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Authors

Johnson, Kevin S
Ottemann, Karen M

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Colonization, localization, and inflammation: The roles of *H. pylori* chemotaxis *in vivo*

Short title: Chemotaxis and infection in *Helicobacter pylori*

Kevin S. Johnson and Karen M. Ottemann*

Department of Microbiology and Environmental Toxicology, University of California Santa Cruz, Santa Cruz, CA 95064 USA

* To whom correspondence should be addressed at Ottemann@ucsc.edu

Abstract

Helicobacter pylori is a gram-negative bacterium that infects half of the world's population, causing gastritis, peptic ulcers, and gastric cancer. To establish chronic stomach infection, *H. pylori* utilizes chemotaxis, driven by a conserved signal transduction system. Chemotaxis allows *H. pylori* to sense an array of environmental and bacterial signals within the stomach, guiding its motility towards its preferred niche within the gastric mucosa and glands. Fine-tuned localization, regulated by the chemotaxis system, enables robust colonization during the acute stage of infection. During chronic infection, chemotaxis helps maintain bacterial populations and modulates the host immune response. Given its importance in host colonization and disease, chemotaxis is an attractive target for future treatments against *H. pylori* infections.

Highlights

- The *H. pylori* chemotaxis system includes classical and auxiliary chemotaxis proteins
- *H. pylori* senses several host and bacterial ligands through four chemoreceptors
- Chemotaxis is critical for colonization during acute infection
- Chemotaxis modulates host inflammation during chronic infection

Introduction

Helicobacter pylori is a gram-negative bacterium that has evolved a keen ability to chronically colonize the stomach, infecting roughly half of the world's population [1]. Colonization can lead to the development of chronic gastritis, gastric and duodenal ulcers,

and gastric cancers [2,3]. Colonization is also linked to potential health benefits, including protection against allergic asthma, and inflammatory bowel and esophageal diseases, presumably though modulating effector T-cell populations or gastric acid production [4–7]. The beneficial and pathogenic aspects of *H. pylori* require colonization, therefore an important goal is to understand the molecular basis of the bacterial factors that promote chronic infection.

H. pylori colonizes the primate stomach, a harsh environment. The stomach lumen ranges between pH 1-5 [8], conditions at which *H. pylori* is viable for only ~30min [9]. Additionally, stomach contents are cleared regularly, and the gastric mucosa undergoes constant turnover [10]. Accordingly, *H. pylori* must rapidly initiate colonization and localize where the environment is more hospitable: within 15μm from the gastric epithelial cells [11], and deep within gastric glands [12].

H. pylori colonization is promoted by chemotaxis, the focus of this review. Chemotaxis is a process that enables *H. pylori* to sense environmental signals and regulate its motility to move away from harmful conditions and towards favorable ones [13]. Chemotaxis promotes colonization and plays a role in modulating host immune responses [14–16]. In addition to chemotaxis, *H. pylori* utilizes a suite of colonization factors including urease, cell shape, adhesins, the Cag pathogenicity island type four secretion system, and the toxin VacA. Readers are referred to several excellent recent reviews on these topics [7,17–19].

The *H. pylori* signal transduction system transforms ligand presence into a swimming response

