Chapter 19
Helicobacter pylori, Cancer, and the Gastric Microbiota

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Gastric Cancer

Gastric adenocarcinoma is the third leading cause of cancer-related death in the world, resulting in approximately 723,000 deaths in 2012, and the 5-year survival rate in the United States is less than 15 % [1–3].

The most common type of cancer that affects the stomach is adenocarcinoma, but lymphoma and leiomyosarcoma may also occur. Two distinct variants of gastric adenocarcinoma can be differentiated histologically; diffuse-type gastric cancer, which consists of individually infiltrating neoplastic cells that do not form glandular structures, and intestinal-type adenocarcinoma, which progresses through a series of well-defined histological steps [4]. Recent comprehensive molecular analysis of almost 300 primary gastric adenocarcinomas suggested a molecular classification dividing gastric cancer into four subtypes [5]. Cristescu et al. used gene expression data to classify gastric cancer into four molecular subtypes. The first are microsatellite unstable tumors (MSI), which occur in the antrum and possess the best overall prognosis with the lowest rate of reoccurrence. Tumor protein 53 (TP53)-active and TP53-inactive types have an intermediate prognosis, with the latter yielding worse prognosis than the former. Mesenchymal-like type forms the fourth subtype, and predicts the worst prognosis and highest frequency of recurrence [6].

The incidence of gastric adenocarcinoma in developed countries has significantly decreased over the past century, primarily due to a decline in intestinal-type adenocarcinomas in the distal stomach [7, 8]. Conversely, the incidence rates of proximal gastric adenocarcinomas as well as those originating within the gastroesophageal junction have been increasing in both the United States and Europe [9, 10].

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The strongest known risk factor for developing gastric adenocarcinoma is chronic infection with *H. pylori*. The degree to which *H. pylori* increases the risk for gastric adenocarcinoma can vary between studies and is likely dependent on several factors including patient age, selection of controls, and the site and stage of gastric cancer. In one study *H. pylori* infection accounted for 6.2 % of all cancers [11], and in another study the combined incidence of intestinal and diffuse-type gastric cancer in *H. pylori*-colonized individuals was reported to be approximately 3 %, compared with 0 % in uninfected persons [12]. To date it is not possible to accurately predict which infected individuals will develop gastric cancer.

H. pylori

H. pylori is a Gram-negative bacterial species that selectively colonizes the gastric epithelium. In 1994, *H. pylori* was recognized as a Type I carcinogen by the WHO, and chronic infection with this organism is the strongest known risk factor for distal gastric adenocarcinoma [13, 14]. *H. pylori* is usually acquired in childhood and in the absence of combined antibiotic therapy can persist for the lifetime of the host, despite the harsh gastric environment [15]. Interestingly, genetic studies indicate that *H. pylori* has colonized humans for at least 58,000 years [16], and approximately half of the world's population is infected with *H. pylori* leading some to speculate that *H. pylori* is an endogenous member of the gastric microbiota. Between 1 and 3 % of persons colonized with *H. pylori* develop gastric adenocarcinoma [17] and factors that play a role in the pathologic outcome of *H. pylori* infection are multifactorial, including strain-specific bacterial constituents, host genetic factors, alterations of the stem niche and host microbiota, and environmental influences including diet [18].

H. pylori Virulence Factors That Influence Gastric Pathogenesis

Bacterial virulence factors play a key role in determining the risk of developing gastric adenocarcinoma following colonization with *H. pylori*. One *H. pylori* virulence factor that clearly influences cancer risk is the *cag* pathogenicity island (*cag*-PAI), a 40-kB DNA insertion element containing genes which encode proteins that form a type IV bacterial secretion system (T4SS). The *cag* T4SS exports CagA from adherent *H. pylori* across the bacterial and epithelial membranes into host cells [19–22].

H. pylori strains that contain CagA are associated with a 5.8-fold increased risk of developing intestinal and diffuse gastric adenocarcinoma compared with uninfected persons. *H. pylori* strains that lack CagA induce only a 2.2-fold increased risk of developing distal gastric adenocarcinoma compared to uninfected persons

[23]. A meta-analysis of studies examining cancer risk suggests that *H. pylori* strains harboring CagA increase the risk of developing distal gastric adenocarcinoma twofold over the risk incurred by CagA-negative strains of *H. pylori* [24].

