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Original article

Gnotobiotic transgenic mice reveal that transmission of *Helicobacter pylori* is facilitated by loss of acid-producing parietal cells in donors and recipients

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Abstract

Helicobacter pylori is acquired during childhood, but its mode of transmission remains unclear. A genotyped *H. pylori* isolate (Hp1) that expresses two classes of adhesins was introduced into the stomachs of three types of germ-free FVB/N mice to model factors that may affect spread of *H. pylori* in humans. Normal mice represented human hosts with normal gastric acid production. Transgenic animals expressing human α-1,3/4-fucosyltransferase in their gastric pit cells represented humans with normal acid production and the commonly encountered Lewis^b histo-blood group receptor for the bacterium's BabA adhesin. *tox*176 transgenic mice have a genetically engineered ablation of their acid-producing parietal cells and increased proliferation of gastric epithelial lineage progenitors that express sialylated glycan receptors for the bacterium's SabA adhesin. These mice mimic features encountered in humans with *H. pylori*-associated chronic atrophic gastritis (CAG). Different combinations and numbers of 6-week-old germ-free normal and transgenic mice were housed together. At least one donor mouse per cage was infected with a single gavage of 10^7 colony–forming units of Hp1. All cagemates were sacrificed 8 weeks later. Cultures of gastric and cecal contents, plus quantitative PCR assays of cecal contents harvested from donors and potential recipients, revealed that transmission only occurred between *tox*176 donors and *tox*176 recipients, and that the distribution of Hp1 along the gastrointestinal tract was significantly broader in mice without parietal cells ($P < 0.001$). Transmission between *tox*176 mice was not attributable to any significant difference in the density of Hp1 colonization of the stomachs of *tox*176 versus normal donors. Our findings lead to the testable hypothesis that the relative hypochlorhydria of young children, and conditions that promote reduced acid production in infected adults (e.g. CAG), represent risk factors for spread of *H. pylori*.

Keywords: *Helicobacter pylori*; Parietal cell; Transmission

1. Introduction

Helicobacter pylori is a microaerophilic Gram-negative bacterium that colonizes the stomachs of approximately half of all humans [1]. Although gastritis inevitably accompanies initial infection, in most cases the host–microbial relationship is relatively benign and not accompanied by overt symptoms. However, a subset of carriers develops severe pathology, including gastric and duodenal ulcers, gastric adenocarcinoma, and gastric B-cell mucosa-associated lymphoid tissue (MALT) lymphoma. The mode of transmission remains unclear. Identifying factors in both the microbe and the host that affect transmissibility is a prerequisite for designing effective public health interventions that prevent or limit the spread of this organism [2,3].

H. pylori is typically acquired in childhood [1,4]. Although evidence for spontaneous clearance in children has been reported [5,6], once acquired, in absence of antibiotic therapy, *H. pylori* generally persists for the lifetime of its host.

Abbreviations: CAG, chronic atrophic gastritis; CFU, colony–forming units; GEP, gastric epithelial lineage progenitors; Le^b, Lewis^b histo-blood group antigen; *tox*176, attenuated diphtheria toxin A fragment.

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To date, no significant reservoirs for *H. pylori* have been found other than the human stomach. Earlier reports of zoonotic transmission of *H. pylori* have been questioned (reviewed in Ref. [7]). Studies of environmental water supplies from many locales throughout the world have failed to culture the organism, although *Helicobacter* DNA has been detected by PCR [8,9].

These observations have led to the conclusion that direct person-to-person contact is the mode of transmission—a notion supported by the increased prevalence in institutionalized individuals [10,11], in family members of children infected with the bacterium [12], and in families with greater numbers of children [13]. Parsonnet et al. [14] showed that the bacterium could be cultured from the vomitus of 16 of 16 *H. pylori*-infected adult volunteers who had been given an agent that induces vomiting, and from the stools of 7 of 14 of these individuals after they received an agent that induces diarrhea. Samples of air taken 3 dm from the subjects during induced vomiting contained culturable *H. pylori* in 6 of 16 cases (38%), while post-vomiting saliva was culture positive in 56%. This group found that *H. pylori*-infected children have an increased incidence of diarrheal illnesses [15] and that treatment of a small cohort of asymptomatic *H. pylori*-infected adults with an inhibitor of gastric acid production produced a 'trend' toward increased numbers of culture-positive stools after induction of diarrhea [16]. However, the authors of the latter report indicated that a number of methodological issues limited their ability to arrive at a definitive conclusion about the role of acid in shedding, and by extrapolation, transmission of *H. pylori*.