Chemotaxis signal transduction systems allow bacteria to direct their motility [13,20,21]. Such systems are widespread, present in ~50% of bacterial species, highlighting the strong fitness advantage they confer [20]. The chemotaxis system of *H. pylori* contains core chemotaxis proteins found in all chemotaxis systems, and auxiliary ones found in only some (Figure 1). The core chemotaxis proteins are the chemoreceptors (TlpA, TlpB, TlpC, and TlpD), the CheW coupling protein, the CheA kinase, and the CheY response regulator [13,22]. The *H. pylori* auxiliary chemotaxis proteins include three CheV-type coupling proteins (CheV1, CheV2, and CheV3), the CheZ phosphatase, and the unique chemotaxis protein ChePep, which localizes CheZ to the poles [12,23–28]. Environmental signals are sensed either directly or indirectly by chemoreceptors, and are relayed to the histidine kinase CheA via the CheW or CheV1 coupling proteins [13,27]. Currently, the role of CheV2 and CheV3 are unknown. Chemicals sensed as repellents activate CheA's auto-phosphorylation, and this phosphoryl group is subsequently passed to CheY via histidine-to-aspartate phosphorelay [29]. Phosphorylated CheY interacts with the flagellar motor, causing it to rotate clockwise and the bacteria to reverse or change direction [30,31]. Alternatively, attractants squelch CheA's auto-phosphorylation; non-phosphorylated CheY does not interact with the motor, and the bacteria swim straight, without direction changes. Mutants in any one of these proteins are non-chemotactic (Che^-) to varying degrees and with different swimming behavior. Accordingly, *cheW*, *cheA*, *cheY*, *cheV1*, and

cheV2 mutants are all Che$^-$, displaying straight swimming phenotypes, likely because they do not produce phosphorylated CheY [24,25,32,33]. *cheZ*, *chepep*, *cheV3* mutants are also Che$^-$, but display hyper-reversal phenotypes, apparently because they produce high amounts of phosphorylated CheY [12,24–26]. Readers are referred to several excellent reviews of this system for more molecular details [18,34,35].

***H. pylori* senses multiple host and bacterially-generated conditions as repellents and attractants**

H. pylori senses specific chemicals and conditions via three transmembrane chemoreceptors with periplasmic sensing domains—TlpA, TlpB, TlpC— and one cytoplasmic receptor, TlpD [16,36–38]. The signals of these chemoreceptors and their distribution likely play a driving role in the localization of *H. pylori in vivo*. Thus, efforts have been made to determine *H. pylori*'s sensing profile.

H. pylori experiences multiple repellent conditions. Several of these are host generated, including acidic pH, reactive oxygen species (ROS), and bile [37,39,40]. The acidic stomach lumen is toxic to *H. pylori* [8,9]. Accordingly, acid is a potent chemorepellent, with TlpA, TlpB, and TlpD playing roles in sensing [37,41,42], and TlpC playing a role in modulating the acid response [43]. Currently, there is only a mechanistic proposal for how TlpB senses acid, via amino acids that are variably protonated [41]. Another signal, ROS [39], is produced by host epithelial and immune cells [44–46]. TlpD senses ROS via an unknown mechanism [39]. Bile acids are toxic to *H. pylori* and are sensed via unknown chemoreceptor(s) [40,47]. Bile is released from the gallbladder, into the

duodenum [48]. The repellent and toxic properties of bile may explain why *H. pylori* does not colonize this region [40,47,49].

H. pylori is also repelled by the self-generated, quorum-sensing molecule autoinducer-2 (AI-2), and responds to its own electron transport chain (ETC) [38,50]. AI-2 is sensed by TlpB as a chemorepellent, in a manner dependent on the periplasmic proteins AibA and AibB [50,51]. A repellent response to AI-2 may promote dispersion of *H. pylori in vivo*. *H. pylori* also senses disruptions to the ETC via TlpD, but the physiological change required to induce this sensing is unknown [38,52,53]

In addition to chemorepellents, many of which are harmful to the bacterium, *H. pylori* also senses several beneficial chemoattractants. One of these chemoattractants, urea, is sensed by TlpB [40,54–56]. In non-infected individuals, urea is available at concentrations between 5-21mM within the stomach [57], and is hydrolyzed by *H. pylori* urease into ammonia and bicarbonate to buffer its local environment [7]. Arginine is sensed as a chemoattractant by TlpA [40,58,59], and is an essential amino acid for *H. pylori* [60,61]. In both urea and arginine chemotaxis, chemoattraction may help *H. pylori* find key nutrients, while also directing it toward the epithelial surface.

