Published in final edited form as:
Methods Mol Biol. 2012 ; 921: 89–98. doi:10.1007/978-1-62703-005-2_12.

Rodent models of *Helicobacter* infection, inflammation and disease

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Abstract

Establishing a reproducible rodent model of persistent *Helicobacter pylori* infection that resembles the *H. pylori*-associated gastritis observed in humans was a considerable challenge until Lee et al (1) successfully adapted a clinical Cag A and Vac A-expressing strain for the mouse stomach. This so-called SS1 (Sydney) strain has since been extensively used for *H. pylori* research; other rodent-adapted *Helicobacter* strains have subsequently been developed and utilized in wild type and genetically engineered rodent models. These bacteria include both *H. pylori* and the larger but related species *H. felis* (originally isolated from cats). In this chapter we focus mainly on these two *Helicobacter* strains and review the rodent models that have been employed to investigate how *Helicobacter* species induce gastric inflammation and disease.

Mice

Most investigators have chosen to use mouse models because of their widespread availability (including multiple inbred strains and genetically engineered variants), short breeding cycles and the accessibility of experimental reagents. The importance of the host response in determining disease outcome is evident from the diverse range of inflammatory and epithelial responses to experimental *Helicobacter* infections in different murine strains (2, 3, 4).

It is important to note that the morphology of the gastric neoplasia in mice is similar to but not identical to that in humans. To ensure uniformity in reporting criteria, investigators are encouraged to follow standard definitions of gastrointestinal malignancy in rodents (5) and to use established scoring systems to enumerate differences between experimental groups (6).

1. Wild type mice

(1) C57BL/6 mice—This strain has been particularly extensively studied for investigating *Helicobacter pylori's* role in gastric carcinogenesis, due in part to the many genetically engineered knockouts available on this background. Following *H. felis* or *H. pylori* infection, the immune response is predominantly Th1-skewed, with a relatively high level of epithelial cell damage and compensatory hyperproliferative response, associated with low bacterial loads (1, 7). *H. felis* induces more severe gastric inflammation in C57BL/6 mice than is observed with *H. pylori* (4).

Whereas in humans the gastritis caused by *H. pylori* inflammation is characterized by the accumulation of neutrophils and mononuclear cells in the mucosa (8), neutrophil recruitment

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is much less prominent in *H. felis* or *H. pylori*-infected mice. Both *H. pylori*-infected C57BL/6 mice and BALB/c mice show a marked influx of mononuclear cells (3, 4).

H. felis infection can eventually progress via metaplasia and dysplasia to cancer in C57BL/6 mice (9), thus mimicking the morphological sequence of changes observed during gastric carcinogenesis in humans (10). The development of high grade dysplastic lesions was not observed in C57BL/6 mice infected with *H. pylori* SS1, even up to 80 weeks post-infection, the longest term study reported to date (11)

The origins of the neoplastic cells in C57BL/6 mice infected by *H felis* appears to be from bone marrow-derived cells recruited to the site of chronic gastric inflammation induced by *H. felis*. Compelling experimental data point to such bone-marrow derived populations repopulating the gastric niche normally occupied by gastric epithelial progenitors and contributing directly to gastric cancer development (12).

(2) BALB/c mice—BALB/c mice are widely used in both cancer and immunology studies. In contrast to the C57BL/6 strain, BALB/c mice exhibit a Th2-predominant response to *Helicobacter* infection, characterized by higher bacterial colonization levels but fewer epithelial lesions (2, 3). Lymphocytic aggregation is a characteristic feature in BALB/c mice after long term *H. pylori* infection (3), thus these mice have been used as a model of *Helicobacter*-induced MALT lymphoma. Following 22 months *H. felis* infection, 38% of BALB/c mice developed this neoplasm, which was not observed in uninfected controls (13). As in humans, antibiotic therapy can eradicate both the infection and the associated lymphoma, demonstrating that chronic *Helicobacter* infection can stimulate antigen-dependent formation of B cell MALT lymphoma in susceptible hosts (14). MALT lymphomas can also be induced by infection with certain isolates of *H. pylori* in Balb/c mice, though not with SS1 (15).

