Comparative Medicine
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Vol 59, No 1
February 2009
Pages 10–17

Effects of *Helicobacter* Infection on Research: The Case for Eradication of *Helicobacter* from Rodent Research Colonies

Maciej Chichlowski and Laura P Hale*

Infection of mouse colonies with *Helicobacter* spp. has become an increasing concern for the research community. Although *Helicobacter* infection may cause clinical disease, investigators may be unaware that their laboratory mice are infected because the pathology of *Helicobacter* species is host-dependent and may not be recognized clinically. The effects of *Helicobacter* infections are not limited to the gastrointestinal system and can affect reproduction, the development of cancers in gastrointestinal organs and remote organs such as the breast, responses to vaccines, and other areas of research. The data we present in this review show clearly that unintentional *Helicobacter* infection has the potential to significantly interfere with the reliability of research studies based on murine models. Therefore, frequent screening of rodent research colonies for *Helicobacter* spp. and the eradication of these pathogens should be key goals of the research community.

The reliability of an experiment that uses an in vivo model system depends on understanding and controlling all variables that can influence the experimental outcome. Infections of mouse colonies are important to the scientific community because they can introduce such harmful variables. Therefore, the ultimate goal of laboratory animal facilities is to maintain disease-free animals, to eliminate those unwanted variables.

Numerous pathogenic microbes can interfere with animal research (reviewed in reference 57), and colonization of mouse colonies with members of the family *Helicobacteriaceae* is an increasing concern for the research community. Naturally acquired *Helicobacter* infections have been reported in all commonly used laboratory rodent species.[3,10,36,44,45,49,82,124] A study of mice derived from 34 commercial and academic institutions in Canada, Europe, Asia, Australia, and the United States showed that 88% of these institutions had mouse colonies infected with 1 or more *Helicobacter* spp.[109] Approximately 59% of these mice were infected with *Helicobacter hepaticus*; however monoinfections with other species also were encountered. In another study, at least 1 of 5 *Helicobacter* spp. was detected in 88% of the 40 mouse strains tested.[4]

Surveys such as these have established that a broad range of *Helicobacter* spp. may be present in mouse research colonies. Several of those *Helicobacter* species cause disease in laboratory mice. *H. hepaticus* first was identified as a pathogen when it was discovered to be the cause of chronic hepatitis and hepatocellular carcinoma in mice,[26,31,116] either alone or in combination with other *Helicobacter* spp.[78] In addition, *H. typhlonius* causes intestinal inflammation in mice with immunodeficiency or defects in immune regulation;[28,37] *H. muridarum* has been associated with gastritis,[86] and *H. bilis* has been associated with hepatitis[35,38] and colitis.[60,61] Although, *H. rodentium* appears to be relatively nonpathogenic in wild-type and SCID mice,[78] combined infection with *H. rodentium* and *H. typhlonius* results in a high incidence of inflammation-associated neoplasia in IL10$^{-/-}$ mice.[9,46] Further, it is becoming increasingly clear that the effects of *Helicobacter* infections are not limited to the gastrointestinal system. *Helicobacter* infections have been documented to directly or indirectly affect responses as diverse as reproduction, development of breast cancer, and altered immune responses to vaccines.[65,95,99] In addition to effects on rodents, *Helicobacter* spp. can infect other laboratory animals[2,5,27,29,33,36,107] and can colonize different anatomic regions of the gastrointestinal system.[35] This review focuses on the potential effect of these organisms on in vivo experiments and biomedical research. The results summarized here emphasize the importance of knowledge of colony infection status and prevention of unintentional infections to achieve the goal of providing a consistent and reliable environment for research studies.

Biologic characteristics of *Helicobacter* organisms

Helicobacter spp. are gram-negative bacteria that vary in their morphology, growth requirements, biochemical profiles, antibiotic susceptibility, and sequence of conserved 16S rRNA genes.[121] Most *Helicobacter* organisms are long, narrow, slightly curved rods with bipolar sheathed flagella. Detailed data on genus characteristics and methods of detection have been published.[3,57,63,88,121] The species that typically infect rodents and their sites of infection are listed in Table 1. Although it does not naturally infect rodents, *H. pylori* is also included in this list due to its common use in research mouse models. Several *Helicobacter* spp. (*H. pylori, H. hepaticus*, *H. bilis, H. muridarum*) are urease-positive, that is, capable

Received: 24 Jun 2008. Revision requested: 01 Aug 2008. Accepted: 16 Sep 2008.
Department of Pathology, Duke University Medical Center, Durham, North Carolina.
**Corresponding Author. Email: laura.hale@duke.edu*

Table 1. Rodent host species and sites of *Helicobacter* infection.