There are several additional chemotactic signals that have not been extensively studied. For example, *H. pylori* uses chemotaxis to migrate to the site of gastric damage [62]. However, the exact host-derived chemicals that direct this response are unknown. Another attractant that has not been mapped to a chemoreceptor is cholesterol [63].

Chemotaxis during *in vivo* colonization

Che$^-$ *H. pylori* mutants have severe colonization defects (Figure 2), and appear to interact with host tissue differently based on their aberrant inflammatory responses (Figure 2). These aspects are discussed in the following sections.

Chemotaxis is required for wild-type colonization

Chemotaxis is required for wild-type (WT) level colonization of the stomach, based on studies using a variety of Che$^-$ mutants, lacking CheA, CheY, CheW, or ChePep. In some cases, Che$^-$ mutants did not colonize at all [16,32], and in others they colonized poorly [14,15,33,49,62,64]. Che$^-$ mutants require 100-fold more bacteria to initiate colonization [33], thus some studies might have employed *H. pylori* doses that were below the infectious dose. Overall, these results show that chemotaxis is critical during the initial stages of infection, particularly with low infectious doses, such as could be experienced during human infection.

Chemotaxis during early infection

For roughly the first three months of an infection, chemotaxis is critical. During this period, Che$^-$ mutants display significant colonization defects that are greater in one region, the antrum [33,49]. The stomach is extensively invaginated into over 25,000 glands, and Che$^-$ mutants fail to robustly colonize these niches, especially within the antrum [12,64,65]. During the first month of a WT infection, the number of glands infected and the amount of

H. pylori per gland increases. Che- mutants, however, infect few glands initially and fail to promote this same population expansion between and within glands [64].

Chemotaxis during chronic infection

A hallmark of *H. pylori* colonization is its ability to achieve chronic infection. The role of chemotaxis during the chronic stage has been more difficult to evaluate. However, it seems that in the absence of challenge by WT, Che- *H. pylori* are able to achieve WT levels of colonization as early as one month post infection, maintaining comparable levels up to six months post infection and presumably longer [14,33,49,64]. During the chronic stage of infection, Che- *H. pylori* colonize gastric glands in the corpus and antrum, while WT populations also colonize the mucus layer of the corpus [64]. Additionally, Che- mutants have been observed to be less closely associated with the gastric epithelium during this stage [14].

Competition

Che- *H. pylori* have general colonization defects when they are the sole infecting strain, with even greater colonization defects observed during competition experiments. When mice are infected with equal doses of WT and Che- *H. pylori*, the Che- mutant is outcompeted by over 1000-fold [33,36]. If the infections are given sequentially, a secondary WT infection can displace a primary population of Che- *H. pylori*, nearly displacing the entire gland population of Che- bacteria. In contrast, a secondary WT infection is unable to displace a primary population of WT [33,64]. This finding suggests

150 that chemotaxis is needed to maintain colonization during chronic colonization, especially
151 when WT *H. pylori* are present.

152 Role of chemoreceptors for colonization

153 Chemoreceptors head the chemotaxis signal transduction system. Accordingly,
154 several studies have examined the *in vivo* phenotypes of chemoreceptor mutants. Loss of
155 individual chemoreceptors alters the sensing profile of *H. pylori*, but does not cause a
156 complete loss of chemotactic ability. Specifically, bacteria would lose the ability to sense
157 some compounds, perhaps biasing bacteria towards or away from signals sensed by the
158 remaining chemoreceptors. Such a change could lead *H. pylori* towards more inhospitable
159 environments.

160 *tlpA* and *tlpC* mutants colonize mice to WT levels during single strain infections, but
161 are outcompeted by WT during competition infections [36]. This phenotype suggests that
162 WT exacerbates the mutant's defect in some way, possibly by competing for nutrients or
163 causing a host response that the mutant cannot avoid. This idea would fit well with the
164 function of TlpA for finding arginine [58,59].