Despite these observations, evidence for oral–oral, gastro-oral, and/or fecal–oral transmission has been conflicting, and epidemiologic studies of populations living in developing or developed countries have failed to detect a significant association between the prevalence of *H. pylori* and hepatitis A (known fecal–oral transmission) [17–19].

With this uncertainty in mind, we turned to germ-free mice belonging to the FVB/N inbred strain to analyze two factors that might affect transmission in humans: acid production and the presence of epithelial glycan receptors for bacterial adhesins. The clinical isolate selected for our studies was recovered from a Peruvian patient with gastritis [20]. The isolate, Hp1, expresses adhesins that recognize at least two types of carbohydrate receptors produced by the human gastric epithelium. One type of receptor, the histo-blood group antigen Lewis^b (Le^b; Fucα1,2Galβ1,3[Fucα1,4] GlcNAcβ), is synthesized by mucus-producing pit and surface epithelial cells in a majority of humans, but is absent in normal FVB/N mice. It binds the product of the *H. pylori babA2* gene [21–23]. The other type of receptor contains NeuAcα2,3Galβ1,4 epitopes (e.g. sialyl-Lewis^x) and binds the product of the *sabA* gene [24]. In normal adult germ-free FVB/N mice, these sialylated epitopes are expressed by two minor cell populations: (i) multi- and oligo-potential epithelial progenitors that fuel continuous epithelial renewal in each of the stomach's many tubular mucosal invaginations (gastric units) [25,26]; and (ii) a narrow band of differentiated pit cells positioned at the junction between the forestomach (squamous epithelium) and the proximal portion of the glandular epithelium [26]. In humans, these sialylated glycans are also expressed in pathologic states that are precursors to *H. pylori*-associated human gastric adenocarcinoma: i.e. chronic atrophic gastritis (CAG), a condition associated with loss of parietal cells, dysplasia, and metaplasia [27].

Hp1 was introduced into the stomachs of three types of germ-free FVB/N mice to model factors that may affect transmission of *H. pylori* in humans. Normal mice represented human hosts with normal gastric acid production without Le^b glycan receptors for the bacterial BabA adhesin. Transgenic animals expressing human α-1,3/4-fucosyltransferase in their gastric pit cells represented human hosts with normal acid production that also express the Le^b receptor. Engineered production of Le^b in these transgenic mice has no discernible effects on the differentiation programs of other gastric epithelial lineages [28]. The third type of germ-free mouse had features of CAG. A genetically engineered ablation of their acid-producing parietal cells was accomplished by forced expression of an attenuated diphtheria toxin A fragment (*tox*176) [29]. Parietal cell ablation produced a fasting gastric pH ≥ 5.5 (versus <3 in their normal non-transgenic littermates), plus a marked amplification of gastric epithelial lineage progenitors (GEPs) that synthesize NeuAcα2,3Galβ1,4 glycan receptors for the SabA adhesin [25,26,30].

We reasoned that by comparing transmission between various combinations of these genetically and environmentally defined mouse models, we would be able to identify the contributions of parietal cells and adhesin receptors to the spread of *H. pylori*. We had previously shown that a single gavage of 10^7 colony forming units (CFU) of Hp1 results in persistent colonization (>4 weeks) in 95–100% of each type of FVB/N mouse [26,31]. Hp1 colonizes the parietal-cell-deficient, NeuAcα2,3Galβ1,4 pit-cell-positive niche located at the junction between the forestomach and the proximal portion of the glandular epithelium of the normal germ-free FVB/N mouse stomach (i.e. the 'forestomach/zymogenic' transition). Colonization leads to formation of lymphoid aggregates and diffuse mild gastritis in this region of the gastric ecosystem [26]. Parietal cell ablation in *tox*176 mice does not affect the density of colonization. However, the area of colonization and lymphoid aggregate formation/gastritis expand from the forestomach/zymogenic transition to the normally acid-protected and now GEP-enriched middle third of the stomach [26]. These findings demonstrate that parietal cells and sialylated glycan receptors for bacterial adhesins play an important role determining the positioning of *H. pylori* within the gastric ecosystem and the geographic patterns of the immuno-inflammatory response. The studies described below now demonstrate that parietal cell loss in *tox*176 mice promotes transmission.