Following translocation, CagA can be tyrosine phosphorylated at N-terminal glutamate-proline-isoleucine-tyrosine-alanine (EPIYA) motifs. Four different EPIYA motifs (EPIYA-A, -B, -C, or –D) have been identified within CagA and can be used as indicators of pathologic outcome [25–27]. An elevated risk of developing gastric cancer is associated with an increased burden of CagA EPIYA-C sites [28], and strains that contain the EPIYA-D motif are associated with increased pathogenesis compared with strains harboring C-type CagA [25, 29]. Nonphosphorylated CagA also exerts effects within host cells that contribute to pathogenesis and has multiple effects on the apical-junctional complex. Specifically, unmodified CagA targets β-catenin, E-cadherin, the hepatocyte growth factor receptor c-Met, the phospholipase PLC-γ, the adaptor protein Grb2, and the kinase PAR1b/MARK2, leading to pro-inflammatory and mitogenic responses, disruption of cell-cell junctions, and loss of cellular polarity [30–37]. In addition, nonphosphorylated CagA also associates with the epithelial tight-junction scaffolding protein ZO-1, and the transmembrane protein junctional adhesion molecule (JAM)-A, leading to ineffective assembly of tight junctions in regions where *H. pylori* is attached [34]. In a CagA-independent manner *H. pylori* can also dysregulate the tight junction proteins occludin and claudin-7 and may alter barrier function [38, 39].

Another *H. pylori* constituent linked to the development of gastric cancer is VacA [40, 41]. VacA is a secreted toxin that causes multiple alterations in host gastric epithelial cells, including vacuolation, altered plasma and mitochondrial membrane permeability, autophagy, and apoptosis [40]. All strains of *H. pylori* contain *vacA*, but there are considerable differences in *vacA* sequences among strains. The regions of greatest diversity are localized to the 5′ region of the gene, which encodes the signal sequence and amino-terminus of the secreted toxin (allele types s1a, s1b, s1c, or s2), an intermediate region (allele types i1 or i2), and a mid-region (allele types m1 or m2) [42, 43]. Strains containing type s1, i1, or m1 alleles are strongly associated with gastric cancer [42, 44, 45]. New studies suggest the association between type i1 alleles and gastric cancer may even be stronger than the risk incurred by *vacA* s- or m-types, or even *cag* status [43, 46, 47].

Intriguing new insights suggest that VacA and CagA may counter-regulate each other to manipulate host cell responses. Specifically, CagA antagonizes VacA-induced apoptosis and activates a cell survival pathway mediated by MAPK and the antiapoptotic protein MCL1 [48]. It has recently been reported that the opposing effects of CagA and VacA may be cell lineage specific. In vivo lineage tracing of the gastric epithelium has demonstrated that Lgr5 (leucine-rich repeat-containing G protein-coupled receptor 5) positive cells are self-renewing, multipotent stem cells responsible for long-term renewal of the gastric epithelium [49]. In *H. pylori*-infected persons with gastric cancer the population of $Lgr5^+$ epithelial cells is expanded compared to uninfected persons with cancer. Furthermore, these $Lgr5^+$ epithelial cells are more susceptible to oxidative DNA damage than Lgr5-negative cells [50], indicating that *H. pylori* specifically targets $Lgr5^+$ epithelial cells.

In differentiated gastric epithelial cells, autophagy is induced in order to degrade intracellular CagA, and binding of VacA to the epithelial cell receptor LRP1 leads to a decrease in intracellular glutathione and allows accumulation of reactive oxygen species, which subsequently induced autophagy [51]. Interestingly, CagA was found to accumulate in gastric epithelial cells that express a stem cell marker, CD44 variant 9. These cancer stem-like cells are resistant to reactive oxygen species and as a result CagA is not degraded by autophagy. Collectively these data suggest that the bacterial oncoprotein CagA is able to persist in a subpopulation of host cells with progenitor-like features, which may confer long-term detrimental effects on the host that may lower the threshold for carcinogenesis [51].

