Marked Liver Tumorigenesis by Helicobacter hepaticus Requires Perinatal Exposure

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BACKGROUND: Although severe hepatitis and liver tumors occur in a high percentage of A/J male mice naturally infected with Helicobacter hepaticus, these effects have not been observed after injection of adult mice with the bacteria.

OBJECTIVES: We tested the hypothesis that perinatal exposure to the bacteria is required for liver tumorigenesis.

METHODS: A/J female mice were infected by intragastric (ig) or intraperitoneal (ip) treatment with 1.5 × 107 H. hepaticus before pregnancy. We examined offspring at progressive time intervals, including some kept until natural death in old age. A/J, BALB/c, and C57BL/6 weaning male mice were similarly treated with the bacteria and observed for up to 2 years.

RESULTS: After ip bacterial infection of A/J females, 41% of their male offspring developed hepatitis and 33% had hepatocellular tumors, including 18% with hepatocellular carcinoma. Treatment by the ig route resulted in a similar incidence of hepatitis in offspring (35%) but fewer total liver tumors (8%) and carcinomas (4%). By contrast, ig instillation of H. hepaticus in weaning A/J, C57BL/6, or BALB/c mice resulted in low incidence of hepatitis (0–20%) and few liver tumors, despite presence of bacteria confirmed in feces.

CONCLUSIONS: Results indicate that a high incidence of liver tumors in mice infected with H. hepaticus requires perinatal exposure. Contributing perinatal factors could include known high sensitivity of neonatal liver to tumor initiation, and/or modulation of immune response to the bacterium or its toxins. Mechanisms of human perinatal sensitivity to such phenomena can be studied with this model.

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Helicobacter hepaticus is a murine microaerophilic bacterium first noted in association with unexplained cases of hepatitis and liver tumors in our animal colony (Ward et al. 1994b) and subsequently isolated and characterized as a new species (Fox et al. 1994). Koch’s postulates were fulfilled with regard to causation of persistent chronic hepatitis (Fox et al. 1996b). H. hepaticus selectively colonizes the hepatic bile canaliculi of mice, causing a morphologically distinctive pattern of chronic active hepatitis progressis and, also persistently infects the large bowel. H. hepaticus infection is readily established in mice by intragastric (ig) or intraperitoneal (ip) treatment with bacterial suspensions, and this method is commonly used to study the disease.

In our initial study of A/J male mice naturally infected with H. hepaticus, we observed hepatitis of increasing severity with age, with most mice exhibiting hepatitis by 1 year (Rice 1995; Ward et al. 1994a). Liver tumors occurred in 6% of the mice at 1 year of age, in 50% at 15 months, and in 92% at 18 months. This high incidence of liver tumorigenesis was striking, in view of human gastric cancer and lymphoma associated with infection by Helicobacter pylori. A later study with another sample of the naturally infected A/J males gave similar quantitative results, with liver tumors in 40% of the males at 12–15 months of age (Diwan et al. 1997). Similarly, another laboratory reported liver tumors in 75% of naturally infected A/J males at 15–18 months of age (Fox et al. 1996a). Thus, the model seemed promising as an experimental system for study of bacterial carcinogenesis.

However, studies with experimental infection by injection of cultured bacteria have yielded lower liver tumor incidences. In an investigation involving ig treatment of mice with H. hepaticus at 10 weeks of age, and including both sexes and a selection of recombinant inbred strains, liver preneoplasia/neoplasia incidence at 14 months of age was, at most, 20% (Ihrig et al. 1999). Avena et al. (2003) exposed A/J mice at 3–4 weeks of age to a relatively low titer of H. hepaticus (107) ig and examined small groups of mice at ages up to 17 months. Although all mice tested positive for the bacterium, hepatitis was relatively mild, and no liver tumors were observed. Possible insight into the reason for the low yield of liver tumors in these studies was offered by Rogers et al. (2004), who exposed A/J mice to H. hepaticus at various perinatal stages and at 12 weeks of age and evaluated the livers at time points up to 12 months of age. Although the limited numbers of mice in each group constrained statistical certainty, the results suggested that exposure before 12 weeks of age was required for development of hepatitis and liver dysplasia: none of the four male mice treated at 12 weeks had these lesions, whereas such changes occurred in 30–70% of those exposed by treatment of the dams at conception or on fetal day 10, or of offspring at 3 weeks of age.