2. Materials and methods

2.1. Mice

All experiments involving mice were conducted using protocols approved by the Animal Studies Committee of Washington University. Le^b transgenic mice contain a human α-1,3/4-fucosyltransferase ORF under the control of nucleotides –596 to +21 of a fatty acid binding protein gene (*Fabpl*) [28]. *tox*176 mice have an attenuated diphtheria toxin A fragment ORF [32] under the control of nucleotides –1035 to +34 of the non-catalytic β-subunit of mouse H^+/K^+ ATPase. *tox*176-mediated parietal cell ablation is >99% complete in 1–52-week-old mice belonging to the pedigree studied [7,25,26,29].

Germ-free transgenic mice and their normal littermates were maintained in plastic gnotobiotic isolators (Standard Safety Equipment). All animals were given an autoclaved chow diet (NIH R and M/AUTO; Purina Mills, Inc.) ad libitum and maintained under a strict 12-h light cycle. All animals were sacrificed at the same time of day (10:00–12:00 h). Transgenic mice were identified using previously described PCR assays of tail DNA [25,28,29].

2.2. Inoculation of germ-free mice with Hp1

Hp1 was cultured in a microaerophilic environment (CampyPak Plus, Becton-Dickinson) for 3 d at 37 °C on brain heart infusion (BHI) agar, supplemented with 10% calf blood, vancomycin (6 μg/ml), trimethoprim (5 μg/ml) and amphotericin B (8 μg/ml). Bacteria were collected [20], concentrated to 10^8 CFU/ml PBS (pH 7.4), brought into the gnotobiotic isolator, and each donor mouse was inoculated with a single gavage of 10^7 CFU. Donors (normal, Le^b or *tox*176) were then placed in cages containing different numbers of germ-free normal, Le^b, and/or *tox*176 recipients. Bedding was replaced in each cage in each gnotobiotic isolator every 3 d. Used bedding was immediately placed in sealed plastic bags within the isolator to prevent spread from cage to cage. Food and water supplies were kept separate for each cage to avoid cross-contamination.

2.3. Assays for Hp1 colonization

2.3.1. Culture

All animals in each cage were sacrificed 8 weeks after Hp1 inoculation of donors. Half of each stomach was homogenized in 0.5 ml of BHI medium. Cecal contents were harvested by extrusion and diluted 1:1 with BHI medium. One hundred microliter aliquots of stomach homogenates and cecal contents were plated on selective BHI/blood agar to assay for the presence of cultivatable Hp1. CFU were scored after a 3–6-d incubation.

2.3.2. Serum ELISA

Sera were obtained from mice at the time of sacrifice and tested for circulating antibodies to Hp1 proteins using a previously described assay [31]. Each serum sample was assayed in duplicate.

2.3.3. Quantitative PCR

A qPCR assay was developed to quantify the number of Hp1 genomes in the cecum. DNA was extracted from cecal contents using the QiaAmp DNA Stool mini kit and the manufacturer's protocol (Qiagen). HP0073 (*ureA*) encoding the urease α-subunit [33] was amplified in 25-μl reactions, containing 300 nM gene-specific primers (HP0073F: 5′-AAACATCGCTTCAATACCCACTTC-3′, HP0073R: 5′-CGGCTGAATTGATGCAAGAA-3′), 12.5 μl 2× SYBR Green PCR Master Mix buffer (Applied Biosystems), 0.25 U UDP-*N*-glycosidase (Life Technologies), and cecal DNA (5 μl of a 1:10 dilution in distilled water). A melting curve was used to identify a temperature where only amplicon, and not primer dimers accounted for SYBR Green-bound fluorescence. All assays were performed in triplicate (ABI Prism 7700 Sequence Detector, Applied Biosystems). The results were referenced to a standard curve generated from cecal contents, recovered from uninfected germ-free normal and *tox*176 mice, that had been spiked with varying numbers of Hp1 (10^3–10^6 CFU) prior to DNA isolation.