Helicobacter spp.	Infected species[a]	Site of infection	References
H. aurati	Hamster	Stomach, intestine	82, 83
H. bilis	Mouse, rat	Intestine	35
H. cholecystus	Hamster	Gallbladder	36
H. cinaedi	Hamster	Intestine	108
H. ganmani	Mouse, rat	Intestine	89
H. hepaticus	Mouse, rat, gerbil	Intestine	25, 44, 97
H. mesocricetorum	Hamster	Intestine	104
H. mastomyrinus	*Mastomys natalensis*	Liver	101
H. muridarium	Mouse, rat	Intestine, stomach	54
H. pylori (experimental infections only)	Mouse	Stomach	93
H. rodentium	Mouse, rat	Intestine	100
H. trogontum	Rat, mouse	Intestine	73, 75
H. typhlonius	Mouse, rat	Intestine	39

[a]Unless noted, all mice listed are *Mus musculus*.

of producing ammonia to neutralize gastric acid, whereas others (for example *H. ganmani*, *H. rodentium*, *H. trogontum*, *H. typhlonius*, and others) are urease-negative.[39,100] Production of urease allows microorganisms to survive in the very acidic gastric environment.[96] Interestingly, recent study has shown that *H. hepaticus* urease is not required for intestinal colonization but promotes hepatic inflammation in male A/JCr mice.[42] Most rodent *Helicobacter* species are urease-negative and thus preferentially colonize the intestine, although in some cases they may translocate to the liver and biliary system, stomach,[95] or other tissues.[95,99]

PCR-based techniques, mainly genus-specific PCR and quantitative PCR, typically are used for identification and detection of most *Helicobacter* spp. Molecular detection of *Helicobacter* DNA by using PCR is rapid and sensitive to the early phases of infection. Further enhanced sensitivity is achieved by using nested primers.[63] One of the most important features of the PCR assay is that it can be performed noninvasively on fecal pellets.[3,25,57] Thus analysis for the presence of *Helicobacter* spp. does not require euthanasia of the animals being tested, an especially important aspect when monitoring valuable rodents, including transgenic and knockout mice.

Transmission

Data regarding the transmission of *Helicobacter* spp. are not consistent. For example, *H. hepaticus*—which is morphologically similar to 3 other intestinal *Helicobacter* spp.: *H. fennelliae*, *H. cinaedi*, and *H. canis*[26]—was transmitted from colony to sentinel mice quickly by means of contaminated bedding.[58] Furthermore, *H. hepaticus* was established as a member of the cecal mucosal-associated microbiota rapidly when administered by gavage to adult mice and eventually grow to become a dominant member of the bacterial community by 14 d.[52] Other researchers reported delayed and inconsistent transmission of *H. bilis* to sentinel mice.[120] Clearly, transmission of *Helicobacter* spp. depends heavily on the species involved and on environmental factors, including husbandry practices. Fecal–oral spread is the primary route of natural acquisition of infection in rodents. Once acquired, *Helicobacter* infections are persistent, with long-term shedding of the organisms in feces. Endemic infections therefore are very common in research facilities, unless specific steps are taken to prevent and eradicate these microorganisms. Several studies[21,22,51,95] have reported successful eradication of *Helicobacter* infections. Methods included depopulation followed by restocking with *Helicobacter*-free mice, embryo transfer, cross-fostering, and antibiotic therapy,[21,22,51,95] but the efficiency of these methods may vary depending on species and strain of rodent or *Helicobacter* organism and experimental conditions.