Host and Environmental Factors That Influence Gastric Pathogenesis

Host polymorphisms also influence the propensity towards gastric cancer development. IL-1ß is a pro-inflammatory molecule that inhibits acid secretion and is increased within the gastric mucosa of *H. pylori*-infected persons. In the context of *H. pylori* infection, individuals with high-expressing IL-1ß polymorphisms have a significantly increased risk for hypochlorhydria, gastric atrophy, and distal gastric adenocarcinoma compared to individuals with genotypes that limit IL-1ß expression [52]. The combination of a more virulent strain of *H. pylori* in a genetically susceptible person further increases the risk of developing gastric cancer. Individuals harboring high-expressing IL-1ß polymorphisms who are infected with *H. pylori* *cagA*$^+$ or *vacA* s1-type strains have a 25-fold or 87-fold increase in risk, respectively, for developing gastric cancer compared to uninfected individuals [53]. Similar to IL-1ß, TNF-α is also a pro-inflammatory cytokine that inhibits acid secretion, and polymorphisms that increase TNF-α expression are also associated with augmenting the risk of developing gastric cancer and its precursors in the presence of *H. pylori* [54].

Environmental factors such as diet also increase the risk of developing gastric carcinoma. Diets high in salted, pickled, or smoked, or poorly preserved foods, those with a high meat content, and those with low fruit and vegetable content are most commonly associated with an increased risk for developing gastric cancer [55–61]. Within the context of *H. pylori* infection, high dietary salt intake and low iron levels are most highly associated with increased risk for developing gastric cancer [62–64].

To date, infection with *H. pylori* is the strongest identified risk factor for developing gastric cancer; however, human trials have indicated that other components of the gastric microbiota may influence gastric disease progression. In a 15-year follow-up study of 3365 subjects, it was reported that antibiotic therapy directed against *H. pylori* significantly reduced the incidence of gastric cancer. What is especially interesting about this study is that less than half of the individuals who

received antibiotics remained free of *H. pylori* at the 15-year follow-up [65]. This suggests that treatment with antibiotics may modify the non-*H. pylori* microbiota in such a way that the development of gastric cancer is attenuated.

The Human Gastric Microbiota in Gastric Pathogenesis

The acidic environment inherent to the stomach in combination with low levels of cultured bacteria from this site led to assumptions that the stomach was a somewhat sterile environment; however, data now show that the stomach harbors a large and diverse bacterial community with colonization densities ranging from 10^1 to 10^3 colony forming units/g [66]. Moreover, recent advancements in molecular techniques and computational analysis have provided evidence that the complex microbiota colonizing the gastric epithelium may influence gastric homeostasis and disease in combination with *H. pylori* [67].

H. pylori-negative individuals possess highly diverse gastric microbiomes (Fig. 19.1). Sequencing of 1833 bacterial clones from 23 gastric biopsy samples identified 128 phylotypes within 8 bacterial phyla; the 5 most abundant phyla were Proteobacteria, Firmicutes, Bacteroidetes, Fusobacteria, and Actinobacteria [68, 69]. Interestingly, Bik et al. did not detect any *H. pylori*-induced alterations in the composition of the gastric microbiota, although *H. pylori* DNA was detected in 7 individuals who were considered to be *H. pylori* negative by traditional diagnostic technologies [68]. An independent study using tagged 454 pyrosequencing analysis of 3 *H. pylori*-negative gastric biopsy samples identified 262 phylotypes representing 13 phyla [70], supporting the notion of a highly diverse gastric microbiota despite substantial variability in the composition of the microbiota between individuals [68, 70]. In contrast, among *H. pylori*-infected individuals, *H. pylori* was found to be the single most abundant phylotype present in the stomach of persons testing positive for this organism [68, 70]. Among the three *H. pylori*-colonized persons tested, *H. pylori* accounted for 93–97 % of all sequence reads and only 33 phylotypes were detected; 229 fewer phylotypes than were detected in *H. pylori*-negative persons [70]. These data suggest that colonization with *H. pylori* greatly reduces the overall diversity of the gastric microbiota. In a more recent study using DNA microarrays to characterize the gastric microbiota in 12 corpus biopsy samples (8 of which were *H. pylori* positive), Maldonado-Contreras et al. detected 44 phyla with four dominant phyla: Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes. *H. pylori* infection increased the relative abundance of non-*H. pylori*-Proteobacteria, Spirochaetes, and Acidobacteria while decreasing the relative abundance of Actinobacteria, Bacteroidetes, and Firmicutes, compared to patterns seen in uninfected stomachs [71]. In this study, *H. pylori* infection was found to account for 28 % of the variance in the microbiota; however, the bacterial communities in both *H. pylori*-negative and -positive individuals remained highly complex [71].