3. Results

An initial set of experiments was designed to test transmission among animals in which parietal cell census was normal in donors and potential recipients. Various combinations of 6-week-old germ-free FVB/N normal and Le^b transgenic mice were housed together. At least one mouse per cage was infected with a single gavage of 10^7 CFU Hp1. The variables tested for their impact on transmission were (i) the presence or absence of epithelial Le^b glycan receptors for the bacterial BabA adhesin in donor or recipient; (ii) the ratio of infected donors to naïve recipients (range 1:5 to 5:1); and (iii) the total population density per cage (range two to seven mice) (cages 1–8 in Table 1). Eight conditions for transmission were evaluated in eight cages containing a total of 20 infected donors (defined by positive culture of their stomach at the time of sacrifice) and 21 germ-free recipients. All animals were sacrificed 8 weeks after gavage of the donor(s), and stomach homogenates were cultured on selective medium. Transmission, as defined by recovery of Hp1 CFU, was not observed in any potential recipient.

We next surveyed the impact of parietal cell loss on transmission by gavaging germ-free normal and/or parietal cell-deficient *tox*176 transgenic donors with Hp1, and housing them with germ-free normal and/or *tox*176 potential recipients (cages 9–12 in Table 1). Transmission was observed in one cage containing a high donor/recipient ratio (four infected *tox*176 and one infected normal animal and one naïve *tox*176 recipient; see cage 10 in Table 1).

Since this cage contained infected *tox*176 and normal donors, a follow-up study, involving 21 mice, sought to

Table 1
Transmission experiment involving gnotobiotic normal, Le[b] and *tox*176 donors and recipients

Cage	Donors; number of mice			Recipients; number of mice		
	Genotype	Positive cultures (CFU), stomach	Positive qPCR assays [a], cecum	Genotype	Positive cultures (CFU), stomach	Positive qPCR assays, cecum
1	1 Normal	1 (10^5)	– [b]	1 Normal	0	–
2	3 Normal	3 (10^4–10^5)	0	1 Normal, 1 Le[b]	0	0
3	1 Normal	1 (10^6)	–	1 Normal, 4 Le[b]	0	–
4	2 Le[b]	2 (10^5, 10^6)	–	1 Le[b], 3 Normal	0	–
5	3 Le[b]	3 (10^5–10^7)	0	1 Le[b], 1 Normal	0	0
6	2 Le[b], 1 Normal	3 (10^5–10^7)	0	1 Normal	0	0
7	1 Le[b], 4 Normal	5 (10^4–10^6)	1 Le[b]	1 Le[b]	0	0
8	1 Le[b], 1 Normal	2 (10^5, 10^6)	–	2 Le[b], 3 Normal	0	–
9	2 *tox*176	2 (10^6)	0	1 Normal	0	0
10	**4*tox*176, 1 Normal**	**5 (10^4–10^7)**	**1*tox*176**	**1*tox*176**	**1 (10^7)**	**0**
11	2 *tox*176, 2 Normal	4 (10^5–10^6)	2 *tox*176	1 *tox*176	0	0
12	2 *tox*176, 2 Normal	4 (10^4–10^7)	1 *tox*176	1 *tox*176	0	0

Donors refer to animals that were given a single gavage of 10^7 CFU of Hp1 at 6 weeks of age. Recipients are germ-free cagemates. All animals were maintained in gnotobiotic isolators prior to and following gavage of donors. No positive cultures from cecal material were observed in this experiment. Cage 10 is highlighted in bold to denote that transmission was observed under these conditions.