In many cases, investigators can be unaware of the infections, because the pathology of most *Helicobacter* species is host-dependent, and infection may be subclinical.[119] A recent study on prevalence and spread of enterohepatic *Helicobacter* species (*H. ganmani*, *H. hepaticus*, *H. typhlonius*, and others)[4] showed that infections can be prevented effectively by harboring mice in individually ventilated cages even if infected mice are reared in neighboring cages. The same study[4] showed that mice reared in open-air cages were very likely to become infected. However, the use of filter tops on conventional cages was sufficient to prevent *Helicobacter* transmission between cages,[4] probably by preventing spread of contaminated stool and bedding.

Helicobacter infections in humans and mice

Presence of *Helicobacter* spp. profoundly affects the host organism. A recent study[77] profiled the changes in gene expression in cecal tissue from disease-susceptible A/JCr and disease-resistant C57BL/6 mice longitudinally, from the acute to the chronic phases of *H. hepaticus* colonization. The results demonstrated that disease-susceptible A/JCr mice exhibit a sustained or biphasic Th1-mediated inflammatory response, whereas disease-resistant C57BL/6 mice exhibit a transient regulated response that was predominated by immunoglobulin-related gene expression. The immunologic mechanisms that regulate this balance may be related to disruption of cytokine signaling and control of the cell cycle.[77] In addition to dysregulated inflammation, disruption of the epithelial barrier may play a role. Overall, *Helicobacter* organisms in mice[14-16,46,99] have been linked to inflammatory bowel disease[6,48] and breast,[87] liver,[105] gastric[1] and colon cancers.

Colonic inflammation and neoplasia. The first reports of pathogenic intestinal *Helicobacter* infections appeared in 1994.[26,116] Most scientists consider that the pathogenic potential of intestinal *Helicobacter* spp. varies. *Helicobacter hepaticus* or *H. bilis* have been used most frequently to model microbial triggers of colon inflammation, because these species were the first to be linked to the development of inflammatory bowel disease and inflammation-associated neoplasia.[6,37,60,62] A study[20] investigating the association between colonization of the lower intestinal tract with *H. hepaticus* of multiple genetically altered lines, including *Rag*$^{-/-}$ and p53$^{+/-}$ mice, showed rectal prolapse and histologic evidence of proliferative typhlitis. Colitis or proctitis was present in 65% of the animals examined, 89% of which were positive for *H. hepaticus* as detected by species-specific PCR.[20] However, *H. typhlonius*, *H. rodentium*, *H. muridarum*, *H. ganmani*, *H. trogontum*, and other species have also been shown to trigger intestinal inflammation in susceptible mice[28,37,89,103,121,125] and are commonly endemic within research animal facilities. Table 2 lists mouse models of *Helicobacter* infection associated with gastrointestinal and liver cancer.

Table 2. Rodent models of *Helicobacter*-associated gastrointestinal and liver cancer

Mouse strain	*Helicobacter* spp. involved	Tumor	References
INS-GAS	*H. pylori*, *H. felis*	Gastric adenocarcinoma	50,55
BALB/c-IL10$^{-/-}$	*H. hepaticus*	Colon carcinoma	79
IL10$^{-/-}$ (C57BL/6)	*H. typhlonius*, *H. rodentium*, *H. hepaticus*	Colon carcinoma	9,46,53
Mdr1a$^{-/-}$	*H. hepaticus*, *H. bilis*	Colon carcinoma	60,111
Rag2$^{-/-}$	*H. hepaticus*	Colon carcinoma	15
A/JCr	*H. hepaticus*	Hepatocellular carcinoma	92,110
Smad3	*H. hepaticus*, *H. bilis*	Colon carcinoma	62

Infections with *H. hepaticus* and *H. bilis* have been established firmly as a trigger of the colonic inflammation in IL10$^{-/-}$ adult mice susceptible to inflammatory bowel disease[6,60] and will not be considered in this section of the review. Genetically, *H. typhlonius* is very closely related to *H. hepaticus*, having only 2.36% difference in the 16S rRNA gene sequence, but *H. typhlonius* has a unique intervening sequence in this gene that makes it easily recognizable by PCR.[18,39] *H. typhlonius* previously was shown to cause an enteric disease characterized by mucosal hyperplasia and associated inflammation in the cecum and colon in immunodeficient mice[39] and IL10$^{-/-}$ mice.[28] In those studies, colitis was relatively mild, with no development of inflammation-associated neoplasia. Another organism from the *Helicobacter* genus, *H. rodentium*, was the first urease-negative, murine *Helicobacter* species isolated from intestines.[100] *H. rodentium* has been described to be nonpathogenic in adult wild-type mice, but this species did enhance cytokine production in mice also infected with *H. hepaticus*.[78] Coinfection with *H. hepaticus* and *H. rodentium* was associated with augmented cecal gene expression and with clinical diarrheal disease in immunodeficient mice.[78,102]