We therefore tested whether progressive hepatitis and a high incidence of liver tumors with aging requires perinatal exposure, as would occur in naturally infected mouse colonies. Our finding that such is, in fact, the case may have bearing on the potential importance of human perinatal exposure to infectious agents on later health consequences. A preliminary report of this work has appeared in abstract form (Diwan et al. 2001).

Materials and Methods

H. hepaticus culturing, treatment, and detection. We used H. hepaticus, Fredrick strain 1A (passage 7), which was isolated contemporaneously from the same colony of mice as those providing the tissues used for cloning and genetic analysis of the bacterium and deposition in the American Type Culture Collection (Fox et al. 1994, 1996b). We grew the bacteria under microaerophilic conditions at 37°C on plates of Brucella agar with horse blood and trimethoprim, vancomycin, and polymyxin B (TVP) (Remel Co., Lenexa, KS) for 3 days. This bacterial strain has subsequently been shown to be detected by H. hepaticus–specific polymerase chain reaction (PCR) primers and to be negative for all PCR-based tests for all.

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other Helicobacter species. To check for motility and morphology, the organism was harvested, suspended in brain heart infusion broth with horse serum and yeast extract (Remel Co.), and visualized by phase-contrast microscopy.

For the in vivo studies, the organism was harvested and suspended in sterile phosphate-buffered saline (PBS), and the concentration was adjusted to a McFarland equivalence turbidity standard of 1.0 (3 × 10^8 bacteria/mL). We tested viability of the suspended organisms by phase-contrast microscopy to determine motility and by subculturing onto Brucella blood agar plates with TVP as described above, both before and after animal inoculations.

All mice were obtained from the Animal Production Area at NCI Frederick. The National Cancer Institute (NCI)-Frederick is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and follows the U.S. Public Health Service policy for the care and use of laboratory animals. We provided animal care in accordance with the procedures as outlined by the Institute of Laboratory Animal Resources (1996). Animals were treated humanely and with regard for alleviation of suffering. Animal treatments consisted of a single dose of 1.5 × 10^8 bacteria in 0.5 mL PBS.

Treated mice were maintained in semi-rigid isolators (Charles River, Baton Rouge, LA), and controls were housed in rooms free of the bacteria. Mice were kept in polycarbonate cages with cellulose fiber chips as bedding. We assayed feces samples for the presence of Helicobacter spp. by passing the samples through a 0.45-µm filter and then streaking them onto Brucella agar with horse blood and TVP at 37°C under microaerophilic conditions. We compared appearance of growth of these cultures with that of starting cultures of Helicobacter.

Perinatal exposure by maternal injection with Helicobacter. We used uninfect ed female and female A/JCr (A/J) mice for this study. We injected 4-week-old females ip with 0.5 mL bacterial suspension. Feces samples from these mice were checked for presence of the bacteria after 3 weeks, and females positive for fecal bacteria were bred with uninfected males. We allowed some females to produce offspring to test their capability for infection of subsequent generations. We obtained a total of 18 male F2 offspring.