[a] All animals with a positive HP0073 (*ureA*) qPCR test of their cecal contents contained *H. pylori* DNA at levels equivalent to 10^5 CFU (genomes) per cecum.

[b] '–' not done; entire sample devoted to culture assay.

determine whether transmission could occur between *tox*176 mice. The ratio of *tox*176 donors to potential *tox*176 recipients was varied between 1:2 and 3:1 (Table 2). Transmission was observed in two of the six cages: one containing two infected *tox*176 donors and two *tox*176 recipients (cage 15), and the other, three infected donors and one recipient (cage 18).

Each of the three colonized recipients in these two experiments contained ≥10^5 CFU of Hp1 in their stomachs. Cultures of gastric homogenates prepared from all 47 infected donor mice from cages 1–18 indicated that loss of acid-producing parietal cells in *tox*176 animals was not associated with a significantly higher density of colonization compared to age-matched normal or Le[b] mice (P = 0.21 and 0.18, respectively; Student's t-test).

To establish whether loss of parietal cells enhances the probability of transmission at the level of the donor and/or the recipient, we conducted a third experiment involving nine cages and 45 mice (Table 3). Housing density was set at five animals per cage (n = 3 donors with a common genotype plus two recipients that also shared a common genotype, except in cage 21 where the ratio was 2:3). This arrangement allowed us to test four conditions: normal (donor) → normal (recipient), normal → *tox*176, *tox*176 → normal, and *tox*176 → *tox*176.

Transmission was defined by recovery of Hp1 CFU in recipient stomachs, and a positive ELISA assay where titers of circulating antibodies to *H. pylori* proteins were two standard deviations above the mean for groups of uninfected normal and *tox*176 mice (n = 6 reference controls). We also used a quantitative (q) PCR assay of DNA prepared from cecal contents to ascertain whether transmission was associated with an expanded distribution of Hp1 along the length of the donor's gastrointestinal tract. The assay was targeted to the Hp1 ORF encoding the α-subunit of urease (HP0073; *ureA*). By adding defined numbers of viable Hp1 (10^0–10^6) to the cecal contents of germ-free normal and *tox*176 mice prior to DNA extraction, we established the limit of sensitivity of this assay to be 10^3 CFU (genomes) per cecum. We used this approach to generate a standard curve so that the amount of HP0073 amplicon produced from total cecal DNA preparations could be calculated (Fig. 1S). The assay was specific: no PCR product was detected using DNA prepared from germ-free normal and *tox*176 cecal contents without added bacteria. Aliquots of the same cecal contents used for DNA extraction were also assayed for Hp1 CFU to determine

Table 2
H. pylori can be transmitted between *tox*176 mice housed in the same cage

Cage	Donors; number of mice				Recipients, number of mice			
	Genotype	Positive cultures (CFU)		Positive qPCR assays [a], cecum	Genotype	Positive cultures (CFU)		Positive qPCR assays [a], cecum
		Stomach	Cecum			Stomach	Cecum	
13	1 *tox*176	1 (10^5)	0	1	2 *tox*176	0	0	1
14	2 *tox*176	2 (10^4, 10^5)	1 (10^4)	2	2 *tox*176	0	0	1
15	**2*tox*176**	**2 (10^4, 10^5)**	**0**	**1**	**2*tox*176**	**1 (10^5)**	**0**	**2**
16	2 *tox*176	2 (10^5)	0	2	1 *tox*176	0	0	0
17	2 *tox*176	2 (10^5)	0	2	1 *tox*176	0	0	0
18	**3*tox*176**	**3 (10^5)**	**0**	**1**	**1*tox*176**	**1 (10^5)**	**0**	**0**

Cages 15 and 18 are highlighted in bold to denote that transmission occurred under these conditions.

[a] All animals with a positive HP0073 (*ureA*) qPCR test contained *H. pylori* DNA at levels equivalent to 10^5 genomes per cecum.