H. rodentium and *H. typhlonius* caused rapid onset of severe colon inflammation and multiple neoplastic lesions in the colons of IL10$^{-/-}$ mice neonatally coinfected with those 2 strains.[46] Those mice rapidly developed severe colitis with a high rate of rectal prolapse (this condition was the indication for euthanasia in 50% of the mice and occurred as early as 5 wk of age, with a mean of 21 wk). Colonic neoplasia was present in 13 of 14 *Helicobacter*-infected IL10$^{-/-}$ mice (compared with 0 of 8 with spontaneous colitis), with a mean of 4 neoplastic lesions per colon and invasive adenocarcinoma in 57%. In addition, rapid onset of severe inflammatory bowel disease and a high incidence of inflammation-associated neoplasia occurred in IL10$^{-/-}$ mice that were monoinfected with either *H. typhlonius* or *H. rodentium*.[9] In that study, *Helicobacter* organisms were detected in the stomach and all portions of the colon, and *H. rodentium* DNA was detected in the small intestine and in 1 mesenteric lymph node sample. Other researchers have reported the presence of *H. typhlonius* in sex organs and gastric tissue of C57BL/6 mice.[95] Overall, both mixed and monoinfections with *H. rodentium* and *H. typhlonius* suggest a synergistic role of those 2 in their inflammatory mechanism, but the exact mechanism is still unknown.

After infection with *H. hepaticus*, 129/SvEv *Rag2*$^{-/-}$ mice lacking mature lymphocytes developed several different types of colon cancer associated with colitis,[15,16] whereas sham-dosed mice did not show any signs of the disease. Inflammatory bowel disease and carcinoma that developed in *H. hepaticus*-infected *Rag2*$^{-/-}$ mice were abrogated by treatment with IL10-competent regulatory T cells.[16] Interestingly, sites of *H. hepaticus*-induced cancer in that study coincided with the primary sites of colonization of *H. hepaticus* in other inbred strains of mice.[43,119] Other studies using immunodeficient mice have revealed similar protective and therapeutic effects mediated by regulatory T cells in mice with colitis.[64,85] Those authors proposed that a signaling pathway involving IL10 and IL6 is essential in maintaining epithelial homeostasis and modulating epithelial invasion during bacterially driven inflammatory diseases.

Recent studies demonstrated that mice lacking *Smad3*, a signaling molecule in the transforming growth factor β pathway, do not manifest the colon cancer phenotype when maintained in a *Helicobacter*-free environment for as long as 9 mo.[62] Infection of the same mouse strain with *H. hepaticus*, *H. bilis*, and a novel *Helicobacter* species triggered colon cancer in 50% to 66% of the animals.[62] These results add to the numerous reports suggesting that bacterial triggers may be important in colorectal cancer, especially in the context of genetic loss of antiinflammatory and antiproliferative signals, such as those provided by transforming growth factor β. In addition, the same research group demonstrated that *H. bilis* causes inflammatory bowel disease in *Mdr1*$^{-/-}$ mice that are deficient in P-glycoprotein transporter[61] and that coinfection with *H. hepaticus* and *H. bilis* results in colitis and eventually progresses to dysplasia.[60]