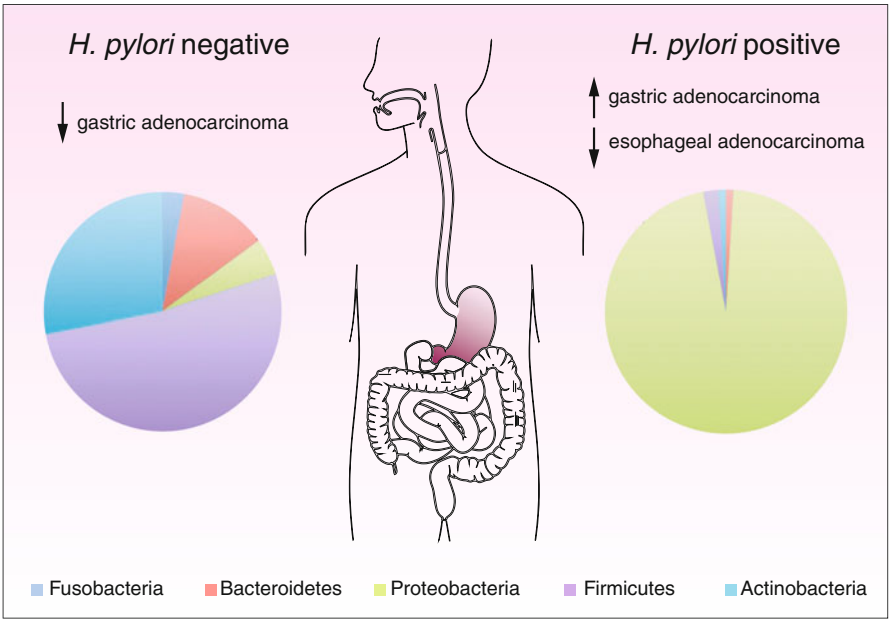

Fig. 19.1 Schematic representation showing the differences in the composition of the human gastric microbiota based on *H. pylori* status. *H. pylori*-negative individuals possess a highly diverse gastric microbiota and exhibit decreased risk of developing gastric adenocarcinoma when compared to *H. pylori* positive individuals who harbor a less diverse microbiota, possess an increased risk for developing gastric adenocarcinoma and concomitant decreased risk for developing esophageal adenocarcinoma

Currently, there are very few studies that have examined differences in microbial composition and outcomes of gastric cancer. One of the key steps in the histologic progression to intestinal-type gastric cancer is the development of atrophic gastritis, a condition that predisposes the stomach to an increase in gastric pH due to loss of parietal cells and overgrowth of non-*Helicobacter* microbiota [4]. A hypochlorhydric environment in the stomach facilitates colonization of other bacteria and may promote the progression towards gastric cancer. In a study focused on the microbiota in ten gastric cancer patients and 5 dysplastic controls using terminal restriction fragment length polymorphism (T-RFLP) in combination with 16S rRNA gene cloning and sequencing, Dicksved et al. found no significant differences between the composition of the gastric microbiota of patients with and without gastric cancer [72]. Specifically, the microbiota of patients with gastric cancer was as complex as the microbiota of dysplastic patients, with five bacterial phyla identified; Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Fusobacteria. *H. pylori* was present in relatively low abundance and the microbiota was, instead, dominated by species of *Streptococcus*, *Lactobacillus*, *Veillonella*, and *Prevotella* [72]. In a more recent study using pyrosequencing to compare the microbiota in gastric mucosa from persons with chronic gastritis, intestinal metaplasia, and gastric cancer, ten bacterial