Table 3
Evidence that parietal cell ablation in donors and recipients enhances transmission of *H. pylori*

Cage	Donors; number of mice					Recipients; number of mice				
	Genotype	Positive cultures (CFU)		Positive qPCR assays [a], cecum	Positive ELISA, serum	Genotype	Positive cultures (CFU)		Positive qPCR assays [a], cecum	Positive ELISA, serum
		Stomach	Cecum				Stomach	Cecum		
19	3 Normal	3 (10^2–10^4)	0	1	3	2 Normal	0	0	0	0
20	3 Normal	3 (10^2–10^6)	1 (10^3)	1	3	2 Normal	0	0	0	0
21	**2*tox*176**	**2 (10^2–10^5)**	**1 (10^4)**	**2**	**2**	**3*tox*176**	**1 (10^4)**	**0**	**1**	**1**
22	**3*tox*176**	**3 (10^2–10^7)**	**1 (10^4)**	**3**	**3**	**2*tox*176**	**1 (10^5)**	**0**	**1**	**1**
23	3 *tox*176	3 (10^5–10^6)	1 (10^2)	3	3	2 *tox*176	0	0	2	0
24	3 Normal	3 (10^2–10^6)	0	0	3	2 *tox*176	0	0	0	0
25	3 Normal	3 (10^6–10^8)	0	0	3	2 *tox*176	0	0	0	0
26	3 *tox*176	1 (10^7)	1 (10^2)	1	3	2 Normal	0	0	0	0
27	3 *tox*176	2 (10^2–10^8)	0	0	3	2 Normal	0	0	0	0

Cages 21 and 22 are highlighted in bold to denote that transmission occurred under these conditions.
[a] All animals with a positive HP0073 (*ureA*) qPCR test contained *H. pylori* DNA at levels equivalent to 10^5 genomes per cecum.

whether a positive qPCR assay, and/or transmission, was associated with the presence of cultivatable bacteria in the distal gut.

This experiment produced two instances of transmission, both occurring in cages containing *tox*176 donors and *tox*176 recipients (numbers 21 and 22 in Table 3). The two recipients had 10^4 and 10^5 CFU of Hp1 in their stomachs and positive serum ELISA assays. All five of the *tox*176 donors in the two cages, and both positive *tox*176 recipients, had a positive cecal qPCR test (10^5 Hp1 genomes per cecum in each case).

We proceeded to perform qPCR assays of cecal contents harvested from all infected *tox*176 donors and all potential *tox*176 recipients in experiments 1–3, plus the infected normal donors and potential normal recipients noted in Tables 1 and 3. The results revealed a statistically significant higher incidence of positive HP0073 qPCR tests in *tox*176 donor cecums than in normal donor cecums (22/36 (61%) versus 2/25 (8%); $P = 0.0006$, χ^2 analysis; Table 4). In addition, the frequency of positive qPCR assays in the cecums of all potential *tox*176 recipients was significantly higher than in all potential normal recipients (8/23 (35%) versus 0/12; $P = 0.021$, Fisher's exact test). The number of cecums with cultivatable Hp1 was also higher in infected *tox*176 donors than in infected normal donors (5/36 (14%) versus 1/25 (4%); Table 4), although the difference between groups did not achieve statistical significance ($P = 0.17$; Fisher's exact test). The expanded distribution of Hp1 in the GI tracts of *tox*176 versus normal donors and recipients was not attributable to significant differences in the density of colonization of their stomachs ($P = 0.79$, Student's *t*-test) (Tables 1–3).

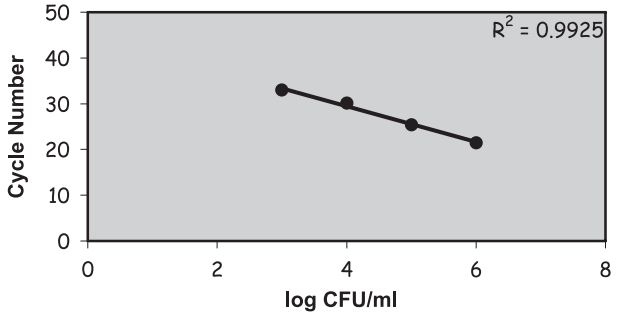

Fig. 1S. Standard curve used for qPCR assay of HP0073 (*ureA*) in cecal contents. Linear regression analysis of PCR cycle number versus log concentration of Hp1 CFU added to a sample of cecal contents harvested from uninfected normal germ-free mice ($n = 3$) prior to extraction of total DNA. All assays were done in triplicate. Mean values are shown. Addition of $<10^3$ CFU did not produce a detectable amplicon signal above background.

