalence of infection and occurrence of ulcers is the possible existence of "ulcerogenic" strains of *H. pylori*. To date, efforts to identify ulcerogenic bacteria have concentrated on those strains which possess the vacuolating cytotoxin (VacA) and cytotoxin-associated gene product (CagA) (5, 6). There are many more people infected with the purported ulcerogenic strains of bacteria than the number of people with ulcers, however. In one recent study, the presence or absence of vacuolating toxin was directly determined for *H. pylori* strains isolated from patients. This study failed to confirm earlier apparent strong correlations based on serology between the presence of CagA- or VacA-positive *H. pylori* strains and duodenal ulcers (38). Thus, while the hypothesis that ulcerogenic strains of *H. pylori* are important in the development of ulcers remains an attractive one, this hypothesis probably represents an oversimplification. An alternative but not mutually exclusive hypothesis to explain the variation in clinical outcome of *Helicobacter* infections is that some genetic component(s) of the host inflammatory-immune response to *Helicobacter* infection, possibly in concert with an environmental factor(s), may be equally important in the development of peptic ulcer disease. Although we believe that CagA and VacA are important virulence factors for *H. pylori*, we have produced evidence with a mouse model that the host can also play an important role. It should be noted, however, that even though *H. pylori* and *H. felis* are closely related (8), there are important differences such as lack of adherence and *cagA* and *vacA*; thus, complete extrapolation to human *H. pylori* infection is not possible.

The present report, examining the inflammatory response in different inbred strains of mice after infection by a single well-characterized strain of *H. felis*, considerably strengthens the idea that the host response to infection is important in disease outcome. In this regard, it is also noteworthy that recently fresh clinical isolates of *H. pylori* have been reported to colonize immunocompetent mice (25). In that study, BALB/c and outbred mice were infected, and although epithelial erosion was

FIG. 2. Distinctive histologic features of *H. felis* gastritis in different mouse strains. (a) Surface epithelial erosion in a C3H/HeN mouse with severe active gastritis at 2 weeks postinoculation (periodic acid-Schiff stain; magnification, ×80). (b) Active chronic gastritis characterized by a predominance of activated mononuclear cells in the lamina propria and the presence of crypt abscesses (arrowhead). C3H/HeN mouse at 2 weeks postinoculation (H&E; magnification, ×175). (c) Prevalence of mast cells with large Giemsa-positive metachromatic granules (arrowheads). Magnification, ×320. (d) Acute inflammation of the lamina propria involving predominantly polymorphonuclear leukocytes in a C57BL/6 mouse at 2 weeks postinoculation (H&E; magnification, ×150). (e) Mucous cell hyperplasia affecting fundic pits in a severely inflamed C57BL/6 mouse at 11 weeks postinoculation (H&E; magnification, ×200) (inset: PAS positivity of mucous cells; magnification, ×400).

observed with some *H. pylori* isolates, similar to the result reported here, only a mild inflammatory response was observed. Whether it will be possible to successfully infect other inbred strains of mice with fresh isolates of *H. pylori* and characterize the resulting inflammatory response remains to be investigated.

In this study, three strains of mice (BALB/c, C3H, and C57BL/6) were infected with a single strain of *H. felis*. These three strains of mice exhibited differences in both the extent and magnitude of infection as well as differences in the magnitude and character of inflammation. As previously observed for outbred animals (12), the fundus was the site of the most intense inflammation in all strains of mice, and BALB/c mice had minimal inflammation and low-level infection of this site at 2 weeks but had the greatest increase in bacterial levels by 11 weeks. C3H mice had severe active chronic gastritis early in the course of infection and a relatively low level of colonization at both time points. C57BL/6 mice had a large number of organisms at 2 weeks, and even though the numbers of bacteria declined somewhat by 11 weeks, the numbers of organisms still remained relatively large at the latter time point. C57BL/6 mice also exhibited a severe inflammatory response dominated by polymorphonuclear cells at 2 weeks which increased in severity concomitant with the decreased numbers of organisms at 11 weeks. Differences in the response of several inbred strains of mice to *H. felis* infection were also recently reported by Sakagami et al. (33).