165 *tlpB* mutants have either no or subtle defects that appear more pronounced later in
166 infection [14,37,49,56]. Interestingly, *tlpB* mutants are not outcompeted by WT [14,16],
167 suggesting that WT does not exacerbate the mutant's defect. This outcome suggests TlpB's
168 signal, urea, is not a limiting substance for *H. pylori*, and fits well with its relatively high
169 (mM) levels [57]. The late infection defects observed for *tlpB* mutants could be related to
170 enhanced inflammation (see below) or to defects associated with AI-2 chemotaxis [14,50]

In single strain infections, *tlpD* mutants have substantial colonization defects early in infection, greater than Che$^-$ *H. pylori* [49,52]. Additionally, *tlpAD* double mutants have an even greater colonization defect, and are defective in antral gland colonization [42]. Treatment of *tlpAD*-infected mice with omeprazole, which blocks acid production, rescues *H. pylori* levels to that seen in *tlpD* single strain infections [42,49]. This result suggest the inability to sense acid via TlpA and D reduces *in vivo* fitness, and indicates the remaining colonization defect in *tlpD* infections is potentially due to an inability to sense ROS or ETC conditions [38,39].

Role of auxiliary chemotaxis proteins in colonization

Loss of the auxiliary chemotaxis proteins creates *H. pylori* mutants that are either fully or partially Che$^-$ [12,23,25,26]. Likewise, mutants lacking the CheVs or ChePep display colonization defects that range from severe, similar to fully Che$^-$ mutants, to less severe [12,25,65]. Overall, data supports that auxiliary proteins can have as substantial an effect as core proteins.

Chemotaxis modulates host inflammation

Host inflammation is a major disease outcome of *H. pylori* colonization. Inflammation occurs upon recognition of microbial-associated molecular patterns (MAMPs) or damage associated molecular patterns (DAMPs) by local monocytes, macrophages, and epithelial cells. These cells release pro-inflammatory cytokines and chemokines, the latter of which recruits neutrophils and antigen presenting cells, including macrophages and dendritic cells, to the site of infection [66,67]. Dendritic cells release

192 cytokines and, process and present *H. pylori* antigens, priming T-cell differentiation [7,66–
193 71]. *H. pylori* colonization induces the differentiation of pro-inflammatory T-helper 1 (Th1)
194 and T-helper 17 (Th17) cells and anti-inflammatory T-helper 2 (Th2) and T-regulatory
195 cells (Tregs) [7,66,70,71]. The degree of host inflammation is modulated by the balance of
196 effector T-cell populations. *H. pylori* induces inflammation via its intimate interaction with
197 the gastric epithelium and the production of virulence factors such as the type 4 secretion
198 system, CagA, NapA, and VacA [7,66,70]. The inflammatory response to *H. pylori*
199 colonization, however, is also modulated by chemotaxis [14–16].

200 Chemotaxis was first shown to modulate inflammation using a *tlpB* mutant in a
201 gerbil model of infection [16]. *tlpB* mutants colonized gerbils to WT levels at four weeks
202 post infection, but induced only low gastric inflammation, as measured using histological
203 enumeration of lymphocytes. While *tlpB* mutants induced less inflammation than WT, *tlpB*
204 infected gerbils had high neutrophil recruitment to the gastric tissue, such as seen a few
205 days after *H. pylori* infection. This phenotype suggested they were mimicking an early stage
206 of infection [16].

207 Subsequent studies extended this work to the mouse model, examining the roles of
208 all chemoreceptors [14]. Three months post-infection, Che$^-$, *tlpA*, and *tlpB* mutants induced
209 modestly less inflammation than WT. At later time points, six months post-infection, *tlpA*
210 and *tlpB* mutants caused high inflammation, while Che$^-$ mutants induced low inflammation.
211 At both time points, all strains colonized to WT levels. Overall, these results made it clear

212 that the inflammatory response was modulated by bacterial properties controlled by
213 chemotaxis, and was affected independent of overall bacterial load [14].