Gastric inflammation and inflammation-associated cancer. *Helicobacter* spp. isolated from the stomachs of humans and animals have become a subject of intense research because of their association with gastric disease.[23,66] Infection with *H. pylori* in humans has been associated with progression from gastritis to gastric adenocarcinoma[12] and is recognized as a key risk factor for the development of gastric mucosa-associated lymphoid tissue (MALT) lymphoma.[17] Although *H. pylori* does not naturally infect mice, this species is the best studied member of the *Helicobacter* genus and frequently is used in mice to generate experimental models of gastric inflammation and cancer. In a recent study, INS-GAS transgenic mice overexpressing amidated gastrin were used to elucidate the effect of *H. pylori* eradication therapy conducted at different stages of *H. pylori*-associated gastric pathology.[55] In that study, all *H. pylori*-infected INS-GAS mice had developed gastrointestinal intraepithelial neoplasia or gastric cancer at 28 wk after infection, accompanied by inflammation, loss of parietal and chief cells, and hypertrophy of foveolar glands. When *H. pylori* antimicrobial eradication therapy was instituted at 8 wk after infection, the risk of gastrointestinal intraepithelial neoplasia was reduced to a level comparable to that of uninfected mice. Several clinical trials have examined the efficacy of *H. pylori* eradication in preventing the development of preneoplastic gastric lesions and gastric cancer in humans[74,122,123] and have proven that such eradication is a successful prevention therapy against gastric cancer.

Lee and colleagues[54] were the first to describe *H. muridarum*, a spiral bacterium morphologically and biochemically associated with an inflammatory response in both antral and oxyntic gastric mucosa of mice. The histologic changes in animals infected with *H. muridarum*, characterized by mononuclear and polymorphonuclear leukocytes, closely resembled those in human antral mucosa colonized by *H. pylori*. *H. muridarum* shares various characteristics (morphologically and biochemically) with other microorganisms that colonize gastric mucosa in the normal noninfected host; however, *H. muridarum* provoked a gastric inflammatory reaction. Another research group independently isolated the same bacterium[86] and confirmed that *H. muridarum* has a potential to colonize both the stomach and intestinal tract of rodents. The authors concluded that this bacterium naturally colonizes the ileum and cecum but can also elicit gastritis after colonizing the gastric mucosa of older animals. Currently, no publications report the occurrence of carcinoma associated with *H. muridarum* infection.

Hepatocarcinoma and hepatic inflammation *H. hepaticus* is an enterohepatic species that persistently colonizes the colon and cecum.[98] This bacterium originally was discovered as the causative agent for the development of chronic hepatitis and hepatocellular cancer in A/JCr mice and other mouse strains.[26,34,56,92,116] In addition, *H. hepaticus* reportedly produces hepatocarcinoma with a male bias.[30,91] Tumors usually arise after 18 mo, whereas after 1 y, susceptible mice exhibit chronic hepatitis.[90] Other studies[41,77] have shown that *H. hepaticus* efficiently colonizes the colon of C57BL/6 mice; however substrains vary in their propensity to develop chronic hepatitis after infection. The isolation of *H. hepaticus* from the spleen of 1 mouse 3 wk after infection suggested hematogenous spread of *H. hepaticus* early in the course of disease, but *H. hepaticus* was not isolated from the spleen at later time points. In another carcinogenesis study,[45] B6C3F1 mice that were infected with *H. hepaticus* developed hepatitis, hepatocellular carcinoma, and hemangiosarcoma of the liver. In addition, increases in cell proliferation rates and apoptosis were observed in the livers of male mice with *H. hepaticus*-associated hepatitis.

In an experiment using F1 hybrid mice derived from A/J and C57BL/6 matings,[41] mice from both groups were infected with *H. hepaticus*. Significant hepatitis was noticed in all *H. hepaticus*-infected parental strains 18 mo after infection. Overall, hepatocellular carcinomas or dysplastic liver lesions were present in 69% of *H. hepaticus*-infected F1 male mice, and *H. hepaticus* was isolated from hepatic tissues of all F1 mice with liver tumors. Chronic active hepatitis was prevalent in other strains of mice as well, including C3H/HeNCr, SJL/NCr, BALB/cAnNCr, and SCID/NCr.[115,116] Other *Helicobacter* spp. associated with the rodent models of liver cancer and hepatobiliary system are *H. bilis*,[32,35] *H. mastomyrinus*,[101] and *H. cholecystus*.[36,84]