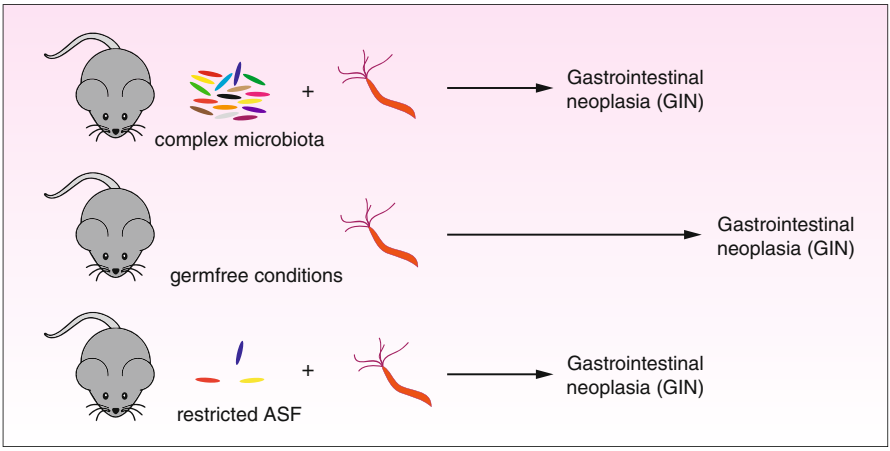

Fig. 19.2 Schematic representation showing the role of the gastric microbiota in the progression towards gastric neoplasia in the context of *H. pylori* infection. Gastrointestinal intraepithelial neoplasia (GIN) spontaneously developed in specific pathogen free (SPF) mice harboring a complex microbiota. In contrast, in germ-free mice, development of GIN in response to *H. pylori* (Hp) infection was over a year slower. In mice harboring a restricted microbiota containing only three species of commensal bacteria (restricted ASF), GIN developed at a rate indistinguishable from SPF mice [89, 92]. Interaction between *H. pylori* and the microbiota influences gastric disease progression

phyla were identified, suggesting the gastric microbiota is even more complex than previously thought [73]. Moreover, significant differences were observed in both the composition and diversity of the gastric microbiota along the distinct histological steps towards gastric cancer. Specifically, Bacilli and members of the Streptococcaceae family were significantly increased in gastric cancer samples compared with chronic gastritis and intestinal metaplasia samples and Epsilonproteobacteria and members of the Helicobacteraceae family were decreased [73].

An interesting dichotomy in disease outcome is that gastric colonization with *H. pylori* appears to confer protection against esophageal adenocarcinoma (Fig. 19.1) [74]. This may be due to *H. pylori*-induced hypochlorhydria as a result of loss of parietal cell function, especially in individuals who possess high expression IL-1ß polymorphisms (see Host factors that influence gastric pathogenesis for further details), or from loss of parietal cells in atrophic gastritis [67]. An alternative hypothesis is that perturbations in the gastric microbiota resulting from the absence of *H. pylori* may increase the propensity for an individual to develop esophageal adenocarcinoma [67].

In order to determine whether changes in the gastric microbiota play a role in the development of gastric cancer or are secondary to the changing gastric environment, further detailed molecular studies to define the composition of the gastric microbiota in well-characterized human populations, with and without gastric cancer, will need to be conducted. Studies of rodent model systems should help identify important drivers and modifiers of diseases related to the microbiome.

The Mongolian Gerbil Gastric Microbiota and Gastric Pathogenesis

The Mongolian gerbil has frequently been used to study *H. pylori*-induced disease and *H. pylori* infection in this model can lead to gastric adenocarcinoma without the co-administration of carcinogens [35, 75–77]. Similar to humans, gastric cancer develops in the distal stomach of gerbils, and another advantage of this model is that several *H. pylori* wild-type and mutant strains colonize well [78–80], thus allowing for the investigation of the role of virulence determinants on parameters of gastric injury. A drawback to this model is that Mongolian gerbils are outbred, which increases the variability of responses to any stimulus and does not allow for genetic manipulation (Fig. 19.3).

Currently, very little is known about the composition of the gerbil gastric microbiota, but most commonly represented phyla include Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes [81, 82]. Similar to mice, the genus *Lactobacillus* dominates the gastric microbiota of uninfected gerbils [81–83].