214 To gain insight into why Che$^-$ mutants induce less inflammation, Rolig *et al*. (2011)
215 examined the specific immune cell populations recruited to the stomach. While Che$^-$
216 mutants induce less histologically-evident inflammation, the total number of CD4+ T-cells
217 recruited was equivalent between mice infected with WT or Che$^-$ *H. pylori* two months post
218 infection [15]. WT infections, however, were found to have higher amounts of pro-
219 inflammatory Th17 cells, as assessed by expression of associated genes, and an overall
220 elevated ratio of Th17/Treg cells compared with Che$^-$ infections. A possible explanation for
221 the ability of WT to trigger a Th17 response came from the observation that WT infections
222 induced more gastric tissue apoptosis than did Che$^-$ infections [15]. Because apoptosis is a
223 host response that can lead to the differentiation of Th17 cells [72], it seemed plausible that
224 the inability to trigger apoptosis partially explains why Che$^-$ infections lack Th17 cells. It is
225 still not fully understood why WT *H. pylori* induces more apoptosis and a more robust Th17
226 response, however, it is now known that Che$^-$ mutants are mis-localized [12,14,64]. Mis-
227 localization possibly induces modified interactions with distinct cell types compared to WT
228 infections, modulating the resulting inflammatory response. The reason for the elevated
229 inflammation of *tlpA* and *tlpB* mutants is still under study.

230 **Conclusions**

231 Chemotaxis is one of several colonization factors utilized by *H. pylori* to promote
232 chronic infection of the stomach [7,12,14,16,25,32,33,49]. Work over the past 20 years

strongly supports that chemotaxis helps *H. pylori* find nutrients such as urea and arginine, and avoid toxic substances such as acid and ROS. The list of critical compounds sensed by *H. pylori* will undoubtedly grow as more work is done to better understand the sensing profile of each chemoreceptor. Chemotaxis plays roles in localization and modulating the host immune response. The stomach landscape is comprised of multiple niches, and studies support that chemotaxis is particularly critical for colonization during early infection, especially in the antrum, and for spread into new glands [12,49,64]. During established infections, chemotaxis is less critical for colonization, perhaps because *H. pylori* has been able to stochastically access the glands and does not demand chemotaxis for growth in these pockets. Instead, in late-stage infections, the role of chemotaxis in inflammation control becomes apparent [14–16]. Understanding the mechanisms by which chemotaxis modulates host inflammation will facilitate our understanding of how bacteria control this important process, and could lead to future treatments to modify disease outcomes. Several pathogens are motile and chemotactic. *H. pylori* is leading our understanding of the roles for this important process *in vivo*, and it will be of great interest to assess how many of the same principles translate across bacterial systems and organs.

Conflicts of interest

None.

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255 study design, data collection and interpretation, or the decision to submit the work for
256 publication.

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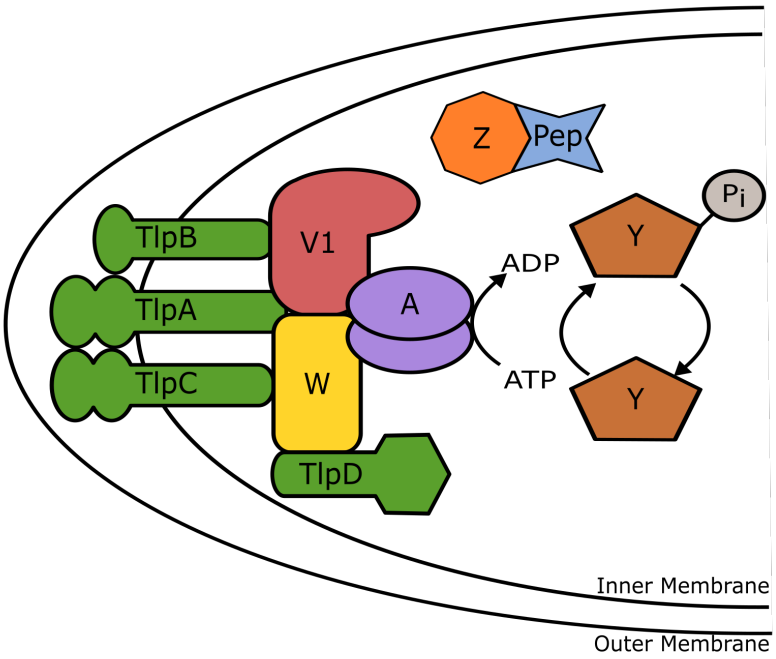
259 **Figure Legends, Figures, and Tables**