In humans, hepatic *Helicobacter* species DNA has been identified in liver tissue, bile and gallbladder tissue from patients with primary sclerosing cholangitis, primary biliary cirrhosis, chronic cholecystitis, and biliary tract malignancies.[24,67,81] Increased serum antibodies to enterohepatic *Helicobacter* species have also been detected in humans with chronic liver diseases.[114] In another study,[71] coinfection with both *H. hepaticus* and *H. rodentium* and monoinfection with *H. bilis* promoted cholesterol gallstone formation in C57BL mice. Those researchers suggested that this process was mediated by the species-specific bacterial products or a reaction of the host to these products, possibly through cytokines and other proinflammatory mediators. Interestingly, mice infected with both *H. rodentium* and *H. hepaticus* developed cholesterol gallstones at the higher rate (78%) than did those infected with either *H. rodentium* or *H. hepaticus* alone (30 and 40%, respectively).

Breast cancer. Inflammation induced in the gut by proinflammatory microbial infection possibly could have systemic effects, which would then influence carcinogenic events in distant organs. Adenomatous polyposis coli (*Apc*) is a gene responsible for multiple intestinal neoplasia in human and mice.[80,112] Infecting *Rag2*-deficient C57BL/6 $Apc^{Min/+}$ (multiple intestinal neoplasia) mice with *H. hepaticus* significantly promotes mammary carcinoma in female mice and enhances intestinal adenoma multiplicity by a TNF-dependent mechanism.[87] In that study, mammary tumors from *H. hepaticus*-infected $Rag2^{-/-}Apc^{Min/+}$ mice showed an 18-fold increase in TNF expression when compared with mammary tissue of uninfected mice. In addition, neutralization of TNF by antibody significantly suppressed both intestinal and mammary tumors in *H. hepaticus*-infected mice, suggesting that TNF or its downstream signaling mediators are required to sustain tumors. The authors of that study were the first to report that an enteric microbial infection promotes cancer in the mammary gland. However, whether this outcome is a direct or indirect effect of infection is unknown, given that the presence of *Helicobacter* organisms in the mammary tissue was not analyzed in the study.

Effects on reproduction. Thus far, the information regarding effects of *Helicobacter* spp. on reproduction is scarce. In one study, *H. typhlonius* was detected by PCR in the sex organs of 3 mouse strains: immunodeficient athymic nude-*nu* (*nu-nu*), *Helicobacter*-sensitive C3H/HeJ and *Helicobacter*-resistant C57BL/6 wild type mice.[95] Sentinel mice of these 3 strains became infected at different times after first exposure to an infected cagemate. *H. typhlonius* DNA was documented to be present in testes, epididymis, uterus, and ovaries in all 3 mouse strains tested for a period of 1 to 2 wk at different times after exposure. Most tissues from C3H/HeJ mice were positive during the first round of PCR, whereas most of the samples derived from the C57BL/6J mice needed a second round of PCR with nested primers to detect the positivity. These results suggest that mice experience a transient bacteremia after *Helicobacter* infection, with clearing of microorganisms rapidly from nontarget tissues followed by preferential long-term colonization of the gastrointestinal tract. In addition, *H. hepaticus* was cultured from fetal viscera of 2 of 11 pups sampled late in gestation from infected SCID/NCr females, suggesting transplacental infection of *H. hepaticus*.[56] Apparent differences among different mouse strains and animal facilities in the detection of the *Helicobacter* in reproductive tissues might reflect quantification issues and not by the presence (or absence) of infection itself.

Experimental infection with *H. pylori* influenced murine pregnancy by increasing the number of fetal resorptions and producing decreased fetal weights when compared with those of noninfected CD1 mice.[93] Induction of Th1-type responses at the endometrial level was 1 mechanism suggested for these phenomena, but this notion was not investigated further. We recently showed that the reproductive success of C57BL/6 $IL10^{-/-}$ female mice intentionally infected with *H. typhlonius* or *H. rodentium* (or both organisms) was decreased compared with that of noninfected mice.[99] Pregnancy rates and the number of pups surviving to weaning were decreased in infected dams. Quantitative PCR detected *Helicobacter* DNA in the reproductive organs of 1 infected mouse on day 82 after infection and in 4 of 5 mice on day 7 after infection. Treatment with a 4-drug anti*Helicobacter* therapy elimi-

nated PCR-detectable excretion of *Helicobacter* DNA, improved fecundity, and enhanced survival of pups born to previously infected dams. Clearly, additional studies are required to investigate the influence of *Helicobacter* spp. infection on reproductive success in other mouse strains. However, numerous published studies performed with mice that were infected unintentionally or unknown to be infected might have to be reevaluated, because the breeding results in those strains could have been due to *Helicobacter* infection and not to the genetic manipulations or treatments used.