Sun et al. used molecular techniques to compare alterations in the gerbil gastric microbiota before and after 12 weeks of *H. pylori* infection [83]. Using temporal temperature gradient gel electrophoresis and pyrosequencing of gastric mucosal samples, Sun et al. reported that *Lactobacillus* was the dominant bacteria in the stomach of *H. pylori*-infected as well as in uninfected gerbils [83]. *Bacillus subtilis, Acinetobacter species, Pseudomonas species, Corynebacterium species, Enterococcus species, Paenibacillus species, Staphylococcus species,* along with unidentified bacteria, were also represented in the gerbil gastric microbiota [83]. In a longer-term study of uninfected and *H. pylori*-infected animals, quantitative PCR was used to track the relative abundance of 15 species of microbes in the gerbil stomach following

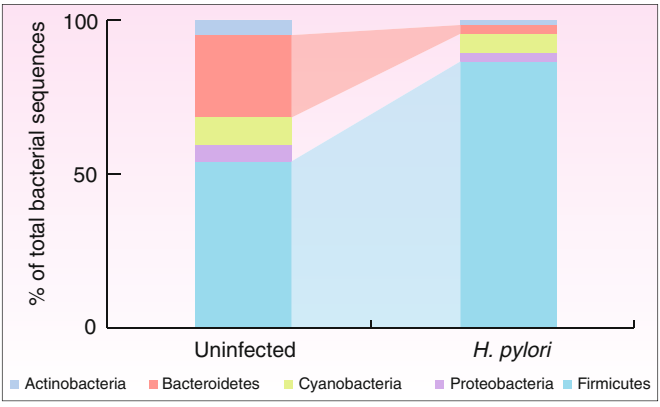

Fig. 19.3 Differences in the composition of the gastric microbiota in *H. pylori*-infected and uninfected mice. There are variations in the relative abundance of phyla in the stomach of *H. pylori*-infected and uninfected INS-GAS mice. *H. pylori* infection significantly increases the relative abundance of *Firmicutes* and decreases *Bacteroidetes* [89]

1 year of infection [84]. In uninfected gerbils, the most abundant genera were *Lactobacillus* and *Enterococcus,* followed by equivalent levels of *Atopobium* and *Clostridium*. In gerbils that were challenged and successfully colonized with *H. pylori*, the relative abundance of *Clostridium coccoides* increased when compared to uninfected gerbils. In gerbils that were challenged with *H. pylori* but not successfully colonized, the proportion of *C. coccoides*, *C. leptum*, and *Bifidobacterium* species was reduced when compared to noninfected gerbils [84]. Another recent analysis of the microbiota in gerbils with and without *H. pylori* infection revealed the presence of *Lactobacillus reuteri, Lactobacillus johnsonii,* and *Lactobacillus murinus* using genomic sequencing and interestingly these strains exerted an inhibitory effect on the growth of *H. pylori* in vitro [85]. The overall importance of differences in microbial composition and the development of gastric cancer; however, have not yet been determined in this model.

The Mouse Gastric Microbiota and Disease

Inbred mice with defined genotypes are another commonly used model of gastric carcinogenesis. In contrast to Mongolian gerbils, transgenic mice can be generated which allows for in-depth analyses of host responses. However, similar to the Mongolian gerbil model, standard inbred mice are frequently limited by their uncontrolled microbial diversity. Gnotobiotic animals are a powerful tool to be able to control the microbiome and add back individual or collections of microorganisms. To date, generation of germ-free gerbils has not been possible; however, gnotobiotic mice can be generated with any required gene mutation to test how genetic alterations in the host may be involved in establishing or controlling the microbiota. The limitations of this model are that it can be very expensive and specialized facilities and expertise are required, limiting their widespread use.

Using 16S rRNA gene cloning and a microarray-based Phylochip microbial profiling system, Rolig et al. identified 10,207 species groups in the mouse stomach and over 2000 of these were identified in all five mice that were analyzed. The Firmicutes phylum accounted for over 50 % of the isolates, with *Clostridia* being the most common class, followed by *Mollicutes* and *Bacilli* respectively. Members of the Bacteroidetes and Verrucomicrobia phyla were the second and third most common phyla, followed by Proteobacteria and Actinobacteria. Similar to what has been reported in the human stomach, the phylotypes with the most members were the *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria* [86].