Experimental infection of weaning mice. Uninfected A/J mice were treated at 3–4 weeks of age with H. hepaticus suspension ip or PBS only. Mice exposed to the bacteria were maintained in isolators. At 2, 4, 12, 24, and 54 weeks after treatment, fecal samples were cultured to determine the presence of H. hepaticus, and mice from each group were euthanized as indicated in Table 1. Mice that died naturally or were moribund during the course of the study were included for analysis with the end point group closest in time. A small number of mice were allowed to live until natural morbidity; their average ages are given in Table 1. We collected livers from all animals up to 54 weeks and divided them into lobes; we either froze portions of each lobe in liquid nitrogen or fixed them in 10% neutral formalin. Liver, stomach, and ceca, as appropriate, were included here. Steiner’s silver stain was used for histologic confirmation of the presence of helical bacteria in liver sections.

Statistical analysis. We analyzed data using GraphPad InStat, version 3.00 (GraphPad Software, San Diego, CA). Tests included a t-test for parametric data and Mann-Whitney Kruskal-Wallis tests for nonparametric data as appropriate.

Results

Liver tumorogenesis in male A/J offspring after maternal exposure to H. hepaticus. Females treated with H. hepaticus suspensions ip and...
found to have positive fecal cultures were mated. Four females produced a total of 27 male offspring, which were housed in groups of two to four. All cages gave positive results for *H. hepaticus* in feces, and the number of mice per cage had no obvious relationship to outcomes (data not shown). At an average age of about 22 months, 11 (41%) of these male offspring had *Helicobacter* hepatitis. Of these, 9 (33%) had at least one liver tumor, 7 (26%) had multiple tumors, and 5 (18%) had hepatocellular carcinoma (Table 2). Hepatobiliary tumors, an adenoma and a carcinoma, occurred in 2 mice. We found tumors in offspring of each of the mothers. All of the hepatocellular tumors occurred in livers that also had *Helicobacter* hepatitis, which was moderate to severe in all cases except for one, which was categorized as mild. Three livers that did not have a tumor had minimal or mild chronic active hepatitis. The four mothers were euthanized when moribund at 49–87 weeks of age; of the four livers, three had chronic inflammation of moderate intensity, but no hepatitis characteristic of *Helicobacter* infection.

Female mice treated ig with the same *H. hepaticus* preparations as the ip-treated females and found to have positive fecal cultures were bred. Five mothers produced a total of 26 male offspring. Fecal cultures from both the mothers and the male offspring were positive for *Helicobacter*. Among these male offspring, 35% had *Helicobacter* hepatitis, an incidence similar to that in male offspring of the ip-treated females (Table 2). However, despite greater average age of these mice, the average severity of the hepatitis was less (close to significance). Only two mice (8%) had hepatocellular tumors, and no mouse had multiple liver tumors. These values are significantly different from those in the male offspring of ip-treated females. The five ig-treated mothers were euthanized at 67–105 weeks of age. We did not observe chronic inflammation of the liver (*p* = 0.048 compared with chronic inflammation in three of four ip-treated mothers).

To test the efficacy of bacterial transmission through feces, we exposed pregnant mice and their offspring up to the time of weaning to feces from *Helicobacter*-positive male mice. Eight litters yielded a total of 32 male offspring. Incidence of males with *Helicobacter* hepatitis (19%) was less than that in offspring of mothers exposed ip (41%), but this difference was only of borderline significance (*p* = 0.086), and average severity grade was similar (Table 2). We observed only one liver tumor, a hepatocellular carcinoma in a male of advanced age without hepatitis. Feces from four of the nine cages housing these mice were positive for *H. hepaticus* at approximately monthly samplings between 1 month and 1 year of age.

We further tested fecal transmission by mating *Helicobacter*-positive male offspring from the above study with uninfected females. We obtained a total of 20 male offspring from nine litters. Although these F2 mice were terminated at earlier ages than mice in the other studies, the incidence and average grade of *Helicobacter* hepatitis were very similar to those for the F1 males exposed directly to contaminated feces during gestation and infancy (Table 2). We found no liver tumors in the F2 males.

We noted typhlitis and hyperplasia of gut-associated lymphoid tissue (GALT) in 4–19% of the male offspring in the various perinatal exposure groups, with no significant differences among them.