260

261 Figure 1- *H. pylori* chemotaxis system. Chemotactic signals are sensed by the
262 chemoreceptors TlpA, TlpB, TlpC, and TlpD. Signals are relayed through the coupling
263 protein CheW (W) or the auxiliary CheV-type coupling protein CheV1 (V1) to the histidine
264 kinase CheA (A). Repellents promote CheA auto-phosphorylation, while attractants squelch
265 CheA auto-phosphorylation. Phosphorylated CheA passes its phosphoryl group to the CheY
266 (Y) response regulator via histidine to aspartate phosphorelay. Phosphorylated CheY
267 interacts with the flagellar motor and is dephosphorylated by CheZ phosphatase (Z), in

complex with ChePep (Pep). CheV2 and CheV3 are not depicted as their role is this system is unknown.

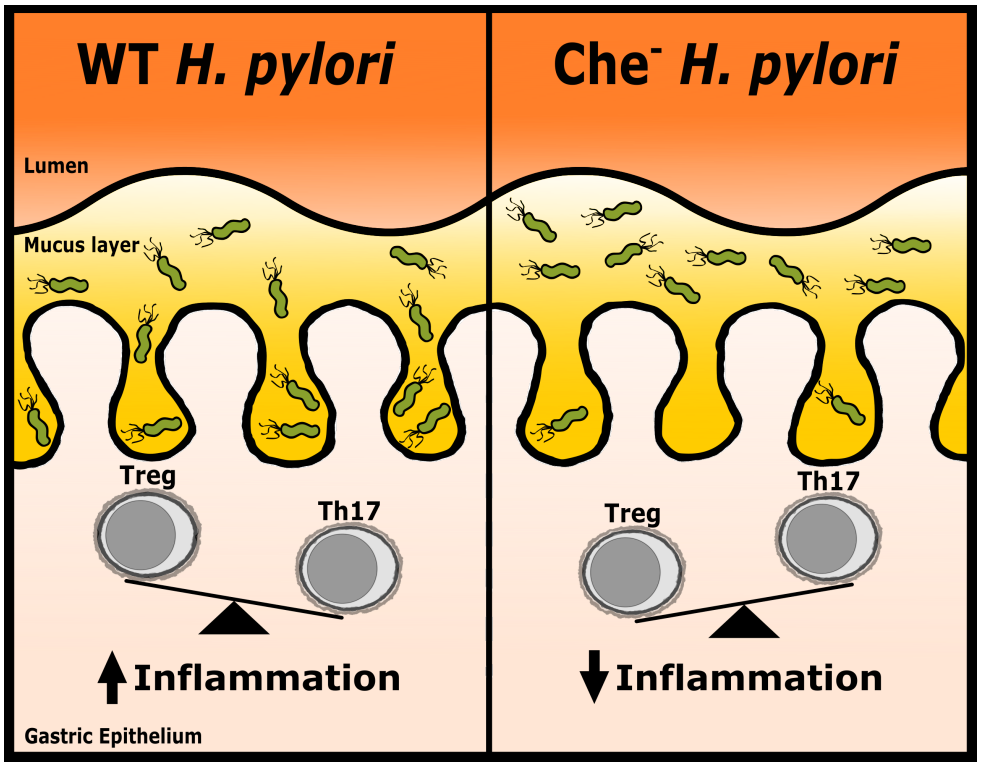


Figure 2- Chemotaxis promotes localization and modulates inflammation *in vivo*. Wild-type (WT) *H. pylori* robustly colonizes gastric glands early during infection, and can replicate and spread between glands. Conversely, non-chemotactic (Che$^-$) *H. pylori* fail to colonize gastric glands to the same degree. Concurrently, WT *H. pylori* initiates a proinflammatory immune response, with a substantial T-helper 17 cell (Th17) response, while non-chemotactic *H. pylori* promotes a dampened immune response, skewed toward T-regulatory cells (Tregs).

Papers of significance

(*= Of special interest, **= Of outstanding interest)

*Lertsethtakarn et al. 2012- A review covering the *H. pylroi* chemotaxis system in greater molecular detail.

**Goers Sweeney et al. 2012- This paper solved the first crystal structure of the sensing portion of the *H. pylori* chemoreceptor, TlpB. Additionally, a proposed mechanism for pH sensing by TlpB is reported.

**Huang et al. 2015- This study describes the mechanism of urea sensing by TlpB.

*Huang et al. 2017- This study uncovers the role of TlpA, TlpB, and TlpD in pH sensing and showed TlpAD mutants have sever colonization defects *in vivo*.

*Croxen et al. 2007- The first paper to demonstrate *H. pylori* senses acidic pH through TlpB.

*Rader et al. 2011- This study uncovers the role of TlpB in sensing the bacterial derived signaling molecule, autoinducer-2.