H. pylori induces chronic atrophic gastritis in the mouse stomach; however, bacteria other than *Helicobacter* species have also been found to induce gastritis in mice. Oral challenge of mice with *Acinetobacter lwoffii* in the absence of *H. pylori* can induce gastric inflammation and metaplastic changes similar to that induced by *H. pylori* [87]. The dominant genus in the uninfected mouse stomach is *Lactobacillus* [81, 82]; however, it is now becoming evident that despite mice having identical genetic backgrounds, their commercial source vendor can affect the composition of

the gastric microbial populations [86, 88]. Despite equal levels of colonization, C57BL/6 mice from two independent vendors developed different grades of inflammation in response to infection with *H. pylori*. Rolig et al. determined that different ratios of *Lactobacillus* species ASF360 and ASF361 were present in the gastric microbiota of mice obtained from two different vendors, and these variations accounted for the differences in inflammation and injury responses when challenged with *H. pylori* [86].

Infection of mice with *H. pylori* can alter the gastric microbiota, which appears to depend on the strain of mouse and duration of infection. In one study using Phylochip analysis, the microbiota of mice infected with *H. pylori* for 4 weeks was not significantly altered overall; however, the abundance of *Firmicutes, Bacteroidetes*, and *Proteobacteria* was decreased and of *Firmicutes* (class *Clostridia*), *Proteobacteria* (genus *Helicobacter*), and *Verrucomicrobia* was increased [86]. Perhaps not surprisingly, administration of antibiotics to mice prior to *H. pylori* infection dramatically altered the composition of the gastric microbiota, changing over 4400 species groups. Members of the *Firmicutes* phylum changed most profoundly and the severity of gastric inflammation in response to *H. pylori* infection was reduced. The inflammatory response was reversed when the gastric microbiota from antibiotic-naïve mice was transferred to mice given antibiotics and was comparable to the inflammatory response observed in an untreated normal mouse [86].

In specific pathogen free (SPF) female Balb/c mice, a 2-month infection with *H. pylori* was found to alter the gastric microbiota by reducing the number of *Lactobacillus* species and increasing bacterial diversity [89]. Of interest in this study is that immunizing mice with *Salmonella enterica* expressing *H. pylori* urease prevented *H. pylori*-induced changes in the gastric microbiota. It should be noted however that *H. pylori* colonization levels were two orders of magnitude lower in vaccinated mice compared to unvaccinated mice [89]. In contrast, studies in SPF C57BL/6 mice have produced conflicting results. In one study using T-RFLP analysis and culture, both acute and chronic infection of C57BL/6 mice with *H. pylori* did not cause significant shifts in the bacterial composition of the gastric microbiota [90].

In other studies using transgenic hypergastrinemic INS-GAS mice that are genetically predisposed to gastric cancer, chronic interaction between *H. pylori* and the gastric microbiota influenced disease progression [91]. In SPF INS-GAS mice harboring a complex microbiota, gastric cancer spontaneously developed [92, 93]. However, in germ-free INS-GAS mice, it took over a year longer for the development of gastric cancer [91] (Fig. 19.2). In addition, germ-free INS-GAS mice that were infected with *H. pylori* developed less severe lesions and were slower to progress to gastrointestinal intraepithelial neoplasia than *H. pylori*-infected SPF INS-GAS mice with a complex microbiota [91]. When the composition of the gastric microbiota was characterized using 454 sequencing of partial 16S ribosomal DNA amplicons, specific differences in phyla were observed between *H. pylori*-infected and uninfected SPF INS-GAS mice. A 12-week infection with *H. pylori* led to an expansion in the proportion of Firmicutes and decreased numbers of Bacteroidetes while causing an overall increase in species diversity [91]. A more recent study demonstrated that a

restricted microbiota containing only three species of commensal bacteria (ASF356 *Clostridium* species, ASF361 *Lactobacillus murinus*, and ASF519 *Bacteroides species*) was sufficient to promote gastric neoplasia in *H. pylori*-infected INS-GAS mice to the same extent as observed in *H. pylori*-infected SPF INS-GAS mice [94].