4. Discussion

Comparisons of germ-free mice with and without a genetically engineered ablation of the parietal cell lineage indicate that removal of these cells in donor and recipient represents a significant risk factor for transmission, and that this transmission may occur through the fecal–oral route. Several key findings support our conclusions. *First*, a sensitive qPCR assay revealed that the distribution of Hp1 along the gastrointestinal tract was significantly broader in infected parietal cell-deficient *tox*176 donors than in infected mice with a normal parietal cell census. *Second*, transmission was only observed when cages contained Hp1 mono-associated *tox*176 donors and germ-free *tox*176 recipients. Transmission between *tox*176 donors and recipients could not be ascribed to significant differences in the density of coloniza-

Table 4
Parietal cell ablation increases the representation of *H. pylori* in the cecum

Genotype	Number of animals tested	Number of animals with positive assay in the cecum	
		Culture	qPCR
Donors			
*tox*176	36	5 (14%)	22 (61%)
Normal	25	1 (4%)	2 (8%)
Recipients			
*Tox*176	23	0	8 (35%)
Normal	12	0	0

tion of the stomachs of the *tox*176 versus normal donors. Moreover, the ratio of *tox*176 donors to *tox*176 recipients in cages where transmission occurred was not significantly different from the ratio of normal donors to normal (or *tox*176) recipients in cages where transmission was not observed. *Third*, although *tox*176 mice have an expanded population of gastric epithelial progenitors (GEPs) that express sialylated glycan receptors for known *H. pylori* adhesins (i.e. SabA), transmission between these mice cannot simply be attributed to the increased availability of epithelial sites for Hp1 attachment, since Le^b transgenic mice also have an expanded repertoire of adhesin receptors but did not transmit or receive the bacterium. Thus, it appears that parietal cell loss is a key factor in regulating transmission: the presence of adhesin receptors may be contributory but it is not sufficient.

There have only been a few reported studies of *H. pylori* transmission in mice. None involved tests of the role of parietal cells and by extrapolation, acid. Our conclusion that transmission occurs through the fecal–oral route is consistent with a report by Yoshimatsu et al. [34] that examined transmission of a *cagA*+, *vacA*+ *H. pylori* strain (CPY2052) in cages containing a single, conventionally raised athymic nude (*nu/nu*) mouse donor and four *nu/nu* recipients. Animals were housed for 2–4 weeks in ordinary cages, or in cages with steel mesh floors through which feces could pass. Transmission was documented by culture and/or PCR of gastric contents only when animals were raised in ordinary cages and had access to their excreta. Fox et al. [35] have recovered another *Helicobacter* species, *H. mustelae*, from the feces of infected ferrets, leading them to suggest that transmission may occur via the fecal–oral route. Chronic *H. mustelae* infection results in transient elevation of gastric pH [36].

4.1. Hypothesis: in the absence of parietal cells, H. pylori assumes a transmissible state

Based on our findings, we propose the following hypothesis. The enhanced transmission associated with *tox*176 animals is linked to their markedly reduced parietal cell census. Exposure to parietal cell products (e.g. acid) allows *H. pylori* to assume a differentiated state in its niche. Differentiation promotes persistence in this niche, and limits survival (transmissibility) during egress from this milieu. In contrast, we postulate that another state, characterized by enhanced transmissibility, is established and maintained by *H. pylori* in the *tox*176 donor stomach, which lacks parietal cells throughout its glandular epithelium.