**Table 2. Histopathologic findings in A/J mice exposed to *H. hepaticus* perinatally and in controls (n [%]).**

| Characteristic                        | Mothers exposed ip | Mothers exposed ig | Mothers and newborns exposed through feces | F2 offspring of feces-exposed males |
|---------------------------------------|--------------------|--------------------|-------------------------------------------|-------------------------------------|
| No.                                   | 27                 | 26                 | 32                                        | 20                                  |
| Average age (days)                    | 674 ± 18           | 674 ± 28           | 647 ± 22                                  | 458 ± 16                            |
| *Helicobacter* hepatitis positive    | 11 (41)            | 9 (35)             | 6 (19)                                    | 5 (25)                              |
| Average grade                         | 3.0 ± 0.4*         | 2.0 ± 0.3*         | 2.5 ± 0.2                                 | 2.6 ± 0.8                           |
| Average age (days)                    | 669 ± 20           | 713 ± 18           | 675 ± 62                                  | 441 ± 40                            |
| Hepatocellular tumors present         | 9* (33)**,†        | 2 (8)**            | 1 (3)*                                    | 0                                   |
| Multiple hepatocellular tumors        | 7 (20)**,‡         | 0†                 | 0                                         | 0                                   |
| Hepatocellular carcinoma              | 5 (18)             | 1 (4)              | 1 (3)                                     | 0                                   |
| Typhlitis                             | 1 (4)              | 3 (12)             | 3 (9)                                     | 1 (6)                               |
| Hyperplasia of GALT                   | 1 (4)              | 3 (12)             | 6 (19)                                    | 3 (15)                              |

Values shown are mean ± SE or number (%) except where indicated.

*Eight had hepatocellular tumors (three with adenomas only, five with adenoma and carcinoma, one each with hepatocellular adenoma and carcinoma and either cholangioma or cholangiocarcinoma), and one had cholangioma only. The liver with hepatobiliary adenoma was the only liver with tumor that did not have *Helicobacter* hepatitis.*

**Multiple multiplicity could not be quantified because of extensive tumor involvement throughout many of the livers. Significance of differences between values with matched superscripts: *p* = 0.07, **p** = 0.039, *p* = 0.0035, *p* = 0.01, *p* = 0.0026.
cages were positive and one was negative. No bacterial growth occurred for the control fecal samples at any time point.

With regard to the gastrointestinal tract, we found hyperplasia of the glandular stomach in 17 of 81 (21%) exposed mice and in 11 of 82 (13%) controls, not a significant difference ($p = 0.22$). Histopathologic examination of ceca revealed hyperplasia of GALT in 7–40% of mice, with no significant differences in incidence between exposed and control mice. Proliferative typhlitis occurred predominantly in the exposed mice (Table 1); incidence was highest at 12–54 weeks after exposure and was significantly greater than controls at 12, 24, and 36 weeks, with an apparent decrease thereafter. The incidence in all exposed mice at 12–36 weeks (21 of 40) was significantly greater than the incidence at > 54 weeks (0 of 6, $p = 0.025$).

**Confirmation of lack of tumorigenic effectiveness of experimental infection with *H. hepaticus* in A/J, BALB/c, and C57BL/6 as weanlings.** To confirm the results noted above for A/J mice and to test whether other mouse strains might be more susceptible, we repeated the experiment and included BALB/c and C57BL/6 mice (Table 3). Small subsets of mice were euthanized at interim time points and all mice remaining in 1 year of age were maintained until natural morbidity. Fecal samples throughout the course of the study confirmed the presence of helical bacteria shed from all of the treated mice and from none of the controls. We observed few age-dependent differences. A summary of lesions for all mice in the study is presented in Table 3.

We diagnosed chronic active hepatitis rarely in two exposed BALB/c mice and one exposed C57BL/6 mouse (Table 3). Liver tumors occurred in low incidence in old mice and were not significantly different in treated versus control groups. Among the experimentally infected mice presenting *H. hepaticus*-associated hepatitis, we found one hepatocellular carcinoma in a BALB/c mouse at 778 days of age, and three adenomas in a C57BL/6 mouse at 720 days.