There are several other examples of susceptibility to infectious agents which have been observed with the three strains of mice employed in this study. In general, disease susceptibility has not been an inherent characteristic of the mouse strain, as the same strain of mouse may be susceptible to one infectious agent but resistant to another. For example, BALB/c and C3H mice are susceptible to *Listeria monocytogenes* infection while C57BL/6 mice are resistant (14). However, in response to infection with *Salmonella typhimurium*, BALB/c and C57BL/6 mice show susceptibility and C3H mice show resistance (15). Although one recent report suggested that the host response to *Helicobacter* infections may be linked to a class II MHC gene (1), in most cases susceptibility to bacterial diseases has been linked to non-MHC autosomal genes (24). Consistent with these reports, our results also suggest that autosomal non-MHC genes are the primary determinants of disease susceptibility. However, in agreement with the recent human studies, MHC genes may also influence disease susceptibility in *H. felis* infection of congenic mice.

Our observation that *H. felis* could lead to surface epithelial erosions in C3H mice, along with the reports of gastric erosions in mice colonized with either the nonculturable *Helicobacter gastrospirillum* sp., reclassified as *H. helmanii* (34) or with *H. pylori* (10, 25) and the report that mice chronically infected with *H. felis* developed atrophic gastritis (21), indicates that mouse models of *Helicobacter* infections may be able to replicate the full spectrum of disease observed in humans. Since we and others (38) have reported *H. felis* to be deficient in the production of the *Helicobacter* cytotoxin VacA and its associated gene product, CagA, epithelial erosions in C3H mice after *H. felis* infection is a provocative observation. It is possible that *H. felis* has an alternate non-cross-reacting gene product responsible for this reaction or that the change occurs as a result of destructive cytokines such as tumor necrosis factor alpha, produced by activated macrophages.

All three strains of mice developed lymphoid aggregates to different extents by 11 weeks after infection. Normally the stomach does not contain organized lymphoid tissue as do other mucosal sites such as the intestines. The development of lymphoid aggregates in response to *Helicobacter* infection in all three strains suggests that these aggregates are a direct response to a chronic gastric mucosal infection, which could be responsible for antigen uptake, presentation, and the development of humoral and cellular immune response seen in *Helicobacter* infection. If this is true, the stomach may also be considered a mucosal effector organ, as also suggested by Pappo et al. (32).

The appearance of mucosal mast cells and that of mucous cell hyperplasia to varying degrees in all three strains of infected mice are novel observations. *Helicobacter*-associated gastritis has generally been described as having two major components, neutrophils and lymphocytes. Although there have been some suggestions that mast cells might play a role in *Helicobacter*-associated gastritis (20, 37), to our knowledge this is the first time that mast cells have been reported to constitute a significant proportion of the surface inflammatory cells associated with *Helicobacter* infections. The presence of mast cells and mucous cell hyperplasia has been well characterized in the parasitology literature where they have been shown to correlate with elimination of distinct pathogens (30). Both responses are at least in part T cell dependent (30). In our study, mucosal mast cells seem to correlate best with the number of bacteria, while mucous cell hyperplasia was most related to intensity of inflammation. Whether gastric mastocytosis in response to *Helicobacter* infection is a defense mechanism, has deleterious effects, or is merely a side effect of local cytokine production such as interleukin 3 and interleukin 4 is an issue which requires further investigation.

In conclusion, the majority of murine *Helicobacter* investigations to date have utilized either outbred animals or BALB/c mice. The results of the present study suggest, however, that when studying the possible mechanisms of severe disease associated with *Helicobacter* infections, other strains of mice such as C57BL/6 (severe inflammation) or C3H (gastric erosions) may be more appropriate. The results also suggest that in addition to bacterial virulence factors, the genetic background of the host may play an important role in the clinical outcome of *Helicobacter* infections. Differences among mouse strains observed in the present study and, by analogy, among infected humans may represent either direct differences in the character of the inflammatory response to gastric colonization by *Helicobacter* organisms or indirect effects related to genetic differences in elements of the *Helicobacter*-specific immune response, including cytokines secreted by activated T cells and/or antibody responses or immune complexes.

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