We have attempted to determine whether organisms detectable by qPCR in the cecums of colonized *tox*176 mice are infectious by pooling cecal contents, positive for Hp1 CFU, from *tox*176 donors 8 weeks after mono-association with Hp1, and inoculating aliquots of the pool into 6-week-old germ-free *tox*176 (and normal) recipients. Six weeks after inoculation, we were unable to detect CFUs in the stomachs of any recipients ($n = 7$–13 animals per group). Our failure to achieve transmission with a single inoculation of cecal contents may indicate that (i) viability in this transmissible state was perturbed by the conditions encountered during preparation of the inoculum; (ii) full assumption of the transmissible state requires passage to more distal regions of the gut; and/or (iii) the number of organisms in the inoculum was quite low (<100 CFU or five orders of magnitude lower than the dose of ex vivo cultured bacteria used to infect donors). In addition, this type of experiment cannot reproduce the continuous access coprophagic *tox*176 recipients have to the feces of *tox*176 donors, nor does the infectious dose in mice necessarily correspond to the dose required to colonize humans (the normal host).

A corollary to our hypothesis is that the transmissible state increases survival in the stomachs of hypochlorhydric recipients. This would help explain the observed pairing of *tox*176 donors with positive *tox*176 recipients. Of course, we cannot formally rule out the possibility that parietal cell-derived factors other than acid affect the ability of the organism to assume the proposed transmissible phenotype.

Clinical isolates of *H. pylori* show considerable genotypic variation [31,37–43]. Such variation could affect responsiveness to environmental changes in pH. The clinical isolate used for our gnotobiotic transgenic mouse experiments (Hp1) is one of 34 reported strains that have been subjected to whole genome genotyping with DNA microarrays containing ORFs from the fully sequenced 26695 and J99 strains [31,38,41,43,44]. This method identifies genes as 'present' if they share >85% sequence identity, over 60% of the length of an ORF, with the corresponding 26695/J99 ORFs. Based on these criteria, DNA microarray genotyping of Hp1 indicates that it lacks only seven of the organism's 207 reported acid-regulated genes [HP0880, HP0641/JHP0584, and HP1002 (hypothetical proteins without homologies to known proteins; http://genolist.pasteur.fr/PyloriGene/), HP0547 (CagA); JHP0584 (putative 3-hydroxyacid dehydrogenase); HP722/JHP0659 (putative OMP, hopO; homolog of SabA sialyl-$Lewis^x$ binding adhesin); and HP1561/JHP1469 (iron (III) ABC transporter; periplasmic iron-binding protein, ceuE1). These findings suggest that Hp1 should be well equipped to respond to the presence or absence of acid in its environment.

4.2. Humans, hypochlorhydria and H. pylori transmission

Our studies of *tox*176 gnotobiotic mice suggest that hypochlorhydria may be a risk factor for transmission of *H. pylori* via the fecal–oral route in humans. Hypochlorhydria occurs during the acute phases of infection [45] and in the setting of CAG. In patients with CAG, destruction of parietal cells leads to colonization of the stomach with various microbial species, and the potential for eradication of *H. pylori* through competition [46]. Under these circumstances, to guarantee survival of its genotype, a colonizing strain of *H. pylori* is pressed to transmit itself to new susceptible hosts. Loss of the acid barrier also increases the risk of

developing diarrheal illnesses from enteropathogens, thereby increasing the potential for shedding of *H. pylori* [16,47]. Levels of acid production are lower in infants and children than in adults [48]. Thus, *H. pylori*-infected individuals subjected to continuous acid suppression therapy, and/or patients with CAG, may be at increased risk for transmission, and children in their families at increased risk to serve as recipients. Assumption of the transmissible state may require considerable time. The development of CAG in *H. pylori*-infected hosts would provide an opportunity for such microbial adaptation. Conversely, assumption of a differentiated state from a transmissible state may be a process that requires slow evolution of the new host's acid-producing capabilities. Children satisfy these recipient requirements. Intriguingly, in a randomized controlled study, Manes et al. [49] claimed that there was an increased risk for recurrent infection with continuous acid suppression therapy. However, strains were not genotyped in this study, making it impossible to distinguish re-infection from recrudescence. Future studies, conducted in gnotobiotic normal and transgenic mice, should be useful for modeling the contributions of specified microbial genes, as well as parietal cell products (acid), in facilitating or impeding the transfer of *H. pylori* between humans.

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