*Collins et al. 2016- This study demonstrates *H. pylori* senses reactive oxygen species through TlpD.

*Schweinitzer et al. 2008- This study demonstrates *H. pylori* is capable of sensing changes in electron transport chain activity through TlpD.

*Aihara et al. 2014- This study determined *H. pylori* is attracted to the site of gastric injury *in vivo* via two-photon microcopy, in a manner dependent on chemotaxis.

**Terry et al. 2005- This is a seminal paper investigating the role of *H. pylori* chemotaxis *in vivo*. This study demonstrated that non-chemotactic *H. pylori* infect to lower levels and are defective in antral colonization, determined the infectious dose of non-chemotactic *H. pylori*, and preformed competition experiments with wild-type and non-chemotactic *H. pylori*.

**Keilberg et al. 2016, MBio- A comprehensive study of wild-type and non-chemotactic *H. pylori* population dynamics within the gastric glands and mucus layer of the antrum and corpus of the stomach over a 6-month period. Additionally, competition experiments with wild-type and non-chemotactic *H. pylori* were performed.

**Howitt et al. 2011- This study determined the auxiliary chemotaxis protein, ChePep, is required for chemotaxis, and *ChePep* mutants are unable to colonize gastric glands within the antrum. It was the first demonstration of the use of chemotaxis to colonize the glands.

*Rolig et al. 2012- This work demonstrated that non-chemotactic *H. pylroi* have a general colonization defect within the antrum, and that TlpD mutants, overall, have a more severe colonization defect than non-chemotactic *H. pylori*.

**Williams et al. 2007- A comprehensive study investigating the role of each chemoreceptor *in vivo* over a 6-month infection. Inflammation, as scored by histology, and overall bacterial loads are measured. This work identified differential immune responses elicited by TlpA, TlpB, and Che$^-$ *H. pylori*.

317 **Rolig et al. 2011- This study investigates the host-immune responses elicited during
318 infection with wild-type and non-chemotactic *H. pylori*. Host gene expression and flow
319 cytometry is used to uncover the unique effector T-cell populations recruited during each
320 infection.

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