Several histopathologic changes were significantly associated with treatment in at least one of the mouse strains. We found hyperplasia of the nonglandular stomach in 6 of 30 (20%) infected A/J mice, compared with 0 of 29 controls ($p = 0.024$). Five of the cases were in old mice, and four were moderate or severe. In the other strains, gastric hyperplasia was uncommon and not associated with treatment. Table 3 shows other significant alterations. In A/J mice, proliferative typhlitis was more common in ceca of infected versus control mice at all end points, and these differences were significant for all mice combined (Table 3). However, the incidence of proliferative typhlitis was reduced to 7% in infected A/J mice at 2 years of age. Significant differences in these parameters were also observed for BALB/c mice; in this strain, frequency of hyperplasia of GALT was also higher in the mice infected with *H. hepaticus*: 15 of 23 (65%) versus 9 of 33 (27%) ($p = 0.0066$).

Fecal samples confirmed that all three strains of experimentally infected mice, but no controls, were shedding *H. hepaticus* at all time points.

**Discussion**

The results of this study confirm the findings of other investigations (Avenaud et al. 2003; Ihrig et al. 1999); we found that ig injection of weanling or young adult A/J mice with *H. hepaticus* reliably leads to early hepatic changes—in 40% of the mice in our study at 12 weeks after exposure—as well as marked increases in oxidative DNA damage, but liver tumors are rarely an outcome. With increasing time after exposure, fecal samples became less uniformly positive, a lower proportion of livers exhibited Helicobacter-related damage, and the elevated oxidative DNA damage relative to controls disappeared. We discovered few hepatitis-related tumors or preneoplastic foci, none in A/J mice. These results are in contrast to those reported for naturally infected mice; for example, Siwordewicz et al. (1997) measured a steady increase in 8-oxo-dG at 10, 12, and 18 months of age. Such mice had high incidence (92%) of liver tumors at 18 months of age (Ward et al. 1999b). Together, the results are consistent with host immune response successfully suppressing *H. hepaticus* in mice infected after weaning.

In the A/J and BALB/c mouse strains, infection with *H. hepaticus* resulted in several hepatic and cecal changes, including proliferative typhlitis, as observed in other studies. We observed no effects in C57BL/6 mice, confirming the resistance of this strain to hepatic effects of the bacteria (Ihrig et al. 1999; Ward et al. 1994a, 1994b), despite the greater concentration of bacteria in the ceca of C57BL/6 than in A/J mice (Whary et al. 2001).

In contrast, perinatal infection of A/J mice by ip injection of their mothers with *H. hepaticus* before breeding led to a high incidence of progressive hepatitis and significant numbers of multiple liver tumors, including hepatocellular carcinomas, in their male offspring. This confirms the previous finding of Rogers et al. (2004). The present study partially reproduces the high incidence of liver tumors in naturally infected A/J males and provides an experimental paradigm for generating *H. hepaticus*-associated liver tumors. We observed lesser effects in male offspring of mothers treated with *H. hepaticus* ig before conception. Intragastric treatment might be expected to be less effective for establishing a systemic infection with *H. hepaticus* than ip treatment. We confirmed this by the presence of chronic inflammation in the livers of ip-treated mothers but not ig-treated mothers. In a study with *H. pylori* and a novel *Helicobacter* species, McCathey et al. (1999) found that ip-administered bacteria, but not ig administration at the same titer, resulted in hepatitis and elicited a much greater immune response. Thus, the likelihood of transmission of progressive disease to offspring may be a function of degree of infection of the mother, and the very high incidences of hepatitis and tumors in male offspring of naturally infected mothers, seen in our original population of infected mice, may reflect the level of bacterial burden accumulated over generations. However, the possibility of attenuation of bacterial virulence with serial passaging cannot be ruled out at present.