(3) C3H mice—C3H/HeJ mice carry a mutation in their toll-like receptor 4 gene, rendering them insensitive to lipopolysaccharide. They are relatively easy to colonize with *H. pylori*, including with the fully sequenced strain 26695 or by isogenic mutants deficient in Lewis X or Y expression (16).

Antral colonization with *H. pylori* SS1 was found to be only moderate in C3H/HeJ mice and, compared with infection in C57BL/6 mice, induced relatively little gastric body atrophy after six months infection (4). In contrast, others have reported relatively high bacterial colonization levels in C3H/HeJ mice infection with the same *H. pylori* strain SS1 (17).

2. Knockout or transgenic mice

Many types of genetically engineered mouse models have been used to gain experimental insights into the immunopathogenesis of *Helicobacter* infection and to develop models of *H. pylori*-associated gastric cancer. Most have been employed on the C57BL/6 background. Some of the more informative models that typify the approaches used to dissect the pathogenesis of the response to *H. pylori* are described below:

(1) INS-GAS mice—*Helicobacter* infection in humans is accompanied by mild hypergastrinemia. INS-GAS mice have been engineered on the inbred FVB/N strain to overexpress the human gastrin gene under control of an insulin promoter, resulting in sustained hypergastrinemia. These mice spontaneously develop gastric atrophy and eventually gastric adenocarcinoma, a process that can be rapidly accelerated by experimental infection with *Helicobacter* species (18). This has proven to be a valuable model to investigate the possible synergistic effects of hypergastrinemia and *Helicobacter* infection in gastric carcinogenesis.

Interestingly, male INS-GAS mice have a much more rapid and significant inflammatory and neoplastic responses to *H. pylori* infection, to a high-salt diet (7.5%), and the combination of both diet and infection compared with female INS-GAS mice (18, 19, 20). There is now considerable evidence that concurrent extragastric inflammation in mice can impact *Helicobacter*-induced gastric mucosal damage and also other pathological changes in the stomach (21, 22, 23). However, whereas intestinal *Helminth* infection tends to reduce inflammatory response to *Helicobacter* species (21), Houghton *et al* have reported that coexisting infection of *Toxoplasma gondii* and *H. felis* in BALB/c mice altered the specific *H. felis* immune response and increased IFN-γ, IL-12 with reduced IL-10 expression, leading to a more severe gastric inflammatory response (23). Other gastric species may also modulate the inflammatory response to *H. pylori*. For example, compared to a pure monoculture of *H. pylori* in male germ free INS-GAS mice, *H. pylori*-infected INS-GAS mice with complex gastric microbiota led to more severe gastritis and accelerated intraepithelial neoplasia (24).

(2) Interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) knockout mice—The severity of gastritis and epithelial changes are significantly reduced following *H. felis* or *H. pylori* infection in IFN-γ deficient mice compared with wild type C57BL/6 mice (25, 26), demonstrating the critical role of IFN-γ in *Helicobacter*-induced gastric inflammation and epithelial cell damage. Studies in TNF-α knockout mice have given less clear-cut results: one group reporting TNF-α to be required for *Helicobacter felis*-induced gastritis (25) while another found that although *H. pylori* colonization was increased in both IFN-γ and in TNF-α deficient mice, the gastric inflammatory response was not decreased in the absence of TNF-α (27).

(3) Interleukin-1 beta (IL-1β) transgenic mice—Since El-Omar *et al* in 2000 first demonstrated a link between host genetic factors affecting IL-1β function and the risk of cancer following *H. pylori* infection (28), there has been considerable interest in exploring the role of this acid-inhibiting, proinflammatory cytokine in gastric atrophy and adenocarcinoma development. Synergism between *Helicobacter* infection and IL-1β expression in the promotion of gastric neoplasia was demonstrated by Tu et al (29) in studies of aging *Helicobacter felis*-infected C57BL/6 mice transgenic for IL-1β overexpression in gastric parietal cells.