***Helicobacter* and immunity.** Oral tolerance is a systemic unresponsiveness (that is, lack of specific antibody production or cell-mediated response) to the same antigens subsequently delivered systemically.[7] In some conditions, oral tolerance can be inhibited, and this effect can be considered equivalent to promotion of sensitization. Such suppression involves signaling by an array of facultative antigen-presenting cells, dendritic cells, and regulatory T cells, as well as lymphocyte anergy or deletion. The inhibitory effect of *Helicobacter* infection on oral tolerance potentially could contribute to the persistent, chronic gastric inflammation and development of allergic response in the laboratory animal models. Indeed, the association between *H. pylori* infection and food allergy[11,19] as well as with other allergic diseases like chronic urticaria,[8,118] atopic dermatitis,[76] and hereditary angioneurotic edema[113] has been suggested. Infection with *H. pylori* or *H. felis* has been shown to increase the absorption of antigens across the digestive epithelium in vitro[69] and across the gastric mucosa in vivo in mice.[68] In 1 study,[117] scientists studied gastric inflammation and T cell response in *H. pylori*-challenged mice after intraperitoneal immunization using *H. pylori* whole-cell lysates in the absence of adjuvants. *H. pylori*-challenged mice without immunization developed moderate to severe gastric inflammation, whereas mice inoculated with *H. pylori* after immunization had little or no gastric inflammation despite persistent colonization with this *Helicobacter* species. The mechanism described by the authors might lead to a state of controlled inflammation in subjects harboring *H. pylori*.

Bacterial flagellin, the primary structural component of flagella, is a dominant target of humoral immunity in response to infection with flagellated pathogenic bacteria[94] including many *Helicobacter* spp. Briefly, flagellin monomers released by bacteria are recognized by receptor molecules and activate innate immunity, in particular triggering a rapid induction of proinflammatory gene expression.[47] Flagellin monomers are a target of the heightened adaptive immune response associated with Crohn disease,[59,106] and purified flagellin functions as a T cell adjuvant.[13,72] Generation of flagellin-specific immunoglobulins in response to intraperitoneal injection with flagellin requires activation of innate immunity, is T-cell–dependent, and can originate from *Helicobacter* flagellin present in the intestinal tract during inflammatory conditions.[94]

C3H/He mice have been used as an experimental model for oral tolerance to ovalbumin.[40] These mice are colonized readily by *H. felis* and develop gastric inflammation in response. Colonization with *H. felis* can inhibit the development of oral tolerance to ovalbumin in mice;[70] development of this tolerance was preserved by treatment rebamipide, a gastroprotective agent used in the treatment of gastritis and ulcerative colitis. The mechanisms may involve the increase in antigenic absorption across the epithelium and the presence of bacterial adjuvants in *H. felis*-infected mice.[68]

Conclusions

The potential influence of *Helicobacter* spp. on *in vivo* experiments continues to be an important issue regarding rodent models used for biomedical research. The data presented here show clearly that infection with *Helicobacter spp*. can affect numerous areas of research. This review focuses on effects of *Helicobacter* infection on the development of cancer (including those of the gastrointestinal tract and breast), immune response, and reproduction of mouse colonies, as well as on the progress of chronic and acute inflammation. Many investigators may be unaware of the infections present in their colonies because the pathology of the infection is host-dependent and can be subclinical. Therefore, frequent screening and the eradication of *Helicobacter* spp. from laboratory animal facilities should be key goals of the research community. The findings we have presented stress the importance of appropriate husbandry practices and the prevention of infection to provide a reliable environment for future research studies and results.

Acknowledgment

This work was supported by grant 5R01CA115480 from the National Institutes of Health.

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