The bacteria might pass from mother to offspring transplacentally, through milk, or through feces. Li et al. (1998) were able to culture *H. hepaticus* from about 20% of fetal viscera in severe combined immunodeficiency (SCID) mice, but not from any of 14 A/J fetuses. This indicates that transplacental transmission is at least possible. However, Singletary et al. (2003) eliminated mother-to-pup transmission of *H. hepaticus* for C57BL/6 females experimentally infected ig with this bacterium by foster nursing the newborns within 24 hr after birth. Thus, under these conditions, at least, transmission was not possible.
This tumorigenic effect was suppressed by colon and mammary tumor incidence in Rag2 and deficient in lymphocytes because of lack of mice heterozygous for the Apc gene (ApcMin/+). Furthermore, the CDT also causes DNA damage by its DNaSE activity (Avenaud et al. 2004) and thus could contribute to tumor initiation. It is possible that this property of CDT explains the special sensitivity of perinatal, because liver tumors are initiated by genotoxicants with higher efficiency in neonatal than in adult rodents (reviewed by Anderson et al. 2000). In this scenario, chronic hepatitis from H. hepaticus might lead to liver tumors effectively only when these have been initiated perinatally. These hypotheses may be readily tested with the model that we have established. In view of the enhanced virulence of infectious agents encountered in early life in humans, with regard to hepatic cancer caused by hepatitis viruses (Suver 1998), and gastric cancers related to H. pylori (Blaser et al. 1995) and possibly to Epstein-Barr virus (Abdirad et al. 2007), pursuit of these mechanisms is important.

**References**

Abdirad A, Ghaderi-Sohi S, Shuyama K, Koriyama C, Nadimi-Barforoosh H, Emami S, et al. 2007. Epstein-Barr virus associated gastric carcinoma: a report from Iran in the last four decades. Diagn Pathol 2:23; doi:10.1186/1746-1596-2-23 [Online 19 July 2007].

Anderson LM, Diwan BA, Fear NT, Roman E. 2000. Critical windows of exposure for children's health: cancer in human and mouse models. Environ Health Perspect 108(suppl 1):573–594.

Arnold B, Schulter T, Hammerling GJ. 2005. Control of peripheral T-lymphocyte tolerance in neonates and adults. Trends Immunol 26:406–411.

Avenaud P, Castroviejo M, Claret S, Rosenbaum J, Megraud F, Avenaud P, Le Bal B, Mayou K, Aras S, Fawzi R, Bioucas-Silva P, et al. 2003. Natural history of Helicobacter hepaticus infection in conventional A/J mice, with special reference to liver involvement. Infect Immun 71:3687–3692.

Anderson LM, Diwan BA, Fear NT, Roman E. 2000. Critical windows of exposure for children's health: cancer in human and mouse models. Environ Health Perspect 108(suppl 1):573–594.

Blaser MJ, Chyu PH, Nomura A. 1995. Age at establishment of Helicobacter pylori infection and gastric carcinoma, gastric ulcer, and duodenal ulcer risk. Cancer Res 55:562–565.

Chen CC, Taylor NS, Ge Z, Schauer DB, Young VB, Fox JG. 2000. Identification of cdtB homologues and cytotoxic distending toxin activity in enteroheltepatbic spp. J Med Microbiol 49:525–534.

Diwan BA, Ramjijk D, Arner M, Aurelic PL, Ward JM, Hursting SD, et al. 2001. Persistent transmission of Helicobacter hepaticus via transplacental and/or transsectional routes: induction of hepatitis and liver tumors in infected mice (Abstract). Int J Med Microbiol 291(suppl 1):131.

Diwan BA, Ward JM, Ramjijk D, Anderson LM. 1997. Promotion by Helicobacter hepaticus-induced hepatitis of hepatic tumors initiated by N-nitrosodimethylamine in male A/Jc mice. Toxicol Pathol 25:597–605.