(4) Interleukin-10 (IL-10) knock out mice—As an anti-inflammatory and immunoregulatory cytokine, IL-10 is an important regulator of the mucosal immune response *in vivo*. IL-10 deficient 129/EvSv mice infected with *H. felis* developed severe hyperplastic gastritis with epithelial cell hyperproliferation and dedifferentiation within 4 weeks of infection. This was associated with a *Helicobacter*-specific Th1 immune response (30). Thus inhibitory mechanisms including IL-10, probably serve to regulate over-exuberant immune and inflammatory reponses to *Helicobacter* species in vivo.

(5) Fas antigen transgenic mice—Epithelial cell apoptosis is an important component of the gastric mucosal response to *H. pylori* infection. To investigate the role of the Fas antigen signaling pathway on *Helicobacter*-infected gastric mucosal growth alterations, Houghton *et al* (31) infected Fas antigen-deficient (lpr) mice on a C57BL/6 background with *H. felis*. Although the Fas knockouts exhibited similar inflammatory responses to wild type C57BL/6 mice, Fas-deficient mice did not undergo mucosal cell apoptosis or gastric atrophy. However, when Fas-deficient mice were reconstituted with wild type bone marrow cells (to obviate early death in the lpr model), gastric cancer were frequently observed after several months of *H. felis* infection (32). This suggests a critical role for Fas-induced

signaling in the gastric mucosa in the prevention of a neoplastic response to a chronic *Helicobacter* infection

(6) p27-deficient mice—Loss of the cyclin-dependent kinase inhibitor $p27^{kip1}$ is a common finding in many human cancers, and is associated with poor prognosis, including in gastric cancer (33). *H. pylori* infection is associated with decreased expression of p27 in human gastric epithelial cells (34), and mice lacking the p27 tumor suppressor protein are tumor-prone when exposed to environmental carcinogens (35). After infection with *H. pylori* SS1, p27-deficient mice develop metaplasia, dysplasia and then gastric cancer after 60 weeks (36).

(7) Cag A-transgenic mice—Murine models of *Helicobacter* infection are not helpful for elucidating the function of the putative *H.pylori* oncogenes CagA since *H. felis* lacks the cag pathogenicity island encoding many genes important for the function of *H. pylori's* type IV secretory system. Although these genes are present in the genome of SS1, they are functionally deficient for CagA translocation. To circumvent this problem CagA transgenic mice have been engineered to express CagA ubiquitously or predominantly in the stomach resulting in hematological and gastrointestinal malignancies, albeit at low frequency (37).

Mongolian gerbils

Mongolian gerbils have been used to study the effects of *H. pylori* infection by several groups worldwide. Severe gastritis, intestinal metaplasia, gastric ulcers and gastric carcinoma have all been reported in *H. pylori*-infected gerbils (38, 39, 40), though some investigators have not been able to reproduce these findings, most likely because the rodents are outbred and the colonies used for experimental manipulations therefore not genetically identical.

As early as 3 weeks after infection with *H. pylori*, inflammatory cells are recruited to the gastric mucosa; consequently there is foveolar hyperplasia, parietal cell loss and the development of mucous cells expressing trefoil factor 2. Inflammation is more prominent in the gastric antrum than in the fundus (41). This distribution of inflammation in gerbils is similar to that observed early in humans, but contrasts with the corpus-predominant bacterial colonization and inflammation reported in either *H. felis* or *H. pylori* infected C57BL/6, BALB/c and C3H mice (4).

The first report of any gastric malignancy developing in experimentally infected animals was published in 1998 by Watanabe and colleagues (42). After 62 weeks of infection with *H. pylori* strain TN2GF4, 10 of 27 (37%) of the infected Mongolian gerbils developed gastric carcinoma with histological similarity to human intestinal type gastric cancer. In the same year Honda *et al* reported similar findings in a small cohort of gerbils infected by the *H. pylori* type strain NCTC 11637 (ATCC 43504) (43). However, other investigators who could not reproduce these findings have questioned whether some of these apparently malignant neoplasms may represent instead heterotopic proliferative glands (44).