Extragastric constituents of the microbiota may also influence outcomes of *H. pylori*-induced disease in mice. Co-infection of C57BL/6 mice with the enterohepatic *Helicobacter* species *H. bilis* or *H. muridarum* significantly attenuated *H. pylori*-induced gastric pathology despite chronic inflammation and efficient colonization of *H. pylori* [95, 96]. Mechanistically this was thought to be mediated through an attenuated T helper 1-associated IgG2c response [96]. In contrast, pre-existing infection with *H. hepaticus* increased *H. pylori*-induced gastric injury at 6 months of infection [95]. The mechanism was not thought to involve a T helper 1-type cell response but, was instead, thought to be mediated by a T helper 17-type cell response to the combined infection [95].

Interestingly, a study suggests that Helminth infections may prevent *H. pylori*-induced changes in the microbiota of INS-GAS mice and may attenuate the severity of *H. pylori*-induced disease [97].

Limitations of Current Models and Alternatives to Investigate the Gastric Microbiota in Gastric Pathogenesis

Although great advances are being made in understanding the complex interplay between the microbiota and *H. pylori* in the development of gastric cancer in animal models, detailed molecular studies are still needed in well-defined human populations to examine differences in the microbiota of *H. pylori*-infected persons with and without gastric cancer [66]. Further, rodent models have several limitations including the finding that the phyla present in *H pylori*-infected human stomachs are not the same as those that predominate in a *H. pylori*-infected rodent stomach. Rodents are not naturally infected with *H. pylori* and need to be experimentally infected with rodent adapted strains. In addition, both the density and topography of *H. pylori* colonization in rodent stomachs does not precisely reflect that of humans [67]. Rodents possess a nonglandular forestomach, which is densely colonized by lactobacilli and can dramatically alter the composition of the rodent gastric microbiome, in contrast to what is present in the human stomach. In addition, some bacteria identified in the mouse stomach may be transient due to coprophagia, further confounding results [81].

The rhesus monkey (*Macaca mulatta*) is an exciting new model for studying the interactions between *H. pylori* and constituents of the gastric microbiota. Similar to humans, rhesus monkeys are naturally infected early in life with *H. pylori* strains which are indistinguishable from human strains. In addition, the anatomy of the rhesus monkey stomach is similar to humans, and multiple samples can be obtained over time by endoscopy [98]. A recent study in SPF rhesus monkeys using 454-pyrosequencing of the hyper-variable region of microbial 16S rRNA gene in

combination with high-throughput analysis of corpus and antral gastric biopsies reported that *Helicobacter* species dominate the gastric microbial community when present, although it should be noted that another 220 phylotypes were also detected. *Helicobacter* dominated the corpus to a larger degree than the antrum perhaps due to the lower pH found in the gastric corpus. However, infection with *H. pylori* was not found to significantly alter the relative abundance of other taxa [98].

Conclusions and Outlook

Gastric adenocarcinoma results in a high number of cancer-related deaths throughout the world and understanding the risk factors for this disease is crucial to identify individuals who are at highest risk for developing disease. Approximately half of the world's population is infected with *H. pylori*; however, 97–99 % of colonized persons will never develop gastric cancer. The risk of developing gastric cancer is multifactorial and recently, the role of the gastric microbiota as an important contributing factor in the progression towards gastric cancer has been identified. The role that the microbiota plays in obesity has been extensively studied and microbial genes can predict obesity with 90 % accuracy [99]. It is tempting to speculate that in the future, it may be possible to identify groups of bacterial taxa present in the stomach that are predictive of gastric disease outcome at specific stages along the Correa cascade. Indeed, it may also be possible to manipulate an individual's specific microbiota to proffer more favorable outcomes following infection with *H. pylori*. Detailed analyses of the human gastric microbiome still need to be conducted and it will be critical to carefully dissect causal versus effect changes. Importantly, delineation of the gastric microbiome is a rapidly evolving and exciting new area for research into the prevention and management of gastric disease.

Disclosures/Conflict of Interest The authors declare there are no conflicts of interest.

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