Erdman SE, Rao VP, Pouthahidis T, Ihrig MM, Ge Z, Feng Y, et al. 2003. CD4-CD25- regulatory lymphocytes require interleukin 10 to interrupt colon carcinoma in mice. Cancer Res 63:1238–1245.

Fox JG, Dewhirst FE, Tully JG, Paster BJ, Yen L, Taylor NS, et al. 1994. Helicobacter hepaticus sp nov, a macrophagic bacteria isolated from liver and intestinal mucosal scrapings from mice. J Clin Microbiol 32:5477–5484.

Fox JG, Li X, Yan L, Cailhill RJ, Hurley R, Lewis R, et al. 1998a. Chronic proliferative hepatitis in A/J mice associated with persistent Helicobacter hepaticus infection: a model of Helicobacter-induced carcinogenesis. Infect Immun 66:1548–1558.

Fox JG, Yan L, Shames B, Campbell J, Murphy JC, Li X. 1996a. Persistent helicobacter and enterococci in germfree mice infected with Helicobacter hepaticus. Infect Immun 64:3673–3681.

Ge Z, Feng Y, Whary MT, Nambiar PR, Xu S, Ng V, et al. 2005. Cytolethal distending toxin is essential for Helicobacter hepaticus colonization in outbred Swiss Webster mice. Infect Immun 73:3559–3567.

Ge Z, Rogers AB, Feng Y, Lee A, Xu S, Taylor NS, et al. 2007. Bacterial cytolethal distending toxin promotes the development of dysplasia in a model of microbially induced hepatocarcinogenesis. Cell Microbiol 9:2070–2080.

Ihrig M, Schrenzel MD, Fox JG. 1999. Differential susceptibility to hepatic inflammation and proliferation in A/Jb recombinant inbred mice. Helicobacter 4:249–259.

Pratt JS, Sachen KL, Wood HD, Eaton KA, Young VB. 2008. Effects of modulation of host immune responses by the cytotoxic helicobacter of Helicobacter hepaticus. Infect Immun 74:4496–4504.

Rao VP, Pouthahidis T, Ge Z, Nambiar PR, Boussaha M, Wan YY, et al. 2006a. Innate immune inflammatory response against enteric bacteria Helicobacter hepaticus induces mammary adenocarcinomas in mice. Cancer Res 66:7395–7400.

Rao VP, Pouthahidis T, Ge Z, Nambiar PR, Horwitz BH, Fox JG, et al. 2006b. Proinflammatory CD4-CD25- lymphocytes promote mammary and intestinal carcinogenesis in ApcMin/+ mice. Cancer Res 66:57–61.

Rice JN. 1995. Helicobacter hepaticus, a recently recognized bacterial pathogen, associated with chronic hepatitis and hepatocellular neoplasia in laboratory mice. Emer Infect Dis 1:119–131.

Ward JM, Arner MR, Haines DC, Benveniste RE. 1994a. Chronic active hepatitis in mice caused by Helicobacter hepaticus. Am J Pathol 145:959–968.

Ward JM, Fox JG, Arner MR, Haines DC, George CV, Collins MJ, et al. 1994b. Chronic active hepatitis and associated liver tumors in mice caused by a persistent bacterial infection with a novel Helicobacter species. J Natl Cancer Inst 86:1222–1227.

Whary MT, Cline J, King A, Ge Z, Shen Z, Shepard B, et al. 2001. Long-term colonization levels of Helicobacter hepaticus in the cecum of hepatitis-prone A/Jc mice are significantly lower than those in hepatitis-resistant C57BL/6j mice. Comp Med 51:413–417.

Young VB, Knox KA, Pratt JS, Cortez JS, Mansfield LS, Rogers AB, et al. 2004. In vivo and in vitro characterization of Helicobacter hepaticus cytolethal distending toxin mutants. Infect Immun 72:2521–2527.