Peek *et al* infected outbred Mongolian gerbils with wild type, CagA- deleted, or Vac A-deleted isogenic mutants of *H. pylori* strain G1.1. Increased apoptosis in the gastric antrum was evident early in infection (2–4 weeks post infection) and *H. pylori* induced-inflammation was accompanied by altered gastric epithelial cell cycling and antral epithelial growth which was associated with elevated serum gastrin levels (45). Using a *H. pylori* whole genome microarray, the intact cag pathogenicity island was implicated as being critical in the differential host inflammatory responses of the gastric ulcer-associated B128 strain and the duodenal ulcer strain G1.1 (46).

While Mongolian gerbils have provided a practical model for studying the pathogenesis of *Helicobacter* infection, especially gastric cancer, the lack of transgenic or specific gene knock out strains and limited commercially available immunological reagents, have limited the utilization of these animals in comparison with mice.

Chemical as gastric co-carcinogens with *H. pylori* infection

Gastric carcinoma is a multistep and multifactorial disease (47). Epidemiological evidence points to a role for dietary components, particularly salt and nitrate intake in conjunction with *H. pylori* as increasing the risk for gastric cancer development (47, 48). Therefore many groups have co-administered certain chemical carcinogens to enhance or accelerate the effects of experimental *Helicobacter* infections in studies of gastric carcinogenesis in rodent models.

Synergy between N-methyl-N-Nitrosourea (MNU) or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and *H. pylori* has been shown to increased gastric tumor incidence in Mongolian gerbils (49, 50) and in mice (51, 52).

Use of rodent models to develop gastric cancer prevention strategies

The strong link between *H. pylori* infection and the subsequent development of distal gastric cancer has spurred many intervention studies in humans. Meta-analysis of the diverse and generally underpowered individual trials reported to date indicate that *H. pylori* eradication will probably reduce gastric cancer incidence by about 50%, especially if given relatively early in disease progression (53). Problems with recruitment and the enormous expense of undertaking these large and lengthy clinical studies have made insights from murine models attractive for the investigation of interventions to reduce gastric cancer incidence. Questions regarding the optimal timing of eradication therapy, the advantage of combined eradication/chemoprevention strategies and the utility of therapeutic or preventive targeted *H. pylori* vaccines as an anti-cancer strategy all lend themselves to investigation in rodent models.

For example, *H. pylori* eradication has been shown to markedly reduce stomach cancer incidence in C57BL/6 mice, hypergastrinemic INS-GAS mice and in Mongolian gerbils (8, 54, 55). Majority of gastric MALT lymphoma can similarly be cured by antibiotics at an early stage in mice (13), as it can be in most humans (56).

In *H. pylori* infected, cancer-prone, male hypergastrinemic INS-GAS mice a recent study has shown that a combination of the cyclo-oxygenase 2 selective anti-inflammatory drug sulindac at a dose of 400 ppm in the drinking water and an antimicrobial antibiotic eradication regimen significantly decreased proinflammatory cytokine expression in the stomach and the development of gastric carcinoma (57).

Rodents may also be helpful in evaluating *H. pylori* vaccines as cancer-preventing strategies. For example, Delyria *et al* recently achieved a high immunization rate in C57BL/6 mice and granulocyte colony–stimulating factor (G-CSF) knockout C57BL/6 mice by intranasal administration of *H. pylori* SS1 lysate with cholera toxin as an adjuvant. Strong IFN-γ expression and enhanced Th17 producing T-cell responses were observed in the immunized mice, which may be important for neutrophil recruitment and subsequent phagocytosis of *H. pylori* bacteria (58).

Conclusions

The development of robust murine and Mongolian gerbil models to investigate the effects of *Helicobacter* infection has allowed investigators to examine the importance of host factors,

environmental factors and bacterial strain virulence in the outcome of gastric diseases. This has greatly enhanced our knowledge of the pathogenesis of chronic gastritis and gastric cancer. These rodent models are also being explored to develop novel therapeutic strategies against *H. pylori* infection and thereby limit the related diseases. Consideration of the host, the *Helicobacter* strain and environmental microbial and chemical co-factors are all important for optimal translation of these findings to the clinic.

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